THESIS

A PROOF OF CONCEPT TO DIFFERENTIATE AMONG DIFFERENCES IN FLAVOR OF AMERICAN LAMB USING VOLATILE FLAVOR COMPOUND ANALYSIS

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ABSTRACT

A PROOF OF CONCEPT TO DIFFERENTIATE AMONG DIFFERENCES IN FLAVOR OF AMERICAN LAMB USING VOLATILE FLAVOR COMPOUND ANALYSIS

Experiments were conducted on lamb legs (n=25 per treatment) from 3 dentition groups [young lambs (0 permanent incisors), yearlings (2 permanent incisors) and mature sheep or mutton (>2 permanent incisors)] to establish a proof of concept for differentiating the inherent differences in flavor that exist in meat from ovine animals of various age classes using volatile flavor compound analysis. The legs were selected from commercial processing facilities. Differences among age group, breed type, sex and production background were evaluated for sensory analysis and volatile compound analysis. Trained panelists evaluated ground meat patties from each leg for lamb flavor intensity and off flavor intensity. In addition, samples were analyzed to determine percentages of lipid, moisture, protein, and ash as well as to identify volatiles produced during cooking of a raw composite of lean and fat from the external surface of the leg.

Analysis of variance was conducted for sensory flavor attributes relative to animal age and production background (grain vs grass) helped to describe the experimental samples. Ratings for lamb flavor intensity were higher ($P < 0.05$) for lamb carcass samples than for yearling carcass samples, and lamb flavor intensity scores were similar for lamb and mature age classes. Off-flavor intensity ratings were highest ($P < 0.05$) for samples from mature lamb carcasses, while lamb and yearling samples produced the lowest ($P < 0.05$) off-flavor intensity ratings. Lamb flavor intensity and off-flavor intensity ratings were higher ($P < 0.05$) for grass-fed lamb samples compared to grain-fed lamb samples. Mature samples had the greatest ($P < 0.05$) off-flavor intensity, while lamb and yearling samples had the least ($P < 0.05$) off-flavor intensity. Grass-fed

lamb samples had the higher ($P < 0.05$) lamb flavor intensity scores and higher ($P < 0.05$) off-flavor intensity scores. Correlations between sensory attributes and metabolites helped to narrow the 500+ to 50 of significance. Findings indicated that metabolites (volatile compounds) were related to flavor of sheep meat. Finally, regression techniques helped to predict lamb flavor intensity, off flavor intensity and proof-of-concept for classifying lamb flavor.

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CHAPTER I

INTRODUCTION

The American Sheep Industry has faced declining supplies of sheep and lambs since the mid 1940's, from 56 million sheep in 1942 to 4 million today. Many blame the decline on repercussions of World War II and soldiers having to eat canned mutton. These soldiers returned from war and never wanted to eat "lamb" again and the next generation was never introduced to it. Blame is also shed on the repeal of the Wool Act in 1993; taking away direct price support payments to producers of wool and mohair. Due to the repeal the market value of sheep, lambs, wool and mohair fell, increasing the difficulty to raise sheep for a living. In truth, declining numbers of sheep resulted from a culmination of these factors including competition from abroad. Australia and New Zealand provide 50% of the U.S. consumer lamb supply and are huge competitors in the fine wool market. In recent years, data has shown that the U.S. sheep inventory is leveling off and is even increasing in some parts of the country. The United States has been investing in technologies and improved efficiencies to stay competitive in the global market. The future of the U.S. sheep industry depends on demand and profitability for lamb (Stillman, Crawford, & Aldrich, 1990.).

Gazdziak (2015) reported that lamb may be a growing dietary protein source. Lamb was the fastest growing protein on menus from 2010 to 2014, and that lamb sales were increasing dramatically. Even with increased in sales, average American consumers are only consuming 0.18 kg of lamb a year. Perceived dissatisfaction with lamb flavor is thought to be an obstacle for the sheep industry. Lamb is considered a specialty or niche protein, and it is one of the most expensive proteins on menus nationwide (Gazdziak, 2015).

Since consumers are expected to pay more for lamb, flavor must be consistent and/or improved allowing for lamb consumers to be continually satisfied. The importance of lamb flavor in the marketplace is underscored by the fact that consumers' flavor preferences are reflected in their lamb purchase decisions (Hoffman, 2016). The most recent National Lamb Quality Audit (NLQA) identified eating satisfaction at the most prominent factor defining lamb quality, and eating satisfaction was generally defined as lamb flavor and/or taste. Additionally, 71.7% of U.S. lamb purchasers surveyed in the NLQA indicated that they were willing to pay a premium for guaranteed eating satisfaction, and they also indicated, on average, that they would be willing to pay a 18.6% premium for this guarantee (Hoffman, 2016). This information serves as an indication that differentiating lamb based on flavor may result in considerable premiums and allow for the American lamb industry to capitalize on opportunities with lamb flavor to increase demand.

Lamb provides a unique flavor, and there are several factors that affect the flavor or taste of lamb. Differences in production background, days on feed, sex and animal age all influence flavor of lamb by altering composition of lean and fat. It has been well established that production practices and animal age influence lamb flavor by altering fat and lean composition. Specifically, as lambs mature, and even if lambs are grain-fed for extended/excessive periods of time, there is an increase in the concentration of branched-chain fatty acids (BCFA) resulting in undesirable, "mutton-like" off-flavors (Tatum, Zerby, & Belk, 2014). A flavor profile that has had negative connotations for consumers is a pastoral flavor which usually is associated with lambs that were pasture finished. Pastoral flavor has been attributed to high concentrations of indoles and amplified with high concentrations of BCFA, specifically 4-methyloctanoic acid and 4-methylnonanoic acids (Owen A Young, Lane, Priolo, & Fraser, 2003). The concentration of

indoles and BCFA produced in the liver during propionate metabolism. When an animal is on a grain-based diet, they produce higher levels of propionate in the rumen. As an animal ages, BCFA concentrations in fat increase gradually and the greatest concentrations are seen in sheep 2 years of age and older (O.A. Young, Berdagué, Viallon, Rousset-Akrim, & Theriez, 1997). Meat from grain-fed lambs is known to have a milder aroma and flavor compared to pasture finished lamb (Tatum et al., 2014).

Fat and lean composition differences can be measured using volatile flavor compound analysis, and researchers focusing on the flavor of meat have been able to associate meat flavor attributes with these methods. The purpose of this research was to conduct a proof-of-concept study to use measures of volatile flavor compounds in lamb to differentiate flavor and identify opportunities to use these technologies at production speeds to segregate lamb carcasses into groups differing in eating quality.

LITERATURE CITED

Gazdziak, S. (2015). Lamb's "wow" factor. Retrieved January 20, 2017, from http://www.provisioneronline.com/articles/102685-lambs-wow-factor?v=preview

Hoffman, T. (2016). *BENCHMARK OF LAMB QUALITY IN U.S. RETAIL AND FOODSERVICE MARKETS*.

Stillman, R., Crawford, T., & Aldrich, L. (n.d.). The U.S. Sheep Industry.

Tatum, J. D., Zerby, H. N., & Belk, K. E. (2014). A Brief Overview of Factors Affecting Lamb Flavor Prepared for the Lamb Industry Roadmap Product Characteristics Team.

Young, O. A., Berdagué, J.-L., Viallon, C., Rousset-Akrim, S., & Theriez, M. (1997). Fat-borne volatiles and sheepmeat odour. *Meat Science*, *45*(2), 183–200. https://doi.org/10.1016/S0309-1740(96)00100-3

Young, O. A., Lane, G. A., Priolo, A., & Fraser, K. (2003). Pastoral and species flavour in lambs raised on pasture, lucerne or maize. *Journal of the Science of Food and Agriculture*, *83*(2), 93–104. https://doi.org/10.1002/jsfa.1282

CHAPTER II

REVIEW OF LITERATURE

Flavor

Flavor is defined as a blend of taste and smell sensations evoked by a substance in the mouth. Another way to describe flavor is that it provides a certain quality to food (Merriam Webster, 2016). Flavor is the combination of aroma (retronasal) and taste experience on the tongue. The importance of aroma often is thought to be a myth until people are asked to block air to their nose as they consume a food. Many will say that they lose the ability to taste as there is a strong relationship between smell and taste and the receptors in the nose. This is because flavor and aroma are thought to work separately (Auvray & Spence, 2008). When food is consumed, flavor molecules that are being experienced on the tongue travel from the mouth to the nasal cavity (retronasal aroma) and interact in determining flavor. Orthonasal aroma is when the nasal cavity is the only receptor of the aroma (Neethling, Hoffman, & Muller, 2016).

Flavor is highly correlated to three sensory channels: gustation (or taste proper), olfaction, and somatosensation. Gustation is the sensory channel that is associated with the stimulation of receptors on the tongue and oral cavity which decipher qualities of sweet, salty, bitter, savory and umami. These five basic tastes are comprised of how different volatile compounds are perceived. The olfactory sense is stimulated by airborne molecules that enter the mouth or retronasal cavity through the nasopharynx and hits the olfactory mucosa. Odors and aromas in the nose and throat are detected by the olfactory sense. Aromas have the ability to alter perception of taste (Veldhuizen, Shepard, Wang, & Marks, 2010).

Somatosensory contributes to flavor perception as it stimulates the proprioceptors in the jaw and mechanoreceptors in the oral cavity. Both receptors can detect texture, detect temperature from warm and cold receptors on the tongue, oral cavity and from nociceptors than can depict pungency and spiciness (Veldhuizen et al., 2010).

Importance of Flavor and Human Preference

Meat consists of micronutrients (vitamins, sugars and nucleotides) and macronutrients (water, proteins and lipids), allowing it to have a large reservoir of flavor precursors ready to undergo various reactions. Meat flavor is a large attribute of meat eating satisfaction (Pegg & Shahidi, 2014). In lamb specifically, flavor is defined as the most prominent factor affecting lamb quality (Hoffman, 2016). Flavor is a major contributor to consumers buying habits. It is important to understand flavor to provide a consistent and naturally flavorful product for the consumer.

Meeting consumer expectations is the ultimate goal, but the consumer is the last step of the production chain. It is important to remember the average consumer's education on meat and meat quality is through advertisements, labels or brands. This is what a customer uses to create their quality expectations and, in turn, to arrive at purchasing decisions (Font-i-Furnols & Guerrero, 2014). Willingness to pay or price is another large driver in consumer preference. Most consumers are willing to pay a premium for guaranteed quality (Hoffman, 2016). Even though there is willingness to pay for quality, lower prices are still preferred and t is a good explanation to low lamb consumption (Font-i-Furnols & Guerrero, 2014).

Naivety aside, consumers consider flavor one of the main facets in the selection and ingestion of a food. Flavor development of the meat product is not the only complex factor contributing to a desired or undesired taste, human preference also has a large role in desirability. Research in other countries has demonstrated that consumers differ in their acceptance of various sheep-

specific meat flavor notes depending upon past eating experiences. Consumers who are more accustomed to eating lamb or mutton with a particular flavor profile seem to prefer ovine meat products with a familiar flavor (Sañudo et al., 2000), whereas consumers who have never experienced lamb or mutton tend to show the greatest aversion and sensitivity to lamb specific flavors.

Perception is a strong driver of dietary intake, it is essential to have a basic understanding to provide a consistent, high quality product to the consumer. For a consumer to willingly ingest a food, the sensory attributes must be appealing but, ingestion also is influenced by hunger level, nutritional state, past eating experience, and good feelings or beliefs of the food. Fig 2.1. demonstrates the complexity of food preference beyond flavor perception.

The ability to differentiate or perceive flavors begins in utero as gustatory and olfactory systems begin to develop and function. Both breast milk and amniotic fluid contain molecules derived from the mother's diet and introduces flavors. Studies have shown that a sweet taste is innate where a salty taste is learned. At an early age, through dietary experience there is an association made of what should or should not be sweet. There are two shifts in early development from indifference to relative (milk to water). Infants are slowly introduced to new foods and hedonic responses are measured through facial expressions for acceptance or negative affect. The second shift happens around age two to three and is acceptance or rejection, which is a result of the development of neophobia as well as a lack of experience of the food (Beauchamp & Cowart, 1985). Neophobia is defined as an unwillingness to eat novel foods and is thought to be an adaptive behavior. Food neophobia is usually overcome with a positive social environment while consuming a novel food. Children who show aversion toward the new food are rewarded with a treat after finishing the novel food item. Early development of an infant is a crucial time

to introduce a variety of different tastes and not over familiarize certain flavors (Ventura & Worobey, 2013).

Beyond development of the gustatory and olfactory systems a portion of flavor preference can be attributed to genetics. Research has identified several genes related to difference in sweet, umami, and bitter taste perception. Perception of taste comes from G-coupled protein receptors encoded with taste receptor gene families. Polymorphisms occur in the gene families and cause a variation perception. The genetic sensitivity to sweet, bitter or umami may influence sensitivity or preference to other tastes (Beauchamp & Cowart, 1985).

Food preferences continuously change during adolescence and adulthood; studies have found a strong correlation between race/ethnicity and preference, suggesting culture and experience may override genetic differences later in life. Preference continues to become more complex to understand as individuals mature (Beauchamp & Cowart, 1985). Preference is influenced by age, sex, health status, education and income as well as the nutritional status of the food. There is a large shift of a primarily hedonic based preference (pleasant or unpleasant sensations) to a preference of health, social, and economic impacts of foods later in life. Advanced aging can cause a decline in normal taste or smell functions attributed to normal aging and disease (Font-i-Furnols & Guerrero, 2014). Every individual has unique preferences and aversions that can only partly be understood.

Development of Flavor

Many factors that contribute to development of lamb flavor. Major precursors can be divided into 2 categories; water soluble flavor components and lipids (Mottram, 1998). Studies have suggested that fatty tissues provide the species-specific characteristics of flavor while lean provide precursors for generic meat flavor. An oversimplified statement but characteristic

species differences are explained in part through differences in lipid derived volatiles. There are hundreds of volatile compounds formed through lipid degradation in cooked meat including, aliphatic hydrocarbons, aldehydes, ketones, alcohols, carboxylic acids and esters. These compounds are derived from oxidation of fatty acids of lipids (lipid oxidation). Most of the meat flavor precursors are water soluble in nature and are thought to be sugars, sugar phosphate, nucleotide bound sugars, peptides, free amino acids, nucleotides and other nitrogenous components. Meat flavor is thought to be thermally derived through two main reactions; lipid oxidation and the Maillard reaction. When heated, reductions were most commonly found in carbohydrates and amino acids most significantly for cysteine and ribose and volatile compounds are formed. These reactions result in meat being essentially flavorless or bloody tasting and having little to no odor, and then hundreds are of volatiles being released through the cooking process creating a distinct flavor or flavors (Farmer, 1994).

Lipids constitute several purposes in meat flavor formation, acting as solvents for volatile compounds during processing or products produced during thermal oxidation providing distinct flavors following reactions with lean meat tissue. Lipids are responsible for desired and undesired flavors and aromas in meat (Calkins & Hodgen, 2007). Lipid oxidation has a poor reputation for ruining meat quality during storage and processing, but it has a large role in the meaty aroma in many meat products. Oxidation of lipids most commonly deteriorates quality of meat. There are several mechanisms that can cause lipids to be oxidized in meat whether non-enzymatic or enzymatic reactions. The most important in meat is autoxidation from continuous free radical chain cascades. A free radical is an atom or molecule with an unpaired electron; thus, free radicals are unstable and reactive. Free radicals cause a chain reaction as the unpaired electron attracts a free electron from another compound and then that compound becomes free

radicals and this is continuous always attracting new electrons and creating new free radicals. This reaction can cause biological damage to lipids, proteins, enzymes and nucleic acids changing the chemical composition (JH, 2016). There are 3 phases to autoxidation; initiation, propagation, and termination resulting in the degradation of desirable flavors in meat. Lipids, and especially phospholipids, degrade to produce volatile compounds. Hydroperoxides are products of lipid oxidation that are odorless and tasteless, but when degraded, they form several types of secondary products such as aldehydes, hydrocarbons ketones and alcohols. Aldehydes are known to be flavor active, possessing low threshold values in the parts per million or parts per billion range and most commonly creating the warmed-over flavor (Frankel, 1980). These compounds alter the flavor profile of the muscle and mask desired flavors and aromas of the meat. Lipid oxidation does not stop at decreasing quality just by creating a warmed-over flavor; it will also change color, texture and other functional properties (Kerry, 2002).

The Maillard reaction occurs between amino acids and reducing sugars with the addition of heat. The Maillard reaction is one of the most important reactions for flavor development and usually begins around 140˚C. This non-enzymatic browning reactions is caused by a reactive carbonyl group in sugar interacting with a nucleophilic amino group of an amino acid. The carbonyl group of the sugar reacts with the amino group of the amino acid producing nitrogen substituted glycosylamine and water. The glycosylamine forms ketosamines and they react further (van Boekel, 2006). An environment is created where the amino group does not neutralize and produces a characteristic flavor and odor dependent on the amino acid. Hundreds of flavor compounds are created during this reaction and then the compounds break down to form new flavor compounds. Most of the volatiles produced from Maillard reactions include heterocyclic nitrogen and sulfur compound (Mottram, 1998).

Factors Affecting Lamb Flavor

The 2015 National Lamb Quality Audit concluded that the flavor of lamb is the reason why consumers both decide to purchase lamb and why they do not (Hoffman, 2016). Many consumers prefer the flavor and quality of domesticated animals over game meats. Sheep are a domesticated species but the flavor is perceived very similarly to that of game meat. It is an acquired taste as it can have a string aroma and intense flavor (Neethling et al., 2016).

Unfortunately, there is no single attribute that simply defines lamb flavor. Most studies have shown that age is the main contributor to quality of lamb or mutton (Tatum et al., 2014). A review of the literature (Tatum et al., 2014) showed that sheep meat flavor intensity increases with age. As a lamb ages, tenderness decreases and flavor intensity increases, often causing a "mutton flavor" (Sink & Caporaso, 1977). Increasing levels of excess fat also increases concentrations of undesirable branched chain fatty acids. The highest concentration of lipids are most commonly found in sheep over 2 years of age (Tatum et al., 2014).

It should seem important to segregate sheep based on age for optimal eating quality. Currently, the Australian sheep industry uses dentition to separate into 3 categories defining quality lambs (no erupted permanent incisors), hogget (2 erupted incisors) or mutton (greater than 2 erupted incisors) (Pethick et al., 2005). In the United States the sheep industry is segregating lambs based on presence of spool or break joints in the commercial processing facilities. The epiphyseal cartilage on the metacarpal (front cannon bones) ossifies as sheep age lambs 3-14 months of age should present break joints and mature sheep greater than 14 months should present a spool joint (ossified epiphyseal cartilage). These 2 categories are subdivided into 2 groups for grading purposes based on maturity using width, shape and color of rib bones, as well as lean color and

texture; Lamb (A maturity 3-months of age) and Older lamb (B maturity 9-14 months of age). These classes or groups of carcasses are only used for grading and market news reporting by the USDA-AMS as USDA-FSIS does not have a current definition for lamb. The problem is meat from ungraded carcasses regardless of age can be labeled as "lamb". In a review by Tatum (2014) both classifications of age (epiphyseal ossification and dentition) have shown to vary based on sex and rate of maturity of the individual animal. Estrogen is the main regulator of growth plate closure. Estrogen in males can be biosynthesized from testosterone through a reaction called aromatase. There is support that castration of males and a reduction in testosterone delays the closure of the growth plate. This leads to a problem when trying to accurately age lambs at slaughter. Ewe lambs have been found to had growth plate closure between 12-16 months, rams were similar at 13-16 months but some wethers weren't exhibiting the epiphyseal closure at 22 months of age (J.Daryl Tatum, Henry N.Zerby, 2014).

Lipid filling can increase in fat due to age but, may also be affected by sexual maturation. Age and fat, have been found to decrease meat quality and in turn, decrease live animal and carcass value. Lambs harvested at a younger age provide a higher quality product to consumers. However, most studies conducted on lamb quality have shown there is no significant difference between lambs (12 mo and younger) and USDA classification yearling mutton (12 to 24 mo). The older the animal becomes, the coarser the lean texture becomes and the stronger the flavor, which all decrease sensory quality and price of product. It is vital that lambs are harvested at an optimal weight and age to achieve a mild, desired product (Pethick et al., 2005).

Compounds associated with mutton flavor or aroma have been attributed to branched chain fatty acids and unsaturated fatty acids with 8- 10 carbon atoms. Wong et al. (1975) identified 6-methylheptanoic acid, n-octenoic acid, 4methyloctanoic acid, 6-methyloctanoic acid, 2-octenoic

acid, n-nonenoic, 4-methylnonanoic acid and 8-methylnonanoic acid as compounds associated with mutton flavor. BCFAs are different for ruminants, but are thought to be a product of methylmalonyl CoA from propionate metabolism. BCFAs are produced when propionate levels exceed the capacity of the liver and methylmalonate then compensates with malonate for fatty acid synthesis. The fatty acids produced accumulate in the subcutaneous fat leading to various volatiles forming during cooking (Wong, Johnson, & Nixon, 1975).

Differences in production systems affect flavor profile of lamb. Animal genetics, management and diet all can contribute to a unique regional flavor profile that could increase market appeal as a branded product. Unfortunately, studies on effects of breed and management on lamb flavor have been so inconsistent that no conclusions appear to arrive at consensus.

A focus in the beef industry has been to understand the difference between beef derived from grain or grass fed cattle; and the same is true for lamb (Calkins & Hodgen, 2007). There is a different flavor profile for grass-fed lamb and there have been negative connotations from consumers of a pastoral flavor (Owen A Young et al., 2003). Meat from grain-fed lambs is known to have a milder aroma and flavor compared to pasture finished lamb (Tatum et al., 2014). Concentrate diets or high-energy diets result in higher concentrations of BCFA than pasture diets. Meat from grass fed animals also has higher concentrations of vitamin E (α-tocopherol) that is naturally present in grass and acts as an antioxidant. Vitamin E will protect phospholipids in the cellular membrane from free radicals, thus decreasing the rate of lipid oxidation. This is one factor that contributes to distinct flavor differences among lamb from grass and grain fed animals (Calkins & Hodgen, 2007).

Lamb fat is harder than any other meat animal's fat because of higher levels of saturated fat, and this may explain why consumers are averse to lamb fat. Cramer and Marchello found the concentration of oleic acid to be a major contributor to fat hardness. High levels of linoleic acids also reduce the softening point of lamb fat. Due to the high concentration of stearic acid, lamb fat has a higher melting point, and contributes to an undesirable mouth coating. Lamb fat also has relatively high levels of oleic acid and low levels of linoleic and linolenic acid. As the animal ages, the proportion of triacyclglycerols to phospholipids increase and the simultaneously synthesized fatty acids including myristic, palmitic, stearic, capric and oleic acids increase and contributes to firmer lamb fat (Channon, Lyons, & Bruce, 2003).

Aromatics

Aroma is the sensory attribute that easily defines a cooked meat species especially lamb. Lamb flavor is unique as is the aromatics that are produced form the volatile compounds, specific branched chain fatty acids form triacyclglycerols. Olfactory cells work in a similar manner to auditory and photoreceptor cells which allow humans to hear and see. An odor is made up of very small molecules that are less than 1 kDa. The odors are bound in the membranes of the nerve cilia in the nose to receptor cells. Once the odor is bound to the receptor cell a series of chemical reactions occur which collect calcium and sodium and in turn release chloride which depolarizes the cell nerve and sends an impulse to the brain. Odor of a food while in the mouth stimulates the nasal epithelium. Thousands of volatile compounds are responsible for odor of food. There are both aliphatic and aromatic compounds; they usually contain a heteroatom which is oxygen, nitrogen and sulfur. Enzymatic action of these compounds can cause fermentation and unpleasant odor of meat. This enzymatic activity can be caused by spoilage or the metabolism of an animal. In cooked meat, the aromatics occurs when chemical reactions of

the volatile compounds are released in the cooking process. Meat flavor is directly related to the concentrations of the volatile aroma compounds and their odor threshold values. Without aroma the four primary tastes (bitter, sour, sweet and salty) would dominate foods (Neethling et al., 2016).

Conclusion

Lamb provides a unique flavor, but can vary tremendously. Consumers desire a positive dining experience; however, many may not know what creates a pleasurable eating experience with lamb (Hoffman, 2016). Desired aroma, taste and flavor are critical aspects of eating satisfaction. Most studies on lamb flavor have shown that meat sourced from various ages of sheep provide differing aroma and flavor profiles. Mutton characteristic and variation of flavor in general of sheep meat limits both the marketability and the acceptability of sheep meat (Channon et al., 2003). There is a relationship between age, diet and fat but human preference also plays a role perceived desirability of lamb. Preference for lamb flavor is dependent on characteristics associated with the consumer, their cultural and ethnical background and past eating experience (Font-i-Furnols & Guerrero, 2014). Following the National Lamb Quality Audit in 2015, this became the mission of the American Sheep Industry to have a consumer driven focus of improving consistency of American lamb through quality, cutability and marketability. Producers have the ability to place emphasis on quality attributes in their operations to improve eating experience and optimal flavor profile of their product (Hoffman, 2016). With the lack of research on the relationship of dentition or epiphyseal ossification on eating quality of sheep meat there is an opportunity to change the segregation of lamb at the processing facility. The inconsistency in consumer preferences allow the American Sheep Industry to segregate lamb based on regional or cultural preferences (Font-i-Furnols & Guerrero, 2014).

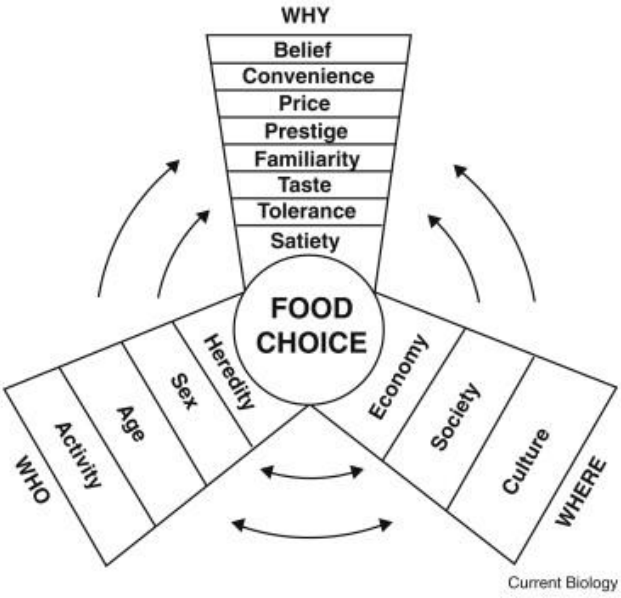

Figure 2.1.The Influence on Food Preference and Choice (Ventura & Worobey, 2013).

LITERATURE CITED

Arsenos, G., Banos, G., Fortomaris, P., Katsaounis, N., Stamataris, C., Tsaras, L., & Zygoyiannis, D. (2002). Eating quality of lamb meat: effects of breed, sex, degree of maturity and nutritional management. *Meat Science*, *60*(4), 379–387. https://doi.org/10.1016/S0309-1740(01)00147-4

Auvray, M., & Spence, C. (2008). The multisensory perception of flavor. *Consciousness and Cognition*, *17*(3), 1016–1031. https://doi.org/10.1016/j.concog.2007.06.005

Beauchamp, G. K., & Cowart, B. J. (1985). Congenital and experiential factors in the development of human flavor preferences. *Appetite*, *6*(4), 357–372. https://doi.org/10.1016/S0195-6663(85)80004-0

Broeckling, C. D., Ganna, A., Layer, M., Brown, K., Sutton, B., Ingelsson, E., … Prenni, J. E. (n.d.). Enabling Efficient and Confident Annotation of LC−MS Metabolomics Data through MS1 Spectrum and Time Prediction. https://doi.org/10.1021/acs.analchem.6b02479

Bueno, M., Resconi, V. C., Campo, M. M., Cacho, J., Ferreira, V., & Escudero, A. (2011). Gas chromatographic–olfactometric characterisation of headspace and mouthspace key aroma compounds in fresh and frozen lamb meat. *Food Chemistry*, *129*(4), 1909–1918. https://doi.org/10.1016/j.foodchem.2011.06.001

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

Channon, H., Lyons, R., & Bruce, H. (2003). Sheepmeat flavour and odour: a review Project number: Sheep CRC 1.3.2.

Font-i-Furnols, M., & Guerrero, L. (2014). Consumer preference, behavior and perception about meat and meat products: An overview. *Meat Science*, *98*(3), 361–371. https://doi.org/10.1016/j.meatsci.2014.06.025

Frankel, E. N. (1980). Lipid oxidation. *Progress in Lipid Research*, *19*(1), 1–22. https://doi.org/10.1016/0163-7827(80)90006-5

Gazdziak, S. (2015). Lamb's "wow" factor. Retrieved January 20, 2017, from http://www.provisioneronline.com/articles/102685-lambs-wow-factor?v=preview

Hoffman, T. (2016). *BENCHMARK OF LAMB QUALITY IN U.S. RETAIL AND FOODSERVICE MARKETS*.

J.Daryl Tatum, Henry N.Zerby, K. E. B. (2014). Physiological Indicators of Ovine Age. *Prepared for the Lamb Industry Raodmap Product Characteristics Team*.

JH, C. (2016). Lipid Oxidation in Meat. *Journal of Nutrition & Food Sciences*, *6*(3), 1–3. https://doi.org/10.4172/2155-9600.1000494

Merriam Webster. (2016). Flavor | Definition of Flavor by Merriam-Webster. Retrieved January 20, 2017, from https://www.merriam-webster.com/dictionary/flavor

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

Neethling, J., Hoffman, L. C., & Muller, M. (2016). Factors influencing the flavour of game meat: A review. *Meat Science*, *113*, 139–153. https://doi.org/10.1016/j.meatsci.2015.11.022

Pegg, R. B., & Shahidi, F. (2014). *Encyclopedia of Meat Sciences*. *Encyclopedia of Meat Sciences*. Elsevier. https://doi.org/10.1016/B978-0-12-384731-7.00129-X

Pethick, D. W., Hopkins, D. L., D'Souza, D. N., Thompson, J. M., Walker, P. J., Batcher, O., … Braggins, T. (2005). Effects of animal age on the eating quality of sheep meat. *Australian Journal of Experimental Agriculture*, *45*(5), 491. https://doi.org/10.1071/EA03256

Rousset-Akrim, S., Young, O. A., & Berdagué, J.-L. (1997). Diet and growth effects in panel assessment of sheepmeat odour and flavour. *Meat Science*, *45*(2), 169–181. https://doi.org/10.1016/S0309-1740(96)00099-X

Sañudo, C., Enser, M. E., Campo, M. M., Nute, G. R., María, G., Sierra, I., & Wood, J. D. (2000). Fatty acid composition and sensory characteristics of lamb carcasses from Britain and Spain. *Meat Science*, *54*(4), 339–346. https://doi.org/10.1016/S0309-1740(99)00108-4

Sink, J. D., & Caporaso, F. (1977). Lamb and mutton flavour: Contributing factors and chemical aspects. *Meat Science*, *1*(2), 119–127. https://doi.org/10.1016/0309-1740(77)90013-4

Stillman, R., Crawford, T., & Aldrich, L. (n.d.). The U.S. Sheep Industry.

Tatum, J. D., Zerby, H. N., & Belk, K. E. (2014). A Brief Overview of Factors Affecting Lamb Flavor Prepared for the Lamb Industry Roadmap Product Characteristics Team.

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

Veldhuizen, M. G., Shepard, T. G., Wang, M.-F., & Marks, L. E. (2010). Coactivation of Gustatory and Olfactory Signals in Flavor Perception. *Chemical Senses*, *35*(2), 121–133. https://doi.org/10.1093/chemse/bjp089

Ventura, A. K., & Worobey, J. (2013). Early Influences on the Development of Food Preferences. *Current Biology*, *23*(9), R401–R408. https://doi.org/10.1016/j.cub.2013.02.037

Wong, E., Johnson, C. B., & Nixon, L. N. (1975). The contribution of 4-methyloctanoic (hircinoic) acid to mutton and goat meat flavour. *New Zealand Journal of Agricultural Research*, *18*(3), 261–266. https://doi.org/10.1080/00288233.1975.10423642

Young, O. A., Berdagué, J.-L., Viallon, C., Rousset-Akrim, S., & Theriez, M. (1997). Fat-borne volatiles and sheepmeat odour. *Meat Science*, *45*(2), 183–200. https://doi.org/10.1016/S0309-1740(96)00100-3

Young, O. A., Lane, G. A., Priolo, A., & Fraser, K. (2003). Pastoral and species flavour in lambs raised on pasture, lucerne or maize. *Journal of the Science of Food and Agriculture*, *83*(2), 93–104. https://doi.org/10.1002/jsfa.1282

CHAPTER III

MATERIALS AND METHODS

Product Selection

Lamb legs were chosen as the sample for volatile flavor analysis as studies have shown that legs have high concentrations of compounds associated with lamb flavor compounds, especially negative flavor notes. The 75 carcasses included in this study were selected from October 2015 to the end of March 2016. LAMB carcasses were selected in each month of the collection period with carcasses representing processing operations in California and Colorado. Due to limited availability in the fall months, YEARLING and MATURE carcasses were exclusively selected in the spring season, and all YEARLING and MATURE carcasses were collected from Colorado processing operations. The genetic variation of the LAMB and YEARLING sample was very diverse, with the clear majority (47 of 50) of sheep being finewool x medium wool crosses. Three hair-type sheep were also included in the LAMB sample. Also, the LAMB and YEARLING sample included lambs resulting from operations in 8 states (CO, NE, CA, KS, SD, OR, IA, and ID) with a mixture of grass-fed and grain-fed lambs. Due to very limited availability, the entire MATURE sample was collected in March at a single Colorado processing facility, and was made up entirely of mature, black-face ewes that were fed a high energy ration in a Colorado feedlot for 45 d prior to harvest. Boneless lamb legs (NAMP 234; N=75) were collected from sheep carcasses in commercial processing facilities according to age class. Animal age (determined via dentition) served as the primary selection criteria for inclusion in the sample. Sheep were selected to represent three dentition groups: 1) young lambs (LAMB; 0 permanent incisors); 2) yearlings (YEARLING; 2 permanent incisors); 3) mature sheep or mutton (MATURE; >2 permanent incisors). Twenty-five (n = 25) carcasses in each dentition

group were identified for inclusion in the study, and associated legs were collected. Data pertaining to production background, production seasons, animal breed, and sex classification were recorded for each carcass. At the time of harvest, the lambs were evaluated for dentition, breed appearance, sex class and production background. USDA yield grade, USDA quality grade, ribeye area, hot carcass weight, 12th rib fat thickness, body wall thickness, flank streakings, visual flank color score, carcass conformation score, leg score, longissimus muscle (LM) marbling score, visual fat color score, and subjective odor scores were collected prior to carcasses fabrication and leg collection. Objective fat and lean color measurements (L*, a*, b*) were obtained from the external surface of each leg using a spectrophotometer (Model # 45/0 MSXE Hunter Labs, Reston, VA). Once the color measurements were obtained, 100 g of lean and fat from the outside surface of the leg were trimmed and placed in a pre-labeled Whirl Pak bag for volatile flavor compound analysis. The remainder of the boneless leg was vacuum packaged and transported under refrigeration (2˚C) to Colorado State University Meat Laboratory for further analysis. Upon arrival, the vacuum-sealed legs were frozen and stored (-20˚C) until sample preparation.

Sample Preparation

Individual legs were tempered for 18 h at 2˚C prior to grinding. Legs were trimmed to 1/8-inch maximum external fat thickness to provide a more uniform composition across samples. Some legs were practically devoid of fat, so no trimming occurred. Each individual leg was twice-ground. First, legs were coarse ground through a 9.5 mm grind plate, then, each sample was homogenized in a hand mixer for 5 m and fine ground using a 3 mm grind plate. The fine grind was then formed into 28.35 g patties using a Patty-O-Matic® Eazy Slider (at least 36 patties per leg) with 24 patties packaged for sensory training and sensory panels. Twelve patties were

vacuum sealed and placed on metal sheet pans in the freezer (-20˚C) to hold form and thickness of patty. From each leg, 100 g was collected for proximate analysis to determine the fat, protein, moisture and ash content of each leg and all other patties were packaged and frozen. After 24 hours, the packages were boxed, sealed and frozen (-20˚C) in the absence of light to minimize photo-oxidation until sensory panels or other analysis.

Volatile Flavor Compound Sample Preparation

Following sample collection at the plant, the tissue sample from the outside surface of each leg was cut in small chunks and submerged in liquid nitrogen until the sample was completely frozen. A stainless-steel spoon was used to transport the frozen tissue sample from the bowl into a commercial food processor (6.62 L; Blixer 6V, Robot Coupe USA Inc., Ridgeland MS). The samples were pulverized until they were finely powdered and homogenous, immediately placed in pre-labeled Whirl Pak bags, and stored in a -80˚C freezer until volatile flavor compound analysis.

Proximate Analysis

Proximate analysis was conducted for each ground leg sample. All samples were analyzed in duplicates at Colorado State University. After individual legs were finely ground, 100 g of the sample were set aside for proximate analysis. The 100 g were crumbled into a stainless-steel bowl, submerged in liquid nitrogen and a stainless steel spoon was used to further break up the crumbled sample, as well as transport the frozen sample from the bowl to a commercial food processor (6.62 L; Blixer 6V, Robot Coupe USA Inc., Ridgeland MS). The samples were pulverized until they appeared finely powdered and homogenized, immediately place in pre-labeled Whirl Pak bags and stored in a -80˚ C freezer until proximate analysis was conducted. Fat content of each sample was extracted using the Folch Method (Fold et al. 1957; AOAC,

2000). Approximately 1 g of sample was homogenized in 2:1 chloroform to methanol solution, then placed on an orbital shaker at room temperature for 20 m. The homogenized sample was filtered through ashless filter paper and 4 ml of 0.9% NaCl was added, followed by a 24 hour refrigeration period. When the filtrate separated into two phases, the low phase was then aspirated and placed into a pre-weighed scintillation vial. The vial was then dried under N2 gas followed by the vial air drying under the hood for 2 h. Following the 2 h the vials were placed in a forced air drying oven for 12 h at 100˚C. Percent total fat was calculated from the formula: %TF= [((Total volume of chloroform: methanol) / 10) x (final lipid weight / initial weight)] x 100. Moisture content of each sample was analyzed using the AOAC oven drying method 950.46 and 934.04 (AOAC, 1995). Approximately 1 g of sample was weighed into aluminum tins and placed in a forced air drying oven for a 24 h period at 100˚C**.** Percent moisture content was determined from the formula: % MC= [(initial weight – dry weight) / initial weight] x 100. Crude protein of each sample was analyzed using the AOAC Official Method 992.15 (2006) with a nitrogen determinator (Leco TruSpec CN or Leco FP-2000; Leco Corporation, St. Joseph, MI and Rapid N cube, Elmentar, Hanau, Germany). Percent protein was calculated using the formula: % Protein = Total % nitrogen (TPN) x 6.25. As content of each sample was analyzed using the ashing method described in the AOAC 923.03 and 920.153 (1995). Approximately 1 g of sample was placed into a pre-weighed, dry crucible. Samples were placed into a Thermolyne box furnace at 600˚C for 18 h. Percent ash was calculated using the formula: % Ash= (ash weight/ wet weight) x 100.

Trained Sensory Analysis

Trained sensory analysis of flavor and aroma was conducted at Colorado State University. Panelists were trained to objectively quantify; lamb flavor intensity, off flavor intensity, and

lamb aroma intensity using an unstructured line scale anchored at both ends (0 = absence or low intensity of specified attribute, 100 = extreme intensity of specified flavor attribute). The panelists were trained using extra patties from the study. After the two-week training, panelists were excused if they still could not quantify and differentiate lamb intensity.

Samples for sensory analysis were assigned to sensory sessions to have an equal representation of each dentition group in each panel. Two panel sessions were conducted each day with at least a 3 hour break between sessions and a minimum of 8 panelists. There were 6 samples per session until the last 3 sessions having 7 samples to complete the sensory panels in 12 sessions.

Frozen Patties for sensory analysis were tempered in a chilled environment of 2˚C for 12 h. Twelve patties were evenly spaced on a griddle and cooked in a combination oven (Rational 5 Senses, Rational USA Inc., Rolling Meadows, IL). The combination oven was preheated to 204^0C with 0% humidity. The twelve patties were cooked for 7 m which allowed for an internal temperature of 70^0C to be reached, temperature was monitored with a Type 5 Thermocouple Thermometer (AccuTuff 340, model 34040, Cooper-Atkins Corporation, Middlefield, CT). Immediately after the temperature was checked, the patties were placed in glass bowls and covered with pre-labeled aluminum foil. The patties were held at 70˚C and transported to the panel room in a warming oven and served within 30 m of cooking. Each panelist had a private booth with a red incandescent light to mask color differences and coffee beans to cleanse the olfactory senses. For palate cleansing between samples, panelists were given unsalted saltine crackers, unsweetened apple juice, and distilled water. Each panelist received 1 patty from each sample to evaluate lamb flavor intensity and off flavor intensity sensory attributes, with the opportunity for another sample if needed. The panelists quantified these attributes using

Qualtrics survey on a tablet or laptop. After each panel, the panelist ratings were evaluated on Qualtrics and a single average for each sample was obtained for each attribute.

Aroma Analysis

Aroma analysis occurred immediately after the flavor analysis panels. Five raw patties were split in half (14.2 g) and split between two 100 ml labeled glass test tubes. The outside of the test tube was cleaned of any sample residue and placed in an 18 slot test tube rack. The test tubes were placed in the water bath (Fisher Scientific™ Isotemp™ Heated Immersion Circulators: Model 6200 H24) set at 70˚C for 30 m. At the conclusion of the flavor sensory panel, panelists received 6 test tubes representing individual samples. Each panelist had a vortex and coffee beans to cleanse the olfactory sense between each sample. Panelists were instructed to vortex the sample for 20 s and quickly open the lid and open mouth experience the sample. Each sample was evaluated for lamb odor intensity using an unstructured scale, anchored at both ends (0 = absence or low intensity of lamb aroma, 100 = extreme intensity of lamb aroma). After each panel the panelist ratings were evaluated on Qualtrics and a single average for each sample was obtained.

Volatile Flavor Compound Analysis

Homogenized samples stored in the -80˚C freezer were transported on dry ice to the metabolomics lab at Colorado State University. One sample was prepped at a time to minimize oxidation. Five g of frozen, homogenized sample were weighed into a 20 ml headspace vial (Thermo Scientific Chromacol 18 MSC-ST201 18 mm Langerwehe, Germany) and a saturated NaCl solution (2 ml) was added, the sample was then kept on dry ice until it was ready to be picked up the SPME fiber. After a 10 m equilibration at ambient temperature, the sample was incubated at 65˚C for 5 m with agitation. Then the volatile compounds were extracted by a 75

µm Carboxen/PDMS SPME fiber (Sigma-Aldrich) at 65°C for 20 m with agitation, and then desorbed at 245˚C for 2 m into a DB-WAXUI column (30 m x 0.25 mm x 0.25 µm, Agilent) in a Trace1310 GC (Thermo) coupled to a Thermo ISQ-LT MS. The inlet was set on splitless mode during desorption. The oven temperature program started at 40˚C held for 3 m, increased to 200˚C at 6˚C/m, and then increased to 220˚C at 20˚C/m. Detection was completed under electron impact mode, with a scan range of 40-350 amu (atomic mass unit) and a scan rate 5 scans/s. Transfer line and source temperatures were 240 and 250˚C, respectively.

Statistical Analysis

Least squares ANOVA were conducted using the PROC GLM procedures of SAS (Version 9.4; SAS Inst. Inc., Cary, NC). Individual leg (animal) served as the experimental unit and fixed effects of dentition class and production background were analyzed at $\alpha = 0.05$. The PROC CORR procedure in SAS (Version 9.4; SAS Inst. Inc., Cary, NC) was utilized to establish Pearson's correlations for sensory attributes, and carcass measurements. PROC CORR procedures were utilized to select metabolites that were significantly correlated ($P < 0.05$) to lamb flavor intensity for subsequent regression modeling procedures. The RandomForest package in R (R 3.2.2, Vienna, Austria) was utilized to identify metabolites of importance for modeling. The PROC REG procedures of SAS (Version 9.4; SAS Inst. Inc., Cary, NC) were used for regression modeling and prediction equation development. A single model was developed for the prediction of lamb flavor intensity using stepwise model selection with the significance level set at $\alpha = 0.10$ to select variables for the model. Three additional models were developed for the prediction of lamb flavor intensity and off flavor intensity using AIC (Akaike Information Criterion) model selection techniques with selection criteria for C(p), AIC, and R^2 statistics and 'best = 100'. AIC is a measure of the relative quality of statistical models. The

effectiveness of the models developed for segregating lamb flavor intensity was tested by applying the developed models to the development data set to determine predictive ability. Thresholds for categorizing lamb flavor intensity into Mild, Medium, and Bold intensities were scores of < 18, 19 – 34, and > 35, respectively, and % accuracy was determined by computing the percentage of correct classification of lamb flavor intensity according to actual sensory panel means. Models for the prediction of off flavor intensity were also developed using PROC REG procedures in SAS (Version 9.4; SAS Inst. Inc., Cary, NC)

CHAPTER IV

RESULTS AND DISCUSSION

Carcass Characteristic Differences

Carcass characteristics for the sheep carcasses included in the sample are presented in Table 4.1. LAMB and YEARLING carcasses had similar ($P > 0.05$) REA, 12th rib fat thickness, YG, and marbling scores, while MATURE carcasses were the leanest and least marbled and possessed a stronger subjective raw odor($P < 0.05$).

Proximate Composition and Relationship to Sensory Attributes

The proximate composition of samples by age class are presented in Table 4.2. Crude fat % was different for each class with YEARLING leg samples having the highest fat %, while MATURE samples were the leanest ($P < 0.0001$). This was primarily an indication of differences in internal seam fat within the legs that were collected for sensory analysis, due to the exterior of all legs being trimmed to a maximum of 0.32 cm of fat. LAMB samples had higher crude protein % than YEARLING samples, but had similar crude protein % as MATURE samples. Differences in crude protein are most easily attributed to proportional differences in crude fat %. Ash % varied by age class with MATURE samples having the highest % ash and YEARLING samples having the lowest ash %.

Relationship of Carcass Characteristics, Sensory Attribute and Metabolites

Correlations for measured carcass traits and sensory attributes are presented in Table 4.3. None of the carcass measurements were significantly ($P < 0.05$) correlated with lamb flavor intensity scores. Dentition score was significantly ($P < 0.05$) and positively correlated ($r = 0.38$) with sensory panel ratings for off-flavor intensity, and carcass measures of 12^{th} rib fat thickness, body

wall thickness, leg score, marbling score, and YG were negatively correlated ($P < 0.05$) with off-flavor intensity. These correlations indicate that as carcasses become fatter and heavier muscled, the intensity of off-flavors declines. This is most likely due the fact that MATURE carcasses were the leanest and lightest muscled, and they also had the greatest off-flavor intensity scores ($P < 0.05$; Table 2.). Similar studies would agree that with more fat, off-flavor decreases however, flavor intensity should increase with maturity (Tatum et al., 2014). The 12th rib fat thickness was significantly and positively correlated to body wall thickness, leg score, marbling, yield grade (stamped) and crude fat percentage unfortunately the yield grade and 12th rib fat thickness should be more highly correlated (0.69).

Correlations for metabolites that were detected from fat and lean samples that were removed from the outer portion of the leg of each sheep carcass with sensory panel attributes are presented in Table 4.4. Fifty metabolites of significance and importance were identified using tests correlation and RandomForest techniques from some 500+ metabolites identified using mass-spectroscopy. Off the 50 metabolites identified of significance, 18 were significantly ($P < 0.05$) correlated with lamb flavor intensity scores and 4 were correlated ($P < 0.05$) with off-flavor intensity scores. These findings indicate that metabolites (volatile compounds) are related to the flavor of sheep meat. Similar metabolites were found for lamb flavor by Bueno and Young using the GC-MS technology.

Relative values of metabolites by age classification are presented in Table 4.5. Once the metabolites of significance were identified, the annotation process began. Using Ramsearch, a graphical user interface that uses the NIST Library msPepSearch tools to search and retrieve spectra. The spectra were imported to RAMSEARH in an mspfile and using the NIST library the spectra is searched using GC-MS in source search using low mass accuracy. The search

results are compiled and through visual and interactive evaluation of similar spectras a match is determined. There is slight subjectivity to this process as it is opinion based which is the best match especially as there were no retention times to compare back to. Once a match is determined in Ramsearch, a Confidence level 1-4 (1 unsure – 4 match) is selected. The data is saved for confident annotations and notes on the questionable annotations. This theoretical library approach has value in annotation of unknown in-source spectra like with lamb (Broeckling et al., n.d.). Even without retention times the findings of this study have similar metabolites for metabolites that contribute to sheep meat flavor that were found by Bueno and Young.

Significant differences in the concentration of the metabolites existed among age classifications of carcasses, indicating that animal age does influence fat and lean composition; however, these data alone are not sufficient for establishing a proof of concept for metabolites or age for predicting lamb flavor.

Differences in Age and Production Background as Quantified by Trained Sensory Panel Ratings

Even though it was not a primary objective in this study, an analysis of variance was conducted for sensory flavor attributes relative to animal age to better describe the experimental sample. Trained sensory panel ratings for ground lamb patties by age class are presented in Table 4.6. LAMB samples had higher ($P < 0.05$) ratings for lamb flavor intensity than YEARLING samples, and, surprisingly, lamb flavor intensity scores were similar for LAMB and MATURE age classes. YEARLING samples had similar lamb flavor intensity scores as MATURE samples, but had the lowest numerical ratings for lamb flavor intensity overall. It should be noted that lamb flavor intensity scores are representative of the intensity of the characteristic flavor of lamb

without off-flavor notes. Pethick (2005) found that as sheep mature the flavor intensifies and consumers find it undesirable. Flavor intensity of lamb has been attributed in large part to branched chain fatty acids (BCFA) and as the animal ages the concentration of BCFAs gradually increase (Sink & Caporaso, 1977). However, the BCFA is found in triglycerides in ovine fat and this could explain the reason why the MATURE ewes were not the highest in lamb flavor intensity as they were the leanest in the study (Rousset-Akrim, Young, & Berdagué, 1997) shown in Table 4.2 . One could also theorize that the YEARLING lambs in this sample may have been fed a corn-based diet for a longer period (as indicated by greater fatness; Table 4.1) which may have contributed to a milder lamb flavor. Also, MATURE samples were lower ($P < 0.05$; Table 4.2) in crude fat than the younger age classes, which may help to explain the similarity in flavor between the LAMB and MATURE samples, seeing that lamb flavor intensity has been widely attributed to sheep fat (Tatum et al., 2014). As expected, MATURE samples had the highest ($P < 0.05$) level of off-flavor intensity, while LAMB and YEARLING samples had the lowest ($P < 0.05$) level of off-flavor intensity. Numerically, LAMB samples had and intermediate level of off-flavor intensity, but much lower (9.42 vs. 22.56) off-flavor intensity ratings than MATURE samples. Therefore, even though lamb flavor intensity ratings for LAMB and MATURE samples were similar, the MATURE samples had a much higher level of off-flavor. This supports previous studies that suggest that as the animal matures the level of off flavor increases (Sink & Caporaso, 1977).

Aroma ratings did not differ ($P > 0.05$) by age class, but it should be noted that, in general, the intensity of aroma from the cooked samples was very low, and panelists had difficulty deciphering differences between samples. Aroma is of huge importance when it comes to flavor unfortunately it is hard to capture. Several studies have tried other techniques of capturing

aroma from cooked lamb samples, and they have been successful in isolating the aroma of the sample. Bueno et al. (2011) used two different techniques; in the first, they tried capturing the aroma right off the cooked sample and in the second they captured the aroma as it was released from the mouth of the panelist as they chewed the sample and was collected from the exhaled air. (Bueno et al., 2011).

Trained sensory panel ratings for ground lamb patties by animal background and averaging over age class are presented in Table 4.7 MATURE samples were not included in this comparison. Grass-fed lamb samples had the higher ($P < 0.05$) lamb flavor intensity and a higher off flavor intensity however it was not statistically significant (P = 0.36). Most literature would suggest this is attributed to the fact that a grain based diet decreases the amount of tryptophan, an amino acid that is degraded in the rumen. In grass fed animals, tryptophan is degraded in the rumen and forms to major compounds that attributes to pastoral flavor; 3-methylindole (skatole) and indole. When excess amounts are absorbed they accumulate in the adipose tissue. Young (1997) also concluded that the basic lamb flavor is produced from the presence of 3- methylindole and alkyl phenols. Rousset et al. (1997), found similar results that grass finished lambs most often has a more intense flavor with a greater incidence of off flavors.

Lamb Flavor and Off Flavor Prediction Models

Stepwise regression techniques were utilized to develop a prediction model for lamb flavor techniques. All 50 metabolites of significance were considered for the final predictive model along with all carcass measurements and animal dentition classification. From the 75 sheep carcasses included in the present study sample, a single predictive equation was developed using stepwise regression (Table 4.8). This prediction equation includes 7 metabolites; C490, C75,

C455, C129, C274, C22, and C494 to achieve an R^2 of 0.59 indicating that 59% of the variation in lamb flavor intensity was explained with this prediction model, refer to Table 4.13 for putative annotations. When tested back on the data set, this equation demonstrated the ability to accurately classify individual sheep carcasses into Mild, Medium, or Bold flavor classes with 84% accuracy. More specifically, it was 67%, 75%, and 92% accurate at classifying carcasses in Mild, Medium, and Bold classes, respectively. The predictive capacity of this equation is illustrated in Figure 4.1. These data indicated a positive proof of concept for using external tissue samples and GC-MS derived metabolites for the classification of sheep carcasses into meaningful flavor groups.

Additionally, AIC regression methods were utilized to develop prediction models and further establish proof of concept for classifying lamb flavor. Three additional models developed using AIC are presented in Table 4.9. These models utilized a greater number of factors, including indicators of fat color, for prediction, and a higher percentage of the variation in lamb flavor intensity was explained; however, despite using a greater number of factors, the overall accuracy of these models were not better than the model developed using the stepwise technique. Nonetheless, these models do support the concept that metabolites and carcass measures can be utilized to classify lamb flavor intensity.

These predictive capabilities are still a novelty to the sheep industry. The idea of predicting eating quality is overwhelming for most, but their focus of currently improving the lamb industry is by segregating the lambs with off flavor attributes first. Stepwise, backward and forward regression techniques were utilized to develop prediction models for off flavor attributes in lamb. The 50 significant metabolites were used for consideration in the model, in conjunction with all carcass measurements and animal dentition classification. Three prediction equations were

developed. The stepwise prediction model (Table 4.10) utilized 10 metabolites as well as 12th rib fat thickness, body wall thickness and marbling to achieve a R^2 of 0.77, representing that 77% of the variation in off flavor intensity was explained in this model. The backward selection technique (Table 4.11) achieved an R^2 of 0.94, using, leg score, L*, b* and 32 metabolites. This model utilizes several more metabolites but with this R^2 value this is the model of best fit. The final predictive model technique was a forward selection model (Table 4.12) utilized 14 metabolites, leg score, body wall thickness and marbling to explain off flavor intensity of the 74 lamb samples. Any of these models could be utilized to accurately segregate the lambs with off flavor attributes.

Conclusions

In conclusion, a proof of concept was established for utilizing a tissue sample from the exterior of carcasses of varying composition and flavor and GS-MS technology to quantify differences in metabolites contributing to lamb flavor intensity. Further research is warranted to pursue instrument development for classifying individual carcasses on the basis of flavor. This is in the best interest of the American Sheep Industry's survival as the meat industry is a competitive market and lamb is continually facing new challenges to maintain or increase their market share (Arsenos et al., 2002).

Table 4.1. Least squares means for carcass measurements between age class (corresponding range for lamb intensity of ground cooked patties).

Age[1]	HCW (kg)	REA (cm^2)	12^{th} Rib Fat (cm)	Body Wall (cm)	Yield Grade	Marbling	Subjective Odor	Visual Color	L*	a*	b*
Lamb	33.94[b] (26.2-42.6)	17.61[a] (12.26-23.87)	0.37[a] (0.51-2.8)	1.08[b] (1.78-4.6)	4.12[a] (2.4-5)	368.75[a] (Sl10-Mt60)	2.21[b] (0-6)	7.13[a] (1-10)	69.91[a] (57.68-77.24)	0.33[b] (-2.42-0.82)	6.42 (2.63-10.25)
Yearling	40.44[a] (27.8-54.9)	16.9[a] (11.61-23.23)	0.40[a] (0.51-2.54)	1.38[a] (2.03-5.08)	4.36[a] (2.4-5)	476.8[a] (Sl00-MAB80)	1.92[b] (0-9)	2.92[b] (1-7)	70.93[a] (60.25-82.58)	1.56[b] (-1.93-7.2)	7.30 (3.05-5.87)
Mature	35.36[b] (22-62.3)	15.03[b] (8.39-23.87)	0.15[b] (0.25-1.27)	0.51[c] (0.51-2.54)	1.92[b] (1.4-3)	179.6[b] (Tr00-Sm60)	3.52[a] (1-7)	3.00[b] (1-5)	65.14[b] (47.48-74.65)	4.88[a] (0.71-8.49)	7.19 (2.43-13.15)
SEM	3.36	0.11	0.03	0.05	0.33	50.44	0.47	0.31	1.17	0.47	3.36
P-value	**0.009**	**0.041**	**<0.0001**	**<0.0001**	**0.0003**	**<0.0001**	**0.0394**	**<0.0001**	**0.0016**	**<0.0001**	0.3861

[a, b, c] Means within column lacking common superscripts differ ($P < 0.05$).

[1] Lamb= 0 permanent incisors; Yearling = 2 permanent incisors; Mature = 2+ permanent incisors

Table 4.2. Proximate analysis composition of raw ground lamb by age class.

Age[1]	Lipid, %	Protein, %	Ash, %
Lamb	9.23[a]	17.43[a]	0.88[b]
Yearling	10.37[a]	16.89[b]	0.85[ab]
Mature	5.06[b]	17.76[a]	0.93[a]
SEM	0.39	0.172	0.02
P-value	**<0.0001**	**0.0023**	**0.0073**

[a, b, c] Means within column lacking common superscripts differ (P < 0.05).

[1]Age Lamb= 0 permanent incisors; Yearling = 2 permanent incisors; Mature = 2+ permanent incisors.

Table 4.3. Simple correlation coefficients comparing carcass measurements and sensory attributes.

	Carcass Measurements[1]															
Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1.Lamb Intensity																
2. Off-Flavor	0.18															
3.Aroma	0.05	-0.02														
4.HCW	-0.15	-0.18	0.06													
5.REA	0.20	-0.22	-0.01	**0.52**												
6.12th Rib Fat	-0.15	**-0.41**	0.07	**0.38**	0.11											
7. Body Wall	-0.18	**-0.61**	0.14	**0.49**	**0.31**	**0.77**										
8.Leg Score	-0.03	**-0.61**	0.12	0.14	**0.29**	**0.56**	**0.79**									
9.LM Marb	-0.01	**-0.37**	0.12	0.23	0.03	**0.38**	**0.45**	**0.41**								
10.YG	-0.11	**-0.56**	0.06	0.21	**0.24**	**0.69**	**0.80**	**0.88**	**0.47**							
11. Odor	0.03	0.12	**-0.24**	-0.07	-0.13	-0.06	-0.22	**-0.33**	-0.15	**-0.27**						
12.Visual Color	0.26	-0.17	-0.03	**-0.25**	0.16	0.12	0.04	**0.35**	0.15	**0.38**	-0.18					
13. Fat L*	-0.11	-0.27	0.10	0.12	0.02	**0.29**	**0.31**	**0.38**	**0.36**	**0.34**	-0.08	0.09				
14.Fat a*	-0.03	0.29	0.05	0.05	**-0.29**	-0.22	**-0.40**	**-0.62**	-0.04	**-0.54**	**0.34**	**-0.35**	-0.04			
15.Fat b*	-0.18	-0.23	-0.10	0.19	-0.05	0.07	0.10	-0.06	0.15	0.01	0.05	-0.11	**0.25**			
16.Crude Fat %	-0.14	0.06	**-0.62**	**0.51**	**0.42**	**0.68**	**0.81**	**0.76**	**0.44**	**0.74**	**-0.27**	0.13	**0.34**	**-0.42**		
17.Crude Prot.	0.15	-0.15	**0.27**	-0.08	0.09	**-0.33**	**-0.37**	**-0.33**	-0.20	**-0.39**	0.07	-0.02	-0.11	**0.24**	0.04	

|r| ($P < 0.05$).

Table 4.4. Simple correlation coefficients comparing metabolites and sensory attributes

	Sensory Attributes		
Variables	$R^{Lamb\ Intensity}$	$R^{Off\text{-}flavor}$	R^{Aroma}
C6	0.246	**0.314**	0.077
C10	**0.348**	**0.301**	-0.012
C11	**0.304**	**0.425**	-0.034
C12	0.240	**0.428**	-0.052
C15	0.293	0.229	-0.104
C22	-0.244	0.033	-0.081
C25	-0.278	-0.197	-0.080
C26	**0.350**	-0.268	0.071
C30	0.246	0.222	-0.030
C31	0.288	0.197	-0.054
C33	**0.305**	0.229	-0.114
C35	0.239	**0.392**	-0.068
C36	0.293	0.205	-0.069
C59	-0.271	0.077	-0.049
C75	**0.420**	-0.141	0.088
C76	0.254	0.055	-0.106
C79	0.239	0.237	-0.097
C85	**-0.306**	-0.176	-0.028
C97	0.268	0.227	-0.071
C129	**0.309**	0.206	-0.075
C146	-0.237	0.141	-0.001
C153	0.289	0.239	-0.136
C156	**0.308**	0.209	-0.075
C180	0.273	0.125	-0.104
C227	0.085	0.145	-0.088
C228	**0.311**	0.222	-0.096
C246	0.232	0.228	-0.066
C248	0.233	0.236	-0.048
C264	0.243	0.455	-0.024
C274	0.290	0.219	-0.096
C285	0.297	0.253	-0.169
C312	0.263	0.161	-0.052
C341	**0.299**	0.194	-0.238
C342	**0.324**	0.195	-0.068
C378	**0.304**	0.097	-0.068

$|r|$ ($P < 0.05$).

Table 4.4. Continued

	Sensory Attributes		
Variables	R $^{\text{Lamb Intensity}}$	R $^{\text{Off-flavor}}$	R $^{\text{Aroma}}$
C403	0.285	0.225	-0.027
C404	0.092	0.184	-0.140
C418	0.277	0.279	-0.068
C423	0.257	0.243	-0.096
C449	**0.357**	0.151	-0.148
C452	**0.336**	0.217	-0.107
C455	**0.317**	0.125	-0.011
C462	-0.239	-0.098	-0.052
C487	0.297	0.224	-0.059
C490	**0.436**	0.132	-0.045
C494	0.269	0.014	-0.019
C501	**0.372**	-0.047	0.004
C505	0.283	0.287	-0.038
C531	**-0.324**	-0.041	-0.086
C537	-0.289	-0.217	-0.063
C544	0.282	0.147	0.078

|r| ($P < 0.05$).

Table 4.5. Metabolite values in ground lamb between age[2] class.

Metabolite	Lamb	Yearling	Mature	*P*-value[1]	Overall Average
C6	455102.76	339553.99	609088.28	0.85	468088.2
C10	70020.34	60301.16	80341.73	0.34	70959.82
C11	472149.93	408375.70	54672.05	0.10	70223.79
C12	93046.13	105632.31	303960.80	0.11	474767.5
C15	8628149.1	17423486.6	13927411.2	0.20	168553.2
C22	44560284.6	75683262.1	47840171.7	**0.01**	13389838
C25	809924.46	469807.49	304926.89	**0.002**	56182874
C26	11794.38	18058.01	34534.95	**<0.0001**	524412.8
C30	4934.10	10907.89	43976.69	0.33	21593.1
C31	2535.57	2719.41	9138.37	0.39	20142.34
C33	2702861.42	2264661.93	3194692	0.24	4828.354
C35	3491.18	5977.92	22278.08	**0.05**	2720980
C36	74394.17	218232.26	178823.32	0.35	10678.22
C59	74394.17	218232.26	178823.324	**<0.0001**	158268.2
C75	49545952.0	25297328.2	28635766.9	**0.0006**	34289598
C76	103370.11	86407.68	103959.22	0.92	97838.58
C79	5267.08	5739.79	11105.92	0.20	7399.361
C85	44988.02	2597067.38	1446855.94	**<0.0001**	1511808
C97	78565.96	94815.2	179369.30	0.21	118110.8
C129	63664.2	100165.11	507985.115	0.33	226104
C146	1614633.8	2028795.27	2140064.83	0.22	1932064
C153	2795.89	2975.56	10808.95	0.16	5563.703
C156	50154.15	73057.07	332724.78	0.32	153354.7
C180	46979.34	12977.02	46258.80	0.50	35248.64
C228	6439.80	7092.17	26769.89	0.26	13528.47
C246	92521.05	98564.29	123432.06	0.21	105005.6
C248	784.95	763.62	898.47	0.37	816.0931
C264	4610.60	4155.72	8085.56	**0.05**	5630.896
C274	814.75	1120.39	3102.34	0.28	1690.843
C285	388.31	365.03	793.11	0.15	517.2018
C312	552079072	303858425	519683685	0.10	4.57E+08
C341	440.18	363.76	584.49	0.17	463.1145
C342	39994.33	35418.60	50490.35	0.66	41994.43
C378	70057857.6	35187262.1	60248246.7	0.21	54963193
C403	1583.16	1913.44	3497.75	0.44	2341.559
C404	251.17	197.45	327.89	0.14	258.9402
C418	6050001.98	597622.847	742208.5	0.13	648862.6
C423	289.09	263.03	356.11	0.24	302.928
C449	49970.76	35639.26	50381.95	0.03	45267.95
C452	114240239	71752191	113939710	0.06	99784639
C455	18110.87	16383.28	19670.89	0.33	18054.26

[1], (*P* < 0.05) are in bold text.

[2] Lamb= 0 permanent incisors; Yearling = 2 permanent incisors; Mature = 2+ permanent incisors.

Table 4.5. Continued

Metabolite	Lamb	Yearling	Mature	*P*-value[1]	Overall Average
C462	82784.35	151083.02	101402.65	**0.04**	112148.2
C487	5067.83	5682.57	11553.04	0.33	7466.461
C490	143.18	92.89	127.09	0.18	120.7539
C494	286.91	287.10	258.41	0.73	277.3473
C501	1732666.29	1400665.93	1300835.75	0.14	1474615
C505	454.49	410.07	548.94	0.26	471.395
C531	1258883.61	3720879.87	2719626.19	**<0.0001**	2584133
C537	4899651.69	6672827.67	4636704.34	0.03	5409864
C544	45518041.1	36834312.8	41845033.5	0.26	41343468

[1], (*P* < 0.05) are in bold text.

[2]Lamb= 0 permanent incisors; Yearling = 2 permanent incisors; Mature = 2+ permanent incisors.

Table 4.6. Least squares means for lamb flavor attributes between age class (corresponding range of ground cooked patties).

Age[1]	Lamb flavor Intensity	Off-flavor	Aroma
Lamb	27.38[a] (16-43)	9.42[b] (0-28)	29.65 (19-42)
Yearling	21.44[b] (12-35)	5.32[b] (0-26)	31.76 (16-45)
Mature	24.56[ab] (14-44)	22.56[a] (1-63)	29.0 (22-53)
SEM	1.40	1.84	1.40
P-Value	**0.0151**	**<0.0001**	0.3423

[a, b, c] Means within column lacking common superscripts differ (P < 0.05).
[1]Age Lamb= 0 permanent incisors; Yearling = 2 permanent incisors; Mature = 2+ permanent incisors

Table 4.7. Least squares means for lamb flavor attributes between background (corresponding range of ground cooked patties).

Background	Lamb flavor intensity	Off-flavor	Aroma
Grass	27.67[a] (16-43)	8.25 (0-28)	30.86 (22-42)
Feedlot	21.16[b] (12-35)	6.44 (0-26)	30.64 (19-45)
SEM	1.41	1.39	1.34
P-Value	**0.002**	0.36	0.904

[0.90a, b, c] Means within column lacking common superscripts differ (P < 0.05).

Table 4.8. Independent variables, R^2, C (p), stepwise procedure for best-fit regression equations developed to predict lamb flavor.

Dependent variable	R^2	C(p)	Variables in model (partial R^2)	% Accuracy
Lamb Flavor Intensity	0.59	5.2850	C490 (0.1901)	84 % Overall
			C75 (0.1186)	67% Mild
			C455 (0.0763)	75% Medium
			C129 (0.0478)	92% Bold
			C274 (0.0987)	
			C22 (0.0372)	
			C494 (0.0213)	

Table 4.9. Independent variables, R^2, C (p), AIC procedure for best-fit regression equations developed to predict lamb flavor.

Dependent variable	R^2	C(p)	AIC	Variables in model (partial R^2)	% accuracy
Lamb Flavor Intensity #1	0.77	-5.07	217.39	L* (0.01164) a* (0.00139) C30 (0.05991) C31 (0.03213) C36 (0.01062) C85 (0.12201) C153 (0.01539) C342 (0.02711) C403 (0.05794) C404 (0.00011647) C423 (0.02774) C455 (0.14602) C490 (0.15514) C494 (0.05750) C501 (0.04153)	83% Overall 67% Mild 92% Medium 63% Bold
Lamb Flavor Intensity #2	0.75	-5.88	219.44	a*(0.00110) C10(0.12203) C12(0.02346) C31(0.00007740) C59(0.07590) C85(0.00109) C129(0.10190) C153(0.00953) C156 (0.00645) C285(0.00626) C423(0.05294) C455(0.12231) C490(0.17067) C494(0.05932)	59% Overall 17% Mild 82% Medium 13% Bold

Table 4.9. Continued.

Dependent variable	R^2	C(p)	AIC	Variables in model (partial R^2)	% Accuracy
Lamb Flavor Intensity #3	0.76	-5.71	219.76	a* (0.0110)	0.57 Overall
				C30 (0.06381)	0.29 Mild
				C31(0.03403)	0.75 Medium
				C36(0.01174)	0.13 Bold
				C85 (0.11290)	
				C153(0.00948)	
				C342(0.02896)	
				C403(0.05086)	
				C404(0.00007970)	
				C423(0.02883)	
				C455(0.13029)	
				C490(0.17377)	
				C494(0.05648)	
				C501(0.04961)	

Table 4.10. Independent variables, R^2, C (p), stepwise procedure for best-fit regression equations developed to predict off-flavor.

Dependent variable	R^2	C(p)	Variables in model (partial R^2)
Off Flavor Intensity	0.77	28.19	12^{th} rib fat (0.37)
			Body Wall (0.02)
			Marbling (0.02)
			C6 (0.02)
			C25 (0.02)
			C26(0.02)
			C30 (0.02)
			C180 (0.04)
			C264 (0.08)
			C449 (0.02)
			C452 (0.02)
			C537(0.03)
			C544 (0.02)

Table 4.11. Independent variables, R^2, C (p), backward procedure for best-fit regression equations developed to predict off-flavor.

Dependent variable	R^2	C(p)	Variables in model (partial R^2)
Off Flavor Intensity	0.94	17.47	Leg Score
			L*
			b*
			C6
			C10
			C11
			C12
			C15
			C22
			C25
			C26
			C33
			C35
			C75
			C76
			C146
			C180
			C246
			C248
			C264
			C274
			C312
			C341
			C403
			C449
			C452
			C455
			C462
			C487
			C490
			C501
			C505
			C531
			C537
			C544

Table 4.12. Independent variables, R^2, C (p), forward selection procedure for best-fit regression equations developed to predict off-flavor.

Dependent variable	R^2	C(p)	Variables in model (partial R^2)
Off Flavor Intensity	0.81	20.06	Leg Score (0.37)
			Body Wall (0.03)
			Marbling (0.02)
			C6 (0.02)
			C25 (0.01)
			C26 (0.02)
			C30 (0.02)
			C59 (0.01)
			C79 (0.06)
			C85 (0.03)
			C180 (0.04)
			C248(0.02)
			C264(0.08)
			C449(0.02)
			C452(0.02)
			C537(0.03)
			C544(0.02)

Table 4.13. Annotated Metabolites

Metabolite	Name
6	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane
10	Silane, dimethyl(dimethyl(dimethyl(2-isopropylphenoxy)silyloxy)silyloxy)(2-isopropylphenoxy)-
11	Fluoren-9-ol, 3,6-dimethoxy-9-(2-phenylethynyl)-
12	2-[(Trimethylsilyl)oxy]-2-{4-[(trimethylsilyl)oxy]phenyl}ethanamine
15	Phenolic putative
22	1-Octen-3-ol
25	Hexanal
26	2-[(Trimethylsilyl)oxy]-2-{4-[(trimethylsilyl)oxy]phenyl}ethanamine
30	Ethylene, 1-trichlorosilyl-2-trimethylsilyl-
33	2,2-Dimethyl-1-oxa-2-silacyclotridecanone-13
35	Phenyl-pentamethyl-disiloxane
36	Tonalid/Versalide
59	Glutarimide, N-(2-(4-methoxyphenyl)ethyl)-
75	2,3-Butanediol
76	2-Methyl-4-propylphenol
85	Chloroethylene
129	2-[(2-Methyl-endo-2-norbornyl)oxy]-tetrahydropyran
146	is-1,3-Cyclohexanedicarbonitrile
153	Indole-2,3(2H,3H)-dione, 4-bromo-5-methyl-
156	1H-Imidazole, 1-methyl-2-vinyl-
180	Tetracyanopyrrole
227	1,4-Dibenzyloxybenzene
228	1H-Dipyrido[2,3-b:3',2'-d]pyrrole
246	Thiophene, 2,5-dimethyl-
248	1-(Trihexylsilyloxy)heptane
264	4'-Amino-2-hydroxystilbene

Table 4.13. Continued

Metabolite	Name
274	1H-Indene-4-acetic acid, 6-(1,1-dimethylethyl)-2,3-dihydro-1,1-dimethyl-
285	3-Pyridinemethanol, .alpha.-[3-[bis(1-methylethyl)amino]-1-propynyl]-.alpha.-methyl-
312	Carbon Dioxide
341	1H-Naphtho(2,3-c)pyran-5,10-dione, 3,4-dihydro-3,6,9-trihydroxy-7-methoxy-3-methyl-
342	Phosphorus P4
378	Phenol, 4-[2-(methylamino)ethyl]-
403	3-Methyl-6-phenyl-thiazolo(3,2-b)-1,2,4-triazole
404	d(-)-5,6,7,8-Tetrahydronaphthaleno[8,7-j]isoquinoline, 2-hydroxy-3-methoxy-
418	2-Isopropoxyethylamine
423	6, 21-Cyclo-4, 5-secoakuammilan-17-oic acid, 1, 2-dihydro-4, 5-dihydroxy-, methyl ester, (2.xi. 6.alpha.)-
449	6, 21-Cyclo-4, 5-secoakuammilan-17-oic acid, 1, 2-dihydro-4, 5-dihydroxy-, methyl ester, (2.xi. 6.alpha.)-
452	Nitrous Oxide
455	4(1H)-Quinazolinone, 2,3-dihydro-1,3-dimethyl-2-thioxo-
462	1-Buten-3-yne, 1-(1,1-dimethylethoxy)-, (Z)-
487	1-Butanol, 4-(trimethylstannyl)-
490	Pymetrozin, tert-butyldimethylsilyl deriv.
494	10H-Naphtho[2,1-b]pyran-10-one, 3-ethyldodecahydro-3,4a,7,7,10a-pentamethyl-
501	Urea
505	2,4' Dimethoxydephenyl
531	Methyl Isobutyl Ketone
537	Cyanic acid ethyl ester
544	Acetone

LITERATURE CITED

Arsenos, G., Banos, G., Fortomaris, P., Katsaounis, N., Stamataris, C., Tsaras, L., & Zygoyiannis, D. (2002). Eating quality of lamb meat: effects of breed, sex, degree of maturity and nutritional management. *Meat Science*, *60*(4), 379–387. https://doi.org/10.1016/S0309-1740(01)00147-4

Auvray, M., & Spence, C. (2008). The multisensory perception of flavor. *Consciousness and Cognition*, *17*(3), 1016–1031. https://doi.org/10.1016/j.concog.2007.06.005

Beauchamp, G. K., & Cowart, B. J. (1985). Congenital and experiential factors in the development of human flavor preferences. *Appetite*, *6*(4), 357–372. https://doi.org/10.1016/S0195-6663(85)80004-0

Broeckling, C. D., Ganna, A., Layer, M., Brown, K., Sutton, B., Ingelsson, E., … Prenni, J. E. (n.d.). Enabling Efficient and Confident Annotation of LC−MS Metabolomics Data through MS1 Spectrum and Time Prediction. https://doi.org/10.1021/acs.analchem.6b02479

Bueno, M., Resconi, V. C., Campo, M. M., Cacho, J., Ferreira, V., & Escudero, A. (2011). Gas chromatographic–olfactometric characterisation of headspace and mouthspace key aroma compounds in fresh and frozen lamb meat. *Food Chemistry*, *129*(4), 1909–1918. https://doi.org/10.1016/j.foodchem.2011.06.001

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

Channon, H., Lyons, R., & Bruce, H. (2003). Sheepmeat flavour and odour: a review Project number: Sheep CRC 1.3.2.

Font-i-Furnols, M., & Guerrero, L. (2014). Consumer preference, behavior and perception about meat and meat products: An overview. *Meat Science*, *98*(3), 361–371. https://doi.org/10.1016/j.meatsci.2014.06.025

Frankel, E. N. (1980). Lipid oxidation. *Progress in Lipid Research*, *19*(1), 1–22. https://doi.org/10.1016/0163-7827(80)90006-5

Gazdziak, S. (2015). Lamb's "wow" factor. Retrieved January 20, 2017, from http://www.provisioneronline.com/articles/102685-lambs-wow-factor?v=preview

Hoffman, T. (2016). *BENCHMARK OF LAMB QUALITY IN U.S. RETAIL AND FOODSERVICE MARKETS*.

J.Daryl Tatum, Henry N.Zerby, K. E. B. (2014). Physiological Indicators of Ovine Age. *Prepared for the Lamb Industry Raodmap Product Characteristics Team*.

JH, C. (2016). Lipid Oxidation in Meat. *Journal of Nutrition & Food Sciences*, *6*(3), 1–3. https://doi.org/10.4172/2155-9600.1000494

Merriam Webster. (2016). Flavor | Definition of Flavor by Merriam-Webster. Retrieved January

20, 2017, from https://www.merriam-webster.com/dictionary/flavor

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

Neethling, J., Hoffman, L. C., & Muller, M. (2016). Factors influencing the flavour of game meat: A review. *Meat Science*, *113*, 139–153. https://doi.org/10.1016/j.meatsci.2015.11.022

Pegg, R. B., & Shahidi, F. (2014). *Encyclopedia of Meat Sciences*. *Encyclopedia of Meat Sciences*. Elsevier. https://doi.org/10.1016/B978-0-12-384731-7.00129-X

Pethick, D. W., Hopkins, D. L., D'Souza, D. N., Thompson, J. M., Walker, P. J., Batcher, O., … Braggins, T. (2005). Effects of animal age on the eating quality of sheep meat. *Australian Journal of Experimental Agriculture*, *45*(5), 491. https://doi.org/10.1071/EA03256

Rousset-Akrim, S., Young, O. A., & Berdagué, J.-L. (1997). Diet and growth effects in panel assessment of sheepmeat odour and flavour. *Meat Science*, *45*(2), 169–181. https://doi.org/10.1016/S0309-1740(96)00099-X

Sañudo, C., Enser, M. E., Campo, M. M., Nute, G. R., María, G., Sierra, I., & Wood, J. D. (2000). Fatty acid composition and sensory characteristics of lamb carcasses from Britain and Spain. *Meat Science*, *54*(4), 339–346. https://doi.org/10.1016/S0309-1740(99)00108-4

Sink, J. D., & Caporaso, F. (1977). Lamb and mutton flavour: Contributing factors and chemical aspects. *Meat Science*, *1*(2), 119–127. https://doi.org/10.1016/0309-1740(77)90013-4

Stillman, R., Crawford, T., & Aldrich, L. (n.d.). The U.S. Sheep Industry.

Tatum, J. D., Zerby, H. N., & Belk, K. E. (2014). A Brief Overview of Factors Affecting Lamb Flavor Prepared for the Lamb Industry Roadmap Product Characteristics Team.

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

Veldhuizen, M. G., Shepard, T. G., Wang, M.-F., & Marks, L. E. (2010). Coactivation of Gustatory and Olfactory Signals in Flavor Perception. *Chemical Senses*, *35*(2), 121–133. https://doi.org/10.1093/chemse/bjp089

Ventura, A. K., & Worobey, J. (2013). Early Influences on the Development of Food Preferences. *Current Biology*, *23*(9), R401–R408. https://doi.org/10.1016/j.cub.2013.02.037

Wong, E., Johnson, C. B., & Nixon, L. N. (1975). The contribution of 4-methyloctanoic (hircinoic) acid to mutton and goat meat flavour. *New Zealand Journal of Agricultural Research*, *18*(3), 261–266. https://doi.org/10.1080/00288233.1975.10423642

Young, O. A., Berdagué, J.-L., Viallon, C., Rousset-Akrim, S., & Theriez, M. (1997). Fat-borne volatiles and sheepmeat odour. *Meat Science*, *45*(2), 183–200. https://doi.org/10.1016/S0309-1740(96)00100-3

Young, O. A., Lane, G. A., Priolo, A., & Fraser, K. (2003). Pastoral and species flavour in lambs

raised on pasture, lucerne or maize. *Journal of the Science of Food and Agriculture*, *83*(2), 93–104. https://doi.org/10.1002/jsfa.1282

APPENDIX

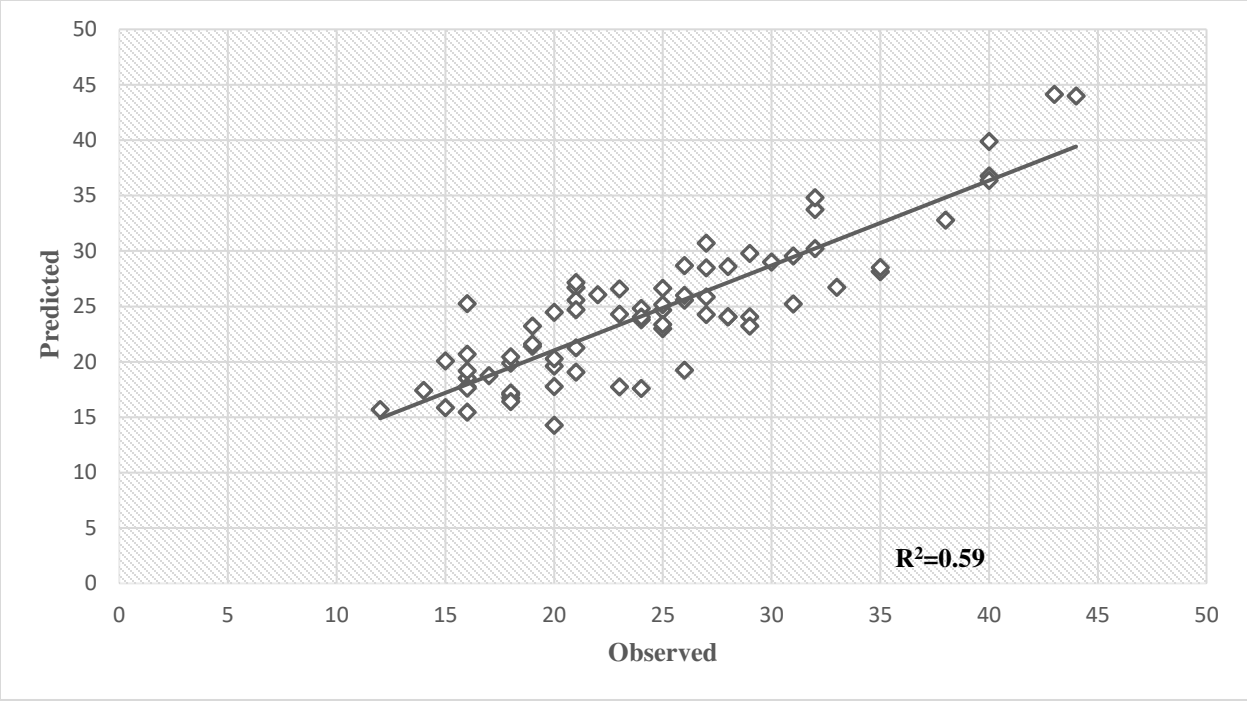

Figure A.4.1. Stepwise regression model of best fit for lamb flavor prediction.

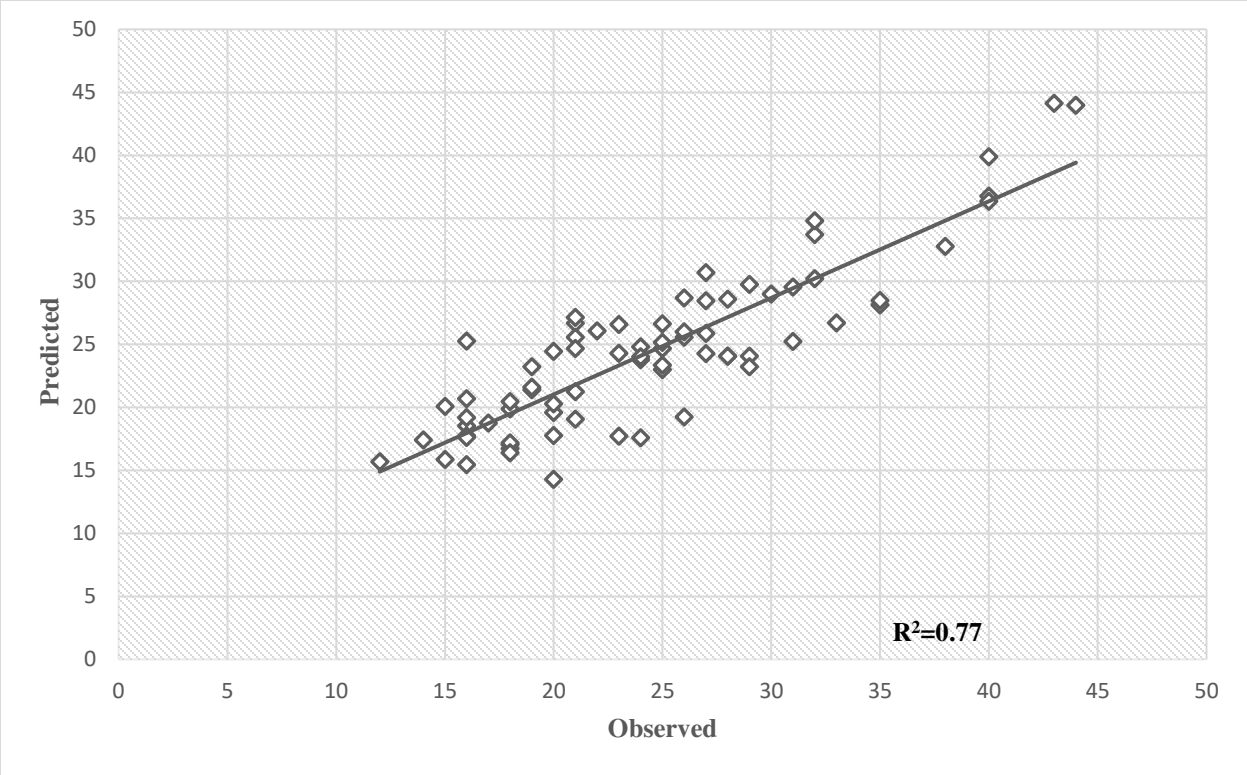

Figure A.4.2. AIC regression #1 model of best fit for lamb flavor prediction.

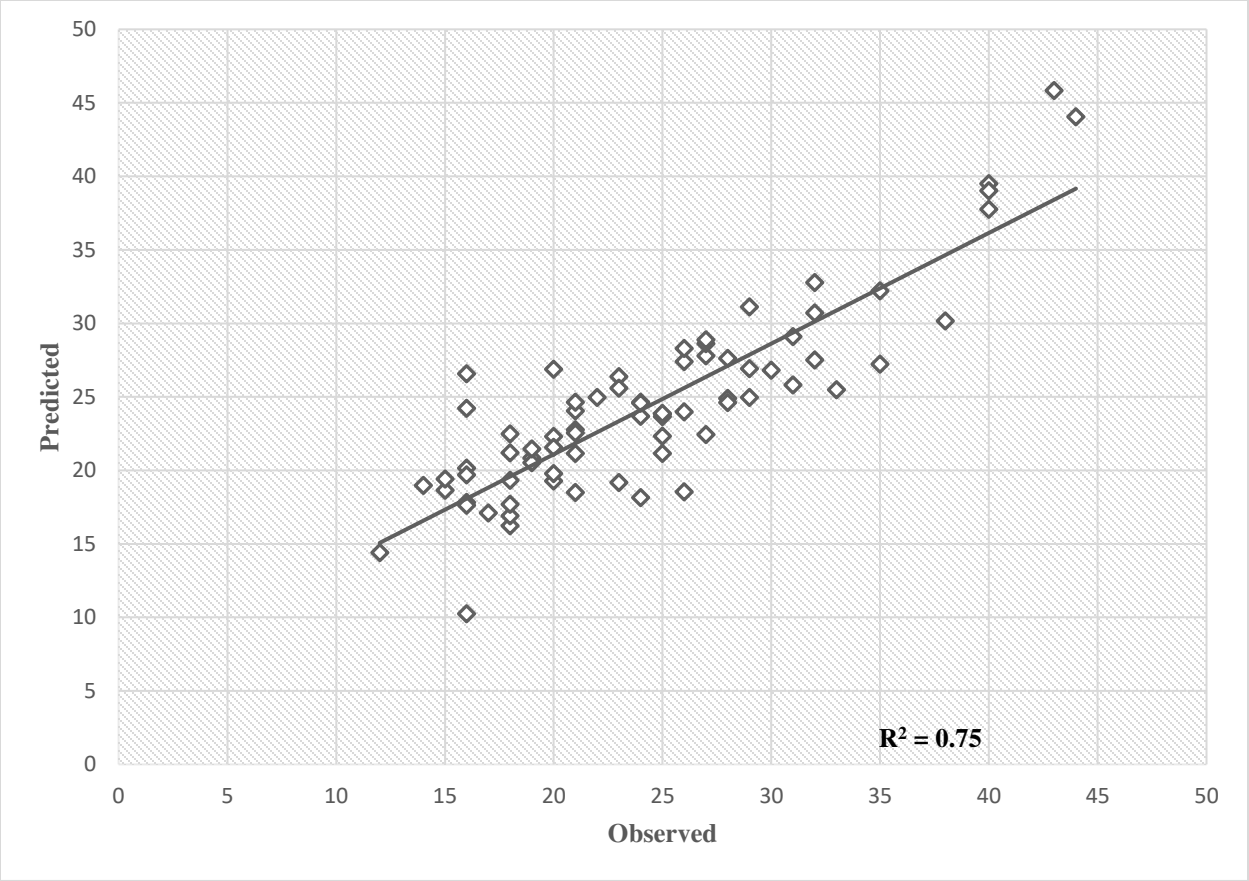

Figure A.4.3. AIC regression #2 model of best fit for lamb flavor prediction.

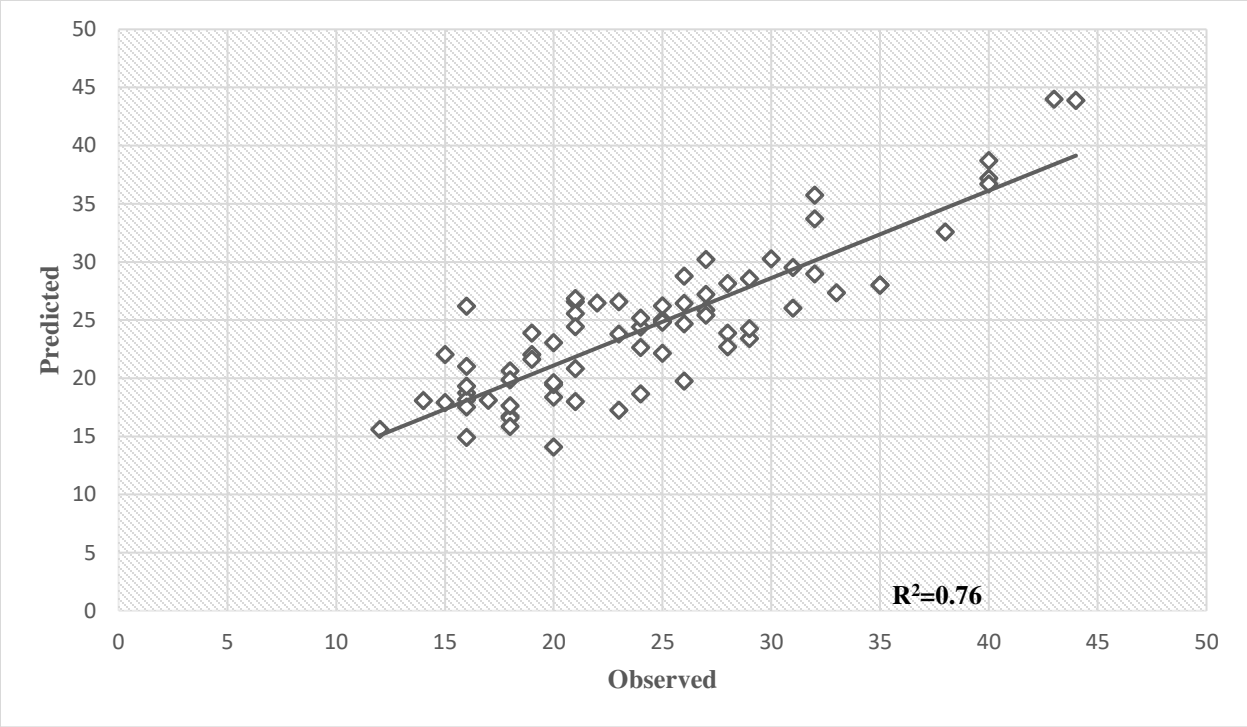

Figure A.4.4. AIC regression #3 model of best fit for lamb flavor prediction.

Figure A.4.5. Example Sensory Ballot