

IMPROVING THE FLAVOR OF GROUND BEEF BY SELECTING TRIMMINGS
FROM SPECIFIC LOCATIONS

A Thesis

by

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ABSTRACT

We hypothesized that carcass subcutaneous fat location would affect sensory and quality traits. Five carcass fat sources were tested: brisket, chuck, plate, flank, and round. Ground beef was formulated using each fat source and extra-lean beef trim (>95% lean) to contain 80% lean trim and 20% fat trim. Patties (100 g) were evaluated for color, lipid oxidation, fatty acid composition and consumer evaluation. Flavor was analyzed using a Gas chromatography with mass spectrometry (GC/MS) on the headspace above a cooked (74°C) patty in a heated (60°C) 473 mL glass jar with a solid phase micro-extraction (SPME) fiber. Color, thiobarbituric acid reactive substance assay (TBARS), consumer sensory, and cook/freezer loss data showed no differences ($P > 0.05$) among carcass locations. Percentage stearic acid was lower ($P = 0.044$) in the brisket than in the chuck and flank. The brisket was higher in percentage cis-vaccenic acid ($P = 0.016$) and in the saturated fatty acid to monounsaturated fatty acid ratio ($P = 0.018$), and lower ($P = 0.004$) in the percentage of total saturated fatty acids than all other sources of subcutaneous fat. Butanedione was highest ($P = 0.013$) in the flank and plate fat. Brisket tended to be higher ($P = 0.054$) than flank, plate, and round in 1-octen-3-ol. Brisket was higher ($P = 0.008$) than chuck, flank, and round, but not different ($P > 0.05$) than plate in octanedione. Brisket was higher ($P = 0.003$) than all other sources for beefy aroma. Flank was higher ($P = 0.047$) than chuck and round for chemical aroma. Brisket was higher ($P = 0.004$) than all other sources except flank for floral aromas. Plate was higher ($P = 0.029$) than all other sources for heated oil aromas. For secondary aroma descriptor, round was higher ($P < 0.001$) than flank, plate, and chuck for dairy. While differences

in some key fatty acids and aromatics existed among carcass locations, when the fat was diluted with a common lean source, fat source did not have a negative effect on sensory or quality traits. Therefore, formulating ground beef using subcutaneous fat from specific locations on a carcass may improve the beef aromatics without negatively affecting sensory or quality traits.

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1. INTRODUCTION AND LITERATURE REVIEW

Introduction

Ground beef represents a major portion of the beef sales in the United States. More than 8.2 billion burgers or cheeseburgers were served in commercial restaurants in 2001 and burgers accounted for 75% of all beef entrees served (National Cattlemen's Beef Association [NCBA], 2002). In fact, ground beef represented 63% of the total volume in the foodservice industry and ground beef was present in 60% of all in-home beef servings (Cattlemen's Beef Board and NCBA, 2009).

Beef flavor is a very important component of the eating quality of beef. Finding methods to naturally enhance the flavor of beef products utilizing existing raw materials could insure palatability of beef economically. According to Mottram (1998a), a major precursor to the development of beef flavors lies in the thermal degradation of lipids. In a study by Baublits et al. (2009), the concentration of monounsaturated fatty acids was positively correlated with beefy/brothy and beef fat flavors, which indicated that as the percentage of these fatty acids increase the flavor profile could be improved. In a study done by Turk and Smith (2009), subcutaneous fat sources were evaluated for fatty acid composition. According to that study, there were significant differences in fatty acid composition among the eight fat depots that were analyzed. Brisket fat had the highest amount of oleic acid (43.1 g/100 g of total fatty acids) and flank fat had the lowest amount (36.8 g/100 g of total fatty acids). Brisket also had the highest concentration of monounsaturated fatty acids and the lowest concentration of saturated fatty acids. This

review will cover the literature published related to the relationship between beef flavor, fatty acid composition, shelf-life stability, and consumer acceptance.

Flavor

Flavor is an important component in the eating quality of meat. Flavor is thermally derived, and without the application of heating, meat has little, to no, flavor. There are two main types of analyzable aromatic flavor precursors, water-soluble and lipid-soluble compounds. Both of these play a role in making up the meat flavor in response to the different cooking reactions (Mottram, 1998a).

Lipid-derived flavors have a higher odor threshold compared to the water-soluble components (Mottram, 1998a). When analyzing the lipid-soluble aromatic components, species differences play a huge role. Lipids from different animal species greatly influence the type of flavor given off during cooking because the adipose tissue acts as a solvent and traps the aromas that can be released upon the application of heat (Wasserman, 1972). Aldehydes are formed from the thermal degradation of the fatty acid at the double bond, and are considered to be major volatiles of cooked meat flavor. Since various species have different levels of unsaturation and dissimilar double bond locations of lipids, unique compounds are formed throughout the degradation process (Mottram, 1998a).

Another contributor to the lipid-soluble component of aromatic flavor is phospholipids. Phospholipids have a high concentration of unsaturated fatty acids and therefore are more susceptible to oxidation and warmed over flavor, which has a negative impact on meat flavor (Mottram, 1998a; Pearson, Love, and Shorland, 1977).

Although a large amount of lipid oxidation is negative, phospholipids are involved in lipid oxidation during initial cooking, which has a positive effect on flavor formation (Mottram, 1998a).

The lipid-soluble component of flavor has two main types of degradation: thermal and oxidative, which will be discussed later (Calkins and Hodgen, 2007). According to Mottram (1998a), a major precursor to the development of beef flavor lies in the thermal degradation of lipids. Thermal degradation is the oxidation of acyl chains of the lipids and produces over half of the volatiles reported in meat flavor (Mottram, 1998b). Most thermal degradation occurs at temperatures of 200-300°C, but if temperatures exceed this, acrid or bitter components can form (Wasserman, 1972). For example, in beef flavor from thermal oxidation, butenal produces a malty, green, roast flavor (Calkins and Hodgen, 2007).

Another important precursor to the development of meat flavor is the water-soluble component, which includes amino acids, peptides, carbohydrates, nucleotides, and thiamine. In water-soluble aromatic flavor, there are two major precursors, cysteine and ribose. Cysteine is a sulfur-containing amino acid. According to Morton, Akroyd, and May (1960), when cysteine compounds are heated, a meat-like flavor is formed. Sulfur compounds are involved in the flavor of cooked meats, and produce an acceptable aroma at low concentrations (Wasserman, 1972). Sulfhydryl groups are located on myosin heads, which can play a role in flavor formation. Cysteine also plays an important role in the Strecker degradation, which involves the oxidative deamination and decarboxylation of an α -amino acid in the presence of a dicarbonyl compound, where it

can lead to the production of ammonia and acetaldehyde (Mottram, 1998aandb). Unlike lipid-soluble aromatic flavor components, cysteine will more readily participate in flavor reactions via the Maillard reaction and Strecker degradation. The other main water-soluble component is ribose. Ribose is a five-carbon structure and is one of the main sugars associated with ribonucleotides, in particular, adenosine triphosphate, which is essential for muscle function (Mottram, 1998a).

While both lipid- and water-soluble aromatic flavor components are different, they have an important interaction, where the lipid-derived aldehydes contribute to the Maillard reaction during cooking. Their interaction leads to a number of heterocyclic compounds with long chain alkyl substituents, such as, pyridines, pyrazines, thiophenes, thiazoles, and thiazolines (Mottram, 1998b). Their reactions compete to influence the overall aroma profiles of cooked meat.

When analyzing meat flavor, one of the most important reactions is the Maillard reaction, which has three stages. In both of the initial stages as well as the later stages, reactions between carbonyl compounds and amino and thiol groups are important steps (Mottram, 1998a). The initial stage is where the amino acid reacts with a reducing sugar to form a Schiff base that cyclizes to give the corresponding glycosylamine (Romero and Ho, 2007). In other words, the sugar and amino acid can be considered as a source of dehydrated sugar products, principally furfurals, furans, and dicarbonyl compounds (Mottram, 1998b). This is followed by a rearrangement reaction to produce Amadori (1-amino-1-deoxy-2-ketose) or Heyns (2-amino-2-deoxyaldose) products (Romero and Ho, 2007). The final stage of this reaction is the formation of polymeric substances that leads

to color development. This reaction produces volatiles from both lipid-Maillard and water-soluble-Maillard interactions. One of the major compounds produced in cysteine-ribose system is furan, a four carbon ring with two double bonds and oxygen, which is said to have a meaty, roasty aroma (Mottram, 1998b; Romero and Ho, 2007). Aldehydes formed during lipid oxidation have been shown to participate in the Maillard reaction (Mottram, 1998b). With this interaction of lipids, it indicates that lipids control the production of sulfur compounds during cooking of meat, which suggests a mechanism by which the concentration of important sulfur compounds is maintained at optimum levels in the cooked product (Mottram, 1998b).

Within the Maillard reaction, there are five main conditions that affect the reaction: temperature, time, pH, water activity, and pressure. Temperature and time contribute in determining what type of end products will be created within this reaction. For example, extended time and higher temperatures increases levels of pyrazines (Romero and Ho, 2007). Also, with temperatures higher than 180° C, an overall increase in volatile compounds have been discovered (Ames, Guy, and Kipping, 2001). The volume of compounds is decreased with an increased amount of storage time (Ames, Bailey, Monti, and Bunn, 1996). Additionally, a higher pH condition/environment intensifies Maillard browning (Romero and Ho, 2007). Water activity (a_w) is positively correlated to the rate of browning, with maximum a_w values of 0.5-0.8, but browning decreased at any higher a_w and a maximum amount of volatiles were observed at a_w 0.72 (Romero and Ho, 2007). Moreno, Molinam, Olano, and Lopez-Fandiño (2003) indicated

that Amadori rearrangement products formed faster and degraded under high pressure, causing an increase of intermediate and advanced reaction products.

Lipid oxidation

Lipid oxidation produces a negative effect on meat flavor. It typically follows the same route as thermal oxidation, but subtle changes produce different volatiles (Mottram, 1998a). The fats in meat are susceptible to oxidation when exposed to molecular oxygen in the air, which results in the production of strong, unpleasant odors and flavors (Aberle et al., 2001). The rate of autoxidation can be affected by pro-oxidants, such as metal ions, heat, ultraviolet light, and low pH (Aberle, Forrest, Gerrard, and Mills, 2001). Upon further processing, e.g. grinding, oxidation is accelerated by the incorporation of oxygen during grinding (Aberle et al., 2001).

The major reaction in the formation of off-flavors in meat is a free-radical chain reaction referred to as autoxidation (oxidative rancidity; Pegg and Shahidi, 2007; Aberle et al., 2001). Lipid oxidation involves a free radical mechanism that produces hydroperoxides (Frankel, 1980). First, there is the degradation of hydroperoxides, which initially involves homolysis to give an alkoxy radical (Mottram, 1998a). This is then followed by cleavage of the fatty acid next to this radical. The nature of the volatile product depends on the alkyl chain and the position of cleavage. If the alkyl group is saturated, a saturated aldehyde group is produced. Depending on location of cleavage, an alkyl radical can be formed, which can either give an alkane or can react with oxygen to produce a hydroperoxide, which furthers rancid oxidation (Mottram, 1998a). The secondary products of lipid oxidation principally include alkanes, alkenes, aldehydes,

ketones, alcohols, esters, furans, and many more (Shahidi, Rubin, and D'Souza 1986; Shahidi 1989; Pegg and Shahidi, 2007).

One of the most common methods to measuring the amount of oxidation in meat is by using thiobarbituric acid. The presence and concentration of total aldehydes in meat, which is the primary product of oxidation, can be expressed as “malonaldehyde equivalents” as an indicator of lipid oxidation by 2-thiobarbituric acid (TBA) test (Wang, Pace, Dessai, Bovell-Benjamin, and Phillips, 2002). This assay involves the reaction of aldehydes in oxidized foods with the TBA reagent under acidic conditions, where a pink liquid forms with a distinctive absorption maximum at 532 nm (Tarladgis, Watts, Youathan, and Dugan, 1960; Siu and Draper 1978). The assay is now known as thiobarbituric acid reactive substances (TBARS; Ke, Cervantes, and Robles-Martinez, 1984, Gray and Pearson 1987).

Meat color

When purchasing meat products in retail, the primary influence is meat color because consumers use the bright, cherry-red color of fresh beef as an indicator of product freshness (Cassens, Faustman, and Jimenez-Colmenero, 1988; Kennedy, Buckley, and Kerry, 2004). This influence can result in an annual revenue loss of \$1 billion to the beef industry (Smith, Belk, Sofos, Tatum, and Williams, 2000). With this type of impact on the beef industry, understanding all the principles associated with myoglobin redox chemistry is crucial to recover from the economic loss due to color instability. Myoglobin, the primary protein in meat color, is a water-soluble protein containing eight helices joined by short non-helical strands (Mancini and Hunt, 2005). It

consists of two main portions, a protein and a non-protein portion, globin and heme, respectively. Within the heme-ring, iron has the ability to form six bonds (ligands; Pérez-Alvarez and Fernández-López, 2007). Of the six binding sites on iron, four of these sites bind directly to the heme-ring. The fifth site is typically bound to an amino acid (typically histidine). Lastly, the sixth binding site is open to bind to oxygen when present. The opportunity of the ligand to bind oxygen results in different states of myoglobin color seen in meat. Primarily, iron is found in two different chemical states: as the ferric (oxidized; Fe^{3+}) and ferrous (reduced; Fe^{2+}) forms. The chemical state of iron affects the opportunity for oxygen to bind to myoglobin. There are three major stages of myoglobin can become in fresh meat: deoxymyoglobin, oxymyoglobin, and metmyoglobin.

Deoxymyoglobin is the pigment formed with the iron atom being in the ferrous form, where the sixth binding site on the iron in the reduced chemical state, is unoccupied (Mancini and Hunt, 2005; Pérez-Alvarez and Fernández-López, 2007). This results in a purplish-red meat color, which is normal of fresh meat immediately after initial cutting. For this pigment to remain, there must be a very low oxygen threshold kept (<1.4 mm Hg; Brooks, 1935). As the oxygen threshold increases, the meat begins to take on a bright, cherry-red color, also known as bloom. This is the color consumers associate with fresh meat, also known as oxymyoglobin. During oxygenation of the meat product, there is no change in the chemical state of iron, but diatomic oxygen binds to the sixth binding site. Unlike deoxymyoglobin, the stability of oxymyoglobin depends on many different factors including pH, temperature, and oxygen competition by other

respiratory processes (Mancini and Hunt, 2005). The other major pigment is metmyoglobin, which is oxidation of the iron atom, causing a shift in chemical state from ferrous to ferric ion, also known as oxidation (Livingston and Brown, 1982; Wallace, Houtchens, Maxwell, and Caughey, 1982). This oxidation results in a brown color in the meat and is normal for meat that has been overwrapped in oxygen permeable film, where it has been placed in retail display for days. The brown color of meat is not considered to be very acceptable to consumers. Like oxymyoglobin, there are numerous factors associated with the formation of metmyoglobin, such as pH, oxygen partial pressure, temperature, light, and reducing activity of the meat (Mancini and Hunt, 2005).

There are typically two different ways to analyze meat color: instrumental and trained panel. A spectrophotometer measures L^* , a^* , b^* , which represent the color range of black to white (L^*), red to green (a^*), and yellow to blue (b^* ; Mancini and Hunt, 2005). For this project, objective instruments were utilized. In addition to characterizing surface color, reflectance can be used to estimate the amounts of each myoglobin redox form on the surface of meat according to a procedure in AMSA (1991). This methodology is a non-invasive, rapid estimation of surface myoglobin redox using certain wavelengths. For this project, wavelengths of 525 and 572 nm were used, to determine amount of metmyoglobin on the surface, due to their isobestic points in meat (Hunt, 1980).

Fatty acids

Lipids are broadly categorized and defined as organic compounds containing hydrogen, carbon, nitrogen, oxygen, and phosphorus. Lipids include several different

types of compounds including fatty acids, triacylglycerols, waxes, glycerophospholipids, sphingolipids, sterols, and terpene-derived molecules. Lipids are unique in the fact that they are insoluble in aqueous solutions but very soluble in organic solvents, such as, dichloromethane, chloroform, hexane, and diethyl ether. The type of solvent used to extract the lipid determines which portion of the lipid will be removed. Hexane has been used to remove the triglycerides, where as chloroform-methanol (polar) solvent is used to remove phospholipids (Mottram, 1998b). One of the most popular extraction methods results in FAME (fatty acid methyl esters), where the fatty acid is converted to fatty acid methyl esters and then separated using gas chromatography. During methylation, the fatty acids are cleaved from triacylglycerols, phospholipids, or any other lipid compounds using a hydrolysis reaction. The fatty acids are then referred to as free fatty acids and are acetylated to a methylene group, which creates a FAME. Lipids are categorized based upon the number of carbons and the presence or absence of double bonds, i.e., whether there is one bond or multiple double bonds, monounsaturated (MUFA) or polyunsaturated fatty acids (PUFA; Rawn, 1989).

With ground beef being such a large portion of red meat that is consumed, there is great benefit in mapping out the fatty acid traits of different fat depots throughout the carcass. In a study by Turk and Smith (2009), clear differences were found in subcutaneous fat depots throughout the carcass, including brisket, plate, chuck, flank, loin, rib, sirloin, and round. The brisket was lower in palmitic (16:0) and stearic (18:0) acid than the other subcutaneous locations (Turk and Smith, 2009). Also, brisket was significantly higher in the concentration of MUFA and the lowest concentration of trans-

vaccenic acid and saturated fatty acids (SFA; Turk and Smith, 2009). All eight depots were significantly different in the MUFA:SFA ratio (Turk and Smith, 2009). The melting point (slip point) of fat affects overall consumer acceptance of products (Turk and Smith, 2009). Wood et al. (2003) stated that as the unsaturation of a meat product increases, the slip point decreases. Smith, Yang, Larsen, and Tume, (1998), Wood et al. (2003), and Chung et al. (2006) demonstrated that fat hardness is dictated primarily by the concentration of stearic acid. Of the eight fat depots studied, brisket had the lowest slip point, while flank had the highest (Turk and Smith, 2009). Brisket is also the highest of all the fat depots in oleic acid (18:1 n-9), with flank having the lowest concentration (Turk and Smith, 2009).

Consumer sensory

Sensory evaluation is a scientific discipline used to evoke, measure, analyze, and interpret reactions to attributes of foods and materials as perceived by all the senses (Institute of Food Technologist, 1975). Appearance is typically the only attribute, which greatly affects the purchasing decision of a consumer (Schilling, 2007). According to Kauffman (1993), meat quality includes seven variables: wholesomeness, nutrition, processing yield, convenience, consistency, appearance, and palatability. Palatability has five components: tenderness, texture, juiciness and flavor (odor and taste; Kauffman, Sybesma, and Eikelenboom, 1990). According to Daley, Abbott, Doyle, Nader, and Larson (2010), there is a significant difference in the fatty acid profile of grass-fed beef when compared to grain-fed beef. The fatty acid differences of grass-fed beef cause a distinct difference in flavor (Daley et al., 2010). Also, with the differences of grain-

versus grass-fed beef, there has also been a greater overall consumer acceptance of grain-fed beef when compared to grass-fed beef (Cox et al., 2006). Therefore, fatty acid profiles can cause a distinct difference in acceptability of meat samples.

Gas chromatography with mass spectrometry

In recent years, flavor research has become more common with the addition of an olfactometry port connected to a gas chromatograph (GC-O) device for sniffing compounds after they are separated from each other. Gas chromatography (GC) is used for separation and detection of volatile compounds, and it is also very helpful with identifying flavor compounds. In a standard setup, a gas chromatograph is fitted with a flame ionization detector (FID) and an odor port, where a splitter is placed at the end of the GC column to split the column products between the FID and the sniff port. Within the sniff port, there is usually a small water reservoir to humidify the effluent and prevent drying out of the nasal passages. A trained panelist sniffs the carrier gas as it flows through the column and records the smell as it comes through, which creates an aromagram. As an aromagram is being recorded, the FID also records a chromatogram that can be compared back to the aromagram to match the odors with chemical peaks. Also, GC-O can be used with mass spectrometry (MS) to further identify the chemicals that are odor-active. The GC-O technology has been used to identify different odor thresholds of flavor compounds by looking at different dilutions (Drawert and Christoph, 1984; Leland, Schieberle, Buettner, and Acree, 2001; van Ruth, 2001). A mass spectrometer has three parts: an ion source, a mass analyzer, and an ion detector. Once the molecules enter the spectrometer they enter an ion source, where they are fragmented

into ions. Each ion has its own mass and a charge, which is often put together as the mass-to-charge ratio (m/z). With this ratio, generated ionic fragments are separated by the mass analyzer and focused toward the ion detector. Upon reaching the detector, a signal that provides a measure of abundance of the ions is produced. At the end of each MS cycle, a total ion chromatogram (TIC) is generated, which shows the abundance and the fragmentation fingerprint. This is a representation of the ions observed by the MS, where the intensity is set at 100% for the most abundant fragment. Within a MS, there are different libraries that help to accurately identify each compound. With this type of technology there are many uses, but the majority of these uses range from single compound identification to detection of active odor compounds, along with quantifying these compounds. There are several common methods used for extraction like solvent extraction, static headspace sampling, solid-phase micro-extraction, simultaneous steam distillation/extraction, and supercritical fluid extraction. These different types of extraction methods can influence the volatiles that are extracted, for example, with solvent extraction, organic solvents are used and this can cause water-soluble compounds to be incompletely extracted. Solid-phase micro-extraction (SPME) uses a short length of fused silica fiber coated with a thin layer of absorptive material. SPME can be used in both static headspace and liquid collection. While using this type of technology has been useful, there are a few problems to overcome. First, the threshold can vary between panelists, along with between machines. Secondly, humidity can affect the perception and should be controlled if possible. Also, panelist issues may cause problems, such as, adaptation, fatigue, and breathing patterns. Panelist issues are

typically one of the biggest problems due to the large amount of variance that can occur. Lastly, the dilution at which the GC-O is carried out can affect the sensory detection of compounds, which can easily be fixed with running multiple concentrations of each sample (Bett-Garber, 2007; Da Costa and Eri, 2007).

There are a few different ways to report the concentrations of the compounds, like Osme, nasal impact frequency, and the charm method. The Osme method is a quantitative bioassay method used to measure the intensity of the compounds. The nasal impact frequency method records the detection frequency. And lastly, the charm method records the beginning and the end of each odor. Along with these methods, typically a trained panelist is used to evaluate the sample (Bett-Garber, 2007).

Objectives

Over the past few years, there has been an increasing interest in the production of premium ground beef products. Most recently, studies looked at differences among subcutaneous fat based on beef carcass locations. With these significant differences found, the importance of analyzing the differences when using a common lean source to dilute these fat sources was apparent. There were two primary objectives of this study. First, determine the flavor profile and fatty acid composition of ground beef generated from subcutaneous fat trimmings taken from five different beef primal cuts, including: brisket, chuck, plate, flank, and round. Secondly, determine the consumer acceptance and/or preference of ground beef generated from these fat trimmings. We hypothesized that carcass subcutaneous fat location would affect quality and sensory traits.

2. MATERIALS AND METHODS

Sample preparation

A common source of lean trim was obtained from a commercial meat distributor in Bryan, Tx. Subcutaneous fat was collected from five different carcass locations (brisket, chuck, plate, flank, and round) during several Texas AandM University extension activities, with a minimum of 20 different animals being represented for each carcass location. Fat collection took place in 3 different collection days. The lean and fat were formulated to contain 80% of the lean source and 20% subcutaneous fat. Once formulated, a final grind was performed and ground beef was formed into 100 g patties (12.07 cm x 1.27 cm) with a stainless steel patty press and patty paper. The patties for the shelf-life portion of the study were placed on a Genpak 17S Styrofoam tray (20.96 cm x 12.07 cm x 1.27 cm, Alliance Paper and Food Service, Franklin Park, IL) and overwrapped with polyvinyl chloride and stored at 4°C. The other patties were placed in the -10°C freezer for 30 min and then vacuum packaged and stored in the freezer at -10°C. The ground beef production was divided into three different replications.

Color measurements

Patties for simulated retail display measurements were placed in a retail display with a temperature of 4°C and lights (F 40T 12; 40 WATT, Alto Collection, Philips Electronics America Corporation, Andover, MA) adjusted to give 1,000 lux illumination at the patty surface. Color measurements were taken daily for 5 d using a Hunter Miniscan XE Plus (Hunter Laboratories Model MSXEt, Reston, VA) using a 10° observation angle, D65 illuminant and 3.5-cm aperture. Color measurements were taken

in two locations for an average measurement of L*, a*, and b* color values.

Additionally, the ratio of 525/572 was calculated according to Hunt (1980), with 572 nm being the isobestic point for both myoglobin and oxymyoglobin, and 525 nm the isobestic point for myoglobin, oxymyoglobin, and metmyoglobin; therefore, this ratio is a measure of the percentage of metmyoglobin on the surface. Hue and chroma were calculated using the formulas for hue angle ($\tan^{-1}(b^*/a^*)$, where larger angles are more yellow and discolored) and saturation index ($((a^2+b^2)^{1/2}$; where larger values represent more intense color).

Fatty acids

Fatty acid methyl esters (FAME) were prepared from the lipid extracts as described by the Morrison and Smith (1964) procedure shown in Appendix A. Individual FAME were quantified as a percentage of total FAME analyzed. All fatty acids normally occurring in beef lean and fat trim, including isomers of conjugated linoleic acid, were identified by this procedure.

Thiobarbituric acid reactive substance (TBARS)

The modified method of Wang et al. (2002) was used to perform TBARS. Standards (0, 2, 4, 8, 10, 20 nM/mL) TEP/TCA were formulated to calculate a regression line in order to calculate μM aldehydes for all samples. Thiobarbituric acid reactive substances were performed on fresh and a display patty from day 1, 3, and 5. The procedure is shown in Appendix B.

Consumer sensory and freezer loss/cook loss

An untrained, 147-member consumer panel was solicited from the local community through random calling and consumer database. Panel members signed up for one of the five nights that were scheduled for sensory tasting at Texas AandM University's sensory facilities. Each panelist was required to provide demographic information including: age, income, gender, and weekly ground beef consumption rates. Demographics and directions for the evaluation are shown in Appendix C. Each panelist evaluated five different samples, each coming from a different source of subcutaneous fat location. Samples were randomly assigned to a different order for each night of sensory evaluation. Each sample was presented with a three-digit random code and placed in a small clear plastic serving cup. Consumers were seated in breadbox style booths, where the consumers were given salt-free saltine crackers and double-distilled, deionized water as palate cleansers. Consumers were also asked to remain silent throughout the evaluation to prevent any biasing. The patties were thawed for 4 h in a cooler at 4°C on each day of the evaluation. The patties were grilled (177°C grill temperature) on clam-shell style grills (George Foreman Clam Shell GRP99, Bedford Heights, Ohio) to an average of 74°C internal temperature using a constant time of 4 min. Each patty was cut into eight individual pieces, providing samples for four consumers per patty. Consumers were provided with a different ballot for each sample. The ballot is shown in Appendix D. The consumer evaluated each sample using a 9-point hedonic scale with a score of 1 being either dislike extremely or extremely bland and a score of 9 was like extremely or extremely flavorful. The samples were evaluated

on overall like/dislike, like/dislike of flavor, level of flavor, like/dislike of beefy flavor, level of beefy flavor, like/dislike of texture, like/dislike of juiciness, level of juiciness. There was also an open-ended question asking for positive or good flavors, along with negative or bad flavors found in the meat sample. Freezer loss and cook loss samples were prepared with the same guidelines as the consumer sensory preparation for both thawing and cooking. The samples were weighed prior to freezing, before cooking, and after cooking to determine both freezer loss and cook loss.

Gas chromatography with mass spectrometry

Samples were prepared the same as consumer sensory samples for thawing and cooking. The samples used for GC analyses, were also used to calculate cook loss and freezer loss. Once samples were cooked, they were placed in a glass jar (473 mL) with a Teflon piece under the metal lid and then placed in a water bath at 60°C, where the headspace was collected with a solid-phase micro-extraction (SPME) Portable Field Sampler (Supelco 504831, 75 µm Carboxen/ polydimethylsiloxane, Sigma-Aldrich, St. Louis, Mo). Upon first receiving the SPME fibers, each fiber was conditioned for one hour at 280°C in the GC injection port. The headspace above each meat sample in the glass jar was collected for 2 h on the SPME. Upon completion of collection, the SPME was injected in the injection port, where the sample was desorbed at 280°C. The sample was then loaded onto the multi-dimensional gas chromatograph into the first column (30 m x 0.53 mm ID/ BPX5 [5% phenyl polysilphenylene-siloxane] x 0.5 µm, SGE Analytical Sciences, Austin, TX), which is non-polar and separates compounds based on boiling point. Through the first column, the temperature started at 40°C and increased at

a rate of 7°/min until reaching 260°C. Upon passing through the first column, a program was designed to leave the heart-cut and cryo-trap open to forward the compounds to the second column (30 m x 0.53 mm ID [BP20- polyethylene glycol] x 0.50 µm, SGE Analytical Sciences), which separates compounds due to polarity. The gas chromatography column was then split at a three-way valve with one column going to the mass spectrometer (Agilent Technologies 5975 series MSD, Santa Clara, CA) and one column going to each of the two sniff ports, which were heated to a temperature of 115° C, and fitted with glass nose pieces. The sniff ports and software for determining flavor and aroma are a part of the AromaTrax program (MicroAnalytics-Aromatrx, Round Rock, Tx). Two panelists were trained to accurately use the Aromatrx software, after they had also been trained according to the beef lexicon aromas (Adhikari et al., 2011).

Statistical design

Analyses of TBARS, cook loss, fatty acids, gas chromatography with mass spectrometry and color data were analyzed for a randomized complete block design using generalized linear models in SAS (9.3 SAS Institute, Cary, NC) with subcutaneous fat source as the main effect and day of processing as the block (n=3). Color data from the retail display period was analyzed as a repeated measure over the 5 d. When analysis of variance indicated a significant F-test ($P < 0.05$), least squares means were separated using Fischer's protected LSD. An analysis of consumer sensory was done using the PROC MIXED procedure in SAS with date and order as random effects. Consumer data were also transformed to normal distribution using Proc TRANSREG where data was

placed into a box-cox model with a lambda of -3 to 3. Least square means were separated with Fishers protected LSD using the PDIFF function of SAS for significant main and interaction effects.

3. RESULTS

Color and TBARS

Fat source main effects on L*, a*, b*, reflectance, hue, and chroma were all found to be not significant ($P > 0.24$; data shown in Table 1). However, there was an interaction ($P = 0.003$) between source and day for L*, where fat source was dependent on day, depicted in Figure 1. The round had the highest ($P < 0.05$) L* of all sources at d 0, but by d 5, brisket had become the highest and round the lowest ($P < 0.05$) L* of all the sources, indicating a more rapid increase in brisket L* values than other sources. Subcutaneous fat source did not have any effect ($P = 0.71$; data shown in Table 1) on TBARS values. All fat sources increased ($P < 0.05$, data not shown) at d 1, 3, and 5.

Fatty acids

Fat source effects for C14:0, 15:0, 15:1, 16:0, 16:1, 17:0, 18:1 trans-9, 18:1 trans-10, 18:1 trans-11, and 18:1 cis-9 were not affected by subcutaneous fat source ($P > 0.05$; data shown in Table 2). Percentage stearic acid (C18:0) was lower ($P < 0.05$) in the brisket than the chuck and flank. The brisket was higher in percentage cis-vaccenic acid (C18:1 cis-11; $P < 0.05$) and in the saturated fatty acid to monounsaturated fatty acid ratio ($P < 0.05$), and lower ($P < 0.05$) in the percentage of total saturated fatty acid than all the other sources of subcutaneous fat. Additionally, SFA percentage was higher ($P < 0.05$) in the flank compared to the round. For C 17:1, MUFA, and MUFA:SFA ratio ($P < 0.05$), brisket was higher than all other fat sources. Brisket was also significantly ($P < 0.05$) lower than all other sources for SFA.

Consumer sensory and freezer loss/cook loss

The consumer demographics are shown in Table 3. The majority of the consumers (64%) were less than 35 years of age. Over half of the panelists had an income of less than 20,000. Gender of panelists were almost evenly divided. Majority of consumers consumed ground beef two to three times per week. Overall like, flavor, level of flavor, beefy, level of beefy, texture, juiciness, level of juiciness, cook loss, and freezer loss were all similar ($P > 0.13$, data shown in Table 4) across beef fat sources.

Gas chromatography with mass spectrometry

Gas chromatography with mass spectrometry results are shown in Table 5. Gas chromatography with mass spectrometry found significant differences in four different compounds. Butanedione was highest in the flank and plate fat source and lowest in the brisket and chuck than all other fat sources ($P < 0.05$). Brisket was higher ($P < 0.05$) than chuck, flank, and round, but not different ($P > 0.05$) than plate in Octanedione. Benzene tended to be higher ($P = 0.097$) in brisket and chuck than round, but it was not different than plate and flank. A tendency existed ($P = 0.054$) for 1-Octen-3-ol to be higher in the brisket than flank, plate, and round. All other compounds were found to be similar ($P > 0.05$) among fat sources. The Aromatrax aroma intensity data was analyzed by both first and second descriptor. For the first descriptor, brisket was higher ($P < 0.05$) than all other sources for beefy aroma, while flank was lower ($P < 0.05$) in beef aroma than plate. Brisket was higher ($P < 0.05$) than all other sources except flank for floral aromas. Flank was highest and brisket and plate were lowest ($P < 0.05$) for chemical aromas. Plate was higher ($P = 0.029$) than all other sources for heated oil aromas. Flank

was lowest and plate was highest ($P < 0.05$) for heated oil aromas. Plate tended to be the lowest ($P = 0.054$) for animal hair aromas. For the secondary aroma descriptor, round was highest and brisket was lowest ($P < 0.05$) in dairy aromas. All other first and second descriptors were not significant ($P > 0.05$) among subcutaneous fat sources.

4. CONCLUSIONS AND DISCUSSION

Color

The main factor used for consumer purchasing decisions is meat color (Faustman and Cassens, 1989). Because of this, the rate of color decline in the retail market becomes one of the most important factors of meat color. The rate of discoloration is monitored in shelf life studies to determine the amount of metmyoglobin and oxidation processes (Faustman and Cassens, 1989). In this study, L^* , a^* , b^* , reflectance, hue, and chroma were all found to be similar across subcutaneous fat sources. However, there was an interaction for L^* , with source interacting with day. In a study by Troutt, Hunt, Johnson, Claus, Kastner, Kropf, and Stroda, (1992), L^* and a^* values decreased with storage, implying patties became darker and less red. In the same study, reflectance increased during d 0 and 2, but no further change occurred after d 2. While these studies tested different components of ground beef, when comparing the results, it may be that fat percentage had more of an affect on meat color than the location of fat source. This study showed contradicting results with the current study having increasing L^* , while the study by Troutt et al. (1992) had decreasing L^* . However, in a study by Sledge (2008), when ground beef patties were overwrapped and placed in retail display, L^* values increased over time.

Fatty acids

With ground beef being such a large portion of red meat that is consumed, there is great benefit in mapping out the fatty acid traits of different fat depots throughout the carcass. In the study by Turk and Smith (2009), clear differences were found in

subcutaneous fat depots throughout the carcass, including brisket, plate, chuck, flank, loin, rib, sirloin, and round. In the current study, C17:1, stearic acid, cis-vaccenic, MUFA, SFA, and the MUFA:SFA ratio were found to be significantly different among fat sources. Also, in the current study, C14:0, 15:1, 16:0, 16:1, 17:0, 18:1 trans-10, 18:1 trans-11, and oleic acid (18:1 cis-9) were all approaching significance. These results are similar to the study by Turk and Smith (2009). In a study by Waldman, Suess, and Brundgardt, (1968), concentration of SFA increased from external to internal sample locations. In a review by Banskalieva, Sahlu, and Goetsch, (2000), different data were combined from different studies into one table to show there are differences in fatty acid composition among the various muscles of goats. Therefore, fatty acid composition has been found to vary among locations across carcasses, which can possibly play a role in different flavor formations based off of fatty acids.

TBARS

TBARS are typically used to monitor the oxidation of meat in retail studies. In this study, there was no differences among subcutaneous fat source. As expected there was a difference in TBARS between d 1, 3, and 5. In a study by Lee, Decker, Faustman, and Mancini, (2005), there were significant differences in TBARS with a treatment by time interaction. In a study by Rhee, Krahl, Lucia, and Acuff (1997), TBARS had similar values across the retail display similar to this study. With time under retail environments, the meat begins to oxidize due to being in the presences of oxygen; therefore, we would expect these values to increase with increased storage time.

Consumer sensory and freezer loss/cook loss

According to Kauffman (1993), meat quality includes seven variables: wholesomeness, nutrition, processing yield, convenience, consistency, appearance, and palatability. In the current study, a consumer panel evaluated ground beef from different subcutaneous fat sources for the following attributes: overall like, flavor, level of flavor, beefy, level of beefy, texture, juiciness, and level of juiciness, where all attributes were found to be similar among sources. In a study by Cross, Berry, and Wells (1980), a trained panel evaluated the ground beef patties with varying fat levels, along with different fat sources and found no significant differences in the following attributes: tenderness, juiciness, ground beef flavor, intensity, connective tissue amount, and mouth coating effect. In a different study by Troutt et al. (1992), a trained panel evaluated ground beef patties with differing fat percentages on the following attributes: moistness, moisture release, juiciness, beef flavor intensity, oily coating of the mouth, firmness, cohesiveness, cohesiveness of mass, and sustained cohesiveness. The trained panel found all attributes to be significant except sustained cohesiveness. Cook loss has always been an issue that is affected by the percentage of fat in the ground beef. In the current study, subcutaneous fat source had no effect on cook loss or freezer loss. In a study by Cross et al. (1980), different fat sources were used, along with varying fat percentages, to formulate ground beef patties. In the Cross study, similar to the current study, cook loss was not significantly affected by fat source or level of fat. Therefore, the Cross study supports the results from the current study, stating fat source has no effect on percentage of cook loss.

Gas chromatography with mass spectrometry

In recent years, flavor research has become more important with the addition of an olfactometry port connected to a gas chromatograph (GC-O) device for sniffing compounds after they are separated from each other. In this current study, gas chromatography with mass spectrometry found two compounds (butanedione and octanedione) to be significantly different among fat sources, and two other compounds (1-octen-3-ol and benzene) were approaching significance. In a study by Larick, Hedrick, Bailey, Williams, Hancock, Garner, and Morrow (1987), gas chromatography with mass spectrometry was used to analyze beef fat, where the mass spectrometry discovered a peak as octanedione. Octanedione is a methyl ketone, which in other studies is thought to be from the autoxidation of fatty acids, particularly C18 unsaturates (via hydroperoxides; Thomas, Dimick, and McNeil, 1971). According to Mottram (1998a), butanedione was thought to be produced by the Maillard reaction. Therefore, both of these compounds would be expected to be present in these samples. As for the Aromatrax data, for the first descriptor there were five descriptors (animal hair, beefy, chemical, floral, heated oil) that were significantly different among fat sources. Also with the aromatrax data, there were two descriptors (barnyard and dairy) found to be different among fat sources within the second descriptor.

Implications

The current study shows that the fatty acid differences among subcutaneous fat sources become diluted when incorporated with a common lean. The lack of differences among subcutaneous fat sources is likely due to the relatively low concentration of fat

being formulated into ground beef. However, this study does reveal that with about 15% fat in the formulation, the fatty acid composition can be altered without negatively impacting the sensory and quality traits of the ground beef patties. For implication within industry, this study could help to find a way to make more profit from typical fat trimmings by creating a premium ground beef.

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APPENDIX A
TABLES AND FIGURE

Table 1. Least squares means (SEM) for the effects of subcutaneous fat source on color and TBARS values of ground beef patties

Trait	Brisket	Chuck	Plate	Flank	Round	P-value
L*	47.15 (0.548)	45.99 (0.548)	46.80 (0.548)	47.90 (0.548)	46.80 (0.548)	0.25
a*	11.26 (0.452)	11.87 (0.452)	11.31 (0.452)	12.15 (0.452)	11.79 (0.452)	0.60
b*	15.74 (0.452)	15.91 (0.452)	15.88 (0.452)	16.61 (0.452)	16.38 (0.452)	0.63
Reflectance (572/525)	100.74 (2.745)	100.42 (2.745)	99.27 (2.745)	94.51 (2.745)	97.66 (2.745)	0.52
Hue	56.06 (0.978)	54.81 (0.978)	55.91 (0.978)	54.85 (0.978)	55.50 (0.978)	0.83
Chroma	19.55 (0.558)	20.05 (0.558)	19.69 (0.558)	20.71 (0.558)	20.33 (0.558)	0.60
TBARS	5.84 (0.695)	6.10 (0.695)	5.48 (0.695)	4.99 (0.695)	4.95 (0.695)	0.71

Table 2. Least squares means (SEM) for the effects of subcutaneous fat source on fatty acid and total lipid content of ground beef patties

Trait	Brisket	Chuck	Plate	Flank	Round	P value
C14:0	3.38 (0.058)	3.47 (0.074)	3.52 (0.058)	3.65 (0.058)	3.62 (0.074)	0.077
C15:0	0.55 (0.014)	0.54 (0.018)	0.53 (0.014)	0.53 (0.014)	0.54 (0.018)	0.27
C15:1	0.13 (0.013)	0.034 (0.022)	0 (0)	0.036 (0.016)	0.051 (0.021)	0.098
C16:0	24.00 (0.25)	25.00 (0.32)	24.96 (0.25)	25.16 (0.25)	24.78 (0.32)	0.086
C16:1	4.63 (0.32)	3.47 (0.41)	3.66 (0.32)	3.15 (0.32)	3.47 (0.41)	0.095
C17:0	1.01 (0.32)	1.17 (0.040)	1.13 (0.032)	1.15 (0.032)	1.14 (0.040)	0.065
C17:1	0.92 ^a (0.033)	0.78 ^b (0.042)	0.78 ^b (0.033)	0.69 ^b (0.033)	0.77 ^b (0.042)	0.020
C18:0	11.36 ^b (0.64)	14.22 ^a (0.81)	13.57 ^a (0.64)	15.37 ^a (0.64)	13.44 ^{ab} (0.81)	0.031
C18:1,t9	0.93 (0.070)	1.065 (0.088)	1.007 (0.070)	1.061 (0.070)	1.012 (0.088)	0.70
C18:1,t10	3.96 (0.57)	4.93 (0.73)	4.55 (0.57)	4.82 (0.57)	4.60 (0.73)	0.081
C18:1, t11	1.039 (0.062)	1.30 (0.079)	1.013 (0.062)	1.23 (0.062)	1.18 (0.079)	0.075
C18:1, c9	37.79 (0.81)	34.66 (1.025)	35.76 (0.81)	33.64 (0.81)	35.58 (1.25)	0.071
C18:1, c11	1.84 ^a (0.074)	1.47 ^b (0.094)	1.52 ^b (0.074)	1.38 ^b (0.074)	1.44 ^b (0.094)	0.023
C18:2	2.87 (0.089)	3.12 (0.11)	3.03 (0.089)	3.067 (0.089)	3.13 (0.11)	0.37
α 18:3	0.14 (0.025)	0.15 (0.033)	0.15 (0.025)	0.17 (0.032)	0.16 (0.032)	0.95
CLA;c9,t11	0.63 (0.060)	0.43 (0.076)	0.39 (0.060)	0.47 (0.060)	0.61 (0.076)	0.13
C20:1	0.19 (0.014)	0.20 (0.017)	0.19 (0.013)	0.16 (0.013)	0.16 (0.017)	0.31

Table 2: Continued

C20:3	0.098 (0.011)	0.084 (0.014)	0.087 (0.014)	0.086 (0.014)	0.093 (0.014)	0.91
C20:4	0.21 (0.020)	0.21 (0.026)	0.18 (0.020)	0.18 (0.020)	0.23 (0.026)	0.54
MUFA	51.42 ^a (0.58)	47.91 ^{bc} (0.74)	48.49 ^b (0.58)	46.17 ^c (0.58)	48.23 ^{bc} (0.74)	0.0044
SFA	39.75 ^b (0.65)	43.85 ^a (0.82)	43.19 ^a (0.65)	45.32 ^a (0.65)	42.99 ^a (0.82)	0.0053
MUFA:SFA	1.30 ^a (0.031)	1.092 ^{bc} (0.039)	1.12 ^b (0.031)	1.019 ^c (0.031)	1.12 ^{bc} (0.040)	0.0044
Total Lipids	14.61 (0.083)	13.56 (1.056)	16.57 (0.83)	16.48 (0.83)	15.31 (1.056)	0.31

Table 3. Consumer panelist demographic information

Item	Percentage of Respondents
Age	
25-29	12.98
30-34	51.15
35-39	8.40
40-44	8.40
45-49	5.34
50-55	13.74
Income	
<20,000	52.67
20,000-29,999	4.58
30,000-39,999	3.05
40,000-49,999	4.58
50,000-59,999	3.82
60,00 or greater	31.30
Gender	
Male	51.91
Female	48.09
Consumption	
Less than once a week	9.92
2-3 times per week	67.18
5 or more times per week	22.90

Table 4. Least squares means (SEM) for the effects of subcutaneous fat source on consumer sensory and freezer/cook loss values of ground beef patties

Trait	Brisket	Chuck	Plate	Flank	Round	P value
Overall Like	5.97 (0.206)	6.48 (0.207)	6.48 (2.06)	6.23 (0.207)	6.34 (0.206)	0.15
Flavor	5.84 (0.237)	6.30 (0.237)	6.02 (0.237)	6.40 (0.238)	6.20 (0.237)	0.13
Level of Flavor	5.50 (0.323)	5.96 (0.323)	5.77 (0.323)	6.10 (0.324)	5.60 (0.324)	0.13
Beefy Flavor	6.05 (0.238)	6.53 (0.238)	6.04 (0.238)	6.41 (0.239)	6.26 (0.238)	0.16
Level of Beefy	5.96 (0.258)	6.21 (0.258)	6.04 (0.258)	6.40 (0.260)	6.10 (0.260)	0.41
Texture	6.25 (0.178)	6.51 (0.178)	6.45 (0.178)	6.61 (0.179)	6.48 (0.178)	0.66
Juiciness	6.67 (0.176)	6.69 (0.176)	6.52 (0.176)	6.78 (0.177)	6.77 (0.176)	0.77
Level of Juiciness	6.69 (0.173)	6.55 (0.173)	6.58 (0.173)	6.71 (0.174)	6.92 (0.173)	0.49
Freezer Loss	4.27 (0.498)	4.13 (0.498)	3.27 (0.498)	2.87 (0.498)	3.63 (0.498)	0.31
Cook Loss	22.81 (2.230)	21.76 (2.230)	25.51 (2.230)	25.58 (2.230)	24.00 (2.230)	0.70

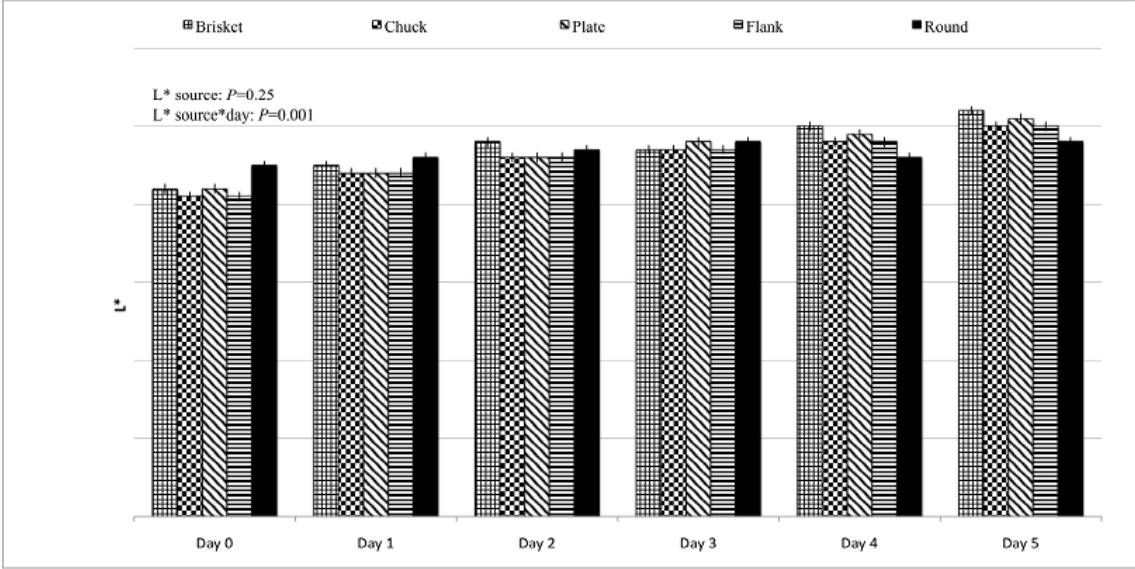
Table 5. Least squares means (SEM) for the effects of subcutaneous fat source on aromatic chemical compounds in the headspace above cooked ground beef patties using GC/MS and Aromatrx

Trait	Brisket	Chuck	Plate	Flank	Round	P-value
Chemical Compounds:						
Butanedione	289541.00 ^b (157955.03)	183322.00 ^b (146237.94)	783769.00 ^a (157955.03)	878332.17 ^a (157955.03)	428539.83 ^{ab} (157955.03)	0.013
Octanedione	113665.50 ^a (19413.91)	33100.71 ^c (17973.79)	98589.50 ^{ab} (19413.91)	42815.00 ^{bc} (19413.91)	27092.83 ^c (19413.91)	0.0079
Benzene	118852.17 (27852.83)	101631.29 (25786.71)	60755.67 (27852.83)	49344.50 (27852.83)	17514.17 (27852.83)	0.097
1-Octen-3-ol	113337.83 (19134.91)	61424.00 (17715.48)	56003.33 (19134.91)	31430.33 (19134.91)	44325.33 (19134.91)	0.054

Table 5: Continued

1 st Descriptors:						
Beefy	23.39 ^a	18.93 ^{bc}	19.44 ^b	15.86 ^c	18.35 ^{bc}	0.0003
	(1.125)	(1.231)	(1.220)	(1.209)	(1.358)	
Floral	13.00 ^a	3.60 ^c	4.67 ^{bc}	11.67 ^{ab}	3.67 ^c	0.0038
	(1.545)	(1.838)	(2.359)	(2.359)	(2.359)	
Chemical	0	3.00 ^b	0	20.00 ^a	2.50 ^b	0.047
	0	(0.707)	0	(0.707)	(0.500)	
Heated oil	3.67 ^{bc}	4.50 ^b	8.00 ^a	1.00 ^c	3.00 ^{bc}	0.029
	(0.514)	(0.629)	(0.890)	(0.890)	(0.629)	
2 nd Descriptors:						
Animal Hair	22.25 ^b	29.00 ^{ab}	0	16.00 ^b	31.67 ^a	0.089
	(2.669)	(3.082)	0	(5.337)	(3.082)	
Dairy	0	9.00 ^c	24.00 ^b	24.00 ^b	28.00 ^a	0.0001
	0	(9.424 e-8)	(9.424 e-8)	(6.664e-8)	(9.424 e-8)	

Figure 1: Least squares means and SEM for subcutaneous fat source by display day interaction for L* values at d 0 thru d 5



APPENDIX B

FAMES EXPERIMENTAL PROCEDURES

Extraction of Total Lipids

- a. Weigh 500 mg adipose tissue or 1 – 5 g muscle and add to a labeled 50-mL glass tube. (More can be used but only saponify 100 μ L of the extracted lipid)
- b. Add 5 mL of chloroform:methanol ($\text{CHCl}_3:\text{CH}_3\text{OH}$, 2:1, v/v).
- c. Homogenize each sample with Polytron homogenizer on medium setting for ~30 s. After homogenization, rinse the probe with $\text{CHCl}_3:\text{CH}_3\text{OH}$ until you have a final volume in the tube of ~15 mL.
- d. Rinse probe in warm water. Spray with clean water into waste beaker. Rinse probe with $\text{CHCl}_3:\text{CH}_3\text{OH}$ into another waste beaker. Dry with Kimwipes.
- e. Let sample sit for 30 min to extract lipids. If stopping at this point, flush with nitrogen, cap and store in cooler.
- f. Filter homogenate through sintered glass filter funnel (or Whatman filter apparatus using 2.4 cm GF/C filters) into a 2nd 50-mL centrifuge tube. Rinse 1st tube 2 to 3 times with $\text{CHCl}_3:\text{CH}_3\text{OH}$. Also rinse filter funnel 1 to 2 times with $\text{CHCl}_3:\text{CH}_3\text{OH}$.
- g. Q.S. filtered homogenate to a convenient volume (20 to 30 mL)
- h. Add 8 mL of 0.74% KCl and vortex 1 min.

- i. Let sit 2 h to separate phases or centrifuge until you get two distinct phases. If stopping at this point then flush with nitrogen, cap, and let sit in refrigerator overnight.
- j. Carefully remove the upper phase and discard. (Do not aspirate any buffer coat at the interface). If you want to stop at this point, then flush with nitrogen, cap and store in -20°C .
- k. Transfer all lower phase to 2-mL glass scintillation vial. Rinse 50-mL tube 2 to 3 times with $\text{CHCl}_3:\text{CH}_3\text{OH}$ into the glass tube.
- l. Evaporate the sample to dryness with nitrogen using the N-Evap set at 40°C .

Saponification and Methylation of Lipids

(If you used 0.5 g adipose tissue or other lipid source, only saponify 100 μL of lipid.)

- a. Add 1 mL of 0.5 N KOH in MeOH; heat in 70°C water bath for 10 min.
- b. Add 1 mL of 14% BF_3 in MeOH; flush with N_2 ; loosely cap; place in 70°C water bath for 30 min.

This procedure saponifies the lipids, i.e., it liberates the fatty acids from the glycerol backbone. The fatty acid is methylated in the process, removing the net negative charge of this group.

- c. Remove the tubes and allow them to cool. Add 2 mL HPLC grade hexane and 2 mL saturated NaCl; vortex for 1 min.
- d. Pipet off upper layer with transfer pipette; place in a 20-mL glass tube containing $\sim 800\text{ mg Na}_2\text{SO}_4$. Add 2 mL of hexane to the tube containing saturated NaCl, vortex, allow phases to separate, and pipette the upper hexane layer into the 20-

mL tube with Na₂SO₄. You should now have ~4 mL of hexane in this tube.

Vortex this tube briefly. The Na₂SO₄ removes any moisture in the hexane.

- e. Pipet the hexane into a labeled glass scintillation vial.
- f. Add 1 mL hexane to the 20 mL tube with Na₂SO₄ in it. Vortex briefly. Transfer this hexane to the scintillation vial.
- g. Evaporate the hexane completely with the N-Evap set at 40°C.
- h. Reconstitute the lipid with the appropriate amount of hexane to obtain approximately 50 mg/mL.

Bring up samples as follows:

500 µL hexane for plasma and digesta

1 mL hexane for adipose tissue

- i. Pipet 400 µL of this solution into a 2-mL autosampler vial containing 1.6 mL of HPLC grade hexane. Only dilute adipose tissue samples. For plasma and digesta use a glass insert and pipet 100 µL undiluted to run on the GC.

0.5 N KOH in MeOH

2.81 g KOH

100 mL of MeOH

Saturated NaCl

31.7 g NaCl

100 mL ddH₂O

0.74% KCl

7.4 g KCl

1 L ddH₂O

GC Analysis:

FAME are analyzed with a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 autosampler, Varian Inc., Walnut Creek, CA). Separation of FAME is accomplished on a fused silica capillary column CP-Sil88 [100m x 0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands), with helium as the carrier gas (flow rate = 1.0 mL/min). Column oven temperature is increased from 150 to 160°C at 1°C per min, from 160 to 167°C at 0.2°C per min, from 167 to 225°C at 1.5°C per min, and then held at 225°C for 16 min. The injector and detector are maintained at 250°C. Total run time is 100 min.

APPENDIX C

THIOBARBITURIC REACTIVE SUBSTANCES ASSAY

Reference

Wang, B., Pace, R. D., Dessai, A. P., Bovell-Benjamin, A., Phillips, B. 2002. Modified extraction method for determining 2-thiobarbituric acid values in meat with increase specificity and simplicity. J. Food Sci. 67:2833-2836.

A. Solutions

a. TCA Extraction solution

7.5% (w/v) trichloroacetic acid

0.1% (w/v) EDTA

0.1% (w/v) Propyl Gallate

b. 80 mM TBA solution

1.15 g Thiobarbituric acid into 100 mL ddH₂O

c. TEP (malondialdehyde or MDA) Stock Solution

Make a 1 mM solution by adding 240 μL of tetraethoxypropane to 1L

H₂O

B. Standards

a. Dilute 1mM TEP stock solution to 80 uM (80uM = .08mM, so 1,000 mL x

0.08mM = 80 mL of 1mM TEP in 1L water)

b. Then make standards following the table below in individual tubes

uM TEP	80nM TEP (μL)	TCA (μL)	Pipette Setting
0	0	2000	1000 x 2
2	50	1950	975 x 2
4	100	1900	950 x 2
6	150	1850	925 x 2
8	200	1800	900 x 2
10	250	1750	875 x 2
20	500	1500	750 x 2
30	750	1250	625 x 2

C. Sample preparation and extraction procedure

a. Mince meat sample and weigh out 5 g

b. Place meat into a 50 mL centrifuge tube and add 15 mL TCA Extraction solution

c. Homogenize meat for 20-30 sec using a blender

d. Place lid back on centrifuge tube

e. Centrifuge at 1,500 x g for 15 min

f. Remove from centrifuge and filter through No. 4 Whatman paper

D. Incubation and Reading

a. Load 96-well microplate

b. Each sample should be loaded in triplicate with 125 μ L / well (See diagram below for details)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	A	A	A	I	I	I	Q	Q	Q
B	2	2	2	B	B	B	J	J	J	R	R	R
C	4	4	4	C	C	C	K	K	K	S	S	S
D	6	6	6	D	D	D	L	L	L	T	T	T
E	8	8	8	E	E	E	M	M	M	U	U	U
F	10	10	10	F	F	F	N	N	N	V	V	V
G	20	20	20	G	G	G	O	O	O	W	W	W
H	30	30	30	H	H	H	P	P	P	X	X	X

c. After sample are loaded, pipette 125 μ L of TBA Solution into each well

d. Incubate for 130 min at 40° C

e. Remove plates from incubator and read at 532 nm on plate reader

APPENDIX D
CONSUMER STUDY

DATE _____

INSTRUCTIONS

PANELIST NO. _____

Thank you for your participation in this study. Your assistance is very much appreciated.

The objective of this study is to evaluate ground beef. Please take your time and evaluate the samples given to you carefully. Please proceed at your own rate.

This sampling will take you between 30 to 45 minutes.

Please answer the following questions as completely as possible. If you have any questions, please ask the monitor for assistance.

Begin by filling out the basic demographic questions on the first page. This information is confidential and will not be used to solicit advertising nor will this information be published with your name associated with it.

After filling out the demographic information you are ready to start the evaluation.

BOLD LETTERS throughout the questionnaire will give you directions on how to complete the evaluation.

Thank you very much for your help and opinions.

PANELIST DEMOGRAPHIC INFORMATION

FILL OUT THE FOLLOWING INFORMATION.

1. Please indicate your age by marking the appropriate blank:

_____ 25-29 years _____ 30-34 years _____ 35-39 years

_____ 40-44 years _____ 45-49 years _____ 50-55 years

2. Please indicate your income (combined income if both you and your spouse are employed) by marking the appropriate blank:

_____ Under \$20,000 _____ \$30,000-\$39,000 _____ \$50,000-\$59,000

_____ \$20,000-\$29,000 _____ \$40,000-\$49,000 _____ \$60,000 or more

3. Please indicate your sex:

_____ Male _____ Female

4. Please indicate how often you consume beef:

_____ less than 1 time a week _____ 2-3 times a week

_____ 5 or more times a week

DIRECTIONS

YOU WILL BE EVALUATING THE SENSORY PROPERTIES OF DIFFERENT GROUND BEEF PRODUCTS.

ANSWER QUESTIONS 1 THROUGH 10 FOR EACH SAMPLE THAT YOU ARE SERVED.

EACH PRODUCT WILL HAVE A NUMBER DESIGNATION ON THE CONTAINER THAT IT IS SERVED IN. PLEASE IDENTIFY THE NUMBER ON EACH SAMPLE AS IT IS SERVED AND MAKE SURE THAT THE NUMBER ON THE SAMPLE MATCHES THE NUMBER ON THE TOP OF THE PAGE OF THE BALLOT.

LET YOUR MONITOR KNOW WHEN YOU WANT TO BEGIN.

PRIOR TO TASTING EACH SAMPLE, PLEASE TAKE A BITE OF THE CRACKER AND THEN RINSE WITH THE WATER PROVIDED IN THE CUP.

APPENDIX E

CONSUMER SENSORY BALLOT

1. Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** of the meat sample.

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------

Dislike	No	Like
Extremely	Preference	Extremely

2. Indicate by placing a mark in the box your **LIKE/DISLIKE** for the **FLAVOR** of the meat sample.

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------

Dislike	No	Like
Extremely	Preference	Extremely

3. Indicate by placing a mark in the box how you feel about the **LEVEL** of **FLAVOR**.

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------

Extremely	No	Extremely
Bland or No Flavor	Difference	Flavorful

4. Indicate by placing a mark in the box your **LIKE/DISLIKE** for the **BEEFY FLAVOR** of the meat sample.

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------

8. Indicate by placing a mark in the box how you feel about the **LEVEL** of **JUICINESS** of the meat product.

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------

Extremely

Neither

Extremely

Dry

Dry or Juicy

Juicy

9. Write down any words that describe the **POSITIVE OR GOOD FLAVORS** in this meat sample.

10. Write down any words that describe the **NEGATIVE OR BAD FLAVORS** in this meat sample.
