QUALITY CHARACTERISTICS OF GROUND ROUND
FORMULATED WITH THREE FAT SOURCES

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by

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

EVALUATING QUALITY CHARACTERISTICS OF GROUND ROUND FORMULATED WITH THREE FAT SOURCES

Presented by Zachary D. Callahan
A candidate for the degree of Master of Science
And hereby certify that in their opinion it is worthy of acceptance

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EVALUATING QUALITY CHARACTERISTICS OF GROUND ROUND FORMULATED WITH THREE FAT SOURCES

Zachary D. Callahan
Dr. Bryon R. Wiegand, Thesis Supervisor

ABSTRACT

This research was performed to determine if changing the source of fat for inclusion in ground beef from the round will cause a change in the number of days that the ground beef has a viable shelf-life as influenced by oxidation. The objectives were to evaluate the color stability and degree of fat oxidation during retail storage of ground beef patties and to determine the influence of fatty acid profile on the extent of ground beef lipid oxidation and shelf-life of ground beef patties over 7 d of refrigerated retail storage. Beef carcasses (n = 30) were chilled for two days and then fabricated. Semimembranosus muscles (IMPS # 168), commonly referred to as top or inside round, were removed and closely trimmed. Within each carcass, three sources of fat (kidney and pelvic = KP, subcutaneous = S, or seam = I) 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. 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 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 to be used for the shelf study. Patties were placed on Styrofoam® trays and overwrapped with oxygen permeable, polyvinyl chloride and placed in refrigerated retail storage (4°C) where an instrumental measurement of color (L*, a*, b*) and thiobarbituric acid reactive substances (TBARS) were collected on d 1, 3, 5, and 7 of the study. The additional 447 g of sample was placed in a whirl-pack bag, stored at 4°C, and used for fat and moisture determination, oxymyoglobin concentration, fatty acid analysis, and calculated iodine value (IV). Data analysis indicated different (P = 0.0004) saturated fat (SFA) percentages (KP > I > S with means of 57.9, 53.5, and 52.1%, respectively). Calculated IV differed (P = 0.003) where KP < I = S with means of 33.9, 37.1, and 38.3, respectively. Changes in fat profile likely explain differences (P = 0.007) in TBA values where KP = S < I with means of 0.110, 0.118, and 0.120 mg/kg, respectively. However, fat percentage of ground beef did not change (P = 0.83) TBA values (75% = 0.116 mg/kg and 93% = 0.116 mg/kg). Minolta a* value differed (P = 0.004) where S > KP with I not differing from S or KP (S = 14.78, I = 14.52, and KP = 14.46). However, these differences in Minolta a* reflectance are not likely discernible by visual appraisal of ground beef patties. Fat source is a significant contributor to quality indicators in ground beef patties and should be considered when formulating products intended for fresh, refrigerated retail sale.
CHAPTER I

LITERATURE REVIEW

TRENDS IN GROUND BEEF CONSUMPTION AND VALUE

Beef is the second most consumed meat and is the most consumed red meat in the United States. Beef is also a primary protein source for Americans. Since 2010 there has been a spike in beef prices, especially in ground beef, which reached its highest recorded price of $3.41/ pound in January of 2013, compared to the 2003 average of $1.95/ pound (Bureau of Labor Statistics Data, 2013). With the spike in beef prices and economic factors, consumption of beef is on the decline. The highest per capita consumption of beef on record was 88.8 pounds in 1976 and has fallen to 58.7 pounds in 2009 (American Meat Institute, 2009). Consumers are either trading out, switching to a less expensive meat, such as chicken, or eating more meatless meals; or trading down, staying in the category by buying less expensive beef cuts (McCarty, 2011). Trading down is the driving factor why there is an increased demand for ground beef while overall consumption of beef is declining. Consumers are purchasing fewer expensive cuts of beef such as steaks and more inexpensive cuts, ground beef being the front runner. Consumers are saving money by purchasing less food that they consider to be luxury foods or foods for special occasions and also by eating out less or at less expensive restaurants. In a study on cost-saving strategies,
consumers were asked what foods were considered luxury foods or foods for special occasions; 62% of the respondents mentioned beef, 42% said steak, 13% mentioned a specific steak, and 7% mentioned beef generically (McCarty, 2011). As consumers move away from eating out as much and more towards at home meals for cost savings, ground beef consumption increases. Ground beef is used in many quick and easy at-home meals such as hamburgers, spaghetti, and meat pizzas just to name a few. In 2005, nearly 65% of all beef was purchased at retail stores and thus is considered to be at-home food and ground beef had the highest at-home consumption per capita (Davis and Lin, 2005). Consumers that still eat away from home are ordering less expensive items on the menu or eating at less expensive restaurants, both leading to greater ground beef consumption.

According to the 1994-98 Continuing Survey of Food Intakes by Individuals, household income, the region of the United States, and rural versus urban living can also effect ground beef consumption. The poverty guidelines used for this study were developed by the U.S. Department of Health and Human Services for implementation of Federal food programs. At the time of the study the low income category accounted for about 19% of U.S. households, the middle income category accounted for 42% of U.S. households, and the high income category accounted for 39% of U.S. households. The study revealed that low income households consumed more kg of beef per capita, 71.94, than did middle and high income households, 67.63 and 62.61, respectively (Davis and Lin, 2005). It also showed that ground beef was the highest kg per capita
consumption for all income categories compared to other cuts of beef (Davis and Lin, 2005). Within the ground beef category, low income households consumed the highest kg per capita of 14.43 followed by middle income households at 12.73 and high income households at 11.72 (Davis and Lin, 2005). This shows that ground beef consumption increases as income decreases and with a downturn in the economy since this study was conducted a higher percentage of households are in the low and middle income categories and consuming more kg of ground beef per capita. Of the ground beef consumed, lower income households consumed the highest percentage, 53.03, at home compared to 51.05 and 45.80 percent for middle and high income households, respectively (Davis and Lin, 2005). Lower income consumers are more likely to purchase ground beef for at home use rather than paying higher prices for a prepared meal at a restaurant. Beef consumption also varies between the geographical regions of the U.S. The dominant beef product consumed in all regions was ground beef but the individual regions varied on kg per capita (Davis and Lin, 2005). The Midwest consumed the greatest amount of kg per capita at 14.17, followed by the South consuming 13.15 kg per capita, the West consuming 12.40 kg per capita, and the least amount was the Northeast consuming 10.27 kg per capita (Davis and Lin, 2005). The amount of the population living in urban, suburban, and rural areas within these regions leads to the differences in consumption. Again, the dominant beef product consumed in all three areas was ground beef (Davis and Lin, 2005). Consumers in rural areas ate the greatest amount of ground beef per
capita at 15.15 kg, and suburban and urban areas were much less at 11.61 and 12.56 kg per capita, respectively (Davis and Lin, 2005).

With ground beef consumption and price increasing, and more consumers purchasing ground beef for at home use it would be beneficial to supermarkets to extend shelf-life to decrease lost value. Most of this fresh ground beef is lost to oxidation and discoloration. Ground beef sold at retail or supermarkets is sold fresh, meaning that it is refrigerated not frozen and only has a few days of viable shelf-life before it is deemed unusable. The meat is normally placed on a Styrofoam® tray and wrapped with a clear film. This allows the consumer to view the meat and make their purchase on quality. If the meat begins to lose its cherry red color and become dull or start to oxidize and turn brown supermarkets will have to put that product on sale to move it or deem it unusable as a total loss. According to Kichang et al. (2012), “The loss of value due to discoloration in beef at the retail level in the U. S. could be over 700 million dollars per year”. In a study conducted by Perishables Group, Inc., supermarket loss estimates were determined by comparing supplier shipment data with point of sale data from six large national and regional supermarket retailers in 2005 and 2006. The percentage of beef loss was 4.3 in 2005 and 4.4 in 2006 (Buzby et al., 2009). This is a relatively low percentage compared to other products such as seafood, lamb and veal at 9.0, 12.3, and 25.4 percent respectively, however, in that large of an industry there is a significant amount of lost value (Buzby et al., 2009).
GROUND BEEF BLENDS

Ground beef is a cut that can be identified by numerous names in a retail shelf. Some of the labels are meant to reveal to the consumer exactly what they are purchasing while others can be more misleading. The USDA recognizes the categories of ground chuck, ground round, chopped sirloin, ground beef, hamburger, pure beef patties, pure beef patty mix, beef patties, and beef patty mix on labels for ground beef products (USDA Ingredient and Labeling Requirements, 2013). Each one of these categories is distinguished by the ingredients they contain. To be considered ground chuck, ground round, or chopped sirloin the product can only contain the ingredients of skeletal muscle and skeletal trimmings (USDA Ingredient and Labeling Requirements, 2013). To be considered ground beef the product can only contain the ingredients of skeletal muscle, skeletal trimmings, head meat, and up to 25% cheek meat that must be listed on the label (USDA Ingredient and Labeling Requirements, 2013). To be considered hamburger, the product can only contain the ingredients of skeletal muscle, skeletal trimmings, head meat, up to 25% cheek meat that must be listed on the label, and added beef fat (USDA Ingredient and Labeling Requirements, 2013). To be considered pure beef patties the product can only contain the ingredients of skeletal muscle, skeletal trimmings, head meat, up to 25% cheek meat that must be listed on the label, and partially defatted chopped beef (USDA Ingredient and Labeling Requirements, 2013). Pure beef patty mix
can only contain the same ingredients as pure beef patties except the partially defatted chopped beef is also required to be on the label (USDA Ingredient and Labeling Requirements, 2013). Beef patties and beef patty mix can contain skeletal muscle, skeletal trimmings, head meat, up to 25% cheek meat, partially defatted chopped beef, partially defatted beef fatty tissue, beef hearts, water, binders, extenders, fillers, and organ meats (USDA Ingredient and Labeling Requirements, 2013). For beef patties, the ingredients of cheek meat, partially defatted beef fatty tissue, beef hearts, water, binders, extenders, fillers, and organ meats are required to be on the label (USDA Ingredient and Labeling Requirements, 2013). For beef patty mix, the ingredients of cheek meat, partially defatted chopped beef, partially defatted beef fatty tissue, beef hearts, water, binders, extenders, fillers, and organ meats are required to be on the label (USDA Ingredient and Labeling Requirements, 2013).

The total fat percentage and how that is represented on the label complicates the product further. According to the USDA, the maximum fat content in any ground beef product is 30%. It is also important to consider that adipose tissue is not exclusively comprised of lipids. By definition, fat is lipid cells suspended in a connective tissue matrix. Nurnberg et al. (1998), explains that adipose tissue can contain between 70 to 90% fat, 5 to 20% water and roughly 5% connective tissue. Griffin (2013) states, “Ground beef in most retail outlets contains from the maximum 30% fat down to approximately 5% fat with the majority being 75 to 85% lean”. That being said the fat content is not required on the label and may just be described by one of the ground beef product categories.
such as ground round. Ground round is considered one of the leaner cuts and would suggest to consumers that the product would have a low fat content but according to regulations on that label it could contain up to 30% fat. This led to a revision to the nutritional labeling requirement in 1994, allowing a percent lean/ percent fat descriptor on ground beef products (Griffin, 2013). Most retail ground beef products will contain this percent lean/ percent fat descriptor to allow consumers to select specific fat content in their ground beef products for the intended use of the product or health considerations.

In today’s market there is a need for leaner meat with increasing numbers of Americans becoming more health conscious in their food choices. It is advised that Americans need to decrease their intake of total fat, saturated fat, and cholesterol while increasing the amount of monounsaturated and polyunsaturated fat (Dietary Guidelines Advisory Committee, 2010). Survey data collected in 2009 revealed that 63% of consumers are trying to consume less animal fat (International Food Information Council Foundation, 2009). For most beef cuts, the easiest ways to decrease the amount of external or subcutaneous fat is to start by producing a carcass with less external fat (lower yield grade), or more closely trimming during butchering. The amount of external fat on retail cuts has dropped from 1.3 cm in the 1980s to 0.24 cm in 2005 (Cross et al., 1986; Savell et al., 2005). Cross et al. (1986) also suggested that consumers would be willing to pay a slightly higher price per pound for closely trimmed cuts. This is seen in the current market with lean ground beef costing more than ground beef with a higher fat content.
Ground beef has the advantage of ranging from the maximum of 30% fat content as previously stated, to an extremely lean product to meet consumer demands. However, decreasing the fat content may also negatively affect consumer acceptance. Fats are included in ground products to develop texture, mouth feel, and assist in the lubricity of the product (Giese, 1996). Egbert et al. (1991) reported that reducing fat content reduces overall acceptability due to flavor intensity, juiciness, and tenderness being directly correlated to fat content. Wong and Maga (1995) also showed higher juiciness and tenderness scores as well as lower cooking yields with increasing fat levels in ground beef. However, their results also indicated no statistical differences in the sensory characteristics of aroma and flavor at different fat levels. Carpenter and King (1969) used a taste panel to compare ground beef samples containing 5% to 30% fat in 5% increments. Their results showed that ground beef containing 15% fat had the best flavor, those containing 20% fat had the best texture and were rated highest for overall satisfaction, and those containing 30% fat were juiciest. Brewer (2012) states, “Over half of the volatiles identified in cooked meat result from lipids which serve as primary sources of flavor compounds. The formation of aldehydes, free fatty acids, ketones, alcohols, and hydrocarbon compounds in lipids are important contributors to meat flavor (Mottram, 1998). Tobin et al. (2012) reported that off-flavor correlated with patties containing 50 and 60% fat and was significantly lower (P < 0.001) for patties containing 40% fat. They concluded that the higher fat level increased the propensity for lipid oxidation which in turn created an off-flavor in the ground beef patties.
There are multiple proven methods to measure the fat content in ground beef. *Dow et al.* (2011) compared three methods to determine their effectiveness of fat determination on 119 ground, beef steak samples. The three methods consisted of a 2:1 chloroform:methanol (Folch) extraction performed according to Folch *et al.* (1957), an ether extraction performed according to AOAC method 960.39 (AOAC, 2007), and a microwave drying and nuclear magnetic resonance (CEM) extraction performed according to Keeton *et al.* (2003). The results indicated that Folch detected a greater amount of fat than CEM (P < 0.05) and ether was not different (P > 0.05) from CEM or Folch (*Dow et al.*, 2011). Numerically, CEM detected the smallest amount of fat, followed by ether, and Folch detected the greatest amount of fat for each treatment group of ground steak (*Dow et al.*, 2011). Differences can be explained by the methods used for extraction. Shahidi and Wanasundara (2002) explained that the Folch method also extracts phospholipids which are not detected by ether and CEM methods. They also explained that ether can extract lipid soluble vitamins, flavor compounds, and color compounds that should not be included in a total lipid determination. Keeton *et al.* (2003) explains in the methodology that the CEM uses nuclear magnetic resonance to detect radio frequencies emitted from hydrogen nuclei and that the total fat percentage is determined by the intensity of the signal. Keeton *et al.* (2003) also explains that signal intensity is directly
proportional to the number of lipid protons and lipids emit the slowest signal of all other food components. Dow et al. (2011) concluded that any of the three methods, Folch, ether, or CEM can be used to determine total fat percentage. It was also suggested that without the use of chemicals the CEM method was more environmentally safe and was much faster at determining total fat percentage than Folch or ether (Dow et al., 2011).

**FAT DEPOTS AND SOURCES**

Fat is a late maturing tissue in cattle as it follows the development of organs, bone, and muscle. Growth in cattle is initially slow and then increases as muscle is deposited. Once muscle growth declines and fat deposition begins the rate of gain normally declines (Rouse and Wilson, 2013). Fat deposition is separated into four depots that are deposited at different times and have been shown by Aldai et al. (2007) to have different fatty acid profiles. Visceral fat or internal fat surrounds the major organs and is usually deposited first. This is also commonly called kidney, pelvis, heart, or mesenteric fat. It is also normally a waste fat that is unwanted and goes to rendering. The kidney, pelvic and heart fat is much easier to remove while warm, so this normally occurs during slaughter following evisceration. The mesenteric fat is removed with the
intestines during evisceration. In today’s market the value of this fat is not significant enough to be collected, but if there was an added value it would be beneficial to save. Intermuscular fat or seam fat is the next depot to be laid down and is deposited between the muscles. Following that is subcutaneous fat which is deposited on the surface of the animal under the skin. Intermuscular and subcutaneous fat are the two major fat depots commonly added to ground beef to achieve a certain blend. Lastly, intramuscular fat is deposited between muscle fibers and is referred to as marbling. Intramuscular fat also contributes to ground beef blends but at a much lower percentage than that of subcutaneous or intermuscular for most ground beef blends. The exception would be extremely lean blends that may not have any additional fat added.

**Factors influencing cellularity and composition of adipose tissue in beef**

The age of the animal is a main factor that contributes to the influencing of fat cellularity and composition. In all livestock species, the amount of adipose tissue increases as the animal ages. This increase can be contributed to either cell proliferation where the cells increase in number or lipid filling where the cells increase in volume. Wegner *et al.* (1998) showed rapid growth in the fat cell diameter of subcutaneous fat in cattle up to 12 months of age. After this peak growth they found a reduced increase up to 2 years of age (*Wegner et al.*, 1998). Nurnberg *et al.* (1998) explains that in 24 month old Black Pied cattle, the intermuscular fat depot contains the largest percent of total fat (45%) at
slaughter, followed by internal and subcutaneous (38 and 17%, respectively).

Robelin (1981) examined the cellularity of bovine adipose tissues at 15, 25, 35, 45, 55, and 65% mature body weight. Examining all the adipose tissues as a whole, cell size increased steadily and significantly (P < 0.05) from 15 to 45% mature weight, stabilized between 45 and 55% mature weight, and later increased numerically but not significantly (P > 0.05) (Robelin, 1981). Total cell number increased slightly numerically but not significantly (P > 0.05) between 15 and 25% mature weight, remained constant up to 45% mature weight, and increased significantly (P < 0.05) from 45 to 55% mature weight (Robelin, 1981). They also found that the individual fat depots have different growth patterns. They found subcutaneous fat to have a 100 fold increase (0.1 to 10.3 kg) in lipid weight between 15 and 65% mature weight, followed by internal fat with a 33 fold increase (0.9 to 29.6 kg), and intermuscular fat with only a 20 fold increase (1.3 to 26.0 kg) (Robelin, 1981). They also found apparent cell proliferation in subcutaneous fat that occurred between 45 and 55% mature weight where there was a significant increase (P < 0.05) in both small and medium sized cells (525 x 10^7 to 1,077 x 10^7; 956 x 10^7 to 1,379 x 10^7, respectively) (Robelin, 1981). Both intermuscular and internal fat showed apparent proliferation earlier, between 35 and 45% mature weight (Robelin, 1981). This was contributed to subcutaneous fat being a late developing tissue leading to higher relative growth and younger cells later in the animal’s growth cycle (Robelin, 1981). All three fat depots showed lipid filling, a significant increase (P < 0.05) in cell volume, between 15 and 45% mature weight (Robelin, 1981). There was no increase (P > 0.05) in cell
volume of any of the fat depots from 45 to 65% mature weight (Robelin, 1981).

Age differences in cattle are also known to be associated with fatty acid composition of both fat depots and phospholipids in muscle. Malau-Aduli et al. (1997) compared Limousin cows (43 months) to heifers and steers (12 months) and Jersey cows (37 months) to heifers and steers (12 months) to test how age would affect the fatty acid composition of adipose tissue. They determined that for both breeds there was a decrease in total saturated fatty acids in adipose tissue with increasing age (Malau-Aduli et al., 1997). They also showed that the total of monounsaturated fatty acids decreased with increasing age in the same animals (Malau-Aduli et al., 1997). Malau-Aduli et al. (1998) compared the same animals but focused on the effect of fatty acid composition in phospholipids in muscle (intramuscular fat). They showed a decrease (P < 0.05) in percentages of the saturated phospholipids 16:0 and 18:0 (20.00 vs 16.65%; 17.60 vs 13.85%, respectively) as well as the monounsaturated phospholipid 18:1n9 (23.15 vs 17.60%) with increasing age (Malau-Aduli et al., 1998). They also showed an increase (P < 0.05) in the total percentage of monounsaturated fatty acids (18.30 vs 22.65) with increasing age (Malau-Aduli et al., 1998).

Breed can also contribute to the influencing of fat cellularity and composition in beef cattle. This can also be amplified by comparing breeds noted for different types of production such as comparing beef production cattle to milk production cattle. Robelin (1981) compared Friesian and Charolais bulls and showed that they have a different propensity to fatten. He reported that both breeds contained approximately the same amount of total fat at 15% mature
body weight but at 65% mature body weight the Friesian bulls had accumulated nearly twice as much total fat than the Charolais bulls (21 vs 13% body weight) (Robelin, 1981). Hood and Allen (1973) compared the cellularity of adipose tissue in Holstein and Hereford Angus crossed steers and found that the cell diameter and volume of subcutaneous adipose tissue was smaller (P < 0.05) in the Holstein steers. They also found significantly less (P < 0.05) adipose cells in the subcutaneous fat in the Holstein steers (Hood and Allen, 1973). The smaller cell size and cell number led to less total subcutaneous fat in the Holstein steers compared to the Hereford Angus crossed steers. This is supported by Truscott et al. (1983) who also showed that Herefords had more subcutaneous fat and less abdominal fat than Friesians at the same total fat weight. Maturing rate of individual breeds can also lead to differences in fat concentrations. Aldai et al. (2007) showed that early maturing breeds had higher concentrations of fat than late maturing breeds. Breed differences in cattle are also known to be associated with fatty acid composition of both fat depots and phospholipids in muscle. Limousin cows have shown to contain higher levels of palmitic acid (16:0) (17.8%) than Jersey cows (15.5%) in their muscle phospholipids compared to 30.2 and 28.2% found in their adipose tissues (Malau-Aduli et al., 1997; 1998). Zembayashi et al. (1995) compared breed type on the fatty acid composition of subcutaneous and intramuscular lipids of finishing steers. Results showed that Japanese Black steers contained significantly (P < 0.05) less saturated fatty acids (37.4% vs 44.9%) and more monounsaturated fatty acids (58.9 vs 52.0%) in the subcutaneous lipids when compared to Holstein steers adjusted for the
same degree of carcass fat (Zembayashi et al., 1995). The same results were observed in intramuscular lipids where Japanese Black steers also contained significantly (P < 0.05) less saturated fatty acids (37.0 vs 44.3%) and more monounsaturated fatty acids (59.3 vs 53.0%) when compared to Holstein steers adjusted for the same degree of carcass fat (Zembayashi et al., 1995). These findings with an adjustment for carcass fatness suggest that breed differences in fatty acid composition were not caused by differences in carcass fatness due to breed type.

Gender can also contribute to the influencing of fat cellularity and composition in beef cattle. It is well known that at equal slaughter weights, bulls are leaner than heifers which are leaner than steers and that gender contributes to differences in fatty acid composition (Zembayashi et al., 1995; Malau-Aduli et al., 1998). Malau-Aduli et al. (1998) reported differences in muscle phospholipid (intramuscular fat) fatty acids between steers and heifers. Their results showed steers have greater quantities (P < 0.05) of myristic (14:0), palmitoleic (16:1), stearic (18:0), oleic (18:1n9), vaccenic (18:1n7), erucic (22:1), and total monounsaturated fatty acids than heifers (Malau-Aduli et al., 1998). They also showed that heifers have significantly (P < 0.05) more total polyunsaturated fatty acids and a higher ratio of polyunsaturated to saturated fatty acids than steers in the same study (Malau-Aduli et al., 1998). Zembayashi et al. (1995) compared fatty acid composition in intramuscular neutral lipid (IMNL), intramuscular phospholipid (IMPL), and total subcutaneous lipid (SNL) of both steers and heifers. They explained a total lipid was used for subcutaneous fat because it
contains very small quantities of phospholipids (Zembayashi et al., 1995). Steers showed a significant (P < 0.05) increase of saturated fatty acids in both SNL (38.5 vs 35.0) and IMNL (39.3 vs 34.0) and a significant (P < 0.05) decrease of monounsaturated fatty acids in both SNL (58.2 vs 62.2) and IMNL (57.8 vs 63.1) when compared to heifers (Zembayashi et al., 1995). No differences (P > 0.05) of polyunsaturated fatty acids were found between genders, however, steers had a decrease (P < 0.05) of total unsaturated fatty acids in both SNL (61.5 vs 65.1) and IMNL (60.7 vs 66.0) when compared to heifers (Zembayashi et al., 1995). No differences (P > 0.05) were shown between genders in any fatty acid groups for IMPL (Zembayashi et al., 1995). It is expected that hormonal differences caused these gender differences in fatty acid composition (Malau-Aduli et al., 1998).

Diet is another factor that can contribute to the influencing of fat cellularity and composition in beef cattle. However, Nurnberg et al. (1998) explains that unlike monogastrics, which are able to allow fatty acids through the digestive system and deposit them in tissues such as adipose without changing them, the potential for dietary manipulation of fatty acid composition in the ruminant is much less. Costa et al. (2012) conducted a study that assessed the effect of feeding diets with distinct forage to concentrate ratios on the cellularity of both subcutaneous and visceral fat depots in two Portuguese bovine breeds. Results showed that neither breed nor diet determines cellularity (Costa et al., 2012). However, subcutaneous fat had larger adiposities and a lower number of cells than visceral fat (6,677 vs 5,584 µm² and 78.0 vs 94.0 cells in 560 x 10³ µm², respectively) meaning the two fats were distinct (Costa et al., 2012). A diet effect
(P < 0.05) in subcutaneous fat revealed lower frequencies of medium sized adiposities (7,200-9,000 µm²) in bulls fed the low silage diets (Costa et al., 2012). They also concluded that ruminal transformation of dietary lipids are able to effect the fatty acid composition of adipose tissue by showing higher branched chain fatty acid percentages in high silage diets compared to low silage diets (Costa et al., 2012). Costa et al. (2012) states, “Diets containing high proportions of non-structural carbohydrates, such as starch, but low amount of fiber promote less extensive biohydrogenation”. Biohydrogenation causes fatty acids to become more saturated or solidify, so promoting less extensive biohydrogenation the fatty acid profile increases the percentage of unsaturated fatty acids.

**FATTY ACIDS**

Fatty acids are the simplest form of lipids. They normally are derived from triglycerides or phospholipids and are made up of a carboxylic acid with a long hydrocarbon chain (Ophandt, 2003). These hydrocarbon chains can vary from short chains as little as four carbons to long chains with as many as 30 carbons (Ophandt, 2003). Fatty acids are classified as saturated or unsaturated. Saturated fatty acids (SFA) hold all the possible hydrogen atoms at each carbon
atom in the chain, therefore containing no double bonds (Ophandt, 2003). This makes the structure liner and more stable. Unsaturated fatty acids (UFA) contain one or more carbon-carbon double bonds in the chain (Ophandt, 2003). Those containing one carbon-carbon double bond are referred to as monounsaturated fatty acids (MUFA) and those containing more than one carbon-carbon double bond are referred to as polyunsaturated fatty acids (PUFA) (Ophandt, 2003). The two carbon atoms bound by the double bond can occur in a cis or trans configuration. A cis configuration means that adjacent hydrogen atoms are on the same side of the double bond (Ophandt, 2003). Each double bond in the cis configuration causes a kink or bend in the chain. As the number of cis double bonds increase, such as in alpha-linoleic acid with three double bonds, the more curved the structure becomes and prohibits the fatty acids from being able to tightly pack together (Ophandt, 2003). A trans configuration means that the two hydrogen atoms are bound to opposite sides of the double bond. This results in the chain not bending as much and resembles the straight structure of SFA (Ophandt, 2003). Fatty acids in the trans configuration tend to be man made or artificial and those in the cis configuration tend to be naturally occurring (Ophandt, 2003).

*Health concerns surrounding fatty acids*

Fats are a major energy source and help absorb certain vitamins and nutrients making them essential to the human body. Ophandt (2003) reveals
when fatty acids are metabolized they yield large quantities of ATP which many cell types use as an energy source. The Harvard Medical School Family Health Guide tells us that there are some types of fat that can be detrimental to human health. SFA have been implicated in diseases such as various cancers and especially coronary heart disease (Wood et al, 2002). Jiang et al. (2011) reported that beef has a greater proportion of SFA than non-ruminant meat giving it a negative image to consumers. Not all saturated fats are equally as bad for health. Williams (2000) reported that lauric acid (C20:0), myristic acid (C14:0), and palmitic acid (C16:0) were considered to have hypercholesterolemic effects. The saturated fats found in dairy products are the most detrimental in increasing low-density lipoproteins (LDL) followed by the saturated fat found in beef; however, some SFA such as steric acid (C18:0) acts more like UFA in that it lowers LDL levels (The Harvard Medical School Family Health Guide, 2007). Hunter et al. (2010) states, “Steric acid (C18:0), a major SFA in beef, had a neutral effect on HDL cholesterol and lowered LDL cholesterol in humans, compared to other SFAs”. Higher LDL levels put you at greater risk for a heart attack because LDL collects in the walls of blood vessels causing blockage. Trans fats are even worse because not only do they increase your LDL cholesterol, they also reduce your beneficial high-density lipoprotein (HDL) cholesterol. Trans fats occur naturally in meat but they were also artificially created in the laboratory to provide cheap alternatives to butter (The Harvard Medical School Family Health Guide, 2007).
Both categories of MUFA and PUFA are considered good fats and primarily come from vegetable and fish products. The Harvard Medical School Family Health Guide reports PUFA are vital to blood clotting, muscle contraction and relaxation, and inflammation. They also lower triglycerides and lower LDL more than they lower HDL, improving cholesterol. Kolanowski and Laufenberg (2006) suggest that omega-3 PUFAs may play a role in the prevention and treatment of certain types of cancers and diseases with an immunoinflammatory component. The International Society for the Study of Fatty Acids and Lipids (2004) recommends a minimum intake of 500 mg/day of Eicosapentaenoic acid (EPA; C20:5n3) and Docosahexaenoic acid (DHA; C22:6n3) combined, for cardiovascular health. Jiang et al. (2011) conducted a study examining the effects of direct supplementation of EPA and DHA in ground beef on eating quality. Results indicated that direct supplementation of EPA and DHA successfully enriched their levels in ground beef; however, it had negative effects on palatability. The DHA had a greater negative effect on ground beef flavor whereas; EPA had a greater negative impact on off-aroma and off-flavor of ground beef. Conjugated linoleic acids (CLA) have also shown to have beneficial health effects. Ruminants naturally produce CLA and De la Torre (2006) reported extracted CLA from beef lipids decreased cancer cell growth more than synthetic CLA. Noci et al. (2005) states, “Linoleic acid (C18:3n3) is a predominant fatty acid in pasture grasses and can be converted to C18:1trans-11 by ruminal bio-hydrogenation, which will be ultimately converted to CLA in adipose tissue by
delta-9 desaturase”. Therefore, grass finished beef tend to contain higher levels of CLA in their adipose tissue.

**Effect of adipose tissue location on fatty acid profiles**

Numerous studies have reported differences in fatty acid composition from different fat depots (Costa *et al.*, 2012; Aldai *et al.*, 2007; Zembayashi *et al.*, 1995). Aldai *et al.* (2007) compared the fatty acid composition of intramuscular, intermuscular, and subcutaneous fat depots in yearling bulls of different genetic groups. Results showed differences (P < 0.05) for SFA concentrations where intermuscular > subcutaneous > intramuscular (54.43, 51.21, and 46.31 g/100 g, respectively) (Aldai *et al.*, 2007). Differences (P < 0.05) were also shown for MUFA concentrations where subcutaneous > intermuscular > intramuscular (43.53, 39.93, and 33.31 g/100 g, respectively) (Aldai *et al.*, 2007). Conversely, PUFA showed differences (P < 0.05) in the reverse order where intramuscular > intermuscular = subcutaneous (19.76, 4.44, and 4.13 g/100 g, respectively) (Aldai *et al.*, 2007). Total UFA showed differences (P < 0.05) where intramuscular > subcutaneous > intermuscular (53.07, 47.66, and 44.37 g/100 g, respectively) (Aldai *et al.*, 2007). Results also showed differences (P < 0.05) for the ratio of UFA: SFA where intramuscular > subcutaneous > intermuscular (1.17, 0.94, and 0.82 g/100 g, respectively) (Aldai *et al.*, 2007).

Location on the beef carcass can also effect fatty acid concentration in adipose tissue. Turk and Smith (2008) examined external adipose tissue
(subcutaneous) from eight carcass locations: round, sirloin, loin, rib, chuck, brisket, plate, and flank. When comparing SFA, the brisket was lower ($P = 0.001$) in palmitic acid (C16:0) and stearic acid (C18:0) than the other sampling sites (24.3 and 8.35 g/100 g, respectively) and myristic acid (C14:0) was lower ($P = 0.002$) (3.22 g/100 g) than all other sites except the chuck (Turk and Smith, 2008). The highest concentrations of these SFA varied among the locations (Turk and Smith, 2008). The highest concentration of C14:0 was in the loin (3.68 g/100 g), the highest concentration of C16:0 was in the rib (27.2 g/100 g), and the highest concentration of C18:0 was in the flank (16.3 g/100 g) (Turk and Smith, 2008). Conversely, when comparing MUFA, brisket was higher ($P = 0.001$) in myristoleic acid (C14:1n5), palmitoleic acid (C16:1n7), oleic acid (C18:1n9), and the ratio of MUFA: SFA (1.91, 7.22, 43.1, and 1.47 g/100 g, respectively) than all other sampling sites (Turk and Smith, 2008). The flank was lower ($P = 0.001$) in C14:1n5, C16:1n7, C18:1n9, and the ratio of MUFA:SFA (1.07, 3.19, 36.8, and 0.88 g/100 g, respectively) than all other sampling sites (Turk and Smith, 2008). When comparing PUFA, the plate contained the highest concentration (1.98 g/100 g) and the brisket contained the lowest concentration (1.63 g/100 g) of linoleic acid (C18:2n6) compared to all other sampling sites (Turk and Smith, 2008). The brisket contained the highest concentration (0.70 g/100 g) and the rib contained the lowest concentration (0.52 g/100 g) of C18:2c9,t11 compared to all other sampling sites (Turk and Smith, 2008). There was no difference ($P > 0.05$) of C18:2t10,c12 concentration among all eight sampling locations (Turk and Smith, 2008).
Due to the variations of fatty acids in adipose tissues and location on the carcass, fatty acid composition differs in various types and brands of ground beef. Turk and Smith (2008) surveyed area ground beef and compared fatty acid composition. They purchased ground beef (chub pack), ground round, ground chuck, and branded ground beef (Angus, Wagyu, guaranteed pasture-fed, grain-fed, and tender) from three major retailers in the vicinity of Texas A and M University. Results showed the lowest MUFA:SFA ratio within the variations of ground beef to be branded tender (0.73) and the highest MUFA:SFA ratio to be grain-fed Wagyu (1.45). The lowest concentration of C18:1n11 was in branded grain-fed Wagyu (1.5) and the highest concentration was in chub pack ground beef and branded tender (7.2 and 8.2, respectively) (Turk and Smith, 2008). The lowest concentration of C18:1n9 was in branded tender (31.5) and the highest concentration was in branded grain-fed Wagyu (45.1) (Turk and Smith, 2008). The lowest concentration of C18:0 was in branded grain-fed Wagyu (9.1) and the highest concentration was in branded pasture-fed (18.4) (Turk and Smith, 2008). There was no difference (P > 0.05) in C16:0 concentrations between the variations of ground beef (Turk and Smith, 2008).

*Melting point*

The firmness of fat is determined by the fatty acid composition, as different fatty acids have different melting points (Wood *et al.*, 2008). Ophandt (2003) reveals that as a group, UFA have lower melting points than SFA and SFA tend
to be solids at room temperature (25°C) compared to UFA that tend to be liquids at room temperature. As previously stated, SFA molecular structure is relatively linear which allows many fatty acids to be closely stacked together. These close intermolecular interactions result in high melting points. The UFA, especially in the cis configuration, contains one or more bond angles that result in bends in the molecular structure. These bends make UFA harder to stack together and much weaker. As a result, the melting points are much lower. Melting point of fatty acids can also play a role in fat color. Wood (2002) states, “Groups of fat cells containing solidified fat with a high melting point appear whiter than when liquid fat with a lower melting point is present”.

**Iodine value**

Iodine value is a measurement to estimate the amount of unsaturation of fatty acids found in carcass fat (DeRouchey *et al*, 2011). The higher the iodine value 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, iodine value can be used as an indirect indicator of lipid oxidation and carcass fat firmness (DeRouchey *et al*, 2011). Iodine value can be calculated by chemical analysis which involves iodine binding to unsaturated or double bonds in fatty acids (AOCS, 1998), from a fatty acid analysis (AOCS, 1998), or near-infrared analysis. The calculation from fatty acid analysis has been the most widely used process by researchers and processors to determine carcass fat iodine. The
near-infrared method is beginning to be more widely adopted by processors due to it being a more rapid method; however, there is very little research to show how this method correlates to the other commonly used methods (DeRouchey et al., 2011).

The pork industry has put an enormous emphasis on using iodine value as a measure of carcass fat quality. Some packing plants have set a maximum iodine value of 72 g per 100 g, rejecting or discounting carcasses that exceed this limit. This maximum value was chosen because a transition occurs around 70 g per 100 g from fat to oil (DeRouchey et al., 2011). Exceeding this maximum iodine value results in decreased slice ability of bellies for bacon, shelf life, and export market acceptance. Feeding dried distillers grains (DDGS), a co-product of ethanol production, has been a leading factor in the increased incidence of soft fat or unsaturation. DeRouchey et al. (2011) reports carcass fat iodine value increases 2 g per 100 g for every 10 percent DDGS that is fed throughout finishing. Studies have also shown that belly firmness decreased when DDGS levels increased from 10 to 20 to 30% in the diet (Cromwell et al., 2011, Weimer et al., 2008). Cromwell et al. (2011) showed that PUFA levels increased while SAT and MUFA levels decreased in subcutaneous fat with increasing (15, 30 and 45%) levels DDGS in the diet revealing that increased belly softness can be explained by an increase in polyunsaturated fatty acids in fat.

Beef on the other hand are not able to absorb fatty acids from the diet as freely because they are broken down in the rumen, resulting in lower overall unsaturated fatty acid composition when compared to swine. Due to this,
processors are not as concerned with iodine value in beef as they are in pork. Feeding DDGS has shown to increase PUFA concentrations in beef; however, the concentrations are still well below levels where you would encounter problems with firmness (Popowski et al., 2012). Popowski et al. (2012) compared fatty acid profiles from 12th rib back fat of steers fed either a 35 percent DDGS diet or a control diet that contained 0 percent corn milling co-products. Results showed a significant increase (P < 0.001) in PUFA (1.84 vs 4.12 percent) when steers were fed the DDGS diet compared to the control. There was also a significant increase (P = 0.01) in iodine value (46.20 vs 48.28) when steers were fed the DDGS diet compared to the control. They showed no significant difference (P > 0.05) in SFA or MUFA (43.46 vs 43.78 percent; 47.30 vs 48.09 percent, respectively) when comparing the control diet to the DDGS diet.

GROUND BEEF QUALITY AND SHELF LIFE

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). Additionally, there is evidence that muscles with high myoglobin concentrations can be subject to oxidation during retail display (Raines et al., 2010). Lipid oxidation is the oxidative degradation of lipids where free radicals steal electrons from the lipids

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in cell membranes, resulting in cell damage. It most often affects PUFA, because they contain multiple double bonds that possess especially reactive hydrogens. Transition metal ions such as iron have been shown to initiate lipid oxidation by generating free radicals capable of abstracting a proton from UFA (Kanner, 1994). Sherbeck et al. (1995) states, “Discoloration in retail packaged meats during display conditions may occur as a combined function of muscle pigment oxidation (oxymyoglobin to metmyoglobin) and lipid oxidation in membrane phospholipids”. The protein responsible for meat color is myoglobin. Uncut meat is in the deoxymyoglobin state where ferrous iron (Fe$^{2+}$) is available and there is no O$_2$ present resulting in a purple color. Once the meat is cut it is exposed to O$_2$ and becomes oxymyoglobin and changes to the bright cherry red color that consumers associate with high quality meat. Once oxidation occurs, ferrous iron (Fe$^{2+}$) is converted to ferric iron (Fe$^{3+}$). Increased Fe$^{3+}$ coupled with decreased O$_2$ causes oxymyoglobin to become metmyoglobin and turn brown (Troy and Kerry, 2010). Taylor (1996) reported that the color of fresh meat is not well correlated with the eating quality; however, the consumer still demands the meat to be bright cherry red.

**Oxidative Mechanisms and Reactions**

Autoxidation is any oxidation that occurs in open air or the presence of oxygen and forms peroxides and hydroperoxides which produce free radicals (Aberle et al., 2012). This process includes three distinct stages: initiation,
propagation, and termination. Initiation occurs when oxygen attacks a fatty acid double bond and cleaves it (Aberle et al., 2012). Each hydrocarbon loses a hydrogen to form a free radical (RH - R◦ + H◦) (Aberle et al., 2012). According to Aberle et al. (2012), “The free radicals made during initiation quickly produce additional activated oxygen species which migrate to other unsaturated fatty acids where they each attack and cleave another double bond producing still more free radicals” (R◦ + O2 - ROO◦; ROO◦ + RH – ROOH + R◦). This is referred to as the propagation stage and continues producing peroxyradicals, hydroperoxyradicals, and new hydrocarbon radicals until either oxygen or double bonds are no longer available for the reaction (Aberle et al., 2012). As the concentrations of oxygen and double bonds decrease, free radical concentration increase leading to one free radical reacting with another (R◦ + R – RR; ROO◦ + ROO◦ - ROOR + O2; RO◦ + R◦ - ROR; ROO◦ + R◦ - ROOR; 2RO◦ + 2ROO◦ - 2ROOR + O2) (Aberle et al., 2012). This is referred to as termination because it produces a non-reactive product that naturally terminates the oxidation process (Aberle et al., 2012). However, Aberle et al. (2012) states, “This only occurs after considerable oxidation has occurred and intense rancid flavor has developed”. Ground beef is more susceptible to autoxidation due to the incorporation of oxygen during the grinding process (Aberle et al., 2012).

Oxidation Determination
The most common test found in literature to determine lipid oxidation is the thiobarbituric acid reactive substances (TBARS) extraction method described by Pegg (2001). This method determines the TBARS of a food sample by an extraction methodology. Malonaldehyde and other TBARS are extracted using a trichloroacetic acid / o-phosphoric acid solution (Pegg, 2001). An aliquot of the filtered extract is reacted with excess TBA. Based on a standard curve derived from spectrophotometric measurements, results are expressed as mg malonaldehyde/ kg of sample. A TBARS value of at least 2.0 mg malonaldehyde/kg is necessary for the sensory detection of rancid off-odors by an untrained typical consumer (Green and Cumuze, 1981). Iodine is also used as an indicator of lipid oxidation. Iodine value estimates the amount of unsaturation of fatty acids found in carcass fat and UFA, especially PUFA, are most susceptible to lipid oxidation.

**Promoters of oxidation and discoloration**

The main promoters of oxidation and discoloration during retail display are fat level, storage time, and storage temperature. As fat percentage decreases protein increases causing ground beef formulations with decreased fat levels to have a greater moisture and protein content (Martin et al., 2013). Martin et al. (2013) examined the effect of three different lean:fat formulations of ground beef (73:27, 81:19, and 91:9) in a shelf life study. Results indicated the 73:27 ground beef was lighter (greater L* value; P < 0.05), than 81:19 and 91:9 ground beef at
all storage intervals (Martin et al., 2013). Increasing the levels of fat increase L* values due to its light color. All formulations maintained similar redness (a*) through 14 d storage, regardless of lean level; however, after 21 d, 81:19 and 91:9 ground beef had greater a* values than 73:27 (P < 0.05) (Martin et al., 2013). Martin et al. (2013) showed TBARS increased as storage lengthened; however, no trend was observed among lean formulations. They concluded that the variation in lipid oxidation could be attributed to the variation in UFA content of the lean sources used in each formulation (Martin et al., 2013).

Storage time can be detrimental to the quality of fresh and frozen retail products. It is well documented that oxidation increases as storage time lengthens (Martin et al., 2013; Hoyle Parks et al., 2012). Hoyle Parks et al. (2012) showed a steady increase in TBARS of fresh ground beef from 0 to 84 h. The most significant (P < 0.05) spikes were from 0 to 24 h (1.30 to 2.24 mg malonaldehyde/kg meat) and 72 to 84 h (2.57 to 3.60 mg malonaldehyde/kg meat). Martin et al. (2013) showed TBARS increased through 21 d frozen storage but declined after 28 d. They concluded that the decline after 28 d was not indicative of decreased oxidation but rather a decline in oxidative reactive substances (Martin, 2013). This is supported by Smith and Alford (1968) who found that bacteria, yeasts, and molds were capable of decomposing peroxides, thereby reducing the amount of substrates reacting with TBARS. Length of storage can also affect color scores. Hoyle Parks et al. (2012) reported lean color scores of fresh beef patties evaluated by sensory panelists significantly increased (P < 0.0001) from 0 to 84 h (1.75 to 5.55). They also showed no
significant 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 (46.44 to 47.44) (Hoyle Parks et al., 2012).

According to Jakobsen and Bertelsen (2000), “The most important factor for maintaining the red oxymyoglobin color and keeping lipid oxidation to a minimum is the temperature”. Jakobsen and Bertelsen (2000) reveal that a low temperature (below 4°C) and an oxygen level of 20% are needed to maintain a cherry red meat color. Raising the oxygen above 55% does not improve the color stability further. Storage in 55% oxygen at 2°C maintains a red meat color (a*) for 10 d, but if the temperature is raised slightly to 5°C the color can only be maintained for 6½ d. Hood (1984) found lower storage temperatures promote oxygen penetration and the solubility of oxygen, which increases the depth of oxymyoglobin penetration that is associated with a more desirable red color. Jakobsen and Bertelsen (2000) also found keeping the temperature below 4°C almost prevents lipid oxidation regardless of the oxygen level.

*Inhibitors of oxidation and discoloration*

Minimizing lipid oxidation and color change is crucial to improve consumer acceptance of ground beef. Antioxidants and acids are used in fresh and further processed meats to prevent oxidative rancidity and improve color stability. Kichang et al. (2012) conducted a study where they electrostatic sprayed both phenolic antioxidants and ascorbic acid on to ground beef patties. Phenolic antioxidants have free radical-scavenging properties and terminate free radical
reaction in meat during storage and ascorbic acid is a reducing agent, which inhibits myoglobin oxidation and brown color development in beef. Results showed ascorbic acid was the most effective in preventing discoloration and had lower TBARS values during aerobic storage at 4°C; however, it had no effect on L* values. Kichang et al. (2012) concluded, “The spray of ascorbic acid was more effective in stabilizing beef color than any other antioxidants, but the combined spray of ascorbic acid with antioxidant would be more beneficial in controlling lipid oxidation as well as color oxidation of ground beef during storage”.

Movileanu et al. (2013) showed that inclusion of phenol derivatives, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), retarded the oxidative process and significantly lowered (P < 0.05) TBARS when compared to control ground beef patties (0.42 vs 1.56 mg malanaldehyde/kg sample). There have also been studies that evaluated the antioxidant activities of edible plant extracts when applied to fresh ground beef. Kim et al. (2013) found butterbur leaf extract (0.5%) exhibited a large inhibitory effect on lipid oxidation as well as retarding the discoloration of ground beef patties. They also revealed Crown daisy leaf extract had the highest antioxidant activity of the ten plant extracts they examined (Kim et al., 2013).

**Effect of packaging on oxidation and shelf life**

The stability of ground beef at retail is largely affected by package type and package environment. Plastic-based films are the materials of choice for the
majority of meat products presented at retail level and film choice is largely
determined by moisture and gas permeability (Troy and Kerry, 2010). Traditional
packaging consists of a polystyrene tray overwrapped with an oxygen permeable
polyvinylchloride (PVC) film. Traditionally packaged ground beef patties have
shown a tendency to darken (P = 0.07; decreased L* values) as display time
increases (Hoyle Parks et al., 2012). Based on the fact that consumer purchasing
decisions are historically based on lean appearance, a modified atmosphere
package (MAP) was developed to prolong color stability (Mancini and Hunt,
2005). The MAP consists of placing the product on a plastic tray, flushing it with a
gas mixture, and sealing in the gasses with a gas flush, tray-sealing package
machine. The gas composition normally used for MAP beef is 20 to 30% CO₂
and 70 to 80% O₂ (Blakistone, 1998). Numerous studies have shown that
elevated levels of oxygen prolong color stability (Bartkowski, Dryden, and
Marchello, 1982; Taylor, 1972). However, Zhao et al. (1994) reports increased
rate of lipid oxidation with elevated oxygen levels. The MAP, due to its high
oxygen levels, has been shown to increase lipid oxidation in ground beef and
other meat products (Jayasingh et al., 2001; Hoyle Parks et al., 2012). This
increased lipid oxidation in MAP can also cause discoloration by producing free
radicals, which oxidize and deteriorate heme pigments. While MAP prolongs
color stability it is negated by increased lipid oxidation which can cause
discoloration.
Purchasing trends vary from shopper to shopper. Some consumers shop at the beginning of the week, while others are end of the week or weekend shoppers. Retailers are dealt the task of providing fresh ground beef to fit with consumer demands with as little loss as possible. Ground beef has a shelf life of 3 to 4 days under normal refrigerated retail display conditions before oxidation and discoloration detour consumers from purchasing the product. This coupled with an increase in ground beef sales has provided more pressure for meat retailers. Extending the shelf life of ground beef just one day could alleviate this problem. Fat level and fat source are significant contributors to quality indicators in ground beef patties and in the right combination may be able to extend shelf life.
CHAPTER II

EVALUATING QUALITY CHARACTERISTICS OF GROUND ROUND FORMULATED WITH THREE FAT SOURCES

ABSTRACT

This research was performed to determine if changing the source of fat for inclusion in ground beef from the round will cause a change in the number of days that the ground beef has a viable shelf-life as influenced by oxidation. The objectives were to evaluate the color stability and degree of fat oxidation during retail storage of ground beef patties and to determine the influence of fatty acid profile on the extent of ground beef lipid oxidation and shelf-life of ground beef patties over 7 d of refrigerated retail storage. Beef carcasses (n = 30) were chilled for two days and then fabricated. Top round muscles (IMPS # 168) were removed and closely trimmed. Within each carcass, three sources of fat (kidney and pelvic = KP, subcutaneous = S, or seam = I) 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. 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 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 to be used for the shelf study. Patties were placed on Styrofoam®
trays and overwrapped with oxygen permeable, polyvinyl chloride and placed in
refrigerated retail storage (4°C) where an instrumental measurement of color (L*,
a*, b*) and thiobarbituric acid reactive substances (TBARS) were collected on d
1, 3, 5, and 7 of the study. The additional 447 g of sample was placed in a whirl-
pack bag, stored at 4°C, and used for fat and moisture determination,
oxymyoglobin concentration, fatty acid analysis, and calculated iodine value (IV).
Data analysis indicated different (P = 0.0004) saturated fat (SFA) percentages
(KP > I > S with means of 57.9, 53.5, and 52.1%, respectively). Calculated IV
differed (P = 0.003) where KP < I = S with means of 33.9, 37.1, and 38.3,
respectively. Changes in fat profile likely explain differences (P = 0.007) in TBA
values where KP = S < I with means of 0.110, 0.118, and 0.120 mg/kg,
respectively. However, fat percentage of ground beef did not change (P = 0.83)
TBA values (75% = 0.116 mg/kg and 95% = 0.116 mg/kg). Minolta a* value
differed (P = 0.004) where S > KP with I not differing from S or V (S = 14.78, I =
14.52, and V = 14.46). However, these differences in Minolta a* reflectance are
not likely discernible by visual appraisal of ground beef patties. Fat source is a
significant contributor to quality indicators in ground beef patties and should be
considered when formulating products intended for fresh, refrigerated retail sale.

INTRODUCTION
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). Additionally, there is evidence that muscles with high myoglobin concentrations can be subject to oxidation during retail display (Raines et al., 2010). Shortened shelf-life can be exacerbated by spoilage microbe loads present in meat products that tend to increase logarithmically and interact with other oxidation products over time of refrigerated storage. The recent increase in domestic ground beef demand matched by increased retail ground beef price make this a pertinent time to explore underlying factors that might impact or potentially allow lengthening of retail ground beef shelf life and consumer acceptance. Our hypothesis was that changing the source of fat for inclusion in ground beef from the round will cause a change in the number of days that the ground beef has a viable shelf-life as influenced by sources of oxidation.

**MATERIAL AND METHODS**

**Sample Selection and Preparation**

Beef cattle (n = 30) were received at an average of 363 kg of body weight. They were fed a receiving diet for 7 d and then fed a standard feedlot ration (Table 1) until time of slaughter, averaging 568 kg body weight. Feeding cattle
the same diet removed any differences that may have been caused by the diet. The cattle were slaughtered in groups of six head at the University of Missouri red meats abattoir under USDA/FSIS inspection. Carcasses were chilled for 48 hours followed by fabrication where Semimembranosus muscles (IMPS # 168), also commonly referred to as top or inside round, were removed and closely trimmed. Within each carcass, three sources of fat (kidney and pelvic = KP, subcutaneous = S, or seam = I) were sourced to blend with the top round to achieve either 75 or 95% lean ground beef. These two fat percentages were chosen to give a high and low percentage of fat to see if it caused differences while keeping those percentages at a level you could actually find in a retail case. Trim and fat blocks were individually ground through a coarse 10 mm plate. 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 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 to be used for the shelf study. Patties were placed on Styrofoam® trays and overwrapped with oxygen permeable, polyvinyl chloride and placed in refrigerated retail storage (4°C) where an instrumental measurement of color (L*, a*, b*) and thiobarbituric acid reactive substances (TBA) were collected on d 1, 3, 5, and 7 of the study. The additional 447 g of sample was placed in a whirl-pack bag, stored at 4°C, and used for fat and moisture determination, oxymyoglobin concentration, and fatty acid analysis. All laboratory analysis was conducted on fresh, never frozen ground beef samples to simulate the majority of ground beef sold at retail.
**Fat and Moisture Determination**

Fat and moisture determination was performed in triplicate on each source and fat percentage combination (six 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, U.S.A.). Briefly, two CEM square sample pads were placed into the moisture/solids analyzer, dried, and tared. 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 the moisture percentage was determined by weight using the CEM moisture/solids analyzer. Once the moisture analysis is completed, the dried sample and pads were rolled in TRAC paper, placed into the 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 (NMR) and converted to wet basis.

**Oxymyoglobin Concentration Determination**

Oxymyoglobin concentration of the ground beef samples were determined on d 2 for each group using the method described by Hunt (1980). Briefly, the sample was placed in a chilled grinder cup, submersed with liquid nitrogen, and
ground for approximately 30 seconds. Three grams of the pulverized sample was weighed out and placed back into the grinder cup with ten times the weight (30 ml) of cold buffer (40 mM potassium phosphate buffer, pH 6.8) and ground for one minute. The homogenized sample was then poured into a centrifuge tube and incubated at 4°C for one hour. The sample was then centrifuged at 15,000 x g for 30 minutes at 4°C, filtered, and poured into an acrylic cuvette. Absorbance was measured at 418 nm using a spectrophotometer (Thermo spectronic Genesys 20 4001/4). Oxymyoglobin values were calculated from the absorbance and expressed as mg of myoglobin per g of sample as described by the author (Hunt, 1980).

**Fatty Acid Analysis**

The methodology utilized for fatty acid determination was an adaptation of the methods used by Folch et al. (1957) and Morrison and Smith (1964). Approximately 1 g of each ground beef sample was placed in a glass tube and 5 mL of chloroform:methanol solution (CHCL3:CH3OH, 2:1, v/v) was added to the tube in order to extract lipids. The sample was homogenized for 30 seconds using an Omni International 2000 homogenizer (Waterbury, CT, U.S.A.). 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 two hours to separate the phases and then the upper phase was removed and discarded. The lower phase was then
transferred to a glass tube and 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, U.S.A.). One mL of 0.5 N KOH in CH3OH 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 CH3OH 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 to the tube. Next, the upper layer was removed and placed in a glass tube with approximately 800 mg of Na₂SO₄ in order 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 Na₂SO₄. 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, U.S.A.) was used to analyze fatty acid methyl esters; samples were injected onto a fused silica capillary column (SPTM – 2,560; 100 m x 0.25 mm x 0.2 μm film thickness; Supelco, Bellefonte, PA, U.S.A.). 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 which means 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 (1998): IV = (0.95 × C16:1) + (0.86 × C18:1n9) +
(1.732 × C18:2n6) + (2.616 × C18:3n3) + (0.785 × C20:1). An internal standard
fatty acid methyl ester was used and all fatty acid values are expressed as the
percentage of fatty acids detected.

Objective Color Determination

Ground beef patty surface color measurements (L*, a*, b*) were taken at d
1, 3, 5, and 7 of retail display utilizing a Minolta Chroma Meter CR-410 (Minolta
Camera Co., Osaka, Japan) with a D65 light source and 10 degree observer.
The instrument was calibrated before each analysis with an identical wrap as
used for the samples placed over a white tile standard. Samples were evaluated
in triplicate to achieve a more accurate representation of each ground beef patty.
The day 7 patty for each treatment within animal was used for comparison over
time because it was measured on all data collection days; whereas the day 1, 3,
and 5 patties were removed on their respective days for oxidation analysis.

Lipid Oxidation Determination

Lipid oxidation was determined using the thiobarbituric acid reactive
substances (TBAR) extraction method described by Pegg (2001). One of the four
patties from each fat source and fat percentage combination of each cattle was
removed on d 1, 3, 5, and 7 of the shelf-life study to be used for lipid oxidation
determination. 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 three minutes using a Hamilton Beach 2-speed Handheld Blender (Model # 59760, Southern Pines, NC, U.S.A.). The sample was then filtered and a 5 mL aliquot of sample solution was added along with 5 mL of TBA to a 50 mL conical centrifuge tube. The tubes were capped, vortexed, and heated in a boiling water bath for 35 minutes. The tubes were removed after 35 minutes from the water bath, chilled in ice for 5 minutes 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 electronic Genesys 20 4001/4). TBA values were obtained from the absorbance as described by the authors and showed mg of malonaldehyde/ kg of sample (Pegg, 2001). Each sample was ran in duplicate and averaged for statistical analysis.

**Statistical Analysis**

Statistical Analysis for proximate analysis, myoglobin concentration, and fatty acid profiles were analyzed using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC, U.S.A.) to obtain LS means and SE estimates. The model included the fixed effects of fat source (KP, S, I) and fat percentage (5 and 25%) and all possible interactions. Furthermore, statistical analysis for TBA
values were analyzed using the repeated measures option in the MIXED procedure of SAS and the model included the fixed effects of fat source, fat percentage, day (3, 5, or 7), and all possible interactions. Objective color scores during the retail display period were also analyzed using the repeated measures option in the MIXED procedure of SAS. 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.

Results

Fat and Moisture Determination

The interaction of fat source by percent fat showed differences in in both fat and moisture percentage (P = 0.0259 and P = 0.0231 respectively) in ground beef (Table 2). Fat source showed differences (P = 0.0053) in fat percentage where S > KP = I with means of 15.691, 14.802, and 14.552 %, respectively. Fat source also showed differences (P = 0.0021) in moisture percentage where I > KP > S with means of 64.312, 63.819, and 63.260 %, respectively. Fat percentage showed differences in fat percentage and moisture percentage (P = < 0.0001 and P = < 0.0001 respectively) as expected.

Myoglobin Concentration Determination
Oxymyoglobin concentration values are presented in Table 3. There were no differences (P > 0.05) in oxymyoglobin concentrations due to fat source and no interactions were observed. However, there were differences due to fat percentage (P = <0.0001) where as we would expect the 5% fat > than 25% fat with means of 3.707 and 3.312 mg/ kg, respectively.

**Fatty Acid Analysis**

Fatty acid concentrations are presented in Table 4. Fat percentage showed to have differences (P < 0.05) on all fatty acid categories except omega-3 fatty acids. Inclusion of 25% fat showed to have greater SFA (58.09 vs 51.00%), SFA:PUFA (15.15 vs 10.30), and omega-3:omega-6 (0.08 vs 0.05%) compared to 5% fat inclusion. Inclusion of 25% fat also showed to have lower MUFA (32.93 vs 38.69%), PUFA (4.79 vs 5.96%), omega-6 (3.78 vs 5.06%), and IV (33.21 vs 39.77) compared to 5% fat inclusion. Fat source differed (P < 0.05) in SFA, MUFA, SFA:PUFA, and IV. When observing SFA, KP > I > S with means of 57.959, 53.507, and 52.175%, respectively. When observing MUFA, S = I > KP with means of 37.592, 36.712, and 33.124%, respectively. When observing SFA:PUFA, KP > I = S with means of 15.522, 11.842, and 10.804, respectively. When observing IV, S > I > KP with means of 38.323, 37.190, and 33.950, respectively. The only source x fat% interaction (P = 0.023) was in the
SFA:PUFA ratio, where KPX25 was the highest (20.30) and SX5 was the lowest (9.2913).

**Objective Color Determination**

The objective color scores of $L^*$, $a^*$, and $b^*$ are diagramed in Figure 2, Figure 3, and Figure 4, respectively. Fat source differed ($P = 0.0006$) in $L^*$ values, where $S > I = KP$ with means of 51.924, 51.396, and 51.2544, respectively. Fat percentage differed ($P < 0.0001$) in $L^*$ values, where 25% > 5% with means of 54.432 and 48.618, respectively. Length of time in retail display differed ($P < 0.0001$) in $L^*$ values, where 7 d > 5 D > 3 d = 1 d with means of 52.035, 51.703, 51.231, and 51.130, respectively. The interaction of fat percentage x time also showed differences ($P = 0.031$) in $L^*$, where 25% X 7 d had the greatest value (54.820) and 5% X 1 d had the lowest value (48.137).

Fat source differed ($P = 0.0046$) in $a^*$ values, where $S > I = KP$ with means of 14.783, 14.522, and 14.469, respectively. Fat percentage differed ($P < 0.0001$) in $a^*$ values, where 25% > 5% with means of 14.886 and 14.297, respectively. Length of time in retail display differed ($P < 0.0001$) in $a^*$ values, where 7 d < 5 D < 3 d < 1 d with means of 12.065, 13.355, 15.041, and 17.904, respectively. The interaction of fat % x time also differed ($P = 0.031$) in $a^*$, where 25% X 1 d had the greatest value (18.400) and 25% X 7 d had the lowest value (12.049). No other interactions showed any differences ($P > 0.05$) in $a^*$ values.
Fat Percentage differed (P < 0.0001) in b* values, where 25% > 5% with means of 7.863 and 6.267, respectively. Length of time in retail display differed (P < 0.0001) in b* values, where 1 d > 3 D > 5 d = 7 d with means of 7.812, 7.054, 6.670, and 6.724, respectively. The interaction of fat percentage x time also showed differences (P = 0.031) in b*, where 25% X 1 d had the greatest value (8.673) and 5% X 5 d had the lowest value (5.879).

**Lipid Oxidation Determination**

The TBA values of treatment over time are diagramed in Figure 1. Fat source differed (P = 0.007) in TBA values, where I = S > KP with means of 0.120, 0.119, and 0.1105 mg malonaldehyde/ kg of sample, respectively. Fat percentage and the interaction of source x fat percentage showed no differences (P > 0.05) in TBA values. However, length of time in display differed (P < 0.0001) in TBA values, where 3 d < 5 d < 7 d with means of 0.088, 0.115, and 0.147 mg malonaldehyde/ kg of sample, respectively.

**Discussion**

**Fat and Moisture Determination**
The three fat sources showed to have different \((P = 0.0053)\) fat percentages, where the percentage of \(S > I = KP\). This was an interesting finding. For this study, the adipose tissues were added at the same weight in all treatments, so the different sources of fat varied in lipid consistencies. Fat is lipid cells suspended in a connective tissue matrix and different types of fat can vary in the ratios of lipid, water, and connective tissue (Nurnberg et al., 1998). These different fat depots are also laid down at different stages of development and provide different functions for the animal that may explain differences in lipid concentration. Results also showed that the fat sources varied in moisture content as well, with \(I > KP > S\). This can be explained by Nurnberg et al. (1998), who reported that adipose tissue can contain between 5 to 20% water. This variation in water content among adipose tissues explains differences in moisture percentage. Fat percentage differed in moisture percentage \((P < 0.0001)\), as expected. This is due to the fact that fat contains roughly 20% water while muscle can contain from 70-80% 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.

**Oxymyoglobin Concentration Determination**

As expected, 5 and 25% fat inclusion caused differences in oxymyoglobin concentration. As the percentage of fat increases, the percentage of muscle containing oxymyoglobin decreases causing the concentrations of that ground
beef blend to decreases as well. The same muscle was used for all formulations so we did not see a difference in oxymyoglobin concentrations among the treatments. Studies have shown that muscles can contain different oxymyoglobin concentrations and muscles with high oxymyoglobin concentrations can be more susceptible to oxidation during retail display (Raines et al., 2010). This study only looked at oxymyoglobin concentrations at one time point to be compared to TBA values but it could be advantageous to compare the oxymyoglobin concentrations over time as well.

**Fatty Acid Analysis**

Percent fat inclusion showed to have a major effect on the fatty acid composition of the ground beef blends. Inclusion of 25% fat showed to have greater SFA, SFA:PUFA, and omega-3:omega-6 ratio as well as lower MUFA, PUFA, omega-6, and IV, compared to 5% fat inclusion. Increasing fat percentage increases the SFA:PUFA ratio, making the product more stable and decreasing oxidation. Fat source differed (P < 0.05) in SFA, MUFA, SFA:PUFA, and IV. 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). In the current study, KP showed to contain the highest concentrations of SFA and SFA:PUFA, as well as the lowest concentrations of MUFA and the lowest iodine value. All of these factors tend to decrease oxidation. Lipid oxidation is largely affected by PUFA,
because they contain multiple double bonds that possess especially reactive hydrogen. S showed the opposite effect, with the lowest SFA and SFA:PUFA as well as the highest MUFA and IV. Aldai et al. (2007) also showed S to have lower SFA and higher MUFA and total UFA when compared to I. The variation in lipid oxidation could also be attributed to the variation in UFA 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 there should be little variation. The only source x fat percentage interaction (P = 0.023) occurred in the SFA:PUFA ratio, where KP added at 25% was the highest (20.30) and S added at 5% was the lowest (9.2913). This can be attributed to both the main effects in the interaction and their effect on the SFA:PUFA ratio.

**Objective Color Determination**

Fat percentage differed (P < 0.0001) in L* values. As the percent fat increases from 5 to 25%, the L* values increase, meaning the ground beef patties are lighter. Martin et al. (2013) also showed 73:27 ground beef was lighter (greater L* value; P < 0.05), than 81:19 and 91:9 ground beef at all storage intervals. Increasing the levels of fat increase L* values due to its light color. Fat source differed (P = 0.0006) in L* values as well. S had higher L* values than both I and KP. However, it was a very small difference (51.942 vs 51.254) and would not likely be discernible by visual appraisal of ground beef patties. Length in refrigerated retail display also differed (P < 0.0001) in L* values. There were no
differences (P > 0.05) from day 1 to 3, but as the days of display increase from 3 to 7, the L* values increased. 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 (46.44 to 47.44) (Hoyle Parks et al., 2012). This means ground beef patties become lighter as days in refrigerated retail display increase. The only interaction to differ (P < 0.05) in L* was the interaction of fat percentage x time. The blends of ground beef containing 25% fat tended to have greater L* values than 5% and within the fat percentages the L* value increased with the number of days on retail display. This compliments the findings of both fat percentage and time.

Consumer purchasing decisions are historically based on lean appearance, so a* value plays a critical role in quality of fresh ground beef patties in refrigerated retail display. Like the findings with L*values, fat percentage, fat source, and time differed (P < 0.05) in a* values. Inclusion of 25% fat showed to increase (P < 0.0001) a* values compared to inclusion of 5% fat (14.886 vs 14.297). This result was unexpected because higher fat percentage would mean less muscle, responsible for the red color, and would likely decrease a* values, but that was not the case in this study. The protein responsible for meat color is myoglobin and freshly cut meat is in the state of oxymyoglobin which makes the muscle a dark cherry red (Aldai et al., 2007). However, Martin et al. (2013) showed all formulations of ground beef (73:27, 81:19, and 91:9) maintained similar redness (a*) through 14 d storage, regardless of lean level, confirming our very small increase in a* value between the 5 and 25% fat level (14.297 vs
The S had higher a* values than I and KP. This is likely due to increased vascular properties of S. However, the values only ranged from 14.783 in S to 14.469 in KP. This slight difference would not likely be discernible by visual appraisal of the ground beef patties. The time in refrigerated retail display differed (P < 0.0001) in a* values as well. As days on display increased from one to seven, a* values decreased from 17.904 to 12.065, meaning the patties lost redness. Raines et al., (2010) also reported that a* values decreased during refrigerated retail display, out to 4 days. However, Hoyle Parks et al. (2012) reported lean color scores of fresh beef patties evaluated by sensory panelists significantly increased (P < 0.0001) from 0 to 84 h (1.75 to 5.55). Redness is the driving factor for consumers when purchasing ground beef, so this discoloration may detour consumers from purchasing it and making it a lost value for the retailer. Packaging type can also affect the rate at which a* value decreases. Numerous studies have shown that elevated levels of oxygen, such as in MAP packaging; prolong color stability of meat (Bartkowski, Dryden, and Marchello, 1982; Taylor, 1972).

Values of b* are the least significant contributor to meat quality. Like the findings with L* and a* values, fat percentage and time differed (P < 0.05) in b* values. Inclusion of 25% fat showed to increase (P < 0.0001) b* values compared to inclusion of 5% fat (7.863 vs 6.267). The product became more yellow as the fat percentage increased. Number of days in refrigerated retail display showed b* decreased from day one to five and then leveled off from day five to seven. Raines et al., (2010) also reported that b* values decreased during refrigerated
retail display, out to four days. The only interaction to differ (P < 0.05) was the interaction of fat percentage x time. The 25% X 1 d had the greatest value (8.673) and 5% X 5 d had the lowest value (5.879) of all the treatments.

**Lipid Oxidation Determination**

Fat source differed (P = 0.007) in TBA values. KP had lower TBA values than I and S, meaning it had less oxidation. This reaffirms our findings from the fatty acid profiles, which indicated KP would have less oxidation compared to I and S because it had a greater SFA:PUFA ratio and a lower IV. Fat percentage and the interaction of source x fat percentage showed no differences (P > 0.05) in TBA values. However, length of time in retail display differed (P < 0.0001) in TBA values. As days increased from three to seven the TBA values increased. Martin et al. (2013) also showed TBARS increased as storage lengthened. The highest TBA value (0.147) was on day seven, the end of the shelf life study. Green and Cumuze (1981) reported a TBA value of at least 2.0 mg malonaldehyde/kg is necessary for the sensory detection of rancid off-odors by an untrained typical consumer. At the end of this study (seven days) the patties showed discoloration and increasing TBA values that would have got them pulled from the case in a retail setting, but were not oxidized to the point of rancidity.
Conclusion

KP inclusion showed to decrease oxidation and discoloration in ground beef patties during refrigerated retail display compared to I and S. More work will have to be conducted to see just how much extra shelf life KP inclusion can provide and if increasing levels KP have any effect on palatability or consumer acceptance. Overall, fat source is a significant contributor to quality indicators in ground beef patties and should be considered when formulating products intended for fresh, refrigerated retail sale.
Table 1. Percent composition of steer diet (as fed basis).

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### Receiving Supplement Composition

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### Feedlot Diet Composition

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### Feedlot Supplement Composition

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<tr>
<td>Rumen Trace Mineral</td>
<td>0.85</td>
</tr>
<tr>
<td>Vitamins A, D and E</td>
<td>0.85</td>
</tr>
<tr>
<td>Rumensin</td>
<td>0.05</td>
</tr>
<tr>
<td>Fat (CWG)</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Table 2. Effects of fat source (I\textsuperscript{a}, S\textsuperscript{b}, KP\textsuperscript{c}) added at 5% and 25% on fat and moisture % of ground beef.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Fat, %</th>
<th>Source</th>
<th>%Fat</th>
<th>P-values</th>
<th>Source x %Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6.99</td>
<td>22.12</td>
<td>7.14</td>
<td>24.24</td>
<td>6.63</td>
</tr>
<tr>
<td>S</td>
<td>25.00</td>
<td>58.57</td>
<td>69.77</td>
<td>56.75</td>
<td>70.18</td>
</tr>
</tbody>
</table>

\textsuperscript{a}intermuscular fat
\textsuperscript{b}subcutaneous fat
\textsuperscript{c}kidney and pelvic fat
Table 3. Effects of fat source (I\(^a\), S\(^b\), KP\(^c\)) added at 5% and 25% on oxymyoglobin concentration of ground beef.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>I</th>
<th>S</th>
<th>KP</th>
<th>SEM</th>
<th>Source</th>
<th>%Fat</th>
<th>Source x %Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxymyoglobin, mg/kg</td>
<td>3.73</td>
<td>3.31</td>
<td>3.69</td>
<td>3.30</td>
<td>3.70</td>
<td>0.07</td>
<td>0.81</td>
</tr>
</tbody>
</table>

\(^a\) intermuscular fat
\(^b\) subcutaneous fat
\(^c\) kidney and pelvic fat
Table 4. Effects of fat source (I\(^a\), S\(^b\), KP\(^c\)) added at 5% and 25% on fatty acid composition\(^d\) of ground beef.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>I 5</th>
<th>25</th>
<th>S 5</th>
<th>25</th>
<th>KP 5</th>
<th>25</th>
<th>SEM</th>
<th>Source %Fat</th>
<th>Source x %Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFA, %</td>
<td>51.62</td>
<td>55.40</td>
<td>47.71</td>
<td>56.64</td>
<td>62.23</td>
<td>1.63</td>
<td>0.00</td>
<td>&lt;0.0001</td>
<td>0.17</td>
</tr>
<tr>
<td>MUFA, %</td>
<td>38.58</td>
<td>34.85</td>
<td>41.55</td>
<td>33.63</td>
<td>35.94</td>
<td>30.31</td>
<td>1.69</td>
<td>0.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PUFA, %</td>
<td>5.97</td>
<td>5.00</td>
<td>5.85</td>
<td>5.16</td>
<td>6.07</td>
<td>4.23</td>
<td>0.34</td>
<td>0.43</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Nd(^e)</td>
<td>3.83</td>
<td>4.75</td>
<td>4.89</td>
<td>4.57</td>
<td>4.31</td>
<td>3.23</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SFA:PUFA</td>
<td>10.85</td>
<td>12.83</td>
<td>9.29</td>
<td>12.32</td>
<td>10.74</td>
<td>20.30</td>
<td>1.62</td>
<td>0.00</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>n-3</td>
<td>0.19</td>
<td>0.20</td>
<td>0.24</td>
<td>0.22</td>
<td>0.21</td>
<td>0.17</td>
<td>0.04</td>
<td>0.46</td>
<td>0.48</td>
</tr>
<tr>
<td>n-6</td>
<td>5.05</td>
<td>3.91</td>
<td>4.96</td>
<td>4.09</td>
<td>5.19</td>
<td>3.33</td>
<td>0.33</td>
<td>0.63</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>n-3 : n-6</td>
<td>0.05</td>
<td>0.10</td>
<td>0.06</td>
<td>0.08</td>
<td>0.05</td>
<td>0.09</td>
<td>0.02</td>
<td>0.84</td>
<td>0.01</td>
</tr>
<tr>
<td>IV</td>
<td>39.40</td>
<td>34.98</td>
<td>42.24</td>
<td>34.41</td>
<td>37.67</td>
<td>30.23</td>
<td>1.47</td>
<td>0.00</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(^a\) Intermuscular fat  
\(^b\) Subcutaneous fat  
\(^c\) Kidney and pelvic fat  
\(^d\) Percent of fatty acids detected  
\(^e\) Non-detectable or Unknown
Figure 1. TBA values of ground beef patties formulated with intermuscular (I), subcutaneous (S), and kidney and pelvic (KP) fat as well as 5 and 25% fat inclusion over 7 days refrigerated (4°C) retail display.
Figure 2. L* values of ground beef patties formulated with intermuscular (I), subcutaneous (S), and kidney and pelvic (KP) fat as well as 5 and 25% fat inclusion over 7 days refrigerated (4°C) retail display
Figure 3: Minolta a* values of ground beef patties over 7 days storage

Figure 3. a* values of ground beef patties formulated with intermuscular (I), subcutaneous (S), and kidney and pelvic (KP) fat as well as 5 and 25% fat inclusion over 7 days refrigerated (4°C) retail display
Figure 4. Minolta b* values of ground beef patties over 7 days storage

Figure 4. b* values of ground beef patties formulated with intermuscular (I), subcutaneous (S), and kidney and pelvic (KP) fat as well as 5 and 25% fat inclusion over 7 days refrigerated (4°C) retail display
APPENDIX A: MATERIALS AND METHODS

A.1 CEM Procedure

Method performed according to Keeton et al., 2003.


1. Using the CEM smart TRAC set the method to 0-30 BEEF.
2. Two CEM square sample pads were placed on the balance pan and the tare button was pressed.
3. Approximately 3.5-4.5 g of sample were weighed using the balance pan and then the sample was smeared across one of the pads using a scoopula spatula.
4. The second pad was placed over the sample sandwiching the sample between both pads.
5. The sample and pads were placed on the balance pan within the CEM Moisture/ Solids Analyzer.
6. The start button was pressed and moisture percentage was determined by the CEM Moisture/ Solids Analyzer.
7. The dried sample was then wrapped in CEM TRAC paper.
8. The sample was packed to the bottom of a CEM TRAC plastic tube and the tube was placed into the CEM Rapid Fat Analyzer.
9. The ready button was pressed and then the start button was pressed.
10. Fat percentage was determined using nuclear magnetic resonance and the computer gave a total fat percentage on a dry matter (DM) basis.

11. DM values were converted to wet matter (WM) values using the following equation.
    
    \[ a. \quad \left( \frac{100 - \% \text{ moisture}}{100} \right) \times \% \text{ fat DM} = \% \text{ fat WM} \]

---

**A.2 Oxymyoglobin Concentration Determination**

**Sample Pulverization**

1. A.2 Oxymyoglobin Concentration Determination

2. Sample Pulverization
   - Chop or mince sample into small cubes.

3. Pour liquid nitrogen into grinder cup to cool it.

4. Add sample to cup and add enough liquid nitrogen to submerge the sample.

5. Freeze sample until rapid boiling of liquid nitrogen ceases. Time will be dependent on initial temperature of sample.

6. Fill grinder cup approximately 1/4 full of liquid nitrogen.

7. Grind sample for approximately 30 seconds.

8. Add a small amount of liquid nitrogen to a metal or plastic dish to cool it. The larger the surface area, the faster the dissipation of nitrogen (should be complete in 2 min.). Pour the blended sample into the cold dish.
9. Place sample in plastic bag. Remove as much air as possible and quickly move to freezer.

**Myoglobin Determination**

10. Weigh 3 grams of the pulverized raw sample into a large weigh boat and record the exact weight of the sample.

11. Place the weighed sample into the grinder cup and add ten times the weight (30ml) in cold buffer (40mM potassium phosphate buffer pH 6.8).

12. Grind sample for approximately 1 minute or until the sample is completely homogenized in the buffer.

13. Pour into labeled centrifuge bottles and incubate at 4°C for **one hour**.

14. Centrifuge the samples at 15,000 x g for 30 minutes at 4°C or at 25,000 x g for 15 minutes at 4°C.

15. Pour off the supernatant and filter (Whatman filter No. 1) into a 50ml glass tube.

16. Fill cuvette to mark.

17. Turn on the spectrophotometer and set wavelength at 418 nm (allow 30 minutes to warm up).

18. Prepare a blank (D.I. water) and zero the spectrophotometer.

19. Measure the absorbance of the sample.

**Calculation**

20. After the sample is scanned, it is only in the oxymb form. Take the absorbance (Abs) of the soret peak (416-418nm) and use the following formulas to calculate the mg Mb/g of sample.

   a. \( \text{Abs}_{418}/\varepsilon \times b \) = molar conc. of myoglobin

   Where \( \varepsilon = 128000 \) for oxymb and \( b = \) pathlength of the cuvette usually 1 cm so \( \text{Abs}_{418}/12800 = \) Molar concentration of myoglobin
b. Molar concentration of myoglobin * molecular weight of myoglobin = g/l of myoglobin (16946g/mol for bovine, 16953g/mol for porcine mb)
c. g/l of myoglobin * number of L of buffer you added to pulverized sample * 1000 = mg of myoglobin in the sample.
d. Mg myoglobin/g of sample you blended = mg of mb/g of sample

Solutions:

Potassium buffer

5.44 g/1 Liter D.I. water

A.3 Fatty Acid Extraction

Extraction of Total Lipids

1. Weigh ~100 mg adipose tissue, 1 g muscle, 2 ml plasma, 1 g digesta, or 1 g liver and place in a labeled 10 ml glass tube.
3. Homogenize each sample with Polytron homogenizer for ~30 seconds or until tissue is broken down. Remove any sample that may have got lodged in homogenizer and place back into tube. Rinse probe with chloroform:methanol twice and ddH2O twice. Dry probe with Kimwipes.
4. Transfer homogenate to 50ml glass tube. Rinse small tube 2-3 times and also place in same 50ml tube.
5. Filter homogenate through sintered glass filter funnel (use 934-AH filter paper) into a second 50ml glass tube. Rinse first tube 2-3 times with chloroform:methanol. In between each sample discard filter paper, rinse filter angled downward with chloroform:methanol, and wipe inside of funnel with kimwhipes. Repeat for all samples.
6. Add 8 ml of 0.74% KCL and vortex ~30s.
7. Let sit 2 hr to separate phases. If stopping at this point flush with Nitrogen, cap, and let sit in refrigerator overnight.
8. Carefully remove upper phase and discard by using vacuum suction apparatus.
9. Transfer all of lower phase to 34 ml glass tube.
10. Evaporate the sample to dryness with nitrogen using the N-Evap in water bath. Set Nitrogen flow between 6 and 7.

Saponification and Methylation of lipids
11. Leave sample tubes in N-Evap but turn the Nitrogen flow off. Add 1 ml of 0.5 N KOH. Heat in water bath for ten minutes.
12. Remove samples from N-Evap and add 1 ml of 14% BF3 in MeOH. Store BF3 in refrigerator.
13. Flush with nitrogen and loosely cap tubes. Place in water bath for 30 min. *Steps 11-13 saponifies the lipids, i.e., it liberates the fatty acids from the glycerides. The acid group is methylated in this process, removing the net negative charge on the group.
14. Remove the tubes and allow them to cool. Add 2 ml HPLC grade hexanes and 2 ml of saturated NaCl. Vortex for ~30s.
15. Pipet off upper layer with transfer pipet; place in a 20 ml glass tube with 800 mg Na2SO4 in it. Add 2 ml hexanes to the tube with saturated NaCl and vortex.

16. Allow to settle and pipet the upper hexanes layer into the 20 ml glass tube with Na2SO4. You should now have ~4 ml of hexanes in the tube. Vortex this tube briefly. The Na2SO4 removes any moisture from the hexanes.

17. Pipet the hexanes into a labeled scintillation vial.

18. Add 1 ml of hexanes into the 20 ml glass tube with Na2SO4 in it. Vortex briefly and transfer the hexanes to the scintillation vial.

19. Evaporate the hexanes completely with the N-Evap using Nitrogen with the flow set between 6 and 7. *Be extremely careful when lowering the samples into the water bath to prevent water from splashing into the vials.

20. Add 1 ml of hexanes back into scintillation vial.

21. Pipet hexanes from the scintillation vial to GC vials. *Avoid pipetting any contaminants such as salt in the bottom of the vial.

22. Refrigerate until ready to load onto the GC.

**Solutions**

**chloroform:methanol** (CHCL3:CH3OH, 2:1, V:V)

**0.5N KOH in Methanol**

- 2.81g KOH / 100ml MeOH

**Saturated NaCl**

- 31.7g NaCl / 100ml ddH2O
0.74% KCl
- 7.4g KCl / 1L ddH2O

**A.4 TBAR Method**

**Materials:**
Food sample (5 grams) homogenized
Antioxidant solution (prepare fresh)
TCA reagent (store 4-6 weeks-refrigerate)
1:1 (v/v) TCA reagent/water
.02 M TBA (prepare fresh)
0.2 mM TMP (store 1 month-refrigerate)
Homogenizer
100 mL volumetric flasks
Whatman no 1 filter paper (12.5 cm)
50 mL centrifuge tubes
Boiling water bath
Spectrophotometer
Glass cuvettes

**Method:**
1) Weigh (record weight) of 5 g homogenized food sample
2) Add 2.5 mL antioxidant solution and 50 mL ice-cold TCA reagent and mix for 2 minutes

3) Add 50 mL ice-cold distilled water and mix for another minute

4) Place a funnel over the top of a 100 mL volumetric flask and line with filter paper. Prewet the filter paper with 1:1 TCA reagent:water

5) Pour the contents into the funnel and filter the extract.

6) Fill the flask to mark with 1:1 TCA reagent/water. Mix thoroughly

**Perform TBA reaction**

7) Pipet a 5.0 mL aliquot from the 100 mL volumetric flask into a 50 mL centrifuge tube. Add 5mL of .02M TBA (Prepare a reagent blank 5 mL 1:1 TCA reagent/water and 5 mL .02M TBA)

8) Cap the tubes, vortex, heat 35 minutes in boiling water bath.

9) Cool by running under tap water for 10 minutes or in ice water for 5 minutes

**Measure sample absorbance**

10) Turn on spectrophotometer and set wavelength to 532nm (allow 30 minutes to warm up before readings)

11) Zero spectrophotometer with the reagent blank and then measure the absorbance of the test sample using a glass cuvette

**Determine Malonaldehyde Recovery**

12) Repeat steps 1-6 with new homogenized food samples, adding 1.5, 2.0, and 4.5 mL of 0.2mM TMP, respectively before step 2. In step 3, reduce the volume of ice-cold distilled water accordingly.
13) Add 1.5, 3.0, and 4.5 mL of 0.2mM TMP to three separate 100 mL volumetric flasks and fill to mark with 1:1 TCA reagent/water

14) Perform TBA reaction and measure absorbance (steps 7-11) using the three TMP spiked samples (step 12) and the three TMP dilutions (step 13).

15) Use the absorbance of the original (unspiked) food sample from step 11 to correct for endogenous malonaldehyde content and then compare the spiked values to those in the TMP dilutions.

\[
\%\text{ recovery} = 100 \times \frac{A_{sp}}{A_{tmp}}
\]

\(A_{sp}\) is the absorbance of the spiked food sample (corrected for endogenous malonaldehyde)

\(A_{tmp}\) is the absorbance of the corresponding TMP dilution

The three resulting percent recoveries are averaged and used in step 16

**Determine TBA value**

16) Calculate \(K\), a constant derived from the assay, using the following equation:

\[
K = \frac{S}{A} \times (\text{MW}) \times (DF) \times (10^6) \times (100/\% \text{ recovery})
\]

\(S = (1 \times 10^{-8})/5\text{ml}\)

\(A = .141\)

MA mol. Wt = 72.03 g/mol

DF = dilution factor = (5/100ml) x (5ml)

\% recovery is the average value determined in step 15
17) Calculate TBA value using the following equation which gives you the mg malonaldehyde eq/kg of food sample:

\[ \text{TBA value} = K \times A_{532} \]

**TBARS Solutions:**

**Antioxidant solution**

Weigh 0.5 g propyl gallate and .5 g EDTA into a 100 mL volumetric flask, dissolve in a small volume of 1:1 ethanol/water and dilute to mark with ethanol/water solution.

*Prepare Fresh Daily.*

**TMP solution-malonaldehyde bis**

Prepare 20mM TMP stock solution by diluting .335 mL TMP into 100mL water or 300mL water with 1 mL TMP (store up to 3 months at 4 degrees C)

(0.2mM solution is used in the assay, so before starting, dilute 1 mL of stock solution in 100mL of water to get a working solution, this can be stored up to 1 month at 4 degrees C)

**TCA reagent**

Weigh 200g TCA in a beaker and dissolve crystals in a small volume of water.

To a volumetric flask, add some water and then add 16 mL of 85% phosphoric acid Swirl solution and then quantitatively transfer TCA mixture from beaker with water. Fill to mark with water and mix contents thoroughly, can be stored 4-6 weeks at 4 degrees C
To calculate how much TBA will be needed for the day: determine the number of samples that will be run and multiple by 5 (5ml per sample). This will give the total volume needed, but make some extra to be safe. 2.883 g TBA/1 liter D.I. water. Prepare fresh daily.
APPENDIX B: SAS PROGRAMS

B.1 Fat Percentage

proc mixed; class source lean rep;
model fat=source|lean;
random rep;
lsmeans source|lean/pdiff;
run;

B.2 Moisture Percentage

proc mixed; class source lean rep;
model moist=source|lean;
random rep;
lsmeans source|lean/pdiff;
run;

B.3 Oxymyoglobin

proc mixed; class source lean rep;
model myo=source|lean;
random rep;
lsmeans source|lean/pdiff;
run;

B.4 TBAR

proc mixed; class rep source lean tm;
model tba=source|lean|tm;
random rep;
repeated tm/sub=rep(source lean) type=cs;
lsmeans source|lean|tm/pdiff;
run;

B.5 Objective Color
proc mixed; class rep source lean tm;
model L=source|lean|tm;
random rep;
repeated tm/sub=rep(source lean) type=cs;
lsmeans source|lean|tm/pdiff;
run;

proc mixed; class rep source lean tm;
model a=source|lean|tm;
random rep;
repeated tm/sub=rep(source lean) type=cs;
lsmeans source|lean|tm/pdiff;
run;

proc mixed; class rep source lean tm;
model b=source|lean|tm;
random rep;
repeated tm/sub=rep(source lean) type=cs;
lsmeans source|lean|tm/pdiff;
run;


