PREDICTING BEEF FLAVOR DIFFERING IN LIPID HEAT DENATURATION AND MAILLARD REACTION PRODUCTS

A Dissertation

by

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ABSTRACT

The objective of this project was to evaluate how initial differences in raw beef lipid oxidation affects the development of beef flavor attributes and volatile aroma compounds using three cooking methods. Our hypothesis was that components of beef flavor in whole muscle beef top loin steaks would be affected by the level of raw lipid oxidation and cooking method. The level of lipid oxidation and cooking method affected flavor attributes of top loin steaks. This is expected because lipid oxidation creates off-flavors that can be detected by trained panelists and the cooking methods used in this project created different levels of Maillard reaction products and they were detected by the trained panelists from brown and roasted flavor descriptors. The grill treatment was responsible for the development of more products from the Maillard reaction and these products were responsible for producing more positive flavor attributes as detected by the trained panelists, and producing more pyrazines and Strecker aldehydes. The steaks from the grill cooking treatment were more tender than the other cooking treatments from the mechanical measurements but the trained panelists did not perceive tenderness differences between the cooking methods. All three cooking methods affected tenderness, with all the treatments being “very tender” and “tender” steaks. However, the panelists described the steaks from the sous-vide treatments as denser. The Maillard products created from the grill cooking methods were able to mask some off-flavors creating positive flavors overall. The raw level of lipid oxidation was responsible for changes in the objective color measurements. This study was able to better understand

how beef flavor is developed in retail and foodservice settings. The low, medium, and high levels of lipid oxidation were responsible for creating a wide range of flavor attributes and volatile compounds. The high level of oxidation was responsible for creating more negative flavor attributes, categorized as off-flavors, developing more aldehydes and alcohols due to the oxidation. The storage time negatively affected redness and luminosity of the steaks. The rapid evaporative ionization mass spectrometry (REIMS) technology was able to predict with high accuracy both the level of lipid oxidation and cooking method.

DEDICATION

My dissertation and degree are dedicated to my husband, family, and friends for all the support during this time. In memory of my mom who always believed in my dreams.

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Contributors

This work was supervised by a dissertation committee consisting of Dr. Rhonda Miller [advisor - chair], Dr. Stephen Smith [co-chair] and Dr. Chris Kerth of the Department of Animal Science, and, Dr. Andreea Botezatu of the Department of Horticulture at Texas A&M University. Another collaborator to this project is Dr. Dale Woerner of Texas Tech University. All work for the dissertation was completed independently by the student.

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NOMENCLATURE

A	Aroma
AMSA	American Meat Science Association
EDTA	Ethylenediaminetetraacetic acid
F	Flavor
GC/MS	Gas Chromatography/Mass Spectrometry
GLM	General Linear Models
IMPS	Institutional Meat Purchase Specifications
IRB	Institutional Review Board
LDA	Linear discriminant
LTLT	Low Temperature Long Time
PROC	Procedure
PVC	Polyvinyl Chloride
REIMS	Rapid Evaporative Ionization Mass Spectrometry
RMSE	Root Mean Square Error
SAS	Statistical Analysis Software
SPME	Solid-Phase Micro-Extraction
SV	Sous-vide
SVAC	Sous-vide Advisory Committee
SVG	Sous-vide plus grill
TBA	2-Thiobarbituric Acid

TBARS	Thiobarbituric Acid Reactive Substances
USDA	United States Department of Agriculture
VIP	Variable Importance in the Projection
WBSF	Warner-Bratzler Shear Force

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1. INTRODUCTION

Flavor, tenderness and color have been shown to be the factors that impact consumers acceptance. Color has been associated with the first impression in the purchase of a meat products. Tenderness has been reported to be one of the consumers as the most important organoleptic characteristic of beef. When tenderness is acceptable, beef flavor is the primary palatability factor. However, tenderness has appreciably improved and decreased in variation since 1991 (Martinez et al., 2015; Morgan et al., 1991))

It has been shown in several studies that multiple factors impact flavor in beef (Calkins & Hodgen, 2007; Farmer & Patterson, 1991; Kerth & Miller, 2015; Legako, Dinh, Miller, & Brooks, 2015). Our research group has been extensively involved in defining what flavors are present in beef whole muscle cuts by developing the beef whole muscle flavor lexicon (Adhikari et al., 2011). Also, effects of cooking method, degree of doneness, and cut have been examined on beef flavor attributes and consumer liking (Bamsey, 2017). Additionally, research to determine perceptions of millennials, non-millennials, heavy beef-eaters, and light beef-eaters has been conducted (Laird, 2015; Luckemeyer, 2015). The impact of grind size, patty thickness, fat level, holding time and cooking method effects on beef flavor attributes and consumer liking in ground beef also have been studied (Beavers, 2017). Effect of cuts purchased in five regions of the USA in beef flavor to has been evaluated (Peña, 2019). Consumer studies have included Central Location Tests and In-Home Use Tests to assess consumer liking

(Berto, 2015; Glasscock, 2014). Most recently, these studies have been used to model beef flavor. Flavor has been shown to be influenced by the development of volatile aroma compounds that are derived from either lipid heat denaturation, Maillard reaction products, or their interaction.

This study has been designed to understand how initial differences in raw beef lipid oxidation (defined using thiobarbituric acid reactive substances (TBARS) values) affects the development of beef flavor attributes and volatile aroma compounds using three cooking methods. The study was conducted using Low Choice top loin steaks subjected to three cooking methods (sous-vide, sous-vide with sear, and flat top grill) and three raw lipid oxidation levels (0.1, 0.5 and >1.0 mg malonaldehyde/kg sample induced by storage environment). These treatments were designed to create differences in raw meat lipid oxidation, simulating how steaks are stored in the retail food service setting. It is well established that if raw meat has higher levels of lipid oxidation, heat denaturation oxidation occurs at a higher rate during cooking and products of lipid oxidation, defined as off-flavors, are stronger and the steak is rated less desirable by consumers.

Three cooking methods were used to induce differences in lipid heat denaturation and Maillard reactions. These cooking methods are sous-vide, sous-vide plus grill (using a high temperature sear after cooking), and grilling. Sous-vide cooking method has been applied to several different food products, but its use for meats is what has popularized this method worldwide (Ruiz, Calvarro, Sánchez del Pulgar, & Roldán, 2013). These are common cooking methods used in the foodservice industry for top loin steaks. Sous-vide

cooking was selected as a low temperature cooking method. In this method, steaks were vacuum-packaged with cook-in film and cooked in water. This is a method that induces lipid heat denaturation. The sous-vide plus grill treatment is commonly used in foodservice. This treatment was used to induce lipid heat denaturation during sous-vide cooking and Maillard reaction products during grilling. The final cooking treatment was to grill steaks on a flat top grill to induce the highest levels of Maillard reaction products.

Steaks were evaluated using an expert, trained beef flavor and texture descriptive attribute panel, for volatile aromatic chemical compounds using a GC/MS system, Warner-Bratzler shear force, and REIMS technology.

The objectives of this project were to understand how the level of lipid oxidation in raw meat in combination with cooking method impacts beef flavor, and then to evaluate a rapid technology to predict beef flavor to better understand how it is created.

Our hypothesis was that components of beef flavor in whole muscle beef top loin steaks would be affected by the level of raw lipid oxidation and cooking method.

2. LITERATURE REVIEW

2.1. Overall Responses to Flavor

Flavor is a combination of taste and aroma. Taste is a biological response to the perception of food in the oral cavity that stimulates receptor cells within taste buds (Breslin, 2013; Meilgaard, Civille, & Carr, 2015). The primary and primitive sense of taste is to measure what is acceptable or unacceptable from what was sampled (Breslin, 2013). Taste is perceived by humans with the edges and dorsal surfaces of the tongue, soft palate, and pharynx (Breslin, 2013; Meilgaard, Civille, & Carr, 2015) by receptors. Gustatory perception is a chemical sense and it detects a stimulus dissolved in water, oil or saliva by the taste buds that are located on the surface of the tongue, mucosa of the palate, and areas of the throat (Meilgaard, Civille, & Carr, 2015). Gustation is a sense that is perceived with taste buds. Sweet, sour, salty, bitter, and umami are the five recognizable tastes, commonly called basic tastes (Kerth & Miller, 2015; Mattes, 2009). The taste receptors then send signals to the sensory nerves.

Caul (1957), for the purpose of practical sensory analysis, restricted the term flavor to the impressions perceived via the chemical senses from a product in the mouth. Flavor includes the aromatic, olfactory sensation caused by volatile aroma compounds; the taste, gustatory perceptions caused by soluble substances in the mouth; and the chemical feeling factors that stimulate nerve endings in membranes of the buccal and nasal cavities (Meilgaard, Civille, & Carr, 2015).

The trigeminal senses are composed of facial nerves responsible for motor and sensory functions of the face and mouth (Kerth & Miller, 2015). This sense is

responsible for the chemical feeling sensations such as burn, heat, cold, and pungency. The feeling stimulates the trigeminal nerve ends that causes the sensation response (Meilgaard, Civille, & Carr, 2015). For most compounds, the trigeminal responses need a higher concentration of the component, or have a higher sensory threshold, when compared to the olfactory and gustatory receptors (Meilgaard, Civille, & Carr, 2015).

2.2. Beef Flavor

When tenderness is acceptable, beef flavor is the primary palatability factor for consumer liking (Behrends et al., 2005a, 2005b; Goodson et al., 2002; Killinger, Calkins, Umberger, Feuz, & Eskridge, 2004). Flavor has also been found to be the most important factor to affect consumers' meat buying habits and preferences when tenderness is not a problem (Sitz, Calkins, Feuz, Umberger, & Eskridge, 2005). Flavor is a complex, multi attribute factor that has positive and negative components (Adhikari et al., 2011; Miller & Kerth, 2012). Beef flavor is identified as a combination of the perception of basic tastes, mouthfeels, aroma, and the interaction of these sensations (Meilgaard, Civille, & Carr, 2015).

Flavor is very important for consumer's repeat purchase. In a study using clod steaks, it was shown that flavor liking had the greatest simple correlation (0.86) to overall liking ratings and was the most important factor for predicting overall like ratings using stepwise regression (Goodson et al., 2002).

Beef flavor and aroma is a combination of hundreds of compounds present in the meat. Most of these compounds are affected by storage and cooking (Calkins & Hodgen,

2007). The flavor of beef is derived upon cooking, since uncooked meat has little or no species-specific aroma, and the most predominant flavor is blood-like taste (Mottram, 1998). The aroma of cooked meat is mainly developed upon heating as well (Pegg & Shahidi, 2004). Flavor is one of the most complex factors that impacts beef because it is the result of various chemical reactions, including lipid degradation, Maillard reactions, and their interactions (Mottram, 1991, 1998). These reactions are highly associated with the formation of positive flavors such as meaty flavor and species-specific flavor (Elmore & Mottram, 2006).

The major beef flavor precursors can be categorized as water-soluble components and lipids (Mottram, 1998). The lean tissue is associated with creating precursors of the meaty/beefy flavor, as categorized in all cooked meats, and the adipose tissue is responsible for providing species-specific characteristics (Hornstein & Crowe, 1960; Kramlich & Pearson, 1960; Koutsidis et al., 2008; Macy, Naumann, & Bailey, 1964; Wasserman & Gray, 1965). The main water-soluble components that are flavor precursors are free sugars, sugar phosphates, nucleotide-bound sugar, free amino acids, peptides, nucleotides, and other nitrogenous components (Mottram, 1998). Cysteine and ribose are the main amino acid and carbohydrate that are lost during the cooking process for the beef flavor formation (Macy, Naumann, & Bailey, 1964; Mottram, 1998). Ribose has been shown to be a heat-labile sugar, and fructose the most heat stable (Koutsidis et al., 2008). An early study (Mulders, 1973) investigated the cysteine and ribose reaction under low water conditions and identified the formation of 40 heterocyclic compounds derived from these reactions.

In 2011 (Adhikari et al., 2011), a study created a library of terms (lexicon) that englobe most of the flavor attributes that can be present in beef. During the development of the lexicon, several different effects such as packaging, cut, storage, meat aging, animal age, cooking method, and spoilage were used to create different flavors to cover beef flavor formation. The study found 38 attributes across the samples that are used as references for beef trained panels.

Beef flavor can be described utilizing lexicon attributes. Adhikari et al. (2011) identified beef identity, brown/roasted, bloody/serumy, fat-like, metallic, sour aromatics, overall sweet flavor, sweet, sour, bitter, salty, and umami as the major attributes present in beef and liver-like, green-hay, green, chemical, burnt, rancid, spoiled, warmed-over, animal hair, cocoa, leather, dairy, sour dairy, and cooked milk as other aroma and flavor notes. Mottram (1998) reported that the most important reactions result in the development of aroma volatiles and contribute to specific-species flavors, increasing beef identity and umami flavors.

One of the most studied off flavors found in cooked meat is warmed over flavor. Lipid oxidation decreases the desirable meaty flavors and there is meat flavor deterioration with an increase in lipid oxidation (Shahidi & Pegg, 1994). This flavor is developed from the oxidized flavors that are present in meat cooked and increase with subsequent age time (Tims & Watts, 1958). Warmed over flavor has been described as stale, cardboard-like, painty, rancid, bitter, and sour, among other flavor descriptors (Love, 1988; St. Angelo et al., 1987). The heating process, among others factors, can

enhance the warmed over flavor present in meat products (Mann et al., 1989; Mielche, 1995; St Angelo, Vercellotti, Jacks, & Legendre, 1996).

2.3. Maillard Reaction

Maillard reaction is one of the most important reactions that contributes to the flavor of cooked meats and meaty compounds. The Maillard reaction is a non-enzymatic browning, responsible for the browning of steaks during the cooking process. In foods, this reaction takes place between monosaccharides, glucose and fructose, or disaccharides, maltose and lactose, as well as reducing pentoses, and amino acids and/or proteins (Zamora & Hidalgo, 2005). Generally, the process occurs between an amino acid and a reducing sugar in the presence of heat. The amine group present in the beginning of the reaction acts as a catalyst, resulting in a faster reaction and higher amounts of reactive intermediate (Van Boekel, 2006). The reaction involves carbonyl groups with free amino acids and takes place when beef is cooked at higher temperatures. The Maillard reaction includes the production of color, flavor and off-flavor, possibly toxic compounds, the development of antioxidant properties and can also decrease nutritional value. The reaction can contribute to innumerable compounds that are involved in the flavors generally described as roasted, browned, meaty, caramelized, and others (Kerth & Miller, 2015). The primary amino acid leads to the final aroma and flavor, and the sugar is responsible for the rate of the reaction (Kiely, Nowlin, & Moriarty, 1960). During the cooking process, most proteins denature between 55 and 80°C (Maillard, 1912).

The Maillard reaction is a complex system that can proceed on different paths depending on the type of amino acid, type of sugar, and temperature. A mechanism was proposed by Hodge (1953) to described the system with seven reactions in three stages: (I) Initial stage – (A) Sugar-amine condensation and (B) Amadori rearrangement; (II) Intermediate stage – (C) Sugar dehydration, (D) Sugar fragmentation, and (E) Amino acid degradation; and (III) Final stage – (F) Aldol condensation and (G) Aldehyde-amine polymerization. The initial stage is colorless, the intermediate stage is colorless to yellow, and the final stage is highly colored.

In the initial stage of the reaction, the amine group condenses with a carbonyl group of a reducing sugar in the presence of heat to produce an *N*-glycosylamine. When the reducing sugar is an aldose it will rearrange to Amadori products (or Heyns product if ketose is the reducing sugar) (Van Boekel, 2006). Glycosylamine is rearranged and dehydrated to form furfural, furanone derivates, hydroxyketones, and dicarbonyl compounds (Calkins & Hodgen, 2007).

Throughout the Amadori rearrangement, Strecker degradation, and Schiff base pathways, the intermediate products can react with other amines, amino acids, aldehydes, hydrogen sulfide, and ammonia (Calkins & Hodgen, 2007). The intermediate stage starts with the Amadori/Heyns products, and proceeds with a sugar fragmentation and the release of an amino group (Van Boekel, 2006). The final stage is comprised of dehydration, fragmentation, cyclization, and polymerization reactions (Van Boekel, 2006). The different paths that the reaction can take are strongly dependent on temperature, pH, and reactants (type of sugar, amino acid, or protein).

Strecker degradation is one of the most important steps for flavor development. During this step, the dicarbonyl compounds, formed in the Maillard reaction, degrade amino acids to form aldehydes (Shahidi, 1998), leading to deamination and decarboxylation of the amino acid (Van Boekel, 2006). Several aldehydes from Strecker degradation are known to have cooked beef aroma, such as acetaldehyde (from alanine), methylpropanal (from valine), 2-methylbutanal (from isoleucine), 3-methylbutanal (from leucine), phenylacetaldehyde (from phenylalanine), and methional that decomposes into dimethyl sulphide, methanethiol, dimethyl disulphide, and propenal (from methionine) (Shahidi, 1998).

2.4. Lipid Thermal Degradation

The next chemical reaction that impacts cooked beef flavor is lipid thermal degradation. The compounds derived from lipid thermal degradation during the cooking process are described as favorable and characteristic of cooked beef (Kerth & Miller, 2015). This reaction is described as the thermal breakdown of lipids and it can be best explained by the disassembly of neutral (triglycerides) and polar (phospholipids) lipids due to the heating process. Thermal reactions during nonoxidative heating of fats include dehydration, decarboxylation, hydrolysis of the ester bond, double bond conjugation, polymerization, dehydrocyclization, aromatization, dehydrogenation, and degradation by carbon-carbon cleavage (Nawar, 1969). This reaction is responsible for the production of hundreds of volatile compounds found in cooked meat, including aliphatic

hydrocarbons, aldehydes, ketones, alcohols, carboxylic acids, and esters (Mottram, 1998), benzaldehyde, benzoic acid, alkylbenzenes, and naphthalene (Shahidi, 1998).

Intramuscular lipids are composed of triglycerides and phospholipids from structural or membrane lipids (Shahidi, 1998). Phospholipids are the major source of volatile components and species-specific flavor precursors that are present in lean beef (Mottram, Edwards, & Macfie, 1982). Triglycerides are not essential (Mottram & Edwards, 1983) in producing meaty aromas. The products from lipid thermal degradation may inhibit the formation of some heterocycles, especially generated from Maillard reactions (Mottram & Edwards, 1983). The removal of all lipids (phospholipids and triglycerides) results in higher roasted aroma, lower lipid oxidation products and higher levels of heterocyclic compounds, predominantly pyrazines (Mottram & Edwards, 1983).

2.5. Lipid Oxidation

Lipid oxidation or autoxidation is also a non-enzymatic reaction that confers a brown color to beef, however it comes from deterioration of the product (Hidalgo & Zamora, 2000). An increase in lipid oxidation in meat decrease flavor, color and acceptability (Cheng, 2016). The oxidation of fatty acids has a great impact on sensory quality attributes (Addis, 1986). Early studies have shown that food products containing high amounts of unsaturated fatty acid, such as beef, have been associated with higher lipid oxidation rates and rapid flavor deterioration (Tims & Watts, 1958). The phospholipid fraction and total lipids become rancid quickly when exposed to air

(Hornstein, Crowe, & Heimberg, 1961). Autoxidation of lipids, occurring during long term storage, may lead to rancid off-flavors, but during the cooking process the oxidation reactions occur quickly and produce desirable flavor volatile profiles (Mottram, 1998).

The oxidation process is initiated by the removal of a hydrogen atom from an individual fatty acid chain, producing a free radical that rapidly forms a peroxy radical with oxygen (Min & Ahn, 2005). The peroxy radical formed subsequently removes a hydrogen atom from another fatty acid chain to form a hydroperoxide (Min & Ahn, 2005). That happens as a chain reaction where the free radicals formed break down the hydrogen molecule and create another radical.

Lipid oxidation has been shown to increase with storage and cooking process (Legako et al., 2015). Light, oxygen concentration, temperature, and degree of unsaturation of the fatty acids are some of the factors that affect lipid oxidation in beef (Skibsted, Mikkelsen, & Bertelsen, 1998).

During the oxidation process of unsaturated fatty acids there is formation of carbonyl compounds and, depending of concentration of these compounds, undesirable off-flavors develop (Shahidi, Rubin, D'Souza, Teranishi, & Buttery, 1986). Lipid oxidation has been commonly associated with unpleasant off-flavors (Mottram, 1998). The degradation of lipids through oxidation is known to cause off-flavors and odors described as rancid and warmed-over flavor in beef (Farmer & Mottram, 1990; Gray & Pearson, 1994; St. Angelo, Crippen, Dupuy, & James, 1990; Tims & Watts, 1958). As rancid flavor develops there is a loss of desirable flavor compounds (Campo et al.,

2006). Oxidized flavor can be detected in a beef sample over a broad range of TBARS values from 0.6 to 2.0 mg of malonaldehyde/kg (Greene & Cumuze, 1981). Compounds like pentanol, hexanal, hexanol, 1-octen-3-ol, nonanol, and malonaldehyde have been associated with lipid oxidation and have been found to influence beef flavor (Hodgen, Cuppett, & Calkins, 2006). The concentration of these compounds also has been used as an indicator for meat flavor deterioration (Bailey, Dupuy, & Legendre, 1980; Dupuy, Bailey, St. Angelo, Vercellotti, & Legendre, 1987; Shahidi, 1989; Shahidi, 1992).

There are many assays available for assessing lipid oxidation values of meat and meat products, however the thiobarbituric acid reactive substances (TBARS) test is widely used for this purpose (Melton, 1983; Shahidi, 1992; Tarladgis, Watts, Younathan, & Dugan, 1960). It is well established that there is a correlation between TBARS values and sensory evaluation (Mielche & Bertelsen, 1993; Spanier, Vercellotti, & James, 1992; Stapelfeldt, Bjørn, Skibsted, & Bertelsen, 1993).

2.6. Reaction Product Interactions

As expected, lipid thermal degradation and Maillard reaction can produce hundreds of possible compounds individually and by their interaction. New volatile compounds are produced, and some production can be partially or totally blocked by the interaction of these reactions (Kerth & Miller, 2015).

The Maillard, lipid degradation, and lipid oxidation reaction are interrelated, and the products of these reactions can modify and interfere with the other reactions (Zamora & Hidalgo, 2011).

In general, volatile compounds from lipid–Maillard interactions have weak odor intensities and higher odor thresholds compared with those generated in each of the primary reactions. Lipid oxidation products can modify the Maillard reaction by promoting or preventing the reaction or by reacting with the intermediates and producing different compounds (Zamora & Hidalgo, 2011). The same is valid for the Maillard reaction with lipid oxidation products. Amadori products have been shown to be responsible for increasing phospholipid oxidation (Zamora & Hidalgo, 2005, 2011). Moreover, volatiles produced in the interaction of these two systems may also have indirect impacts on the generation of volatile flavor compounds (Kerth & Miller, 2015).

2.7. Cooking Applications

Processes that increase desirable quality and enhance sensory qualities, is important to increasing beef demand (Banerjee & Verma, 2015; Holdsworth & Simpson, 2007). Heating method defined as cooking method affects beef eating quality, color, texture, and flavor (Aaslyng, Bejerholm, Ertbjerg, Bertram, & Andersen, 2003; Davey & Niederer, 1977; Martens, Stabursvik, & Martens, 1982; Oroszvári, Rocha, Sjöholm, & Tornberg, 2006). Cooking method is the most important extrinsic factor that impacts the production of volatile aroma compounds (Kerth & Miller, 2015). Cooking method is classified by whether the heat is under moist or dry conditions. Cooking methods are moist when the moisture that cooks out of the beef is unable to escape the surface of the beef. The sous-vide cooking technique, sous-vide method is considered as moist method, cooks the beef at a low temperature, lower than the boiling point of water, and the low

temperatures prevent the beef surface from dehydration and the development of Maillard products (Baldwin, 2012). Dry conditions are characterized by high temperatures of direct heat (>177°C) and results in the surface turning brown to black colors (Kerth & Miller, 2015). This cooking method generates Maillard products, that affects flavor and the production of volatile compounds.

As previously described, the cooking method is responsible for the reactions and the generation of the aroma volatile compounds. Among the improvements in the industry, there is a cooking method that consists in heating the product to a low end-point temperature using extended heating times, known as low-temperature long-time (LTLT) or sous-vide (Sánchez del Pulgar, Gázquez, & Ruiz-Carrascal, 2012; Uttaro, Zawadski, & McLeod, 2019). Sous-vide is a moist type of cooking. This technique cooks a product under controlled temperature and time inside a heat-stable vacuum pouch and is a cooking process well established in the literature and industry (Church & Parsons, 1993; Creed & Reeve, 1998; Hrdina-Dubsky, 1989). Sous-vide is used to prevent oxidation by reducing contact with free oxygen in the air and to preserve the quality of the food (Schellekens, 1996).

The Sous-vide Advisory Committee (SVAC) defined sous-vide as an interrupted catering system in which raw or parcooked food is sealed into a vacuumed laminated plastic pouch or container, heat treated by controlled cooking, rapidly cooled and then reheated for service after a period of chilled storage at temperatures around 0–3°C (Creed, 1998). Using this cooking process, there is a preservation of the food sensory quality, due to a reduced amount of water loss and both flavor and aroma volatiles

compounds (Vaudagna et al., 2002), texture and nutrient retention (Unger, 1985), and maintenance of consistent tenderness and juiciness (Bouton & Harris, 1981; Cover, 1943; Laakkonen, Wellington, & Sherbon, 1970; Machlik & Draudt, 1963). Also, this cooking method prevents dehydration of the surface of the steak, preventing the Maillard reaction. There is also a possible increase in shelf life of products during chill storage after LTLT technique is applied (Creed, 1998).

A typical routine for sous-vide cooking submerges and holds raw or minimally processed food products, vacuum-packaged, in circulating water held at a constant temperature, heated to 55-70℃ for hours to multiple days depending on meat type, thickness and degree of connective tissue (Baldwin, 2012). After cooking, meat can be chilled and stored refrigerated for many days (Baldwin, 2012), and prior to serving the meat can be reheated to the desired final internal temperature. Sous-vide cooking has been studied since the 1990s (Mossel & Struijk, 1991; Schellekens, 1996) and its use has increased in the past decade in restaurants and home use (Bañón, Nieto, & Díaz, 2007; Keller, Benno, Lee, & Rouxel, 2008; Myhrvold, Young, & Bilet, 2011).

The main disadvantage of the sous-vide cooking is the lack of extensive Maillard reaction products on the surface of the meat and meat products (Roldán et al., 2015; Roldán, Ruiz, Sánchez del Pulgar, Pérez-Palacios, & Antequera, 2015). The lack of Maillard products is caused by the absence of extremely high temperatures on the surface (around 60℃ in sous-vide, higher than 200℃ in oven, higher than 250℃ on a pan, and higher than 350℃ on a barbecue) (Mitra, Lametsch, Greco, & Ruiz-Carrascal, 2018; Roldán et al., 2015; Roldán, Ruiz, Sánchez del Pulgar, Pérez-Palacios, &

Antequera, 2015). The reduced presence of Maillard products on the surface of meat impacts directly the flavor development and brown surface coloring. To overcome the lack of Maillard products, chefs frequently roast, fry, or grill the surface of sous-vide cooked meats, achieving the brown color and flavor (Myhrvold, Young, & Bilet, 2011).

2.8. Beef Color

Color has been shown to be the primary factor that influences consumer's initial purchasing decision of beef (Aberle et al., 2001; Greene, Hsin, & Zipser, 1971; Jeremiah, 1982; Kropf, 1980). When the beef surface is exposed to oxygen, color turns to a consumer desirable bright cherry-red color (Suman, Hunt, Nair, & Rentfrow, 2014). Consumers often associate the bright cherry-red color to beef freshness (Faustman & Cassens, 1990; Hood & Riordan, 1973), and use the discoloration as an indicator of lack of freshness (Mancini & Hunt, 2005). Carpenter, Cornforth, & Whittier (2001) found that consumers preferred beef steaks with a color more red when compared to purple and brown (least desirable) and the raw color did not affect their taste score. Preferences for beef color does not bias taste scores (Carpenter, Cornforth, & Whittier, 2001).

Chilled storage in retail-display conditions deteriorates desirable color stability in beefwhen stored for extended periods (Greer & Jones, 1991). Myoglobin is the protein primarily responsible for meat color (Faustman & Cassens, 1990). It is a water soluble protein containing 8 α-helices linked by a compound. Four major chemical states of myoglobin are primarily responsible for meat color: deoxymyoglobin, oxymyoglobin, carboxymyoglobin, and metmyoglobin.

Deoxymyoglobin is associated with purplish-red color and it occurs when there is no ligand present at the 6^{th} site and the heme iron is in the ferrous state (Fe^{2+}). This color state is associated with vacuum packaged beef and muscle immediately after cutting because of the low concentration of oxygen present. When the cut is exposed to oxygen, there is a reaction called oxygenation, resulting in the formation of oxymyoglobin. Deoxymyoglobin is very unstable and can change state very quickly. Oxymyoglobin is bright cherry-red color and there is no change in the heme iron state. The oxymyoglobin state has an O_2 ligand on the 6^{th} site and results in a change in the myoglobin structure and stability. It is in this chemical state that consumers have preferred. Metmyoglobin can occur from deoxymyoglobin or oxymyoglobin. Formation of metmyoglobin starts with discoloration resulting from oxidation of ferrous myoglobin (Deoxy or oxymyoglobin) to the ferric state (Livingston & Brown, 1982; Wallace, Houtchens, Maxwell, & Caughey, 1982). Metmyoglobin brown and forms when the heme ring being in the very stable ferric state (Fe^{3+}). Carboxymyoglobin is a myoglobin state used by the retail beef industry to increase consumer acceptability. A carbon monoxide molecule is added to the gaseous environment in modified atmosphere packaging at low levels. Carbon monoxide is bound to the 6^{th} vacant site of the deoxymyoglobin and it forms a bright-red color that is partially stable (Mancini & Hunt, 2005).

Color can be objectively measured using colorimeters, spectrophotometers, or color systems (Hunter, CIE, and tristimulus) (Mancini & Hunt, 2005). CIE color space values are commonly used by researchers to measure color. For beef, L*, a* and b* color spaces values are reported. L* and a* are easily applied to muscle color, while b*

(blue and yellow) is harder to be correlated. The loss of red color, indicated by a decrease in a* value, can be used to estimate pigment oxidation and discoloration by the difference in the chemical state of the myoglobin (Mancini & Hunt, 2005).

2.9. Tenderness

Tenderness is seen by the consumers as an important organoleptic characteristic of meat (Koohmaraie, 1994; Savell et al., 1989). Cooking methods, such as environment, rate, degree of doneness, temperature, and time impact meat tenderness and eating quality (Wheeler, Shackelford, & Koohmaraie, 1997). Additionally, cooking methods also impact the physical changes of meat texture during heating (García-Segovia, Andrés-Bello, & Martínez-Monzó, 2007). Cooking is defined as the heating of meat to a high enough temperature to denature proteins (Davey & Gilbert, 1974). Myofibrillar proteins and connective tissue proteins are the main proteins involved in beef tenderness. During cooking structure changes occur in cell membranes, shrinkage of meat fibers, gel formation of myofibrillar and sarcoplasmic proteins there is solubilization of proteins, and shrinkage of connective tissue (Tornberg, 2005). Beef tenderization during the cooking process occurs in two stages. During the first stage at temperatures below 65°C there is loss of myofibrillar tensile strength (Davey & Gilbert, 1967, 1974). At higher temperatures there is tenderization by the disintegration of the collagen in the interstitial connective tissue, defined as the second stage (Davey & Gilbert, 1974; Laakkonen, 1973). Some morphological changes of beef also happen during heating. When heating up to 50°C there is only slight effect. At 50°C myofibrillar proteins exhibit compression.

At 60℃ there is a coagulation of thin and thick filaments, myofibrillar shrinkage, and granulation of sarcolemma. At 70℃ there is myofibrillar fragmentation at the Z disk and shrinkage of the endomysium. At 80℃ there is more disintegration of thin filaments and gelatinization of collagen fibers in the perimysium. At 90℃ the structure becomes amorphous (Cheng & Parrish Jr., 1976; Jones, Carroll, & Cavanaugh, 1977).

Increase in temperature also affect texture attributes in meat. The main factors considered to affect meat texture are myofibrillar proteins, muscle cytoskeleton, intramuscular connective tissue (Harris, 1976; Jones, Carroll, & Cavanaugh, 1977; Silva, Orcutt, Forrest, Bracker, & Judge, 1993; Greaser, 1997), and intrafibre water (Currie & Wolfe, 1980; Offer et al., 1989). Meat texture changes are highly related to the heat-affected changes (Palka & Daun, 1999). The tenderness can be evaluated by subjective methods such as a consumer panel, objectively, by instruments such as Warner-Bratzler shear force, or sensorial using trained panels (AMSA, 2015).

2.10. Volatile Aroma Compounds

Food flavor is a complex combination of volatile compounds causing a variety of odors, comprising a combination of hundreds of components (Maul, 1998; Pearce & Gardner, 1998). In beef over 1,000 volatile aroma compounds have been identified that influence its flavor (Manley & Choudhury, 1999; Mottram, 1998; Shahidi & Pegg, 1994). The olfactory sensation plays its role by perceiving the volatile aromatic compounds through the aroma of the sample. The compounds are detected by the olfactory epithelium, located in the roof of the nasal cavity. When people chew and

swallow, volatile aromatic compounds are released from the mouth and pass the nasopharyngeal passage where they reach the olfactory epithelium and are then identified by the nerves (Meilgaard, Civille, & Carr, 2015). There are several hundred volatile compounds derived from lipid degradation that have been found in cooked meat, such as aliphatic hydrocarbons, ketones, aldehydes, alcohols, esters, and carboxylic acids, and some aromatic compounds, mainly hydrocarbons (Mottram, 1998).

Trans-2-nonenal, *trans, trans*-2,4-decadienal, and 1-octen-3-one are most likely derived from thermal oxidation of polyunsaturated fatty acids, while methional, phenylacetaldehyde, and 2-methyl-3-furanthiol are products of Maillard reactions (Farmer, 1994). These compounds can be produced from different precursors and different mechanisms or different parts of the same mechanism. The compound 2-methyl-3-furanthiol has a flavor descriptor related to meat-like, sweet, and sulfurous and it is derived from cysteine and ribose or thiamine, a product of the Maillard reaction (Farmer, 1994; Gasser & Grosch, 1988). The compound bis 2-methyl-3-furyl disulphide is also related to meat-like and derived from the same precursors, however this compound is produced from thermal degradation (Farmer, 1994; Farmer & Patterson, 1991; Gasser & Grosch, 1988).

The Maillard reaction is responsible for producing many compounds which contribute to flavor (Mottram, 1994, 1998). Roasted flavors in beef are usually associated with the presence of heterocyclic compounds, such as thiazoles, pyrazines, and oxazoles (Mottram, 1998). Cooked meat has a notable level of sulfur-containing compounds, normally present in low concentrations, however, the odor threshold makes

them a very important contributor to cooked meat aroma (Mottram, 1998). Boiled beef presents a higher number of aliphatic thiols, sulfides, and disulfides (Mottram, 1998). Furans and thiophenes with a thiol group located in the 3 position, and related disulfides, contribute to a strong meat-like aroma and have low odor threshold values (Evers**,** Heinsohn, Mayers, & Sanderson**,** 1976; Van den Ouweland & Peer, 1972). Cooked beef flavor has been identified to contain 2-methyl-3-(methylthio)furan (MacLeod & Ames, 1986), 2-methyl-3-furanthiol and the corresponding disulfide, bis-(2-methyl-3-furanyl) disulfide (Gasser & Grosch, 1988). Some other thiols and disulfides containing 2-furanylmethyl moieties have also been found in cooked meat (Farmer & Patterson, 1991; Madruga & Mottram, 1995). The degradation of sulfur-containing compounds has been associated with loss of meat flavor (Drumm & Spanier, 1991; Spanier, Edwards, & Dupuy, 1988).

The saturated and unsaturated aldehydes produced from lipid oxidation are major contributors to the volatile compounds of cooked meats (Mottram, 1998).

The identification of food odors has been commonly assessed by human perception through trained panelists and headspace/direct gas chromatography/mass spectrometry (GC/MS) (Grigioni, Margaría, Pensel, Sánchez, & Vaudagna, 2000). Trained panels can identify changes in off flavors, but they can have some limitations such as availability and judge fatigue. Trained panel can be used in combination with GS/MS to identify and describe what compounds and descriptors drive beef flavor.

2.11. Flavor contribution from thiamine

Thiamine (B_1) is a hydrosoluble vitamin and it has been found to be one of the main precursors of meat flavor. Thiamine is very susceptible to thermal degradation When thiamine is thermally degraded it produces multiple sulfur compounds such as thiols, furans, sulfides, and disulfides (Grosch, 2001; MacLeod, 1994). Some of these compounds have a very important contribution to cooked meat aroma, having low aroma thresholds (Kerscher & Grosch, 1998).

The volatile aroma compounds 2-methyl-3-furanthiol and 2/3-mercapto-3/2-pentanone can arise from degradation of thiamine (Guentert et al., 2013), but also from Maillard reactions between cysteine and ribose, the Strecker reactions of sulphur amino acids, and the interactions between them contributing to cooked beef flavor.

The duration, temperature, type of cooking, presence of other ingredients, and pH influence the losses of thiamine (Lassen, Kall, Hansen, & Ovesen, 2002).

2.12. REIMS

The thermal ablation by electric current by the ionization method of samples with a high percentage of moisture is called rapid evaporative ionization mass spectrometry (REIMS) (Schäfer et al., 2009). This technique is based on the mechanical or thermal ablation of tissue material leading to the formation of an aerosol containing charged particles (Balog et al., 2016). This technique uses ionization and desorption of molecules by generating a smoke from a sample using a hand-held device (Kosek et al., 2019). REIMS techniques have been widely used for tissue analysis and it generally takes a few

seconds and can provide histological tissue identification with 90-98% accuracy in classification (Balog et al., 2013). REIMS technology does not require sample preparation, improves the time to obtain results and reduces the chance of technical mistakes during sample preparation.

This mass spectrometry technique has a hand-held device called an intelligent knife or "iknife" and is the electrosurgical tool (Rigano et al., 2019). The handheld device burns the sample creating an aerosol of ionized and neutral molecules. The aerosol of molecules reaches a heated impactor in the machine that disrupts the cluster and ionizes the remaining neutral molecules (Golf et al., 2015). REIMS is an ambient mass spectrometry (AMS) technique and it has been mostly investigated for clinical analyses of intraoperative tissue mainly used in biomedical applications and the clinical medicine field (Alexander et al., 2017; Balog et al., 2010; 2013; 2015; Neidert & Bozinov, 2013; St. John et al., 2017). Recently, it has been employed in several food adulteration studies for meat (Balog et al., 2016) to classify fish species (Black et al., 2017), observe lipid changes in porcine muscle tissue (Guitton et al., 2018), aid in segregation of pork carcasses with and without boar taint (Verplanken et al., 2017), and the identification of bacterial colonies (Strittmatter et al., 2014).

The mass spectra collected from REIMS has shown high prediction accuracies of various meat attributes. Balog et al. (2016) demonstrated that data collected from REIMS were able to determine mammalian meat species and beef breeds with 100% and 97% accuracy, respectively. Black et al. (2017) showed the REIMS potential to classify fish species with a prediction rate of 98.99%. Guitton et al. (2018) identified porcine

muscle from experimental groups of animals fed with growth promoters with at least 95% accuracy. Gredell et al. (2019) was able to use REIMS data to predict dark cutter, top choice/prime, low choice/select, Wagyu, grass fed, tender and tough, Angus and not Angus strip loin steaks. They also evaluated different machine learning algorithms to predict model sets varying 81.5 to 99% of final accuracy rate.

This technology has the potential to predict beef flavor by categorizing samples into groups using models with data collected from REIMS spectra. The MS captured from REIMS produce a specific pattern from the compound's profiles. Samples can be divided in groups upon cooking method, flavor attributes intensity and other characteristics that differentiate by compound's profile. The group can be predicted by using REIMS data upon the specific pattern that each sample's compound produces.

3. MATERIALS AND METHODS

Trained sensory panelist training and testing procedures were approved by the Texas A&M Institutional Review Board (IRB2018-0762).

3.1. Sample Selection

Low Choice beef strip loins (n = 18, IMPS 180) were purchased at a commercial beef processing facility (Ruffino Meats and Food service, Bryan, Texas) over three days for three experimental repetitions. Subprimals were stored in a vacuum bag for 14 d at 2°C before they were fabricated into steaks. Full loins were then dipped in a 3% lactic acid solution for one minute for microbiological spoilage control. Each subprimal was fabricated into twelve, 2.54 cm top loin steaks using a band-saw (Butcher Boy, Model B-16 F, Los Angeles, CA) and randomly assigned to treatments (three lipid of oxidation levels and three cooking methods). One steak was randomly assigned to descriptive sensory analysis and volatile aromatic compound GC/MS evaluation, and the second steak was assigned to Warner-Bratzler shear force, REIMS and raw and cooked TBARS values. Both steaks were packed, stored, and cooked together.

Steaks were stored to induce differences in raw lipid oxidation of: low - approximately 0.1 mg of malonaldehyde/kg of sample; medium - approximately 0.5 mg of malonaldehyde/kg of sample; and high > 1.0 mg of malonaldehyde/kg of sample. To create the three levels of lipid oxidation, the low group was vacuum-packed at the processing facility and cooked by sous-vide on the same day. Medium and high group steaks were placed in Styrofoam trays with PVC overwrap (Food Service Film Roll,

Members Mark, Bentonville, AR) and placed under fluorescent lights between 1,500 and 1,700 lux, measured using a light meter (Reed, Model ST-1 301) at 4°C for two days for medium group and five days for high group, these treatments were used to induce TBARS values of 0.5 and >1.0 in the raw steak. Time of light exposure and length of storage for the three groups were determined in preliminary tests.

3.2. Color Measurements

Steaks assigned to sensory panel were evaluated for raw objective color, before and after storage time, using a Minolta Chromameter (Spectro-photocolorimeter Minolta CR-400; Konica Minolta, Sensing, Inc., Osaka, Japan, light source with a 2° Observer) calibrated before testing using a white tile (Y = 96.78, x = - 0.25, y = 2.25). L*, a*, and b* color space values were recorded from each steak from three random surface locations. Surface color was measured after steaks were removed from respective package for thirty minutes (AMSA, 2012). Hue angle was calculated ($HA = \tan^{-1}$ [b*/a*]) and chroma ($C = \sqrt{a^{*2}+b^{*2}}$)

3.3. Cooking Methods

3.3.1. Sous-vide

For sous-vide treatments, steaks were vacuum-packaged in cook-in bags (CN535, Cryovac Sealed Air Corporation, Duncan, SC) with an oxygen transmission rate of 20 cc at 0% RH at 22.8°C (m^2, 1 atm, 24 h) prior to the cooking procedure. Water was placed in a cooking container (APW Wyott W-3V1 classic countertop, 30.48 x 50.8 cm warmer,

Allen, TX) and an immersion water circulating sous-vide cooking system (Anova Culinary, temperature range 25℃ to 99℃, pump speed 1.2 GPM, 8 LPM, San Francisco, CA). The immersion water circulating system was set at 49℃. A needle temperature probe (ThermoWorks, sous-vide mini needle probe, SKU: THS-113-109, Sensor range -50℃ to 250℃, American Fork, UT) was inserted in the geometric center of the steak through the package using a water, air tight foam tape (ThermoWorks, sous-vide foam tape, SKU: THS-600-475, operation temp. -40℃ to 90℃, American Fork, UT). The steaks were cooked until they reached an internal temperature of 49°C. Raw and cooked weights, initial and final temperatures, and time were recorded. After the steaks reached the desired internal temperature, they were placed in a walk-in cooler at 4℃ overnight. The following day, the steaks were placed back in the sous-vide at 70℃ for reheat until reached the final internal temperature of 70℃ for evaluation.

3.3.2. Sous-vide plus Grill

Steaks assigned to sous-vide with searing (Sous-vide + grill) treatment were cooked and chilled in the manner as described above for sous-vide only. The following day, the steaks were reheated until they reached the internal temperature of 70℃ in the sous-vide water bath then the steaks were placed on a dry heat cooking flat solid stainless-steel surface at 204℃ (Star Max 536TGF Countertop Electric Griddle with Snap Action Thermostatic Controls, Star International Holdings Inc. Company, St. Louis, MO) for one minute and then were flipped over for one more minute to create the browning color on the outside. This provided sufficient time and surface contact for

some Maillard reaction. Internal temperatures were monitored and recorded during the searing process.

3.3.3. Grill

Steaks assigned for the grill treatment were vacuum-packaged on the precooking sous-vide day to minimize further lipid oxidation. Steaks assigned to the flat top grilling method were cooked using a dry heat cooking flat solid stainless-steel surface at 204°C (Star Max 536TGF Countertop Electric Griddle with Snap Action Thermostatic Controls, Star International Holdings Inc. Company, St. Louis, MO) to an internal temperature endpoint of 70°C and were flipped at 35°C. Internal temperatures were monitored by an iron-constantan thermocouple probe (Omega Engineering, Stamford, CT) inserted into the geometric center and the temperature were displayed on a reader (HH501BT Type T thermometer, Omega Engineering, Stanford, CT). The two steaks for each treatment were cooked simultaneously.

3.4. Expert, Trained Descriptive Beef Flavor Analysis

A six-member trained descriptive sensory panel (AMSA, 2015; Meilgaard, Civille, & Carr, 2015) at Texas A&M University in College Station, TX, evaluated each cooked steak (n = 108). Prior to analysis, panelists were retrained for 12 days to evaluate samples for standard taste, aroma, and texture using the beef lexicon (Adhikari, et al. 2011) on a 16-point numerical scale with 0 = none and 15 = extremely intense as described in Table 1.

After three days of training, the panelists and the panel leader identified that the sous-vide cooked samples had different texture attributes when compared to the samples that were cooked on the flat surface grill. Denseness was added to describe differences in texture between the treatments. Twelve samples were evaluated per day for nine evaluation days. After steaks were cooked and measurements recorded, steaks were wrapped in foil and placed in a holding oven (Model 750-TH-II, Alto-Shaam, Menomonee Falls, WI) for up to 20 minutes, until served. A warm-up sample was served at the beginning of each sensory day to calibrate the panelists and the panel leader. Panelists came to a consensus for flavor and texture attributes. The warm-up samples were randomized between the treatments. Samples were randomly assigned order by treatment and coded with a three-digit code. Double-distilled, and carbonated water and salt-less saltine crackers (Premium Unsalted Tops Saltine Crackers, Nabisco, East Hanover, NJ) were provided for cleansing the palate between samples. The panelists were seated in table and were provided with all the references from training. Samples were cut into 1.27 cm x 1.27 cm x 2.54 cm (steak thickness) cuboidal, and two to three cuboidals were served to each panelist. The samples were served with a plastic 59 mL soufflé cups (translucent plastic portion cups, Georgia-Pacific, Asheboro, North Carolina). Samples were served at least five minutes apart to minimize fatigue. After warm-up and six samples the panelists had a break for at least ten minutes. Samples were served immediately after being cut, so that serving temperature was at 37°C.

3.5. Warner-Bratzler Shear Force

Warner-Bratzler shear force determinations was conducted as defined by AMSA (2015). The WBSF steaks were cooked with the panel sample. After steaks were cooked, they were placed on a tray at room temperature, overwrapped with plastic film (Food Service Film Roll, Members Mark, Bentonville, AR) to minimize evaporative losses and then stored overnight in a walk-in cooler at 4°C. On the day that the WBSF values were determined, the samples were placed at room temperature for two hours. Up to six cores, 1.3 cm in diameter were removed parallel to the muscle fiber orientation. Each core was sheared once, perpendicular to the muscle fiber orientation using a United Testing Machine (United SSTM-500, Huntington Beach, CA) at crosshead speed 200 mm/min using a 10.0 kg load cell, and a V-shape blade with a 60° angle and a half-round peak. The peak force in kilograms was recorded for each core and the mean for each steak was used for further statistical analysis.

3.6. Thiobarbituric Acid Reactive Substances

A 10 g sample was removed from each raw and cooked steak in duplicate for TBARS value determination from the WBSF steak. The sample was blended using a homogenizer (Polytron) at 15,000 RPM for one minute with 50 mL of distilled water and 5 mL of antioxidant solution (1:1 mixture of 0.5% propyl gallate and 0.5% EDTA). The homogenized sample was added to 2.5 mL of 4N HCl and 31.5 mL of distilled water as described by Tarladgis, Watts, Younathan, & Dugan (1960) and modified by Rhee (1978). The distillate was analyzed in duplicate using TBA reagent (1:1) prepared in

distilled water. The result from the color-development had the absorbance measured at 532 nm and the results were expressed as mg of malonaldehyde per kg of meat.

3.7. Gas Chromatography – Mass Spectrometry (GC/MS)

The samples for GC/MS were collected from representative cubes taken from the same steak as the panelists were served. For chemical volatile aroma analysis, 5 g (+/- 0.05 g) of each sample was weighed and placed in a 20 mL glass vial. To each sample, an aliquot 10 µL of an internal standard (99 v/v% methanol, 1 v/v% 1,2-dichloromethylbenzene) was added. Samples were then placed in a heating block (Block analog 2 120V with block modular 28M, VWR) held at 65℃. The volatile compounds present in the static headspace were collected using a solid-phase micro-extraction (SPME) Portable Field Sampler (Supelco 504831, 75 µm carboxen/ polydimethylsiloxane, Sigma-Aldrich, St. Louis, MO) for 20 minutes. Volatile aroma compounds were eluted from the SPMEs and separated using gas chromatography (GC; Agilent Technologies 7920 series GC, Santa Clara, CA). The sample was desorbed at 280℃ for three minutes.

The sample was loaded onto the multi-dimensional gas chromatograph column (Agilent VF 5MS 30 m × 0.25 mm ID/1µ film thickness, SGE Analytical Sciences, Austin, TX). The temperature started at 40℃ (held for one minute) and increased at a rate of 20℃/min until reaching 250℃. Compounds were identified and quantified with a mass spectrometer (MS; Agilent Technologies 5975 series MSD, Santa Clara, CA) using Wiley Chemical Library.

3.8. Rapid Evaporative Ionization Mass Spectrometry (REIMS)

After the samples were cooked, a 2.54 cm cube sample was removed for REIMS technology analysis from the WBSF steak. The REIMS samples were cut, immediately frozen in liquid nitrogen at -196℃, placed in foil with a tag separated from the meat samples, and stored at -80℃ until all the samples from the three reps were collected and they were taken to Texas Tech University, Lubbock, Texas for the analysis. Before analysis, samples were thawed in a walk-in cooler at 0-4℃ overnight.

The REIMS technology (iKnife; Xevo G2-S quadrupole TOF-MSA, Waters Corp., Wilmslow, UK) aerosol was produced from the surface of each meat sample using a hand-held sampling device. Five burns per sample were collected in negative ion mode. The generated aerosol was transferred to an on-line Q-TOF mass spectrometer (Xevo G2-S quadrupole TOF-MSA, Waters Corp., Wilmslow, UK) using an orthogonal air jet pump and ionized via a heated impactor in the MS source. Negative mode ion mass spectra were acquired in the mass range of m/z 100−1200. All acquired data files were preprocessed using a custom-built software package (Waters Research Centre, Budapest, Hungary) containing standard Masslynx preprocessing algorithms (Waters Corp., Wilmslow, UK). The relative abundance values from the 5 burns were averaged to create a single value for each sample. Data were preprocessed to include lock mass correction (leucine enkephalin), background subtraction, and normalized to the total ion current. Additionally, individual peaks were binned in intervals of 0.5 m/z starting with 100.25 and ending with 1,199.75 for a total of 2,200 variables. Leucine enkephalin has a

molecular weight of 555.632 g/mol and its detection would interfere with neighboring components. Therefore, mass bins in the range of 550-560 were excluded from the data.

3.9. Statistical Analysis

The experimental design was completely randomized block design, with a 3 x 3 interaction. Data were analyzed using lipid oxidation group and cooking method, and their interaction as fixed effects, with Analysis of Variance alpha value at 5% and SAS software (v94, SAS Institute, Inc. Cary, NC). Data was analyzed using PROC GLM using the pdiff function. Replicate was included as a fixed effect in the model and sensory day, block and order was included as a random effect. Least squares mean were determined for main effects and significant two-way interactions. Prior to analysis, GC total ion counts data was transformed using log10(x+1).

To understand the relationship between treatments (level of lipid oxidation and cooking methods), descriptive sensory attributes, and volatile aroma compounds, Principal component analysis (PCA) and partial least squares regression (PLS) was conducted using XLSTAT (2019.2.1, Addinsoft, New York, NY). Data were presented in bi-plots. Variables used in partial least squares regression equations were selected to have variable importance in the projection (VIP) ≥ 0.9.

Several predictive models were evaluated to assess capabilities of REIMS for identification of level of lipid oxidation and cooking method. Models were built and classifications predicted using PLS- linear discriminant (LDA). Using this method, PLS reduced dimensionality and collinearity within the data set before classification with the

LDA model by using individual scores values from a predetermined number of PLS components as input for LDA. Before fitting the model, variables with correlation coefficients ≥ 0.75 were identified. Mass bins were then log transformed to address skewness of data distributions, mean centered, and unit variance scaled so that each variable had a mean of zero and an equal distribution. Eighty percent of the pre-processed data were then randomly selected to train the models, with the remaining 20% set aside to test the prediction accuracy of the newly developed models. Splitting of the data was performed separately for each model so that each classification category could be evenly distributed between training and testing sets. Prediction models were fit using the PLS.LDA function from the PLSGENOMICS package in R (R Core Team, 2019). The number of PLS components used as inputs for LDA was determined to maximize the predictability of the test data set. Several measures were calculated to evaluate predictive ability of each model using the predictions of the model built with the training set on the test set. Sensitivity was determined as the number of true positives divided by the number of true positives plus false negatives. It is essentially the accuracy of each individual class. Sensitivity did not, however, take false positives into consideration; therefore, precision was calculated for each class in conjunction with sensitivity. Precision was equal to the number of true positives divided by the number of true positives plus false positives.

4. RESULTS AND DISCUSSION

4.1. Descriptive Sensory Analysis

Flavor attributes of animal hair, barnyard, beef identity, bitter, bloody/serumy, brown, buttery, burnt, cardboardy, chemical, cooked milk, dairy, fat-like, fishy, green, green-hay like, heated oil, leather, liver-like, metallic, musty-earthy/humus, overall sweet, rancid, refrigerator stale, roasted, salty, smoky charcoal, smoky wood, soapy, sour aromatics, and sour milk/sour dairy (Table 1) and on texture attributes of denseness, muscle fiber tenderness, connective tissue, and juiciness were evaluated (Table 2). During training it was decided to combine some attributes such as smoky charcoal and smoky wood, and cooked milk, dairy, and sour milk/sour dairy. Flavor attributes of animal hair, barnyard, green, green-hay like, leather, rancid, and spoiled putrid were not present and data were not reported.

Level of lipid oxidation affected flavor attributes of top loin steaks ($P < 0.05$). Brown ($P = 0.32$), bitter ($P = 0.26$), fat like ($P = 0.41$), metallic ($P = 0.63$), overall sweet ($P = 0.13$), sour aromatics ($P = 0.05$), burnt ($P = 0.12$), chemical ($P = 0.15$), liver-like ($P = 0.38$), smoky charcoal/wood ($P = 0.35$), and soapy ($P = 0.14$) attributes were not affected by lipid oxidation level. High and medium levels of lipid oxidation had lower intensities of beef identity ($P = 00.0001$), roasted ($P = 0.004$), sweet ($P = 0.0004$), umami ($P = 0.006$), and buttery ($P = 0.0003$) flavor attributes. These results are similar to the results found by Campo et al. (2006) where increase in storage from 4 to 9 days decreased beef identity flavor. St. Angelo et al. (1987) found a negative correlation between cooked beef/brothy flavor attribute, hexanal volatile compound and TBARS

values, these results showed that the increase in lipid oxidation compounds resulted in lower levels of beef identity flavor attribute. Decrease in beef identity flavor has been associated with protein degradation throughout the lipid autoxidation. Lipid oxidation may catalyze degradation of sulfur-containing compounds that contribute to meat flavor changes (Drumm & Spanier, 1991; Kerscher & Grosch, 1988). Additionally, development of off-flavors through lipid oxidation can mask meaty flavor (Van Ba, Hwang, Jeong, & Touseef, 2012).

Steaks from the high level of lipid oxidation had more intense sour ($P = 0.005$), musty earthy/humus ($P = 0.008$), heated oil ($P = 0.02$), painty ($P = 0.004$), and warmed-over ($P < 0.0001$) flavor attributes. Fishy ($P < 0.0001$) and refrigerator stale ($P = 0.004$) flavor attributes were also more intense in the medium and high oxidation level steaks. The production of off-flavors and odors, such as old, stale, oxidized, warmed over, rancid, or painty have been shown to be a result of oxidation of unsaturated fatty acids (Rhee, 1989). Steaks with increased levels of lipid oxidation had increased intensities of painty, warmed-over, fishy, and refrigerator stale flavor attributes.

Cardboardy ($P = 0.001$) and sour milk/dairy/cooked milk ($P = 0.007$) more intense in steaks from medium and high levels. The increase in levels of cardboardy were expected due to the increase of lipid oxidation (St. Angelo et al., 1987). Rhee, Anderson, and Sams (2005) reported a high positive correlation (0.94) between cardboard flavor and levels of lipid oxidation. Campo et al. (2006) also found an increase in abnormal flavor (off-flavor) with increase of storage time from 4 to 9 days. MacDonald, Gray, & Kakuda (1980) found that overall off-flavor formation can be

predicted by TBARS values during the first 7 days of storage. Campo et al. (2006) reported high relationship between increase in abnormal and rancid flavors and decrease in beef flavor identity in beef samples, corroborating the results reported in this study.

Cooking methods affected flavor attributes of top loin steaks ($P < 0.05$). Beef identity ($P < 0.0001$), brown ($P < 0.0001$), sweet ($P < 0.0001$), umami ($P < 0.0001$), overall sweet ($P < 0.0001$), buttery ($P = 0.0006$), heated oil ($P < 0.0001$), and smoky charcoal/wood ($P < 0.0001$) flavor attributes were more intense in grill steaks, followed by steaks from the sous-vide + grill (SVG) and the sous-vide (SV) treatment consecutively. As expected, when dry cooking conditions are applied, there is development of more Maillard reaction products and increase in positive sensory attributes, such as beef identity, brown, umami, and smoky charcoal flavors (Kerth & Miller, 2015). The lower amounts of umami flavor in steaks from the sous-vide treatment were also reported by Shahidi (1994). Glutamate, a flavor precursor compound in umami basic taste is lower in meat cooked in water and boiled (Shahidi, 1994). Boiling and sous-vide are moist cooking methods and would expectantly to have lower levels of Maillard reaction products.

Steaks from grill treatment had lower intensity of cardboardy ($P < 0.0001$), and refrigerator stale ($P < 0.0001$) flavors attributes compared to sous-vide + grill, followed by sous-vide cooking treatment. Steaks from grill treatment had more intense bloody/serumy ($P < 0.0001$), metallic ($P = 0.009$), and burnt ($P < 0.0001$) flavor attributes when compared to sous-vide + grill and sous-vide. The increase of bloody and metallic flavors was expected because the cooking time of the grill treatment was much

lower than sous-vide and sous-vide + grill (Table 5), and the cooking is uneven. The burnt results were also expected because the grill treatment is a direct dry heat method of cooking.

The sous-vide treatment was responsible for having steaks with more intense sour ($P = 0.003$), fishy ($P = 0.02$), liver like ($P = 0.0004$), and warmed-over ($P < 0.0001$) flavor attributes. In study, James and Calkins (2005) reported that the slower cooking and longer hold time tend to improve the dissipation of undesirable volatile flavor compounds. Roasted ($P < 0.0001$) attribute had the less intense flavor for steaks cooked on the grill treatment (7.2), followed by sous-vide (8.1) and sous-vide + grill (8.6), and all the treatments were different. Higher roasted flavor attributes were given to the sous-vide cooking method probably because of the moist heat cookery method and longer time of cooking (Table 5).

Warmed-over flavor is one of the most studied off-flavors in literature, and it is a consequence of lipid oxidation, mainly from the membrane phospholipids (St. Angelo et al, 1987). It is also stated that the threshold for oxidized flavor perception ranges between 0.6 and 2.0 mg of MDA/kg of sample (Greene & Cumuze, 1981). Campo et al. (2006) found in their study that the off-flavors were easily detected at the 2.8 mg of MDA/kg threshold. The increase of the brown flavor descriptor in the grill treatment probably masked some of the off-flavors found in the samples, such as what was found by Byrne et al. (2001), that the warmed-over flavor and the aroma of hexanal was masked by other strong flavors present in the meat samples. The green flavor compounds were detected in small amounts in the samples, however the small amounts

detected were found in the high lipid oxidation group. Green flavor descriptor is associated with the presence of hexanal, a volatile aldehyde derived from the oxidation of omega 6 fatty acids (Campo et al., 2006). Hexanal volatile compound concentration in this study increased 156% in the high level of lipid oxidation group compared to low, and that is likely to have been a primary contributor to the green flavor (Table 10).

The two way interactions for the flavor attributes brown, buttery, and burnt are presented on Figures 1, 2, and 3, respectively. The interaction for the brown flavor is a result of the Maillard reaction products being present in higher amounts in the grill cooking method. From the direct dry heat exposed for a longer amount of time when compared to the sous-vide plus grill treatment that was exposed to the grill for a total of two minutes, and lowest amount of brown in the sous-vide samples that were solely exposed to the LTLT with no direct dry heat. The two way interaction found in the buttery flavor attribute can be explained by the higher amounts in buttery detected in grill cooking method and in the low level of lipid oxidation. Over time with storage, there is a reduction of buttery flavor, probably because the compounds that produce this flavor are oxidized and/or covered by stronger flavors developed during oxidation. The buttery flavor is also more easily identified when dry direct heat is applied such as the treatments that utilized the grill. The burnt flavor interaction is mainly due to the higher amount of this attribute found in the samples that used grill as a form of dry direct heat.

Lipid oxidation level did not affect texture attributes (Table 4), such as denseness ($P = 0.34$), juiciness ($P = 0.65$), muscle fiber tenderness ($P = 0.61$), and connective tissue ($P = 0.30$) on steaks. Cooking methods did not affect muscle fiber tenderness ($P =$

0.10) and connective tissue ($P = 0.41$) in steaks, however it affected denseness ($P < 0.0001$) and juiciness ($P < 0.0001$).

Steaks from SV and SVG treatments were higher in denseness and lower in juiciness compared to the grill treatment. The results from juiciness were not expected because the grill treatment had scores at least one-point higher than the sous-vide treatments. Mortensen, Frøst, Skibsted, and Risbo (2012) found that the samples with the highest amount of juiciness where detected at the lowest temperature and time treated (56℃ at 3 h). The juiciness from their samples decreased when the temperature and time increased (58 and 60℃, 6, 9, and 12 hours). So, the results from this study for juiciness were higher in the grill treatments probably because the steaks designated to this treatment had a very short cooking time (Table 5), not allowing enough time to decrease the juices from the steaks, and the sous-vide treatments, although cooked in a bag (moist cooking conditions), were cooked for a prolonged time and had a large amount of purge loss because the prolonged time to denature and shrink proteins. Grilling after sous-vide may boost the Maillard products but also may have a result of excessive dehydration, modifying texture and juiciness. Tenderness and juiciness changes are highly influenced by the cooking temperature and time applied (Christensen, Bertram, Aaslyng, & Christensen, 2013; Laakkonen, Wellington, & Sherbon, 1970; Sánchez del Pulgar, Gázquez, & Ruiz-Carrascal, 2012). Roldán, Antequera, Martín, Mayoral, & Ruiz (2013) reported a higher cooking loss as the temperature rises, especially above 60℃, when muscle fibers shrink longitudinally normally increasing moisture loss.

The denseness was different in the sous-vide samples probably because the morphological changes of the muscle fibers during the wet cooking conditions. In raw meat, the muscle fibers are more defined, where the gaps between the fibers and the endomysial tubes are more visible (Palka, 1999; Palka & Daun, 1999). Palka & Daun (1999) observed that after cooking to 60°C, a granulation of the sarcolemma and perymisium began, and at 70°C the granulation of components intensified, and larger granules were observed. García-Segovia, Andrés-Bello, and Martínez-Monzó (2007) evaluated steaks cooked at 70°C in sous-vide and observed differences in structure of the samples when analyzed in micrographs. By using the moist cooking method, steam would be present and would cause a greater degree of unfolding and denaturation of sarcoplasmic proteins and myosin (Tornberg, 2005). The sous-vide treatment also caused the connective tissue (endomysium) structures to be more diffused (García-Segovia, Andrés-Bello, & Martínez-Monzó, 2007), causing a change in the structure creating a denser sample when compared to a traditional dry cookery condition, such as the grill cooking method. The panelists from this study were able to observe the presence of visibly and texturally defined muscle fibers in the grill cooking method. They also called the texture of the sous-vide cooked steaks mushy, dense, and spongy. Although the samples were tender, they were not able to detect normal muscle fiber structure such as in the grill treatment.

Principal component analysis (PCA) was used to identify relationships between descriptive flavor and texture attributes and treatments, shown as biplots in Figure 4. The grill cooking treatment was clustered together with most of the positive attributes

such as beef ID, brown, umami, smoky, sweet, and juiciness, and concurrently the sous-vide and high oxidation treatment are in the same quadrant as off-flavors, such as cardboardy, refrigerator stale, warmed-over, fishy, painty, and they were closer to liver-like and denseness, giving more evidence that the samples cooked by sous-vide were denser than the samples cooked on the grill.

Low level of lipid oxidation was also closely associated with positive flavors when compared to medium and high levels. The sous-vide plus grill treatment was clustered together with the roasted flavor attribute.

4.2. Cook yield and time

Steaks from lipid oxidation group did not affect cook yield, pre-cooking, and cooking time, however cooking method affected cook yield (P <0.0001) and cooking time (P <0.0001) (Table 5). The steaks from sous-vide + grill treatment had the lowest values for cook yield compared to sous-vide and grill treatments, which were not different between treatments. Steaks from sous-vide and sous-vide + grill treatments had the highest values of cook time when compared to the grill treatment.

Ruiz-Carrascal, Roldán, Refolio, Perez-Palacios, Antequera (2019) reported similar results using sous-vide cooked lamb loins which the samples that were cooked with a higher Maillard products after sous-vide cooking had a higher cooking loss. They reported around 45% of cooking loss for these samples compared to 12% of cooking loss from the sous-vide cooked samples.

4.3. Color

The initial level of lipid oxidation affected L*, a*, b*, chroma, and hue angle color measurements of top loin steaks (Table 6). The low lipid oxidation group was responsible for the highest values of L* ($P = 0.0005$), a* ($P < 0.0001$), and chroma ($P < 0.0001$) in raw steaks. The samples from the low lipid oxidation group were lighter than the other groups. The a* ($P = 0.74$) and chroma ($P = 0.87$) values were not significantly different between steaks from low and medium group. Steaks from high lipid oxidation level had the lowest values for L*, a*, b*, and chroma, but for L* values medium and high levels were not different ($P = 0.69$), and for b* values, low and medium levels were not different ($P = 0.53$).

Steaks from high level of lipid oxidation had the highest values of hue angle ($P < 0.0001$), followed by medium and low which were not different ($P = 0.07$). Overall, the steaks from higher level of lipid oxidation resulting from an increased storage time decreased the luminosity (L*) and the redness (a*) of steaks, and these results were expected because with the increase of level oxidation there is an increase in metmyoglobin state in the steaks. These results are similar to what Sujiwo, Kim, Song, and Jang (2019) reported, decreasing in a* over time, where they tested 1, 3, 6, 9, 12, 15, and 18 days of storage. The decrease of a* (redness) values are associated with the decrease of freshness as perceived by consumers (Robbins et al., 2013).

The cooking method groups did not affect color measurement in the steaks due to the measurement being done only on the raw samples prior to cooking, in an effort to evaluate the effects of lipid oxidation on raw sample color.

4.4. Tenderness

The initial level of lipid oxidation and cooking methods affected the tenderness values from the WBSF measurements (Table 7). Steaks from medium and high levels of lipid oxidation had the lowest values of WBSF ($P = 0.013$). The higher levels of lipid of oxidation had the most tender samples, probably because the longer storage time and more than likely involving the proteolytic enzymes system. Low and high levels were not different ($P = 0.08$).

The samples from grill cooking treatment had the lowest values of WBSF, followed by sous-vide and sous-vide + grill ($P < 0.0001$). Steaks from sous-vide and sous-vide + grill treatments were not different ($P = 0.13$). The results from tenderness, where samples from the sous-vide treatment were tougher, were not expected because the moist cookery heat treatment and sous-vide cooking have been seen to increase tenderness. Vaudagna et al. (2002) studied sous-vide cooked beef muscles in a range of 50 to 65℃ and a range of 90 to 390 minutes and concluded that 60℃ for 270 minutes treatment resulted in the most tender samples, and the same parameters were used by Botinestean, Keenan, Kerry, & Hamill (2016), who concluded that sous-vide cooked beef samples had an increase in tenderness at 60℃ for 270 minutes.

Although the WBSF values were not expected, the trained panelists did not find differences in tenderness ($P = 0.10$) and they found that the samples from the sous-vide were drier than the grill samples. The results found in juiciness probably influenced the tenderness results. The sous-vide samples were tougher, however all the results found are considered tender. Although the cooking methods were different, the WBSF values

between the samples are lower than 0.8 kg. The sous-vide + grill treatment is categorized as “tender” (3.2 < WBSF < 3.9 kg) and the sous-vide and grill treatments are categorized as “very tender” (WBSF < 3.2 kg) (Belew, Brooks, McKenna, & Savell, 2003). Martinez et al. (2015), in the national beef tenderness survey, reported 95.93% of retail top loins steaks classified as “very tender”, cooked on a non-stick electric grill.

4.5. TBARS

The raw levels of lipid oxidation were validated in Table 8. The lipid oxidation groups were defined as low (0.12 mg of malonaldehyde/kg of sample), medium (0.71), and high (1.83), and they were all different ($P < 0.0001$). The level of lipid oxidation also affected the cooked steaks TBARS values ($P < 0.0001$). Steaks from low lipid oxidation group had the lowest values, followed by the medium and high level.

The cooking process did not affect any of the treatments ($P > 0.05$). The increase between medium and high level is greater than the increase between the low and medium and it was expected because lipid oxidation is a free-radical autocatalytic chain reaction (Rhee, 1988), increasing in a non-linear fashion. The same nonlinear increment in the TBARS values between the medium and high treatments (4 and 9 d) seen when compared to low and medium levels (0 and 4 d) was found in a study evaluating beef samples displayed under lights in retail settings (Campo et al., 2006).

4.6. Volatile Aroma Compounds

The least squares mean of total ion counts of volatile aroma compounds of cooking methods and level of lipid oxidation are presented in Tables 9 and 10. The alcohol functional group of volatile compounds was not affected ($P > 0.05$) by the cooking method (Table 9).

Steaks cooked on SV group had the lowest amount of 2-methyl-butanal ($P < 0.0001$), 3-methyl-butanal ($P < 0.0001$), and 2-methyl-propanal ($P = 0.0002$) compared to SV and SVG cooking groups, and the SVG and grill did not differ ($P = 0.05$) significantly. The cooking method did not affect hexanal/carbon disulfide ($P = 0.29$). Aldehydes can be formed through lipid oxidation or Maillard reaction (Jousse, Jongen, Agterof, Russel, & Braat, 2002).

Acetaldehyde, propanal, 2-methylpropanal, 3-methylpropanal, 2-methylbutanal, methional, and phenylacetaldehyde are Strecker aldehyde products (Resconi, Escudero, & Campo, 2013; Rochat & Chaintreau, 2005). The volatile compound 2-methyl-propanal is associated with having a characteristic sharp, pungent odor (Burdock, 2010). Benzaldehyde is mainly a product of Maillard reaction (Drumm & Spanier, 1991). Benzaldehyde has been identified as a product from the Strecker degradation of phenylglycine (Mottram & Edwards, 1983), however, in the present study, an increase was not found in this compound with the treatments with a higher Maillard reaction.

Although the furan volatile compounds are mainly produced from Maillard reactions (Dashdorj, Amma, & Hwang, 2015) the furan functional group in this study was not affected by the three cooking method treatments. The cooking methods affected

the ketone volatile aroma compounds. The samples cooked on the grill had the highest amount of 2-heptanone ($P = 0.0005$) compared to SV and SVG cooking groups. SV and SVG did not differ ($P = 0.07$) significantly.

The grill cooking group had the highest amount of 2-ethyl-3,5-dimethyl-pyrazine ($P < 0.0001$), and methyl-pyrazine ($P < 0.0001$) compared to SV and SVG cooking groups. SV and SVG did not differ ($P = 0.97$) significantly for methyl-pyrazine compound and all the cooking methods were different ($P < 0.0001$) for 2-ethyl-3,5-dimethyl-pyrazine compound.

The compound methyl-pyrazine has been described by the flavor attributes grilled chicken and savory (Braddock, Sims, & O'keefe, 1995). The volatile 2- ethyl-3,5-dimethyl-pyrazine has been associated with coffee and roasted nuts (Kerth & Miller, 2015), toasted nut, sweet woody, roasted cocoa (Burdock, 2010). On the PLS (Figure 9), this compound is clustered together with brown, beef ID, overall sweet, smoky charcoal, and sweet flavor attributes and with the grill treatment, and methyl-pyrazine is clustered together with buttery and umami, corroborating with the associations found in the literature.

The aroma volatile compound 2-ethyl-3,5-dimethyl-pyrazine has been associated with toasted nut, sweet woody, and roasted cocoa odor flavor attributes (Burdock, 2010). This compound has glucose and glutamate precursors, and it is produced in the Maillard reaction in the presence of sodium hydroxide (Maga, 1992). Umami basic taste is primarily stimulated by L-glutamate, normally in the form of monosodium glutamate (Kerth & Miller, 2015). 2-Ethyl-3,5-dimethyl-pyrazine is clustered together with smoky

charcoal, umami, and sweet flavor attributes and with the grill cooking treatment (Figure 9). The compound 2-ethyl-3,5-dimethyl-pyrazine was not present in the SV treatment, consistent with all other pyrazines (Table 9). This volatile is negatively correlated with umami flavor by the quadrants that they are located in on the PLS (Figure 9), and the panelists attributed less umami flavor to SV compared to SVG and grill treatments (Table 3).

Pyrazine volatile compounds are derived from Maillard reactions (Fors, 1983), from intermediate reactions including Amadori rearrangement (or Heyns rearrangement), or via the Strecker degradation mechanism (Jousse, Jongen, Agterof, Russel, & Braat, 2002), and the presence of these compounds has been associated with the brown flavor descriptor (Watanabe & Sato, 1971).

As expected, the pyrazine functional group was more expressed in the cooking method treatments that had a higher level of Maillard reaction products, such as SVG and grill because of the direct high heat in contact with the steak surface. SV samples did not have any amount of pyrazines expressed (Figure 11). Moist heat cookery, such as sous-vide, prevents the dehydration of the surface of the food product (initial step of the Maillard reaction), subsequently preventing the development of Maillard products (Kerth & Miller, 2015). Pyrazine volatile compounds are produced mainly from the Maillard reaction due to the dry heat cookery method with high, direct heat. The production of these aroma compounds is known to prevent the warmed-over flavor and other off-flavors in beef (Parliament, 1989). The same trend can be seen in this study when comparing Table 3 and 9, where there is an increase in the pyrazine functional

group as a result of the grill cooking method. Methyl-pyrazine does not contribute as much to flavor because it presents a high odor threshold, unlike ethyl-pyrazine, which is very important to flavor due to the low odor threshold (Jousse, Jongen, Agterof, Russel, & Braat, 2002).

Sulfur-containing compound was affected by the cooking methods. The compound 3-(methylthio)-propanal ($P = 0.04$) was highest in the grill and SVG and lowest in the SV cooking treatment. 3-(methylthio)-propanal has a powerful onion, meat-like odor, and it has a pleasant, warm, meat, and soup-like flavor at low levels. This compound seems to be produced in higher amounts with the increase of Maillard reaction (Table 9).

The sulfur-containing compounds are classified as some of the most important contributors to desirable meat flavor (Chang & Peterson, 1977; MacLeod, 1986). These compounds are normally present in low amounts due to their instability, but they contribute highly to flavor due to their low flavor threshold (Drumm & Spanier, 1991; Golovnja & Rothe, 1980; MacLeod, 1986).

The pyrazines compound groups were not affected by level of lipid oxidation group (Table 10). The acetic acid compound was higher in the low and high level of lipid oxidation compared to medium ($P = 0.0007$). The high level of lipid oxidation affected the concentration of alcohol volatile compounds by increasing the levels of 1-octen-3-ol ($P < 0.0001$), 1-hexanol ($P < 0.0001$), and 1-pentanol ($P < 0.0001$) compared to the medium and low levels.

Hydrocarbons, aldehydes, ketones, and alcohols are formed during lipid degradation (Dashdorj, Amma, & Hwang, 2015). Aldehydes and ketones are the most important compounds that affect aroma throughout lipid oxidation, however alcohols and hydrocarbons (such as alkanes, alkenes, and alkylfuranone) can also play a role in aroma (Resconi, Escudero, & Campo, 2013).

The compound 1-octen-3-ol has flavor profile associated with mushroom, earthy, and fungal (Kerth & Miller, 2015), and it has a powerful, sweet odor, with a strong herbaceous, rose and hay, and sweet flavor (Burdock, 2010). The low group of lipid oxidation had zero amounts of this compound (Table 10). 1-Hexanol is found to have a green, fruity, apple skin flavor descriptor (Kerth & Miller, 2015). 1-Pentanol is associated with fusel, fermented, bread, and cereal flavor (Kerth & Miller, 2015), and has a sweet and pleasant odor but a burning taste (Burdock, 2010).

The high level of lipid oxidation affected the concentration of several aldehyde volatile compounds by increasing the levels of benzaldehyde ($P = 0.007$) and heptanal ($P = 0.008$) compared to the medium and low levels, and these levels did not differ between the low and medium treatments. The compounds hexanal ($P = 0.002$) and octanal ($P = 0.02$) were lowest at the steaks on the medium level of oxidation compared to the low and high levels.

Hexanal is a major lipid oxidation product, is used as a measure of lipid autoxidation in foods (Drumm & Spanier, 1991; Melton, 1983), and it is generally the most common volatile compound found in cooked beef (Nawar, 1969). Hexanal can produce desirable beef flavor if present in low concentrations (Melton, 1983). Although

hexanal is used as an indicator of lipid autoxidation, it is not intended to be used alone as the index (Shahidi & Pegg, 1994). Hexanal is an unstable compound and it undergoes oxidation, polymerization, and degradation, resulting in the production of other compounds, such as hexanoic acid (Palamand & Dieckmann, 1994). Hexanal has been associated with fatty, green, grassy, powerful, penetrating, and characteristic fruity odor and taste (Burdock, 2010).

Nonanal and hexanal are two common volatile compounds found resulting from fatty acid heat hydrolysis (Kerth & Miller, 2015). Heptanal and pentanal are also significant contributors. Heptanal has a very strong, harsh, pungent odor and an unpleasant fatty taste (Burdock, 2010; Kerth & Miller, 2015).

The formation of aldehydes is responsible for contributing greatly to the loss of desirable flavor in meats because of the high rate of formation during lipid oxidation and their low flavor thresholds (Ullrich & Grosch, 1987). The alcohol group contributes less to undesirable flavors in meat compared to aldehydes due to the higher flavor thresholds (Drumm & Spanier, 1991).

The alkane functional group was affected by the level of lipid oxidation. The alkane compounds butane ($P = 0.0003$), decane ($P = 0.04$), dodecane ($P = 0.04$), octane ($P = 0.002$), styrene ($P = 0.002$), and pentane ($P = 0.0006$) were higher in the high level of lipid compared to medium and low levels that did not differ between them. Styrene has been associated with having sweet, balsamic, almost floral, and very penetrating odor (Burdock, 2010). The propane volatile aroma compound had the lowest amount in the steaks on the high level of oxidation ($P = 0.02$).

The furan volatile aroma compound 2-pentyl-furan ($P < 0.0001$) was higher in the high level of lipid compared to medium and low. The dihydro-3-methylene-2,5-furandione volatile aroma compound was lower in the high level of lipid oxidation.

The ketone functional group was affected by the level of lipid oxidation. The levels of ketone compounds 2-heptanone ($P < 0.0001$) and 2-pentanone ($P = 0.003$) were greater in the steaks from the high level of lipid oxidation and 3-pentanone compound was lowest in the high level of lipid oxidation.

The sulfur-containing compounds group was affected by the level of lipid oxidation. The carbon disulfide was greater ($P = 0.01$) in the high level of oxidation compared to low and medium levels. The hexanal/carbon disulfide ratio was affected by the initial level of lipid oxidation ($P = 0.03$). The high oxidation led to higher values of the ratio hexanal/carbon disulfide compared to medium and low levels, indicating a more intense lipid oxidation compared to Maillard reaction development. Ruiz-Carrascal, Roldán, Refolio, Perez-Palacios, & Antequera (2019) reported lamb loins oven roasted before sous-vide cooking had a higher values of the ratio hexanal/carbon disulfide compared to oven roasted after sous-vide and solely sous-vide only cooked. The volatile aroma compound 2,5-dimethyl-pyrazine ($P = 0.02$) had significant interactions where higher levels of lipid oxidation and cooking methods with higher levels of Maillard reaction products.

A PLS biplot was created in order to understand how the volatile aroma compounds correlate to the flavor and texture descriptive attributes, cooking methods, and lipid oxidation level treatments (Figure 11). The SV treatment was clustered

together with denseness, liver-like, refrigerator stale, cardboardy, warmed-over, sour, and fishy attributes, and with propanoic acid, ethenyl ester, and 4-octen-3-one volatile aroma compounds.

Products of lipid oxidation are also associated with contributing to liver-like flavor (Calkins & Hodgen, 2007). Although the high level of lipid oxidation did not affect liver-like flavor (Table 3), the higher levels of Maillard reaction products were able to mask liver-like flavor, and the liver-like flavor attribute was clustered together with the SV treatment group (Figure 11), as the cooking method that did not produce high concentrations of Maillard reaction products.

The high level of lipid oxidation was associated with sour aromatics, sour milk/sour dairy, musty earthy/humus, and painty flavor attributes, and with 4-octen-3-one, styrene, and 3-buten-2-one volatile compounds. The 4-octen-3-one volatile aroma compound has been associated with contributing to fishy and rancid flavor profiles (Jacobsen et al., 2000) and the present study helps the literature to associate these flavors.

The grill cooking method was clustered together with burnt, metallic, bloody/serumy, heated oil, juiciness, smoky wood/charcoal, brown, beef id, and umami sensory attributes and several volatile aroma compounds, such as 2,4-dimethyl-heptane, decane, 3-methyl-butanal, 3-hexanone, 2-ethyl-3,5-dimethyl-pyrazine, 2,5-dimethyl-pyrazine, 2,5-dihydro-3,5-dimethyl-2-furanone, 2-methyl-butanal, and nonanal compounds. The grill cooking method was mostly correlated with positive flavor attributes while SV was associated with negative flavor attributes. This relationship was

probably seen due to the Maillard reaction products being more prevalent in the grill treatment group, developing these positive flavor attributes, compared to the SV treatment which did not develop many of those positive flavors.

The medium lipid oxidation and SVG cooking method were associated with roasted and alpha pinene. Low oxidation was clustered with 2,5-octanedione, 2-(ethenyloxy)-propane, decanal, sulfur dioxide compounds and salty flavor attribute.

4.7. REIMS

The vapor collected from each burn generates a peak with the intensity of the peak collected. Each peak has an individual spectra pattern with the compound weights collected. There is a visual difference in the spectra between the level of lipid oxidation and cooking methods (Figures 10, 11, and 12). The data collected from REIMS was used to predict groups for the samples into level of lipid oxidation and cooking method, positive flavor attributes (beef ID, brown, and roasted) and negative flavor attributes (cardboardy and warmed-over). The level of lipid oxidation model had a prediction accuracy of 97.2% (Table 11). The classification of the samples by the REIMS had the highest sensitivity with the low and high groups, having the most extreme values in the study. The classification misplaced 12% of high oxidation level samples into the medium level of oxidation group as illustrated on Figure 13. The cooking method group model had a prediction accuracy of 99% (Table 12). The model had a higher accuracy of assigning the samples in the correct cooking group when compared to the lipid oxidation

group. The only misplacement of the samples was from the SV cooking method. The model misplaced 3% of the SV samples into the SV+G group as shown on Figure 14.

The beef identity flavor attribute model had a prediction accuracy of 100% (Table 13). The model had a higher accuracy of assigning the samples in the correct positive flavor attribute group when compared to the other flavor attributes and treatment groups (Figure 15). The brown flavor attribute model had a prediction accuracy of 99% (Table 14). The only misplacement of the samples was from the low flavor intensity. The model misplaced 3% of the low samples into the mild group as shown on Figure 16. The roasted flavor attribute model had a prediction accuracy of 93.5% (Table 15). The model had the lowest accuracy of assigning the samples in the correct positive flavor attribute when compared to beef identity and brown flavor attribute models (Figure 17).

Cardboardy flavor attribute model had a prediction accuracy of 87% (Table 16). The negative flavor attributes model has lower accuracy of assigning the samples in the correct flavor attribute when compared to positive flavor attribute models (Figure 18). Warmed-over flavor attribute model had a prediction accuracy of 78.7% (Table 17). The model had the lowest accuracy of assigning the samples in the correct negative flavor attribute when compared to the other flavor attribute models (Figure 17). Positive and negative flavor attributes can be predicted by using REIMS data with high accuracy.

The results found in this study corroborates with the literature evaluating the efficacy of using REIMS technology. Gredell et al. (2019) evaluated the comparison of machine learning algorithms for predicting modeling of beef attributes using REIMS

data. They used REIMS to create models to predict dark cutter, top choice/prime, low choice/select, Wagyu, grass fed, tender and tough, Angus and non-Angus strip loin steaks. They also evaluated different machine learning algorithms to predict model sets varying 81.5 to 99% of final accuracy rate. Data collected from REIMS technology was able to predict beef flavor with high accuracy by grouping the samples by the lipid oxidation group, cooking method, positive and negative flavor attributes.

5. CONCLUSION

The SV, SVG, and grill cooking methods were responsible for creating a wide range of flavor attributes and volatile compounds. The grill treatment was responsible for developing more products from the Maillard reaction and these products were responsible for producing more positive flavor attributes from the trained panelists, produce more pyrazines, and more Strecker aldehydes. The grill treatment was also able to mask some off-flavors, and more tender samples. All the three cooking methods affected tenderness, which all the treatments had “very tender” and “tender” steaks.

The low, medium, and high levels of lipid oxidation were responsible for creating a wide range of flavor attributes and volatile compounds. The high level of oxidation was responsible for creating more negative flavor attributes, categorized as off-flavors, developing more aldehydes and alcohols due to the oxidation. The storage time affected negatively redness and luminosity of the steaks.

The REIMS technology was able to predict with high accuracy both level of lipid oxidation and cooking methods, positive and negative flavor attributes.

REFERENCES

Aaslyng, M. D., Bejerholm, C., Ertbjerg, P., Bertram, H. C., & Andersen, H. J. (2003). Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. *Food quality and preference*, *14*(4), 277-288.

Aberle, E. D., Forrest, J. C., Gerrard, D. E., Mille, E. W., Hedrick, H. B., Judge, M. D., & Merkel, R. A. (2001). *Principles of Meat Science*. Dubuque, IA, USA: Kendall/Hunt Co.

Addinsoft (2019). XLSTAT statistical and data analysis solution. Boston, USA.

Addis, P. B. (1986). Occurrence of lipid oxidation products in foods. *Food and Chemical Toxicology*, *24*(10-11), 1021–1030.

Adhikari, K., E. Chambers IV, Miller, R. K., Vazquez, L. A., Bhumiratana, N., & Philips, C. (2011). Development of a lexicon for beef flavor in intact muscle. *Journal of Sensory Studies*, *26*(6), 413-420.

Adhikari, K., E. Chambers IV, Miller, R. K., Vazquez, L. A., Bhumiratana, N., & Philips, C. (2011). Development of a lexicon for beef flavor in intact muscle. *Journal of Sensory Studies*, *26*(6), 413-420.

Alexander, J., Gildea, L., Balog, J., Speller, A., McKenzie, J., Muirhead, L., Scott, A., Kontovounisios, C., Rasheed, S., Teare, J., & Hoare, J. (2017). A novel methodology for in vivo endoscopic phenotyping of colorectal cancer based on real-time analysis of the mucosal lipidome: a prospective observational study of the iKnife. *Surgical Endoscopy*, *31*(3), 1361-1370.

AMSA (2012). Meat color measurement guidelines. American Meat Science

Association. Champaign, IL.

AMSA (2015). Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of meat. American Meat Science Association. Chicago, IL.

AMSA. (2012). Meat color measurement guidelines. Champaign, IL. American Meat Science Association.

Bailey, M. E., Dupuy, H. P., & Legendre, M. G. (1980). Undesirable meat flavor and its control. In G. Charalambous (Ed.), *Analysis and control of less desirable flavors in foods and beverages* (pp. 31−52). New York: Academic Press.

Baldwin, D. E. (2012). Sous-vide cooking: A review. *International Journal of Gastronomy and Food Science*, *1*(1), 15–30.

Balog, J., Kumar, S., Alexander, J., Golf, O., Huang, J., Wiggins, T., Abbassi-Ghadi, N., Enyedi, A., Kacska, S., Kinross, J., & Hanna, G. B. (2015). In vivo endoscopic tissue identification by rapid evaporative ionization mass spectrometry (REIMS). *Angewandte Chemie International Edition*, *54*(38), 11059-11062.

Balog, J., Perenyi, D., Guallar-Hoyas, C., Egri, A., Pringle, S. D., Stead, S., Chevallier, O. P., Elliott, C. T., & Takats, Z. (2016). Identification of the species of origin for meat products by rapid evaporative ionization mass spectrometry. *Journal of Agricultural and Food Chemistry*, *64*(23), 4793-4800.

Balog, J., Sasi-Szabó, L., Kinross, J., Lewis, M. R., Muirhead, L. J., Veselkov, K., Mirnezami, R., Dezső, B., Damjanovich, L., Darzi, A., & Nicholson, J. K. (2013). Intraoperative tissue identification using rapid evaporative ionization mass spectrometry. *Science Translational Medicine*, *5*(194), 194ra93.

Balog, J., Szaniszlo, T., Schaefer, K. C., Denes, J., Lopata, A., Godorhazy, L., Szalay, D., Balogh, L., Sasi-Szabo, L., Toth, M., & Takats, Z. (2010). Identification of biological tissues by rapid evaporative ionization mass spectrometry. *Analytical Chemistry*, *82*(17), 7343-7350.

Bamsey, M. L. (2017). Beef flavor myology. Master's Thesis, Texas A&M University, College Station.

Banerjee, R. & Verma, A. (2015). Minimally processed meat and fish products. In M. W. Siddiqui, & M. S. Rahman (Eds.). *Minimally Processed Foods* (pp. 193-250). Springer International Publishing.

Bañón, S., Nieto, G., & Díaz, P. (2007). Aplicaciones de la tecnología sous-vide en la elaboración de platos preparados cárnicos para catering. *Eurocarne*, *17*(155), 109-115.

Beavers, B. A. (2017). Relationship between descriptive flavor and texture attributes and consumer acceptance in ground beef patties. Master's Thesis, Texas A&M University, College Station.

Behrends, J. M., Goodson, K. J., Koohmaraie, M., Shackelford, S. D., Wheeler, T. L., Morgan, W. W., Reagan, J. O., Gwartney, B. L., Wise, J. W., & Savell, J. W. (2005a). Beef customer satisfaction: Factors affecting consumer evaluations of calcium chloride-injected top sirloin steaks when given instructions for preparation. *Journal of Animal Science*, *83*(12), 2869–2875.

Behrends, J. M., Goodson, K. J., Koohmaraie, M., Shackelford, S. D., Wheeler, T. L., Morgan, W. W., Reagan, J. O., Gwartney, B. L., Wise, J. W., & Savell, J. W.

(2005b). Beef customer satisfaction: USDA quality grade and marination effects on consumer evaluations of top round steaks. *Journal of Animal Science*, *83*(3), 662–670.

Berto, M. C. (2015). Consumer attitudes of predicted flavor aromas in steaks created with different steak thickness, quality grades, and cooking surface temperatures. Master's Thesis, Texas A&M University, College Station.

Black, C., Chevallier, O. P., Haughey, S. A., Balog, J., Stead, S., Pringle, S. D., Riina, M.V., Martucci, F., Acutis, P.L., Morris, M., & Nikolopoulos, D.S. (2017). A real time metabolomic profiling approach to detecting fish fraud using rapid evaporative ionisation mass spectrometry. *Metabolomics*, *13*(12), 153.

Bouton, P. E., & Harris, P. V. (1981). Changes in the tenderness of meat cooked at 50–65°C. *Journal of Food Science*, *46*(2), 475–478.

Braddock, J. C., Sims, C. A., & O'keefe, S. F. (1995). Flavor and oxidative stability of roasted high oleic acid peanuts. *Journal of Food Science*, *60*(3), 489-493.

Breslin, P. A. (2013). An evolutionary perspective on food and human taste. *Current Biology*, *23*(9), 409-418.

Burdock, G. A. (2010). *Fenaroli's handbook of flavor ingredients* (6th ed.). Boca Raton, FL, USA: CRC Press.

Buttery, R. G., Turnbaugh, J. G., & Ling, L. C. (1988). Contribution of volatiles to rice aroma. *Journal of Agricultural and Food Chemistry*, *36*(5), 1006-1009.

Calkins, C. R., & Hodgen, J. M. (2007). A fresh look at meat flavor. *Meat Science*, *77*(1), 63-80.

Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D., & Richardson, R. I. (2006). Flavour perception of oxidation in beef. *Meat Science, 72*(2), 303-311.

Carpenter, C. E., Cornforth, D. P. & Whittier, D. (2001). Consumer preferences for beef color and packaging did not affect eating satisfaction. *Meat Science, 57*(4), 359-363.

Caul, J. F. (1957). The profile method of flavor analysis. *Advances in Food Research*, 7, 1-40.

Chang, S. S., & Peterson, R. J. (1977). Recent developments in the flavor of meat. *Journal of Food Science, 42*, 298-305.

Cheng J. H. (2016). Lipid Oxidation in Meat. *Journal of Nutrition & Food Sciences*, *6*(3) 494.

Cheng, C. S., & Parrish Jr, F. C. (1976). Scanning electron microscopy of bovine muscle: Effect of heating on ultrastructure. *Journal of Food Science*, *41*(6), 1449-1454.

Church, I. J., & Parsons, A. L. (1993). Review: sous-vide cook-chill technology. *International Journal of Food Science and Technology*, *28*(6), 575-586.

Cover, S. (1943). Effect of extremely low rates of heat penetration on tendering of beef. *Food Research*, *8*, 388-394.

Creed, P. G. (1998). Sensory and nutritional aspects for sous-vide processed foods. In S. Ghazala, *Sous-vide and Cook-Chill Processing of the Food Industry* (pp. 57-88). Gaithersburg, MD, USA: Aspen Publishers, Inc

Creed, P. G., & Reeve, W. (1998). Principles and applications of sous-vide processed foods. In S. Ghazala, *Sous-vide and cool-chill processing of the food industry* (pp. 25-56). Gaithersburg, MD, USA: Aspen Publishers, Inc

Currie, R. W., & Wolfe, F. H. (1980). Rigor related changes in mechanical properties (tensile and adhesive) and extracellular space in beef muscle. *Meat Science, 4*(2), 123-143.

Dashdorj, D., Amna, T., & Hwang, I. (2015). Influence of specific taste-active components on meat flavor as affected by intrinsic and extrinsic factors: an overview. *European Food Research and Technology*, 241(2), 157-171.

Davey, C. L., & Gilbert, K. V. (1967). Structural changes in meat during ageing. *International Journal of Food Science & Technology, 2*(1), 57-59.

Davey, C. L., & Gilbert, K. V. (1974). Temperature-dependant toughness in beef. *Journal of Science Food and Agriculture, 25*(8), 93.

Davey, C. L., & Niederer, A. F. (1977). Cooking tenderizing in beef. *Meat science, 1*(4), 271-276.

Drumm, T. D., & Spainer, A. M. (1991). Changes in the content of lipid autoxidation and sulphur-containing compounds in cooked beef during storage. *Journal of Agricultural and Food Chemistry, 39*(2), 336–343.

Dupuy, H. P., Bailey, M. E., St. Angelo, A. J., Vercellotti, J. R., & Legendre, M. G. (1987). Instrumental analyses of volatiles related to warmed-over flavor of cooked meats. In A. J. St. Angelo, & M. E. Bailey (Eds.), *Warmed-over flavor of meat* (pp. 165−191). Orlando, FL: Academic Press.

Elmore, J. S., & Mottram, D. S. (2006). The role of lipid in the flavour of cooked beef. *Developments in Food Science, 43*, 375-378.

Evers, W. J., Heinsohn, H. H., Mayers, B. J. & Sanderson, A. (1976) Furans substituted in the three position with sulfur. In *Phenolic, Suljiir and Nitrogen Compounds in Food Flavors* (pp. 184-193), eds G. Charalambous and I. Katz. American Chemical Society, Washington DC.

Farmer, L. J. (1994). The role of nutrients in meat flavour formation. *Proceedings of the Nutrition Society, 53*(2), 327-333.

Farmer, L. J., & Mottram, D. S. (1990). Recent studies on the formation of meat-like aroma compounds. *Flavour Science and Technology*, 113-116.

Farmer, L. J., & Patterson, R. L. S. (1991). Compounds contributing to meat flavour. *Food Chemistry, 40*(2), 201–205.

Farmer, L. J., Patterson, R. L. S. (1991). Compounds contributing to meat flavour. *Food Chemistry, 40*(2), 201–205.

Faustman, C. & Cassens R. G. (1990). The biochemical basis for discoloration in fresh meat: a review. *Journal of Muscle Foods, 1*(3), 217-243.

Fors, S. (1983). Sensory properties of volatile Maillard reaction products and related compounds: A literature review. *The Maillard reaction in foods and nutrition*, 185-286.

García-Segovia, P., Andrés-Bello, A., & Martínez-Monzó, J. (2007). Effect of cooking method on mechanical properties, color and structure of beef muscle (M. pectoralis). *Journal of Food Engineering, 80*(3), 813-821.

Gasser, U., & Grosch, W. (1988). Identification of volatile flavour compounds with high aroma values from cooked beef. *Zeitschrift fur Lebensmittel Untersuchung und Forschung*, *186*, 489–494.

Glasscock, R. A. (2014). Beef Flavor Attributes and Consumer Perception. Master's Thesis, Texas A&M University, College Station.

Golf, O., Strittmatter, N., Karancsi, T., Pringle, S. D., Speller, A. V., Mroz, A., ... & Takats, Z. (2015). Rapid evaporative ionization mass spectrometry imaging platform for direct mapping from bulk tissue and bacterial growth media. *Analytical Chemistry*, *87*(5), 2527-2534.

Golovnja, R. V., & Rothe, M. (1980). Sulfur containing compounds in the volatile constituents of boiled meat. *Food/Nahrung*, *24*(2), 141-154.

Goodson, K. J., Morgan, W. W., Reagan, J. O., Gwartney, B. L., Courington, S. M., Wise, J. W., & Savell, J. W. (2002). Beef customer satisfaction: factors affecting consumer evaluations of clod steaks. *Journal of Animal Science*, *80*(2), 401–408.

Gray, J. L., & Pearson, A. M. (1994). Lipid-derived off-flavours in meat formation and inhibition. In *Flavour of meat and meat products* (116–143). Springer, Boston, MA.

Gredell, D. A., Schroeder, A. R., Belk, K. E., Broeckling, C. D., Heuberger, A. L., Kim, S. Y., King, D. A., Shackelford, S. D., Sharp, J. L., Wheeler, T. L., Woerner, D. R., & Prenni, J. E. (2019). Comparison of Machine Learning Algorithms for Predictive Modeling of Beef Attributes Using Rapid Evaporative Ionization Mass Spectrometry (REIMS) Data. *Scientific reports*, *9*(1), 5721.

Greene, B. E., & Cumuze, T. H. (1982). Relationship between TBA numbers and inexperienced panelists' assessments of oxidized flavor in cooked beef. *Journal of Food Science*, *47*(1), 52-54.

Greene, B. E., Hsin, I. M. & Zipser, M. Y. W. (1971). Retardation of oxidative color changes in raw ground beef. *Journal of Food Science, 36*(6), 940-942.

Greer, G. G., & Jones, S. D. M. (1991). Effects of lactic acid and vacuum packaging on beef processed in a research abattoir. *Canadian Institute of Food Science and Technology Journal, 24*, 181–185.

Grigioni, G. M., Margaría, C. A., Pensel, N. A., Sánchez, G., & Vaudagna, S. R. (2000). Warmed-over flavour analysis in low temperature–long time processed meat by an "electronic nose". *Meat Science*, *56*(3), 221-228.

Grosch, W. (2001). Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. *Chemical Senses*, *26*(5), 533-545.

Guentert, M., Bruening, J., Emberger, R., Koepsel, M., Kuhn, W., Thielmann, T., & Werkhoff, P. (1990). Identification and formation of some selected sulfur-containing flavor compounds in various meat model systems. *Journal of Agricultural and Food Chemistry*, *38*(11), 2027-2041.

Guitton, Y., Dervilly-Pinel, G., Jandova, R., Stead, S., Takats, Z., & Le Bizec, B. (2018). Rapid evaporative ionisation mass spectrometry and chemometrics for high-throughput screening of growth promoters in meat producing animals. *Food Additives & Contaminants: Part A*, *35*(5), 900-910.

Harris, P. V. (1976). Structural and other aspects of meat tenderness. *Journal of texture studies*, *7*(1), 49-63.

Hidalgo, F. J., & Zamora, R. (2000). The role of lipids in nonenzymatic browning. *Grasas Aceites*, *51*, 35–49.

Hodge, J. E. (1953). Dehydrated foods, chemistry of browning reactions in model systems. *Journal of agricultural and food chemistry*, *1*(15), 928-943.

Hodgen, J. M., Cuppett, S. L., & Calkins, C. R. (2006). Identification of off-flavor compounds in beef. Proceedings of the American Meat Science Association Reciprocal Meat Conference, Champagne-Urbana, IL.

Holdsworth, S. D., & Simpson, R. (2007). *Thermal processing of packaged foods*. New York: Springer.

Hood, D. E., & Riordan, E. B. (1973). Discolouration in pre-packaged beef: Measurement by reflectance spectrophotometry and shopper discrimination. *International Journal of Food Science & Technology*, *8*(3), 333–343.

Hornstein, I., & Crowe, P. F. (1960). Meat flavor chemistry, flavor studies on beef and pork. *Journal of Agricultural and Food Chemistry*, *8*(6), 494-498.

Hornstein, I., Crowe, P. F., & Heimberg, M. J. (1961). Fatty acid composition of meat tissue lipids. *Journal of Food Science*, *26*(6), 581-586.

Hrdina-Dubsky, D. L. (1989). Sous-vide finds its niche. *Food Engineering International*, *14*(7), 40-42, 44, 46, 48.

Jacobsen, C., Hartvigsen, K., Lund, P., Adler-Nissen, J., Hølmer, G., & Meyer, A. S. (2000). Oxidation in fish-oil-enriched mayonnaise. *European Food Research and Technology*, *210*(4), 242-257.

Jeremiah, L. E. (1982). A review of factors influencing consumption, selection, and acceptability of meat purchases. *Journal of Consumer Studies and Home Economics*, *6*, 137–154.

Jones, S. B., Carroll, R. J., & Cavanaugh, J. R. (1977). Structural changes in heated bovine muscle. A scanning electron microscope study. *Journal of Food Science, 42*, 125.

Jousse, F., Jongen, T., Agterof, W., Russell, S., & Braat, P. (2002). Simplified kinetic scheme of flavor formation by the Maillard reaction. *Journal of Food Science*, *67*(7), 2534-2542.

Keller, T., Benno, J., Lee, C., & Rouxel, S. (2008). Under pressure: Cooking Sous-vide. New York, NY: Artisan.

Kerscher, R., & Grosch, W. (1998). Quantification of 2-methyl-3-furanthiol, 2-furfurylthiol, 3-mercapto-2-pentanone, and 2-mercapto-3-pentanone in heated meat. *Journal of Agriculture and Food Chemistry, 46*(5), 1954–1958.

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.

Kiely, P. J., Nowlin, A. C., & Moriarty, J. H. (1960). Bread aromatics from browning systems. *Cereal Science Today*, *5*, 273-274.

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.

Koohmaraie, M. (1994). Muscle proteinases and meat aging. *Meat Science, 36*(1-2), 93–104.

Kosek, V., Uttl, L., Jírů, M., Black, C., Chevallier, O., Tomaniová, M., Elliott, C.T., & Hajšlová, J. (2019). Ambient mass spectrometry based on REIMS for the rapid detection of adulteration of minced meats by the use of a range of additives. *Food Control, 104,* 50-56.

Koutsidis, G., Elmore, J. S., Oruna-Concha, M. J., Campo, M. M., Wood, J. D., & Mottram, D. S. (2008). Water-soluble precursors of beef flavour: I. Effect of diet and breed. *Meat Science, 79*(1), 124-130.

Kramlich, W. E., & Pearson, A. M. (1960). Separation and identification of cooked beef flavor components. *Journal of Food Science, 25*(6), 712-719.

Kropf, D. H. (1980). Effects of retail display conditions on meat color. In Proceedings 33rd reciprocal meat conference (pp. 15–32), 22–25 June 1980, West Lafayette, Indiana, USA.

Laakkonen, E. (1973). Factors affecting tenderness during heating of meat. In *Advances in food Research* (pp. 257-323). New York: Academic Press.

Laakkonen, E., Wellington, G. H., & Sherbon, J. N. (1970). Low-temperature, long-time heating of bovine muscle 1. Changes in tenderness, water-binding capacity, pH and amount of water-soluble components. *Journal of Food Science*, *35*(2), 175-177.

Laird, H. L. (2015). Millennial's Perception of Beef Flavor. Master's Thesis, Texas A&M University, College Station.

Lassen, A., Kall, M., Hansen, K., & Ovesen, L. (2002). A comparison of the retention of vitamins B1, B2 and B6, and cooking yield in pork loin with conventional and enhanced meal-service systems. *European Food Research and Technology*, *215*(3), 194-199.

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.

Legako, J. F., Dinh, T. T. N., Miller, M. F., & Brooks, J. C. (2015). Effects of USDA beef quality grade and cooking on fatty acid composition of neutral and polar lipid fractions. *Meat science, 100,* 246-255.

Livingston, D. J., & Brown, W. D (1982). The chemistry of myoglobin and its reactions. *Food Technology, 35*(5), 244–252.

Love, J. (1988). Sensory analysis of warmed-over flavor in meat. *Food Technology, 42*(6), 140-143.

Luckemeyer, T. J. (2015). Beef flavor attributes and consumer perception of light beef eaters. Master's Thesis, Texas A&M University, College Station.

Machlik, S. M., & Draudt, H. N. (1963). The Effect of Heating Time and Temperature on the Shear of Beef Semitendinosus Muscle. *Journal of Food Science*, *28*(6), 711-718.

MacLeod, G. (1994). The flavor of beef. In F. Shahidi (Ed.), *Flavor of meat and meat products* (pp. 4–37). Glasgow, UK: Blackie Academic and Professional.

MacLeod, G., & Ames, J. (1986). 2-Methyl-3-(methylthio) furan: a meaty character impact aroma compound identified from cooked beef. *Chemistry and Industry, 7*, 175-176.

Macy, R. L., Naumann, H. D., & Bailey, M. E. (1964). Water-soluble flavor and odor precursors of meat. I. Qualitative study of certain amino acids, carbohydrates, nonamino acid nitrogen compounds, and phosphoric acid esters of beef, pork, and lamb. *Journal Food Science, 29*(2), 136-141.

Madruga, M. S., & Mottram, D. S. (1995). The effect of pH on the formation of Maillard-derived aroma volatiles using a cooked meat system. *Journal of the Science of Food and Agriculture*, *68*(3), 305–310.

Maga, J. A. (1992). Pyrazine update. *Food Reviews International*, *8*(4), 479-558.

Maillard, L. C. (1912). Action of amino acids on sugars. Formation of melanoidins in a methodical way. *Compte-Rendu de l'Academie des Sciences, 154*, 66-68.

Mancini, R. A. & Hunt, M. (2005). Current research in meat color. *Meat science*, *71*(1), 100-121.

Manley, C. H., & Choudhury, B. H. (1999). Thermal process flavorings. In P. R. Ashurst (Ed.), *Food flavorings* (3rd ed.) (pp. 283–326). Boston: Kluwer Academic Publisher.

Mann, T. F., Reagan, J. O., Lillard, D. A., Campion, D. R., Lyon, C. E., & Miller, M. F. (1989). Effects of Phosphate in Combination with Nitrite or Maillard Reaction Products upon Warmed-Over Flavor in Precooked, Restructured Beef Chuck Roasts. *Journal of Food Science, 54*(6), 1431-1433.

Martens, H., Stabursvik, E., & Martens, M. (1982). Texture and colour changes in meat during cooking related to thermal denaturation of muscle proteins. *Journal of Texture Studies, 13*(3), 291–309

Mattes, R. D. (2009). Is there a fatty acid taste? *Annual Review of Nutrition, 29,* 305-327.

Maul, F. (1998). Application of an "electronic nose" technology for quality assessment of food. In *Developing premium-quality programs for fresh fruits and vegetables*. Florida Postharvest Horticulture Institute and Industry Tour. Horticultural Sciences Department. Cooperative Extension Service. University of the Florida and The Florida Fruit and Vegetable Association

Meilgaard, M., Civille, G. V., & Carr, B. T. (2015). Sensory evaluation techniques. CRC Press, Boca Raton, FL.

Melton, S. L. (1983). Methodology for following lipid oxidation in muscle foods. *Food Technology, 37*(7), 105-111, 116.

Mielche, M. M. (1995). Development of warmed-over flavour in ground turkey, chicken and pork meat during chill storage. A model of the effects of heating temperature and storage time. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, *200*(3), 186-189.

Mielche, M. M., & Bertelsen, G. (1993). Effects of heat treatment on warmed-over flavour in ground beef during aerobic chill-storage. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, *197*, 8–13.

Miller, R. K., & Kerth, C. R. (2012). Identification of compounds responsible for positive beef flavor. Final report to the National Cattlemen's Beef Association, Centennial, CO.

Min, D. B., & Ahn, D. U. (2005). Mechanism of lipid peroxidation in meat and meat products — A review. *Food Science Biotechnology*, *14*(1), 152–163.

Min, D. B., Ina, K., Peterson, R. J., & Chang, S. S. (1979). Preliminary identification of volatile flavor compounds in the neutral fraction of roast beef. *Journal of Food Science*, *44*(3), 639-642.

Mitra, B., Lametsch, R., Greco, I., & Ruiz-Carrascal, J. (2018). Advanced glycation end products, protein crosslinks and post translational modifications in pork subjected to different heat treatments. *Meat Science*, *145*, 415-424.

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.

Mortensen, L. M., Frøst, M. B., Skibsted, L. H., & Risbo, J. (2012). Effect of time and temperature on sensory properties in low-temperature long-time sous-vide cooking of beef. *Journal of Culinary Science & Technology*, *10*, 75–90.

Mossel, D. A., & Struijk, C. B. (1991). Public health implication of refrigerated pasteurized ('sous-vide') foods. *International Journal of Food Microbiology, 13*(3), 187–206.

Mottram, D. S. (1991). Meat. In H. Maarse, *Volatile compounds in foods and beverages* (pp. 107-177). New York: Marcel Dekker.

Mottram, D. S. (1994). Meat flavour: a review. In J. R. Piggott, & A. Patterson, *Understanding natural flavours* (pp. 140-163). Glasgow: Blackie.

Mottram, D. S. (1998). Flavour formation in meat and meat products: a review. *Food Chemistry, 62*(4), 415–424.

Mottram, D. S., & Edwards, R. A. (1983). The role of triglycerides and phospholipids in the aroma of cooked beef. *Journal of the Science of Food and Agriculture, 34*(5), 517-522.

Mottram, D. S., Edwards, R. A., & Macfie, J. H. (1982). A comparison of the flavour volatiles from cooked beef and pork meat systems. *Journal of the Science of Food and Agriculture*, *33*(9), 934-944.

Mulders, E. J. (1973). Volatile components from the non-enzymic browning reaction of the cysteine/cystine-ribose system. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, *152*(4), 193-201.

Myhrvold, N., Young, C., & Bilet, M. (2011). *Modernist cuisine: The art and science of cooking*. Bellevue, WA: The Cooking Lab.

Nawar, W. W. (1969). Thermal degradation of lipids. *Journal of Agricultural and Food Chemistry*, *17*(1), 18-21.

Neidert, M. C., & Bozinov, O. (2013). Mass spectrometry-based intraoperative tissue identification in neurosurgery. *World neurosurgery*, *80*(6), 683.

Offer, G., Knight, P., Jeacocke, R., Almond, R., Cousins, T., Elsey, J., Parsons, N., Sharp, A., Starr, R., & Purslow, P. (1989). The structural basis of the water-holding, appearance and toughness of meat and meat products. *Food Structure*, *8*(1), 17.

Oroszvári, B. K., Rocha, C. S., Sjöholm, I., & Tornberg, E. (2006). Permeability and mass transfer as a function of the cooking temperature during the frying of beef burgers. *Journal of Food Engineering*, *74*(1), 1-12.

Palamand, S. R., & Dieckmann, R. H. (1974). Autoxidation of n-hexanal. Identification and flavor properties of some products of autoxidation. *Journal of Agricultural and Food Chemistry*, *22*(3), 503-506.

Palka, K., & Daun, H. (1999). Changes in texture, cooking losses, and myofibrillar structure of bovine M. semitendinosus during heating. *Meat Science*, *51*(3), 237-243.

Parliament, T. H. (1989). Thermal generation of aromas. In Thermal generation of aromas. *American Chemical Society*, *409*, 2-11.

Pearce, T., & Gardner, J. (1998). Predicting organoleptic scores of sub-ppm flavour notes. Part 1. Theoretical and experimental details. Part 2. Computational analysis and results. *Analyst, 123*, 2047-2066.

Pegg, R. B., & Shahidi, F. (2004). Heat effects on meat. Flavour development (1st ed.). Encyclopedia of Meat Sciences. Oxford: Academic Press.

Peña, C. A. (2019). Beef flavor audit. Master's Thesis, Texas A&M University, College Station.

Resconi, V., Escudero, A., & Campo, M. (2013). The development of aromas in ruminant meat. *Molecules, 18*(6), 6748-6781.

Rhee, K. S. (1978). Minimization of further lipid peroxidation in the distillation 2-thiobarbituric acid test of fish and meat. *Journal of Food Science, 43*(6), 1776-1778.

Rhee, K. S. (1989). Chemistry of meat flavor. In D. B. Min, & Smouse, T. (Eds.), *Flavor chemistry of lipid foods* (pp. 166–189). Champaign, Illinois: American Oil Chemists' Society.

Rigano, F., Stead, S., Mangraviti, D., Jandova, R., Petit, D., Marino, N., & Mondello, L. (2019). Use of an "Intelligent Knife"(iknife), Based on the Rapid Evaporative Ionization Mass Spectrometry Technology, for Authenticity Assessment of Pistachio Samples. *Food Analytical Methods*, *12*(2), 558-568.

Robbins, K., Jensen, J., Ryan, K. J., Homco-Ryan, C., McKeith, F. K., & Brewer, M.S. (2003). Dietary vitamin E supplementation effects on the color and sensory characteristics of enhanced beef steaks. *Meat Science*, *64*(3), 279-285.

Rochat, S., & Chaintreau, A. (2005). Carbonyl odorants contributing to the in-oven roast beef top note. *Journal of Agricultural and Food Chemistry*, *53*(24), 9578-9585.

Roldán, M., Antequera, T., Martín, A., Mayoral, A. I., & Ruiz, J. (2013). Effect of different temperature–time combinations on physicochemical, microbiological, textural and structural features of sous-vide cooked lamb loins. *Meat Science*, *93*(3), 572-578.

Roldán, M., Loebner, J., Degen, J., Henle, T., Antequera, T., & Ruiz-Carrascal, J. (2015). Advanced glycation end products, physico-chemical and sensory

characteristics of cooked lamb loins affected by cooking method and addition of flavour precursors. *Food Chemistry, 168*, 487–495

Roldán, M., Ruiz, J., Sánchez del Pulgar, J., Pérez-Palacios, T., & Antequera, T. (2015). Volatile compound profile of sous-vide cooked lamb loins at different temperature–time combinations. *Meat Science, 100*, 52-57.

Ruiz, J., Calvarro, J., Sánchez del Pulgar, J., & Roldán, M. (2013). Science and technology for new culinary techniques. *Journal of Culinary Science & Technology, 11*(1), 66-79.

Ruiz-Carrascal, J., Roldán, M., Refolio, F., Perez-Palacios, T., & Antequera, T. (2019). Sous-vide cooking of meat: a Maillarized approach. *International Journal of Gastronomy and Food Science*, 100138.

Sánchez del Pulgar, J., Gázquez, A., & Ruiz-Carrascal, J. (2012). Physico-chemical, textural and structural characteristics of sous-vide cooked pork cheeks as affected by vacuum, cooking temperature, and cooking time. *Meat Science, 90*, 828-835.

Savell, J. W., Cross, H. R., Francis, J. J., Wise, J. W., Hale, D. S., Wilkes, D. L., & Smith, G. C. (1989). National consumer retail beef study: Interaction of trim level, price and grade on consumer acceptance of beef steaks and roasts. *Journal of Food Quality, 12*(4), 251-274.

Schäfer, K. C., Dénes, J., Albrecht, K., Szaniszló, T., Balog, J., Skoumal, R., Katona, M., Tóth, M., Balogh, L., & Takáts, Z. (2009). In vivo, in situ tissue analysis using rapid evaporative ionization mass spectrometry. *Angewandte Chemie International Edition, 48*(44), 8240-8242.

Schellekens, M. (1996). New research issues in sous-vide cooking. *Trends in Food Science and Technology*, 7(8), 256–262.

Shahidi, F. (1989). Flavor of cooked meat. In R. Teranishi, R. G. Buttery, & F. Shahidi (Eds.), *Flavor chemistry trends and developments* (pp. 188–201). Washington, DC: American Chemical Society.

Shahidi, F. (1992). Prevention of lipid oxidation in muscle foods by nitrite and nitrite-free compositions. In A. J. St. Angelo (Ed.), *Lipid oxidation in food* (pp. 161–182). Washington DC, USA: ACS Symposium Series 500.

Shahidi, F. (1998). Flavor of muscle foods: An overview. In F. Shahidi (Ed.), *Flavor of meat, meat products, and seafoods* (pp. 1–4). London: Blackie Academic and Professional.

Shahidi, F., & Pegg, R. B. (1994). Hexanal as an indicator of meat flavor deterioration. *Journal of Food Lipids*, *1*(3), 177-186.

Shahidi, F., Rubin, L. J., D'Souza, L. A., Teranishi, R., & Buttery, R. G. (1986). Meat flavor volatiles: a review of the composition, techniques of analysis, and sensory evaluation. *Critical Reviews in Food Science & Nutrition*, *24*(2), 141-243.

Silva, T. J. P., Orcutt, M. W., Forrest, J. C., Bracker, C. E., & Judge, M. D. (1993). Effect of heating rate on shortening, ultrastructure and fracture behaviour of prerigor beef muscle. *Meat Science 33*(1), 1-24.

Sitz, B. M., Calkins, C. R., Feuz, D. M., Umberger, W. J., & Eskridge, K. M. (2005). Consumer sensory acceptance and value of domestic, Canadian, and Australian grass-fed beef steaks. *Journal of Animal Science*, *83*(12), 2863-2868.

Skibsted, L. H., Mikkelsen, A., & Bertelsen, G. (1998). Lipid-derived of flavours in meat. In F. Shahidi, *Flavour of meat, meat products and seafoods* (2nd ed., pp. 217-248). London: Blackie Academic & Professional.

Spanier, A. M., Edwards, J. V., & Dupuy, H. P. (1988). The warmed-over flavor process in beef: a study of meat proteins and peptides. *Food Technology, 42*(6), 110–118.

Spanier, A. M., Vercellotti, J. R., & James, J. R. (1992). Correlation of sensory, instrumental and chemical attributes of beef as influenced by meat structure and oxygen exclusion. *Journal Food Science, 57,* 10–15.

St. Angelo, A. J., Crippen, K. L., Dupuy, H. P., & James Jr, C. (1990). Chemical and sensory studies of antioxidant-treated beef. *Journal of Food Science*, *55*(6), 1501-1505.

St. Angelo, A. J., Vercellotti, J. R., Legendre, M. G., Vinnet, C. H., Kuan, J. W., James, C. Jr., & Dupuy, H. P. (1987). Chemical and instrumental analyses of warmed-over flavor in beef. *Journal of Food Science, 52*(3), 1163–1168.

St. Angelo, A. J., Vercellotti, J., Jacks, T., & Legendre, M. (1996). Lipid oxidation in foods. *Critical Reviews in Food Science & Nutrition*, *36*(3), 175-224.

St. John, E. R., Balog, J., McKenzie, J. S., Rossi, M., Covington, A., Muirhead, L., Bodai, Z., Rosini, F., Speller, A.V., Shousha, S., & Ramakrishnan, R. (2017). Rapid evaporative ionisation mass spectrometry of electrosurgical vapours for the identification of breast pathology: towards an intelligent knife for breast cancer surgery. *Breast Cancer Research*, *19*(1), 59.

Stapelfeldt, H., Bjørn, H., Skibsted, L. H., & Bertelsen, G. (1993). Effect of packaging and storage conditions on development of warmed-over flavour in sliced, cooked meat. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, *196*(2), 131-136.

Strittmatter, N., Rebec, M., Jones, E. A., Golf, O., Abdolrasouli, A., Balog, J., Behrends, V., Veselkov, K. A., & Takats, Z. (2014). Characterization and identification of clinically relevant microorganisms using rapid evaporative ionization mass spectrometry. *Analytical chemistry*, *86*(13), 6555-6562.

Sujiwo, J., Kim, H. J., Song, S. O., & Jang, A. (2019). Relationship between quality and freshness traits and torrymeter value of beef loin during cold storage. *Meat science*, *149*, 120-125.

Suman, S. P., Hunt, M. C., Nair, M. N. & Rentfrow, G. (2014). Improving beef color stability: Practical strategies and underlying mechanisms. *Meat Science, 98*(3), 490-504.

SVAC (Sous-vide Advisory Committee) (1991). Code of Practice for Sous-vide Catering Systems. Tetbury, Gloucestershire: SVAC.

Tarladgis, B. G., Watts, B. M., Younathan, M. T., & Dugan, L. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of the American Oil Chemists Society*, *37*(1), 44-48.

Tims, M. J., & Watts, B. M. (1958). Protection of cooked meats with phosphates. *Food Technology, 12*(5), 240.

Tornberg, E. (2005). Effects of heat on meat proteins — Implications on structure and quality of meat products. *Meat Science, 70*(3), 493–508.

Ullrich, F., & Grosch, W. (1987). Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, *184*(4), 277-282.

Unger, H. (1985). Microwaveable packs and boil in the bag pouches. *Food Processing*, *54*(9), 31-33.

Uttaro, B., Zawadski, S., & McLeod, B. (2019). Efficacy of multi-stage sous-vide cooking on tenderness of low value beef muscles. *Meat science*, *149*, 40-46.

Van Ba, H., Hwang, I., Jeong, D., & Touseef, A. (2012). Principle of meat aroma flavors and future prospect. In Isin Akyar (Ed.), *Latest research into quality control* (pp. 145–176). Rijeka Croatia: InTech.

Van Boekel, M. A. J. S. (2006). Formation of flavour compounds in the Maillard reaction. *Biotechnology advances*, *24*(2), 230-233.

Van den Ouweland, G. A., & Peer, H. G. (1975). Components contributing to beef flavor. Volatile compounds produced by the reaction of 4-hydroxy-5-methyl-3 (2H)-furanone and its thio analog with hydrogen sulfide. *Journal of Agricultural and Food Chemistry*, *23*(3), 501-505.

Vaudagna, S. R., Sánchez, G., Neira, M. S., Insani, E. M., Picallo, A. B., Gallinger, M. M., & Lasta, J. A. (2002). Sous-vide cooked beef muscles: effects of low temperature–long time (LT–LT) treatments on their quality characteristics and storage stability. *International Journal of Food science & Technology*, 37(4), 425-441.

Verplanken, K., Stead, S., Jandova, R., Van Poucke, C., Claereboudt, J., Bussche, J. V., De Saeger, S., Takats, Z., Wauters, J., & Vanhaecke, L. (2017). Rapid evaporative ionization mass spectrometry for high-throughput screening in food analysis: The case of boar taint. *Talanta, 169,* 30-36.

Wallace, W. J., Houtchens, R. A., Maxwell, J. C., & Caughey, S. (1982). Mechanism of autoxidation for hemoglobins and myoglobins. *Journal of Biological Chemistry, 257*(9), 4966–4977.

Wasserman, A. E., & Gray, N. (1965). Meat flavor. I. Fractionation of water-soluble flavor precursors of beef. *Journal of Food Science, 30*(5), 801-807.

Watanabe, K., & Sato, Y. (1971). Alkyl-substituted pyrazines and pyridines in the flavor components of shallow fried beef. *Journal of Agricultural and Food Chemistry, 19*(5), 1017-1019.

Wheeler, T. L., Shackelford, S. D., & Koohmaraie, M. (1997). Standardizing collection and interpretation of Warner–Bratzler shear force and sensory tenderness data. In *Proceedings 50th annual Reciprocal Meat Conference* (pp. 68–77), Ames, IA.

Zamora, R., & Hidalgo, F. J. (2005). Coordinate contribution of lipid oxidation and Maillard reaction to the nonenzymatic food browning. *Food Science and Nutrition, 45*(1), 49-59.

Zamora, R., & Hidalgo, F. J. (2011). The Maillard reaction and lipid oxidation. *Lipid Technology, 23*(3), 59-62.

APPENDIX A

FIGURES AND TABLES

Table 1. Definition and reference standards for meat descriptive flavor aromatics and basic taste sensory attributes and their intensities where 0 = none; 15 = extremely intense adapted from Adhikari et al. (2011).

Attributes	Definition	Reference
Animal hair	The aromatics perceived when raw wool is saturate with water.	Caproic acid = 12.0
Barnyard	Combination of pungent, slightly sour, hay-like aromatics, associated with farm animals and the inside of a horn.	White pepper in water = 4.0 (F); 4.5 (A) Tinture of civet = 6.0 (A)
Beef identity	Amount of beef flavor identity in the sample.	Swanson's beef broth = 5.0 80% lean ground beef = 7.0 Beef brisket = 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 = 5.5 Beef brisket = 6.0
Brown	A round, full aromatic generally associated with beef suet that has been broiled.	Beef suet = 8.0 80% lean ground beef = 10.0
Buttery	Sweet, dairy-like aromatic associated with natural butter	Land O'Lakes unsalted butter = 7.0
Burnt	The sharp/acrid flavor note associate with over-roasted beef muscle, something over-baked or excessively browned in oil.	Arrowhead Mills Puffed Barley Cereal = 3.0
Cardboardy	Aromatic associated with slightly oxidized fats and oils, reminiscent of wet cardboard packaging	Dry cardboard = 5.0 Wet cardboard = 7.0
Chemical	The aromatics associated with garden hose, hot Teflon pan, plastic packaging and petroleum-based product such as charcoal	Zip-Loc sandwich bag =13.0 Clorox in water = 6.5
Cooked milk	A combination of sweet, brown flavor notes and aromatics	Babybel original Swiss cheese = 2.5

Table 1. Continued.

Attributes	Definition	Reference
Dairy	The aromatics associated with products made from cow's milk, Such as cream, milk, sour cream or buttermilk.	Dillon's reduced fat milk (2%) = 8.0
Fat-like	The aromatics associated with cooked animal fat.	Hillshire farms Lit'l beef smokies = 7.0 Beef suet = 12.0
Fishy	Characteristic of fresh fish.	Ground beef with tuna = 4 (F), 6 (A) Patty made with 100g ground beef and 20g of tuna.
Green	Sharp, slightly pungent aromatics associated with green/plant/ vegetable matters such as parsley, spinach, pea pod, fresh cut grass, etc.	Hexanal in propylene glycol (5,000 ppm) = 6.5 (A) Fresh parsley water = 9.0
Green-hay like	Brown/green dusty aromatics associated with dry grasses, hay, dry parsley and tea leaves.	Dry parsley in medium snifter = 5.0 (A) Dry parsley in ~30-mL cup = 6.0
Heated Oil	The aromatics associated with oil heated to a high temperature.	Wesson Oil, microwaved 3 min = 7.0 Lay's Potato Chips = 4.0 (A)
Leather	Musty, old leather (like old book bindings).	2,3,4-Trimethoxybenzaldehyde= 3.0(A)
Liver-like	The aromatics associated with cooked organ meat/liver.	Beef liver = 7.5 Oscar Mayer Braunschweiger liver sausage = 10.0
Metallic	The impression of slightly oxidized metal, such as iron, copper and silver spoons.	0.10% potassium chloride Solution = 1.5 USDA choice strip steak = 4.0 Dole canned pineapple juice = 6.0
Musty-Earthy/ Humus	Musty, sweet, decaying vegetation.	Sliced button mushrooms = 3.0 (F & A) 1000ppm of 2,6- Dimethycyclohexanol in propylene glycol = 9.0 (A)

Table 1. Continued.

Attributes	Definition	Reference
Overall sweet	A combination of sweet taste and sweet aromatics. The aromatics associated with the impression of sweet.	Post-shredded wheat spoon size=1.5 (F) Hillshire farms Lit'l beef smokies = 3.0
Rancid	The aromatics commonly associated with oxidized fat and oils. These aromatics may include cardboard, painty, varnish and fishy	Microwaved Wesson vegetable oil (3 min) = 7.0 Microwaved Wesson vegetable oil (5 min) = 9.0
Refrigerator stale	Aromatics associated with products left in refrigerator for an extended period of time and absorbing a combination of odors (lack of freshness/flat)	80% lean ground beef, stored overnight and served at room temperature = 4.5 (F); 5.5 (A)
Roasted	A round, full aromatic generally associated with beef suet that has been broiled/roasted.	80 % Lean Ground Chuck = 10.0 (F)
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
Smoky Charcoal	An aromatic associated with meat juices and fat drippings on hot coats which can be acrid, sour, burned, etc.	Wright's Natural Hickory seasonings in water = 9.0 (A)
Smoky wood	Dry, dusty aromatic reminiscent of burning wood	Wright's Natural Hickory seasoning in water = 7.5 (A)
Soapy	An aromatic commonly found in unscented hand soap	Ivory bar soap in water = 6.5 (A)
Sour aromatics	The aromatics associated with sour substances.	Dillon's buttermilk = 5.0
Sour milk/ Sour dairy	Sour, fermented aromatics associated with dairy products such as buttermilk and sour cream.	Laughing cow light Swiss cheese= 7.0 Dillon's buttermilk = 9.0

Table 2. Definition and reference standards for meat descriptive texture attributes and their intensities where 0 = none, 15 = extremely intense adapted from Meilgaard, Civille, & Carr, 2015.

Attributes	Definition	Reference
Denseness	Compactness of the cross section of the sample after biting completely through with the molars.	Nougat = 4.0 Malted milk balls = 6.0 Fruit jellies = 15.0
Muscle Fiber Tenderness	The ease in which the muscle fiber fragments during mastication.	Eye of round = 9.0 Tenderloin = 14.0
Connective Tissue	The structural component of the muscle surrounding the tissue amount during mastication.	Brisket steak = 7.0 Tenderloin = 14.0
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

Table 3. Flavor and basic tastes descriptive attributes least squares mean for strip loin steaks segmented by lipid oxidation group and cooking method where 0 = none and 15 = extremely intense.

	Beef			Bloody/	Basic taste				
Treatments	identity	Brown	Roasted	Serumy	Bitter	Salty	Sweet	Sour	Umami
Lipid oxidation group[1]	0.0001	0.32	0.04	0.21	0.26	0.27	0.0004	0.005	0.006
Low	9.3[b]	8.3	8.4[b]	0.7	2.5	2.0	1.5[b]	2.3[a]	4.3[b]
Medium	8.5[a]	8.0	7.9[a]	0.8	2.5	2.0	1.2[a]	2.3[a]	4.1[ab]
High	8.6[a]	8.0	7.6[a]	1.0	2.7	1.9	1.1[a]	2.6[b]	3.8[a]
Cooking method[1]	<0.0001	<0.0001	<0.0001	<0.0001	0.07	0.001	<0.0001	0.003	<0.0001
Sous-vide	7.9[a]	6.0[a]	8.1[b]	0.6[a]	2.7	1.9[a]	0.9[a]	2.6[b]	3.2[a]
Sous-vide + grill	8.5[b]	8.0[b]	8.6[c]	0.4[a]	2.5	2.0[b]	1.3[b]	2.3[a]	4.0[b]
Grill	10.1[c]	10.4[c]	7.2[a]	1.5[b]	2.6	2.0[b]	1.7[c]	2.3[a]	5.0[c]
RMSE[2]	0.69	0.96	1.11	0.57	0.35	0.17	0.38	0.33	0.57

[1]P-value from Analysis of Variance table.

[abc] Mean values within a column and interaction followed by the same letter are not significantly different ($P < 0.05$).

[2]RMSE = Root Mean Square Error.

Table 3. Continued.

Treatments	Fat like	Metallic	Overall Sweet	Sour aromatics	Musty/Earthy Humus	Cardboardy	Burnt	Buttery	Chemical
Lipid oxidation group[1]	0.41	0.63	0.13	0.05	0.008	0.001	0.12	0.0003	0.15
Low	2.4	2.4	0.5	0.7	1.9[a]	1.6[ba]	0.1	0.4[b]	0.0
Medium	2.4	2.3	0.5	0.7	1.8[a]	1.9[ab]	0.2	0.2[a]	0.1
High	2.5	2.4	0.4	0.9	2.2[b]	2.3[b]	0.1	0.1[a]	0.2
Cooking method[1]	0.05	0.009	<0.0001	0.01	0.67	<0.0001	<0.0001	0.0006	0.21
Sous-vide	2.3	2.3[a]	0.2[a]	0.9[b]	2.0	2.6[c]	0.0[a]	0.1[a]	0.1
Sous-vide + grill	2.4	2.2[a]	0.4[b]	0.8[ab]	1.9	2.1[b]	0.0[a]	0.2[b]	0.1
Grill	2.6	2.5[b]	0.7[c]	0.6[a]	2.0	1.1[a]	0.3[b]	0.4[c]	0.1
RMSE[2]	0.44	0.37	0.29	0.42	0.43	0.70	0.18	0.29	0.25

[1]P-value from Analysis of Variance table.

[abc] Mean values within a column and interaction followed by the same letter are not significantly different ($P < 0.05$).

[2]RMSE = Root Mean Square Error.

Table 3. Continued.

Treatments	Sour Milk/Dairy/ Cooked Milk	Fishy	Heated oil	Liver like	Painty	Refrigerator Stale	Smoky/Wood Charcoal	Soapy	Warmed over
Lipid oxidation group[1]	0.007	<0.0001	0.02	0.38	0.004	0.004	0.35	0.14	<0.0001
Low	0.3[a]	0.0[a]	0.5[a]	0.2	0.0[a]	0.5[a]	0.6	0.0	0.2[a]
Medium	0.5[ab]	0.4[b]	0.5[a]	0.4	0.0[a]	0.9[b]	0.5	0.2	0.5[a]
High	0.7[b]	0.7[b]	0.8[b]	0.2	0.2[b]	0.9[b]	0.5	0.1	1.1[b]
Cooking method[1]	0.11	0.02	<0.0001	0.0004	0.95	<0.0001	<0.0001	0.48	<0.0001
Sous-vide	0.6	0.6[b]	0.2[a]	0.5[b]	0.1	1.2[c]	0.0[a]	0.1	0.9[b]
Sous-vide + grill	0.5	0.4[a]	0.6[b]	0.3[a]	0.0	0.8[b]	0.3[b]	0.1	0.8[b]
Grill	0.4	0.2[a]	1.0[c]	0.1[a]	0.1	0.3[a]	1.2[c]	0.0	0.2[a]
RMSE[2]	0.43	0.56	0.49	0.35	0.24	0.48	0.41	0.28	0.58

[1]P-value from Analysis of Variance table.

[abc] Mean values within a column and interaction followed by the same letter are not significantly different ($P < 0.05$).

[2]RMSE = Root Mean Square Error.

Table 4. Texture descriptive attributes least squares mean for strip loin steaks segmented by lipid oxidation group and cooking method where 0 = none and 15 = extremely intense.

Treatments	Denseness	Juiciness	Muscle Fiber Tenderness	Connective Tissue
Lipid oxidation group[1]	0.34	0.65	0.61	0.30
Low	6.2	9.0	11.5	11.9
Medium	6.6	9.2	11.3	11.9
High	6.2	9.0	11.4	11.7
Cooking Method[1]	<0.0001	<0.0001	0.10	0.41
Sous-vide	6.9[b]	8.8[a]	11.5	11.9
Sous-vide + grill	6.8[b]	8.5[a]	11.2	11.8
Grill	5.2[a]	9.8[b]	11.5	12.0
RMSE[2]	1.01	0.80	0.67	0.62

[1]P-value from Analysis of Variance table.
[ab] Mean values within a column and interaction followed by the same letter are not significantly different ($P < 0.05$).
[2]RMSE = Root Mean Square Error.

Table 5. Cook yield and cook time least squares mean for strip loin steaks segmented by lipid oxidation group and cooking method.

Treatments	Cook Yield, %	Cook Time, min Pre-cooking	Cook Time, min Cooking
Lipid oxidation group[1]	0.258	0.709	0.175
Low	77.19	54.18	69.55
Medium	77.62	56.76	76.02
High	78.50	55.19	65.39
Cooking Method[1]	<0.0001	0.498	<0.0001
Sous-vide	78.77[b]	54.66	100.35[b]
Sous-vide + grill	75.27[a]	56.09	96.03[b]
Grill	79.26[b]	.	14.57[a]
RMSE[2]	2.985	8.366	18.74

[1]P-value from Analysis of Variance table.

[ab] Mean values within a column and interaction followed by the same letter are not significantly different ($P < 0.05$).

[2]RMSE = Root Mean Square Error.

Table 6. Least squares mean for raw Minolta CIE L*, a*, and b*color space values, Chroma, and Hue angle for strip loin steaks segmented by lipid oxidation group and cooking method.

	CIE Color space value				
Treatments	L*	a*	b*	Chroma	Hue angle
Lipid oxidation group[1]	0.0005	<0.0001	0.006	<0.0001	<0.0001
Low	42.98[b]	12.80[b]	6.23[a]	14.22[b]	25.61[a]
Medium	41.57[a]	12.59[b]	6.72[b]	14.31[b]	28.73[a]
High	41.38[a]	8.68[a]	6.13[a]	10.77[a]	36.93[b]
Cooking method[1]	0.858	0.620	0.372	0.518	0.990
Sous-vide	41.85	11.56	6.41	13.30	30.35
Sous-vide + grill	42.00	11.45	6.44	13.21	30.39
Grill	42.07	11.07	6.24	12.79	30.53
RMSE[2]	1.606	2.119	0.622	1.944	5.692

[1]P-value from Analysis of Variance table.
[ab] Mean values within a column and interaction followed by the same letter are not significantly different (P<0.05).
[2]RMSE = Root Mean Square Error.

Table 7. Least squares mean for Warner-Bratzler Shear Force values, for strip loin steaks by lipid oxidation groups and cooking method.

Treatments	WBSF values, kg
Lipid oxidation group[1]	0.013
Low	3.23[b]
Medium	2.86[a]
High	3.04[ab]
Cooking Method[1]	<0.0001
Sous-vide	3.20[b]
Sous-vide + grill	3.36[b]
Grill	2.60[a]
RMSE[2]	0.418

[1]P-value from Analysis of Variance table.
[ab]Mean values within a column and interaction followed by the same letter are not significantly different ($P < 0.05$).
[2]RMSE = Root Mean Square Error.

Table 8. Raw and cooked least squares mean for TBARS values, for strip loin steaks by lipid oxidation groups and cooking method.

Treatments	TBARS, raw	TBARS, cooked	
Lipid oxidation group[1]	<0.0001	<0.0001	*P*-value[2]
Low	0.12[a]	0.22[a]	0.225
Medium	0.71[b]	0.66[b]	0.848
High	1.83[c]	1.71[c]	0.103
Cooking Method[1]	0.523	0.383	
Sous-vide	0.85	0.94	0.328
Sous-vide + grill	0.87	0.85	0.943
Grill	0.95	0.79	0.169
RMSE[3]	0.386	0.392	0.450

[1]P-value from Analysis of Variance table for columns.
[2]P-value from Analysis of Variance table for rows.
[abc]Mean values within a column and interaction followed by the same letter are not significantly different ($P < 0.05$).
[3]RMSE = Root Mean Square Error.

Table 9. Least squares mean of concentration for volatile aroma compounds for cooking methods.

Volatile Compound	SV	SVG	Grill	RMSE[2]	*P*-value[1]
Acids					
Acetic acid	2.60	1.71	2.40	2.440	0.30
Alcohol					
1-Octen-3-ol	1.83	1.75	1.80	2.075	0.98
1-Hexanol	1.01	1.17	0.99	1.903	0.91
1-Pentanol	2.64	2.49	2.40	2.411	0.91
Aldehyde					
Acetaldehyde	4.22	4.19	4.21	0.961	0.99
Benzaldehyde	2.71	3.14	3.01	2.520	0.78
Butanal	1.97	1.61	1.17	2.198	0.33
2-Methyl-butanal	0.41[a]	4.77[b]	5.39[b]	1.331	<0.0001
3-Methyl-butanal	3.46[a]	4.74[b]	5.11[b]	1.425	<0.0001
Decanal	0.72	1.43	1.83	2.213	0.12
Hexanal	4.77	4.21	4.28	2.142	0.53
Heptanal	2.80	3.20	3.32	2.585	0.68
Nonanal	3.53	3.33	4.20	2.645	0.36
Octanal	3.07	3.25	3.42	2.619	0.86
2-Methyl-propanal	1.03[a]	2.75[b]	3.66[b]	2.500	0.0002
Pentanal	1.21	1.14	1.06	1.905	0.94
Alkane					
Butane	2.38	2.88	2.05	2.244	0.31
Decane	3.29	2.95	2.85	2.446	0.74
Dodecane	3.15	2.82	2.97	2.422	0.85
Octane	2.84	2.86	2.91	2.502	0.99
Styrene	2.22	2.27	2.15	2.383	0.97
Propane	1.81	2.59	1.90	1.714	0.13
Pentane	2.18	2.00	1.05	2.081	0.06
Furan					
2-Pentyl-furan	2.18	2.36	2.81	1.933	0.39
2,5-Dihydro-3,5-dimethyl-2-furanone	1.02	0.59	0.76	1.311	0.41
Dihydro-3-methylene-2,5-furandione	0.89	0.76	1.12	1.333	0.53

[1]P-value from Analysis of Variance table for rows.

[abc]Mean values within a column and interaction followed by the same letter are not significantly different ($P < 0.05$).

[2]RMSE = Root Mean Square Error.

Table 9. Continued.

Volatile Compound	SV	SVG	Grill	RMSE[d]	*P*-value[a]
Ketone					
2-Butanone	4.04	4.25	3.91	2.548	0.85
3-Hydroxy-2-butanone	4.82	5.00	5.64	1.799	0.15
2-Heptanone	1.96[a]	2.85[a]	3.97[b]	2.022	0.0005
2-Pentanone	2.10	2.83	2.43	2.253	0.43
2-Propanone	4.68	4.28	4.93	1.911	0.37
2,3-Butanedione	3.07	3.30	2.39	2.524	0.31
3-Pentanone	1.65	0.72	0.91	1.656	0.06
Pyrazine					
3-Ethyl-2,5dimethyl-pyrazine	0.00[a]	1.17[b]	2.30[c]	1.893	<0.0001
Methyl-pyrazine	0.00[a]	1.92[b]	1.93[b]	1.942	<0.0001
Sulfur-containing compounds					
Carbon disulfide	6.07	6.11	5.94	0.424	0.22
Dimethyl disulfide	0.29[a]	1.71[b]	0.90[ab]	1.715	0.004
3-(methylthio)-propanal	0.32[a]	1.43[b]	1.87[b]	1.823	0.002
Thiobis-methane	3.13	3.02	2.35	2.500	0.38
Ratio					
Hexanal/Carbon disulfide	0.56	0.38	0.67	0.786	0.29

[1]P-value from Analysis of Variance table for rows.

[abc]Mean values within a column and interaction followed by the same letter are not significantly different ($P < 0.05$).

[2]RMSE = Root Mean Square Error.

Table 10. Least squares mean of concentration for volatile aroma compounds for level of lipid oxidation.

Volatile Compound	Low	Medium	High	RMSE[2]	*P*-value[1]
Acids					
Acetic acid	2.44[b]	0.92[a]	3.35[b]	2.440	0.0007
Alcohol					
1-Octen-3-ol	0.00[a]	1.69[b]	3.69[c]	2.075	<0.0001
1-Hexanol	0.00[a]	0.86[a]	2.31[b]	1.903	<0.0001
1-Pentanol	1.05[a]	2.27[a]	4.20[b]	2.411	<0.0001
Aldehyde					
Acetaldehyde	4.41	4.08	4.12	0.961	0.29
Benzaldehyde	2.66[a]	2.19[a]	4.01[b]	2.520	0.007
Butanal	1.21	1.87	1.66	2.198	0.48
2-Methyl-butanal	3.42	3.37	3.79	1.331	0.32
3-Methyl-butanal	4.45	2.30	4.56	1.425	0.76
Decanal	1.28	0.80	1.89	2.213	0.13
Hexanal	4.45[ab]	3.45[a]	5.36[b]	2.142	0.002
Heptanal	2.79[a]	2.37[a]	4.17[b]	2.585	0.008
Nonanal	3.64	2.96	4.46	2.645	0.06
Octanal	3.15[ab]	2.40a	4.19[b]	2.619	0.02
2-Methyl-propanal	2.47	2.22	2.75	2.500	0.68
Pentanal	1.77	0.68	0.97	1.905	0.06
Alkane					
Butane	0.69[a]	2.63[b]	3.99[c]	2.244	<0.0001
Decane	2.41[a]	2.79[ab]	3.89[b]	2.446	0.01
Dodecane	2.44[a]	2.76[ab]	3.74[b]	2.422	0.04
Octane	2.19[a]	2.31[a]	4.11[b]	2.502	0.0009
Styrene	1.46[a]	1.78[a]	3.41[b]	2.383	0.0006
Propane	2.10[ab]	2.70[b]	1.50[a]	1.714	0.02
Pentane	0[a]	1.60[b]	3.63[c]	2.081	<0.0001
Furan					
2-Pentyl-furan	0.66[a]	2.01[b]	4.68[c]	1.933	<0.0001
2,5-Dihydro-3,5-dimethyl-2-furanone	0.70	1.17	0.49	1.311	0.12
Dihydro-3-methylene-2,5-furandione	1.28[b]	1.17[b]	0.32[a]	1.333	0.002

[1]P-value from Analysis of Variance table for rows.

[abc]Mean values within a column and interaction followed by the same letter are not significantly different ($P < 0.05$).

[2]RMSE = Root Mean Square Error.

Table 10. Continued.

Volatile Compound	Low	Medium	High	RMSE[d]	*P*-value[a]
Ketone					
2-Butanone	4.34	4.32	3.56	2.548	0.29
3-Hydroxy-2-butanone	5.62	4.68	5.15	1.799	0.14
2-Heptanone	1.07[a]	3.04[b]	4.66[c]	2.022	<0.0001
2-Pentanone	1.98[a]	1.91[a]	3.47[b]	2.253	0.003
2-Propanone	4.82	4.36	4.72	1.911	0.64
2,3-Butanedione	3.16	2.46	3.14	2.524	0.49
3-Pentanone	1.33[b]	1.47[b]	0.49[a]	1.656	0.02
Pyrazine					
3-Ethyl-2,5dimethyl-pyrazine	1.11	0.69	1.67	1.893	0.10
Methyl-pyrazine	1.39	0.84	1.61	1.942	0.29
Sulfur-containing compounds					
Carbon disulfide	6.01[a]	5.90[a]	6.21[b]	0.424	0.01
Dimethyl disulfide	1.09	0.96	0.84	1.715	0.80
3-(methylthio)-propanal	1.30	0.96	1.36	1.823	0.67
Thiobis-methane	3.10	2.44	2.96	2.500	0.58
Ratio					
Hexanal/Carbon disulfide	0.35[b]	0.47[b]	0.79[c]	0.786	0.03

[1]P-value from Analysis of Variance table for rows.

[abc]Mean values within a column and interaction followed by the same letter are not significantly different ($P < 0.05$).

[2]RMSE = Root Mean Square Error.

Table 11. Misclassification matrix[1] of initial level of lipid oxidation groups predicted[2] by Partial Least Squares-Linear Discriminant Analysis using molecular profiles of beef strip loin steaks collected using rapid evaporative ionization mass spectrometry.

	Predicted Class					
Reference Class	Low	Medium	High	Total	Sensitivity	Precision
Low	36	0	0	36	100%	100%
Medium	0	24	3	27	88.8%	100%
High	0	0	45	45	100%	93.7%
Total	36	24	48	108		

Overall accuracy[3] 97.2%

[1] Number of samples falling into each respective classification category after prediction.
[2] Models were built using 80% of the original data and tested using the remaining 20%.
[3] Percentage from RStudio software statistical analysis.

Table 12. Misclassification matrix[1] of cooking method groups predicted[2] by Partial Least Squares-Linear Discriminant Analysis using molecular profiles of beef strip loin steaks collected using rapid evaporative ionization mass spectrometry.

	Predicted Class					
Reference Class	SV	SVG	Grill	Total	Sensitivity	Precision
SV	35	0	0	35	100%	97.2%
SVG	1	36	0	37	97.2%	100%
Grill	0	0	36	36	100%	100%
Total	36	36	36	108		

Overall accuracy[3] 99.0%

[1] Number of samples falling into each respective classification category after prediction.
[2] Models were built using 80% of the original data and tested using the remaining 20%.
[3] Percentage from RStudio software statistical analysis.

Table 13. Misclassification matrix[1] of beef identity flavor attribute predicted[2] by Partial Least Squares-Linear Discriminant Analysis using molecular profiles of beef strip loin steaks collected using rapid evaporative ionization mass spectrometry.

	Predicted Class					
Reference Class	Low	Mild	Intense	Total	Sensitivity	Precision
Low	33	0	0	33	100%	100%
Mild	0	47	0	47	100%	100%
Intense	0	0	28	28	100%	100%
Total	33	47	28	108		

Overall accuracy[3] 100.0%

[1] Number of samples falling into each respective classification category after prediction.
[2] Models were built using 80% of the original data and tested using the remaining 20%.
[3] Percentage from RStudio software statistical analysis.

Table 14. Misclassification matrix[1] of brown flavor attribute predicted[2] by Partial Least Squares-Linear Discriminant Analysis using molecular profiles of beef strip loin steaks collected using rapid evaporative ionization mass spectrometry.

	Predicted Class					
Reference Class	Low	Mild	Intense	Total	Sensitivity	Precision
Low	30	1	0	31	96.8%	100%
Mild	0	44	0	44	100%	100%
Intense	0	0	33	33	100%	100%
Total	30	45	33	108		

Overall accuracy[3] 99.0%

[1] Number of samples falling into each respective classification category after prediction.
[2] Models were built using 80% of the original data and tested using the remaining 20%.
[3] Percentage from RStudio software statistical analysis.

Table 15. Misclassification matrix[1] of roasted flavor attribute predicted[2] by Partial Least Squares-Linear Discriminant Analysis using molecular profiles of beef strip loin steaks collected using rapid evaporative ionization mass spectrometry.

	Predicted Class					
Reference Class	Low	Mild	Intense	Total	Sensitivity	Precision
Low	28	3	0	31	90.3%	100%
Mild	0	45	3	48	93.7%	91.8%
Intense	0	1	28	29	96.5%	90.3%
Total	28	49	31	108		

Overall accuracy[3] 93.5%

[1] Number of samples falling into each respective classification category after prediction.
[2] Models were built using 80% of the original data and tested using the remaining 20%.
[3] Percentage from RStudio software statistical analysis.

Table 16. Misclassification matrix[1] of cardboardy flavor attribute predicted[2] by Partial Least Squares-Linear Discriminant Analysis using molecular profiles of beef strip loin steaks collected using rapid evaporative ionization mass spectrometry.

	Predicted Class					
Reference Class	Low	Mild	Intense	Total	Sensitivity	Precision
Low	36	3	2	41	87.8%	87.8%
Mild	3	37	1	41	90.2%	86.0%
Intense	2	3	21	26	80.8%	87.5%
Total	41	43	24	108		

Overall accuracy[3] 87.0%

[1] Number of samples falling into each respective classification category after prediction.
[2] Models were built using 80% of the original data and tested using the remaining 20%.
[3] Percentage from RStudio software statistical analysis.

Table 17. Misclassification matrix[1] of warmed-over flavor attribute predicted[2] by Partial Least Squares-Linear Discriminant Analysis using molecular profiles of beef strip loin steaks collected using rapid evaporative ionization mass spectrometry.

	Predicted Class					
Reference Class	Low	Mild	Intense	Total	Sensitivity	Precision
Low	35	4	7	46	76.1%	79.5%
Mild	4	23	1	28	82.1%	79.3%
Intense	5	2	27	34	79.4%	77.1%
Total	44	29	35	108		

Overall accuracy[3] 78.7%

[1] Number of samples falling into each respective classification category after prediction.
[2] Models were built using 80% of the original data and tested using the remaining 20%.
[3] Percentage from RStudio software statistical analysis.

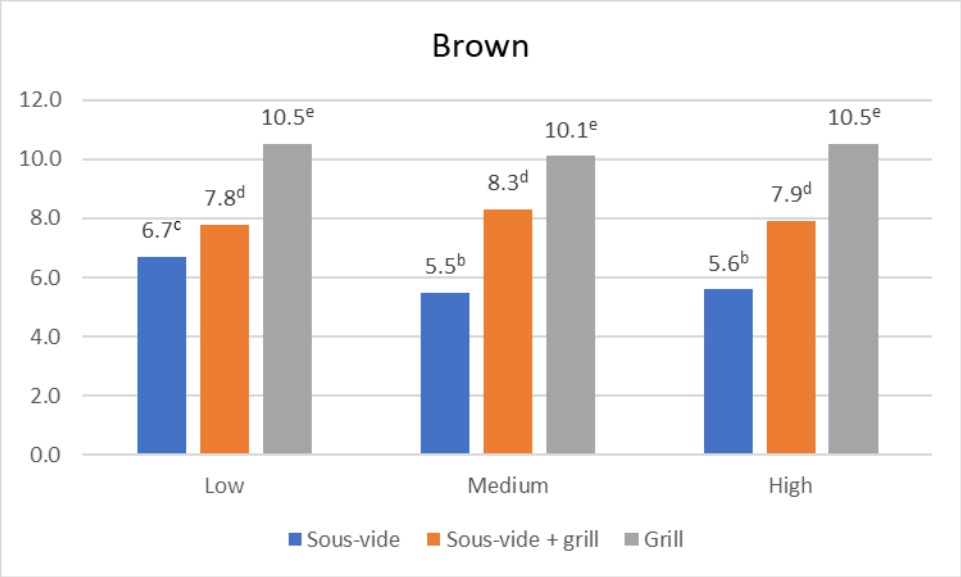


Figure 1. Cooking method by lipid oxidation level interaction least squares mean for brown (P = 0.03) descriptive flavor attribute where 0 = none and 15 = extremely intense.

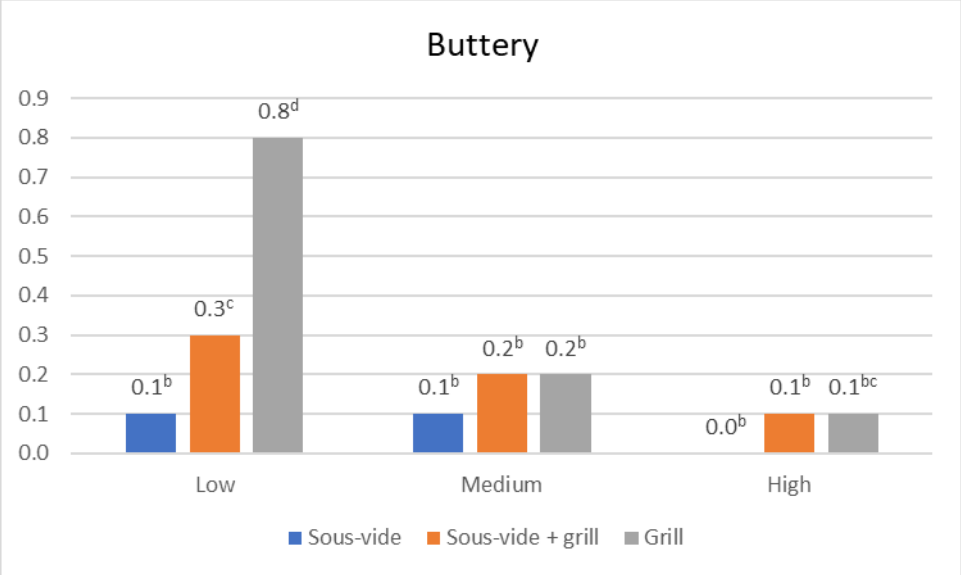


Figure 2. Cooking method by lipid oxidation level interaction least squares mean for buttery (P = 0.006) descriptive flavor attribute where 0 = none and 15 = extremely intense.

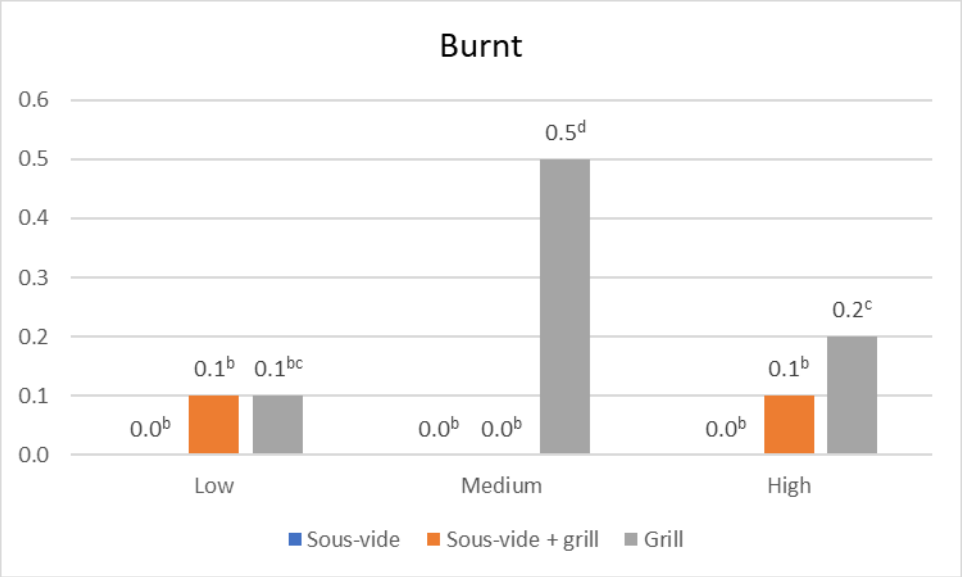


Figure 3. Cooking method by lipid oxidation level interaction least squares mean for burnt (P = 0.003) descriptive flavor attribute where 0 = none and 15 = extremely intense.

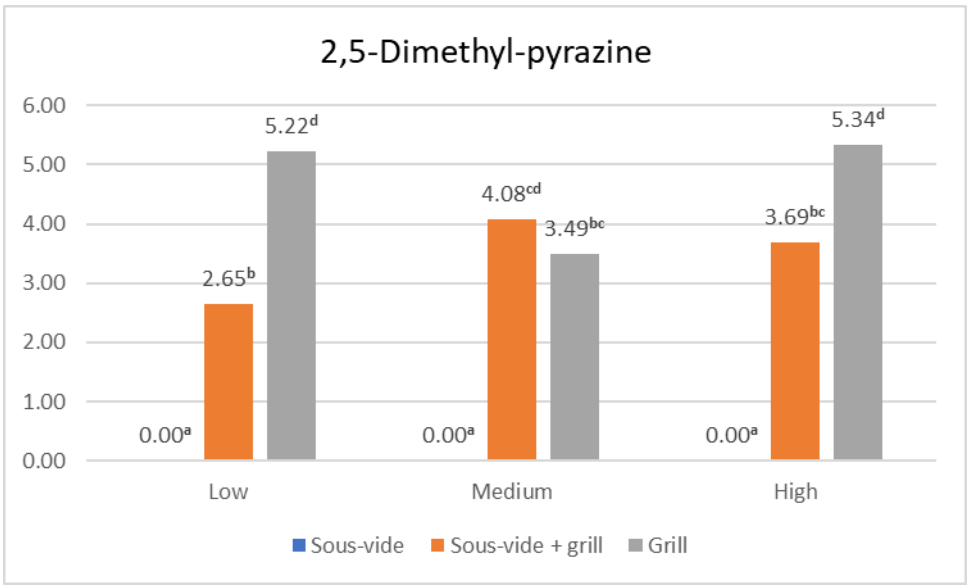


Figure 4. Cooking method by lipid oxidation level interaction least squares mean for 2,5-Dimethyl-pyrazine (P = 0.02) volatile aroma compound.

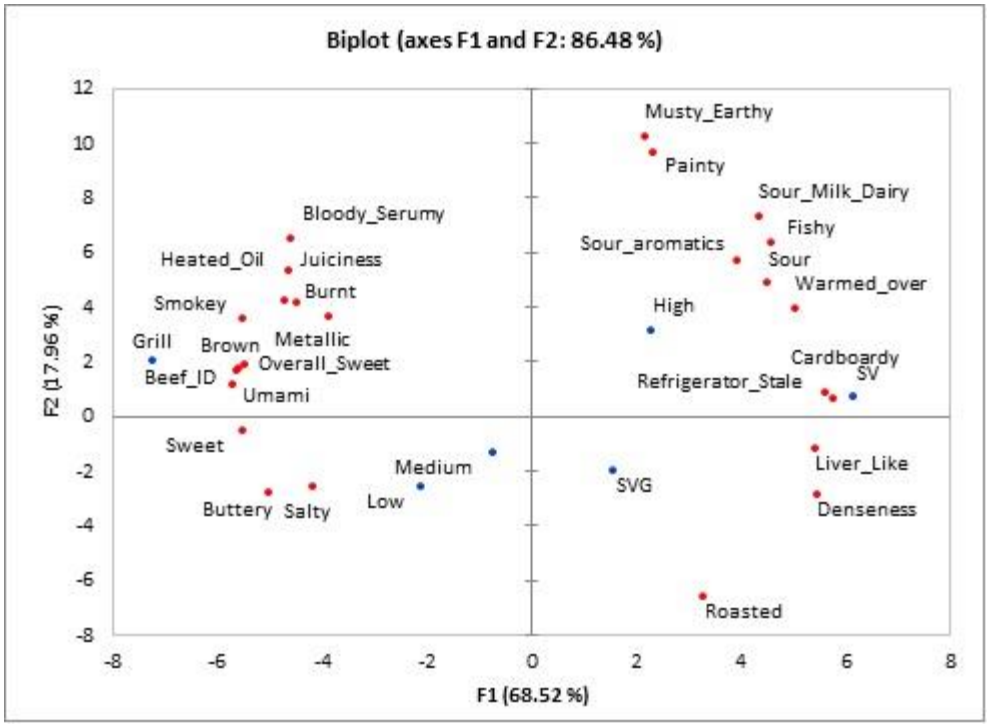


Figure 5. Principle Component Analysis of lipid oxidation level, cooking method (•), and descriptive flavor and texture attributes (•).

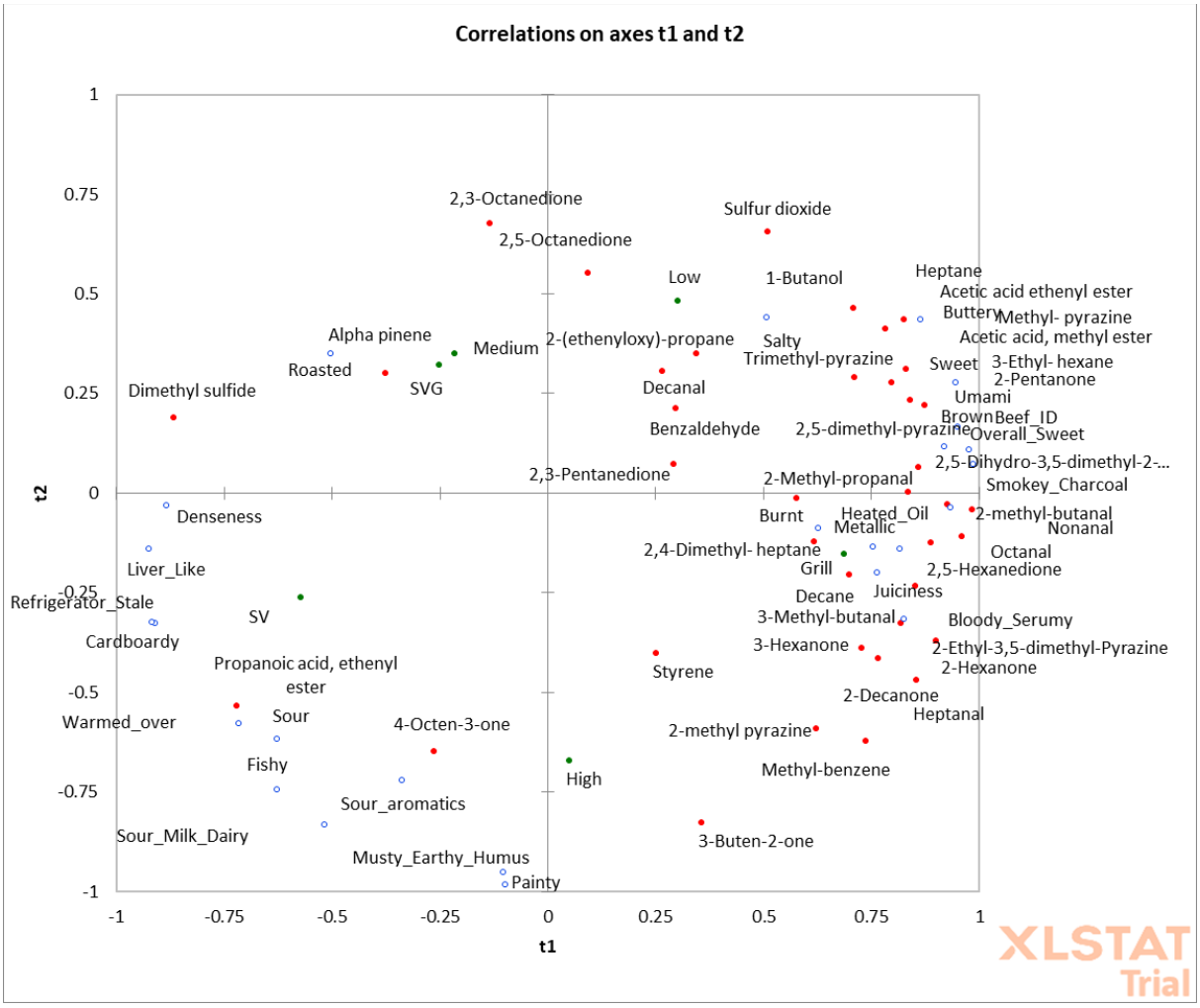
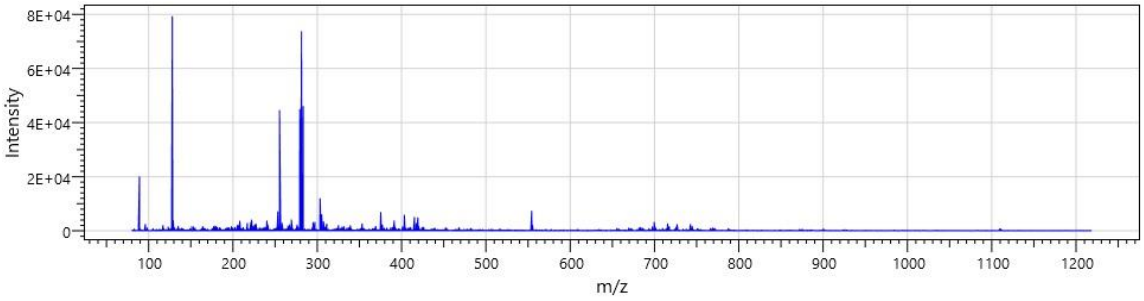
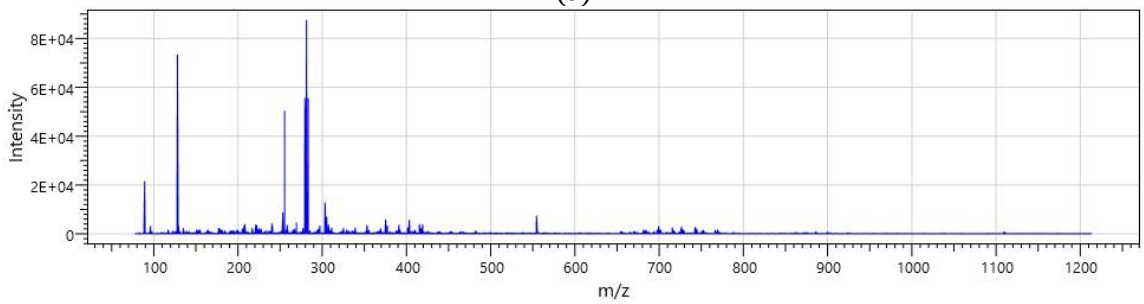


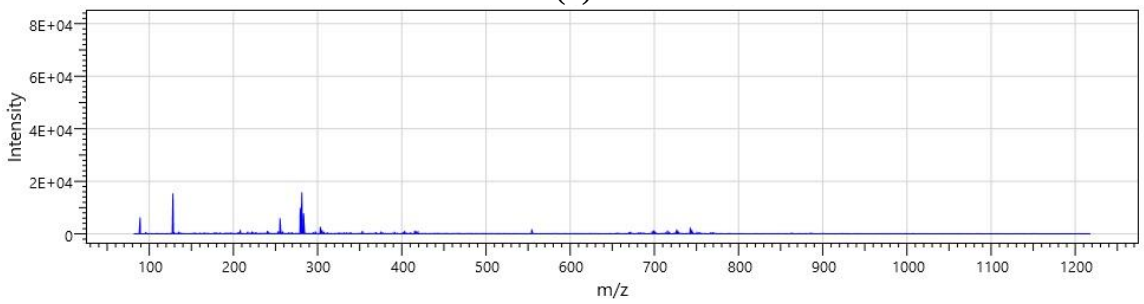
Figure 6. Partial least squares regression biplot for volatile aroma compounds (•), treatments (•), and descriptive flavor and texture attributes (•).



(a)

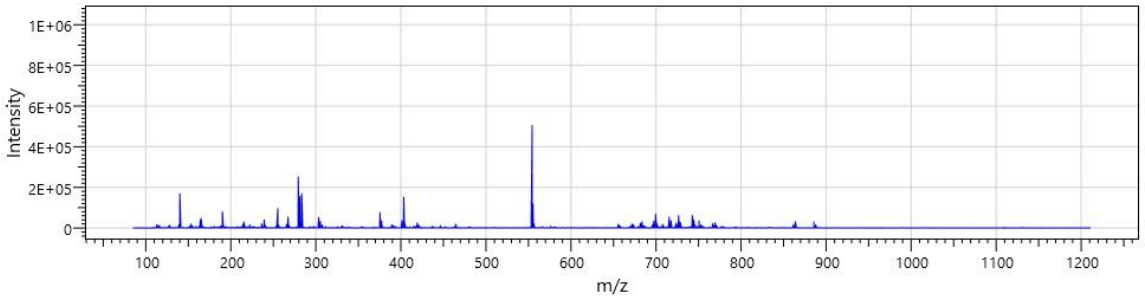


(b)

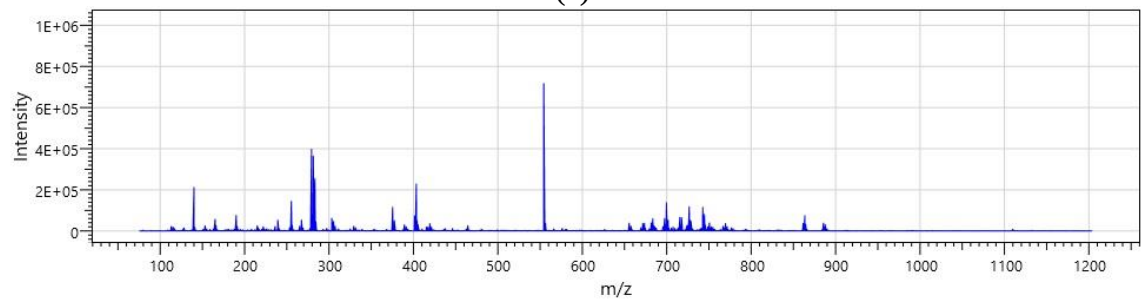


(c)

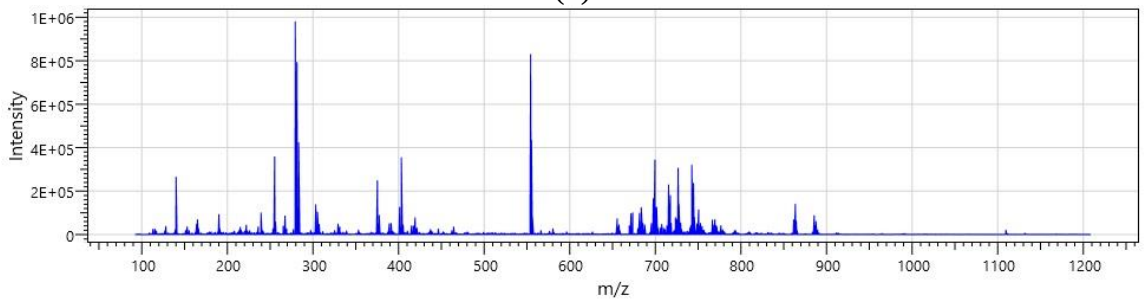
Figure 7. Example of spectra generated from rapid evaporative ionization mass spectrometry from low level of lipid oxidation samples cooked at sous-vide (a), sous-vide plus grill (b) and grill (c).



(a)

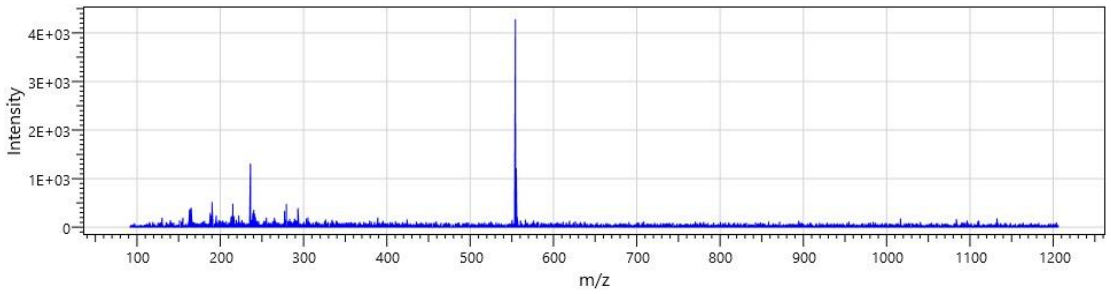


(b)

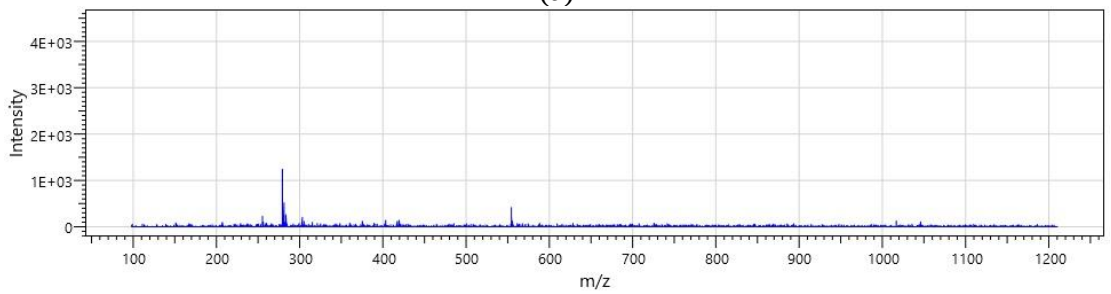


(c)

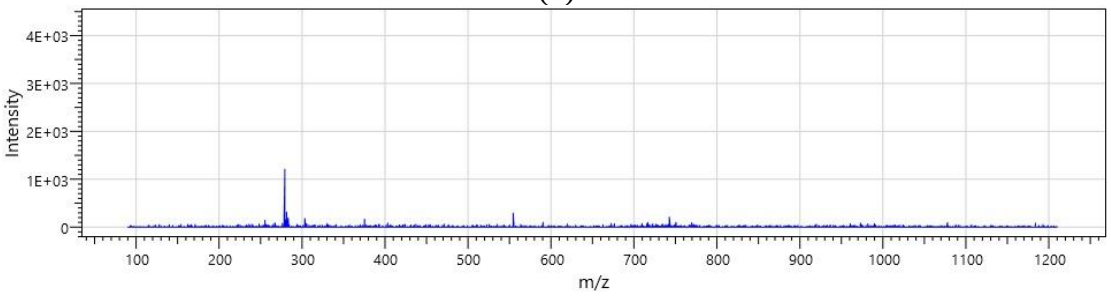
Figure 8. Example of spectra generated from rapid evaporative ionization mass spectrometry from medium level of lipid oxidation samples cooked at sous-vide (a), sous-vide plus grill (b) and grill (c).



(a)



(b)



(c)

Figure 9. Example of spectra generated from rapid evaporative ionization mass spectrometry from high level of lipid oxidation samples cooked at sous-vide (a), sous-vide plus grill (b) and grill (c).

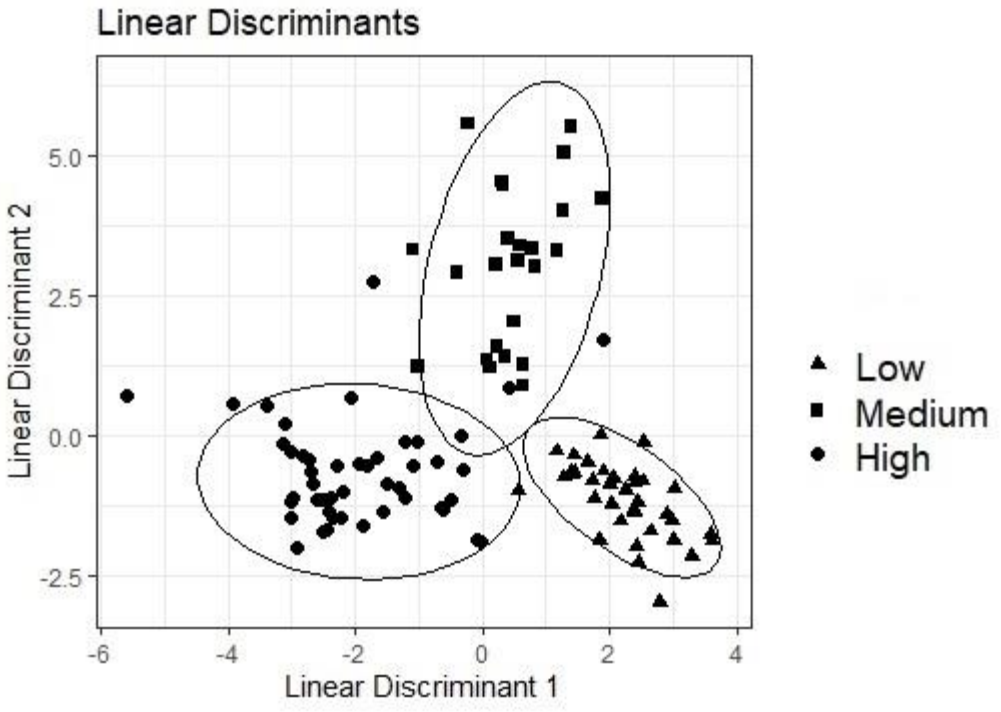


Figure 10. Projection of partial least squares-linear discriminant scores of the model built from rapid evaporative ionization mass spectrometry (REIMS) mass bins to predict level of lipid oxidation.

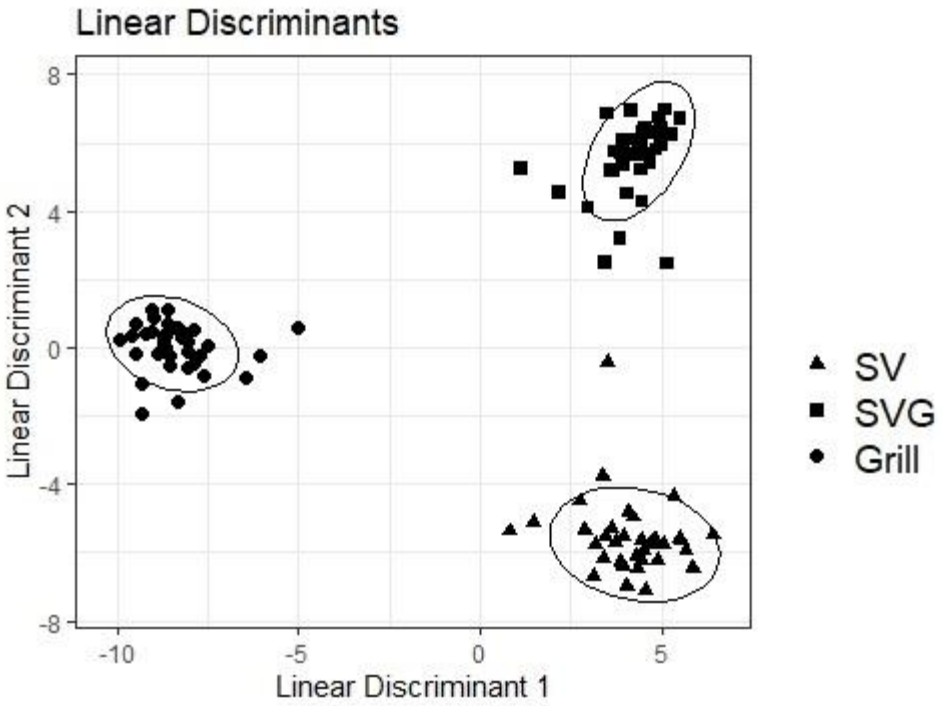


Figure 11. Projection of partial least squares-linear discriminant scores of the model built from rapid evaporative ionization mass spectrometry (REIMS) mass bins to predict cooking method.

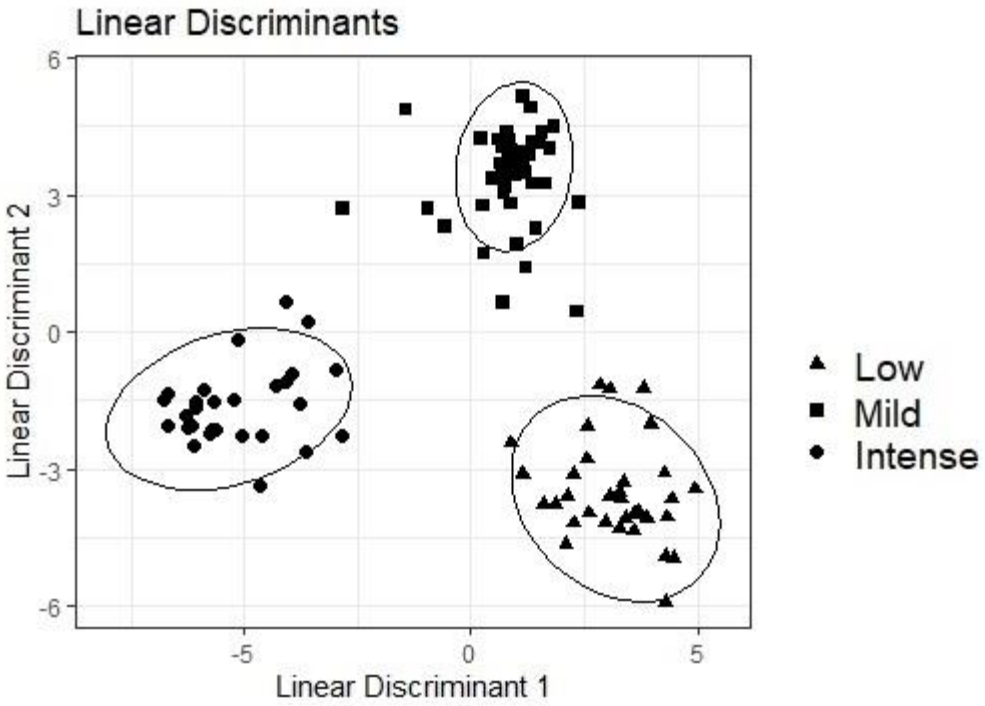


Figure 12. Projection of partial least squares-linear discriminant scores of the model built from rapid evaporative ionization mass spectrometry (REIMS) mass bins to predict beef identity flavor attribute.

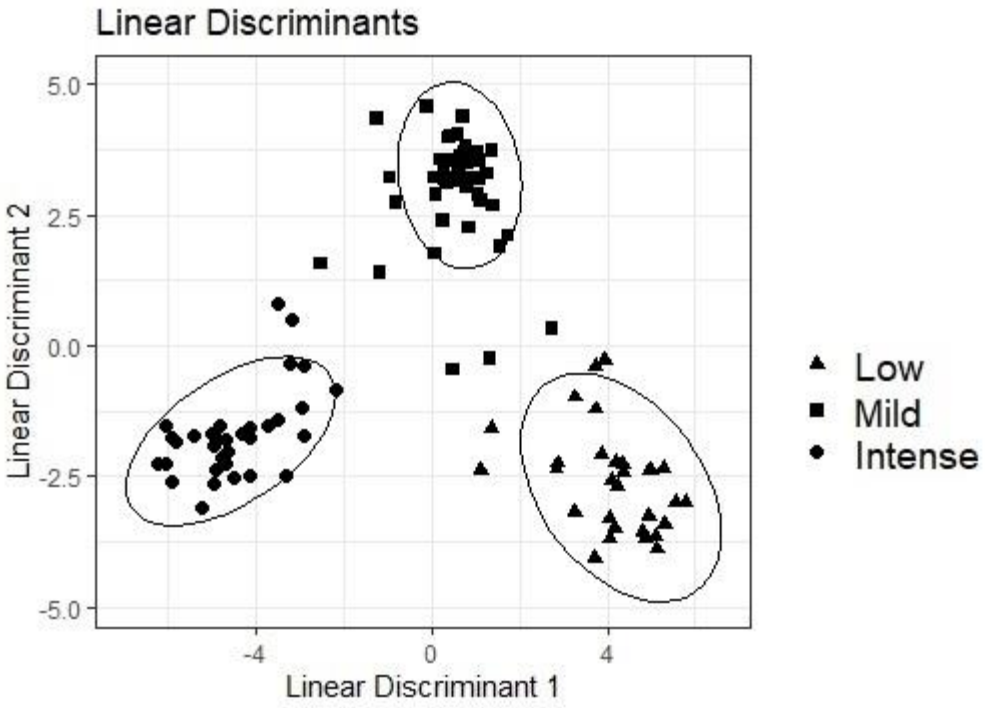


Figure 13. Projection of partial least squares-linear discriminant scores of the model built from rapid evaporative ionization mass spectrometry (REIMS) mass bins to predict brown flavor attribute.

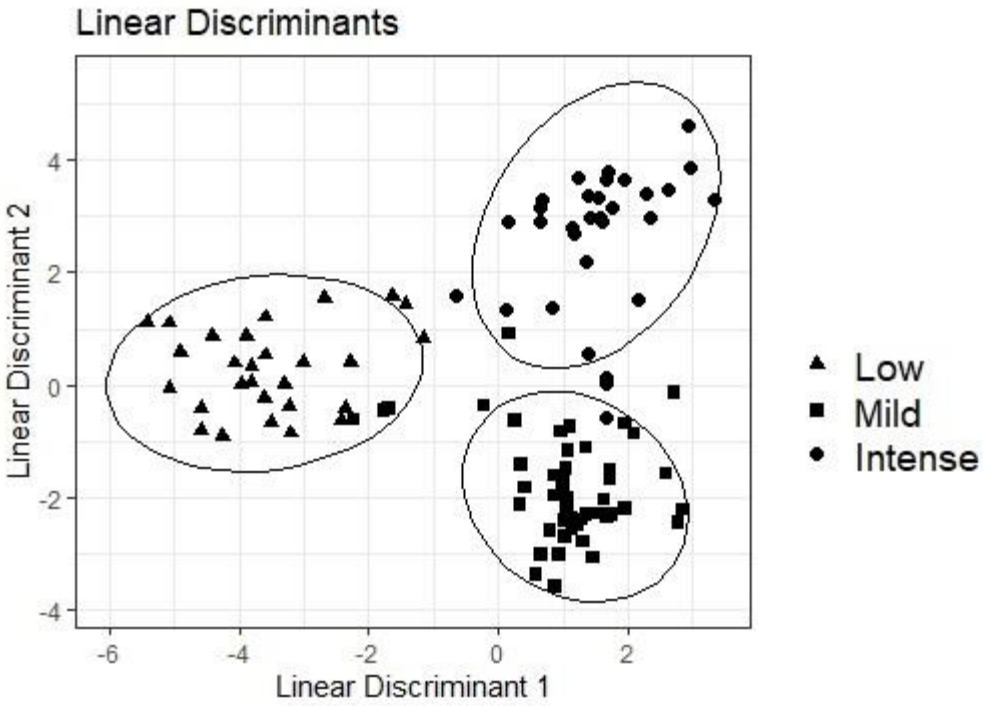


Figure 14. Projection of partial least squares-linear discriminant scores of the model built from rapid evaporative ionization mass spectrometry (REIMS) mass bins to predict roasted flavor attribute.

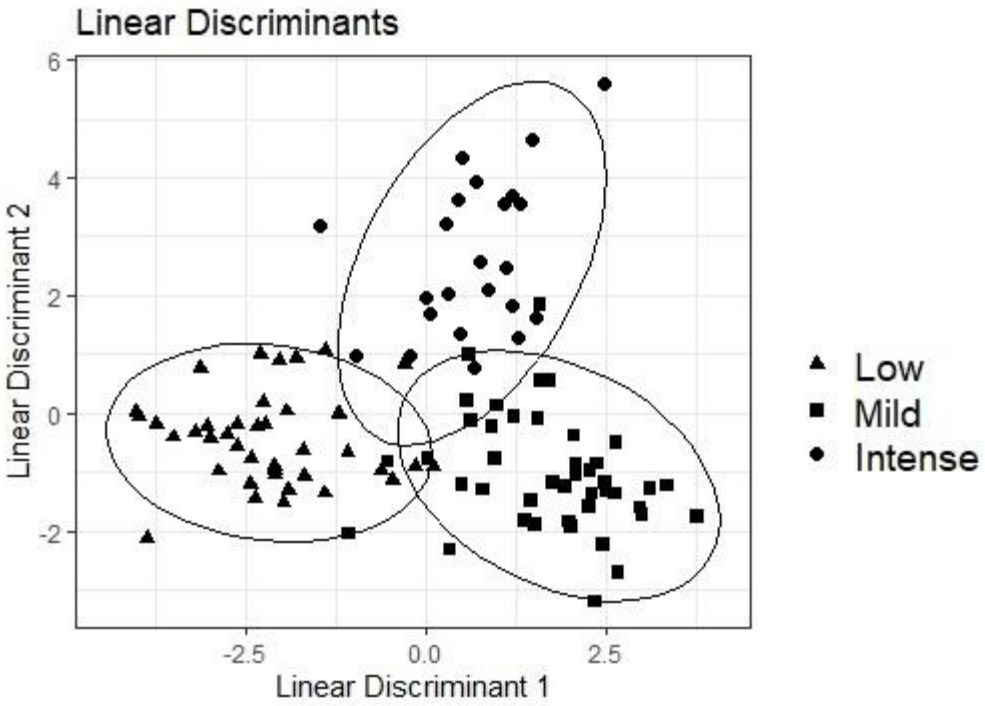


Figure 15. Projection of partial least squares-linear discriminant scores of the model built from rapid evaporative ionization mass spectrometry (REIMS) mass bins to predict cardboardy flavor attribute.

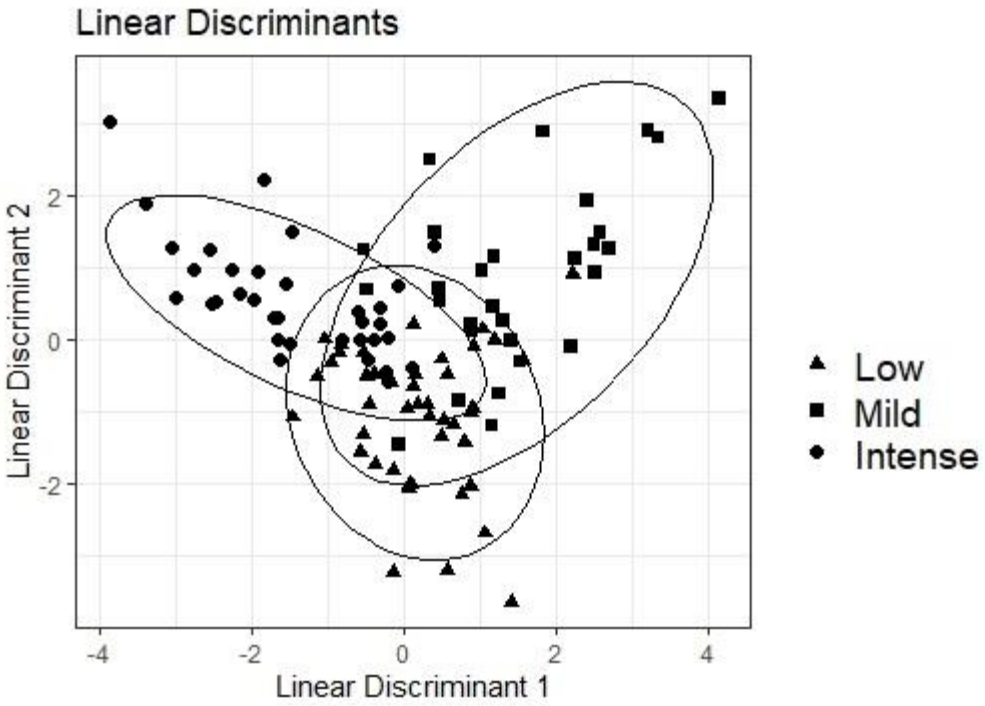


Figure 16. Projection of partial least squares-linear discriminant scores of the model built from rapid evaporative ionization mass spectrometry (REIMS) mass bins to predict warmed-over flavor attribute.

APPENDIX B

TRAINED DESCRIPTIVE ANALYSIS TRAINING GUIDELINES

Day 1

- Introduced Universal Scale for flavor intensity.
  - Soda flavor in Saltine Crackers = 2.0
  - Apple flavor in Motts Apple Sauce = 5.0
  - Orange flavor in Minute Maid Frozen Orange Juice = 7.0
  - Grape flavor in Welch's Grape Juice = 10.0
  - Cinnamon flavor in Big Red Chewing Gum = 12.0
- Introduced basic tastes (salty, sweet, bitter, sour and umami), beef flavor identity, fat-like, brown and roasted flavor attributes.
- Sample evaluation for the introduced attributes.
  - 862 – Choice strip loin steak grilled 70℃ at 204℃.
  - 223 – Choice strip loin steak cooked in the sous-vide to 70℃ at 70℃.
  - 756 – Prime strip loin steak grilled 70℃ at 204℃.
  - 544 – Choice strip loin steak cooked in the sous-vide to 70℃ at 70℃ and finish in grill (30 seconds each side) at 204℃.

Day 2

- Reviewed previously introduced attributes.
- Introduced bloody/serumy, metallic, liver-like and overall sweet flavor attributes.
- Sample evaluation for the introduced attributes.
  - 245 – Choice strip loin steak grilled to 70℃ at 204℃.
  - 398 – Choice strip loin steak grilled to 70℃ at 204℃.
  - 954 – Choice strip loin steak grilled to 60℃ at 204℃.
  - 537 – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃ and finished in grill (30 seconds each side) at 204℃.
  - 541 – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃.

Day 3

- Reviewed previously introduced attributes.
- Introduced warmed over, refrigerator stale, cardboardy, green, green-haylike, soapy, buttery flavor attributes.
- Sample evaluation for the introduced attributes.
  - **624** – Frozen cooked sous-vide (frozen for a week) choice strip loin steak – reheated in sous-vide to 70℃ at 70℃.

- **135** – Choice strip loin steak grilled to 70℃ at 204℃.
- **952** – Prime strip loin steak grilled to 70℃ at 177℃.
- **214** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃. Stored 4 days in cooler.
- **245** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃ and finished in grill (1 min each side) at 204℃.
- **574** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃ and finished in grill (1 min each side) at 204℃.

Day 4

- Reviewed previously introduced attributes.
- Introduced flavor attributes musty-earthy/humus, heated oil, rancid, fishy and painty.
- Sample evaluation for the introduced attributes.
    - **798** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃ and finished in grill (1 min each side) at 204℃.
    - **552** – Choice strip loin steak grilled to 70℃ at 204℃.
    - **642** – Frozen cooked sous-vide (frozen for a week) choice strip loin steak – reheated in sous-vide to 70℃ at 70℃.
    - **168** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃.
    - **683** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃ and finished in grill (1 min each side) at 204℃.
    - **527** - Choice strip loin steak grilled to 70℃ at 177℃.
    - **175** - Choice strip loin steak grilled to 70℃ at 204℃.
    - **031** - Choice strip loin steak grilled to 70℃ at 204℃.

Day 5

- Reviewed previously introduced attributes.
- Introduced flavor attributes refrigerator stale, fishy, smoky wood, smoky charcoal, leather, animal hair and barnyard.
- Sample evaluation for the introduced attributes.
    - **421** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃. Stored 6 days in walk in cooler.
    - **158** – Choice strip loin steak grilled to 70℃ at 204℃.
    - **563** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃.
    - **114** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃ and finished in grill (1 min each side) at 204℃.
    - **998** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃.
    - **878** – Choice strip loin steak grilled to 70℃ at 177℃.
    - **975** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃ and finished in grill (1 min each side) at 204℃.

- **687** – Choice strip loin steak grilled to 65℃ at 204℃.

Day 6

- Reviewed previously introduced attributes.
- Introduced flavor attributes sour aromatics, sour milk/sour dairy, dairy, cooked milk, chemical and spoiled putrid.
- Sample evaluation for the introduced attributes.
    - **212** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃.
    - **235** – Choice strip loin steak grilled to 70℃ at 204℃.
    - **557** – Choice strip loin steak grilled to 65℃ at 177℃.
    - **864** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃.
    - **111** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃ and finished in grill (1 min each side) at 204℃.
    - **655** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃ and finished in grill (1 min each side) at 204℃.
    - **980** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃ and finished in grill (1 min each side) at 204℃.
    - **021** - Choice strip loin steak grilled to 70℃ at 204℃.

Day 7

- Reviewed previously introduced attributes.
- Introduced texture denseness, juiciness, muscle fiber tenderness and connective tissue amount.
- Sample evaluation for the introduced attributes.
    - **122** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃ and finished in grill (1 min each side) at 204℃. Prior cooked in sous-vide to 48.9℃ (one day before and stored overnight in a walk-in cooler at 4℃).
    - **654** – Choice strip loin steak grilled to 70℃ at 204℃.
    - **121** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃. Prior cooked in sous-vide to 48.9℃ (one day before and stored overnight in a walk-in cooler at 4℃).
    - **897** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃. Prior cooked in sous-vide to 48.9℃ (one day before and stored overnight in a walk-in cooler at 4℃).
    - **014** – Choice strip loin steak grilled to 70℃ at 204℃. Stored 11 days in walk in cooler.

- **095** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃ and finished in grill (1 min each side) at 204℃. Prior cooked in sous-vide to 48.9℃ (one day before and stored overnight in a walk-in cooler at 4℃).
- **612** – Prime strip loin steak grilled to 70℃ at 204℃.
- **844** - Choice strip loin steak cooked in sous-vide to 70℃ at 70℃ and finished in grill (1 min each side) at 204℃. Prior cooked in sous-vide to 48.9℃ (one day before and stored overnight in a walk-in cooler at 4℃).
- **970** - Choice strip loin steak grilled to 65℃ at 177℃.
- **940** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃.

Day 8

- Reviewed previously introduced attributes.
- Sample evaluation.
    - **544** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃.
    - **021** – Choice strip loin steak grilled to 70℃ at 204℃.
    - **109** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃ and finished in grill (1 min each side) at 204℃. Prior cooked in sous-vide to 48.9℃ (one day before and stored overnight in a walk-in cooler at 4℃).
    - **500** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃.
    - **650** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃ and finished in grill (1 min each side) at 204℃. Prior cooked in sous-vide to 48.9℃ (one day before and stored overnight in a walk-in cooler at 4℃).
    - **167** - Choice strip loin steak grilled to 65℃ at 177℃.
    - **511** – Choice strip loin steak grilled to 70℃ at 204℃.
    - **619** - Choice strip loin steak cook in the sous-vide to 70℃ at 70℃. *It may take closer to 2 hours.
    - **678** - Choice strip loin steak grilled to 70℃ at 204℃.
    - **855** – Choice strip loin steak grilled to 70℃ at 204℃.
    - **122** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃ and finished in grill (1 min each side) at 204℃. Prior cooked in sous-vide to 48.9℃ (one day before and stored overnight in a walk-in cooler at 4℃).
    - **834** – Choice strip loin steak cooked in sous-vide to 70℃ at 70℃.

Day 9

- Reviewed a few attributes after first replication texture denseness, and flavor attributes roasted, soapy, fishy, cardboardy.
- Sample evaluation for all of the introduced attributes.
    - **224** – Choice strip loin steak cook in the sous-vide to 158°F (70°C) at 158°F. Stored in the cooler. *It may take closer to 2 hours.

- **854** – Choice strip loin steak grill 158°F (70°C) at 400°F. Stored in the cooler.
- **174** – Choice strip loin steak sous-vide 158°F (70°C) at 158°F and finish in grill (1 min each side) at 400°F. Stored in the cooler.*It may take closer to 2 hours.
- **050** – Choice strip loin steak cook in the sous-vide to 158°F (70°C) at 158°F. *It may take closer to 2 hours.
- **710** – Choice strip loin steak sous-vide 158°F (70°C) at 158°F and finish in grill (1 min each side) at 400°F.*It may take closer to 2 hours.

Day 10

- Reviewed a few attributes after first replication texture muscle fiber tenderness, and flavor attributes cardboardy, heated oil.
- Sample evaluation for all of the introduced attributes.
  - **541** – Choice strip loin steak cook in the sous-vide to 158°F (70°C) at 158°F. Stored in the cooler. *It may take closer to 2 hours.
  - **877** – Choice strip loin steak grill 158°F (70°C) at 400°F. Stored in the cooler.
  - **704** – Choice strip loin steak sous-vide 158°F (70°C) at 158°F and finish in grill (1 min each side) at 400°F. Stored in the cooler.*It may take closer to 2 hours.
  - **510** – Choice strip loin steak cook in the sous-vide to 158°F (70°C) at 158°F. Stored in the cooler. *It may take closer to 2 hours.
  - **870** – Choice strip loin steak sous-vide 158°F (70°C) at 158°F and finish in grill (1 min each side) at 400°F.*It may take closer to 2 hours.
  - **774 -** Choice strip loin steak grill 158°F (70°C) at 400°F.
  - **564** – Choice strip loin steak grill 158°F (70°C) at 400°F. Stored in the cooler.

Day 11

- Reviewed a few attributes after first replication texture denseness, and flavor attributes burnt, musty-earthy/humus.
- Sample evaluation for all of the introduced attributes.
  - **145** – Choice strip loin steak grill 158°F (70°C) at 400°F. Stored in the cooler.
  - **544** – Choice strip loin steak cook in the sous-vide to 158°F (70°C) at 158°F. Stored in the cooler. *It may take closer to 2 hours.
  - **541** – Choice strip loin steak sous-vide 158°F (70°C) at 158°F and finish in grill (1 min each side) at 400°F. Stored in the cooler.*It may take closer to 2 hours.

- **124** – Choice strip loin steak grill 158°F (70°C) at 400°F. Stored in the cooler.
- **021** – Choice strip loin steak cook in the sous-vide to 158°F (70°C) at 158°F. Stored in the cooler. *It may take closer to 2 hours.
- **874** – Choice strip loin steak sous-vide 158°F (70°C) at 158°F and finish in grill (1 min each side) at 400°F.*It may take closer to 2 hours.
- **900** – Choice strip loin steak grill 158°F (70°C) at 400°F.
- **810** – Choice strip loin steak sous-vide 158°F (70°C) at 158°F and finish in grill (1 min each side) at 400°F.*It may take closer to 2 hours.
- **111** – Choice strip loin steak grill 158°F (70°C) at 400°F.
- **901** – Choice strip loin steak grill 158°F (70°C) at 400°F. Stored in the cooler.