THESIS

NUTRIENT ANALYSIS OF TEN RAW U.S. BEEF VARIETY MEAT ITEMS AND BEEF FLAVOR MYOLOGY

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ABSTRACT

NUTRIENT ANALYSIS OF TEN RAW U.S. BEEF VARIETY MEAT ITEMS AND BEEF FLAVOR MYOLOGY

Many factors play a role in decision-making related to food and diet; these are closely linked to preferences and personal values in populations with access to a safe and affordable food supply. Many consumers value both nutrition and flavor preferences when making individual choices that ultimately comprise their overall diet pattern. Therefore, it is critical to maintain current, valid information regarding both the sensory profile and nutrient content of foods in the marketplace.

Two studies were performed on edible portions of beef carcasses; the first evaluated nutritional value of beef variety meat items in order to update the United States Department of Agriculture (USDA) Food Composition Database. Nutrition information in databases maintained by the USDA is used as groundwork by various groups for several purposes including nutrition monitoring activities, research, policy creation, and nutritional labeling. However, up-to-date nutrition information is not available for beef variety meat items. Therefore, the objective of this study was to expand availability of nutrient data for beef variety meat items. Beef heart, liver, kidney, tongue, honeycomb tripe, oxtail, marrow bones, testicles, blood, and bone broth were obtained from facilities in the United States. Standardized procedures were used to dissect and homogenize samples. Nutrient analysis occurred at USDA-Agricultural Research Service (ARS) approved laboratories using validated methods and standards. Each of the variety meat items in this study qualifies for at least one “Good Source” or “Excellent Source” labeling claim as

defined by the USDA based on the proportion of separable lean component. “Good source” indicates that a product contains 10-19% of the Daily Value (DV) or Recommended Daily Intake (RDI) per Reference Amount Customarily Consumed (RACC) for that nutrient, while “Excellent Source” designates that the food contains at least 20% of the DV or RDI per RACC for that nutrient. Additionally, Vitamin K_2 has been studied recently to ascertain beneficial effects on human health, and this nutrient was present in all samples analyzed. This study provides current, analytically-derived nutrient information for U.S. beef variety meat items. Results reflect that these variety meat items could be beneficial in providing essential vitamins and minerals as a component of a healthy diet. This data will be valuable for use by the meat industry, those selling variety meats, researchers, dietetic professionals, and consumers.

The objective of the second experiment was to evaluate effects of quality grade, final internal temperature, and cooking method on sensory profile of five beef muscles: rectus femoris, gluteus medius, infraspinatus, triceps brachii, and teres major, in order to characterize sensory characteristics of these cuts. Two quality grades (USDA Select, Upper 2/3 Choice/Top Choice), three cooking methods (grill, pan grill, oven roast), and three final internal temperatures (58.3°C, 70°C, and 80°C) were included; each of 102 unique treatment combinations were replicated six times. Vacuum packaged beef was purchased directly from a commercial beef harvest facility, fabricated 14 days post-production, and frozen at -20°C until analysis. Each sample was rated by a trained sensory panel for flavor, tenderness, and juiciness factors. Although muscles were not compared directly, muscle differences did exist relative to treatment effects. Degree of doneness had the greatest impact across all muscles evaluated, with higher final temperatures related to greater ($P < 0.05$) beef ID, browned, and roasted notes in most muscles and decreased ($P < 0.05$) tenderness. Additionally, panelists rated samples as having

greater amounts of bloody/serumy, metallic, and sour flavors ($P < 0.05$) when cooked to lower end-point temperatures. Cooking method affected flavor note ratings for all muscles, with oven roasting producing increased ($P < 0.05$) cardboardy, earthy/musty, and sour flavors, whereas pan grilling resulted in more intense bitter and burnt flavors ($P < 0.05$). Quality grade had a minimal impact on the muscles included in the study. Association of volatile aromatic compounds with specific treatments also varied based on muscle. Overall, the 80°C and pan grilling treatments were related to the most volatile compounds compared to other treatments; primarily pyrazines, alkanes, and alkenes. These results highlighted the importance of understanding the properties of individual cuts in order to best utilize them for a positive eating experience. In combination with previous research, these data will be used to develop a resource that characterizes sensory characteristics of lesser-utilized beef cuts to benefit the meat industry, foodservice operations, in-home cooks, and ultimately beef consumers.

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CHAPTER 1

INTRODUCTION

Chronic diseases, including obesity, heart disease, and type 2 diabetes, are leading causes of death in the United States (CDC, 2017). However, these conditions are often preventable through lifestyle choices, including healthy eating habits. The 2015 Dietary Guidelines for Americans Advisory Committee placed emphasis on this idea and recommended a healthy overall eating pattern, rather than focusing on the elimination of specific foods or nutrients as previous guidelines had. A major message of the 2015 USDA Guidelines was to eat a variety of nutrient dense foods using appropriate serving sizes (DGAC, 2015). Doing so will aid in meeting nutrient needs while maintaining an appropriate caloric intake. In order to make informed nutrition-related decisions, it is essential to have an accurate source of current nutrient data.

The USDA maintains nutrient databases in order to provide nutritional information for foods in the marketplace. This information is used as groundwork by various groups for several purposes including nutrition monitoring activities, research, policy creation, and nutrition labeling (Ahuja, Moshfegh, Holden, & Harris, 2013). Partnership with industry groups, academic institutions, and federal agencies have allowed for the expansion and improvement of the databases for the benefit of all stakeholders. Previously, the National Cattlemen's Beef Association collaborated with the Nutrient Data Laboratory to update nutrition information for beef muscle cuts for inclusion in the USDA National Nutrient Database for Standard Reference (Patterson, Duvall, Howe, & Holden, 2009). However, current nutrition information was not available for beef variety meat items, which are defined as edible parts of the animal other than skeletal muscle. The objective of the first study was to expand the availability of nutrient data for

beef variety meat items and to provide relevant data to update the USDA Food Composition Database. Upon inclusion of the data in the Nutrient Database for Standard Reference, this information will be publicly available.

The second experiment focused on beef flavor, which is a major driver of consumer acceptance, especially as tenderness of beef available in the retail and foodservice sectors has improved (Hunt et al., 2014; Legako et al., 2015). Huffman et al. (1996) found that when meat was prepared in the home, flavor was the major driver of overall palatability, outweighing tenderness and juiciness. Despite general use of flavor as a singular and straightforward term, it is actually a complex attribute of beef acceptability that is influenced by many factors. Pre- and post-harvest elements, including feeding regime, marbling level, internal temperature, cooking method and others are related to the flavor development of meat (Calkins & Hodgen, 2007). Additionally, due to variation in function and composition of different muscles, chemical characteristics of meat cuts differ. Therefore, various cuts of beef respond differently to unique preparation techniques.

Research has been performed for many of the most popular items to understand the chemical and sensory characteristics of beef cuts. However, there are other, lesser consumed cuts of meat available in the marketplace that have not been studied as extensively. Additionally, changes in beef fabrication procedures have allowed specific muscles from the round and chuck to be separated to be merchandized individually to deliver a more consistent product for consumers (Hildrum et al., 2009; Von Seggern, Calkins, Johnson, Brickler, & Gwartney, 2005; Yeh, Omaye, Ribeiro, Calkins, & de Mello, 2018) . These innovative beef cuts have not been analyzed extensively with regard to how marbling level, cooking method, and internal temperature play a role in flavor and palatability.

Therefore, the objective of the study was to evaluate the influence of quality grade, degree of doneness, and cooking method on the sensory profile (including flavor, tenderness, and juiciness) of five beef muscles: rectus femoris, gluteus medius, infraspinatus, triceps brachii, and teres major. This information will be beneficial in developing a consumer resource that establishes flavor of beef cuts for maximized value as a protein source.

CHAPTER 2

REVIEW OF LITERATURE PART 1

The issue of the Dietary Goals for the American people in 1977 was the foundation for the *Dietary Guidelines for Americans* that have been released every five years since 1980. Nutrition recommendations had historically focused on nutrient adequacy, whereas the Dietary Goals took into account the associations between food intake and chronic disease, leading to an increased emphasis on moderation of food consumption (USDA & USHHS, 2013). To a greater extent than previous editions, the 2015 Dietary Guidelines focused on eating patterns as a whole, rather than on individual foods and nutrients (DGAC, 2015). As scientific research emerges in the field of nutrition, it is becoming more apparent that interactions between foods in the diet result in a cumulative effect on health that should be considered in addition to the individual effects of dietary components (DGAC, 2015; Freeland-Graves & Nitzke, 2013). Nonetheless, the 2015 Dietary Guidelines recommended that saturated fat should make up no more than 10% of total caloric intake. While limiting or eliminating *trans* fat from processed food sources is advised, the guidelines state that animal products containing *trans* fat in small amounts may be included as part of a healthy diet. Unlike previous editions, no upper limit was provided for dietary cholesterol in the most recent release, acknowledging inadequate evidence related to its effect on blood cholesterol levels (DGAC, 2015). Although from a practical standpoint it is critical to consider entire eating patterns when developing nutrition recommendations, it is also important to understand the function of single macro- and micro-nutrients in the body.

Protein

Amino acids are covalently linked by peptide bonds to form polypeptides, or proteins. There are twenty amino acids which differ in regard to shape, size, and characteristics based on the unique structural component called the "R" group, or "side chain". Classification of amino acids is often based on these R groups due to the relationship with functional properties. The role of proteins in the body are numerous and vital; proteins function as enzymes, transporters, signaling molecules, transcription factors, and structural components. They are essential for metabolism, immunity, tissue support, and muscle function (Stapanik & Caudill, 2013).

Individual dietary protein and amino acid requirements vary due to differences in activity level, physiological maturity, non-protein energy availability, and disease conditions. This requirement represents the body's need for amino acids, and could be divided into needs for individual amino acids. However, the body can synthesize 11 of the twenty amino acids and thus the entire amount required for functional processes does not need to be consumed in the diet. Amino acids can be classified into two groups based on this fact: indispensable (essential) and dispensable (nonessential). Indispensable amino acids are those which cannot be synthesized de novo from compounds normally available in cells and therefore must be obtained through nutrient intake; the nine indispensable amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Animal sources of protein are considered "complete" due to the presence of all essential amino acids, whereas the majority of plant protein sources lack one or more essential amino acids (Hoffman & Falvo, 2004). The eleven dispensable amino acids (alalnine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, tyrosine) can be synthesized in the body under ordinary conditions. The formation of these compounds depends on availability of precursor amino acids

and the condition of the organism. Additionally, utilization rate has been shown to rise above synthesis rate for some of the indispensable amino acids in specific stages of life or illnesses. Consequently, arginine, cysteine, glutamine, glycine, proline, tyrosine can be classified as semidispensable and conditionally indispensable, signifying that they may be required in the diet under certain circumstances (Stapanik & Caudill, 2013).

The RDA for this macronutrient is 0.8 grams of protein per kilogram body weight per day, and is based on the needs of a minimally active but healthy adult. Protein needs increase as physical activity level rises, with 1.6 grams/kilogram per day recommended for individuals with intense daily activity (Wu, 2016). An important consideration related to protein intake and food source is protein quality. Determination of protein quality is based on two factors: amino acid composition and digestibility of the protein. The protein digestibility-corrected amino acid score (PDCAAS) takes into account the proportion of limiting amino acid and the digestion and absorption of the protein from the digestive tract (Stapanik & Caudill, 2013). The PDCAAS serves as the standard for evaluation of protein quality (FAO, 2011; Stapanik & Caudill, 2013). Protein sources of animal origin provide the highest quality rating of all foods, and consumption of animal protein has been associated with positive health outcomes in various populations (Godfrey, Robinson, Barker, Osmond, & Cox, 1996; Hoffman & Falvo, 2004).

Lipids

A diverse collection of molecules make up the lipid macronutrient class with the common factor being solubility in nonpolar solvents. Lipids are essential for membrane structure and function, activation of numerous signaling pathways, lubrication of body surfaces, and energy storage, among other functions in the body. Approximately 35% of all calories consumed by American adults are in the form of dietary fat, or lipids (Stapanik & Caudill, 2013). The

classification of lipids into subgroups provides insight to structural and metabolic relationships throughout this diverse macronutrient class.

Fatty Acids

Fatty acids are composed of a carboxylic head group and a hydrocarbon chain tail, which varies in regard to the number of carbons present, with chains of 12 to 22 carbons being most prevalent. Fatty acids are often categorized by the length of the carbon chain and the number of double bonds in the chain, which is referred to as the degree of saturation. Although not rigid standards, the chain-length classification system refers to fatty acids with less than 6 carbons as short-chain, 8 to 14 carbons as medium chain, and more than 14 carbons as long-chain fatty acids. Saturated fatty acids are those that do not contain any double bonds in the carbon chain to which hydrogen could be added. Conversely, fatty acids with one or more double bond are referred to as unsaturated because hydrogen atoms could covalently bond to the compound at these sites. Further categorization of unsaturated fatty acids leads to monounsaturated fatty acids (MUFAs) with a single double bond, and polyunsaturated fatty acids (PUFAs) with two or more double bonds. Dietary guidelines have recommended reducing saturated fat intake based on evidence that intake of these lipids generally raise blood cholesterol concentrations (German & Dillard, 2004).

The only essential fatty acids are two classes of PUFAs: omega-3 and omega-6, referring to the location of the last double bond in relation to the methyl carbon of the hydrocarbon tail. Both of these are required for physiological function and must be consumed in the diet because the body cannot completely synthesize them, nor interconvert them. Omega-3 fatty acids, found in fish and fish oil, are seen as more favorable due to the anti-inflammatory effects of the eicosanoid metabolic products created from these fatty acids. Eicosanoids are oxygenated, non-

esterified fatty acids that act as hormones to influence cellular functions. When omega-6 fatty acids are consumed in higher amounts, as is common in an American diet, eicosanoid products formed from omega-6 fatty acids outnumber the eicosanoids derived from omega-3 fatty acids. The larger quantities of omega-6 derivatives has been shown to lead to increased amounts of prostaglandins, thromboxins, and lipoxins, potentially contributing to development of inflammatory disorders and cell proliferation (Simopoulos, 1999).

The term *trans* fat refers to fats containing unsaturated fatty acids with double bond(s) in the *trans* configuration, being geometric isomers to unsaturated fats with *cis* double bonds. *Trans* fatty acids are formed through hydrogenation of vegetable oils to create partially hydrogenated oil, but are also found in small amounts in dairy and meat products. Most regulatory definitions of *trans* fats refer to the partially hydrogenated oils used in food products. The FDA now requires *trans* fat content to be declared on food labels due to the negative health implications associated with these fatty acids (CFR 21 101.9). However, several health benefits have been attributed to the predominant *trans* fatty acid present in meat products: conjugated linoleic acids (CLA). Physiological effects of CLA in animal models, as well as human studies include improved blood lipid profile, reduced body fat mass, and suppression of carcinogenesis (Belury, 2002; Ip, Scimeca, & Thompson, 1994; Smedman & Vessby, 2001; Thom, Wadstein, & Gudmundsen, 2001).

In meat products, the most common fats are MUFAs and saturated fatty acids, with oleic, palmitic, and stearic acids typically accounting for the greatest proportion of total fatty acids (Valsta, Tapanainen, & Männistö, 2005). Compared to other saturated fatty acids, stearic acid has been shown to have a neutral effect on blood cholesterol as it does not raise LDL cholesterol

concentrations (Grundy, 1994; Mensink, 2005). This is a result of the body's ability to convert stearic acid to oleic acid, an unsaturated fatty acid.

Triacylglycerols

The predominant form of lipids in both plant oils and animal fats are triacylglycerols (TAG), also called triglycerides. These are formed when the three hydroxyl groups of glycerol form ester bonds with three fatty acids. These TAGs constitute about 90% of the dietary macronutrient called fat. Due to differences in melting point, TAGs exist in foods in both solid and liquid forms at room temperature, with fats being solid and oils liquid at room temperature (Stapanik & Caudill, 2013).

Glycerophospholipids

Similar in structure to triacyglycerols, glycerophospholipids consist of a glycerol backbone bonded to at least one fatty acid and to a phosphate group with a polar head group attached. Characterization of phospholipids is based on the head group present. The presence of a polar head group and fatty acyl chains provides the molecules with amphipathic properties that are suited for membrane formation, the major function of glycerophospholipids in the body. Diversity exists in this lipid subclass due to the many combinations of fatty acids, head groups, and bond types, leading to variability in the specific function of phospholipids for membrane structure and signaling properties (Stapanik & Caudill, 2013).

Cholesterol

Steroids are lipid compounds united by their 4 ring structure, but vary in function with regard to the side chains and oxidation state of the ring. In animal tissues, including humans, cholesterol is the predominant form of sterol and is necessary for cell membrane structure, in addition to being essential for steroid hormone synthesis and protein modification. Bile acids are

also synthesized from cholesterol in the liver. Cholesterol is provided in the diet through animal foods and is synthesized endogenously in the human body to maintain adequate levels. Excretion of cholesterol occurs through formation of high density lipo-proteins (HDL), eventually being converted to low-density lipoproteins (LDL) that can be removed from circulation mainly by the liver (Stapanik & Caudill, 2013).

Vitamins

Vitamins are organic compounds that are essential to physiological function but required in small amounts compared to the macronutrients as they are not used for energy metabolism. As a group, vitamins most commonly act as components of coenzymes, but their roles are not limited to this function. The 13 essential vitamins are often classified based on their solubility, with A, D, E, and K falling into the fat-soluble category and all others being soluble in water.

Vitamin A

All compounds that demonstrate activity similar to retinol, the alcoholic form of this vitamin, are commonly classified as vitamin A. Consumption of both plant and animal foods can provide precursors to vitamin A, albeit in different forms: retinyl esters from animal sources and primarily beta-carotene from plant sources. This fat soluble nutrient plays many biological roles, making it essential to vision, immune function, and reproduction (Stapanik & Caudill, 2013). The recommended daily dietary allowance is 700 micrograms for women ages 19 – 70 years, and 900 micrograms for men of the same age range (IOM, 2001).

Riboflavin (Vitamin B_2)

The roles of riboflavin in the human body are diverse and vital to numerous systems. Niacin derivatives, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), are versatile redox cofactors in the cell that are involved in energy production and numerous other

pathways. Fatty acid oxidation, DNA replication and repair, clearance of neurotransmitters, blood pressure regulation, and activation of vitamin B_6 are all dependent on niacin (Stapanik & Caudill, 2013). Dairy products generally provide the greatest proportion of this nutrient in the diet, but meat and fatty fish are rich sources as well (Powers, 2003). The recommended daily intake of riboflavin for individuals over 18 years is 1.1 milligrams for women, while slightly higher for men at 1.3 milligrams (IOM, 1998).

Niacin (Vitamin B_3)

Dietary niacin is used endogenously to produce nicotinamide adenine dinucleotide (NAD), which is utilized by over 400 known proteins; more physiological reactions require this molecule than any other vitamin-derived compound. The role of niacin in energy metabolism and genomic regulation make it extremely important to human health. Meat, fish, and fortified grain products are good dietary sources of this nutrient (Stapanik & Caudill, 2013). Current nutritional data for beef variety meats suggest that these items could contribute significantly to dietary niacin requirements (USDA SR, 2018). Daily intake of niacin is recommended to be 14 milligrams for females and 16 milligrams for males above the age of 13 years (IOM, 1998).

Pantothenic Acid

A wide range of foods provide pantothenic acid to the diet, including organ meats, fish, egg yolk, broccoli, milk, legumes, and whole grains. As a precursor to coenzyme A and acyl carrier protein (ACP), pantothenic acid is required for energy regulation through fatty acid synthesis and oxidation, citric acid cycle involvement, and roles in metabolism of organic acids such as those from amino acid catabolism (Stapanik & Caudill, 2013). Accounts of either deficiency or toxicity have not been common for this water soluble vitamin. Although a

Recommended Dietary Intake is not available for pantothenic acid, the adequate intake level is set at 5 milligrams for all individuals over the age of 13 years (IOM, 1998).

Vitamin B_6

There are six major forms of the compound that is termed vitamin B_6. Pyridoxal 5'-phosphate (PLP) and pyridoxamine 5'-phosphate (PMP) are the major derivatives contained in foods of animal origin. Meat and fish products are major contributors of this nutrient in the diet of Americans, but fortified cereals also provide vitamin B_6 in the form of pyridoxine (Stapanik & Caudill, 2013). More than 100 enzymes in the human body require PLP as a coenzyme, many of which are involved in the metabolism of amino acids as well as glycogen. Vitamin B_6 intake has also been inversely linked to risk of cardiovascular disease risk and associated mortality, as well as risk for Parkinson's Disease (Cui, Iso, Date, Kikuchi, & Tamakoshi, 2010; Lau, De, Koudstaal, Witteman, Hofman, & Breteler, 2006; Rimm, 1998). For both men and women ages 19 – 50 years, the recommended daily intake for vitamin B_6 is 1.3 milligrams, increasing to 1.7 and 1.5 milligrams for men and women respectively at ages over 50 years (IOM, 1998).

Vitamin B_{12}

Cyanobalamin, as well as other bioactive members of the cobalamin family that contain cobalt, are considered vitamin B_{12}. Neurological function and blood formation depend on adequate concentrations of these compounds in the body. However, intrinsic factor, which is a protein secreted in the gastric mucosa, is also required for vitamin B_{12} to be properly absorbed for use (Stapanik & Caudill, 2013). These complex-structured cobalamin compounds are only synthesized by anaerobic microorganisms, but are present in beef and other meat products due to their production by ruminant gut microflora. While a limited number of plant foods contain vitamin B_{12} in amounts that would be beneficial to meeting dietary requirements, most provide

only traces or an inactive form of the nutrient (Watanabe, 2007). However, fortified cereals are another source of vitamin B_{12} in the United States. The recommended dietary allowance for vitamin B_{12} is 2.4 milligrams for people over the age of 13 years (IOM, 1998).

Vitamin D and 25-Hydroxy Vitamin D

Vitamin D_2 (ergocalciferol) and D_3 (cholecalciferol) differ slightly in regard to chemical structure, but are considered similar in terms of biological activity. Ergocalciferol is synthesized in yeast, invertebrates, and fungi in response to ultraviolet (UV) light exposure, while cholecalciferol is produced in the skin of vertebrate animals, including humans, upon exposure to UV light. In the liver, vitamin D is hydroxylated to form 25-hydroxycholecalciferol (25-hydroxy vitamin D), which can circulate in plasma before conversion to 1,25-dihydroxyvitamin D. This final biologically active compound is responsible for the main functions of vitamin D in the body, including homeostatic regulation of calcium and phosphorus concentrations to maintain bone health as well as function of extraskeletal tissues (Stapanik & Caudill, 2013).

Vitamin D is available through dietary supplements formulated with synthetic compounds, and also from a limited number of food sources. In the United States, fortification of foods such as milk and grain products occurs to enhance vitamin D intake, but populations in developing regions may be at risk for deficiency due to a lack of reliable sources of this nutrient. Men and women ages 19 to 70 years are recommended to obtain 15 micrograms of vitamin D on a daily basis (IOM, 2011).

Vitamin K

Differing slightly in structure, phylloquinone (vitamin K_1) and menaquinones (vitamin K_2) are both classified as vitamin K due to similar activity. First discovered and most recognized is their role in blood coagulation, but vitamin K-dependent proteins exist in other pathways such

as bone metabolism (Stapanik & Caudill, 2013). Since menaquinones are obtained from animal sources, they may be more bioavailable for absorption than plant-derived phylloquinones (Beulens et al., 2013). Recent work suggests that vitamin K_2 in particular may also mitigate health condition risk through various mechanisms including preventing vascular calcification and preventing bone loss (Knapen, Drummen, Smit, Vermeer, & Theuwissen, 2013; Maresz, 2015; Yamaguchi & Weitzmann, 2011).

However, menaquinones contribute minimal amounts to the overall consumption of vitamin K (Booth & Suttie, 1997). Additionally, evidence as to their health effects has not been conclusive. Therefore, no recommendations exist for vitamin K_2 separate from the overall vitamin K guidelines which are based on requirements for phylloquinone related to its role in coagulation (Beulens et al., 2013; National Research Council, 2000). The adequate intake for vitamin K has been set at 90 micrograms for females and 120 micrograms for males ages 19 years and over (IOM, 2001).

Minerals

Opposed to the organic constituents of the diet, minerals are single elements found in nature that are required for fundamental human functioning. Minerals can be categorized as macroelements, required at levels of 100 milligrams/day; trace elements, needed between 1 milligram to 100 milligrams/day; and ultra-trace elements, of which less than 1 milligram is needed per day. Minerals serve diverse functions in the body, ranging from skeletal formation to cellular signaling and osmotic balance (Stapanik & Caudill, 2013).

Calcium

Calcium serves numerous roles in the body, from playing a structural role in bone formation to acting as a second messenger through alterations in systolic calcium concentrations.

Many processes are regulated to some degree by intracellular calcium, including muscle contraction, movement of substances across membranes, neurotransmitter release, and DNA synthesis initiation (Stapanik & Caudill, 2013). For adults ages 19 – 50 years, 1,000 milligrams of calcium are recommended per day, increasing to 1,200 milligrams for those over the age of 50 (IOM, 2011).

Copper

Copper is a trace element that performs functions in the body as a component of numerous enzyme systemsserving as an electron-pair acceptor (Lewis base) for enzymatic and oxidation-reduction reactions. Copper in biological systems is part of assist in metabolizing iron; synthesizing neuropeptides, neurotransmitters, and collagen; and protecting compounds from reactive oxygen species. The recommended daily intake. for copper is 900 micrograms for individuals over the age of 19 years (IOM, 2001).

Iron

Iron exists biologically in several oxidation states, primarily ferrous (Fe^{2+}) and ferric (Fe^{3+}). Iron is a constituent of many proteins that can be classified as heme and non-heme proteins. Hemoglobin and myoglobin are iron-containing heme proteins that are essential for oxygen transport and metabolism. Many enzymes also require heme as a structural component, including peroxidases, catalases, and cytochrome P450s (Stapanik & Caudill, 2013).

Iron deficiency is a global health concern, causing anemia and preventing proper physical and mental development in children and adolescents. Additionally, many other health conditions or disease states can be worsened by inadequate iron intake (West & Oates, 2008). Iron is available from a variety of food sources, such as red meat, dry beans, and fortified grains.

However, differences exist between the types of iron obtained from meat versus other food products. Red meat and dark poultry contain heme iron, whereas other food sources do not. Heme iron is more bioavailable, so although this type usually makes up a smaller percentage of iron intake, it contributes a greater amount to total iron absorption for functional use (Hurrell & Egli, 2010). The recommended iron intake for females between 19 and 50 years of age is 18 milligrams per day, while adult males and older females require less than half that amount, 8 milligrams (IOM, 2001).

Manganese

Manganese is involved in cartilage formation through proteoglycan synthesis, explaining why structural and bone defects are characteristic of this nutrient deficiency. As a component of enzymes, manganese also plays a role in carbohydrate metabolism, formation of urea, and defense against reactive oxygen species (Stapanik & Caudill, 2013). The adequate intake for manganese is set at 1.8 milligrams per day for females and 2.3 milligrams per day for males (IOM).

Phosphorus

Free phosphate, also called inorganic phosphate, is the form of phosphorus found in biological systems. However, most of the phosphate in the body is concentrated in bones, where it binds with calcium to produce hydroxyapatite, the chief component of the skeleton. Phosphate serves other purposes as well, both in phosphorylating compounds to prevent movement of neutral molecules such as glucose, and playing a role in pH balance through urinary excretion as dihydrogen phosphate. While individuals aged 19 years and over are recommended to consume 700 milligrams of phosphorus per day, the guidelines for 9 – 18 year old people are nearly double that at 1,250 milligrams per day (IOM, 1997).

Zinc

Zinc is essential for a wide variety of body functions; these can be separated into three categories: structural, regulatory, and catalytic. Through its inclusion in hundreds of metalloenzymes it plays a role in many unique biological functions. Zinc is essential for growth and maturation in children, so deficiencies can cause stunting of varying severity, as well as lack of intellectual development (Chasapis, Spiliopoulou, Loutsidou, & Stefanidou, 2012). Both innate and adaptive immune system function are also affected by intracellular levels of zinc. The mineral is necessary for signaling pathways and protein activation that lead to secretion of anti-inflammatory cytokines and antigen presentation, among other mechanisms (Stapanik & Caudill, 2013). Genomic regulation, connective tissue growth, taste acuity, wound healing, cognitive function, and many other essential processes are dependent on consistently having adequate levels of zinc (Chasapis et al., 2012).

Zinc can be obtained through the diet from a variety of foods including meat, dairy products, and legumes. National dietary surveys reflect that beef contributes the greatest percentage of zinc in the American diet (Cotton, Subar, Friday, & Cook, 2004). It is recommended that women between the ages of 14 and 18 years consume 9 milligrams of zinc per day, which can decrease to 8 milligrams for those 19 years of age and above. The guidelines for men above age 13 suggest 11 milligrams per day (IOM, 2001).

USDA Food and Nutrient Databases

The goal of USDA nutritional databases is to provide nutrient information for foods in the marketplace; since the 1890s, the USDA has been compiling and publishing this data (Ahuja et al., 2013). In order to expand and update these tools, the USDA forms partnerships with different sectors, including industry, academia, and government organizations. In this way,

nutrient data for many foods, including beef muscle products, has been updated and is available for use by broad audiences to serve a variety of functions to ultimately benefit the health of the population. USDA food and nutrient databases provide the basic infrastructure for many types of dietary research and play a role in nutrition monitoring related activities that are critical for assessing dietary pattern shifts and health of the population. Additionally, development of public policy including the U.S. Dietary Guidelines for Americans and intake recommendations for specific nutrients relies on the information gained from maintaining and revising USDA databases (Ahuja et al., 2013). These resources also provide the underlying information for many commercial nutrient analysis software systems used by nutrition professionals to provide dietary recommendations to patients and clients in various settings. Labeling of both single ingredient foods, such as meat items, as well as other products may rely on data provided through the USDA National Nutrient Database for Standard Reference (SR), especially when laboratory analysis is cost-prohibitive.

Although currently undergoing modernization procedures, the USDA SR is a stand-alone resource updated each year that has been the main source of food composition data in the country. The most recent release of this document is the SR-Legacy, which was released in April of 2018. Nutrient data for over 7,000 food items is included, and is available for public use through the Nutrient Data Laboratory through the United States Department of Agriculture (USDA SR 2018).

Currently, nutrient data provided in the USDA SR originated from a study performed through a collaboration between the USDA and the University of Wisconsin in 2003. This provided nutritional information about beef heart, kidney, tripe, and brain, but consisted of a limited sample size that lacked geographical representation (Showell et al., 2012). Due to a lack

of analytically derived data, other nutrition information for other variety meat items has been imputed (USDA SR 2018).

Meat Product Labeling

The USDA Food Safety and Inspection Service (FSIS) mandates a universal labeling system for food products that functions to provide consistent nutrition information to consumers, dietary professionals, and food purveyors. The publication of the Nutrition Labeling of Single-Ingredient Products and Ground or Chopped Meat and Poultry Products Final Rule in 2012 shifted the nutritional labeling of major meat cuts from voluntary to mandatory. This guideline specifies the meat products that require a nutrition label, excluding variety meat items among other meat cuts from the labeling regulations (9 CFR 317.344).

Although the USDA FSIS does not dictate that variety meat items report nutrient content on the package, a traditional nutritional label is required when health or nutrient claims are displayed on food products (21 CFR 101.54). A product can present a "good source", "contains", or "provides" labeling claim if it contains 10 to 19 percent of the reference daily intake (RDI) established for that nutrient per reference amount customarily consumed (RACC) for the food item (CFR 9 381.454). An "excellent source", "high", or "rich in" claim can be made if 20 percent or more of the RDI for a nutrient is provided in a single RACC.

Role of Meat in a Healthy Diet

Due to increasing evidence that the eating pattern as a whole influences health outcomes, the 2015 – 2020 United States Dietary Guidelines focus on a whole diet approach in order to promote sufficient nutrient intake as well as minimizing risk for chronic disease. For these outcomes to be achieved, emphasis on consumption of nutrient-dense foods is critical (USDA & USHHS, 2010). Along with other food groups, it is advised to incorporate a variety of proteins

foods, including lean meats. Despite a shift from single nutrient recommendations, the dietary guidelines do present quantitative limits for saturated fats and sodium, as well as added sugars. Saturated fats should be restricted to 10 percent or less of total daily calories, while sodium intake should not exceed 2,300 milligrams per day (DGAC, 2015).

Obesity is an individual and public health issue that affects more than a third of U.S. adults, and is associated with increased risk for heart disease, type 2 diabetes, stroke, and various types of cancer (Goodwin & Chlebowski, 2016; Ogden, Carroll, Fryar, & Flegal, 2015; U.S. Department of Health and Human Services, 2013). Obesity is defined in the U.S. as having a body mass index value of greater than 30 kilograms per meter^2. Although the obesity epidemic is multifactorial, researchers have attributed the problem in part to high availability of energy-dense foods that are often highly processed, which may contribute to weight gain due to a lack of satiation (Crino, Sacks, Vandevijvere, Swinburn, & Neal, 2015; Duncan, Bacon, & Weinsier, 1983). Conversely, diets high in protein foods can contribute to increased satiety and eating satisfaction and have been associated with greater success related to weight loss and weight management (Leidy, Carnell, Mattes, & Campbell, 2007; Weigle, Breen, & Matthys, 2005; Westerterp-Plantenga, Lemmens, & Westerterp, 2012). Moreover, the consumption of protein foods such as lean meat supplies, not only this macronutrient, but other essential nutrients as well (USDA SR 2018). Vitamins and minerals contained in lean beef contribute considerably to recommended daily intakes, especially for vitamin B_{12}, iron, potassium, zinc, and vitamin B_6 (Phillips et al., 2015). Considering these factors, a diet including lean meat can be beneficial in balancing energy intake and nutrient intake to align with the Dietary Guidelines for Americans to promote weight management and health.

Coronary heart disease (CHD) is the leading cause of death in the world, and similar to many non-communicable diseases, diet is a key risk factor (World Health Organization, 2014). Due to the association with CHD, saturated fat is a common concern related to meat consumption; however, recent evidence has initiated controversy about the traditional claims regarding this nutrient and disease risk. Nonetheless, saturated fats including lauric, myristic, and palmitic acids, have been shown to effect blood lipid profiles, increasing low-density lipoprotein cholesterol (LDL) concentrations, which are detrimental, and raising the beneficial high-density lipoprotein cholesterol (HDL) when substituted for carbohydrates (Katan, Zock, & Mensink, 1994). Another saturated fatty acid, stearic acid, has been termed neutral in terms of blood lipids, having little effect on blood cholesterol concentration (NCBA, 2007; Hunter et al., 2010). Stearic acid is prevalent in beef, making up a significant percentage of the total saturated fat content. Additionally, monounsaturated and polyunsaturated fatty acids make up more than half of the total fat in beef, despite the traditional classification of animal fat as saturated (USDA SR, 2018). Favorable changes in lipid profile and inflammatory factors have been associated with the unsaturated fats oleic acid and linoleic acid, both present in beef items (Covas, 2007; Willett, 2012). Dietary fat is necessary for biological function, so conscious consideration of intake, both type and amount, rather than exclusion, is likely to benefit individuals.

The Dietary Approaches to Stop Hypertension (DASH) diet pattern is highlighted in the 2015 dietary guidelines as a favorable eating strategy due to clinical evidence supporting its role in lowering cardiovascular disease risk (DGAC, 2015; Pao-Hwa, 2001). While the DASH diet focuses on plant foods and recommends limiting red meat intake, the Beef in an Optimal Lean Diet (BOLD) strategy includes red meat in an otherwise similar eating pattern. Results from a randomized, controlled trial demonstrated that effects on lipid and lipoprotein risk factors for

cardiovascular disease were comparable between a heart healthy diet including lean beef and the DASH diet encouraged by many health professionals (Roussell et al., 2012). Nonetheless, it is important to consider portion size as well as contribution to overall energy intake.

Variety Meat Consumption and Utilization

Variety meat items, falling under the category of edible offal, represent a significant portion of the U.S. export value for beef products, comprising 25% of the total volume and 12% of the value of beef exports in 2017 (USMEF, 2017). Edible byproducts from beef make up approximately 12% of the live weight of cattle (Ockerman & Hansen, 2000). As demand for muscle products increases globally, a larger amount of lower-value co-products will be produced as well, although demand may not increase to match this increased production (Mullen et al., 2017). Therefore, it is essential to utilize these raw materials effectively to optimize nutritional significance, maximize value of the carcass, and maintain sustainability of the industry.

Factors Influencing Consumption

Consumption of variety meat items, also termed edible offal, is highly dependent on cultural practices and traditions, and therefore varies widely based on country and ethnicity. Products that are under-valued in some countries are deemed delicacies that demand a premium in other regions (Aberle, Forrest, Gerrard, & Mills, 2001; Spoonger, 1988). Due to vast differences in consumption patterns of variety meats, food neophobia likely plays a role as well. Food neophobia is defined as a physiological and behavioral tendency to avoid unfamiliar foods that developed as a defense mechanism; this phenomenon has been shown to be stronger in response to animal products compared to plant foods (Al-Shawaf, Lewis, Alley, & Buss, 2015). A study exploring consumer attitudes related to beef variety meats found that acceptance is often highly related to past life experiences, but also to the form in which the product is presented

(Henchion, McCarthy, & O'Callaghan, 2016). Additionally, results provide insight into potential use of edible offal as ingredients, with individuals perceiving the inclusion of offal into food products as more tolerable than eating these products alone. However, an intrinsic desire for novelty in regard to food choices in part of the population (Knaapila et al., 2007) may be favorable to variety meat consumption as well, especially if marketed appropriately. Perceived naturalness and potential benefits may play an important role in influencing variety meat consumption patterns in regions where it is not customary practice (Henchion et al., 2016).

Innovative Applications

The chemical and nutritional properties of beef by-products, including variety meats, have lead researchers to investigate innovative ways to use these items. Henchion (2016) describes three levels of processing that could be applied to beef by-products to improve carcass utilization: low, intermediate, and high. At the lowest level, products are mixed in as ingredients; an intermediate processing level relates to incorporation of freeze-dried powders derived from by-products; at the highest level, vitamins and minerals are extracted and included in a concentrate form (Henchion et al., 2016).

Bioactive peptides are protein fragments that have unique physiological impacts which positively influence health; these compounds can be generated from carcass products through post-mortem proteolytic activity, or with the use of exogenous proteases (Kitts & Weiler, 2003; Toldrá, Aristoy, Mora, & Reig, 2012). A multitude of peptides exist with only a portion of them exhibiting beneficial activity, such as antihypertensive or antioxidant properties, so filtration or other methods can be used to concentrate specific types (Toldrá et al., 2012; Toldrá et al., 2016). Since the amino acid chains do not present these unique functions when contained as part of the

protein from which they originate, special processing of variety meats could provide distinct benefits for human health.

Certain proteins, specifically collagen and blood plasma, have functional properties that can be useful for food production including acting as binding, gelation, and emulsification agents. In addition to being used for these characteristics, proteins in variety meats can also be a source of essential and limiting amino acids (Mullen et al., 2017). As a rich source of macro- and micro-nutrients, variety meats could provide a medium for extraction of amino acids, vitamins, and minerals that are nutritionally relevant. Both the raw materials and isolated compounds may be especially beneficial for target populations with unique nutrient demands (Margarita, Castillo, Alejandro, & Ligardo, 2015). Product development opportunities exist for using food technology to maximize potential for variety meat products. As consumers in some regions of the world become increasingly concerned with naturalness of products, use of food extracts for supplemental nutrition materials may be desired (Henchion et al., 2016).

International Perspective

In addition to novel applications, variety meat items provide opportunity for underdeveloped countries, in which food accessibility is the major priority. Both over-nutrition and under-nutrition fall under the term malnutrition, according to the World Health Organization (WHO, 2000). This imbalance between the body's supply and demand for nutrients is especially prevalent in regions where safe and nutritional food is not available. As discussed, affinity for edible offal items is largely dependent on an individual's culture; these items are consumed regularly by many populations. Generally, these items are less expensive than skeletal muscle meat products, and would therefore be a more accessible form of protein and other nutrients when cost is a prohibiting factor to obtaining adequate nourishment. Researchers have

recognized to some extent the prospect that exists for utilizing variety meat items to serve these needs. Fayemi et al. (2018) recently investigated the potential of organ meats in providing an affordable and nutrient dense product to underserved populations. Findings suggest that protein, macronutrients, and bioactive molecules in edible offal could benefit individuals experiencing malnutrition if variety meat items were processed to generate a functional and palatable end product (Fayemi, Yetim, & Ahhmed, 2018).

Aside from further-processed items, offal products are a major component of the diet in many countries. Egypt is the main importer of beef liver from the United States, purchasing 76% of those sold in 2016, but Mexico, South Africa, and several South American nations are significant importers as well (Schaefer & Arp, 2017). Accruing comprehensive nutrient data for variety meat items may assist researchers and health professionals in understanding the contribution of these items to the diet of people around the world, and in developing strategies that would improve health and nutrition status by increasing consumption of these nutritionally significant products.

CHAPTER 3

NUTRIENT ANALYSIS OF TEN RAW U.S. BEEF VARIETY MEAT ITEMS

Materials and Methods

Institutional Animal Care and Use Committee approval was not required for this study as samples were obtained from federally inspected harvest facilities. Dissection and analysis procedures used for this study were nearly identical to methods described by Acheson et al. (Acheson et al., 2015).

Experimental Design

Product sampling was designed to be representative of the U.S. supply merchandised in retail markets. Retail packages of raw beef heart, liver, kidney, tongue, honeycomb tripe, oxtail, and marrow bones were obtained from three different processing facilities (Texas, Nebraska, and Kansas) in the United States, to provide national representation of retail-ready beef variety meat items. Beef bone broth was obtained from the National Cattlemen's Beef Association Culinary Team in Centennial, CO. Beef Rocky Mountain oysters (testicles) were obtained from a single supplier in Colorado. Beef blood was obtained from one processing facility in Pennsylvania.

Description, source, and International Meat Purchasing Specifications (IMPS) identifier for each variety meat item are found in Table 1.1. From each of the three suppliers, beef heart, liver, kidney, tongue, honeycomb tripe, oxtail, and marrow bones were collected two separate days, at least seven days apart. A minimum of four packages per collection date were procured of heart, liver, kidney, tongue, honeycomb tripe, and oxtail for a total of eight packages per item. Eight packages of vacuum sealed retail-ready marrow bone slices were obtained from the Kansas facility; femur bones were obtained from the Texas and Nebraska facilities and were sliced and

vacuum sealed at Colorado State University Meat Lab to match the specifications of those collected from Kansas. Three separate containers of beef blood were utilized, all from the Pennsylvania supplier. Three separate containers of beef bone broth were utilized, all produced by the National Cattlemen's Beef Association Culinary Team. Three packages of testicles were used, all from a single supplier in Colorado. Beef heart, liver, kidney, tongue, honeycomb tripe, oxtail, marrow bones, and beef bone broth were maintained at 0 to 4°C during transportation to Colorado State University Meat Laboratory.

Upon arrival, packages were inspected for packaging integrity, and any packages with lack of a preserved seal were vacuum sealed in the meat laboratory. Excluding bone broth, all packages and containers were stored in a dark environment at 0 to 4°C for seven days post-production prior to being frozen at -20° C for a minimum of forty-eight hours, until dissection. Bone broth was stored in a dark environment at 0 to 4°C for 3 days prior post-production, then frozen at -20° C for a minimum of forty-eight hours, until dissection. Beef blood and Rocky Mountain oysters were frozen to below 0°C at each processing facility, maintained a temperature of below 0° C during transport to Colorado State University Meat Laboratory, and stored at -20° C upon arrival to Colorado State University Meat Laboratory until dissection.

Cut Dissections

Beef variety meat items were tempered in a single layer at 0 to 4° C for 24 to 72 hours, depending on item thickness, until internal temperature reached 0 to 4° C. After thawing, each individual sample was weighed with the packaging to the nearest 0.1 g, then removed from the package and weighed to the nearest 0.1 g. The sample was then blotted to remove any surface moisture and weighed again to the nearest 0.1 g. Internal temperature and start dissection time were recorded for each sample. The entire piece or pieces within a package were utilized for

dissection. Post-dissection separable component weights and end dissection times and temperatures were recorded for each item. Dissections were performed using standard methods, including limited exposure to light, and use of powder-free nitrile gloves to protect nutrients from degradation. Dissections were performed by CSU personnel in a 5 to 7°C environment using disposable stainless steel scalpels (Integra Miltex, York, PA) to yield separable components.

Separable components were defined as follows: separable lean tissue included any lean muscle or organ tissue, intramuscular fat and light connective tissue deemed edible; external fat included adipose tissue located on the outer surface of the cut; internal fat included adipose tissue deposited between lean tissue; and refuse included any waste including bone and heavy inedible connective tissue. For liquid items and items requiring no dissection (tripe, testicles blood, bone broth), separable lean tissue was used to describe the total sample. A yield tolerance of 97.0 to 100.0% was established prior to dissection. Any samples not meeting yield tolerance were removed from the study and replaced with a new sample of the same item, origin, and collection date. For dissected items, a total of three packages of each item from a single origin and collection date were used for homogenization, after meeting yield tolerance. Each of the separable components from each sample, excluding refuse, were homogenized individually immediately following dissection. Honeycomb tripe was procured following specification criteria of being practically devoid of external fat resulting in no dissection. Any tripe samples not meeting this criterion were trimmed at Colorado State University Meat Lab to match this specification before being homogenized. Testicles were devoid of fat, and the outer membrane of the testicle was removed at the processing facility, resulting in no dissection. Due

to the liquid and homogenous nature of the blood and bone broth, these items were not altered prior to homogenization.

Homogenization

For non-liquid items (heart, liver, kidney, tongue, oxtail, bone marrow, tripe, testicles), each separable component derived from a single package for were homogenized resulting in one lean sample per package, in addition to one external fat and one internal fat sample for each package when applicable. Standard methods of homogenization were adhered to, including homogenizing with use of powder-free nitrile gloves and in the absence of direct light to protect samples from contamination and nutrient degradation (Acheson et al., 2015). Separable lean tissue from each package was cut into 2.5 cm^3 pieces and placed into a stainless steel strainer inside a stainless steel bowl containing liquid nitrogen until all pieces were completely frozen. The pieces were transferred into a 6.62-liter Robot Coupe BLIXER 6V (Robot Coupe USA Inc., Ridgeland, MS). Samples were blended for approximately 10 seconds at 1500 rpm and 30 seconds at 3500 rpm until a fine-powder consistency was reached. Immediately following homogenization, each sample was placed into a 710 mL Whirl-Pak bag using a stainless steel spoon that was dipped in liquid nitrogen for 10 seconds before use. Each sample bag was placed into a -20° C freezer immediately. External and internal fat samples were frozen following the same procedures as with lean tissue. After samples were frozen, samples were placed into a 3.79-liter Robot Coupe BLIXER 4V (Robot Coupe USA Inc., Ridgeland, MS) and blended into a finely-powdered consistency under the same time and speed protocols as with lean homogenization. Fat samples were immediately placed into 532 mL Whirl-Pak bags using a stainless steel spoon dipped in liquid nitrogen for 10 seconds before use. Sample bags were immediately placed into a -20° C freezer.

Beef blood and beef bone broth were homogenized using the same technique. One full container, as procured, of each liquid was blended in a 3.79-liter stainless steel blender (Waring, Stamford, CT). After blending, a stainless steel ladle was drawn through the liquid from the bottom of the blender upward, and a 60 mL syringe (Medtronic, Minneapolis, MN) was used to extract the liquid from the ladle. The syringe was used to create droplets that were dropped into a fine mesh strainer inside a stainless steel bowl filled with liquid nitrogen. This procedure was repeated until at least 300 g of the sample was frozen as droplets. Samples were immediately placed into 710 mL Whirl-Pak bags using a stainless steel spoon that was dipped in liquid nitrogen for 10 seconds before use. Each sample bag was immediately placed into a -20° C freezer.

After all samples were homogenized each day, homogenates were double bagged and transferred from a -20° C freezer into a -80° C freezer until compositing and analysis occurred.

Lean Compositing

For heart, liver, kidney, tongue, tripe, oxtail, and bone marrow, all homogenates of separable lean tissue of the same item and origin were combined in equal parts by weight to create three lean composites per item (one from each supplier). For Rocky Mountain oysters, three composites were created, one from each of three packages of product. Three composites of blood were created, one from each of three bottles of blood obtained. For bone broth, three composites were made, consisting of one batch of bone broth per composite. All compositing procedures occurred by combining lean homogenates, blending composites in a 6.62-liter Robot Coupe BLIXER 6V (Robot Coupe USA Inc., Ridgeland, MS), and aliquoting into Whirl-Pak bags in the presence of liquid nitrogen. All samples analyzed at an on-site laboratory were immediately placed back into a -80° C freezer until analysis occurred. All samples analyzed at

off-site laboratories were placed into a -80° C freezer before being shipped in insulated boxes with dry-ice and gel ice packs via overnight shipping.

Fat Compositing

For items containing separable fat (heart, kidney, tongue, oxtail), all fat homogenates of the same item and fat type were combined in equal parts in weight. For oxtail, equal parts in weight of each fat type were then combined for a single composite. Oxtail was the only item containing external and internal fat. All compositing procedures occurred by combining fat homogenates, blending composites in a 3.79-liter Robot Coupe BLIXER 4V (Robot Coupe USA Inc., Ridgeland, MS), and aliquoting into Whirl-Pak bags in the presence of liquid nitrogen. All samples analyzed at an on-site laboratory were immediately placed back into a -80° C freezer until analysis occurred. All samples analyzed at off-site laboratories were placed in a -80° C freezer prior to being shipped in insulated containers with dry-ice and gel ice packs via overnight shipping.

Nutrient Analysis

Nutrient analysis occurred at USDA-ARS approved laboratories including Colorado State University (CSU) and external laboratories. Pre-determined analyses were designated to each laboratory under approval of USDA-NDL (Nutrient Database Laboratory).

For beef heart, liver, kidney, and tongue, the following analyses were conducted: proximate analysis (protein, ash, moisture, fat), fatty acid profile, ICP minerals, cholesterol, B vitamins (thiamin, niacin, riboflavin, pantothenic acid, vitamin B_6, vitamin B_{12}), vitamin A, vitamin E, vitamin D, 25-hydroxy vitamin D, and vitamin K. For beef tripe, oxtail, bone marrow, testicles, and blood, the following analyses were performed: proximate analysis (protein, ash, moisture, fat), fatty acid profile, ICP minerals, cholesterol, vitamin A, vitamin E, vitamin D, 25-

hydroxy vitamin D, and vitamin K. Beef bone broth was analyzed for proximate components (protein, ash, moisture, fat), fatty acid profile, ICP minerals, vitamin K, vitamin A, vitamin E, amino acid profile, and total tryptophan.

National Institute of Standards and Technology (NIST) standard reference material 1849a Adult/Infant Nutritional Supplement (Gaithersburg, MD), and standard materials (Beech Nut Brand Beef and Chicken baby food, ground beef standard, pork and egg standard, bologna standard, and salmon standard) obtained from the Food Analysis Laboratory Control Center (Virginia Polytechnic Institute and State University; Blacksberg, VA), were utilized to validate nutrient determinations to ensure the accuracy and precision of generated data among all laboratories. All standard materials were obtained from (FALCC) Food Analysis Laboratory Control Center (FALCC; Virginia Polytechnic Institute and State University, Blacksburg, VA). Ground beef and bologna standard materials were analyzed with each analysis group to ensure values existed within the acceptable range established by the FALCC for proximate analysis (protein, ash, fat, and dry matter). ICP minerals analyses were validated with use of NIST Adult/Infant Nutritional Supplement and bologna standard material. Beechnut beef baby food was used to validate thiamin, niacin, riboflavin, pantothenic acid, vitamin B_6, and vitamin B_{12} assays. Beechnut chicken baby food was used for validation of vitamin E assay. For cholesterol, vitamin B_{12}, and fatty acid profile assays, ground beef standard material was utilized. Pork and egg standard was used to validate vitamin D, 25-hydroxy vitamin D analyses total thiamin, and vitamin K analyses. Salmon standard was utilized for validation of amino acid profile and vitamin A assays. Chemical analyses were considered valid by the USDA-NDL when the standard value generated was within the standard error of the certified value.

Proximate Analysis

Proximate analysis was conducted to determine percent protein, ash, moisture and fat content for all lean tissue composites for each item from each origin. Proximate analysis was conducted for fat composites for each item that contained separable fat: heart, kidney, tongue, oxtail.

Protein Analysis

Crude protein was determined following the AOAC Official Method 992.15 (AOAC, 2006) using a nitrogen determinator (Leco TruSpec CN or Leco FP-2000; Leco Corporation, St. Joseph, MI and Rapid N Cube, Elementar, Hanau, Germany). Total percentage nitrogen was multiplied by a factor of 6.25 to calculate percent protein. Protein content was determined at Colorado State University.

Ash Analysis

Ash content was determined using the ashing method described by AOAC 923.03 and 920.153 (AOAC, 2000). Approximately 1 gram of sample was placed into a pre-weighed, dry crucible prior to placing the crucible into a Thermolyne box furnace at 600° C for 18 hours. Percent ash was calculated by dividing the ash weight by the initial sample weight and multiplying by 100. Ash analysis was conducted at Colorado State University.

Moisture Analysis

Moisture content was determined using the oven drying method described in AOAC 950.46 and 934.01 (AOAC, 1995). Approximately 1 gram of sample was weighted into aluminum tins prior to placing the tins into a forced air drying oven for 24 hours at 100° C. Percent moisture content was determined from the formula: % Moisture = [(initial weight – dry weight) / initial weight] x 100. Moisture content was analyzed at Colorado State University.

Fat Analysis

Fat content was determined suing the chloroform:methanol method described by Folch, Lees, and Stanley (1957). Approximately 1 gram of sample was homogenized in 2:1 chloroform:methanol solution prior to placing into an orbital shaker at room temperature for 20 minutes. Sample was filtered through ashless filter paper and 4 mL of 0.9% NaCL was added before being refrigerated for 24 h. Upon phase separation of the filtrate, aspirated low phase content was placed into a pre-weighed scintillation vial and dried under N_2 gas followed by vial air drying under a hood for 2 hours. Vials were placed into a forced air drying oven for 12 hours at 100° C. Percent total fat was calculated from the formula: % Total Fat = [((Total volume of chloroform:methanol) / 10) x (final lipid weight / initial weight)] x 100. Total fat content was analyzed at Colorado State University.

Fatty Acid Analysis

Fatty acid methyl esters (FAMES) were prepared as described by Parks and Goins (1994). Analysis of FAMES occurred by liquid chromatography using an Agilent Model 6890 Series II (Avondale, PA) gas chromatograph-fixed with a Series 7683 injector and flame ionization detector in addition to being equipped with a 100-m x 0.25-mm fused silica capillary column (SP-2560 Supelco Inc. Bellefonte, PA). Fatty acid percentages were calculated based on the total FAME analyzed. Fatty acid analysis was conducted at Colorado State University.

ICP Mineral Analysis

Mineral analyses were determined for Ca, Mg, K, Na, Fe, Zn, Cu, Mn and P by using inductively coupled plasma mass spectrometry methods described by the AOAC Official Methods 2011.19 and 993.14 (AOAC, 2000) and USDA wet ashing procedure. ICP mineral determination was conducted at Covance Laboratories (Madison, WI).

Cholesterol Analysis

Cholesterol analysis was performed using saponification, extraction, evaporation, and derivatization as described by AOAC Official Method 994.10 (AOAC, 2000). Cholesterol content was analyzed at Covance Laboratories (Madison, WI).

B Vitamins

B-Vitamins analysis was conducted for thiamin, niacin, riboflavin, pantothenic acid, vitamin B_6 and vitamin B_{12} by using methods described as follows: Total thiamin – AOAC Official Method 942.23, 953.17, 957.17; niacin – AOAC 944.13 and 960.46; riboflavin – AOAC 960.46 and 940.33; pantothenic acid – AOAC 945.74, 992.07, 960.46; vitamin B_6 – AOAC 961.15; vitamin B_{12} – AOAC 952.20 and 960.46 (AOAC, 2000). Analysis of B-vitamins was conducted at Covance Laboratories (Madison, WI).

Vitamin E

Vitamin E analysis was conducted using high performance liquid chromatography (HPLC) with ultraviolet (UV) detection, with external calibration, and internal standard recovery post analysis. Vitamin E analysis was conducted by Craft Technologies Laboratory (Wilson, NC).

Vitamin A

Vitamin A analysis was performed by using HPLC with UV detection of retinol with external calibration, and internal standard recovery post analysis. This method is adapted from AOAC Official Method 2001.13. Vitamin A analysis was conducted by Craft Technologies Laboratory (Wilson, NC).

Vitamin D and 25-Hydroxy-Vitamin D

Vitamin D analysis was conducted by Covance Laboratories (Madison, WI). Analysis was conducted for Vitamin D_2, D_3, and 25-Hydroxy Vitamin D_3, and was determined using the chromatography-mass spectrophotometry method described in AOAC Official Method 2011.11.

Vitamin K

Vitamin K content was analyzed using HPLC with fluorescence detection after post-column reduction. Vitamin K analyses were performed at the Vitamin K Laboratory within the Jean Mayer USDA Human Nutrition Research Center on Aging (HNRCA) at Tufts University (Medford, MA).

Amino Acid Profile

Amino acid profile analysis was conducted using the methods described in AOAC Official Method 982.30 using chromatography with fluorescence and ultra-violet detection. Tryptophan was determined utilizing the total tryptophan method as described by AOAC Official Method 988.15. Amino acid profile and tryptophan analysis were performed at Covance Laboratories (Madison, WI).

Data Analysis

Using R statistical software, mean and standard error of the mean (SEM) of nutrient values were calculated from the three composites of each item for heart, liver, kidney, tongue, tripe, oxtail, and bone marrow. A single mean and SEM, representing a national average, are reported for these items. Values from the three composites of each Rocky Mountain oysters, blood, and bone broth were used to calculate a mean and SEM value for each nutrient per item.

Results and Discussion

The ten beef variety meat items in this experiment are listed and described in Table 1.1. Items were analyzed to determine nutrient content, including proximate analysis, fatty acid profile, ICP minerals, cholesterol, B vitamins (thiamin, niacin, riboflavin, pantothenic acid, vitamin B_6, vitamin B_{12}), vitamin E (alpha-tocopherol, beta-tocopherol, delta-tocopherol, gamma-tocopherol), vitamin A, vitamin D (vitamin D_2 and D_3), 25-hydroxy vitamin D, vitamin K, and amino acid profile, and total tryptophan. For each item, the compositing scheme and analyses performed are presented in Table 1.2.

Separable Components

Heart, liver, kidney, tongue, oxtail, and bone marrow were dissected to obtain individual separable components. Total grams of separable components for each item, as well as the percentages of total pre-dissected weight comprised by each component are listed in Table 1.3. Internal fat was not present for heart, liver, or bone marrow, and external fat was not present for liver, kidney, tongue, or bone marrow. Retail-ready packages of liver contained skinned and sliced portions, resulting in a high percentage of separable lean tissue with a small amount of refuse (thick blood vessels or connective tissue). Internal fat was present on the kidneys surrounding the blood vessels and ureter, which were trimmed flush with the kidney surface at the production facility. Intramuscular fat of the tongue muscle tissue was included in separable lean tissue, while intermuscular fat was measured as internal fat, and refuse included the skin of the tongue. Percentages of separable lean are relatively consistent with findings of Purchas, Wilkinson, and Carruthers (2015), but some variation in fat and refuse values exists between the two studies. Fat content differences in particular are potentially attributable to the country of origin: New Zealand versus United States. Oxtail contained a significant amount of refuse due to

the presence of bones (coccygeal vertebrae), resulting in a relatively small percentage of separable lean tissue. Packages of bone marrow bones contained slices of femur bones, in which the marrow was present. The bone marrow was measured as separable lean tissue, while the bones made up the refuse for this item, which was a considerable percentage of the total weight. Separable component data will be used by the USDA NDL to extrapolate total nutrient profile of each item using the weight of the entire item and the nutrient values obtained for both lean and fat tissue.

Proximate Analysis

Mean and standard error of the mean results from analysis of proximate components and cholesterol content of each item are presented in Table 1.4. Proximate and cholesterol analysis was also performed for fat samples of those items for which external and/or internal fat was present and is displayed in Table 1.5.

Protein

Bone marrow and bone broth contained a significantly lower percentage of protein than the other items in the study. Of the eight remaining items, the lowest protein content on a percentage basis was testicles with approximately 11%, while the highest was found in oxtail and liver at above 19% of the total weight. Some variety meat items include collagen, which is a structural protein with different characteristics than myofibrillar proteins that comprise the majority of skeletal muscle. The collagen content of beef tripe, as a proportion of total protein, has been determined to be about 20 percent, resulting in a unique amino acid pattern when compared to skeletal muscle foods (Zarkadas, Karatzas, & Zarkadas, 1996). Although individual amino acid content was not measured in this study, the protein digestibility of several variety meat items would likely differ from traditionally consumed meat products since amino acid

composition is a factor in determining the protein digestibility corrected amino acid score (PDCAAS).

Fat

Fat content was analyzed for separable lean of each item; values were highly variable, with bone marrow having the highest percentage of lipids by a substantial margin at 77% of total weight. Tongue, tripe, and oxtail contained 11%, 7%, and 6% fat respectively, with the percentage of fat for all other items being less than 5%. A recent experiment aimed at determining nutritional value of South African beef offal showed similar trends for the three items included in both studies (van Heerden & Morey, 2014). Kidney contained the least fat with approximately 3% fat across the two experiments; a greater amount of fat and larger difference between studies (11% and 18%) occurred for tongue, and heart fell between the other items with 3% and 7% of total weight comprised of fat in the current and South African study respectively (van Heerden & Morey, 2014). Bone broth was prepared by the National Cattlemen's Beef Association Culinary Team using a recipe published by The Beef Checkoff which includes directions to strain broth prior to consumption, so the fat content of the broth was marginal at less than 1%. The separable fat composites indicated similar levels of percent fat for each item analyzed, ranging from 43% to 49% as shown in Table 1.5.

Moisture

An inverse relationship has been shown in previous literature between moisture content and fat content in meat products, specifically skeletal muscle (Acheson et al., 2015; Patten et al., 2008; A. M. Smith, Harris, Haneklaus, & Savell, 2011). Consistent with these findings, the items in the current study with the highest percentage of fat, bone marrow and tongue, had the least moisture. Additionally, bone broth had the highest moisture content, over 96%, and the lowest

fat content of all items analyzed. Moisture content of the fat composite samples ranged from 37% to 44%, with fat and moisture combined comprising over 80% of the total weight.

Cholesterol

For the nine items assessed (bone broth was excluded), cholesterol content was variable. Tongue and oxtail contained under 100 milligrams per 100 grams of separable lean. Heart, tripe, and blood had between 116 mg/100g and 128 mg/100g, whereas testicles and liver were higher with 219 mg/100g and 257 mg/100g, respectively. The greatest amount of cholesterol was found in kidney at 400 mg/100 g of lean tissue. The two items lowest in this nutrient are comparable to the cholesterol levels found in beef skeletal muscle cuts, which have been reported in the 80 mg to 100 mg/100g range (Roseland et al., 2018). The 2015 Dietary Guidelines did not provide a quantitative limit for dietary cholesterol due to insufficient evidence, but rather claimed that limiting saturated fat in the diet should lead to lower intake of cholesterol which would benefit health outcomes (DGAC, 2015).

Extra-Labeling Claims

The USDA regulates use of nutrient label claims on food products and provides guidelines for the requirements of these claims (9 C.F.R. 317.354). “Good Source”, equivalent to “Contains” and “Provides”, can be used on a label if the product contains 10 to 19% of the Daily Value (DV) or Recommended Daily Intake (RDI) per RACC (reference amount customarily consumed) for that nutrient. To include a claim of “Excellent Source”, “High”, or “Rich In”, the food must contain at least 20% of the DV or RDI per RACC for that nutrient. Each of the items evaluated in this study, except for bone broth, qualifies for at least one “Good Source” or “Excellent Source” labeling claim; these percentages are presented in Table 1.6 for those items that qualified. Percentages listed in Table 1.6 are based only on separable lean tissue and may be

altered upon extrapolation of values for the entire item including all separable components by USDA NDL.

Vitamin Analysis

Results of vitamin A, D, E, and K analysis for nine of the variety meat items are presented in Table 1.7 and Table 1.8. For the four items evaluated, B-vitamin content is found in Table 1.9. Data for vitamin content of bone broth is displayed in Table 1.10. For fat composites, vitamin analysis results are shown in Table 1.5. To provide greater clarity, discussion focuses on those vitamins that contribute significantly to recommended daily intake levels.

Vitamin A

Vitamin A content was analyzed for all items excluding bone broth, but was highest in the liver, with a mean of 37 mcg per gram of separable lean. This related to 3,702 mcg per 100 grams or 12,340 International Units (IU), which is 247% of the Daily Value for vitamin A. The separable lean component from other items, as well as the fat composites, contained considerably less vitamin A: under 1 mcg/g. These results supported claims that beef liver is a good source of vitamin A. The amount of vitamin A in liver reported in the latest release of the USDA Standard Reference Database was higher, at 26,088 IU (USDA SR 2018). The Tolerable Upper Intake level for vitamin A as established by the Food and Nutrition Board of the Institute of Medicine is 3,000 mcg per day; this value represents the highest amount of a nutrient that most people may consume to result in no adverse health risks (IOM, 2000). However, much higher consistent consumption levels of vitamin A are considered generally toxic: greater than 25,000 IU/day for six years, or greater than 100,000 IU/day for six months (Penniston & Tanumihardjo, 2006). Nonetheless, it is important to have relevant vitamin A content information for liver and other

variety meats to allow for appropriate nutrition recommendations to be made for a range of individuals and populations.

B-Vitamins

Heart, liver, kidney, and tongue were evaluated for B-vitamin content. Riboflavin was present in all four items, ranging from 3.6 mcg/g to 33.8 mcg/g of separable lean. Both niacin and pantothenic acid content were highest in the liver at 141 mcg/g and 57 mcg/g of separable lean respectively. Other items contained between 39 and 63 mcg/g of niacin, and between 5 and 22 mcg/g of pantothenic acid on a separable lean basis. The pyridoxine hydrochloride form of vitamin B_6 was present at levels ranging from 2 to 10 mcg/g of separable lean in the four items analyzed. Vitamin B_{12} was also present in each of the items, with content between .05 and .85 mcg/g. Thiamin was present in the lowest quantities with less than 1 mcg per gram of separable lean.

Although values for several of the B-vitamins appeared to be minimal, daily requirements of these vitamins are less than many other nutrients. Consequently, the B-vitamin content of these variety meat items contributed a significant amount to the Daily Value, exceeding the recommended amount of each vitamin in the case of several the items. Skeletal muscle and other animal products are considered to be a valuable source of B-vitamins, especially vitamin B_{12} (Pereira & Vicente, 2013), and these results provide evidence that variety meat items are included in this claim. Tolerable upper intake levels are not determined for riboflavin, pantothenic acid, or vitamin B_{12}. However, the upper intake value for niacin is 35 mg/day and for vitamin B_6 is 100 mg/day (IOM., 1998), which are significantly greater than the amounts contained in a 100 gram portion any of the items in the study.

Vitamin K

Both phylloquinone (vitamin K_1) and ten forms of menaquinones (vitamin K_2) were analyzed for all samples in this study. Vitamin K_1 was found in bone marrow at the highest level of any item in this study at 166 ng/g of separable lean, representing 14% of the daily value; the content in other items ranged from 0.5 ng/g to 9.7 ng/g. The fat composite samples contained between 27.3 ng/g to 69.1 ng/g of phylloquinone. Menaquinones were present in all samples analyzed aside from bone broth, with menaquinone-4, and menaquinones -9, -10, -11, -12, and -13 present in the largest amount in separable lean. Menaquinones -4, -9, -10, -11, -12, and -13 were present in the fat samples, while the other forms were not detectable.

Current dietary reference values (DRV) for vitamin K are solely based on the phylloquinone form, which is derived mainly from plant sources and plays a role in blood coagulation. However, menaquinones, which are found in higher amounts in animal products and fermented foods, have been studied recently in regard to their effect on human health. A study conducted by Beulens and colleagues found an association between vitamin K_2 intake and decreased coronary calcification, suggesting that it may be able to play a role in preventing cardiovascular disease (Beulens et al., 2009). Studies have also shown vitamin K_2 to be associated with improved bone quality and decreased incidence of bone fractures (Knapen et al., 2013; Knapen, Schurgers, & Vermeer, 2007; Maresz, 2015; Schwalfenberg, 2017). The research on dietary needs, bioavailability, and health impacts of vitamin K_2 are limited, but this area warrants further investigation.

Inductively Coupled Plasma Mineral Analysis

Results of inductively coupled plasma (ICP) mineral analyses for each item analyzed in the study are presented in Table 1.11. For applicable items, Table 1.6 indicates the potential USDA labeling claims for each nutrient. Calcium content was highest in the bone marrow

samples, contributing over 64% of the Daily Value per 100 grams of marrow. The liver samples contained the largest amount of copper by a large margin with 119 mcg per gram of separable lean, followed by heart and kidney with 3.7 mcg/g and 4.8 mcg/g respectively. The amount of copper in 100 grams of liver (11,900 mcg) relates to nearly 600% of the Daily Value for copper, exceeding the tolerable upper limit of 10,000 mcg (IOM, 2001). While gastrointestinal discomfort may occur at copper intake of as low as 5,000 mcg per day, the level at which liver damage may occur is more difficult to establish (Stapanik & Caudill, 2013). Research suggests that daily copper intake of 10,000 mcg for multiple weeks would not result in toxicity in individuals with normal ability to maintain copper homeostasis (Pratt, Omdahl, & Sorenson, 1985). Nonetheless, it may be important to consider copper levels in liver especially for those consistently consuming this product as part of their diet.

Iron was present at significant levels in heart, liver, kidney, tongue, oxtail, and blood. Blood contained the most, with 491.33 mcg/g relating to more than 200% of the Daily Value in a 100 gram portion. It would be important to consider the form in which blood is consumed, as it is typically used as an ingredient and therefore eaten in smaller quantities. However, the high iron content of blood may be able to be applied for nutrition purposes, especially as blood has been tested as a fortification medium for other nutrients (Margarita et al., 2015). Levels of manganese were relatively low in all samples, with kidney containing the most and providing 13% of the Daily Value. Phosphorus was present in all samples and provided from 15% to 36% of the Daily Value for 100 gram portions of liver, kidney, tongue, oxtail, bone marrow, and testicles, with the other items containing lesser amounts. Zinc levels were lowest in bone broth, blood, and testicles, but were sufficient to contribute 10% to 35% of the Daily Value per 100 grams for each of the other items.

Amino Acids

Amino acid profile and total tryptophan analyses were conducted for bone broth prepared by the National Cattlemen's Culinary Team and are presented in Table 1.9. Bone broth has recently been publicized as beneficial to human health, despite a lack of scientific evidence to support this claim. One of the most common arguments for the consumption of bone broth is the presence of glutamine, which is associated with immune system function and therefore has been linked to overall health (Field, Johnson, & Pratt, 2000; Newsholme et al., 1999). Glycine was the amino acid in the highest concentration in the broth analyzed, at a level of 3.18 mg per gram of broth. However, glutamic acid followed, present at 2.25 mg per gram. The broth contained other amino acids as well, including alanine, arginine, aspartic acid, and proline at levels over 1 mg per gram.

Fatty Acid Profile

The fatty acid profiles of fat samples for heart, kidney, tongue, and oxtail are presented in Table 1.12. The fatty acid profiles of separable lean tissue for the ten items evaluated in this study are presented in Table 1.13. The fatty acid found to be most prevalent (as a percentage of total fatty acids in each sample) for both lean tissue and fat samples was oleic acid (C18:1c9), a monounsaturated fatty acid that is most commonly associated with olive oil. Monounsaturated fatty acids have been associated with decreased LDL and total cholesterol and increased HDL cholesterol when they replace other macronutrients in the diet, such as saturated fatty acids and carbohydrates (Vannice & Rasmussen, 2014). Following oleic acid, stearic acid (C18:0) and palmitic acid (C16:0) made up the next highest percentage of total fatty acids for both lean tissue and fat samples. Stearic and palmitic acid are both saturated fatty acids. Research has shown that stearic acid, unlike some saturated fatty acids, does not have a negative impact on serum

cholesterol levels (Grundy, 1994; Vannice & Rasmussen, 2014; Yu, Derr, Etherton, & Kris-Etherton, 1995). Despite differing in chain length by only two carbons, palmitic acid has not been shown to have the same neutral effect on cholesterol levels that stearic acid does but rather a detrimental impact. Linoleic acid (C18:2) was the next most prevalent fatty acid, making up a larger percentage of the fatty acid profile in the separable lean tissue of heart, liver, kidney, and blood compared to other items. Linoleic acid is a polyunsaturated fatty acid that must be obtained from dietary sources, as the body cannot synthesize this fatty acid.

Comparison to USDA Standard Reference Database

Date from the present study were compared to existing nutrient values in the USDA National Nutrient Database for Standard Reference (SR) for heart, liver, kidney, tongue, and tripe; the other items analyzed in this study are not currently included in the database. These comparisons were made using the mean values calculated for each item in the study. The comparisons between USDA SR values and current study values are presented in Table 1.14 and Table 1.15. Much of the data in the USDA database for these items was derived from a 2003 study in which a limited number of samples of beef heart, kidney, and tripe from a single origin were analyzed for nutrient content (Showell et al., 2012). Other data originated from studies with alternative objectives or was imputed.

Conclusions

This study provided current, analytically derived nutrient information for U.S. beef variety meat items including heart, liver, kidney, tongue, honeycomb tripe, oxtail, bone marrow, Rocky Mountain oysters, blood, and bone broth. Data will be used to update and expand the USDA Food and Composition database for use by researchers, consumers, nutrition professionals, and those involved in policy creation. According to percentages calculated from

the separable lean component only, each of the items included in the study, except bone broth, qualify for a “Good Source” or “Excellent Source” extra labeling claim for at least one nutrient. However, results may be altered upon analysis of data for whole items (including fat, refuse) by the USDA Nutrient Data Laboratory. Nonetheless, data suggested that variety meat items could be beneficial in providing essential vitamins and minerals as a component of a healthy diet. Consumption of these items is more prevalent in developing countries, and having comprehensive nutrient data will aid in understanding their contribution to the diet of certain populations. Findings from this study suggest that edible offal products provide potential for increasing the nutrient density of diets that are deficient in protein and certain micronutrients. Additionally, results may present opportunity for food technologists to utilize variety meat items in developing new food or supplement products that would be beneficial to the nutrient needs of certain individuals.

Table 1.1. Description of ten U.S. beef variety meat items and International Meat Purchase Specifications (IMPS) numbers.

Item Name	Description	IMPS Number[1]
Beef heart, cap off	Cap removed (including auricles, arteries, gristly material); bone removed	720
Beef liver, sliced	Fabricated from skinned and deveined liver; sliced to 0.25 – 0.5 inches thick	702
Beef kidney	Blood vessels, pizzle cord, and ureter trimmed flush with kidney surface; capsule membrane surrounding kidney removed	722
Beef tongue, short cut	Tongue removed directly behind base of hyoid bone; hyoid bones, glandular tissue and trachea removed; epiglottis and major blood vessels trimmed flush with surface	716
Beef honeycomb tripe, scalded	Reticulum; dark internal intestinal lining removed; bleached (scalded)	726
Beef oxtail, segmented	Skinned tail; removed at juncture of second and third coccygeal vertebrae; external fat trimmed to no more than .25 inches; cut into segments	721
Beef bone marrow	Femur bones, knobs removed from ends; cut to approximately 1 inch slices	
Beef testicles	Testicles; cremasteric muscle and spermatic cord trimmed flush with surface; membrane surrounding testicle removed	
Beef blood	Edible blood; bottled; frozen	
Beef bone broth	Prepared by National Cattlemen's Beef Association Culinary Team	

[1] IMPS not defined for bone marrow, testicles, blood, or bone broth.

Table 1.2. Description of compositing scheme and analyses performed for each U.S. beef variety meat item.

Item	Composite Level	Number of Suppliers	Analyses
Heart	3 composites (1 from each supplier; composited over 2 collection dates)	3	Proximate analysis (crude fat, crude protein, percent moisture, and ash), fatty acid profile, cholesterol, vitamin A, B vitamins (B_1, B_2, B_3, B_5, B_6, B_{12}), vitamin D/25-hydroxy vitamin D, vitamin E, vitamin K, ICP minerals
Liver	3 composites (1 from each supplier; composited over 2 collection dates)	3	Proximate analysis (crude fat, crude protein, percent moisture, and ash), fatty acid profile, cholesterol, vitamin A, B vitamins (B_1, B_2, B_3, B_5, B_6, B_{12}), vitamin D/25-hydroxy vitamin D, vitamin E, vitamin K, ICP minerals
Kidney	3 composites (1 from each supplier; composited over 2 collection dates)	3	Proximate analysis (crude fat, crude protein, percent moisture, and ash), fatty acid profile, cholesterol, vitamin A, B vitamins (B_1, B_2, B_3, B_5, B_6, B_{12}), vitamin D/25-hydroxy vitamin D, vitamin E, vitamin K, ICP minerals
Tongue	3 composites (1 from each supplier; composited over 2 collection dates)	3	Proximate analysis (crude fat, crude protein, percent moisture, and ash), fatty acid profile, cholesterol, vitamin A, B vitamins (B_1, B_2, B_3, B_5, B_6, B_{12}), vitamin D/25-hydroxy vitamin D, vitamin E, vitamin K, ICP minerals
Honeycomb tripe	3 composites (1 from each supplier; composited over 2 collection dates)	3	Proximate analysis (crude fat, crude protein, percent moisture, and ash), fatty acid profile, cholesterol, vitamin A, vitamin D/25-hydroxy-vitamin D, vitamin E, vitamin K, ICP minerals
Oxtail	3 composites (1 from each supplier; composited over 2 collection dates)	3	Proximate analysis (crude fat, crude protein, percent moisture, and ash), fatty acid profile, cholesterol, vitamin A, vitamin D/25-hydroxy-vitamin D, vitamin E, vitamin K, ICP minerals
Bone marrow	3 composites (1 from each supplier; composited over 2 collection dates)	3	Proximate analysis (crude fat, crude protein, percent moisture, and ash), fatty acid profile, cholesterol, vitamin A, vitamin D/25-hydroxy-vitamin D, vitamin E, vitamin K, ICP minerals
Testicles	3 composites (3 packages, 1 supplier)	1	Proximate analysis (crude fat, crude protein, percent moisture, and ash), fatty acid profile, cholesterol, vitamin A, vitamin D/25-hydroxy-vitamin D, vitamin E, vitamin K, ICP minerals
Blood	3 composites (3 bottles, 1 supplier)	1	Proximate analysis (crude fat, crude protein, percent moisture, and ash), fatty acid profile, cholesterol, vitamin A, vitamin D/25-hydroxy-vitamin D, vitamin E, vitamin K, ICP minerals
Bone broth	3 composites (3 batches, 1 supplier)	1[1]	Proximate analysis (crude fat, crude protein, percent moisture, and ash), fatty acid profile, ICP minerals, amino acid profile, total tryptophan

[1] Prepared by National Cattlemen's Beef Association Culinary Team

Table 1.3. Mean and standard error of the mean of separable components derived from six raw U.S. beef variety meat items expressed as grams and as a percentage of pre-dissected weight.

	Separable tissue[1]		External fat[2]		Internal fat[3]		Refuse[4]	
	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
Item								
Heart	650.58 ± 135.20	88.23 ± 3.27	60.96 ± 24.81	7.95 ± 2.27		0.00	19.32 ± 10.20	3.12 ± 1.69
Liver	480.99 ± 39.23	95.97 ± 1.14		0.00		0.00	11.39 ± 3.77	2.37 ± 0.91
Kidney	678.34 ± 151.09	85.22 ± 2.57		0.00	92.12 ± 35.36	10.99 ± 2.74	13.23 ± 8.32	2.19 ± 1.48
Tongue	1014.77 ± 84.01	73.32 ± 2.28		0.00	139.40 ± 33.98	9.82 ± 1.76	220.62 ± 37.72	15.82 ± 1.96
Oxtail	346.21 ± 34.81	34.82 ± 2.50	136.24 ± 20.40	13.82 ± 2.16	53.44 ± 19.39	5.30 ± 1.84	444.64 ± 47.76	44.76 ± 3.72
Bone Marrow	198.68 ± 20.89	16.30 ± 0.84		0.00		0.00	1015.34 ± 102.1	83.88 ± 7.69

[1]Separable lean tissue weight (g) includes all lean muscle and organ tissue. Separable lean, %: [separable lean tissue (g)/ pre-dissection cut weight (g)] x 100.
[2]External fat weight (g) includes all fat located on the outer surface of the item. External fat, %: [external fat (g)/ pre-dissection cut weight (g)] x 100. External fat was not present for liver, kidney, tongue, and bone marrow.
[3]Internal fat weight (g) includes any fat which lies between lean tissue. Internal fat, %: [internal fat (g)/ pre-dissection cut weight (g)] x 100. Internal fat was not present for heart, liver, and bone marrow.
[4]Refuse weight (g) includes all bone and heavy connective tissue (including tongue skin). Refuse, %: [refuse (g)/ pre-dissection (g)] x 100.
[5]Tripe, testicles, blood, and bone broth were not dissected due to the homogeneous nature of the products; the entire item is considered lean tissue.

Table 1.4. Mean and standard error of the mean of proximate values (% protein, % total fat,% ash, and % moisture) and cholesterol content of 100 grams of separable lean tissue[1] from ten raw U.S. beef variety meat items.

Cut	Protein (%)	Total Fat (%)	Ash (%)	Moisture (%)	Cholesterol mg/g
Heart	17.48 ± 0.19	3.21 ± 0.18	1.19 ± 0.07	78.70 ± 0.36	1.16 ± 0.01
Liver	19.57 ± 0.16	4.50 ± 0.12	1.46 ± 0.04	71.47 ± 0.08	2.57 ± 0.04
Kidney	17.41 ± 0.53	3.31 ± 0.26	1.14 ± 0.08	78.79 ± 0.31	4.00 ± 0.10
Tongue	17.42 ± 0.5	11.20 ± 0.15	1.00 ± 0.08	69.61 ± 0.08	0.87 ± <0.01
Tripe	12.15 ± 0.4	7.23 ± 0.48	0.52 ± 0.02	78.78 ± 0.44	1.24 ± 0.06
Oxtail	19.91 ± 0.22	6.45 ± 0.07	0.82 ± 0.03	71.68 ± 0.34	0.67 ± 0.02
Bone Marrow	1.25 ± 0.08	77.09 ± 0.23	0.62 ± 0.06	11.51 ± 0.31	1.28 ± 0.01
Testicles	10.82 ± 0.04	2.91 ± 0.11	1.20 ± 0.03	86.18 ± 0.05	2.19 ± 0.02
Blood	18.85 ± 0.31	1.15 ± 0.02	3.10 ± 0.08	77.38 ± 0.06	1.57 ± 0.01
Bone Broth	1.92 ± 0.04	0.68 ± 0.09	0.43 ± 0.06	96.23 ± 0.19	– [2]

[1]Separable lean tissue includes all lean muscle and organ tissue.
[2]Cholesterol content of bone broth was not analyzed.

Table 1.5. Proximate values and nutrient content of fat from four[1] raw U.S. beef variety meat items as a single national composite per item[2].

	Heart Fat	Kidney Fat	Tongue Fat	Oxtail Fat[3]
Proximates, units				
Protein, %	8.56	8.03	8.79	7.50
Fat, %	46.33	43.22	49.28	49.28
Ash, %	0.38	0.39	0.40	0.28
Moisture, %	38.83	37.22	43.70	38.55
Nutrients				
Cholesterol, mg/g	1.00	1.32	1.27	0.92
Retinol (Vitamin A), mcg/g	0.37	0.38	0.10	0.27
Vitamin D_2, mcg/g	<0.016	<0.016	<0.016	<0.016
Vitamin D_2, mcg/g	<0.016	<0.016	<0.016	<0.016
25-Hydroxy Vitamin D, mcg/g	<0.016	0.01	<0.016	<0.016
AlphaTocopherol[4] (Vitamin E),	9.25	10.56	6.86	6.95
Phylloquinone (Vitamin K_1), mcg/g	37.60	69.10	28.80	27.30
Vitamin K_2 [5]				
Menaquinone-4, ng/g	300.00	273.00	344.50	183.50
Menaquinone-9, ng/g	11900.00	14200.00	13500.00	14370.00
Menaquinone-10, ng/g	3826.00	2043.00	3568.00	4255.00
Menaquinone-11, ng/g	48540.00	38720.00	29269.00	27200.00
Menaquinone-12, ng/g	88.10	132.50	64.20	54.00
Menaquinone-13, ng/g	0.00	0.00	0.00	0.00
Calcium, mcg/g	<38.506	41.30	43.10	58.50
Copper, mcg/g	1.36	1.06	0.66	<0.396
Iron, mcg/g	20.60	14.40	14.60	7.91
Magnesium, mcg/g	99.40	59.00	90.90	72.30
Manganese, mcg/g	<0.196	0.23	0.21	<0.196
Phosphorus, mcg/g	830.00	605.00	858.00	624.00
Potassium, mcg/g	1290.00	895.00	1440.00	1160.00
Sodium, mcg/g	486.00	747.00	510.00	565.00
Zinc, mcg/g	6.90	6.96	10.40	13.70

[1]Fat was present only on heart, kidney, tongue, and oxtail.
[2]A single fat composite for each item (heart, kidney, tongue, oxtail) included equal weight of fat from each piece from all 3 suppliers.
[3]Oxtail fat is an equal composite of external and internal fat; both were present only for this item.
[4]Beta-, Deta-, and Gamma-Tocopherol were not detectable for any fat samples.
[5]Menaquinone-5, -6, -7, -8 were not detectable for any fat samples.
[6]Values preceded by < reflect the limit of detection; the amount of the nutrient was below the detection limit.

Table 1.6. Percentage of the RDI[1] contributed by 100 grams of separable lean tissue[2] only from nine[3] raw U.S. beef variety meat items qualifying for USDA "Excellent Source of" and "Good Source of" extra labeling claims[4].

Nutrients[5]	Heart	Liver	Kidney	Tongue	Oxtail[7]	Tripe	Bone marrow	Testicles	Blood
Protein (%)	35[a]	39[a]	35[a]	35[a]	40[a]	24[a]		22[a]	38[a]
Vitamins									
Vitamin A (Retinol), %		247[a]							
Riboflavin (B_2), %	67[a]	199[a]	171[a]	21[a]	7	7	7	7	7
Pantothenic Acid, %	13[b]	57[a]	22[a]		7	7	7	7	7
Vitamin B_6 [6], %	18[b]	48[a]	36[a]	13[b]	7	7	7	7	7
Niacin (B_3), %	23[a]	71[a]		20[a]	7	7	7	7	7
Vitamin B_{12}, %	208[a]	1417[a]	713[a]	90[a]	7	7	7	7	7
Vitamin K_1 (phylloquinone), %							14[b]		
Minerals									
Calcium, %							65[a]		
Copper, %	19[b]	597[a]	24[a]						
Iron, %	26[a]	29[a]	29[a]	13[b]	11[b]				273[a]
Manganese, %		13[b]							
Phosphorus, %		36[a]	23[a]	15[b]	15[b]		30[a]	21[a]	
Zinc, %	12[b]	26[a]	14[b]	23[a]	36[a]	11[b]			

[1] Reference daily intakes (RDI) dietary allowance (RDA) is the daily intake level of a nutrient that is considered to be sufficient to meet the requirements of 97-98% of healthy individuals in the United States.

[2] Separable lean tissue includes all lean muscle and organ tissue.

[3] The tenth item in the study, bone broth, did not provide 10% or more of any nutrient it was analyzed for.

[4] Providing over 20% of the RDI qualifies the item to be labeled as an "excellent source" of the nutrient. Providing between 10-19% of the RDI qualifies the item to be labeled as a "good source" of the nutrient.

[5] Percentages are based on the RDI units for each nutrient: Vitamin A (Retinol), IU; Riboflavin (B_2), mg; Pantothenic Acid, mg; Vitamin B_6, mg; Niacin (B_3), mg; Vitamin B_{12}, µg; Vitamin K (phylloquinone), µg; Calcium, mg; Copper, mg; Iron, mg; Manganese, mg; Phosphorus, mg; Zinc, mg.

[6] Percentages based on pyridoxine hydrochloride form.

[7] Oxtail, tripe, bone marrow, testicles, and blood were not analyzed for niacin, riboflavin, pantothenic acid, vitamin B_6, or vitamin B_{12}.

[a] Item qualifies for extra labeling as an "Excellent source" of the nutrient.

[b] Item qualifies for extra labeling as a "Good source" of the nutrient.

Table 1.7. Mean and standard error of the mean of mean vitamin values from separable lean tissue[1] from six[2] raw U.S. beef variety meat items.

Nutrient, units	Heart	Liver	Kidney	Tongue	Tripe	Oxtail
Retinol (Vitamin A), mcg/g	0.02 ± 0.00	37.02 ± 9.39	0.25 ± 0.11	0.05 ± 0.02	0.03 ± 0.01	0.03 ± 0.02
Vitamin D_2 [3], mcg/g	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Vitamin D_3[3], mcg/g	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
25-Hydroxy Vitamin D, mcg/g	<0.01	<0.01	0.01 ± <0.01	<0.01	<0.01	<0.01
Vitamin E						
AlphaTocopherol, mcg/g	8.18 ± 0.83	4.72 ± 0.36	4.36 ± 0.75	4.20 ± 0.15	1.80 ± 0.22	2.96 ± 0.50
BetaTocopherol, mcg/g	[5]	[5]	[5]	[5]	[5]	[5]
DeltaTocopherol, mcg/g	[5]	[5]	[5]	[5]	[5]	[5]
GammaTocopherol, mcg/g	[5]	[5]	[5]	<0.90[4] ± 0.54	[5]	[5]
Phylloquinone (Vitamin K_1), ng/g	3.17 ± 0.23	9.67 ± 2.46	1.27 ± 0.09	8.83 ± 2.46	6.43 ± 0.78	3.43 ± 0.58
Vitamin K_2						
Menaquinone-4, ng/g	16.47 ± 2.28	0.00	45.37 ± 13.68	88.27 ± 13.65	127.83 ± 13.17	47.80 ± 2.29
Menaquinone-5, ng/g	0.00	0.00	0.00	0.00	0.00	0.00
Menaquinone-6, ng/g	4.70 ± 1.06	31.00 ± 3.57	0.00	0.00	0.00	0.00
Menaquinone-7, ng/g	0.00	77.87 ± 13.11	0.00	0.00	0.00	0.00
Menaquinone-8, ng/g	0.00	37.33 ± 5.21	0.00	0.00	0.00	0.00
Menaquinone-9, ng/g	34.57 ± 4.08	71.13 ± 21.90	11.77 ± 0.33	3518.33 ± 281.90	1609.50 ± 495.08	1612.00 ± 99.14
Menaquinone-10, ng/g	79.07 ± 29.57	67.37 ± 15.28	0.00	1306.17 ± 319.70	497.60 ± 194.81	431.63 ± 72.14
Menaquinone-11, ng/g	1897.33 ± 424.33	1340.67 ± 181.80	1104.83 ± 70.84	10224.33 ± 1370.37	14229.00 ± 2916.51	7044.33 ± 521.38
Menaquinone-12, ng/g	5.67 ± 0.70	537.83 ± 55.23	15.03 ± 1.40	16.10 ± 4.75	38.40 ± 7.47	19.77 ± 5.36
Menaquinone-13, ng/g	14.27 ± 1.41	1707.33 ± 126.14	42.33 ± 6.47	0.00	7.90 ± 3.95	0.00

[1]Separable lean tissue includes all lean muscle and organ tissue.
[2]Vitamin content of other items is presented in a separate table.
[3]Vitamin D content was below the limit of detection for all items (limit of detection: 0.001 µg/g).
[4]At least one of the composites included in the mean for these values was below the limit of detection for the corresponding nutrient.
[5]Nutrient was reported as below the limit of detection for this item; the limit of detection was not defined.

Table 1.8. Mean and standard error of the mean of mean vitamin values from separable lean tissue[1] from three[2] raw U.S. beef variety meat items.

Nutrient, units	Bone Marrow	Testicles	Blood
Retinol (Vitamin A), mcg/g	0.48 ± 0.10	0.05 ± 0.02	0.07 ± 0.02
Vitamin D_2 [3], mcg/g	<0.01	<0.01	<0.01
Vitamin D_2[3], mcg/g	<0.01	<0.01	<0.01
25-Hydroxy Vitamin D, mcg/g	<0.01	<0.01	0.02 ± <0.01
Vitamin E			
AlphaTocopherol, mcg/g	8.50 ± 3.61	20.23 ± 3.23	2.21 ± 0.12
BetaTocopherol, mcg/g	[5]	[5]	[5]
DeltaTocopherol, mcg/g	[5]	[5]	[5]
GammaTocopherol, mcg/g	[5]	[5]	0.11 ± 0.02
Phylloquinone (Vitamin K_1), ng/g	166.00 ± 36.87	7.93 ± 0.43	0.53 ± 0.03
Vitamin K_2			
Menaquinone-4, ng/g	338.00 ± 43.42	335.83 ± 26.28	0.00
Menaquinone-5, ng/g	0.00	0.00	0.00
Menaquinone-6, ng/g	0.00	2.27 ± 2.27	0.73 ± 0.15
Menaquinone-7, ng/g	0.00	10.57 ± 5.43	1.50 ± 0.00
Menaquinone-8, ng/g	0.00	0.00	0.00
Menaquinone-9, ng/g	23000.00 ± 1249.00	0.00	0.00
Menaquinone-10, ng/g	6124.67 ± 1453.62	0.00	9.53 ± 0.73
Menaquinone-11, ng/g	52866.67 ± 2273.27	790.67 ± 60.32	27.40 ± 2.59
Menaquinone-12, ng/g	186.00 ± 76.53	9.37 ± 1.53	17.30 ± 0.35
Menaquinone-13, ng/g	0.00	94.53 ± 4.45	26.83 ± 0.93

[1]Separable lean tissue includes all lean muscle and organ tissue.
[2]Vitamin content of other items is presented in a separate table.
[3]Vitamin D content was below the limit of detection for all items (limit of detection: 0.001 µg/g).
[4]At least one of the composites included in the mean for these values was below the limit of detection for the corresponding nutrient.
[5]Nutrient was reported as below the limit of detection for this item; the limit of detection was not defined.

Table 1.9. Mean and standard error of the mean of B-vitamin values from separable lean tissue[1] from four[2] raw U.S. beef variety meat items.

	Heart	Liver	Kidney	Tongue
B-vitamin, units	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Thiamin, mcg/g	0.36 ± 0.01	0.18 ± 0.01	0.36 ± 0.01	0.09 ± <0.01
Thiamin Hydrochloride, mcg/g	0.46 ± 0.01	0.23 ± 0.01	0.45 ± 0.02	0.12 ± 0.00
Riboflavin (Vitamin B_2), mcg/g	11.4 ± 0.04	33.8 ± 0.28	29.1 ± 0.40	3.60 ± 0.36
Niacin (Vitamin B_2), mcg/g	45.13 ± 1.45	141.33 ± 4.37	62.67 ± 1.10	39.47 ± 3.99
Pantothenic Acid (Vitamin B_5), mcg/g	12.63 ± 4.57	56.93 ± 4.66	22.13 ± 8.82	5.03 ± 2.24
D Calcium Pantothenate (Vitamin B_5), mcg/g	13.77 ± 4.95	61.87 ± 5.05	24.03 ± 9.58	5.47 ± 2.46
Pyridoxine Free Base (Vitamin B_6), mcg/g	2.92 ± 0.16	7.87 ± 0.12	5.88 ± 0.99	2.11 ± 0.08
Pyridoxine Hydrochloride (Vitamin B_6), mcg/g	3.55 ± 0.20	9.56 ± 0.14	7.15 ± 1.21	2.57 ± 0.10
Vitamin B_{12}, mcg/g	0.12 ± 0.03	0.85 ± 0.12	0.43 ± 0.03	0.05 ± <0.01

[1]Separable lean tissue includes all lean muscle and organ tissue.
[2]B-vitamin content was analyzed only for heart, liver, kidney, tongue.

Table 1.10. Mean and standard error of the mean of vitamin and amino acid content for beef bone broth[1].

Vitamin, units	
Retinol (Vitamin A), mcg/g	[2]
Phylloquinone (Vitamin K_1), ng/g	2.60 ± 0.89
Vitamin K_2	
Menaquinone-4, ng/g	0.00
Menaquinone-5, ng/g	0.00
Menaquinone-6, ng/g	0.00
Menaquinone-7, ng/g	0.00
Menaquinone-8, ng/g	0.00
Menaquinone-9, ng/g	0.00
Menaquinone-10, ng/g	0.00
Menaquinone-11, ng/g	141.13 ± 37.22
Menaquinone-12, ng/g	0.00
Menaquinone-13, ng/g	0.00
Vitamin E	
AlphaTocopherol, mcg /g	0.94 ± 0.37
BetaTocopherol, mcg /g	[2]
DeltaTocopherol, mcg /g	[2]
GammaTocopherol, mcg /g	<0.06 ± 0.01
Amino Acid, units	
Alanine, mg/g	1.39 ± 0.07
Arginine, mg/g	1.27 ± 0.05
Aspartic Acid, mg/g	1.07 ± 0.03
Cystine, mg/g	<0.11 ± <0.01
Glutamic Acid, mg/g	2.25 ± 0.02
Glycine, mg/g	3.18 ± 0.19
Histidine, mg/g	0.22 ± 0.01
Isoleucine, mg/g	0.26 ± 0.01
Leucine, mg/g	0.54 ± 0.03
Lysine, mg/g	0.55 ± 0.02
Methionine, mg/g	0.16 ± 0.00
Phenylalanine, mg/g	0.35 ± 0.02
Proline, mg/g	1.77 ± 0.10
Serine, mg/g	0.52 ± 0.03
Threonine, mg/g	0.34 ± 0.01
Tryptophan, mg/g	<0.01 ± <0.01
Tyrosine, mg/g	0.15 ± 0.01
Valine, mg/g	0.41 ± 0.01

[1]Prepared by the National Cattlemen's Beef Association Culinary Team

[2]Nutrient was reported as below the limit of detection for this item; the limit of detection was not defined.

Table 1.11. Mean and standard error of the mean values of mineral content from separable lean tissue[1] from ten raw U.S. beef variety meat items.

Cut	Calcium, µg/g	Copper, µg/g	Iron, µg/g	Magnesium, µg/g	Manganese, µg/g	Phosphorous, µg/g	Potassium, µg/g	Sodium, µg/g	Zinc, µg/g
Heart	43.23 ± 1.39	3.70 ± 0.05	46.40 ± 1.01	223.33 ± 2.03	0.34 ± 0.01	2000.00 ± 28.87	2610.00 ± 43.59	951.33 ± 36.61	18.03 ± 0.37
Liver	40.23 ± 0.55	119.47 ± 25.78	51.53 ± 1.45	185.67 ± 1.86	2.60 ± 0.10	3550.00 ± 30.55	2910.00 ± 26.46	616.00 ± 10.58	38.90 ± 2.86
Kidney	84.97 ± 2.64	4.80 ± 0.22	52.13 ± 2.66	165.33 ± 0.88	1.09 ± 0.03	2336.67 ± 6.67	2340.00 ± 69.28	1843.33 ± 52.07	20.73 ± 0.70
Tongue	46.67 ± 1.05	1.09 ± 0.07	22.97 ± 0.98	180.67 ± 5.21	<0.20[3]	1500.00 ± 40.41	2523.33 ± 72.65	784.33 ± 19.89	34.30 ± 0.67
Tripe	181.00 ± 29.31	<0.50 ± 0.07	5.21 ± 0.11	106.23 ± 8.76	<0.54[2] ± 0.23	623.67 ± 16.50	876.67 ± 43.38	929.33 ± 135.93	15.83 ± 0.49
Oxtail	86.27 ± 5.80	0.95 ± 0.19	19.07 ± 1.27	193.33 ± 5.04	<0.20[3]	1500.00 ± 40.41	2456.67 ± 59.25	1106.67 ± 33.83	53.73 ± 2.79
Bone Marrow	6450.00 ± 4175.82	<0.42 ± 0.01	6.75 ± 0.41	<126.97[2] ± 72.07	<0.20[3]	2982.00 ± 1862.48	<197.33[2] ± 1.76	467.33 ± 123.11	2.92 ± 1.23
Testicles	74.53 ± 7.17	0.82 ± 0.11	16.07 ± 0.90	132.67 ± 5.61	0.32 ± 0.02	2096.67 ± 85.11	3013.33 ± 121.29	1166.67 ± 44.85	14.00 ± 0.55
Blood	64.33 ± 0.94	1.06 ± 0.14	491.33 ± 6.01	<39.70[2] ± 0.17	<0.20[3]	191.67 ± 2.33	549.00 ± 4.00	11233.33 ± 218.58	2.97 ± 0.06
Broth	74.00 ± 25.09	<0.59[2] ± 0.11	<3.96[2] ± 0.04	42.53 ± 0.70	<0.20[3]	145.00 ± 9.02	1051.67 ± 48.68	145.00 ± 9.02	<0.79[2] ± 0.01

[1]Separable lean tissue includes all lean muscle and organ tissue.
[2]At least one of the composites included in the mean for these values was below the limit of detection for the corresponding nutrient.
[3]Manganese content was below the limit of detection (limit of detection: .20 µg/g).

Table 1.12. Fatty acid profile of four[1] raw U.S. beef variety meat item fat samples analyzed as a single composite per item[2], listed as fatty acid percentages.

Fatty Acid	Heart Fat	Kidney Fat	Tongue Fat	Oxtail Fat[3]
C10:0	0.02	0.00	0.00	0.00
C12:0	0.05	0.00	0.00	0.00
C12:1	0.01	0.00	0.00	0.00
C14:0	0.59	0.44	0.40	0.39
C14:1	0.49	0.24	0.33	0.26
C16:0	24.63	21.94	22.05	23.14
C16:1	3.24	4.72	4.07	3.92
C17:0	1.32	1.30	1.28	1.23
C17:1	1.12	1.05	1.04	1.05
C18:0	20.71	23.91	23.04	24.09
C18:1t6-8	0.61	0.53	0.63	0.55
C18:1t9	0.49	0.42	0.45	0.45
C18:1t10	2.38	3.05	2.90	2.99
C18:1t11	1.05	1.07	1.22	1.18
C18:1c9	35.91	34.62	35.34	34.38
C18:1c11	1.11	1.02	1.01	1.07
C18:2	5.06	4.54	5.14	4.16
C18:3	0.24	0.21	0.20	0.26
C20:0	0.02	0.01	0.08	0.03
C18:2c9t11	0.15	0.17	0.13	0.17
C18:2t10c12	0.04	0.08	0.02	0.03
C20:1	0.12	0.09	0.10	0.12
C20:4	0.39	0.31	0.38	0.39
C20:5	0.00	0.00	0.00	0.00
C24:0	0.06	0.08	0.03	0.04
Unknown	0.18	0.20	0.13	0.10

[1]Fat was present only on heart, kidney, tongue, and oxtail.
[2]Fat composites for each item (heart, kidney, tongue, oxtail) include fat from all 3 origins.
[3]Oxtail fat is an equal composite of external and internal fat; both were present only for this item.

Table 1.13. Mean and standard error of the mean of fatty acid profiles of separable lean tissue [1] from ten raw U.S. beef variety meat items.

Fatty Acid	Heart	Liver	Kidney	Tongue	Tripe	Oxtail	Bone marrow	Testicles	Blood	Bone broth
C10:0	0.01 ± 0.01	<0.01 ± <0.01	0.00	0.08 ± 0.02	0.02 ± <0.01	0.06 ± 0.01	0.01 ± <0.01	0.06 ± 0.01	0.00	0.03 ± 0.02
C12:0	0.00	0.00	0.00	0.03 ± 0.01	0.00	0.07 ± 0.01	<0.01 ± <0.01	0.07 ± 0.01	0.00	0.01 ± <0.01
C12:1	<0.01 ± <0.01	0.00	0.00	0.03 ± 0.01	0.01 ± 0.01	0.06 ± 0.01	0.00	0.00	0.00	0.00
C14:0	0.29 ± 0.12	0.37 ± 0.02	0.38 ± 0.01	2.49 ± 0.07	2.74 ± 0.10	2.40 ± 0.04	3.36 ± 0.21	1.50 ± 0.03	1.33 ± 0.07	2.63 ± 0.12
C14:1	0.11 ± 0.05	0.13 ± 0.02	0.23 ± 0.01	1.09 ± 0.02	0.44 ± 0.01	0.46 ± 0.03	0.30 ± 0.02	0.45 ± 0.02	0.17 ± 0.02	0.14 ± 0.03
C16:0	15.84 ± 0.42	11.38 ± 1.25	16.67 ± 0.60	25.81 ± 0.27	26.33 ± 0.21	23.65 ± 0.51	26.12 ± 0.73	33.79 ± 0.16	15.57 ± 0.22	15.37 ± 2.36
C16:1	0.49 ± 0.05	0.26 ± 0.07	0.16 ± 0.02	0.11 ± 0.01	0.05 ± 0.01	3.10 ± 0.13	1.69 ± 0.04	1.78 ± 0.01	1.43 ± 0.07	1.29 ± 0.05
C17:0	0.91 ± 0.05	1.06 ± 0.01	0.57 ± 0.01	0.98 ± 0.02	2.30 ± 0.04	1.17 ± 0.03	0.63 ± 0.08	0.64 ± 0.06	0.31 ± 0.01	0.41 ± 0.02
C17:1	0.04 ± 0.03	<0.01 ± <0.01	0.15 ± 0.02	0.40 ± 0.03	0.11 ± 0.01	0.98 ± 0.03	0.72 ± 0.04	0.09 ± <0.01	0.22 ± 0.01	0.81 ± 0.04
C18:0	19.62 ± 0.67	36.61 ± 0.66	16.83 ± 0.37	13.25 ± 0.09	21.8 ± 0.02	15.09 ± 0.03	13.57 ± 0.78	11.33 ± 0.13	21.97 ± 1.60	20.2 ± 0.77
C18:1t6-8	0.16 ± 0.02	0.12 ± 0.01	0.13 ± 0.01	0.18 ± 0.02	0.19 ± 0.01	0.38 ± 0.03	0.12 ± 0.01	0.16 ± 0.01	0.18 ± 0.03	0.16 ± 0.02
C18:1t9	0.13 ± 0.02	0.11 ± 0.02	0.17 ± 0.02	0.28 ± 0.02	0.26 ± 0.02	0.42 ± 0.04	0.11 ± <0.01	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.02
C18:1t10	0.17 ± 0.04	0.17 ± 0.02	0.10 ± 0.01	0.24 ± 0.01	0.14 ± <0.01	2.73 ± 0.06	0.15 ± 0.01	0.17 ± 0.02	0.30 ± 0.03	0.18 ± 0.01
C18:1t11	0.92 ± 0.11	1.59 ± 0.04	0.67 ± 0.09	0.75 ± 0.10	0.20 ± 0.03	0.73 ± 0.06	0.58 ± 0.05	0.43 ± 0.02	0.89 ± 0.06	0.48 ± 0.03
C18:1c9	16.21 ± 0.78	14.74 ± 0.14	19.83 ± 2.07	38.70 ± 0.50	37.86 ± 0.14	38.26 ± 0.58	33.24 ± 0.12	20.86 ± 0.28	29.19 ± 0.48	46.33 ± 1.74
C18:1c11	2.16 ± 0.06	1.92 ± 0.04	3.42 ± 0.19	2.89 ± 0.03	1.72 ± 0.01	2.03 ± 0.04	2.28 ± 0.28	2.63 ± 0.05	1.43 ± 0.03	2.09 ± 0.04
C18:2	26.83 ± 2.22	17.26 ± 0.39	23.10 ± 0.84	7.90 ± 0.06	4.61 ± 0.02	6.03 ± 0.19	12.76 ± 0.08	7.20 ± 0.10	19.85 ± 0.98	4.58 ± 0.29
C18:2c9t11	0.44 ± 0.08	0.55 ± 0.01	0.40 ± 0.05	0.34 ± 0.06	0.23 ± <0.01	0.30 ± 0.03	0.29 ± 0.01	0.27 ± 0.13	0.86 ± 0.06	0.31 ± 0.01
C18:2t10c12	0.08 ± 0.03	0.04 ± 0.03	0.16 ± 0.01	0.30 ± 0.01	0.02 ± <0.01	0.03 ± 0.01	0.10 ± 0.01	0.00	0.04 ± 0.01	0.15 ± 0.02
C18:3	0.61 ± 0.03	1.24 ± 0.07	0.71 ± 0.07	0.39 ± 0.02	0.23 ± 0.02	0.12 ± 0.03	0.58 ± 0.02	0.45 ± 0.03	2.47 ± 0.07	0.62 ± 0.02
C20:0	0.20 ± 0.01	0.06 ± 0.04	0.39 ± 0.01	0.47 ± 0.11	0.21 ± 0.01	0.14 ± 0.02	0.15 ± 0.04	0.16 ± 0.02	0.10 ± 0.01	0.27 ± 0.02
C20:1	0.27 ± 0.03	0.17 ± 0.04	0.34 ± 0.02	0.24 ± <0.01	0.01 ± <0.01	<0.01 ± <0.01	0.17 ± 0.01	0.15 ± 0.01	0.00	0.16 ± 0.02
C20:2	0.12 ± 0.03	0.32 ± 0.03	0.38 ± 0.02	0.19 ± 0.10	0.12 ± 0.01	0.01 ± 0.01	0.60 ± 0.25	0.54 ± 0.04	0.31 ± 0.03	1.11 ± 0.02
C20:4	13.66 ± 0.37	10.91 ± 0.20	14.13 ± 0.50	2.37 ± 0.05	0.23 ± <0.01	1.40 ± 0.02	2.01 ± 0.08	14.41 ± 0.13	2.48 ± 0.14	2.19 ± 0.07
C20:5	0.37 ± 0.04	0.41 ± 0.04	0.43 ± 0.03	0.12 ± 0.02	0.00	0.05 ± <0.01	0.28 ± 0.02	1.06 ± 0.02	0.54 ± 0.03	0.28 ± 0.01
C22:6	0.13 ± 0.04	0.16 ± 0.04	0.32 ± 0.03	0.10 ± 0.00	0.00	0.01 ± 0.01	<0.01 ± <0.01	1.52 ± 0.08	0.00	0.00
C24:0	0.08 ± 0.07	0.22 ± 0.01	0.14 ± 0.01	0.02 ± 0.00	0.00	0.21 ± 0.03	0.00	0.00	0.00	0.00
Unknown	0.16 ± 0.02	0.19 ± <0.01	0.17 ± 0.03	0.25 ± 0.05	0.17 ± 0.02	0.10 ± 0.01	0.18 ± 0.04	0.21 ± 0.01	0.29 ± 0.01	0.17 ± <0.01

[1]Separable lean tissue includes all lean muscle and organ tissue.

Table 1.14. Comparison of current study proximate and mineral mean values from raw separable lean tissue[1] from five[2] raw U.S. beef variety meat items to USDA SR-28 proximate and mineral values from five beef variety meat items.

Nutrient	Heart[3]	Liver[4]	Kidney[5]	Tongue[6]	Tripe[7]
Protein, %					
USDA NDL Value	17.72	20.36	17.40	14.90	12.07
Data Value	17.48	19.57	17.41	17.42	12.15
Fat, %					
USDA NDL Value	3.94	3.63	3.09	16.09	3.69
Data Value	3.21	4.50	3.31	11.20	7.23
Ash, %					
USDA NDL Value	1.10	-[8]	-[8]	-[8]	-[8]
Data Value	1.19	1.46	1.14	1.00	0.52
Moisture, %					
USDA NDL Value	77.11	70.81	77.89	64.53	84.16
Data Value	78.70	71.47	78.79	69.61	78.78
Cholesterol, mg/100g					
USDA NDL Value	124.00	275.00	411.00	87.00	122.00
Data Value	116.00	256.67	400.33	87.07	124.33
Calcium, mg/100g					
USDA NDL Value	7.00	5.00	13.00	6.00	69.00
Data Value	4.32	4.02	8.50	4.67	18.10
Iron, mg/100g					
USDA NDL Value	4.31	4.90	4.60	2.95	0.59
Data Value	4.64	5.15	5.21	2.30	0.52
Magnesium, mg/100g					
USDA NDL Value	21.00	18.00	17.00	16.00	13.00
Data Value	22.33	18.57	16.53	18.07	10.62
Manganese, mg/100g					
USDA NDL Value	0.035	-[8]	-[8]	-[8]	-[8]
Data Value	0.03	0.26	0.11	<0.02	<0.05
Phosphorus, mg/100g					
USDA NDL Value	212.00	387.00	257.00	133.00	64.00
Data Value	200.00	355.00	233.67	150.00	62.37
Potassium, mg/100g					
USDA NDL Value	287.00	313.00	262.00	315.00	67.00
Data Value	261.00	291.00	234.00	252.33	87.67
Sodium, mg/100g					
USDA NDL Value	98.00	69.00	182.00	69.00	97.00
Data Value	95.13	61.60	184.33	78.43	92.93
Zinc, mg/100g					
USDA NDL Value	1.70	4.00	1.92	2.87	1.42
Data Value	1.80	3.89	2.07	3.43	1.58
Copper, mg/100g					
USDA NDL Value	0.396	-[8]	-[8]	-[8]	-[8]
Data Value	0.37	11.95	0.48	0.11	<0.05

[1]Separable lean tissue includes all lean muscle and organ tissue.
[2]Comparisons made for cuts with nutrient data included in USDA SR-28: heart, liver, kidney, tongue, and tripe.
[3]USDA-ARS, Standard Reference number 13321, Beef, variety meats and by-products, heart, raw
[4]USDA-ARS, Standard Reference number 13325, Beef, variety meats and by-products, liver, raw
[5]USDA-ARS, Standard Reference number 13323, Beef, variety meats and by-products, kidneys, raw
[6]USDA-ARS, Standard Reference number 13339, Beef, variety meats and by-products, tongue, raw
[7]USDA-ARS, Standard Reference number 13341, Beef, variety meats and by-products, tripe, raw
[8]Values are not available in the USDA Standard Reference database.

Table 1.15. Comparison of current study mean vitamin values from raw separable lean tissue[1] from five[2] raw U.S. beef variety meat items to USDA SR-28 vitamin values from five beef variety meat items.

Nutrient	Heart[3]	Liver[4]	Kidney[5]	Tongue[6]	Tripe[7]
Retinol, µg/100g					
USDA NDL Value	0.00	4968.00[8]	419.00[8]	0.00[8]	0.00[8]
Data Value	2.00	3702.00	24.67	4.67	2.67
Thiamin, mg/100g					
USDA NDL Value	0.238	0.189	0.357	0.125	0.00
Data Value	0.05[9]	0.02[9]	0.05[9]	0.01[9]	- [12]
Niacin, mg/100g					
USDA NDL Value	7.53	13.18	8.03	4.24	0.88
Data Value	4.51	14.13	6.27	3.95	- [12]
Riboflavin, mg/100g					
USDA NDL Value	0.91	2.76	2.84	0.34	0.06
Data Value	1.14	3.38	2.91	0.36	- [12]
Pantothenic Acid, mg/100g					
USDA NDL Value	1.79	- [11]	- [11]	- [11]	- [11]
Data Value	1.26	5.69	2.21	0.50	- [12]
Vitamin B_6, mg/100g					
USDA NDL Value	0.28	1.08	0.67	0.31	0.01
Data Value	0.36[10]	0.96[10]	0.72[10]	0.26[10]	- [12]
Vitamin B_{12}, µg/100g					
USDA NDL Value	8.55	59.30	27.50	3.79	1.39
Data Value	12.46	84.90	42.77	5.43	- [12]
Vitamin D (D_2 + D_3), µg/100g					
USDA NDL Value	- [11]	1.20	1.10	- [11]	0.00
Data Value	<0.18	<0.20	<0.41	<0.21	<0.17
Vitamin E, alpha-tocopherol), mg/100g					
USDA NDL Value	0.22	0.38	0.22	- [11]	0.09
Data Value	0.82	0.47	0.44	0.42	0.18
Vitamin K (phylloquinone), µg/100g					
USDA NDL Value	0.00	3.10	0.00	- [11]	0.00
Data Value	0.32	0.97	0.13	0.88	0.64

[1]Separable lean tissue includes all lean muscle and organ tissue.
[2]Comparisons made for cuts with nutrient data included in USDA SR-28: heart, liver, kidney, tongue, and tripe.
[3]USDA-ARS, Standard Reference number 13321, Beef, variety meats and by-products, heart, raw
[4]USDA-ARS, Standard Reference number 13325, Beef, variety meats and by-products, liver, raw
[5]USDA-ARS, Standard Reference number 13323, Beef, variety meats and by-products, kidneys, raw
[6]USDA-ARS, Standard Reference number 13339, Beef, variety meats and by-products, tongue, raw
[7]USDA-ARS, Standard Reference number 13341, Beef, variety meats and by-products, tripe, raw
[8]Listed as RAE (retinol activity equivalents).
[9]Values presented are thiamin hydrochloride form.
[10]Values presented are pyridoxine hydrochloride form.
[11]Values are not available in the USDA Standard Reference database.
[12]Tripe was not analyzed for the nutrients denoted.

Nutrient	Heart	Liver	Kidney	Tongue	Oxtail[4]	Tripe[4]	Bone marrow[4]	Testicles[4]	Blood[4]
Protein	★	★	★	★	★	★		★	★
Vitamin A (Retinol)	X	★	X	X					
Riboflavin (B_2)	★	★	★	★					
Pantothenic Acid	✚	★	★	X					
Vitamin B_6	✚	★	★	✚					
Niacin (B_3)	★	★	X	★					
Vitamin B_{12}	★	★	★	★					
Vitamin K_1 (phylloquinone)	X	X	X	X	X	X	✚	X	X
Calcium	X	X	X	X	X	X	★	X	X
Copper	✚	★	★	X	X	X	X	X	X
Iron	★	★	★	✚	✚	X	X	X	X
Manganese	X	✚	X	X	X	X	X	X	X
Phosphorus	X	★	★	✚	✚	X	★	★	X
Zinc	X	★	✚	★	★	✚	X	X	X

[1] ★ = Meets "Excellent Source of" certification; ✚ = Meets "Good Source of" certification; **X** = Does not meet certification

[2] Reference daily intakes (RDI) dietary allowance (RDA) is the daily intake level of a nutrient that is considered to be sufficient to meet the requirements of 97-98% of healthy individuals in the United States.

[3] Providing over 20% of the RDI qualifies the item to be labeled as an "excellent source" of the nutrient. Providing between 10-19% of the RDI qualifies the item to be labeled as a "good source" of the nutrient.

[4] Oxtail, tripe, bone marrow, testicles, and blood were not analyzed for niacin, riboflavin, pantothenic acid, vitamin B_6, or vitamin B_{12}.

Figure 1.1. Percentage of the RDI[2] contributed by 100 grams of separable lean tissue from raw U.S. beef variety meat items qualifying for USDA "Excellent Source of" and "Good Source of" extra labeling claims[3].

REFERENCES

Aberle, E. D., Forrest, J. C., Gerrard, D. E., & Mills, E. W. (2001). *Principles of Meat Science* (4th ed.). Kendall/Hunt Publishing Company.

Acheson, R. J., Woerner, D. R., Martin, J. N., Belk, K. E., Engle, T. E., Brown, T. R., … McNeill, S. H. (2015). Nutrient database improvement project: Separable components and proximate composition of raw and cooked retail cuts from the beef loin and round. *Meat Science*, *110*, 236–244. https://doi.org/10.1016/J.MEATSCI.2015.06.001

Adhikari, K., Chambers IV, E., Miller, R., Vázquez-Araújo, L., Bhumiratana, N., & Philip, C. (2011). Development of a lexicon for beef flavor in intact muscle. *Journal of Sensory Studies*, *26*(6), 413–420. https://doi.org/10.1111/j.1745-459X.2011.00356.x

Ahuja, J. K. C., Moshfegh, A. J., Holden, J. M., & Harris, E. (2013). USDA Food and Nutrient Databases Provide the Infrastructure for Food and Nutrition Research, Policy, and Practice. *Journal of Nutrition*, *143*(2), 241S–249S. https://doi.org/10.3945/jn.112.170043

Al-Shawaf, L., Lewis, D. M. G., Alley, T. R., & Buss, D. M. (2015). Mating strategy, disgust, and food neophobia. *Appetite*, *85*, 30–35. https://doi.org/10.1016/j.appet.2014.10.029

AOAC. (1995). *Removal of Moisture, Official Method 8.2.1.1* (16th ed.). Arlington, VA: Association of Official Analytical Chemists.

AOAC. (2000). *Official Methods of Analysis* (17th ed.). Arlington, VA: Association of Official Analytical Chemists.

AOAC. (2006). *Analysis of Protein, Official Method* (18th ed.). Arlington, VA: Association of Official Analytical Chemists.

Belury, M. A. (2002). Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. *Annual Review of Nutrition*, *22*(1), 505–531. https://doi.org/10.1146/annurev.nutr.22.021302.121842

Berry, B. W. (1994). Fat Level, High Temperature Cooking and Degree of Doneness Affect Sensory, Chemical and Physical Properties of Beef Patties. *Journal of Food Science*, *59*(1), 10–14. https://doi.org/10.1111/j.1365-2621.1994.tb06885.x

Beulens, J. W. J., Booth, S. L., Van Den Heuvel, E. G. H. M., Stoecklin, E., Baka, A., & Vermeer, C. (2013). The role of menaquinones (vitamin K2) in human health. *British Journal of Nutrition*, *110*(8), 1357–1368. https://doi.org/10.1017/S0007114513001013

Beulens, J. W. J., Bots, M. L., Atsma, F., Bartelink, M.-L. E. L., Prokop, M., Geleijnse, J. M., … van der Schouw, Y. T. (2009). High dietary menaquinone intake is associated with reduced coronary calcification. *Atherosclerosis*. https://doi.org/10.1016/j.atherosclerosis.2008.07.010

Booth, S. L., & Suttie, J. W. (1997). Recent Advances in Nutritional Sciences Dietary Intake and Adequacy of Vitamin K. *Nutrition Research*, *128*(5), 785–788.

Bouton, P. E., Harris, P. V, & Hill, C. (1966). The effects of cooking temperature and time on some mechanical properties of meat, *37*, 140–144.

Brooks, J. C., & Savell, J. W. (2004). Perimysium thickness as an indicator of beef tenderness. *Meat Science*, *67*(2), 329–334. https://doi.org/10.1016/j.meatsci.2003.10.019

Calkins, C. R., & Hodgen, J. M. (2007). A fresh look at meat flavor. *Meat Science*, *77*(1 SPEC. ISS.), 63–80. https://doi.org/10.1016/j.meatsci.2007.04.016

CDC. (2017). Chronic Disease Prevention and Health Promotion. Retrieved June 7, 2018, from https://www.cdc.gov/chronicdisease/overview/index.htm

Chandrashekar, J., Hoon, M. A., Ryba, N. J. P., & Zuker, C. S. (2006). The receptors and cells for mammalian taste. *Nature*, *444*(7117), 288–294. https://doi.org/10.1038/nature05401

Chasapis, C. T., Spiliopoulou, C. A., Loutsidou, A. C., & Stefanidou, M. E. (2012). Zinc and human health: An update. *Archives of Toxicology*, *86*(4), 521–534. https://doi.org/10.1007/s00204-011-0775-1

Christensen, K. L., Johnson, D. D., West, R. L., Marshall, T. T., & Hargrove, D. D. (1991). The effect of breed of sire and age at feeding on muscle tenderness in the beef chuck. *Journal of Animal Science*, *69*(9), 3673–3678.

Cotton, P. A., Subar, A. F., Friday, J. E., & Cook, A. (2004). Dietary sources of nutrients among US adults, 1994 to 1996. *Journal of the American Dietetic Association*, *104*(6), 921–930. https://doi.org/10.1016/j.jada.2004.03.019

Covas, M. I. (2007). Olive oil and the cardiovascular system. *Pharmacological Research*. https://doi.org/10.1016/j.phrs.2007.01.010

Crino, M., Sacks, G., Vandevijvere, S., Swinburn, B., & Neal, B. (2015). The Influence on Population Weight Gain and Obesity of the Macronutrient Composition and Energy Density of the Food Supply. *Current Obesity Reports*, *4*(1), 1–10. https://doi.org/10.1007/s13679-014-0134-7

Cross, H. R., Berry, B. W., & Wells, L. H. (1980). Effects of Fat Level and Source on the Chemical, Sensory and Cooking Properties of Ground Beef Patties. *Journal of Food Science*, *45*(4), 791–794. https://doi.org/10.1111/j.1365-2621.1980.tb07450.x

Cross, H. R., Carpenter, Z. L., & Smith, G. C. (1973). Effects of Intramuscular Collagen and Elastin on Bovine Muscle Tenderness. *Journal of Food Science*, *38*(6), 998–1003. https://doi.org/10.1111/j.1365-2621.1973.tb02133.x

Cross, H. R., Stanfield, M. S., & Koch, E. J. (1976). Beef Palatability as Affected by Cooking Rate and Final Internal Temperature. *Journal of Animal Science*, *43*(1), 114–121.

https://doi.org/10.2134/jas1976.431114x

Cui, R., Iso, H., Date, C., Kikuchi, S., & Tamakoshi, A. (2010). Dietary folate and vitamin B6 and B12 intake in relation to mortality from cardiovascular diseases: Japan collaborative cohort study. *Stroke*, *41*(6), 1285–1289. https://doi.org/10.1161/STROKEAHA.110.578906

Davey, C. L., & Gilbert, K. V. (1974). Temperature-dependent cooking toughness in beef. *Journal of the Science of Food and Agriculture*, *25*(8), 931–938. https://doi.org/10.1002/jsfa.2740250808

Dayton, W. R., Goll, D. E., Zeece, M. G., Robson, R. M., & Reville, W. J. (1976). A Ca2+-Activated Protease Possibly Involved in Myofibrillar Protein Turnover. Purification from Porcine Muscle. *Biochemistry*, *15*(10), 2150–2158. https://doi.org/10.1021/bi00655a019

Devine, C. E., Wahlgren, N. M., & Tornberg, E. (1999). Effect of rigor temperature on muscle shortening and tenderisation of restrained and unrestrained beef m. longissimus thoracicus et lumborum. *Meat Science*, *51*(1), 61–72. https://doi.org/10.1016/S0309-1740(98)00098-9

DGAC. (2015). Scientific Report of the 2015 Dietary Guidelines Advisory Committee. *Dietary Guidelines Advisory Committee*. https://doi.org/10.1017/CBO9781107415324.004

Duncan, K. H., Bacon, J. A., & Weinsier, R. L. (1983). The effects of high and low energy density diets on satiety, energy intake, and eating time of obese and nonobese subjects. *American Journal of Clinical Nutrition*, *37*(5), 763–767. https://doi.org/10.1093/ajcn/37.5.763

Fayemi, P. O., Yetim, H., & Ahhmed, A. (2018). Targeting the pains of food insecurity and malnutrition among internally displaced persons with nutrient synergy and analgesics in organ meat. *Food Research International*, *104*, 48–58. https://doi.org/10.1016/J.FOODRES.2016.11.038

Field, C. J., Johnson, I., & Pratt, V. C. (2000). Glutamine and arginine: immunonutrients for improved health. *Medicine and Science in Sports and Exercise*, *32*(7 Suppl), S377-88. https://doi.org/10.1097/00005768-200007001-00002

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of Biological Chemistry*, *226*(1), 497–509. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/13428781

Food and Agriculture Organization of the United Nations. (2011). *Dietary protein quality evaluation in human nutrition: Report of an FAO Expert Consultation*. Retrieved from http://www.fao.org/ag/humannutrition/35978-02317b979a686a57aa4593304ffc17f06.pdf

Freeland-Graves, J., & Nitzke, S. (2013). Position of the Academy of Nutrition and Dietetics: Total Diet Approach to Healthy Eating. *JAND*, *113*, 307–317. https://doi.org/10.1016/j.jand.2012.12.013

German, J. B., & Dillard, C. J. (2004). Saturated fats: what dietary intake? *The American Journal*

of Clinical Nutrition, *80*(3), 550–559. https://doi.org/10.1093/ajcn/80.3.550

Godfrey, K., Robinson, S., Barker, D. J., Osmond, C., & Cox, V. (1996). Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. *BMJ (Clinical Research Ed.)*, *312*(7028), 410–4. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8601112

Goodwin, P. J., & Chlebowski, R. T. (2016). Obesity and cancer: Insights for clinicians. *Journal of Clinical Oncology*, *34*(35), 4197–4202. https://doi.org/10.1200/JCO.2016.70.5327

Grayson, A. L., Shackelford, S. D., King, D. A., McKeith, R. O., Miller, R. K., & Wheeler, T. L. (2016). The effects of degree of dark cutting on tenderness and sensory attributes of beef. *Journal of Animal Science*, *94*(6), 2583–2591. https://doi.org/10.2527/jas2016-0388

Grundy, S. M. (1994). Influence of stearic acid on cholesterol metabolism relative to other long-chain fatty acids. *The American Journal of Clinical Nutrition*, *60*(6), 986S–990S. https://doi.org/10.1093/ajcn/60.6.986S

Guelker, M. R., Haneklaus, A. N., Brooks, J. C., Carr, C. C., Delmore, R. J., Griffin, D. B., … Savell, J. W. (2013). National beef tenderness survey-2010:Warner-Bratzler shear force values and sensory panel ratings for beef steaks from United States retail and food service establishments. *Journal of Animal Science*, *91*(2), 1005–1014. https://doi.org/10.2527/jas.2012-5785

Hall, R. L. (1968). Food flavors: Benefits and problems. *Food Technology*, *22*(54).

Henchion, M., McCarthy, M., & O'Callaghan, J. (2016). Transforming Beef By-products into Valuable Ingredients: Which Spell/Recipe to Use? *Frontiers in Nutrition*, *3*(November), 1–8. https://doi.org/10.3389/fnut.2016.00053

Hildrum, K. I., Rødbotten, R., Høy, M., Berg, J., Narum, B., & Wold, J. P. (2009). Classification of different bovine muscles according to sensory characteristics and Warner Bratzler shear force. *Meat Science*, *83*(2), 302–307. https://doi.org/10.1016/j.meatsci.2009.05.016

Hoffman, J. R., & Falvo, M. J. (2004). Protein - Which is best? *Journal of Sports Science and Medicine*, *3*(3), 118–130.

Huff-Lonergan, E., Mitsuhashi, T., Beekman, D. D., Parrish, F. C., Olson, D. G., & Robson, R. M. (1996). Proteolysis of Specific Muscle Structural Proteins by μ-Calpain at Low pH and Temperature is Similar to Degradation in Postmortem Bovine Muscle. *Journal of Animal Science*, *74*(5), 993–1008. https://doi.org/10.2527/1996.745993x

Huffman, K. L., Miller, M. F., Hoover, L. C., Wu, C. K., Brittin, H. C., & Ramsey, C. B. (1996). Effect of Beef Tenderness on Consumer Satisfaction with Steaks Consumed in the Home and Restaurant. *Journal of Animal Science*, *74*(1), 91–97. https://doi.org/10.2527/1996.74191x

Hunt, M. R., Garmyn, A. J., O'Quinn, T. G., Corbin, C. H., Legako, J. F., Rathmann, R. J., … Miller, M. F. (2014). Consumer assessment of beef palatability from four beef muscles from

USDA Choice and Select graded carcasses. *Meat Science*, *98*(1), 1–8. https://doi.org/10.1016/j.meatsci.2014.04.004

Hunt, M. R., Legako, J. F., Dinh, T. T. N., Garmyn, A. J., O'Quinn, T. G., Corbin, C. H., … Miller, M. F. (2016). Assessment of volatile compounds, neutral and polar lipid fatty acids of four beef muscles from USDA Choice and Select graded carcasses and their relationships with consumer palatability scores and intramuscular fat content. *Meat Science*, *116*, 91–101. https://doi.org/10.1016/j.meatsci.2016.02.010

Hunter, J. E., Zhang, J., & Kris-Etherton, P. M. (2010). Cardiovascular disease risk of dietary stearic acid compared with trans , other saturated , and unsaturated fatty acids : a systematic review 1 – 4. *Am J Clin Nutr, American Society for Nutrition*, *91*(May), 46–63. https://doi.org/10.3945/ajcn.2009.27661.46

Hurrell, R., & Egli, I. (2010). Iron bioavailability and dietary reference values. *The American Journal of Clinical Nutrition*, *91*(5), 1461S–1467S. https://doi.org/10.3945/ajcn.2010.28674F.Am

Institute of Medicine. (1997). *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*. *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*. National Academies Press (US). https://doi.org/10.17226/5776

Institute of Medicine. (1998). *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*. *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*. National Academies Press (US). https://doi.org/10.17226/6015

Institute of Medicine. (2001). *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. National Academies Press (US). https://doi.org/10.17226/10026

Institute of Medicine. (2011). *Dietary Reference Intakes for Calcium and Vitamin D*. *Dietary Reference Intakes for Calcium and Vitamin D*. National Academies Press (US). https://doi.org/10.17226/13050

Institute of Medicine (U.S.). Standing Committee on the Scientific Evaluation of Dietary Reference Intakes., Institute of Medicine (U.S.). Panel on Folate, O. B. V., & Institute of Medicine (U.S.). Subcommittee on Upper Reference Levels of Nutrients. (1998). *Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B_6, folate, vitamin B_{12}, pantothenic acid, biotin, and choline*. National Academy Press.

Ip, C., Scimeca, J. A., & Thompson, H. J. (1994). Conjugated linoleic acid. A powerful anticarcinogen from animal fat sources. *Cancer*, *74*(3 Suppl), 1050–4. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8039138

Johnson, M. H., Calkins, C. R., Huffman, R. D., Johnson, D. D., & Hargrove, D. D. (1990).

Differences in cathepsin B + L and calcium-dependent protease activities among breed type and their relationship to beef tenderness. *Journal of Animal Science*, *68*(8), 2371–2379. https://doi.org//1990.6882371x

Johnson, R. C., Chen, C. M., Muller, T. S., Costello, W. J., Romans, J. R., & Jones, K. W. (1988). Characterization of the Muscles within the Beef Forequarter. *Journal of Food Science*, *53*(5), 1247–1250. https://doi.org/10.1111/j.1365-2621.1988.tb09249.x

Jung, E. Y., Hwang, Y. H., & Joo, S. T. (2016). Muscle profiling to improve the value of retail meat cuts. *Meat Science*, *120*, 47–53. https://doi.org/10.1016/j.meatsci.2016.04.012

Katan, M. B., Zock, P. L., & Mensink, R. P. (1994). Effects of fats and fatty acids on blood lipids in humans: An overview. In *American Journal of Clinical Nutrition* (Vol. 60). https://doi.org/10.1093/ajcn/60.6.1017S

Kemp, C. M., & Parr, T. (2012). Advances in apoptotic mediated proteolysis in meat tenderisation. *Meat Science*, *92*(3), 252–259. https://doi.org/10.1016/j.meatsci.2012.03.013

Kerth, C. (2016). Determination of volatile aroma compounds in beef using differences in steak thickness and cook surface temperature. *Meat Science*, *117*, 27–35. https://doi.org/10.1016/j.meatsci.2016.02.026

Kerth, C. R., & Miller, R. K. (2015). Beef flavor: A review from chemistry to consumer. *Journal of the Science of Food and Agriculture*, *95*(14), 2783–2798. https://doi.org/10.1002/jsfa.7204

Killinger, K. M., Calkins, C. R., Umberger, W. J., Feuz, D. M., & Eskridge, K. M. (2004). Consumer sensory acceptance and value for beef steaks of similar tenderness, but differing in marbling level. *Journal of Animal Science*, *82*(11), 3294–3301. https://doi.org//2004.82113294x

King, D. A., Dikeman, M. E., Wheeler, T. L., Kastner, C. L., & Koohmaraie, M. (2003). Chilling and cooking rate effects on some myofibrillar determinants of tenderness of beef. *Journal of Animal Science*, *81*(6), 1473–1481. https://doi.org//2003.8161473x

Kitts, D., & Weiler, K. (2003). Bioactive Proteins and Peptides from Food Sources. Applications of Bioprocesses used in Isolation and Recovery. *Current Pharmaceutical Design*, *9*(16), 1309–1323. https://doi.org/10.2174/1381612033454883

Knaapila, A., Tuorila, H., Silventoinen, K., Keskitalo, K., Kallela, M., Wessman, M., … Perola, M. (2007). Food neophobia shows heritable variation in humans. *Physiology and Behavior*, *91*(5), 573–578. https://doi.org/10.1016/j.physbeh.2007.03.019

Knapen, M. H. J., Drummen, N. E., Smit, E., Vermeer, C., & Theuwissen, E. (2013). Three-year low-dose menaquinone-7 supplementation helps decrease bone loss in healthy postmenopausal women. *Osteoporosis International*, *24*(9), 2499–2507. https://doi.org/10.1007/s00198-013-2325-6

Knapen, M. H. J., Schurgers, L. J., & Vermeer, C. (2007). Vitamin K2 supplementation improves hip bone geometry and bone strength indices in postmenopausal women. *Osteoporosis International*, *18*(7), 963–972. https://doi.org/10.1007/s00198-007-0337-9

Koch, R., Crouse, J., & Dikeman, M. (1993). Effect of Marbling on Variation and Change in Beef Tenderness In Bos Taurus and Bos Indicus Crosses. Retrieved from http://digitalcommons.unl.edu/hruskareports/127/

Laska, M., Distel, H., & Hudson, R. (1997). Trigeminal perception of odourant quality in congenital anosmic subjects. *Chem Sens*, *22*(June). https://doi.org/10.1093/chemse/22.4.447

Lau, De, L. M. L., Koudstaal, P. ., Witteman, J. C. M., Hofman, A., & Breteler, M. M. B. (2006). Dietary folate, vitamin B12, and vitamin B6 and the risk of Parkinson disease. *Neurology*, *67*(2), 315–318.

Lawrence, T. E., King, D. A., Obuz, E., Yancey, E. J., & Dikeman, M. E. (2001). Evaluation of electric belt grill, forced-air convection oven, and electric broiler cookery methods for beef tenderness research. *Meat Science*, *58*(3), 239–246. https://doi.org/10.1016/S0309-1740(00)00159-5

Legako, J. F., Brooks, J. C., O'Quinn, T. G., Hagan, T. D. J., Polkinghorne, R., Farmer, L. J., & Miller, M. F. (2015). Consumer palatability scores and volatile beef flavor compounds of five USDA quality grades and four muscles. *Meat Science*, *100*, 291–300. https://doi.org/10.1016/j.meatsci.2014.10.026

Legako, J. F., Dinh, T. T. N., Miller, M. F., Adhikari, K., & Brooks, J. C. (2016). Consumer palatability scores, sensory descriptive attributes, and volatile compounds of grilled beef steaks from three USDA Quality Grades. *Meat Science*, *112*, 77–85. https://doi.org/10.1016/j.meatsci.2015.10.018

Leidy, H. J., Carnell, N. S., Mattes, R. D., & Campbell, W. W. (2007). Higher protein intake preserves lean mass and satiety with weight loss in pre-obese and obese women. *Obesity*, *15*(2), 421–429. https://doi.org/10.1038/oby.2007.531

Lorenzen, C. L., Neely, T. R., Miller, R. K., Tatum, J. D., Wise, J. W., Taylor, J. F., … Savell, J. W. (1999). Beef Customer Satisfaction: Cooking Method and Degree of Doneness Effects on the Top Loin Steak. *Journal of Animal Science*, *77*(3), 637–644. https://doi.org/10.2527/1999.773645x

Maresz, K. (2015). Proper Calcium Use: Vitamin K2 as a Promoter of Bone and Cardiovascular Health. *Integrative Medicine (Encinitas, Calif.)*, *14*(1), 34–9. https://doi.org/CliCa0504605610

Margarita, P., Castillo, M., Alejandro, Y., & Ligardo, M. (2015). Protein Quality of Rice Drinks Fortified with Bovine and Porcine Blood Plasma, *68*(39), 7487–7496.

Maughan, C., Tansawat, R., Cornforth, D., Ward, R., & Martini, S. (2012). Development of a beef flavor lexicon and its application to compare the flavor profile and consumer

acceptance of rib steaks from grass- or grain-fed cattle. *Meat Science*, *90*(1), 116–121. https://doi.org/10.1016/j.meatsci.2011.06.006

McCormick, R. J. (1994). The flexibility of the collagen compartment of muscle. *Meat Science*, *36*(1–2), 79–91. https://doi.org/10.1016/0309-1740(94)90035-3

McKeith, F. K., Devol, D. L., Miles, R. S., Bechtel, P. J., & Carr, T. R. (1985). Chemical and Sensory Properties of 13 Major Beef Muscles. *Journal of Food Science*, *50*(4), 869–872.

Mensink, R. P. (2005). Effects of stearic acid on plasma lipid and lipoproteins in humans. *Lipids*, *40*(12), 1201–1205. https://doi.org/10.1007/s11745-005-1486-x

Miller, M. F., Carr, M. A., Ramsey, C. B., Crockett, K. L., & Hoover, L. C. (2001). Consumer thresholds for establishing the value of beef tenderness. *Journal of Animal Science*, *79*(12), 3062–3068. https://doi.org//2001.79123062x

Miller, R. K. (1994). Quality Characteristics. In D. M. Kinsman, A. W. Kotula, & B. C. Breidenstein (Eds.), *Muscle Foods*. Boston, MA: Springer.

Morgan, J. B., Savell, J. W., Hale, D. S., Miller, R. K., Griffin, D. B., Cross, H. R., & Shackelford, S. D. (1991). National beef tenderness survey. *Journal of Animal Science*, *69*(8), 3274–3283. https://doi.org/10.2527/1991.6983274x

Mottram, D. S. (1998). Flavour formation in meat and meat products: A review. *Food Chemistry*, *62*(4), 415–424. https://doi.org/10.1016/S0308-8146(98)00076-4

Mullen, A. M., Álvarez, C., Zeugolis, D. I., Henchion, M., O'Neill, E., & Drummond, L. (2017). Alternative uses for co-products: Harnessing the potential of valuable compounds from meat processing chains. *Meat Science*, *132*(April), 90–98. https://doi.org/10.1016/j.meatsci.2017.04.243

National Academy of Sciences. (2000). *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. Washington, DC: National Academy Press.

National Cattlemen's Beef Association. (2007). *Beef lipids in perspective*. *Beef Facts: Human Nutrition Research*.

National Research Council (Ed.). (2000). *Dietary Reference Intakes*. Washington, DC: National Academy Press.

Newsholme, P., Curi, R., Pithon Curi, T. C., Murphy, C. J., Garcia, C., & Pires de Melo, M. (1999). Glutamine metabolism by lymphocytes, macrophages, and neutrophils: its importance in health and disease. *The Journal of Nutritional Biochemistry*, *10*(6), 316–324. https://doi.org/10.1016/S0955-2863(99)00022-4

Obuz, E., Dikeman, M. E., Grobbel, J. P., Stephens, J. W., & Loughin, T. M. (2004). Beef longissimus lumborum, biceps femoris, and deep pectoralis Warner-Bratzler shear force is

affected differently by endpoint temperature, cooking method, and USDA quality grade. *Meat Science*, *68*(2), 243–248. https://doi.org/10.1016/j.meatsci.2004.03.003

Ockerman, H. W., & Hansen, C. L. (2000). *Animal Byproduct Processing and Utilization* (1st ed.). Lancaster, PA: Technomic.

Ogden, C. L., Carroll, M. D., Fryar, C. D., & Flegal, K. M. (2015). *Prevalence of Obesity Among Adults and Youth, 2011 - 2014*. *NCHS Data Brief*. https://doi.org/10.1017/S1368980017000088

Ohman, C. E., Wiegand, B. R., Gruen, I. U., & Lorenzen, C. L. (2015). Beef muscle isolation has no detrimental effect on premium ground beef programs. *Meat Science*, *106*, 50–54. https://doi.org/10.1016/j.meatsci.2015.03.022

Pao-Hwa, F. M. S. L. P. S. W. M. V. L. J. A. G. A. B. D. H. E. O. P. R. C. E. R. M. I. D. G. S.-M. N. K. (2001). Effects on Blood Pressure of Reduced Dietary Sodium and the Dietary Approaches To Stop Hypertension (Dash) Diet. *The New England Journal of Medicine*, *344*(1), 3–10. https://doi.org/10.1056/NEJM200101043440101

Park, P. ., & Goins, R. E. (1994). In Situ Preparation of Fatty Acid Methyl Esters for Analysis of Fatty Acid Composition in Foods. *Journal of Food Science*, *59*(6), 1262–1266. https://doi.org/10.1111/j.1365-2621.1994.tb14691.x

Patten, L. E., Hodgen, J. M., Stelzleni, A. M., Calkins, C. R., Johnson, D. D., & Gwartney, B. L. (2008). Chemical properties of cow and beef muscles: Benchmarking the differences and similarities1,2. *Journal of Animal Science*, *86*(8), 1904–1916. https://doi.org/10.2527/jas.2007-0478

Patterson, K. Y., Duvall, M. L., Howe, J. C., & Holden, J. M. (2009). *USDA nutrient data set for retail beef cuts, release 1.0*. Beltsville, MD.

Penniston, K. L., & Tanumihardjo, S. A. (2006). The acute and chronic toxic effects of vitamin A. *The American Journal of Clinical Nutrition*, *83*(2), 191–201. https://doi.org/10.1093/ajcn/83.2.191

Pereira, P. M. de C. C., & Vicente, A. F. dos R. B. (2013). Meat nutritional composition and nutritive role in the human diet. *Meat Science*, *93*(3), 586–592. https://doi.org/10.1016/j.meatsci.2012.09.018

Phillips, S. M., Iii, V. L. F., Heaney, R. P., Nicklas, T. A., Slavin, J. L., & Weaver, C. M. (2015). Commonly consumed protein foods contribute to nutrient intake , diet quality , and nutrient adequacy 1 – 7, *101*(May), 1346–1352. https://doi.org/10.3945/ajcn.114.084079.Accumulating

Powers, H. J. (2003). Riboflavin (vitamin B-2) and health. *The American Journal of Clinical Nutrition*, *77*(6), 1352–1360. https://doi.org/10.1093/ajcn/77.6.1352

Pratt, W. B., Omdahl, J. L., & Sorenson, J. R. J. (1985). Lack of effects of copper gluconate

supplementation. *The American Journal of Clinical Nutrition*, *42*(4), 681–682. https://doi.org/10.1093/ajcn/42.4.681

Prost, E., Pelczynska, E., & Kotula, A. W. (1975). Quality characteristics of bovine meat. II. beef tenderness in relation to individual muscles, age and sex of animals and carcass quality grade. *Journal of Animal Science*, *41*(2), 541–547. Retrieved from http://jas.fass.org/cgi/content/abstract/41/2/541

Purchas, R. W., Wilkinson, B. H. P., Carruthers, F., & Jackson, F. (2015). A comparison of the trans fatty acid content of uncooked and cooked lean meat, edible offal and adipose tissue from New Zealand beef and lamb. *Journal of Food Composition and Analysis*, *41*, 151–156. https://doi.org/10.1016/j.jfca.2015.01.016

Purslow, P. P. (2005). Intramuscular connective tissue and its role in meat quality. *Meat Science*, *70*(3 SPEC. ISS.), 435–447. https://doi.org/10.1016/j.meatsci.2004.06.028

Reicks, A. L., Brooks, J. C., Garmyn, A. J., Thompson, L. D., Lyford, C. L., & Miller, M. F. (2011). Demographics and beef preferences affect consumer motivation for purchasing fresh beef steaks and roasts. *Meat Science*, *87*(4), 403–411. https://doi.org/10.1016/j.meatsci.2010.11.018

Resconi, V. C., Escudero, A., & Campo, M. M. (2013). The development of aromas in ruminant meat. *Molecules*, *18*(6), 6748–6781. https://doi.org/10.3390/molecules18066748

Rimm, E. B. (1998). Folate and Vitamin B6 From Diet and Supplements in Relation to Risk of Coronary Heart Disease Among Women. *JAMA: The Journal of the American Medical Association*, *279*(5), 359–364. https://doi.org/10.1001/jama.279.5.359

Roseland, J. M., Nguyen, Q. V., Douglass, L. W., Patterson, K. Y., Howe, J. C., Williams, J. R., … McNeill, S. H. (2018). Fatty acid, cholesterol, vitamin, and mineral content of cooked beef cuts from a national study. *Journal of Food Composition and Analysis*, *66*, 55–64. https://doi.org/10.1016/J.JFCA.2017.12.003

Roussell, M. A., Hill, A. M., Gaugler, T. L., West, S. G., Vanden Heuvel, J. P., Alaupovic, P., … Kris-Etherton, P. M. (2012). Beef in an Optimal Lean Diet study: Effects on lipids, lipoproteins, and apolipoproteins. *American Journal of Clinical Nutrition*, *95*(1), 9–16. https://doi.org/10.3945/ajcn.111.016261

Schaefer, D., & Arp, T. (2017). Importance of variety meat utilization to the meat industry. *Animal Frontiers*, *7*(4), 25–28. https://doi.org/10.2527/af.2017.0439

Schwalfenberg, G. K. (2017). Vitamins K1 and K2: The Emerging Group of Vitamins Required for Human Health. *Journal of Nutrition and Metabolism*. https://doi.org/10.1155/2017/6254836

Seideman, S. C. (1986). Methods of Expressing Collagen Characteristics and Their Relationship to Meat Tenderness and Muscle Fiber Types. *Journal of Food Science*, *51*(2), 273–276. https://doi.org/10.1111/j.1365-2621.1986.tb11107.x

Semler, M. L., Woerner, D. R., Belk, K. E., Enns, K. J., & Tatum, J. D. (2016). Effects of United States Department of Agriculture carcass maturity on sensory attributes of steaks produced by cattle representing two dental age classes. *Journal of Animal Science*, *94*(5), 2207–2217. https://doi.org/10.2527/jas2016-0382

Shackelford, S. D., Koohmaraie, M., & Wheeler, T. L. (1995). Effects of slaughter age on meat tenderness and USDA carcass maturity scores of beef females. *Journal of Animal Science*, *73*(11), 3304–3309. https://doi.org/10.2527/1995.73113304x

Shahidi, F., Samaranayaka, A. G. P., & Pegg, R. B. (2014). Maillard reaction and browning. In M. Dikeman & C. Devine (Eds.), *Encyclopedia of Meat Sciences* (2nd ed., pp. 391–403). London, UK: Elsevier.

Shepherd, G. M. (2005). Outline of a theory of olfactory processing and its relevance to humans. *Chemical Senses*, *30 SUPPL.*(June), 3–5. https://doi.org/10.1093/chemse/bjh085

Showell, B. A., Williams, J. R., Duvall, M., Howe, J. C., Patterson, K. Y., Roseland, J. M., & Holden, J. M. (2012). USDA Table of Cooking Yields for Meat and Poultry Prepared by, 1–30.

Simopoulos, A. P. (1999). Essential fatty acids in health and chronic disease. *The American Journal of Clinical Nutrition*, *70*(3), 560s–569s. https://doi.org/10.1093/ajcn/70.3.560s

Smedman, A., & Vessby, B. (2001). Conjugated linoleic acid supplementation in humans--metabolic effects. *Lipids*, *36*(8), 773–81. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11592727

Smith, A. M., Harris, K. B., Haneklaus, A. N., & Savell, J. W. (2011). Proximate composition and energy content of beef steaks as influenced by USDA quality grade and degree of doneness. *Meat Science*, *89*(2), 228–232. https://doi.org/10.1016/J.MEATSCI.2011.04.027

Smith, G., Savell, J., Cross, H., Carpenter, Z., Murphey, C., Davis, G., … Berry, B. (1987). Relationship of Usda Quality Grades To Palatability of Cooked Beef1. *Journal of Food Quality*, *10*(4), 269–286. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.1745-4557.1987.tb00819.x/abstract

Spoonger, W. F. (1988). Organs and glands as human food. In A. . Pearson & T. R. Dutson (Eds.), *Edible meat by-products* (pp. 197–217). London, UK: Elsevier Applied Science.

Stapanik, M. H., & Caudill, M. A. (2013). *Biochemical, Physiological, and Molecular Aspects of Human Nutrition*. (M. H. Stapanik & M. A. Caudill, Eds.) (3rd ed.). Elsevier.

Stolowski, G. D., Baird, B. E., Miller, R. K., Savell, J. W., Sams, A. R., Taylor, J. F., … Smith, S. B. (2006). Factors influencing the variation in tenderness of seven major beef muscles from three Angus and Brahman breed crosses. *Meat Science*, *73*(3), 475–483. https://doi.org/10.1016/j.meatsci.2006.01.006

Sullivan, G. A., & Calkins, C. R. (2011). Ranking beef muscles for Warner-Bratzler shear force

and trained sensory panel ratings from published literature. *Journal of Food Quality*, *34*(3), 195–203. https://doi.org/10.1111/j.1745-4557.2011.00386.x

The Meat Buyer's Guide. (2014) (8th ed.). Washington, DC: North American Meat Association.

Thom, E., Wadstein, J., & Gudmundsen, O. (2001). Conjugated Linoleic Acid Reduces Body Fat in Healthy Exercising Humans. *Journal of International Medical Research*, *29*(5), 392–396. https://doi.org/10.1177/147323000102900503

ToldrÃ¡, F., Mora, L., & Reig, M. (2016). New insights into meat by-product utilization. *Meat Science*, *120*, 54–59. https://doi.org/10.1016/j.meatsci.2016.04.021

Toldrá, F., Aristoy, M. C., Mora, L., & Reig, M. (2012). Innovations in value-addition of edible meat by-products. *Meat Science*, *92*(3), 290–296. https://doi.org/10.1016/j.meatsci.2012.04.004

U.S. Department of Agriculture, & U.S. Department of Health and Human Services. (2010). *Dietary Guidelines for Americans, 2010*. *7th edition*. Washington, DC: U.S. Government Printing Office. https://doi.org/10.1016/S0300-7073(05)71075-6

U.S. Department of Health and Human Services. (2013). *Managing Overweight and Obesity in Adults*.

United States Meat Export Federation. (n.d.). *Leading Markets for U.S. Beef Variety Meat Exports*.

USDA National Nutrient Database for Standard Reference, Legacy. (2018). US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory.

USDA, & USHHS. (2013). *Dietary Guidelines Advisory Committee Meeting 1*. *History of Dietary Guidance Development in the United States and the Dietary Guidelines for Americans*. Bathesda, MD: USDA, USHHS.

Valsta, L. M., Tapanainen, H., & Männistö, S. (2005). Meat fats in nutrition. *Meat Science*, *70*(3), 525–530. https://doi.org/10.1016/j.meatsci.2004.12.016

Van Ba, H., Hwang, I., Jeong, D., & Touseef, A. (2012). Principle of Meat Aroma Flavors and Future Prospect. *Latest Research into Quality Control*, 145–176. https://doi.org/37707

Van Boekel, M. A. J. S. (2006). Formation of flavour compounds in the Maillard reaction. *Biotechnology Advances*, *24*(2), 230–233. https://doi.org/10.1016/j.biotechadv.2005.11.004

van Heerden, S. M., & Morey, L. (2014). Nutrient content of South African C2 beef offal. *Journal of Food Measurement and Characterization*, *8*(3), 249–258. https://doi.org/10.1007/s11694-014-9198-z

Vannice, G., & Rasmussen, H. (2014). Position of the academy of nutrition and dietetics: Dietary fatty acids for healthy adults. *Journal of the Academy of Nutrition and Dietetics*.

https://doi.org/10.1016/j.jand.2013.11.001

Voges, K. L., Mason, C. L., Brooks, J. C., Delmore, R. J., Griffin, D. B., Hale, D. S., … Savell, J. W. (2007). National beef tenderness survey - 2006: Assessment of Warner-Bratzler shear and sensory panel ratings for beef from US retail and foodservice establishments. *Meat Science*, *77*(3), 357–364. https://doi.org/10.1016/j.meatsci.2007.03.024

Von Seggern, D. D., Calkins, C. R., Johnson, D. D., Brickler, J. E., & Gwartney, B. L. (2005). Muscle profiling: Characterizing the muscles of the beef chuck and round. *Meat Science*, *71*(1), 39–51. https://doi.org/10.1016/j.meatsci.2005.04.010

Watanabe, F. (2007). Vitamin B $_{12}$ Sources and Bioavailability. *Experimental Biology and Medicine*, *232*(10), 1266–1274. https://doi.org/10.3181/0703-MR-67

Weigle, D. S., Breen, P. A., & Matthys, C. C. (2005). A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body we. *American Journal of Clinical Nutrition*, *82*(1), 41–48. https://doi.org/10.1093/ajcn.82.1.41

West, A. R., & Oates, P. S. (2008). Mechanisms of heme iron absorption: Current questions and controversies. *World Journal of Gastroenterology*, *14*(26), 4101–4110. https://doi.org/10.3748/wjg.14.4101

Westerterp-Plantenga, M. S., Lemmens, S. G., & Westerterp, K. R. (2012). Dietary protein - Its role in satiety, energetics, weight loss and health. *British Journal of Nutrition*, *108*(SUPPL. 2). https://doi.org/10.1017/S0007114512002589

Willett, W. C. (2012). Dietary fats and coronary heart disease. *Journal of Internal Medicine*. https://doi.org/10.1111/j.1365-2796.2012.02553.x

World Health Organization. (2000). *Nutrition for health and development: A global agenda for combating malnutrition*.

World Health Organization. (2014). *Global status report on noncommunicable diseases 2014*.

Wu, G. (2016). Dietary protein intake and human health. *Food & Function*, *7*(3), 1251–65. https://doi.org/10.1039/c5fo01530h

Yamaguchi, M., & Weitzmann, M. N. (2011). Vitamin K2 stimulates osteoblastogenesis and suppresses osteoclastogenesis by suppressing NF-κB activation. *International Journal of Molecular Medicine*, *27*(1), 3–14. https://doi.org/10.3892/ijmm.2010.562

Yancey, J. W. S.; Wharton, M. D.; Apple, J. K. ; (2011). Cookery method and end-point temperature can affect the Warner–Bratzler shear. *Meat Science*, *88*(1), 1–7. https://doi.org/10.1016/j.meatsci.2010.11.020

Yeh, Y., Omaye, S. T., Ribeiro, F. A., Calkins, C. R., & de Mello, A. S. (2018). Evaluation of palatability and muscle composition of novel value-added beef cuts. *Meat Science*, *135*(August 2017), 79–83. https://doi.org/10.1016/j.meatsci.2017.08.026

Yu, S., Derr, J., Etherton, T. D., & Kris-Etherton, P. M. (1995). Plasma cholesterol-predictive equations demonstrate that stearic acid is neutral and monounsaturated fatty acids are hypocholesterolemic. *The American Journal of Clinical Nutrition*, *61*(5), 1129–1139. https://doi.org/10.1093/ajcn/61.5.1129

Zarkadas, G. C., Karatzas, C. D., & Zarkadas, C. G. (1996). Assessing the Myofibrillar and Connective Tissue Protein Contents and Protein Quality of Beef Tripe. *Journal of Agricultural Food Chemistry*, *44*, 2563–2572. Retrieved from https://pubs.acs.org/doi/pdf/10.1021/jf950262y

Zinn, D. W., Gaskins, C. T., Gann, G. L., & Hedrick, H. B. (1970). Beef Muscle Tenderness as Influenced by days on feed, sex, maturity and anatomical location. *Journal of Animal Science*, 307–309.

CHAPTER 4

REVIEW OF LITERATURE PART 2

In order to provide a superior eating experience, it is critical to understand both the value consumers place on sensory attributes of beef, as well as the unique characteristics of various meat cuts. Tenderness, flavor, and juiciness attributes are the main drivers influencing consumer acceptability of beef (C. R. Kerth & Miller, 2015; M. F. Miller, Carr, Ramsey, Crockett, & Hoover, 2001; Reicks et al., 2011). However, compositional differences between muscles require that research be conducted on individual meat cuts to accurately describe sensory characteristics. Cooking method, final internal temperature, and quality grade are known to affect the palatability of beef (Berry, 1994; Lorenzen et al., 1999; Obuz, Dikeman, Grobbel, Stephens, & Loughin, 2004), but conclusions should not be generalized to all muscles.

Tenderness

Tenderness has traditionally been recognized as the most important factor influencing consumer acceptability for beef muscle cuts. In light of the significance of this trait, the United States beef industry has made strides to improve quality, increasing tenderness over past decades as monitored by National Beef Tenderness Surveys (Guelker et al., 2013; Morgan et al., 1991; Voges et al., 2007). Pre- and post-harvest factors, including breed-type, production systems, slaughter practices, and preparation techniques, contribute to tenderness outcomes (Shackelford, Koohmaraie, & Wheeler, 1995; Stolowski et al., 2006; Yancey, J. W. S.; Wharton, M. D.; Apple, 2011). Nonetheless, tenderness variation within a carcass also is significant due to differences in composition and function of muscles in the live animal (Sullivan & Calkins, 2011). Gaining knowledge about the tenderness of all beef muscles is critical for providing a consistent and

predictable product to consumers to increase liking and repurchasing. To do so, researchers continue to investigate what is known to be a complex system, with post-mortem proteolysis, sarcomere length, and connective tissue playing key roles.

Myofibrillar Factors

Upon depletion of residual oxygen after slaughter, actin-myosin cross-linkages become permanently linked due to a lack of adenosine triphosphate necessary for the detachment of myosin heads from actin filaments (Aberle et al., 2001). Sarcomere shortening during the development of rigor mortis is associated with a phase of initial toughening and is greatly impacted by the chilling process. Rapid chilling of carcasses can result in "cold shortening", wherein a greater number of actin-myosin cross-bridges form resulting in increased protein overlap that ultimately relates to a tougher product (Devine, Wahlgren, & Tornberg, 1999; King, Dikeman, Wheeler, Kastner, & Koohmaraie, 2003).

Following rigor mortis, proteolytic enzymes begin to break down muscle structure by acting on key myofibrillar proteins during the aging process. Research by Koohmarie (1992) and others suggest that the calpain system is responsible for the majority of post-mortem proteolysis in meat associated with increased tenderness. Several isoforms of calpain exist, with μ-calpain (calpain 1) and m-calpain (calpain 2) being the best characterized. Both cleave myofibrillar proteins but do not act significantly on actin or myosin (Dayton, Goll, Zeece, Robson, & Reville, 1976; Huff-Lonergan et al., 1996). Calpastatins play a role in this system as an endogenous inhibitor of calpains, therefore limiting tenderization. Other enzymes present in meat, including caspases and cathepsins, may also be involved in the natural tenderization process (M. H. Johnson, Calkins, Huffman, Johnson, & Hargrove, 1990; Kemp & Parr, 2012), but controversy about their roles exists due to limited and contradicting evidence.

Connective Tissue

Three types of intramuscular connective tissue exist in muscle to maintain structural integrity: epimysium, surrounding the entire muscle bundle; perimysium, around a bundle of muscle fibers; and endomysium, covering each individual muscle fiber. Collagen and elastin are both classified as connective tissue present in meat, but collagen has been identified as contributing to toughness whereas elastin is not consistently related to tenderness characteristics (Cross, Carpenter, & Smith, 1973). As animals age, heat-stable cross-linkages form between collagen fibers. Both total collagen content and percentage of soluble collagen contribute to tenderness variation, and these characteristics differ considerably with respect to breed, animal age, and muscle (Seideman, 1986; Stolowski et al., 2006; Von Seggern et al., 2005). Because connective tissue properties cannot be manipulated significantly through post-mortem practices, it has been termed "background toughness" (Purslow, 2005).

McKeith et al. (1985) analyzed collagen content of thirteen beef muscles, showing significant variation between cuts, which correlated to a weak association with subjective tenderness ratings. However, other studies have shown stronger correlations between amount or solubility of collagen and tenderness ratings, as well as instrumental tenderness measurements (Cross, Berry, & Wells, 1980; Seideman, 1986). In order to better understand muscle differences, Brooks and Savell (2004) investigated perimysium thickness as a component of meat tenderness. The psoas major contained the thinnest perimysium layer (2.73 mm), with infraspinatus and triceps brachii having intermediate thicknesses (4.56 mm and 4.76 mm, respectively), and the semitendinosus exhibiting the thickest layer (6.65 mm) (Brooks & Savell, 2004). Since epimysium is typically removed from the muscle prior to consumption, and endomysium

comprises a small percentage of intramuscular collagen (McCormick, 1994), perimysium is likely accountable for the majority of connective tissue variation between muscles.

Effect of Cooking

Utilizing cooking techniques to positively impact tenderness is arguably one of the most impactful ways to improve eating quality of tougher muscles. However, inherent muscle characteristics should not be disregarded as they play a role in how cooking method and final internal temperature can be used appropriately to affect palatability. This is exemplified by variation in response to cooking method due to differences in both intramuscular fat content and collagen content, two characteristics that are dissimilar between muscles in the same carcass (Lawrence, King, Obuz, Yancey, & Dikeman, 2001; Obuz et al., 2004). Miller (1994) describes that a higher amount of marbling can be protective against protein denaturation during cooking, as well as decreasing the strength of connective tissue in the meat. However, muscles with low collagen content may be at increased risk for becoming tough at higher cooking temperatures as myofibrillar toughening will override the extent to which collagen solubilization can lead to tenderization (Obuz et al., 2004).

The literature suggests that degree of doneness impacts tenderness the most compared to other cooking factors such as method or heat source temperature. A higher degree of doneness typically results in a tougher end product, often negatively influencing consumer palatability (Cross, Stanfield, & Koch, 1976; Lawrence et al., 2001; Yancey, J. W. S.; Wharton, M. D.; Apple, 2011). Davey and Gilbert (1974) characterized two distinct phases of toughening during the cooking process, hypothesizing that between 40°C and 50°C, an observed decrease in tenderness was due to changes in the contractile system as seen by loss of myosin solubility, while collagen shrinkage resulted in the change seen between 65°C and 75°C. However, this

trend has not been consistent across muscles in other studies; Obuz and colleagues (2004) found a similar toughening phase between 60°C and 80°C in biceps femoris and deep pectoral, but an increase in tenderness between 40° C and 60° C in these muscles, and a general decrease in tenderness with increased degree of doneness in the longissimus lumborum.

Flavor

While tenderness has historically been recognized as the major factor influencing consumer acceptability, flavor is becoming increasingly important as the beef industry has strived to produce more consistently tender products (Killinger, Calkins, Umberger, Feuz, & Eskridge, 2004; Voges et al., 2007). Across all demographics studied in a 2006 survey, flavor was ranked as the highest motivator to beef steak and roast purchasing, followed closely by tenderness and juiciness (Reicks et al., 2011). Although likely perceived as a simple concept with a logical meaning by consumers, flavor is a complex attribute affected by several physiological mechanisms. Flavor has been defined as "the sum of those characteristics of any material taken in the mouth, perceived principally by the senses of taste and smell and also by the general tactile and pain receptors in the mouth" (Hall, 1968). Responses by the gustatory cells, olfactory bulb, and trigeminal nerves each contribute to flavor of meat. Basic tastes, including sweet, sour, bitter, salty, and umami, are detected by taste receptor cells that make up taste buds, which are distributed across papillae on the tongue (Chandrashekar, Hoon, Ryba, & Zuker, 2006). Aromas are sensed by olfactory receptors as what have been described as "odor images", which in combination with taste, motor manipulation, and even vision and hearing, produce flavor perception (Shepherd, 2005). The trigeminal nerve is responsible for sensory and motor functions of the mouth and face, including biting and chewing. This nasal trigeminal system is responsible for the sensations that are associated with different foods, such as the cool

feeling of menthol or the pungency of acetic acid, and consequently play a role in awareness of the flavor of foods (Laska, Distel, & Hudson, 1997).

Flavor Development

The shift from a blood-like taste and minimal odor of raw meat to the characteristic flavor of cooked beef demonstrates that this flavor is thermally derived. Reactions between non-volatile substrates present in raw meat during the cooking process result in production of thousands of volatile compounds that contribute significantly to the sensory perception of meat (Mottram, 1998; Van Ba, Hwang, Jeong, & Touseef, 2012). The Maillard reaction and lipid oxidation are the two major pathways that occur during heating to influence flavor.

The Maillard reaction is often referred to as non-enzymatic browning, and predominantly takes place when meat is cooked at high temperatures (C. R. Kerth & Miller, 2015). The process begins with a condensation reaction between an amino group and a reducing sugar; the product of this reaction is rearranged into an Amadori product if the sugar was an aldose, or a Heyns product in the case of a ketose sugar (Mottram, 1998). The amino group is then released, resulting in sugar fragmentation compounds that can undergo many reactions such as dehydrations, polymerizations, and cyclizations that the released amino acids can again participate in (Resconi, Escudero, & Campo, 2013). The Strecker reaction also takes place, in which amino acids are degraded by dicarbonyls that are formed in the Maillard reaction; this step is often regarded as part of the Maillard reaction but can also occur independently (Resconi et al., 2013) Characteristic of the Maillard reaction, compared to sugar caramelization, is the presence of amino groups that act as catalysts which allow for more intermediate products and a faster reaction rate (Van Boekel, 2006). Final products of these processes include furans, pyrroles, pyrazines, pyrroles, thiophenes, and thiazole among other heterocyclic compounds that generally

result in roasted, browned, meaty, and caramelized flavors (C. R. Kerth & Miller, 2015). Variation in the rate and extent of these reactions can be attributed to differences in the starting compounds, water activity, and pH of the meat matrix (Resconi et al., 2013).

Lipid oxidation during cooking results in the relatively rapid formation of compounds that lead to desirable meat flavor, but these reactions can also produce unfavorable flavor and odor notes during storage of raw or cooked meat when they occur at a much slower rate (Mottram, 1998). Both saturated and unsaturated fatty acids are present in meat products, but the fatty acid profile varies due to differences in breed, and production practices such as feeding regime. Oxidation of these compounds is promoted via heating during the cooking process, but exposure to light, oxygen, and metals can also induce oxidation reactions (Van Ba et al., 2012). Hydrocarbons, aldehydes, ketones, and carboxylic acids constitute some of the hundreds of compounds produced from lipid oxidation, primarily of fatty acids (Mottram, 1998). Compared to Maillard reaction products, compounds resulting from lipid oxidation generally have higher odor detection threshold values (Van Ba et al., 2012). Therefore, lipid-derived volatiles likely impact the overall aroma of meat to a lesser extent than the aforementioned heterocyclic compounds. Nonetheless, lipids are a component of all meat products and contribute to flavor differences between species, animals, and muscles.

Both the Maillard reaction and lipid oxidation can lead to a multitude of compounds, so the interaction of these molecules is to be expected. Kerth and Miller explained that these interactions sometimes means distinct products are formed, but can also lead to inhibition of typical products from being created (2015). Lipid degradation products are reported to block the progression of the Maillard reaction and subsequent production of heterocyclic aroma compounds (Shahidi, Samaranayaka, & Pegg, 2014). However, when new products are formed,

they are typically lower in odor intensity than the compounds resulting from either of the original pathways, and therefore contribute to product aroma to a lesser degree (C. R. Kerth & Miller, 2015).

Beef Flavor Analysis

As discussed, flavor is not a single attribute, although it was defined as such by the American Meat Science Association in 1978 in guidelines for sensory evaluation of meat products. The document and definition were updated in 1995, but a comprehensive tool for evaluating flavor of beef muscle cuts did not exist until Adhikari and colleagues published a lexicon created for this purpose in 2011. Due to the large number of compounds that have been identified and associated with specific flavors in meat (Calkins & Hodgen, 2007), it is critical to be able to characterize samples with respect to individual flavor attributes rather than using broad terms such as flavor desirability.

Various cooking methods, including grilling, broiling, outdoor grilling, and roasting, as well as five final internal temperatures were used in the creation of the lexicon as it is recognized that these impart dissimilar flavor notes to the meat. Additionally, treatments were selected to represent different muscle categories, quality grades, animal ages, aging technique, and packaging type in order to provide comprehensive results that would be applicable across a wide range of research situations (Adhikari et al., 2011). Since the inception of the tool, it has been beneficial in analyzing sensory characteristics to evaluate diverse treatment combinations (Grayson et al., 2016; Legako, Dinh, Miller, Adhikari, & Brooks, 2016; Semler, Woerner, Belk, Enns, & Tatum, 2016).

Beef Carcass Utilization

After observing a disturbing trend of significantly decreasing value of beef chuck and round over five years in the 1990s, the beef industry commenced research to enhance value of cuts within these subprimals (Yeh et al., 2018). Although by no means the first study to characterize beef muscles, an extensive profiling project conducted by Von Seggern and colleagues was part of this response (Von Seggern et al., 2005). Results provided insight that led to innovative fabrication practices to upgrade cuts that had previously been underutilized, with a prime example being the infraspinatus, commonly referred to as the flat iron (Von Seggern et al., 2005). Previous work had evaluated muscle differences within the beef carcass, but focused primarily on tenderness in regard to sensory attributes (Christensen, Johnson, West, Marshall, & Hargrove, 1991; R. C. Johnson et al., 1988; Prost, Pelczynska, & Kotula, 1975; Zinn, Gaskins, Gann, & Hedrick, 1970). Yet, even prior to the 1990s, data was provided regarding flavor, tenderness, and juiciness for thirteen beef muscles including the infraspinatus, rectus femoris, gluteus medius, and triceps brachii (McKeith et al., 1985). This study provided unique data at the time, but the sensory description included only overall desirability as a measure of flavor and did not incorporate variables such as cooking method or degree of doneness. Despite this earlier work, the muscle profiling study performed by Von Seggern lead to a greater industry response than had been experienced previously, likely due in part to the timing that allowed a tangible economic incentive to be realized, and the resources provided by funding agencies to disseminate information and implement changes (2005). This work lead to increased utilization of some cuts of the round and chuck, namely the infraspinatus which is now often sold as a steak cut that is appreciated and marketed for superior tenderness.

Despite characterization of inherent muscle traits, many cuts obtained from the ends of the carcass have still been lesser utilized due to the preference for tender and juicy meat products that are often obtained from the middle of the carcass. A void in the literature warrants further research as to how cooking methods could be applied to further increase the utilization and value of these cuts. While more recent studies have further considered variations in sensory characteristics among muscles (Bouton, Harris, & Hill, 1966; Hildrum et al., 2009; Hunt et al., 2014; Legako et al., 2015), few investigate the role that chefs or home cooks could play in improving quality of more affordable meat cuts. As proper cooking methods can positively influence consumer acceptability, as reflected by increase in palatability of the semitendinosus due to appropriate preparation techniques (Jung, Hwang, & Joo, 2016), it would be worthwhile to gain knowledge in this area. Doing so could benefit consumers as well as those along the beef supply chain by providing cost effective product options and while still increasing revenue (Yeh et al., 2018).

CHAPTER 5

BEEF FLAVOR MYOLOGY

Materials and Methods

Institutional Animal Care and Use Committee approval was not required for this study as samples were obtained from federally inspected harvest facilities.

Sample Collection, Fabrication, and Treatment Designation

The treatment outline is summarized in Table 2.1, and was designated in order to evaluate the effects of quality grade, final internal temperature, and cooking method on sensory profile of five beef muscles: rectus femoris (IMPS 167E), gluteus medius (IMPS 184B), infraspinatus (IMPS 114D PSO1), triceps brachii (IMPS 114E), and teres major (IMPS 114F). Two quality grades (USDA Select, Upper 2/3 Choice/Top Choice), three cooking methods (grill, pan grill, oven roast), and three final internal temperatures (58.3°C, 70°C, and 80°C) were studied. Grill and pan grill cooking methods were applied to 2.54 cm steak or medallion cuts from all muscles. Oven roasting was applied to 5.08 – 10.16 cm roasts from rectus femoris, gluteus medius, and triceps brachii, as well as whole muscles of infraspinatus and teres major. Additionally, whole teres major muscles were subjected to grill and pan grill treatments to represent common cooking practices for this cut. Each of 104 treatment combinations were replicated six times for a total of 612 pieces (N = 612).

Vacuum packaged beef was purchased directly from a commercial beef harvest facility and transported to the Colorado State University meat laboratory under refrigerated conditions (2°C). All product was aged for 14 days post-production prior to fabrication. From each respective muscle and quality grade, cuts were randomly assigned to treatment groups and

vacuum packaged. Rectus femoris, gluteus medius, and triceps brachii selected for oven roast treatments were trimmed of excess fat and portioned into 10.16 cm, 5.08 cm, and 5.08 cm roasts respectively. Infraspinatus muscles selected for grill and pan grill were portioned into two equal sections (IMPS 1114D PSO1), cutting perpendicular to the muscle fiber direction, while muscles selected for oven roast were left whole for the cooking process. Rectus femoris, gluteus medius, and triceps brachii selected for grill and pan grill treatments were trimmed of excess fat and portioned into 2.54 cm thick steaks to produce items 1167E, 1184B, and 1114E, respectively (*The Meat Buyer's Guide*, 2014). For gluteus medius and triceps brachii, two steaks and one roast were fabricated from a single subprimal; these were assigned to the same degree of doneness across each of the three cooking methods. Infraspinatus and rectus femoris subprimals were fabricated into either two steaks, or one roast; one of each steak originating from a single subprimal was assigned to pan grill and grill, within the same final temperature treatment. Due to the difference in treatment scheme for teres major, whole teres major muscles were utilized for all three cooking methods (grill, pan grill, oven roast). A single muscle was used for each separate treatment. Additional teres major muscles were portioned into 2.54 cm medallions (IMPS 1114F) for grill and pan grill treatments only. Samples were frozen at -20°C until analysis.

Cooking Methods

Gas Grill

Steaks were grilled on a Char-Broil® Performance 4 Burner Gas Grill (Model #463376117, Char-Broil, Columbus, GA) to the appropriate internal temperature. The grill was allowed to pre-heat until the external thermometer read 260°C prior to use. A type K thermocouple thermometer (SPLASH-PROOF SUPER-FAST® THERMAPEN®, ThermoWorks,

Lindon, UT) was used to record endpoint temperatures, measured in the geometric center of the cut. Grates were cleaned between samples to prevent residual char from contaminating other samples.

Pan Grill

Cuts were cooked to their assigned degree of doneness using a Le Creuset® Signature Square Skillet Grill (Le Creuset, West Ashley, SC). Before cooking, skillets were preheated on the open gas burner of a Southbend Commercial Range (Model #560 – AA 2TR, Southbend, Fuquay-Varina, NC) until an infrared thermometer (Mastercool, Randolf, NJ) measured the temperature of the skillet to be 204°C. Endpoint temperature was measured in the geometric center of each steak using a probe thermometer (SPLASH-PROOF SUPER-FAST® THERMAPEN®, ThermoWorks, Lindon, UT) and recorded for each cut. A clean skillet was used for each sample to prevent flavor contamination from previous samples.

Oven Roast

Roasts and whole subprimals were oven roasted to their prescribed degree of doneness in a commercial combination oven (Model SCC WE 61 E; Rational, Landsberg am Lech, Germany) preheated and set at 176°C and 0% humidity using default fan settings. Internal temperature was monitored in the geometric center of the piece using the oven core temperature probe (Model SCC WE 61 E; Rational, Landsberg am Lech, Germany). Endpoint internal temperature was measured in the geometric center of each steak using a probe thermometer (SPLASH-PROOF SUPER-FAST® THERMAPEN®, ThermoWorks, Lindon, UT) and recorded.

Trained Sensory Evaluation

Colorado State University graduate students served as taste panel members; training sessions were conducted prior to beginning sensory panels. All panelists were trained to

consistently and objectively evaluate flavor attributes based on the beef flavor lexicon outlined by Adhikari et al. (2011), including beef flavor ID, browned, roasted, bloody/serumy, metallic, fat-like, umami, sweet, sour, salty, bitter, burnt, butter, heated oil, cardboard, livery, green/hay-like, and earthy musty attributes (0 = none; 15 = extremely intense). Additionally, training sessions included instruction on evaluation of myofibrillar tenderness, connective tissue tenderness, overall tenderness, and juiciness (1 = extremely tough, abundant, extremely tough, and extremely dry; 15 = extremely tender, none, extremely tender, and extremely juicy). Training sessions were based on the Table 2.2 displays all attributes and references. Each taste panel session included twelve individual samples evaluated by six panelists; a total of 51 panel sessions were completed. Samples were randomly assigned to panels to such that duplicate treatments were not served on the same panel.

Roasts and steaks for sensory analysis were tempered in a at 0 – 4°C for 24 – 48 hours to reach an internal temperature of 2 – 4°C. Samples were cooked according to assigned to cooking method and degree of doneness treatment. After cooking, each sample was vacuum packaged and stored for 8 – 24 hours at 0 – 4°C until being transferred to a circulating water bath (Fisher Scientific™ Isotemp™ Heated Immersion Circulators: Model 6200 H24; set at 55°C) set at 55°C for 30 to 60 minutes prior to the sensory panel. For each panel, samples were removed from the water bath and the package, trimmed of all external fat and connective tissue, and cut into 1 cm^2 pieces to be served to panelists. For roasts from the rectus femoris, gluteus medius, and triceps brachii, a 2.54 cm thick slice was removed from the center of the roast and portioned into 1 cm^2 pieces. Panelists were served samples in individual booths under red filtered light to reduce bias arising from other panelists and internal meat color. Distilled water and unsalted soda crackers

were served between each sample and panelists were instructed to consume both in order to prevent residual flavor from influencing the subsequent sample.

Volatile Aromatic Compound Analysis

Volatile compound analysis was performed on the same cuts that were cooked for and served to trained panelists. Frozen samples from Colorado State University were shipped to Texas A&M University, where they were stored at -80°C until analysis. An Aroma Trax gas chromatography/mass spectrophotometer system was used for quantification of volatile compounds. To prepare for extraction, samples were placed in heated glass jars (473 mL), capped with a metal screw-top lid above a Teflon lid to prevent off-aromas, and placed in a 60°C water bath to thaw. A solid-phase micro-extraction (SPME) Portable Field Sampler (Supelco 504831, 75 μm Carboxen/ polydimethylsiloxane, Sigma-Aldrich, St. Louis, Mo) was used for headspace sampling, which was performed for 2 hours per sample after the sample reached 60°C. The SPME fiber was then inserted into the injection port of the gas chromatograph (GC). The sample was desorbed at 280°C before being loaded onto the first column ((30m X 0.53mm ID/ BPX5 (5% Phenyl Polysilphenylene-siloxane) X 0.5 μm, SGE Analytical Sciences, Austin, TX), of the multi-dimensional GC. As samples passed through this column, they were exposed to a starting temperature of 40°C, which increased at a rate of 7°C per minute until reaching 260°C in order to separate compounds on the basis of boiling point. Following the first column, compounds flowed to a second column, in which they were separated based on polarity.

At this point, a three-way valve diverted compounds into three separate columns, with one leading to the mass spectrometer (Agilient Technologies 5975 Series MSD, Santa Clara, CA) and the other two leading to two humidified sniff ports with glass nose pieces. These ports were heated to a temperature of 115° C. Panelists were trained to accurately analyze beef lexicon

aromas using the Aroma Trax program and software (program (MicroAnalytics-Aromatrax, Round Rock, TX).

Statistical Analysis

Before analysis of trained sensory panel data, individual panelist ratings were averaged to obtain a single sensory rating for each attribute of each sample. Due to the difference between the treatment scheme for teres major and other muscles, each muscle was analyzed separately. Data from the gluteus medius, infraspinatus, rectus femoris, and triceps brachii were analyzed as a 3-way factorial using quality grade, degree of doneness, and cooking method as fixed effects. Data from the teres major were analyzed utilizing two different statistical procedures due to the unbalanced design within muscle. Data from the teres major, roast thickness only, was analyzed as a 3-way factorial using quality grade, degree of doneness, and cooking method as fixed effects. To analyze the grill and pan grill treatments of teres major, oven roast was excluded and a 4-way factorial was used, with quality grade, thickness, degree of doneness, and cooking method as fixed effects. Panel number and feed order were included as random variables in all models. Data analysis was performed using the procedures of SAS (Version 9.4; SAS Inst. Inc., Cary, NC). For each analysis, main effect and interaction comparisons were tested for significance using PROC GLIMMIX with $\alpha = 0.05$ and the denominator degree of freedom was calculated by the Kenward-Roger method.

Results

Trained Sensory Analysis

Significant interactions were few and inconsistent among all muscles evaluated. To provide greater clarity with regard to the objectives of the study, main effects of quality grade,

degree of doneness, and cooking method are presented, as well as the effect of thickness for teres major.

Infraspinatus

Effects of degree of doneness, cooking method, and quality grade on the sensory characteristics of infraspinatus are displayed in Table 2.3. Degree of doneness had statistically significant influences on flavor, juiciness, and tenderness of infraspinatus steaks and roasts. Cuts cooked to a final temperature of 80°C had greater ($P < 0.01$) beef ID and browned intensity than those cooked to both 58.3°C and 70°C. Additionally, roasted intensity was greater ($P = 0.03$) for infraspinatus cooked to 80°C than samples cooked to 58.3°C. Bloody/serumy decreased ($P < 0.01$), whereas bitter and burnt flavors increased ($P < 0.01$), as degree of doneness increased from 58.3°C to 80°C. Infraspinatus cooked to 80°C had lower ($P < 0.05$) panel ratings for sour, metallic, and buttery flavor intensities than the two lower degrees of doneness. Fat-like and earthy/musty flavor panel ratings were greater ($P < 0.05$) in samples cooked to 70°C than those cooked to 80°C; the inverse ($P = 0.03$) was true for umami intensity. Panelists detected a more intense ($P < 0.01$) salty taste in infraspinatus that reached a final temperature of 80°C than with a final temperature of 58.3°C. Muscle fiber tenderness was higher ($P = 0.01$) and connective tissue tenderness exhibited a similar trend ($P = 0.08$) when infraspinatus was cooked to 58.3°C compared to 80°C, resulting in a more ($P = 0.02$) tender product overall. The final temperature of 80°C corresponded to a less juicy ($P < 0.01$) infraspinatus than either of the lower degrees of doneness.

Cooking method also impacted the flavor attributes and juiciness of infraspinatus cuts. Pan grilled steaks and roasts had greater ($P < 0.01$) panel ratings for salty, bitter, and burnt flavors, but lower intensity of bloody/serumy ($P < 0.01$) than both grilled and oven roasted

treatments. Compared to pan grilling, oven roasting increased ($P = 0.01$) livery flavor but decreased ($P < 0.01$) beef ID. Panel ratings for browned flavor increased ($P < 0.01$) across oven roast, grill, and pan grill treatments respectively. Grilling the infraspinatus produced lower ($P < 0.05$) trained panelist ratings for metallic intensity compared to oven roasting. Umami flavor was less prominent ($P < 0.05$) in oven roasted samples, whereas earthy/musty flavor was greater ($P < 0.01$). Sweet flavor was higher ($P < 0.05$) in the grilling treatment than pan grilling, and the highest ($P < 0.01$) panel ratings for sour were a result of oven roasting. Neither tenderness or juiciness attributes were significantly impacted ($P > 0.10$) by cooking method. No differences were found in infraspinatus sensory attributes corresponding to quality grade variation.

Gluteus Medius

Table 2.4 depicts the effects of quality grade, final temperature, and cook method on gluteus medius steaks and roasts. Quality grade influenced juiciness, as well as salty and buttery flavor notes. Top Choice gluteus medius was more ($P < 0.05$) juicy and buttery, but had a lower ($P < 0.01$) intensity of saltiness than the Select cuts.

Beef ID was not influenced by final temperature, but this treatment level did produce the greatest impact on sensory characteristics of the gluteus medius muscle overall. Panel ratings for browned flavor were higher ($P < 0.01$) when product was cooked to 80°C compared to both 58.3°C and 70°C, while metallic and sour intensities were lower ($P < 0.05$). There was a significant decrease ($P < 0.01$) in panelist ratings for bloody/serumy flavor at each increasing level of degree of doneness. The lowest final temperature treatment, 58.3°C, resulted in the lowest ($P < 0.01$) panel ratings for roasted flavor intensity, but the highest ($P < 0.01$) for buttery. Bitter, and burnt flavors differed ($P < 0.05$) between the 58.3°C and 80°C final temperatures, with greater intensity in the samples cooked to the higher temperature. As degree of doneness

increased, panel ratings for juiciness and muscle fiber tenderness decreased ($P < 0.01$) incrementally. Additionally, higher ($P < 0.01$) ratings for connective tissue tenderness were seen in the 58.3°C treatment compared to the higher final temperature treatments; this combination lead to greatest ($P < 0.01$) overall tenderness for cuts cooked to 58.3°C.

The flavor of gluteus medius was also influenced by the method used to cook the product. Oven roasting resulted in the lowest ($P < 0.01$) panelist ratings for beef ID, browned, and fat-like, but the highest ($P < 0.01$) for roasted intensity. Trained panel ratings for browned flavor were also lower ($P < 0.01$) for grilled than pan grilled cuts. Pan grilled cuts differed from grilled and oven roasted for several attributes; salty, bitter, and burnt ratings were higher ($P < 0.01$) for pan grilling, while sweet intensity was lower ($P < 0.01$). Cooking in the oven was associated with a greater ($P = 0.03$) intensity of cardboardy and livery when compared to pan grilling, but grilling was similar ($P > 0.10$) to both other treatments. The type of cooking method used did not influence ($P > 0.10$) any of the trained panelist ratings for tenderness or juiciness attributes.

Rectus Femoris

Effects of quality grade, final temperature, and cooking method on sensory attributes of rectus femoris are displayed in Table 2.5. Quality grade did impact several flavor attributes of rectus femoris. Panel ratings for fat-like, salty, buttery, and green/hay-like intensities were greater ($P < 0.05$) among the Top Choice cuts than Select. However, neither juiciness nor tenderness characteristics were related to differences in quality grade.

Final temperature again influenced the greatest number of sensory attributes, including flavor, juiciness, and tenderness. Panel ratings for beef ID were higher ($P = 0.03$) in the cuts cooked to 80°C than those cooked to 58.3°C, and browned flavor ratings were highest ($P < 0.01$) among the 80°C treatment. The two higher final temperatures produced higher ($P < 0.01$) roasted

intensity than cooking to 58.3°C. As with previously discussed muscles, ratings for bloody/serumy flavor decreased ($P < 0.01$) as final temperature increased; additionally, fat-like showed the same relationship ($P < 0.01$) with final temperature. Trained panelist ratings for metallic and sour flavor notes were lowest ($P < 0.01$) when samples were cooked to 80°C, while this final temperature lead to more ($P < 0.01$) intense salty, bitter, burnt, and heated oil flavors. Panel ratings for buttery flavor were highest ($P < 0.01$) in samples that were cooked to 58.3°C. Juiciness ratings decreased ($P < 0.01$) with increasing final temperature; consequently, the juiciest product was cooked to 58.3°C. Muscle fiber tenderness, connective tissue tenderness, and therefore overall tenderness, were lower ($P < 0.01$) among the samples cooked to 80°C.

Cooking method had an effect on flavor of rectus femoris cuts. Oven roasting produced the highest ($P < 0.01$) trained panelist ratings for roasted flavor, but the lowest ($P < 0.01$) ratings for beef flavor ID, fat-like, and buttery. As seen with other muscles, pan grilling resulted in the most intense ($P < 0.05$) salty, bitter, and burnt notes; this cooking method also lead to the highest ($P < 0.01$) ratings for umami flavor in rectus femoris. Grill and oven roast treatments did not differ in regard to green/hay-like flavor, but both had higher ratings ($P < 0.01$) than pan grilled samples. Trained panelist ratings for muscle fiber tenderness and overall tenderness approached significance ($P = 0.09$), with oven roasting exhibiting the highest tenderness ratings of the cooking methods.

Triceps Brachii

Effects of quality grade, final temperature, and method of cooking on sensory attributes of triceps brachii are presented in Table 2.6. All three treatment factors had an impact on the sensory characteristics of steaks and roasts from the triceps brachii muscle. Quality grade influenced more attributes of triceps brachii than any other muscle in the study. Cuts graded Top

Choice had greater ($P < 0.05$) intensities of several flavor notes: fat-like, umami, buttery, and green/hay-like. Panelist ratings for cardboardy were detected at higher ($P < 0.05$) levels in Select grade cuts. Additionally, tenderness was affected by quality grade, with Top Choice samples showing greater ($P < 0.01$) ratings for both muscle fiber tenderness and connective tissue amount, leading to a more ($P < 0.01$) tender product overall. These findings suggest that Top Choice triceps brachii will likely provide a more satisfactory eating experience than product graded USDA Select.

Consistent with other muscles evaluated, final temperature had the greatest impact on the sensory development of triceps brachii. Compared to the 58.3°C treatment, cuts cooked to 80°C provided lower ($P < 0.05$) trained panelist ratings for buttery flavor. Additionally, ratings for browned, burnt, and umami flavors were higher ($P < 0.01$) only when samples were cooked to the highest final temperature, 80°C. As degree of doneness increased across the three endpoint temperatures, panelists ratings for roasted and salty flavors increased ($P < 0.01$), while bloody/serumy and sour notes decreased ($P < 0.01$). Fat-like intensity was similar between cuts cooked to 58.3°C and 70°C, but was lower when the highest degree of doneness was reached ($P < 0.01$). Panelists perceived less ($P < 0.01$) bitterness in samples from the 58.3°C treatment than those from either of the higher final temperatures. Juiciness and tenderness were both affected by the temperatures to which steaks and roasts from the triceps brachii muscle were cooked. As final temperatures increased, the samples became less juicy ($P < 0.01$). Higher ($P < 0.01$) panel ratings for muscle fiber tenderness in samples cooked to 58.3°C resulted in a more ($P < 0.01$) tender product, since connective tissue amount did not differ ($P = 0.31$) across any endpoint temperatures.

Of the three cooking methods, oven roasting the triceps brachii resulted in the greatest change to flavor attributes. Panel ratings for roasted, cardboardy, and livery notes were higher ($P < 0.01$) in oven roasted steaks and roasts compared to grilled and pan grilled, while the intensity ratings for beef ID, umami, and saltiness were lower ($P < 0.01$). Oven roasting also lead to an increase ($P = 0.03$) in trained panelist ratings for sourness compared to the pan grilling treatment. Grilling resulted in the highest ($P < 0.01$) browned flavor ratings, followed by pan grilling, with oven roasted samples having the lowest ($P < 0.01$). In the same trend seen among other muscles, pan grilling produced samples with the highest ($P < 0.01$) intensities for bitter and burnt flavor notes. A more ($P < 0.01$) juicy product was achieved by using a roasting method for cooking.

Teres Major: Roast Thickness

In order to represent common preparation techniques for the teres major muscle, all cooking methods were applied to the roast, which consisted of the entire subprimal. Therefore, the sensory attributes resulting from the roast thickness only are presented in Table 2.7. Final temperature affected flavor, juiciness, and tenderness of teres major roasts, while quality grade and cooking method affected only flavor notes. The Top Choice quality grade was associated with higher ($P < 0.05$) ratings for metallic, sour, cardboard, livery, and earthy-musty flavor intensities. Although USDA Choice is often regarded as higher quality and therefore demands a higher price in the marketplace, this data suggested that the Top Choice quality grade of teres major roasts may impart flavor notes that are less desirable to consumers compared to the Select grade.

Roasts cooked to 58.3°C exhibited higher ($P < 0.05$) trained panelist ratings for metallic and sweet flavors compared to those cooked to 80°C. With incremental rises in degree of doneness, from 58.3°C to 80°C, the panelist ratings for intensity of browned and salty notes

increased ($P < 0.01$) and bloody/serumy, fat-like, and buttery flavors were reduced ($P < 0.01$). Although final temperatures of 70°C and 80°C showed similar ratings for bitter and burnt flavors, the roasts cooked to 58.3°C had lower intensities of these attributes. The highest degree of doneness produced samples that were rated higher ($P < 0.01$) for roasted flavor, and had a less ($P < 0.01$) intense sour taste. As with other muscles, degree of doneness had a considerable effect on juiciness and tenderness of teres major roasts. Cooking to increasingly higher internal temperatures resulted in a less juicy ($P < 0.01$) product, and lower ($P < 0.01$) muscle fiber tenderness and connective tissue tenderness ratings. Overall tenderness was not significantly different between the two higher final temperatures, but the most ($P < 0.01$) tender product resulted from the 58.3°C treatment.

Method of cooking impacted many of the flavor attributes measured. The grilled samples had higher ($P < 0.01$) intensity ratings for beef ID and umami. Panel ratings for browned flavor were highest ($P < 0.01$) for pan grilled samples, followed by grilled and oven roasted respectively ($P < 0.01$). The lowest ($P < 0.05$) ratings for roasted and sweet flavors were seen in roasts that were pan grilled, with grilling and oven roasting presenting similar ratings. Fat-like intensity differed ($P < 0.05$) between pan grilled and grilled treatments, with the latter being higher. Compared to oven roasted samples, pan grilled roasts were found to have higher ($P < 0.01$) trained panelist ratings for sour. All three cooking methods differed ($P < 0.01$) in regard to bitter and burnt flavor ratings, ranging from highest to lowest in pan grilled, grilled, and oven roasted samples respectively. A more ($P < 0.05$) buttery final product resulted from grilling than pan grilling. Panel ratings for salty, earthy/musty, and cardboardy were greater ($P < 0.01$) among oven roasted samples than the other two cooking methods, and earthy/musty ratings were also higher ($P < 0.01$) for oven roasting than pan grilling, but grilling was similar to both.

Teres Major: Grilled & Pan Grilled Steaks and Roasts

In order to compare 2.54 cm steaks with roasts (whole subprimals) of the teres major muscle, the oven roast cooking method was excluded from analysis since it was not represented in both thickness levels. Therefore, Table 2.8 displays the sensory characteristic results from two quality grades of teres major steaks and roasts cooked to three final temperatures using grilling and pan grilling cooking methods. Quality grade influenced several flavor attributes, with samples in the Top Choice quality grade category presenting higher ($P < 0.01$) ratings for metallic, sour, cardboard, livery, and earthy/musty notes, but a less ($P < 0.05$) burnt flavor.

Roasts differed from 2.54 cm steaks in regard to flavor, tenderness, and juiciness attributes. The panel ratings for intensity of browned, metallic, salty, and burnt flavors were higher ($P < 0.05$) for roasts, and umami and earthy/musty notes were lower ($P < 0.05$), compared to steaks. Trained panelists found that roasts were more ($P < 0.01$) juicy than steaks, but that muscle fiber tenderness, connective tissue tenderness, and overall tenderness ratings were higher ($P < 0.01$) for the teres major steaks.

Final internal temperature of the teres major influenced many sensory attributes measured in this study. The highest ($P < 0.05$) ratings for browned, roasted, and salty flavors were detected in samples cooked to 80°C, with no difference found between the 58.3°C and 70°C levels. Conversely, the 80°C treatment resulted in lower ($P < 0.05$) sweet, sour, and livery flavor notes than the other two degrees of doneness. Bloody/serumy and fat-like panel ratings differed across all three endpoint temperatures, decreasing ($P < 0.01$) as degree of doneness increased. Panel ratings showed that bitter and burnt flavors were significantly less intense ($P < 0.01$) and buttery was more intense ($P < 0.01$) among the samples cooked to the lowest temperature, 58.3°C. Ratings for juiciness and all tenderness attributes decreased ($P < 0.01$) with increasing final

temperature; subsequently, teres major steaks and roasts cooked to 80°C were the least juicy and tender.

Since only two cooking methods were compared in this analysis, fewer differences were found across methods of cooking, compared to other muscles. However, browned, bitter, and burnt panel ratings were still higher ($P < 0.01$) in the pan grilled samples than the grilled samples. Grilling resulted in a product that tasted more ($P < 0.01$) roasted, as well as having higher ($P < 0.05$) ratings for both sweet and sour notes.

Relationship between Treatments, Sensory Attributes, and Volatile Compounds

Volatile compounds were extracted from the headspace of cooked samples using the aforementioned method; compounds present in one or more of the samples are listed in Table 9.

Infraspinatus

Figure 1 shows a partial least squares regression biplot for trained sensory ratings and volatile aromatic compounds for infraspinatus. Component 1 explained 27.8% of the variation in cooking treatments, while 11.9% was attributed to component 2. The upper right quadrant contains several sensory attributes that were associated with the lowest degree of doneness (58.3°C) by trained panelist ratings, including tenderness, juiciness, and bloody-serumy. The n-aldehydes heptanol and tridecanal are found in this quadrant as well. The 70°C cooking temperature treatment was located in the lower left quadrant, but relatively close to the 58.3°C on both components 1 and 2. Pan grilled cooking treatment and 80°C internal temperature were more closely associated with browned, burnt, beef ID, salty, and bitter flavor notes. Clustering with these attributes were several pyrazine compounds, alkanes and alkenes (decane, decene, heptane), the aromatic hydrocarbon benzene, a Strecker aldehyde (benzaldehyde), and butanone. The oven roast cooking treatment was in closer proximity to attributes including cardboardy,

livery, and musty, which was consistent with trained panelist ratings for this treatment. Benzene (1,3-bis(1,1-dimethylethyl)), limonene, tridecane, and phenol (4-(1,1-dimethylpropyl) were the volatile compounds closest to oven roasting on the biplot. The grilled cooking treatment was farther removed from all volatile compounds than any other treatment.

Gluteus Medius

A partial least squares regression biplot for gluteus medius trained sensory ratings and volatile aroma compounds is presented as Figure 2. Component 1 represented 27.8% of variation seen in cooking treatments as explained by sensory ratings and volatile compounds, whereas component 2 represented 11.9%. The 58.3°C final temperature treatment clustered with tenderness attributes, as well as juiciness, bloody-serumy, sour, fat-like, and buttery. These trends were reflective of the results from trained sensory analysis previously described. Although this grouping was not closely associated with volatile compounds; dodecanal, octanol, and octanal were the nearest, being in the same quadrant. With respect to component 1, the pan grill treatment and 80°C temperature treatment were closely related to the largest cluster of volatile compounds, which included predominantly pyrazines, alkenes, and alkanes. Flavor notes, such as burnt, bitter, salty, and umami were also intermixed with these volatile compounds on the plot. Browned and beef ID flavors were most closely related to the pan grill cooking treatment than any other treatment when considering both components 1 and 2.

Rectus Femoris

Figure 3 displays a partial least squares regression biplot including trained sensory ratings and volatile compounds as independent variables and treatment combinations as dependent variables for rectus femoris. The variation in cooking treatments explained by component 1 of the biplot was 22%; component 2 explained 11.6% of the variation. The degree of doneness

treatments separated with respect to component 1, and volatile compound shifts were seen along this component as well. The left quadrants contained a greater number of volatile compounds as a whole, which was associated with the highest final cooking temperature (80°C); compounds included pyrazines, ketones, Strecker aldehydes, and alkanes. Closer to the 70°C treatment, a higher number of n-aldehydes were found, as well as several alkenes. The 58.3°C cooking treatment was less associated with volatile compounds than the higher degrees of doneness, but was again related to trained sensory ratings for tenderness and juiciness. The pan grill treatment was most closely associated with bitter, beef ID, burnt, and umami flavor notes, in addition to benzene and pyrazine volatile aromatic compounds. The grilling cook method was closest to ethanol, tetradecanal and tetradecane, as well as octanol and octene. Similar to other muscles, the oven roasting cook method was least associated with volatile compounds compared to grilling and pan grilling.

Triceps Brachii

The partial least squares regression biplot shown in Figure 4 contains trained sensory ratings and volatile aromatic compounds evaluated for the triceps brachii. Approximately 30% of the variation seen in cooking treatments was attributed to component 1 in the biplot, whereas 11% was attributed to component 2. As seen for other muscles, the highest degree of doneness (80°C) was associated with the greatest number of volatile compounds, compared to the lower temperatures. Pan grill, grill, and 80°C cooking treatments were located on the right half of the plot, which was also where the majority of the volatile compounds appear. Sensory ratings for tenderness, juiciness, sour, and metallic are most closely associated with the two lower degrees of doneness (58.3°C and 70°C). The volatile compounds most related to these treatments were ethanol, acetic acid, and butanone. The oven roast cook method was separated from most flavor

notes as well as volatile compounds, with cardboardy and benzene 1,3-bis(1,1-dimethylethyl) being the closest, respectively.

Teres Major

A partial least squares regression biplot relating trained sensory attributes and volatile aromatic compounds to cooking treatments for teres major is presented in Figure 5. For this muscle, component 1 explained 19.9% of the variation in treatment differences, while component 2 explained 8.3%. Volatile compounds clustered around the 70°C degree of doneness, with the cluster projecting towards the 80°C treatment, but few in proximity to the 58°C temperature. Similar to other muscles, the 58.3°C was clustered with trained sensory ratings for tenderness and juiciness, as well as metallic. Compounds close to the 80°C degree of doneness included decene, decane, heptane, octane, octanol, and methanethiol. The oven roast cook method again was farthest removed from volatile compound, but was in the same quadrant as several flavor notes including carboardy, livery, and musty, mirroring trained sensory panel analysis results. The pan grilled cooking method was not as closely associated with volatile flavor compounds as was seen in other muscles. However, pyrazines were the compounds in closest proximity on the biplot to this treatment, in addition to bitter and burnt being the closest trained sensory panelist ratings.

Discussion

In the 1990s, a decrease in the value of beef rounds and chucks prompted the industry and researchers to investigate new methods to increase utilization of these subprimals (Von Seggern et al., 2005). This initiative resulted in the development of a muscle profiling database that provides data on the compositional and physical traits of 39 beef muscles, including those studied in this project (Von Seggern et al., 2005). Other researchers have investigated the impact

of separating individual muscles, as well as the marketability of these value-added cuts (Ohman, Wiegand, Gruen, & Lorenzen, 2015; Yeh et al., 2018). Yet, current literature is limited in regard to sensory development in rectus femoris, gluteus medius, infraspinatus, triceps brachii, and teres major beef muscles as a result of various cooking techniques. Although lesser-known by consumers, these cuts could offer more affordable options for both home cooks and foodservice operations. However, in order to optimize their utilization and deliver a satisfactory product that will encourage continued purchase and consumption, more information about their sensory properties is needed. Therefore, this study provides data to describe the influences of cooking factors, as well as quality grade, on the eating characteristics of these cuts.

Across all muscles evaluated, final internal temperature had the largest impact, influencing the flavor profile, juiciness, and tenderness factors of all five cuts. Beef identify flavor was found to increase with increasing degree of doneness, except in the gluteus medius where final temperature did not play a role. Panelist ratings for browned flavor intensity were highest when samples were cooked to 80°C; this same trend was seen for roasted notes only in teres major and triceps brachii. Browned flavor results from the production of flavor compounds through the Maillard reaction and subsequent interactions, which occurs most readily at high temperatures (Van Ba et al., 2012; Van Boekel, 2006). Inherently, higher final temperatures correspond with longer cooking times, which allowed for greater development of positive aromatic flavor compounds associated with beefy, roasted, and browned flavor notes. As expected, longer cooking times needed to achieve a higher internal temperature also correlated a higher burnt intensity, accompanied by bitter flavor notes. The perceived intensity of bloody/serumy, as well as metallic and sour flavors, decreased with levels of increasing end-point temperature. Additionally, the fat-like flavor and mouthfeel was lesser when samples were

cooked to 80°C compared to the lower temperatures. Off-flavor intensities including cardboardy, livery, and green/hay-like were not consistently related to level of doneness, but cooking to a higher temperature did decrease earthy/musty notes in three of the five cuts. These results provide relevant data for flavor attributes of specific cuts, since most studies comparing muscles have focused on tenderness rather than overall flavor or off-flavor notes (Calkins & Hodgen, 2007).

Juiciness was greater at lower degrees of doneness, with samples cooked to high temperatures providing a consistently drier product across all muscles. Panelists rated juiciness for the 58.3°C treatment from 8.41 to 9.10, compared to the lower ratings of 5.82 to 7.50 at the 80°C temperature. Muscles variations were present, with rectus femoris being the least juicy cut at both temperatures. Following the same trend as juiciness, tenderness measures were indirectly related to degree of doneness. These results reflect similarities to data from Yancey et al. (2011), showing that shear force measurements increase with increasing end-point temperatures. To more accurately represent changes in tenderness, both muscle fiber and connective tissue tenderness were rated, and were used to calculate the overall tenderness rating. As described in Table 2.2, muscle fiber tenderness represented the fragmentation of the sample during mastication, whereas connective tissue rating was reflective of the amount of structural tissue that does not break down during chewing. The toughening of beef during thermal treatment has been reported to occur during two phases, the first between 40°C and 50°C and second between 65°C and 75°C (Bouton et al., 1966; Davey & Gilbert, 1974). Alterations in the toughness of beef during these phases has been attributed to collagen shrinkage and denaturation of myofibrillar proteins, but direct links between mechanisms and specific temperature ranges are inconsistent in the literature (Obuz et al., 2004).

Relative to degree of doneness in this study, the magnitude of change in muscle fiber tenderness was slightly greater than that of connective tissue, with both contributing to a general decrease in overall tenderness as internal temperatures rose. While the infraspinatus has gained attention for superior tenderness in recent years, the teres major obtained the highest tenderness ratings in this study. However, a review characterizing tenderness of numerous beef muscles lends support to current results, showing that infraspinatus and teres major are both tender cuts, gluteus medius and triceps brachii are tougher muscles, and rectus femoris falls between the two groups (Sullivan & Calkins, 2011). Just as consumer preference varies in relation to degree of doneness, there were positive and negative aspects associated with sensory attributes at each final temperature that can be taken into consideration when determining the appropriate preparation method for particular consumers.

Although not as influential as degree of doneness across the study as a whole, cooking method did play a role in the flavor development of all muscles, in addition to having several tenderness and juiciness effects. The most prominent beefy and roasted flavors were seen in the oven roasted treatment for three of the five cuts. It is important to keep in mind that the oven roasted treatment was performed on larger cuts, either 5.08 or 10.16 cm roasts or entire subprimals, compared to the 2.54 cm steaks that were subjected to the grill and pan grill treatments for most muscles. Additionally, the oven temperature was lower than the surface of the grill and pans used, resulting in a more gentle transfer of heat. Therefore, the longer cooking time for roasts likely played a role in development of these flavors. Conversely, pan grilling provided the most browned flavor in all muscles except the triceps brachii, where browned was rated higher when grilled. When meat is cooked at high temperatures, such as when pan grilled or grilled, the Maillard reaction is initiated due to the loss of water from the surface, resulting in

a browned color and unique flavor properties (C. R. Kerth & Miller, 2015). Perhaps the largest impact of cooking method among all muscles was the detection by panelists of more intense burnt and bitter flavors detected in the pan grilled samples; this was also related to an increase in salty notes in several of the muscles. Maughan et al. (2012) found bitter notes in meat to be inversely correlated with consumer acceptance, so it would be advised to use care with this preparation technique in order to avoid negative influences on palatability. Several flavors that were not greatly affected by degree of doneness were impacted by cooking method, with oven roasting producing the most differences compared to grilling and pan grilling. Generally, sour, cardboardy, and earthy/musty flavors were found at higher levels in the oven roasted treatment, and the savory taste of umami was lowest when oven roasting was used. Clearly, each method of cookery could have potential positive and negative effects, so desired flavor outcomes should be considered when deciding which may be most appropriate.

Effect of quality grade on sensory attributes varied between muscles, but this treatment factor had a much lower impact than either degree of doneness or cooking method. Although quality grade is often associated with increased desirability, research indicates that production of volatile flavor compounds is not necessarily correlated with higher amounts of intramuscular fat (Cross et al., 1980; Mottram, 1998; Legako et al., 2015). Evidence is conflicting as to whether quality grade is consistently associated with consumer eating satisfaction, with the effects on sensory attributes shown to be muscle dependent (Killinger et al., 2004; Koch, Crouse, & Dikeman, 1993; G. Smith et al., 1987). These factors may explain in part the lack of effect on sensory attributes seen in this study. Buttery was the most consistently altered attribute, with differences due to quality grade seen in three of the muscles. The teres major reflected the greatest flavor changes related to quality grade; several off flavors including metallic, sour,

livery, earthy, and cardboardy were elevated in the Top Choice cuts compared to the Select cuts. An increase in tenderness was associated with higher quality grade in the triceps brachii only, but could play a role in the eating quality of this cut. These results suggest that selecting higher quality grades of beef from the muscles included in this study, with the potential exception of triceps brachii, is unlikely to considerably alter the consumer eating experience. Therefore, purchasers could make more economically sound purchasing decisions through use of Select products rather paying a premium for Top Choice, without sacrificing quality.

Results from the partial least squares regression biplots from each of the five muscles exhibited trends related to treatment effect on volatile aromatic compounds. The lowest degree of doneness treatment (58.3° C) was least closely associated with volatile compounds compared to the two higher final temperatures. However, this treatment was in close proximity to two n-aldehydes in the infraspinatus, including heptanol. The n-aldehydes are primarily products of lipid oxidation, and heptanol in particular has been described as providing an oily or rancid flavor to meat products (C. Kerth, 2016).

As meat is exposed to thermal treatment, the Maillard reaction as well as lipid oxidation result in the formation of volatile compounds that contribute to the aroma and flavor of the end product. Data from this study indicate that cooking to temperatures above 58.3°C results in greater association with these compounds. The 80°C treatment was often associated with a cluster of aromatic compounds, most consistently including pyrazines, alkenes, and alkanes. Mottram explains the formation of pyrazines through the cooking process, and describes this class of compounds as the most common in meat cooked to well-done temperatures (Mottram, 1998). Pyrazines have previously been associated with increased consumer acceptability, as well as browned and nutty flavors (C. R. Kerth & Miller, 2015; Legako et al., 2016). In both

consumer and trained sensory panels, alkanes have been associated with increased ratings for positive attributes including overall liking, beef ID, and browned flavors (Legako et al., 2016).

Of the cooking method treatments, the pan grilled samples were associated with the greatest amount of volatile compounds. Similar to the 80°C treatment, the volatiles most related to pan grilling were pyrazines, alkenes, alkanes, as well as benzaldehyde and butanone in the infraspinatus. This cooking treatment was associated with higher trained sensory ratings for browned, beef ID, bitter, and burnt, which also tended to cluster with the pan grill treatment and associated volatile compounds on the linear regression biplots. Previous research characterizing volatile aromatic compounds in meat has not focused on cooking methods, especially with regard to individual muscles of the beef carcass. The type and concentration of volatile compounds in meat products can vary based on the muscle (Hunt et al., 2016); therefore, it is important to characterize the volatile profiles of individual muscles as was done in this study.

Conclusion

Descriptive sensory data for lesser-utilized beef cuts are necessary to promote beef carcass utilization for optimum consumer eating satisfaction. Quality grade, cooking method, and final temperature are known to affect palatability of beef in general, and results of this study indicate their influence on the infraspinatus, gluteus medius, rectus femoris, triceps brachii, and teres major specifically. Degree of doneness had the greatest impact on sensory attributes of all muscles evaluated, but the three cooking methods used also produced differences, primarily related to flavor. A higher quality grade was not associated with improved sensory characteristics across most muscles in the study, although tenderness was improved in the Top Choice triceps brachii. Muscle differences were evident for most treatment effects, highlighting the importance of understanding the properties of individual cuts in order to utilize them for a positive eating

experience. Doing so will benefit the meat industry, foodservice operations, in-home cooks, and ultimately consumers of beef through more effective beef carcass utilization, marketing, and preparation techniques.

Table 2.1. Treatment outline for five beef muscles incorporating two quality grades, two thickness levels, three cooking methods, and three degrees of doneness.

Muscle	Quality Grades	Thickness	Cooking Method	Degree of Doneness (°C)	Total Treatment Combinations
Rectus Femoris (Knuckle)	Select, Top Choice	1", Roast	Grill (1" only), Pan Grill (1" only), Oven Roast (Roast only)	58.3, 70, 80	18
Gluteus Medius	Select, Top Choice	1", Roast	Grill (1" only), Pan Grill (1" only), Oven Roast (Roast only)	58.3, 70, 80	18
Infraspinatus	Select, Top Choice	1", Roast	Grill (1" only), Pan Grill (1" only), Oven Roast (Roast only)	58.3, 70, 80	18
Triceps Brachii	Select, Top Choice	1", Roast	Grill (1" only), Pan Grill (1" only), Oven Roast (Roast only)	58.3, 70, 80	18
Teres Major	Select, Top Choice	1", Roast	Grill, Pan Grill, Oven Roast (Roast only)	58.3, 70, 80	30

Table 2.2. Definition and reference standards for beef descriptive flavor aromatics and basic taste sensory attributes and intensities from Adhikari et al. (2011) where 0 = none and 15 = extremely intense.

Attributes	Definition	Reference
Flavor		
Beef Flavor ID	Amount of beef flavor identity in the sample.	Swanson's beef broth = 5.0 80% lean ground beef = 7.0 Beef brisket (160 °F)= 11.0
Bitter	The fundamental taste factor associated with a caffeine solution.	0.01% caffeine solution = 2.0 0.02% caffeine solution = 3.5
Bloody/Serumy	The aromatics associated with blood on cooked meat products. Closely related to metallic aromatic.	USDA Choice strip steak (60 °C internal) = 5.5 Beef brisket = 6.0
Browned	Aromatic associated with the outside of grilled or broiled meat; seared but not blackened or burnt.	Steak cooked at high temperature (internal 137 °F, seared on outside)
Burnt	The sharp/acrid flavor note associated with over roasted pork muscle, something over baked or excessively browned in oil.	Arrowhead Mills Puffed Barley Cereal= 3.0
Buttery	Sweet, dairy-like aromatic associated with natural butter.	Land O'Lakes Unsalted butter = 7.0
Cardboardy	Aromatic associated with slightly oxidized fats and oils, reminiscent of wet cardboard packaging.	Dry cardboard (1 in. square) = 5.0 (a) Wet cardboard (1 in. square and 1 cup water) = 7.0 (a)
Fat-Like	The aromatics associated with cooked animal fat.	Hillshire farms Lit'l beef smokies = 7.0 Beef suet = 12.0
Green/Hay-like	Sharp, slightly pungent aromatics associated with green/plant/ vegetable matter such as parsley, spinach, pea pod, fresh cut grass etc.	Hexanal (50 mL) in propylene glycol (10 mL) at 5000ppm = 6.5 (a) Fresh parsley water (25 g steeped in water for 15 min then drained) = 9.0
Heated Oil	The aromatics associated with oil heated to a high temperature.	Wesson Vegetable Oil (1/2 cup, microwaved 3 min) = 7.0 Lay's Potato Chips (4 chips in medium snifter) = 4.0 (a)
Metallic	The impression of slightly oxidized metal, such as iron, copper, and silver spoons.	0.10% Potassium Chloride solution = 1.5 Select strip Steak (60 °C internal) = 4.0

		Dole Canned Pineapple Juice = 6.0
Musty-Earthy/ Humus	Musty, sweet, decaying vegetation.	Mushrooms = 0 1000 ppm of 2,6-Dimethylcyclohexanol = 9.0 (a)
Roasted	Aromatic associated with roasted meat.	Precooked Roast
Salty	The fundamental taste factor of which sodium chloride is typical.	0.15% sodium chloride solution = 1.5 0.25% sodium chloride solution = 3.5
Sour	The fundamental taste factor associated with citric acid.	0.015% citric acid solution = 1.5 0.050% citric acid solution = 3.5
Sweet	The fundamental taste factor associated with sucrose.	2.0% sucrose solution = 2.0
Umami	Flat, salty, somewhat brothy. The taste of glutamate, salts of amino acids and other molecules called nucleotides.	0.035% Accent Flavor Enhancer solution = 7.5
Juiciness		
Juiciness	The amount of perceived juice that is released from the product during mastication.	Carrot = 8.5; Mushroom = 10.0; Cucumber = 12.0 Apple = 13.5; Watermelon = 15.0 Choice top loin steak cooked to 58°C = 11.0 Choice top loin steak cooked to 80°C = 9.0
Tenderness		
Muscle fiber tenderness	The ease in which the muscle fiber fragments during mastication	Select eye of round steak cooked to 70°C = 9.0 Select tenderloin steak cooked to 70°C = 14.0
Connective tissue tenderness	The structural component of the muscle surrounding the muscle fiber that will not break down during mastication	Cross cut beef shank cooked to 70°C= 7.0 Select tenderloin cooked to 70°C = 14.0
Overall tenderness	Average of muscle fiber tenderness and connective tissue amount when connective tissue amount is 12 or less.	If connective tissue amount is 12 to15, then overall tenderness = the value of muscle fiber tenderness; If connective tissue amount is < 12 then overall tenderness is the average of connective tissue amount and muscle fiber tenderness.

Table 2.3. Trained sensory attributes[1] of USDA Select and Upper 2/3 Choice (Top Choice) beef infraspinatus cooked to three degrees of doneness using three cook methods.

	Quality Grade				Final Temperature (°C)					Cook Method				
Attribute	Select	Top Choice	SEM[2]	*P* – Value	58.3	70	80	SEM[2]	*P* – Value	Grill	Pan Grill	Oven Roast	SEM[2]	*P* – Value
Beef Flavor ID	6.93	6.92	0.10	0.93	6.66^{m}	6.78^{m}	7.33^{n}	0.12	<0.01	6.91^{xy}	7.18^{x}	6.68^{y}	0.12	<0.01
Browned	5.66	5.71	0.11	0.74	5.15^{m}	5.56^{m}	6.34^{o}	0.13	<0.01	5.59^{y}	6.39^{x}	5.08^{z}	0.13	<0.01
Roasted	6.51	6.48	0.11	0.81	6.32^{m}	6.47^{mn}	6.69^{n}	0.12	0.03	6.52	6.40	6.57	0.12	0.47
Bloody/Serumy	0.84	0.96	0.06	0.19	1.40^{m}	0.93^{n}	0.38^{o}	0.08	<0.01	0.96^{x}	0.69^{y}	1.06^{x}	0.08	<0.01
Metallic	0.98	1.01	0.06	0.65	1.09^{m}	1.03^{m}	0.86^{n}	0.07	0.02	0.87^{y}	1.03^{xy}	1.08^{x}	0.07	0.04
Fat-Like	1.55	1.61	0.07	0.61	1.60^{m}	1.72^{m}	1.42^{n}	0.09	0.04	1.74	1.54	1.47	0.09	0.06
Umami	0.85	0.86	0.06	0.90	0.82^{mn}	0.76^{n}	1.00^{m}	0.07	0.03	0.92^{x}	0.96^{x}	0.70^{y}	0.07	0.02
Sweet	0.35	0.36	0.04	0.91	0.35^{n}	0.35	0.36	0.05	0.96	0.44^{x}	0.26^{y}	0.36^{xy}	0.05	0.02
Sour	0.67	0.69	0.06	0.64	0.80^{m}	0.73^{m}	0.51^{n}	0.07	<0.01	0.65^{y}	0.53^{y}	0.90^{x}	0.07	<0.01
Salty	0.61	0.59	0.05	0.72	0.49^{n}	0.59^{mn}	0.71^{m}	0.06	<0.01	0.51^{y}	0.75^{x}	0.54^{y}	0.06	<0.01
Bitter	0.54	0.57	0.06	0.66	0.32^{o}	0.53^{n}	0.83^{m}	0.07	<0.01	0.36^{y}	0.92^{x}	0.39^{y}	0.07	<0.01
Burnt	0.34	0.31	0.06	0.68	0.06^{o}	0.28^{n}	0.63^{m}	0.07	<0.01	0.10^{x}	0.80^{y}	0.07^{x}	0.07	<0.01
Buttery	0.61	0.70	0.05	0.24	0.74^{m}	0.72^{m}	0.51^{n}	0.07	0.02	0.76	0.58	0.63	0.06	0.13
Heated Oil	0.07	0.05	0.02	0.31	0.05	0.05	0.08	0.02	0.32	0.06	0.08	0.05	0.02	0.57
Cardboardy	0.44	0.49	0.05	0.44	0.46	0.49	0.45	0.06	0.84	0.44^{y}	0.36^{y}	0.60^{x}	0.06	<0.01
Livery	0.41	0.61	0.07	0.06	0.50	0.55	0.47	0.09	0.80	0.50^{xy}	0.33^{y}	0.71^{x}	0.09	0.01
Green/Hay-Like	0.17	0.25	0.03	0.08	0.20	0.18	0.23	0.04	0.70	0.17	0.16	0.28	0.04	0.07
Earthy/Musty	0.72	0.72	0.05	0.98	0.74^{mn}	0.81^{m}	0.60^{n}	0.06	0.03	0.68^{x}	0.59^{x}	0.89^{y}	0.06	<0.01
Juiciness	8.30	8.30	0.15	0.99	8.81^{m}	8.60^{m}	7.49^{n}	0.18	<0.01	8.51	8.07	8.32	0.18	0.11
MF Tenderness[3]	9.61	9.46	0.18	0.56	10.01^{m}	9.52^{mn}	9.08^{n}	0.22	0.01	9.77	9.26	9.57	0.22	0.24
CT Tenderness[3]	9.81	9.60	0.18	0.39	10.07^{m}	9.68	9.37	0.22	0.08	9.93	9.41	9.78	0.22	0.22
O Tenderness[3]	9.64	9.45	0.17	0.41	9.97^{m}	9.50^{mn}	9.18^{n}	0.21	0.02	9.77	9.29	9.57	0.20	0.24

[a-b]Means in the same column lacking a common superscript differ due to quality grade (*P* – Value < 0.05)
[m-o] Means in the same column lacking a common superscript differ due to final temperature (*P* – Value < 0.05)
[x-z] Means in the same column lacking a common superscript differ due to cook method (*P* – Value < 0.05)
[1]Attributes were scored using a 15-point numerical scale: 0 = none and 15 = extremely intense.
tandard error (largest) of the least squares means
[3]MF Tenderness = Muscle Fiber Tenderness; CT Tenderness = Connective Tissue Tenderness; O Tenderness = Overall Tenderness

Table 2.4. Trained sensory attributes[1] of USDA Select and Upper 2/3 Choice (Top Choice) beef gluteus medius cooked to three degrees of doneness using three cook methods.

	Quality Grade				Final Temperature (°C)					Cook Method				
Attribute	Select	Top Choice	SEM[2]	*P* – Value	58.3	70	80	SEM[2]	*P* – Value	Grill	Pan Grill	Oven Roast	SEM[2]	*P* – Value
Beef Flavor ID	6.93	6.95	0.11	0.83	6.82	6.96	7.05	0.12	0.27	6.99[x]	7.27[x]	6.56[y]	0.13	<0.01
Browned	5.85	5.70	0.14	0.29	5.52[n]	5.62[n]	6.20[m]	0.16	<0.01	5.52[z]	6.20[x]	5.62[y]	0.16	<0.01
Roasted	6.75	6.76	0.12	0.94	6.39[n]	6.82[m]	7.05[m]	0.13	<0.01	6.59[y]	6.53[y]	7.16[x]	0.13	<0.01
Bloody/Serumy	0.60	0.66	0.06	0.40	1.18[m]	0.52[n]	0.19[n]	0.07	<0.01	0.75	0.57	0.58	0.06	0.07
Metallic	1.27	1.28	0.06	0.92	1.44[m]	1.30[m]	1.10[n]	0.07	<0.01	1.25	1.27	1.31	0.07	0.82
Fat-Like	0.96	1.06	0.05	0.12	1.28[m]	0.96[n]	0.80[n]	0.06	<0.01	1.17[x]	1.02[x]	0.86[y]	0.06	<0.01
Umami	0.80	0.85	0.05	0.39	0.80	0.81	0.87	0.06	0.58	0.84	0.89	0.74	0.06	0.09
Sweet	0.28	0.22	0.03	0.14	0.27	0.24	0.23	0.04	0.65	0.27[x]	0.16[y]	0.31[x]	0.03	<0.01
Sour	0.86	0.95	0.06	0.25	1.13[m]	1.02[m]	0.56[n]	0.07	<0.01	0.99	0.82	0.90	0.07	0.28
Salty	0.77[a]	0.63[b]	0.04	<0.01	0.63	0.71	0.76	0.05	0.09	0.69[y]	0.82[x]	0.59[y]	0.05	<0.01
Bitter	0.70	0.65	0.07	0.78	0.45[n]	0.67[mn]	0.87[m]	0.09	<0.01	0.53[y]	1.12[x]	0.35[y]	0.09	<0.01
Burnt	0.47	0.36	0.09	0.38	0.13[n]	0.39[n]	0.72[m]	0.11	<0.01	0.23[y]	0.99[x]	0.02[z]	0.11	<0.01
Buttery	0.25[b]	0.34[a]	0.03	0.04	0.46[m]	0.21[n]	0.21[n]	0.04	<0.01	0.36	0.28	0.24	0.04	0.10
Heated Oil	0.03	0.04	0.01	0.48	0.03	0.03	0.04	0.01	0.67	0.05	0.04	0.02	0.02	0.18
Cardboardy	0.41	0.45	0.04	0.42	0.45	0.44	0.42	0.05	0.90	0.45[xy]	0.25[y]	0.51[x]	0.05	0.03
Livery	0.21	0.23	0.04	0.67	0.28	0.22	0.15	0.04	0.09	0.23[xy]	0.14[y]	0.29[x]	0.04	0.03
Green/Hay-Like	0.23	0.25	0.04	0.68	0.28	0.27	0.17	0.04	0.10	0.26	0.21	0.25	0.04	0.68
Earthy/Musty	0.58	0.54	0.05	0.53	0.62	0.59	0.49	0.06	0.16	0.56	0.56	0.57	0.06	0.99
Juiciness	7.35[b]	7.78[a]	0.16	0.03	8.90[m]	7.52[n]	6.27[n]	0.20	<0.01	7.67	7.46	7.56	0.20	0.69
MF Tenderness[3]	8.63	8.59	0.14	0.85	9.63[m]	8.38[n]	7.83[n]	0.17	<0.01	8.73	8.35	8.76	0.17	0.17
CT Tenderness[3]	9.09	9.12	0.15	0.89	10.00[m]	8.76[n]	8.51[n]	0.18	<0.01	9.18	8.83	9.30	0.18	0.13
O Tenderness[3]	8.82	8.78	0.13	0.31	9.72[m]	8.54[n]	8.14[n]	0.16	<0.01	8.89	8.55	8.95	0.16	0.15

[a-b]Means in the same column lacking a common superscript differ due to quality grade (*P* – Value < 0.05)
[m-o] Means in the same column lacking a common superscript differ due to final temperature (*P* – Value < 0.05)
[x-z] Means in the same column lacking a common superscript differ due to cook method (*P* – Value < 0.05)
[1]Attributes were scored using a 15-point numerical scale: 0 = none and 15 = extremely intense.
[2]Standard error (largest) of the least squares means
[3]MF Tenderness = Muscle Fiber Tenderness; CT Tenderness = Connective Tissue Tenderness ; O Tenderness = Overall Tenderness

Table 2.5. Trained sensory attributes[1] of USDA Select and Upper 2/3 Choice (Top Choice) beef rectus femoris cooked to three degrees of doneness using three cook methods.

	Quality Grade				Final Temperature (°C)					Cook Method				
Attribute	Select	Top Choice	SEM[2]	*P* – Value	58.3	70	80	SEM[2]	*P* – Value	Grill	Pan Grill	Oven Roast	SEM[2]	*P* – Value
Beef Flavor ID	6.94	6.92	0.11	0.85	6.73^{n}	6.97^{mn}	7.10^{m}	0.13	0.03	6.98^{x}	7.18^{x}	6.64^{y}	0.13	<0.01
Browned	5.47	5.52	0.13	0.73	5.24^{n}	5.36^{n}	5.88^{m}	0.16	<0.01	5.61^{y}	6.41^{x}	4.46^{z}	0.16	<0.01
Roasted	6.93	6.74	0.11	0.08	6.45^{n}	6.91^{m}	7.16^{m}	0.12	<0.01	6.68^{y}	6.73^{y}	7.10^{x}	0.12	<0.01
Bloody/Serumy	0.51	0.60	0.07	0.32	1.01^{m}	0.53^{n}	0.12^{o}	0.09	<0.01	0.59	0.50	0.56	0.08	0.69
Metallic	1.09	1.02	0.06	0.40	1.25^{m}	1.09^{m}	0.82^{n}	0.07	<0.01	1.05	1.01	1.11	0.07	0.49
Fat-Like	0.91^{b}	1.08^{a}	0.06	0.01	1.17^{m}	1.00^{n}	0.81^{o}	0.07	<0.01	1.06^{x}	1.13^{x}	0.80^{y}	0.69	<0.01
Umami	0.75	0.82	0.05	0.21	0.74	0.81	0.81	0.06	0.52	0.73^{y}	0.94^{x}	0.69^{y}	0.06	<0.01
Sweet	0.24	0.29	0.03	0.27	0.29	0.27	0.23	0.04	0.52	0.28	0.22	0.29	0.04	0.32
Sour	0.56	0.63	0.05	0.29	0.69^{m}	0.67^{m}	0.43^{n}	0.06	<0.01	0.61	0.53	0.65	0.06	0.30
Salty	0.74^{a}	0.62^{b}	0.05	0.04	0.55^{n}	0.64^{n}	0.85^{m}	0.06	<0.01	0.63^{y}	0.78^{x}	0.63^{y}	0.06	0.05
Bitter	0.56	0.47	0.06	0.25	0.44^{n}	0.41^{n}	0.69^{m}	0.07	<0.01	0.44^{y}	0.80^{x}	0.29^{y}	0.07	<0.01
Burnt	0.23	0.16	0.06	0.36	0.12^{n}	0.04^{n}	0.41^{m}	0.07	<0.01	0.08^{y}	0.50^{x}	<0.01^{y}	0.07	<0.01
Buttery	0.25^{b}	0.40^{a}	0.03	<0.01	0.45^{m}	0.28^{n}	0.23^{n}	0.04	<0.01	0.38^{x}	0.38^{x}	0.20^{y}	0.04	<0.01
Heated Oil	0.04	0.05	0.01	0.38	0.03^{n}	0.02^{n}	0.08^{m}	0.01	0.01	0.06	0.05	0.03	0.01	0.26
Cardboardy	0.41	0.43	0.05	0.79	0.42	0.43	0.41	0.06	0.97	0.37	0.41	0.48	0.06	0.31
Livery	0.21	0.25	0.04	0.53	0.31	0.21	0.18	0.05	0.13	0.18	0.21	0.30	0.05	0.15
Green/Hay-Like	0.19^{b}	0.27^{a}	0.03	0.03	0.28	0.21	0.19	0.03	0.18	0.28^{x}	0.15^{y}	0.25^{x}	0.04	0.01
Earthy/Musty	0.48	0.48	0.04	0.95	0.51	0.52	0.40	0.05	0.09	0.45	0.53	0.45	0.05	0.29
Juiciness	7.23	7.35	0.17	0.56	8.41^{m}	7.64^{n}	5.82^{o}	0.19	<0.01	7.16	7.23	7.49	0.20	0.32
MF Tenderness[3]	8.82	8.68	0.16	0.45	9.28^{m}	8.91^{m}	8.06^{n}	0.18	<0.01	8.52	8.71	9.03	0.18	0.09
CT Tenderness[3]	9.38	9.07	0.12	0.05	9.73^{m}	9.40^{m}	8.54^{n}	0.15	<0.01	9.08	9.14	9.45	0.14	0.13
O Tenderness[3]	9.05	8.82	0.13	0.15	9.42^{m}	9.09^{m}	8.29^{n}	0.15	<0.01	8.75	8.87	9.18	0.15	0.09

[a-b]Means in the same column lacking a common superscript differ due to quality grade (*P* – Value < 0.05)
[m-o] Means in the same column lacking a common superscript differ due to final temperature (*P* – Value < 0.05)
[x-z] Means in the same column lacking a common superscript differ due to cook method (*P* – Value < 0.05)
[1]Attributes were scored using a 15-point numerical scale: 0 = none and 15 = extremely intense.
[2]Standard error (largest) of the least squares means
[3]MF Tenderness = Muscle Fiber Tenderness; CT Tenderness = Connective Tissue Tenderness; O Tenderness = Overall Tenderness

Table 2.6. Trained sensory attributes[1] of USDA Select and Upper 2/3 Choice (Top Choice) beef triceps brachii cooked to three degrees of doneness using three cook methods.

	Quality Grade				Final Temperature (°C)					Cook Method				
Attribute	Select	Top Choice	SEM[2]	*P* – Value	58.3	70	80	SEM[2]	*P* – Value	Grill	Pan Grill	Oven Roast	SEM[2]	*P* – Value
Beef Flavor ID	7.04	7.12	0.09	0.22	6.94	7.12	7.29	0.12	0.07	7.12^{x}	7.20^{x}	6.85^{y}	0.11	<0.01
Browned	5.63	5.69	0.11	0.69	5.41^{n}	5.55^{n}	6.03^{m}	0.14	<0.01	5.84^{x}	6.42^{y}	4.73^{z}	0.14	<0.01
Roasted	6.83	6.83	0.08	0.99	6.42^{o}	6.90^{n}	7.17^{m}	0.10	<0.01	6.69^{y}	6.69^{y}	7.11^{x}	0.10	<0.01
Bloody/Serumy	0.65	0.62	0.06	0.72	1.21^{m}	0.53^{n}	0.17^{o}	0.07	<0.01	0.71	0.57	0.63	0.07	0.34
Metallic	1.14	1.10	0.05	0.51	1.27^{m}	1.18^{n}	0.90^{o}	0.06	<0.01	1.10	1.11	1.15	0.06	0.67
Fat-Like	1.01^{b}	1.13^{a}	0.04	0.01	1.19^{m}	1.12^{m}	0.89^{n}	0.04	<0.01	1.06	1.07	1.07	0.04	0.95
Umami	0.81^{b}	0.96^{a}	0.05	<0.01	0.81^{o}	0.83^{n}	1.02^{m}	0.06	<0.01	0.92^{x}	0.98^{x}	0.75^{y}	0.06	<0.01
Sweet	0.24	0.28	0.03	0.35	0.27	0.28	0.24	0.04	0.69	0.22	0.24	0.32	0.04	0.11
Sour	0.68	0.68	0.08	0.99	0.88^{m}	0.69^{n}	0.47^{o}	0.09	<0.01	0.67^{xy}	0.57^{y}	0.81^{x}	0.09	0.03
Salty	0.67	0.69	0.04	0.55	0.53^{m}	0.67^{n}	0.84^{o}	0.05	<0.01	0.74^{x}	0.78^{x}	0.52^{y}	0.05	<0.01
Bitter	0.46	0.49	0.05	0.65	0.31^{o}	0.49^{m}	0.64^{m}	0.06	<0.01	0.43^{x}	0.71^{y}	0.29^{x}	0.06	<0.01
Burnt	0.24	0.16	0.05	0.23	0.06^{o}	0.17^{n}	0.38^{m}	0.06	<0.01	0.14^{y}	0.46^{x}	<0.01^{y}	0.06	<0.01
Buttery	0.28^{b}	0.40^{a}	0.03	<0.01	0.43^{m}	0.34^{mn}	0.26^{n}	0.04	<0.01	0.35	0.36	0.31	0.04	0.52
Heated Oil	0.05	0.04	0.01	0.28	0.03	0.06	0.06	0.01	0.27	0.04	0.06	0.04	0.01	0.63
Cardboardy	0.50^{a}	0.38^{b}	0.04	0.03	0.46	0.42	0.43	0.05	0.83	0.38^{x}	0.37^{x}	0.55^{y}	0.05	<0.01
Livery	0.22	0.23	0.04	0.98	0.26	0.26	0.16	0.05	0.17	0.17^{y}	0.15^{y}	0.36^{x}	0.05	<0.01
Green/Hay-Like	0.15^{b}	0.28^{a}	0.03	<0.01	0.25	0.24	0.15	0.04	0.09	0.22	0.20	0.23	0.04	0.85
Earthy/Musty	0.43	0.49	0.05	0.29	0.46	0.53	0.40	0.05	0.15	0.47	0.40	0.51	0.05	0.28
Juiciness	7.58	7.86	0.15	0.09	8.82^{m}	7.66^{n}	6.67^{o}	0.17	<0.01	7.56^{y}	7.49^{y}	8.10^{x}	0.17	<0.01
MF Tenderness[3]	8.10^{b}	8.82^{a}	0.14	<0.01	8.94^{m}	8.27^{n}	8.17^{n}	0.17	<0.01	8.56	8.51	8.32	0.17	0.56
CT Tenderness[3]	8.62^{b}	9.22^{a}	0.14	<0.01	9.12	8.85	8.78	0.17	0.31	8.88	9.00	8.88	0.17	0.85
O Tenderness[3]	8.31^{b}	8.97^{a}	0.12	<0.01	8.98^{m}	8.52^{n}	8.43^{o}	0.15	0.02	8.69	8.70	8.54	0.15	0.70

[a-b]Means in the same column lacking a common superscript differ due to quality grade (*P* – Value < 0.05)
[m-o] Means in the same column lacking a common superscript differ due to final temperature (*P* – Value < 0.05)
[x-z] Means in the same column lacking a common superscript differ due to cook method (*P* – Value < 0.05)
[1]Attributes were scored using a 15-point numerical scale: 0 = none and 15 = extremely intense.
[2]Standard error (largest) of the least squares means
[3]MF Tenderness = Muscle Fiber Tenderness; CT Tenderness = Connective Tissue Tenderness; O Tenderness = Overall Tenderness

Table 2.7. Trained sensory attributes[1] of USDA Select and Upper 2/3 Choice (Top Choice) beef teres major, roast thickness only, cooked to three degrees of doneness using three cook methods.

	Quality Grade				Final Temperature (°C)					Cook Method				
Attribute	Select	Top Choice	SEM[2]	*P* – Value	58.3	70	80	SEM[2]	*P* – Value	Grill	Pan Grill	Oven Roast	SEM[2]	*P* – Value
Beef Flavor ID	6.92	6.90	0.11	0.90	6.74	6.89	7.11	0.13	0.07	7.20[x]	6.71[y]	6.81[y]	0.13	<0.01
Browned	5.88	5.93	0.13	0.71	5.53[o]	5.90[n]	6.27[m]	0.15	<0.01	6.00[x]	6.49[y]	5.24[z]	0.15	<0.01
Roasted	6.67	6.73	0.14	0.72	6.42[n]	6.64[n]	7.04[m]	0.16	<0.01	6.85[x]	6.25[y]	7.00[x]	0.16	<0.01
Bloody/Serumy	0.74	0.84	0.08	0.23	1.32[m]	0.79[n]	0.23[o]	0.10	<0.01	0.78	0.74	0.82	0.10	0.78
Metallic	1.17[b]	1.34[a]	0.06	0.02	1.39[m]	1.24[mn]	1.15[n]	0.07	0.02	1.30	1.28	1.20	0.07	0.47
Fat-Like	1.23	1.22	0.06	0.87	1.44[m]	1.25[n]	0.98[o]	0.07	<0.01	1.34[x]	1.13[y]	1.21[xy]	0.07	0.05
Umami	0.74	0.71	0.06	0.64	0.66	0.73	0.78	0.07	0.39	0.87[x]	0.59[y]	0.70[y]	0.07	<0.01
Sweet	0.25	0.24	0.03	0.84	0.30[m]	0.26[mn]	0.18[n]	0.04	0.04	0.30[x]	0.17[y]	0.27[x]	0.04	0.02
Sour	0.61[b]	1.13[a]	0.05	<0.01	0.97[m]	0.95[m]	0.69[n]	0.07	<0.01	0.86[xy]	1.03[x]	0.72[y]	0.07	<0.01
Salty	0.75	0.77	0.04	0.78	0.63[o]	0.76[n]	0.89[m]	0.05	<0.01	0.79[y]	0.59[y]	0.90[x]	0.05	<0.01
Bitter	1.05	0.96	0.10	0.52	0.69[n]	1.13[m]	1.20[m]	0.12	<0.01	0.81[y]	1.88[x]	0.34[z]	0.12	<0.01
Burnt	0.94	0.69	0.12	0.15	0.38[n]	0.96[m]	1.10[m]	0.15	<0.01	0.44[y]	1.99[x]	<0.01[z]	0.15	<0.01
Buttery	0.38	0.35	0.04	0.60	0.51[m]	0.37[n]	0.23[o]	0.05	<0.01	0.46[x]	0.31[y]	0.34[xy]	0.05	0.05
Heated Oil	0.10	0.07	0.02	0.18	0.08	0.12	0.05	0.02	0.08	0.08	0.10	0.08	0.02	0.54
Cardboardy	0.46[b]	0.74[a]	0.06	<0.01	0.65	0.65	0.51	0.07	0.16	0.50[y]	0.49[y]	0.82[x]	0.07	<0.01
Livery	0.26[b]	0.42[a]	0.05	<0.01	0.43	0.30	0.29	0.05	0.06	0.31	0.29	0.43	0.05	0.07
Green/Hay-Like	0.20	0.27	0.03	0.05	0.28	0.24	0.18	0.03	0.07	0.22[xy]	0.17[y]	0.30[x]	0.03	0.02
Earthy/Musty	0.52[b]	0.92[a]	0.06	<0.01	0.79	0.70	0.67	0.07	0.40	0.60[y]	0.62[y]	0.94[x]	0.07	<0.01
Juiciness	8.12	8.43	0.15	0.09	9.10[m]	8.55[n]	7.18[o]	0.17	<0.01	8.21	8.39	8.24	0.17	0.66
MF Tenderness[3]	9.69	9.59	0.16	0.64	10.34[m]	9.54[n]	9.03[o]	0.19	<0.01	9.69	9.60	9.62	0.19	0.92
CT Tenderness[3]	10.02	9.99	0.16	0.88	10.56[m]	9.97[n]	9.48[o]	0.18	<0.01	10.01	9.82	10.17	0.18	0.32
O Tenderness[3]	9.77	9.68	0.15	0.63	10.32[m]	9.63[n]	9.23[n]	0.17	<0.01	9.77	9.62	9.79	0.17	0.71

[a-b]Means in the same column lacking a common superscript differ due to quality grade (*P* – Value < 0.05)
[m-o] Means in the same column lacking a common superscript differ due to final temperature (*P* – Value < 0.05)
[x-z] Means in the same column lacking a common superscript differ due to cook method (*P* – Value < 0.05)
[1]Attributes were scored using a 15-point numerical scale: 0 = none and 15 = extremely intense.
[2]Standard error (largest) of the least squares means
[3]MF Tenderness = Muscle Fiber Tenderness; CT Tenderness = Connective Tissue Tenderness; O Tenderness = Overall Tenderness

Table 2.8. Trained sensory attributes[1] of USDA Select and Upper 2/3 Choice (Top Choice) beef teres major 1 inch steaks and roasts cooked to three degrees of doneness using two cook methods.

	Quality Grade				Thickness				Final Temperature (°C)					Cook Method			
Attribute	Select	Top Choice	SEM[2]	*P* – Value	Roast	2.54 cm	SEM[2]	*P* - Value	58.3	70	80	SEM[2]	*P* – Value	Grill	Pan Grill	SEM[2]	*P* – Value
Beef Flavor ID	7.03	6.95	0.10	0.39	6.98	7.01	0.10	0.75	6.88	6.99	7.11	0.11	0.14	7.05	6.93	0.10	0.20
Browned	6.13	6.14	0.12	0.98	6.26[w]	6.01[x]	0.12	0.05	5.84[y]	6.04[y]	6.51[x]	0.13	<0.01	5.94[y]	6.33[x]	0.12	<0.01
Roasted	6.51	6.58	0.09	0.54	6.55	6.54	0.09	0.90	6.33[y]	6.50[y]	6.81[x]	0.10	<0.01	6.68[x]	6.41[y]	0.09	<0.01
Bloody/Serumy	0.64	0.76	0.06	0.10	0.77	0.63	0.06	0.06	1.16[x]	0.74[y]	0.20[w]	0.07	<0.01	0.74	0.66	0.06	0.32
Metallic	1.11[x]	1.26[x]	0.05	<0.01	1.31[w]	1.07[x]	0.05	<0.01	1.25	1.21	1.10	0.06	0.09	1.19	1.18	0.05	0.90
Fat-Like	1.24	1.20	0.05	0.53	1.20	1.24	0.05	0.56	1.48[x]	1.24[y]	0.95[w]	0.06	<0.01	1.24	1.21	0.05	0.56
Umami	0.81	0.72	0.05	0.08	0.71[x]	0.82[w]	0.05	0.02	0.75	0.75	0.79	0.06	0.77	0.82[x]	0.71[y]	0.05	0.03
Sweet	0.25	0.27	0.03	0.60	0.23	0.29	0.03	0.12	0.30[x]	0.30[x]	0.18[y]	0.04	0.02	0.33[x]	0.19[y]	0.03	<0.01
Sour	0.60[x]	1.11[x]	0.06	<0.01	0.80	0.90	0.06	0.13	0.95[x]	0.90[x]	0.71[y]	0.07	<0.01	0.91	0.79	0.06	0.08
Salty	0.77	0.78	0.04	0.76	0.85[w]	0.69[x]	0.04	<0.01	0.65[y]	0.76[y]	0.90[x]	0.05	<0.01	0.72	0.82	0.04	0.04
Bitter	1.08	0.93	0.08	0.15	1.34	0.66	0.08	<0.01	0.69[y]	1.06[y]	1.26[x]	0.10	<0.01	0.69[y]	1.32[x]	0.08	<0.01
Burnt	0.90[x]	0.61[x]	0.10	0.03	1.22[w]	0.29[x]	0.10	<0.01	0.35[y]	0.82[y]	1.10[x]	0.12	<0.01	0.31[y]	1.20[x]	0.10	<0.01
Buttery	0.42	0.39	0.03	0.54	0.38	0.43	0.03	0.32	0.56[x]	0.39[y]	0.26[y]	0.04	<0.01	0.43	0.38	0.03	0.27
Heated Oil	0.09	0.06	0.02	0.06	0.09	0.06	0.02	0.21	0.08	0.10	0.05	0.02	0.10	0.06	0.09	0.02	0.12
Cardboardy	0.46[x]	0.64[x]	0.05	<0.01	0.50	0.60	0.05	0.09	0.57	0.62	0.46	0.06	0.09	0.57	0.53	0.05	0.51
Livery	0.26[x]	0.41[x]	0.04	<0.01	0.31	0.36	0.04	0.25	0.40[x]	0.36[x]	0.24[y]	0.05	0.01	0.35	0.31	0.04	0.38
Green/Hay-Like	0.19	0.25	0.03	0.07	0.20	0.25	0.03	0.16	0.24	0.25	0.17	0.03	0.11	0.24	0.20	0.03	0.22
Earthy/Musty	0.55[x]	0.83[x]	0.05	<0.01	0.61[x]	0.77[w]	0.05	<0.01	0.76	0.70	0.60	0.06	0.08	0.70	0.68	0.05	0.74
Juiciness	7.81	8.05	0.12	0.11	8.19[w]	7.67[x]	0.12	<0.01	8.89[x]	8.03[y]	6.87[w]	0.14	<0.01	7.89	7.98	0.12	0.46
MF Tenderness[3]	9.83	9.82	0.14	0.96	9.63[x]	10.02[w]	0.14	0.03	10.72[x]	9.77[y]	8.99[w]	0.17	<0.01	9.83	9.82	0.14	0.95
CT Tenderness[3]	10.21	10.21	0.13	0.99	9.90[x]	10.52[w]	0.13	<0.01	10.95[x]	10.17[y]	9.52[w]	0.15	<0.01	10.24	10.18	0.13	0.70
O Tenderness[3]	9.91	9.91	0.13	0.99	9.67[x]	10.15[w]	0.13	<0.01	10.68[x]	9.85[y]	9.21[w]	0.15	<0.01	9.95	9.88	0.13	0.64

[w-x]Means in the same column lacking a common superscript differ due to quality grade (*P* – Value < 0.05)
[w-x] Means in the same column lacking a common superscript differ due to cut thickness (*P* – Value < 0.05)
[w-y] Means in the same column lacking a common superscript differ due to final temperature (*P* – Value < 0.05)
[w-y] Means in the same column lacking a common superscript differ due to cook method (*P* – Value < 0.05)
[1]Attributes were scored using a 15-point numerical scale: 0 = none and 15 = extremely intense.
[2]Standard error (largest) of the least squares means
[3]MF Tenderness = Muscle Fiber Tenderness; CT Tenderness = Connective Tissue Tenderness; O Tenderness = Overall Tenderness

Table 2.9. Volatile aromatic chemical compounds identified in one or more muscles.

Chemical Name
1-Decene
1-Dodecene
1-Heptanol
1-Octanol
1-Octen-3-Ol
1-Octene
2-Butanone
2-Butanone, 3-hydroxy
2-Heptanone
2-Methyl Pyrazine
2-Octene
2-Pentanone
2,3-Octanedione
2,3,5-trimethyl pyrazine
3-Dodecen-1-Al
Acetic acid
Benzaldehyde
Benzene, 1,2-dimethyl
Benzene, 1,3-bis(1,1-dimethylethyl)
Benzene, methyl
Benzeneacetaldehyde
Benzoic acid
Butanal, 2-methyl-
Butanal, 3-methyl-
Carbon disulfide
Decanal
Decane
Disulfide, dimethyl
dl-Limonene
Dodecanal
Dodecane
Ethanol

Ethanol, 2-(hexyloxy)-
Ethanone, 1-(1H-pyrrol-2-yl)
Furan, 2-pentyl
Heptanal
Heptane, 3-methyl
Heptane, 3-methylene
Hexanal
Indole
Methanethiol
Methyllaurte
N-Heptanal
Nonanal
Octanal
Octane
Pentadecane
Pentanal
Phenol
Phenol, 4-(1,1-dimethylpropyl)
Pyrazine, 2-ethyl-6-methyl
Pyrazine, 2,5-dimethyl
Pyrazine, 2,6-dimethyl
Pyrazine, 3-ethyl-2,5-dimethyl
Pyrazine, methyl
Pyrazine, trimethyl
Styrene
Tetradecanal
Tetradecane
Toluene
Tridecanal
Tridecane
Undecanal
Xylene

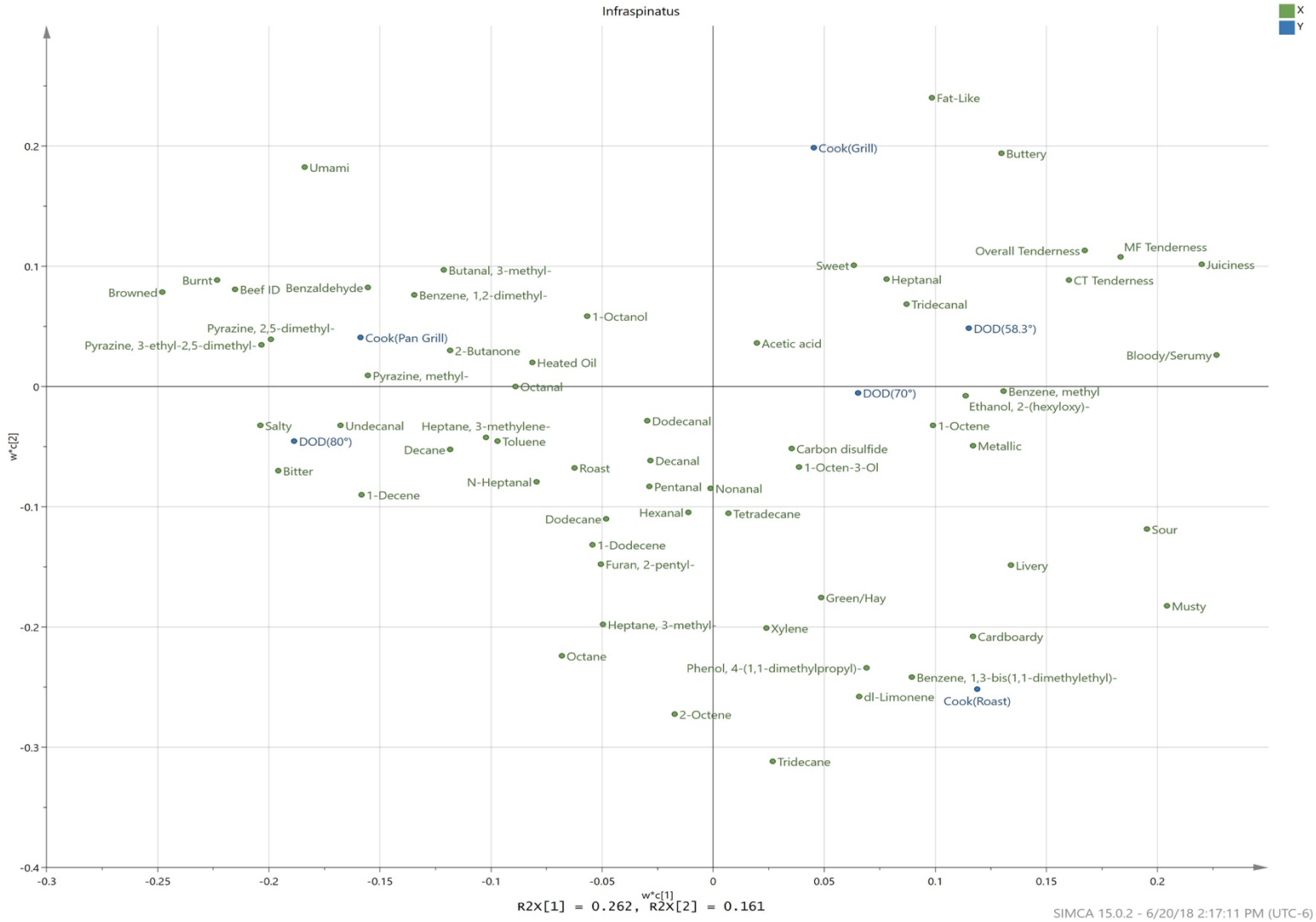

Figure 2.1. Partial least squares regression biplot for trained sensory ratings and volatile aromatic compounds as related to cooking treatments for infraspinatus.

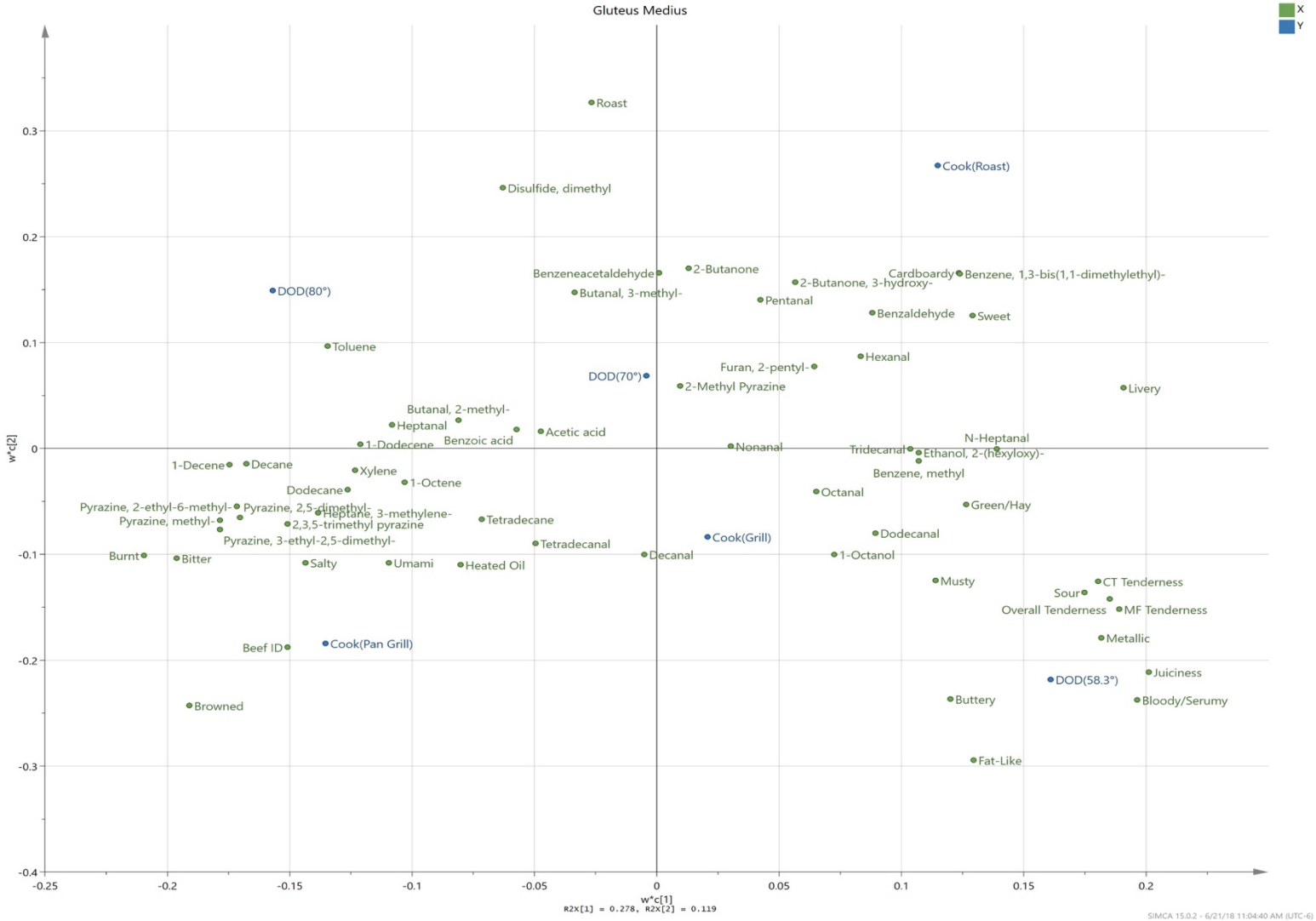

Figure 2.2. Partial least squares regression biplot for trained sensory ratings and volatile aromatic compounds as related to cooking treatments for gluteus medius.

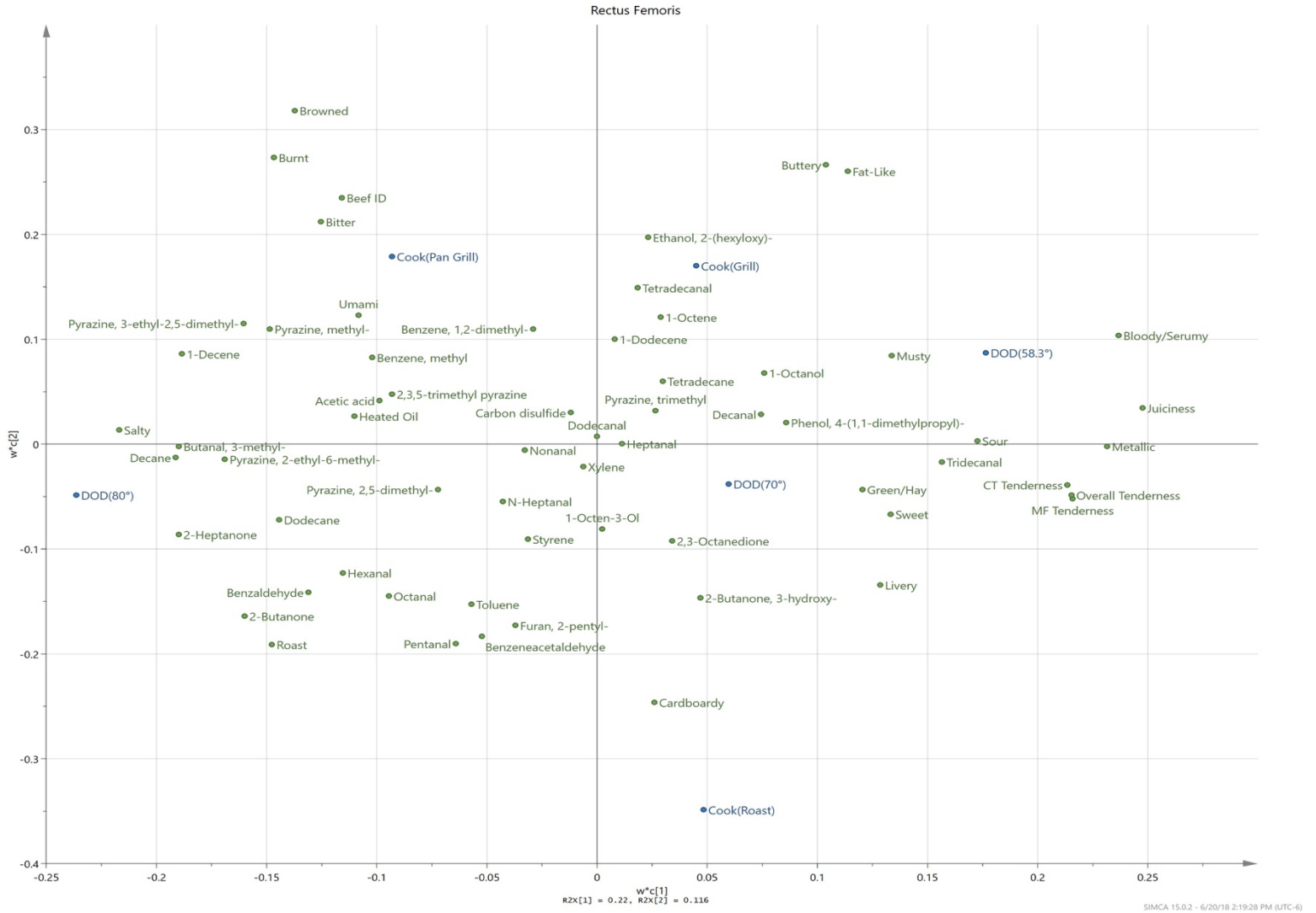

Figure 2.3. Partial least squares regression biplot for trained sensory ratings and volatile aromatic compounds as related to cooking treatments for rectus femoris.

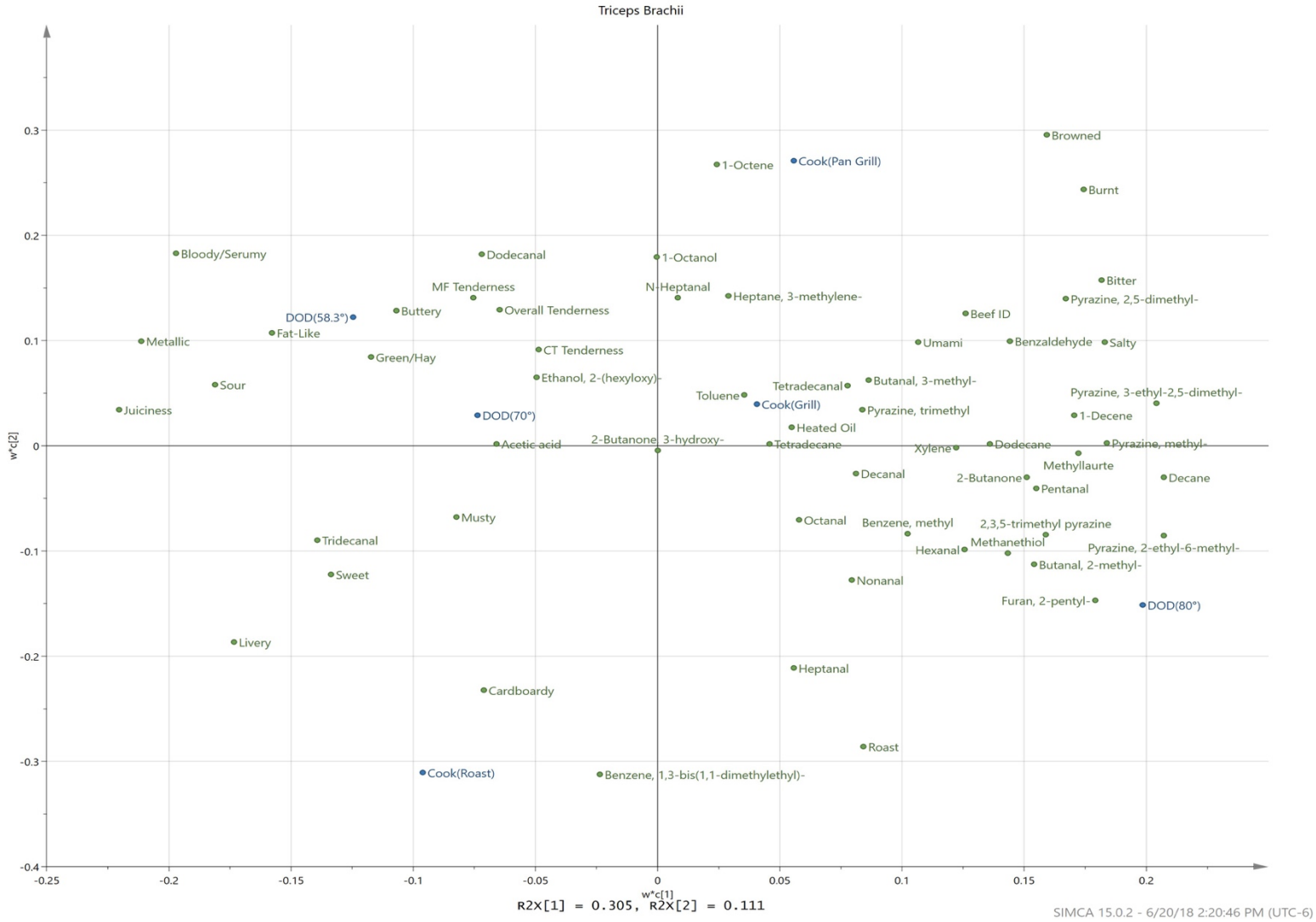

Figure 2.4. Partial least squares regression biplot for trained sensory ratings and volatile aromatic compounds as related to cooking treatments for triceps brachii.

Figure 2.5. Partial least squares regression biplot for trained sensory ratings and volatile aromatic compounds as related to cooking treatments for teres major.

REFERENCES

Aberle, E. D., Forrest, J. C., Gerrard, D. E., & Mills, E. W. (2001). *Principles of Meat Science* (4th ed.). Kendall/Hunt Publishing Company.

Adhikari, K., Chambers IV, E., Miller, R., Vázquez-Araújo, L., Bhumiratana, N., & Philip, C. (2011). Development of a lexicon for beef flavor in intact muscle. *Journal of Sensory Studies*, *26*(6), 413–420. https://doi.org/10.1111/j.1745-459X.2011.00356.x

Berry, B. W. (1994). Fat Level, High Temperature Cooking and Degree of Doneness Affect Sensory, Chemical and Physical Properties of Beef Patties. *Journal of Food Science*, *59*(1), 10–14. https://doi.org/10.1111/j.1365-2621.1994.tb06885.x

Bouton, P. E., Harris, P. V, & Hill, C. (1966). The effects of cooking temperature and time on some mechanical properties of meat, *37*, 140–144.

Brooks, J. C., & Savell, J. W. (2004). Perimysium thickness as an indicator of beef tenderness. *Meat Science*, *67*(2), 329–334. https://doi.org/10.1016/j.meatsci.2003.10.019

Calkins, C. R., & Hodgen, J. M. (2007). A fresh look at meat flavor. *Meat Science*, *77*(1 SPEC. ISS.), 63–80. https://doi.org/10.1016/j.meatsci.2007.04.016

Chandrashekar, J., Hoon, M. A., Ryba, N. J. P., & Zuker, C. S. (2006). The receptors and cells for mammalian taste. *Nature*, *444*(7117), 288–294. https://doi.org/10.1038/nature05401

Christensen, K. L., Johnson, D. D., West, R. L., Marshall, T. T., & Hargrove, D. D. (1991). The effect of breed of sire and age at feeding on muscle tenderness in the beef chuck. *Journal of Animal Science*, *69*(9), 3673–3678.

Cross, H. R., Berry, B. W., & Wells, L. H. (1980). Effects of Fat Level and Source on the Chemical, Sensory and Cooking Properties of Ground Beef Patties. *Journal of Food Science*, *45*(4), 791–794. https://doi.org/10.1111/j.1365-2621.1980.tb07450.x

Cross, H. R., Carpenter, Z. L., & Smith, G. C. (1973). Effects of Intramuscular Collagen and Elastin on Bovine Muscle Tenderness. *Journal of Food Science*, *38*(6), 998–1003. https://doi.org/10.1111/j.1365-2621.1973.tb02133.x

Cross, H. R., Stanfield, M. S., & Koch, E. J. (1976). Beef Palatability as Affected by Cooking Rate and Final Internal Temperature. *Journal of Animal Science*, *43*(1), 114–121. https://doi.org/10.2134/jas1976.431114x

Davey, C. L., & Gilbert, K. V. (1974). Temperature-dependent cooking toughness in beef. *Journal of the Science of Food and Agriculture*, *25*(8), 931–938. https://doi.org/10.1002/jsfa.2740250808

Dayton, W. R., Goll, D. E., Zeece, M. G., Robson, R. M., & Reville, W. J. (1976). A Ca2+-

Activated Protease Possibly Involved in Myofibrillar Protein Turnover. Purification from Porcine Muscle. *Biochemistry*, *15*(10), 2150–2158. https://doi.org/10.1021/bi00655a019

Devine, C. E., Wahlgren, N. M., & Tornberg, E. (1999). Effect of rigor temperature on muscle shortening and tenderisation of restrained and unrestrained beef m. longissimus thoracicus et lumborum. *Meat Science*, *51*(1), 61–72. https://doi.org/10.1016/S0309-1740(98)00098-9

Grayson, A. L., Shackelford, S. D., King, D. A., McKeith, R. O., Miller, R. K., & Wheeler, T. L. (2016). The effects of degree of dark cutting on tenderness and sensory attributes of beef. *Journal of Animal Science*, *94*(6), 2583–2591. https://doi.org/10.2527/jas2016-0388

Guelker, M. R., Haneklaus, A. N., Brooks, J. C., Carr, C. C., Delmore, R. J., Griffin, D. B., … Savell, J. W. (2013). National beef tenderness survey-2010:Warner-Bratzler shear force values and sensory panel ratings for beef steaks from United States retail and food service establishments. *Journal of Animal Science*, *91*(2), 1005–1014. https://doi.org/10.2527/jas.2012-5785

Hall, R. L. (1968). Food flavors: Benefits and problems. *Food Technology*, *22*(54).

Hildrum, K. I., Rødbotten, R., Høy, M., Berg, J., Narum, B., & Wold, J. P. (2009). Classification of different bovine muscles according to sensory characteristics and Warner Bratzler shear force. *Meat Science*, *83*(2), 302–307. https://doi.org/10.1016/j.meatsci.2009.05.016

Huff-Lonergan, E., Mitsuhashi, T., Beekman, D. D., Parrish, F. C., Olson, D. G., & Robson, R. M. (1996). Proteolysis of Specific Muscle Structural Proteins by μ-Calpain at Low pH and Temperature is Similar to Degradation in Postmortem Bovine Muscle. *Journal of Animal Science*, *74*(5), 993–1008. https://doi.org/10.2527/1996.745993x

Hunt, M. R., Garmyn, A. J., O'Quinn, T. G., Corbin, C. H., Legako, J. F., Rathmann, R. J., … Miller, M. F. (2014). Consumer assessment of beef palatability from four beef muscles from USDA Choice and Select graded carcasses. *Meat Science*, *98*(1), 1–8. https://doi.org/10.1016/j.meatsci.2014.04.004

Hunt, M. R., Legako, J. F., Dinh, T. T. N., Garmyn, A. J., O'Quinn, T. G., Corbin, C. H., … Miller, M. F. (2016). Assessment of volatile compounds, neutral and polar lipid fatty acids of four beef muscles from USDA Choice and Select graded carcasses and their relationships with consumer palatability scores and intramuscular fat content. *Meat Science*, *116*, 91–101. https://doi.org/10.1016/j.meatsci.2016.02.010

Johnson, M. H., Calkins, C. R., Huffman, R. D., Johnson, D. D., & Hargrove, D. D. (1990). Differences in cathepsin B + L and calcium-dependent protease activities among breed type and their relationship to beef tenderness. *Journal of Animal Science*, *68*(8), 2371–2379. https://doi.org//1990.6882371x

Johnson, R. C., Chen, C. M., Muller, T. S., Costello, W. J., Romans, J. R., & Jones, K. W. (1988). Characterization of the Muscles within the Beef Forequarter. *Journal of Food Science*, *53*(5), 1247–1250. https://doi.org/10.1111/j.1365-2621.1988.tb09249.x

Jung, E. Y., Hwang, Y. H., & Joo, S. T. (2016). Muscle profiling to improve the value of retail meat cuts. *Meat Science*, *120*, 47–53. https://doi.org/10.1016/j.meatsci.2016.04.012

Kemp, C. M., & Parr, T. (2012). Advances in apoptotic mediated proteolysis in meat tenderisation. *Meat Science*, *92*(3), 252–259. https://doi.org/10.1016/j.meatsci.2012.03.013

Kerth, C. (2016). Determination of volatile aroma compounds in beef using differences in steak thickness and cook surface temperature. *Meat Science*, *117*, 27–35. https://doi.org/10.1016/j.meatsci.2016.02.026

Kerth, C. R., & Miller, R. K. (2015). Beef flavor: A review from chemistry to consumer. *Journal of the Science of Food and Agriculture*, *95*(14), 2783–2798. https://doi.org/10.1002/jsfa.7204

Killinger, K. M., Calkins, C. R., Umberger, W. J., Feuz, D. M., & Eskridge, K. M. (2004). Consumer sensory acceptance and value for beef steaks of similar tenderness, but differing in marbling level. *Journal of Animal Science*, *82*(11), 3294–3301. https://doi.org//2004.82113294x

King, D. A., Dikeman, M. E., Wheeler, T. L., Kastner, C. L., & Koohmaraie, M. (2003). Chilling and cooking rate effects on some myofibrillar determinants of tenderness of beef. *Journal of Animal Science*, *81*(6), 1473–1481. https://doi.org//2003.8161473x

Koch, R., Crouse, J., & Dikeman, M. (1993). Effect of Marbling on Variation and Change in Beef Tenderness In Bos Taurus and Bos Indicus Crosses. Retrieved from http://digitalcommons.unl.edu/hruskareports/127/

Laska, M., Distel, H., & Hudson, R. (1997). Trigeminal perception of odourant quality in congenital anosmic subjects. *Chem Sens*, *22*(June). https://doi.org/10.1093/chemse/22.4.447

Lawrence, T. E., King, D. A., Obuz, E., Yancey, E. J., & Dikeman, M. E. (2001). Evaluation of electric belt grill, forced-air convection oven, and electric broiler cookery methods for beef tenderness research. *Meat Science*, *58*(3), 239–246. https://doi.org/10.1016/S0309-1740(00)00159-5

Legako, J. F., Brooks, J. C., O'Quinn, T. G., Hagan, T. D. J., Polkinghorne, R., Farmer, L. J., & Miller, M. F. (2015). Consumer palatability scores and volatile beef flavor compounds of five USDA quality grades and four muscles. *Meat Science*, *100*, 291–300. https://doi.org/10.1016/j.meatsci.2014.10.026

Legako, J. F., Dinh, T. T. N., Miller, M. F., Adhikari, K., & Brooks, J. C. (2016). Consumer palatability scores, sensory descriptive attributes, and volatile compounds of grilled beef steaks from three USDA Quality Grades. *Meat Science*, *112*, 77–85. https://doi.org/10.1016/j.meatsci.2015.10.018

Lorenzen, C. L., Neely, T. R., Miller, R. K., Tatum, J. D., Wise, J. W., Taylor, J. F., … Savell, J. W. (1999). Beef Customer Satisfaction: Cooking Method and Degree of Doneness Effects on the Top Loin Steak. *Journal of Animal Science*, *77*(3), 637–644.

https://doi.org/10.2527/1999.773645x

Maughan, C., Tansawat, R., Cornforth, D., Ward, R., & Martini, S. (2012). Development of a beef flavor lexicon and its application to compare the flavor profile and consumer acceptance of rib steaks from grass- or grain-fed cattle. *Meat Science*, *90*(1), 116–121. https://doi.org/10.1016/j.meatsci.2011.06.006

McCormick, R. J. (1994). The flexibility of the collagen compartment of muscle. *Meat Science*, *36*(1–2), 79–91. https://doi.org/10.1016/0309-1740(94)90035-3

McKeith, F. K., Devol, D. L., Miles, R. S., Bechtel, P. J., & Carr, T. R. (1985). Chemical and Sensory Properties of 13 Major Beef Muscles. *Journal of Food Science*, *50*(4), 869–872.

Miller, M. F., Carr, M. A., Ramsey, C. B., Crockett, K. L., & Hoover, L. C. (2001). Consumer thresholds for establishing the value of beef tenderness. *Journal of Animal Science*, *79*(12), 3062–3068. https://doi.org//2001.79123062x

Miller, R. K. (1994). Quality Characteristics. In D. M. Kinsman, A. W. Kotula, & B. C. Breidenstein (Eds.), *Muscle Foods*. Boston, MA: Springer.

Morgan, J. B., Savell, J. W., Hale, D. S., Miller, R. K., Griffin, D. B., Cross, H. R., & Shackelford, S. D. (1991). National beef tenderness survey. *Journal of Animal Science*, *69*(8), 3274–3283. https://doi.org/10.2527/1991.6983274x

Mottram, D. S. (1998). Flavour formation in meat and meat products: A review. *Food Chemistry*, *62*(4), 415–424. https://doi.org/10.1016/S0308-8146(98)00076-4

Obuz, E., Dikeman, M. E., Grobbel, J. P., Stephens, J. W., & Loughin, T. M. (2004). Beef longissimus lumborum, biceps femoris, and deep pectoralis Warner-Bratzler shear force is affected differently by endpoint temperature, cooking method, and USDA quality grade. *Meat Science*, *68*(2), 243–248. https://doi.org/10.1016/j.meatsci.2004.03.003

Ohman, C. E., Wiegand, B. R., Gruen, I. U., & Lorenzen, C. L. (2015). Beef muscle isolation has no detrimental effect on premium ground beef programs. *Meat Science*, *106*, 50–54. https://doi.org/10.1016/j.meatsci.2015.03.022

Prost, E., Pelczynska, E., & Kotula, A. W. (1975). Quality characteristics of bovine meat. II. beef tenderness in relation to individual muscles, age and sex of animals and carcass quality grade. *Journal of Animal Science*, *41*(2), 541–547. Retrieved from http://jas.fass.org/cgi/content/abstract/41/2/541

Purslow, P. P. (2005). Intramuscular connective tissue and its role in meat quality. *Meat Science*, *70*(3 SPEC. ISS.), 435–447. https://doi.org/10.1016/j.meatsci.2004.06.028

Reicks, A. L., Brooks, J. C., Garmyn, A. J., Thompson, L. D., Lyford, C. L., & Miller, M. F. (2011). Demographics and beef preferences affect consumer motivation for purchasing fresh beef steaks and roasts. *Meat Science*, *87*(4), 403–411. https://doi.org/10.1016/j.meatsci.2010.11.018

Resconi, V. C., Escudero, A., & Campo, M. M. (2013). The development of aromas in ruminant meat. *Molecules*, *18*(6), 6748–6781. https://doi.org/10.3390/molecules18066748

Seideman, S. C. (1986). Methods of Expressing Collagen Characteristics and Their Relationship to Meat Tenderness and Muscle Fiber Types. *Journal of Food Science*, *51*(2), 273–276. https://doi.org/10.1111/j.1365-2621.1986.tb11107.x

Semler, M. L., Woerner, D. R., Belk, K. E., Enns, K. J., & Tatum, J. D. (2016). Effects of United States Department of Agriculture carcass maturity on sensory attributes of steaks produced by cattle representing two dental age classes. *Journal of Animal Science*, *94*(5), 2207–2217. https://doi.org/10.2527/jas2016-0382

Shackelford, S. D., Koohmaraie, M., & Wheeler, T. L. (1995). Effects of slaughter age on meat tenderness and USDA carcass maturity scores of beef females. *Journal of Animal Science*, *73*(11), 3304–3309. https://doi.org/10.2527/1995.73113304x

Shahidi, F., Samaranayaka, A. G. P., & Pegg, R. B. (2014). Maillard reaction and browning. In M. Dikeman & C. Devine (Eds.), *Encyclopedia of Meat Sciences* (2nd ed., pp. 391–403). London, UK: Elsevier.

Shepherd, G. M. (2005). Outline of a theory of olfactory processing and its relevance to humans. *Chemical Senses*, *30 SUPPL.*(June), 3–5. https://doi.org/10.1093/chemse/bjh085

Smith, G., Savell, J., Cross, H., Carpenter, Z., Murphey, C., Davis, G., … Berry, B. (1987). Relationship of Usda Quality Grades To Palatability of Cooked Beef1. *Journal of Food Quality*, *10*(4), 269–286. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.1745-4557.1987.tb00819.x/abstract

Stolowski, G. D., Baird, B. E., Miller, R. K., Savell, J. W., Sams, A. R., Taylor, J. F., … Smith, S. B. (2006). Factors influencing the variation in tenderness of seven major beef muscles from three Angus and Brahman breed crosses. *Meat Science*, *73*(3), 475–483. https://doi.org/10.1016/j.meatsci.2006.01.006

Sullivan, G. A., & Calkins, C. R. (2011). Ranking beef muscles for Warner-Bratzler shear force and trained sensory panel ratings from published literature. *Journal of Food Quality*, *34*(3), 195–203. https://doi.org/10.1111/j.1745-4557.2011.00386.x

The Meat Buyer's Guide. (2014) (8th ed.). Washington, DC: North American Meat Association.

Van Ba, H., Hwang, I., Jeong, D., & Touseef, A. (2012). Principle of Meat Aroma Flavors and Future Prospect. *Latest Research into Quality Control*, 145–176. https://doi.org/37707

Van Boekel, M. A. J. S. (2006). Formation of flavour compounds in the Maillard reaction. *Biotechnology Advances*, *24*(2), 230–233. https://doi.org/10.1016/j.biotechadv.2005.11.004

Voges, K. L., Mason, C. L., Brooks, J. C., Delmore, R. J., Griffin, D. B., Hale, D. S., … Savell, J. W. (2007). National beef tenderness survey - 2006: Assessment of Warner-Bratzler shear and sensory panel ratings for beef from US retail and foodservice establishments. *Meat*

Science, *77*(3), 357–364. https://doi.org/10.1016/j.meatsci.2007.03.024

Von Seggern, D. D., Calkins, C. R., Johnson, D. D., Brickler, J. E., & Gwartney, B. L. (2005). Muscle profiling: Characterizing the muscles of the beef chuck and round. *Meat Science*, *71*(1), 39–51. https://doi.org/10.1016/j.meatsci.2005.04.010

Yancey, J. W. S.; Wharton, M. D.; Apple, J. K. ; (2011). Cookery method and end-point temperature can affect the Warner–Bratzler shear. *Meat Science*, *88*(1), 1–7. https://doi.org/10.1016/j.meatsci.2010.11.020

Yeh, Y., Omaye, S. T., Ribeiro, F. A., Calkins, C. R., & de Mello, A. S. (2018). Evaluation of palatability and muscle composition of novel value-added beef cuts. *Meat Science*, *135*(August 2017), 79–83. https://doi.org/10.1016/j.meatsci.2017.08.026

Zinn, D. W., Gaskins, C. T., Gann, G. L., & Hedrick, H. B. (1970). Beef Muscle Tenderness as Influenced by days on feed, sex, maturity and anatomical location. *Journal of Animal Science*, 307–309.