

A fresh look at ground beef spoilage

by

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Abstract

The objective of this study was to determine the point at which ground beef reaches spoilage as determined by consumers relative to microbiological, lipid oxidation, color measurements, and the meat microbiome. Retail ground beef packages were delivered to the KSU meat laboratory from a case-ready facility and randomly assigned to a display duration (0 – 14 d). Packages were stored in mother-bags at 2 – 4°C in the absence of light until placed in coffin-style retail cases under fluorescent lighting conditions at 2 – 4°C for 8 different display periods. Consumer sensory analysis of visual, odor, touch, and taste were compared to objective measurements of trained sensory redness scores, discoloration scores, microbiological, lipid oxidation, and meat microbiome analysis. For each organoleptic measurement, consumers were asked if they would purchase the sample, and whether or not they thought the sample was spoiled. Consumers were less ($P < 0.05$) likely to purchase and more ($P < 0.05$) likely to rate samples as spoiled based on visual, touch, and odor evaluation once they reached 8 d of display. Logistic regression models were generated and indicated appearance measurements were the strongest predictors of consumers likeliness to purchase, and spoilage assessment. Threshold values of 50, 75, 90, and 95% were identified for consumer purchase intent, and values of 5, 10, 25, and 50% were identified for consumer spoilage indication. Objective measurements such as trained sensory panel redness score also served as very strong ($P < 0.05$) predictors, explaining 86% of the variation within the consumer purchase intent model with redness scores of 74.8, 64.1, 48.4, and 32.7 corresponding to 50, 75, 90, and 95% purchase likelihood. 77% of the variation within the consumer appearance spoilage rating model was explained by trained sensory redness score, with 74.75, 64.10, 48.40, and 32.7 corresponding to 5, 10, 25, and 50% likelihood of a consumer rating a sample as spoiled. While there are relationships present

between the consumer purchase intent and spoilage rating and other quality measures, the purchase intent and spoilage decision of the consumers was mostly driven by the color and appearance of the sample in the retail case.

Key Words: spoilage, ground beef, color, microbial, consumer

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Dedication

This thesis is dedicated to my parents, Mike and Amy, thank you for allowing me to chase my dreams and supporting me no matter what.

Chapter 1 - A Review of Literature

The global population is projected to increase approximately 10% by the year 2031 (Dohلمان et al., 2022). With this impending increase, safeguarding food products within our supply chain has heightened significance. 23% of the world's produced meat goes to waste, with spoilage standing out as a primary culprit (Karwowska et al., 2021). The repercussions are overwhelming: an annual waste of 780,000 animals in the beef industry alone, coupled with financial losses exceeding \$3.73 billion due to discoloration (Ramanathan, 2022). Notably, much of the meat removed from shelves remains perfectly consumable, falling short only of consumer preferences.

Recognizing that consumers are deterred by the oxidized metmyoglobin (**MMb**) brown color replacing the cherry-red color of fresh meat (Brooks et al., 2007; Lybarger et al., 2023), it's essential to revisit the benchmarks for spoilage. While fresh meat is turning brown in color, there are other changes in intrinsic factors occurring simultaneously such as microbial growth, volatile organic compound (**VOC**) formation, and lipid oxidation which consequently are influenced by extrinsic factors such as animal handling prior to harvest, sanitation practices, storage conditions, packaging methods, and whether the product has been ground or remains in whole muscle form (Wickramasinghe et al., 2019; Pellissery et al., 2020). Historically, the meat industry has relied on a 7-log colony forming unit (**CFU**) /gram microbial growth threshold, a concept originating from the landmark study by Butler, Bratzler, and Mallman in 1953 (Butler et al., 1953). However, the specific point at which ground beef crosses the threshold into spoilage remains inadequately explored and understood. Therefore, the aim of this review is twofold: to evaluate the diverse factors impacting spoilage and to reassess whether the conventional 7-log CFU/gram microbial growth value adequately defines spoilage in traditional ground beef packaging.

Definition of Spoilage

The spoilage of meat can best be defined as a “dynamic process and is affected by the intrinsic and extrinsic attributes of meat” such as chemical composition, water activity, animal husbandry, initial microbiota, and storage rendering it unfit/unappealing for human consumption (Yang and McMullen, 2024). For the last half century, a microbial growth threshold of 7-log CFU/gram has been used to define spoilage in fresh meat products. This standard can be traced back to a 1953 study by Butler, Bratzler, and Mallman. In this study, Butler et al. (1953) measured spoilage by examining days of storage, color fading, and microbial growth using a *Pseudomonas* inoculum on steaks from the *longissimus dorsi* muscle. Steaks were inoculated using two techniques including longitudinal streaking with several loops of culture and a direct spray of a 1:10 dilution of the culture. Butler et al. (1953) found steak color fading corresponded with *Pseudomonas* levels exceeding log 7 CFU/g. Steak color fading pertains to the percent of MMb present in the product and was measured by combining hue value and chroma using the formula $I = \frac{C}{5}(2\Delta H) + 6\Delta V + 3\Delta C$. Hue and chroma are further described in Figure 1.2. When inoculated samples were compared to control samples, shelf-life decreased typically by 3-7 days depending upon the storage conditions. Shelf-life was assessed with the used of trained sensory panels, objective color measurements, and microbial enumeration. Inoculated samples showed the greatest exponential growth at log 7 CFU/gram after 6 days of storage which correlated with high rates of discoloration. At this point, samples became more concentrated in MMb content, exhibited stronger aromas and eventually showed slime formation after 14 days in the storage case. However, their use of an inoculum, lack of sensory analysis, and other chemical attributes pose challenges in applying these results to natural spoilage processes in fresh meat, where a variety of organisms are present (Butler et al., 1953).

Since this study, the 7-log CFU/g growth threshold has been widely used in industry and research, although it hasn't been conclusively shown as the definitive marker of spoilage. For example, Brooks et al. (2007) evaluated ground beef patties within different packaging treatments using log 7 CFU/g as a benchmark for spoilage. The treatments in their study included traditional PVC overwrap as the control, high-oxygen MAP with a gas concentration of 80% O₂ and 20% CO₂, high-oxygen MAP with rosemary, low-oxygen CO MAP with a gas concentration of 0.4% CO, 30% CO₂, 69.6% N₂ and low-oxygen MAP with rosemary (Brooks et al., 2007). MAP packages were displayed in fluorescent lighting and evaluated on day 0, 1, 3, 5, 7, 14, and 21 of display. Brooks et al. (2007) analyzed spoilage using trained and consumer color and odor panels, Thiobarbituric Acid (**TBA**) analysis, and microbial sampling. Psychrophilic aerobic bacteria and total coliform counts indicated samples did not reach log 7 CFU/g growth even after 21 days of display for the MAP treatments. Moreover, their focus on *E. coli* and *Salmonella* counts did not establish 7-log bacteria presence as a spoilage indicator (Brooks et al., 2007). Therefore, while consumers were able to identify noticeable changes in color and smell, the bacterial counts still remained below the established 7-log CFU/g threshold.

Despite being cited for years as a spoilage standard, the 7-log CFU/g threshold has not been verified for ground products and is often referenced in outdated literature. Spoilage is subjectively measured by various factors such as changes in color, texture, odor, taste, and microbial counts (Brooks et al., 2007). It can result from a combination of chemical, environmental, and microbial dynamics that deteriorate fresh meat, rendering it unfit for consumption (Huis in't Veld, 1996; Pellissery et al., 2020).

Meat, with its high water activity (0.99), rich nutrient content, optimal pH (5.6), and the presence of autolytic enzymes, fats, and lipids, is highly perishable (Erkmen and Bozoglu, 2016;

Pellissery et al., 2020). Spoilage can result from microorganisms, browning, protein and lipid oxidation, and physical changes due to storage conditions (Pellissery et al., 2020).

Microorganisms are a common cause of spoilage, breaking down fats, carbohydrates, and proteins, leading to slime production, off-flavors, odors, and discoloration (Pellissery et al., 2020).

Ultimately, the shelf-life of a product is determined by consumer preference (Lybarger et al., 2023). Meat color has been identified to be the number one purchasing motivator for consumers (Killinger et al., 2004; Mancini and Hunt, 2005; Lybarger et al., 2023). In a study conducted by Lybarger et al. (2023) packages of ground beef were evaluated in the retail case by trained sensory panelists and consumers comparing data collected using a HunterLab spectrophotometer. The results of this study showed a^* was the “most simple and robust prediction of beef color acceptability” (Lybarger et al., 2023). Additionally, the percentage of MMb present on the packages aligned with the a^* value indicators (Lybarger et al., 2023). A disconnect remains in literature comparing the consumer opinion in conjunction with objective measurements used to determine spoilage in fresh meat products.

Impact of spoilage on retail food waste and economic loss

The growing global population is significantly increasing the demand for food, particularly animal protein sources (Dohlman et al., 2022). It is, therefore, essential to research methods to reduce food spoilage and decrease the amount of food wasted annually at the retail level. One of the primary reasons red meat products are left on grocery store shelves is due to consumer rejection based on the color of the product.

Ramanathan et al. (2022) conducted a study to evaluate the direct impact of meat discoloration on economic and natural resource losses worldwide. Discoloration often leads consumers to perceive meat as spoiled (Ramanathan, 2022). To assess the economic impact, data

was collected from two national retail grocery chains and one regional grocery store chain. This data included total beef sales, total beef discarded, and discounted sales values over one year. Using the discarded meat value, Ramanathan et al. (2022) calculated the total number of animals wasted (total beef discarded / 249.7 kg), the loss of energy (total meat amount $\times$ energy footprint), and the loss of water (total meat amount $\times$ water footprint) with values reported by Asem-Hiablíe et al. (2019) (Asem-Hiablíe, 2019; Ramanathan, 2022). Additionally, greenhouse gas emissions were measured using the Global Warming Potential (GWP) index ($\text{CO}_2\text{e} = \text{discarded meat amount} \times \text{GWP}$) (Asem-Hiablíe, 2019; Ramanathan, 2022). The study found 13,486,928 kg of beef was discarded due to discoloration, and 11.07% of sales were discounted. This resulted in an economic loss of \$3.73 billion for the beef industry (Ramanathan, 2022). These findings highlight the critical need to address meat discoloration to reduce food waste and its associated economic and environmental impacts.

Meat color chemistry

Myoglobin, an iron porphyrin pigment similar to hemoglobin, is the protein responsible for meat color (Robach and Costilow, 1961). Four different forms of myoglobin can be present in fresh red meat products: deoxymyoglobin (**DMb**), oxymyoglobin (**OMb**), MMb, and carboxymyoglobin (**COMb**). The reactions between different states of myoglobin are shown in Figure 1.1.

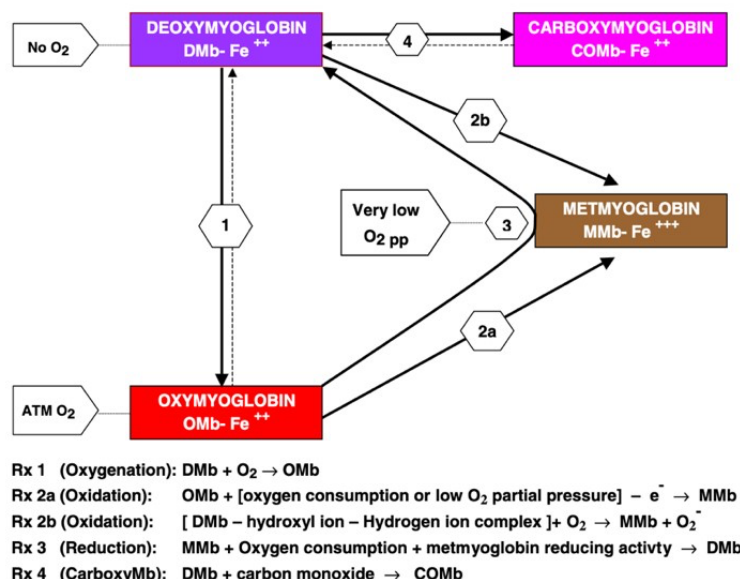


Figure 1.1. Visible myoglobin redox interconversions on the surface of meat (Mancini and Hunt, 2005).

The state of myoglobin changes depending on the ligand binding to the 6th coordination site of the protein (Mancini and Hunt, 2005) and the movement of electrons. Iron exists in two states depending on electron binding within myoglobin: ferrous (Fe^{2+}) and ferric (Fe^{3+}). DMb, which is observed as a purplish-red color, occurs when there is a lack of oxygen and no ligand binding in the 6th coordination site. As seen in reaction 1, OMb, responsible for the bright cherry-red color desired by consumers, forms when oxygen binds to myoglobin and iron remains in the ferrous state. Reaction 2a, when water takes over the 6th ligand binding site, OMb shifts to MMb, and the iron changes from the ferrous state to the ferric state undergoing oxidation (Mancini and Hunt, 2005; Suman and Joseph, 2013; Arkfeld et al., 2015). COMb, a more stable bright red color than OMb, forms when carbon monoxide binds to the myoglobin in the ferrous state of iron as shown by reaction 4 in Figure 1.1 (Mancini and Hunt, 2005; Suman and Joseph, 2013; Arkfeld et al., 2015). Once myoglobin has been completely oxidized, it will remain

permanently in the MMb state presenting a brown-tan color which is highly undesirable by consumers. However, in the presence of some bacteria, such as *Pseudomonas*, a change can occur of brown to red color producing an unidentified red myoglobin derivative (Morita et al., 1996).

Morita et al. (1996) compared the red pigment of Parma hams with the red derivative present in other meat products. In this study, bacteria were isolated from Parma hams and Tipo Parma (Parma-type dry cured ham) and examined for their ability to generate red myoglobin derivatives (Morita et al., 1996). Over 400 bacterial isolates were examined, however the ten isolates with the most pronounced color conversion capacity were selected (Morita et al., 1996). The heme pigments were extracted from red myoglobin derivatives with the use of the water-acetone method. Spectral analysis of cultures where color changed from brown to red were conducted where Parma hams were perceived to be crimson in color and did not receive nitrite or nitrate treatment (Morita et al., 1996). Different treatments were applied to model fermented sausage. Sausages that received treatment were inoculated with the isolates from the Parma ham and Tipo Parma ham while control-1 sausage samples were only treated with sodium nitrite (0.01%) and sodium ascorbate (0.05%), and control-2 samples where only sodium chloride was added (Morita et al., 1996). As a result of this study, *Staphylococcus epidermidis*, *Staphylococcus warneri* and *Staphylococcus lentus* strains were present in the isolates with the most pronounced conversion of MMb to a red myoglobin derivative and are most likely to cause the reddening of the Parma ham when there are no nitrites or nitrates present (Morita et al., 1996). We may see a similar transition in fresh meat products, although the conversion from brown to red after prolonged periods in the retail case has not been evaluated in entirety and may be due to a shift in the microbiome of the product after extended periods of oxygen exposure.

Visual color evaluation

Visual color evaluation in meat products can be conducted using both trained sensory panels and consumer sensory panels, each providing unique insights into color perception and acceptance.

Trained sensory panels consist of a small group of individuals who have undergone rigorous training sessions to accurately quantify color characteristics using specific scales (King et al., 2023). Additionally, trained color panelists must be able to discriminate color differences using the Farnsworth-Musell 100-Hue test (King et al., 2023). Panelists assess attributes such as "discoloration" and "redness" of a product (Mancini and Ramanathan, 2020). Their training allows them to provide precise and consistent evaluations, making them valuable for detailed color analysis. In addition to color, trained sensory panelists can also evaluate touch, odor, and taste of products. For odor evaluation, panelists are trained to pick up on oxidized, rancid, and putrid aromas as well as the formation of slime for touch evaluation. When analyzing the taste of products, trained sensory panelists are well versed on identifying the tenderness, juiciness, flavor, texture, beef flavor, and most importantly any off flavors that may be present such as rancidity, oxidation, or sourness.

Consumer sensory panels involve a larger number of untrained participants who evaluate the product based on their willingness to purchase and overall acceptability (Mancini and Ramanathan, 2020). Consumers are often asked of series of questions asking them to rank the sample based on how much they like the appearance, touch, odor, or taste, followed by yes/no questions asking their overall acceptability and whether they would purchase the product or not. Consumer sensory panels provide insights into the everyday consumer's perspective, which is crucial for understanding market preferences and behavior. Both types of panels are essential in color, odor, touch, and taste studies because they offer complementary information. Trained

panels deliver detailed, technical assessments, while consumer panels reflect the practical and subjective views of the general public. Utilizing both methods together provides a comprehensive understanding of spoilage and its impact on consumer choices (King et al., 2023).

Instrumental color evaluation

Color can be measured using Commission Internationale de l'Eclairage (CIE) values of L^* , a^* , and b^* , which determine the spectrum of white to black (L^*), green to red (a^*), and blue to yellow (b^*) using a spectrophotometer Figure 1.2.

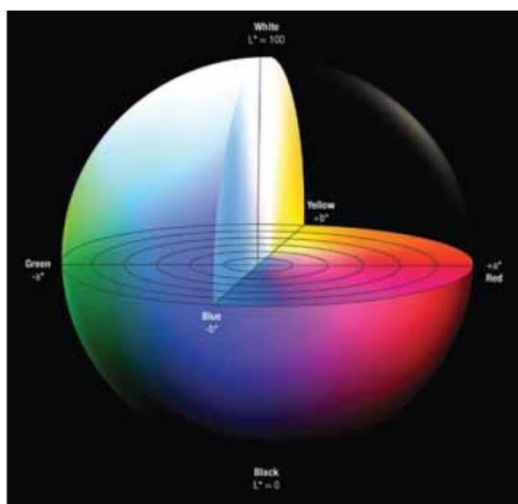


Figure 1.2 Representation of color solid for CIE $L^* a^* b^*$ color space (AMSA, 2012).

These tristimulus values can also be used to calculate different percentages of myoglobin present, such as DMb, OMb, MMb, and COMb, as well as chroma and hue angle (Ramanathan et al., 2020; King et al., 2023). Chroma indicates how vivid the color is, and the hue angle shows the angle at which the sample deviates from the x-axis. Chroma is calculated using the equation $(a^{*2} + b^{*2})^{1/2}$ and the hue angle is calculated as $[\arctangent(b^*/a^*)]$ (King et al., 2023). The spectral data provides data at different wavelengths. With the values determined at different wavelengths, the percentage of each myoglobin redox form can be determined. According to the AMSA Color Guidelines, the isosbestic wavelengths of 572 nm/525nm can be used to calculate

MMb, 610 nm/525nm can be used to calculate OMb, and 474 nm/525 nm can be used to calculate the amount of DMb present in a product (King et al., 2023).

Impact of lipid oxidation on spoilage

The process of lipid oxidation begins during the conversion of muscle to meat and continues to accelerate as the meat ages, affecting color, flavor, and odor (Domínguez et al., 2019). Lipid oxidation is a deterioration reaction that plays a major role in the changes in color and odors observed in raw meat products (Gray et al., 1996; Domínguez et al., 2019). This process involves polyunsaturated fatty acids reacting with oxygen species, triggering a series of secondary reactions that lead to lipid degradation (Amaral et al., 2018). Lipid oxidation occurs in three phases: initiation, propagation, and termination. During these phases, hydroperoxides are produced from the reaction of unsaturated fatty acids with free oxygen (Domínguez et al., 2019). Although hydroperoxides have a short lifespan, they break down into secondary compounds such as hydrocarbons, aldehydes, ketones, alcohols, esters, and acids, which contribute to the off-flavors (rancidity) and odors known as VOCs in fresh meat products (Gray et al., 1996; Domínguez et al., 2019).

Autooxidation, also known as free radical oxidation, commonly occurs in fresh meat when packages are exposed to an oxygen-rich environment, facilitating color changes and producing off-odors and microorganisms (Huang and Ahn, 2019). Oxidation occurs at rapid rates when packages are exposed to an oxygen-rich environment. Lipids significantly contribute to the flavor profile of red meat; therefore, a higher ratio of marbling is likely to increase the flavor profile of a product. According to O'Quinn et al., (2023) intramuscular fat is known to be one of the largest drivers of beef flavor development which has driven the emphasis the industry places on producing high marbling cattle (O'Quinn et al., 2023). However, with a higher lipid profile, the product is susceptible to more rapid oxidation resulting in a faster color change (Domínguez

et al., 2019). Additionally, ground beef is highly prone to lipid oxidation due to increased oxygen exposure and surface area during the mixing and grinding process coupled with the high fat content typically 80% lean and 20% fat (Faustman et al., 2010; Hearn et al., 2021).

The oxidation of fresh meat products can be mitigated by using antioxidants (Gray et al., 1996). Antioxidant compounds often include phenolic antioxidants or compounds derived from natural food products such as fruits, teas, herbs, seeds, spices, and vegetables (Shah et al., 2014; Manassis et al., 2020). Due to their chemical structures, antioxidants scavenge oxidation-initiating radicals, decrease localized oxygen concentrations, decompose peroxides, and chelate chain-reaction-initiating catalysts such as metal ions (Manassis et al., 2020). Extensive research has been conducted on the use of natural antioxidants in ground beef and their effects on shelf-life. Kim et al. (2013) evaluated the effects of a synthetic antioxidant, butylated hydroxytoluene (**BHT**), versus crown daisy leaf, pumpkin leaf, chamnamul, fatsia, leek leaf, bok choy, Acanthopanax, soybean leaf, butterbur leaf and broccoli extracts and their effects on the lipid oxidation of ground beef patties with the used of TBARs and objective color measurements (Kim et al., 2013). Plants were dehydrated, pulverized, and stored at -20°C prior to extraction where water and 70% ethanol were used for extraction. DPPH radical scavenging activity was used to determine the free radical scavenging activity of extracts. Kim et al. (2013) found significant color effects by butterbur leaf and broccoli extracts as well as BHT where a^* values of ground beef depleted at a slower rate when compared to other natural extracts and the control (Kim et al., 2013). In addition to upholding the integrity of ground beef color, BHT and butterbur leaf extract also had the ability to decrease the rate of lipid oxidation (Kim et al., 2013). Nonetheless, this antioxidant study is missing the sensory aspect integrated with the use of antioxidants.

TBA is the main assay used to quantify malondialdehyde (MDA) which is the most important substance produced from the secondary lipid oxidation of fatty acids. TBA is used to quantify lipid oxidation because MDA is a highly TBA reactive substance allowing quantification of the colorimetric measure of the complex formed between TBA and MDA (Dominguez et al., 2019).

Impact of volatile organic compounds on spoilage

Volatile organic compounds (VOCs) are recognized as key indicators of spoilage in raw meat, arising from processes such as lipid oxidation and the activity of various microorganisms present in the product (Bleicher et al., 2022). VOCs are often lipid-derived compounds produced from the oxidation of fatty acids during autooxidation, which can result in an unsuitable aromatic profile if the product's shelf life has expired (Domínguez et al., 2019; Dinh et al., 2021).

Lipid oxidation is not the sole source of VOCs; microorganisms can also contribute to undesirable odors in meat. Stutz et al. (1991) identified *Pseudomonads* and other related gram-negative organisms as the primary microorganisms responsible for producing putrid, fruity, cabbagey, and sulfur-associated odors in refrigerated meats (Stutz et al., 1991). Additionally, Dainty et al. (1983) reported *Pseudomonads* often dominate the microflora in spoiled products and are a common source of "off-odors" (Dainty et al., 1984). In aerobically packaged fresh meat products, the microflora typically includes *Pseudomonas* species such as *P. fragi*, *P. putida*, *P. lundensis*, and *P. fluorescens* (Doulgeraki and Nychas, 2013; Pellissery et al., 2020).

A study conducted by Mansur et al. (2019) used fresh beef round cuts to identify potential spoilage markers in chilled air or vacuum packages. The study used trained sensory panelists to evaluate odor, tenderness, and color, and used headspace solid-phase microextraction coupled with gas chromatography time-of-flight mass spectrometry (HS-SPME-GC-TOF/MS) to analyze VOCs (Mansur et al., 2019). Sensory attributes were analyzed on a five-point scale: 1 =

severely defective (spoiled), 2 = clearly defective (spoiled), 3= moderately/mildly defective (satisfactory), 4 = good, and 5 = excellent. The study found sensory scores for both treatments decreased with increased storage time, becoming unacceptable at 7-11 days of storage. Additionally, the most significant changes in VOCs occurred after seven days of storage, identifying ketones (2-butanone, diacetyl, 2-heptanone, 3-octanone, and acetoin), alcohols (ethanol, 2-methylbuten-1-ol, 3-methylbutan-1-ol, and 2,3-butanediol), and acids (acetic acid) as likely contributors to beef spoilage when exposed to air (Mansur et al., 2019).

Impact of bacteria on spoilage

Not only is microbial evaluation crucial to ensuring food safety but is one if not the most popular method to identify spoilage within a raw meat product due to the ideal environment meat provides for pathogenic growth. Ground beef is known to have a higher initial microbial load than whole muscle cuts as the increased surface area combined with the grinding process allows for a greater contamination rate (Pohlman et al., 2009). Despite the ever-increasing effort to mitigate microbial contamination within a product, inevitable recontamination will occur during the grinding process as newly exposed surfaces will facilitate the spread of microorganisms (Pohlman et al., 2009). The initial microbial load and microflora of a ground product is mainly determined by the physiological status of the animal at the time of slaughter and the hygienic integrity of the processing facility (Pellissery et al., 2020). Spoilage organisms are classified into different groups such as Gram-positive spore formers, Gram-negative rods, lactic acid bacteria (**LAB**), along with yeasts and molds (Pellissery et al., 2020). Moreover, while it is not common for fresh meats to be spoiled by yeasts and molds due to their inability to produce extracellular proteases, *Alternaria*, *Aspergillus*, *Fusarium*, *Rhizopus*, and *Cladosporium* could potentially play a role in mold growth (Jay et al., 2008; Pellissery et al., 2020).

Research has established five categories of ecological determinants known as intrinsic, processing, extrinsic, implicit, and emergent effects (Nychas et al., 2008). The ecological determinants influence the establishment of microbial association within a product and determine the climax population known as the ephemeral of specific spoilage microorganisms allowing for proliferation in available niches (Nychas et al., 2008). Dependent upon the storage condition, different microbes dominate in the fresh meat microflora at 0-4°C. When packages are exposed to the air in an environment, *Pseudomonas* spp. is known to dominate the microflora and typically causes slime formation within the environment (Nychas et al., 2007; Nychas et al., 2008). Wickramasinghe et al. (2019), states slime formation due to *Pseudomonas* typically occurs with the microbial load has reach 10^7 or 10^8 CFU/g however, the time it takes for a product to reach this load of microbes can differ depending on various attributes of the meat product (Nychas et al., 2008; Wickramasinghe et al., 2019). Additionally, packages stored in CO₂ environments have predominantly lactic acid producing bacteria in the microflora which can lead to souring in processed meats such as ham but is not known to have any spoilage effects in fresh meat products (Nychas et al., 2007; Nychas et al., 2008).

As time progresses, the microorganisms present in the product will continue to grow and replicate with nutrients found in the optimal environment provided by the fresh meat product producing undesired organoleptic changes (Hanna et al., 1983; Nassos et al., 1983). In a study conducted by Nassos et al. (1983), eight ground beef samples of different fat to lean ratios packaged with in relatively low oxygen permeable casings were evaluated for bacterial counts (Nassos et al., 1983). The fat content varied from very lean (12 – 14%), lean fat content (15 – 19%) and medium fat content (22 – 25%) (Nassos et al., 1983). Portions of 50g were removed from the packages of ground beef on day 0 and at 3- and 4-day intervals ending with a total of 6

samples for microbiological analysis, sensory analysis. Sensory panelists were asked to evaluate odor and appearance of the samples on a nine-point hedonic scale (1: dislike extremely; 9: like extremely). The study determined as the age of ground beef increased, the microflora shifted from gram-negative to gram-positive organisms. Characteristically, 95% of the gram-positive microorganisms found in the microflora produced lactic acid (Nassos et al., 1983). Lactic acid and bacterial count had a significant correlation. LABs were shown to inhibit the growth of gram-negative bacteria in the absence of oxygen where the microflora was approaching 100% of the total microflora in vacuum-packaged samples (Pierson et al., 1970; Nassos et al., 1983).

Determining the meat microbiome with DNA

In addition to enumeration of microbes within the meat, genomic DNA extraction can also be conducted using various methods in order to determine the microflora of the fresh meat product (Yalçinkaya et al., 2017). A study was conducted demonstrating the benefits and challenges of different DNA extraction methods on fresh meat, one of those being the Qiagen method (Yalçinkaya et al., 2017). For all DNA extraction, the integrity of relative quantity is determined using gel electrophoresis where the Qiagen method exhibited high integrity when demonstrating relative abundance of the DNA. Images of DNA present from the different methods of extraction are shown in Figure 1.3.

With the use of DNA extraction, we are able to study the bacterial communities. Hanlon et al. (2021) conducted a study comparing four packaging treatments as well as different lighting treatments on ground beef (Hanlon et al., 2021). The four packaging treatments consisted off high-oxygen MAP, CO MAP, vacuum roll stock ouches, and traditional overwrap packages. The different lighting treatments consisted of fluorescent, light emitting diode, and darkness. Along with microbial enumeration, Hanlon et al., (2021) conducted 16S ribosomal RNA sequencing of DNA to further investigate the microbial profile (Hanlon et al., 2021). Ground beef was sampled

at fabrication, after a 20 day dark storage period and at the end of a 3 day retail display period in their respective lighting treatments. While Hanlon et al., (2021) was able to determine the predominant microflora of ground beef particularly under fluorescent light conditions is *Carnobacterium* with *Pseudomonas* and *Lactobacillus* also accounting for a good portion of the microbiome, there was no significant shift in the bacteria present dependent upon storage or lighting treatment (Hanlon et al., 2021). The study conducted by Hanlon et al., (2021) is very foundational in evaluating the microbiome of fresh meat with the use of DNA sequencing, however, the challenge occurs with lack of sensory evaluation within the study. More data must be collected evaluating the microbiome and consumer opinion as fresh meat ages in the retail case.

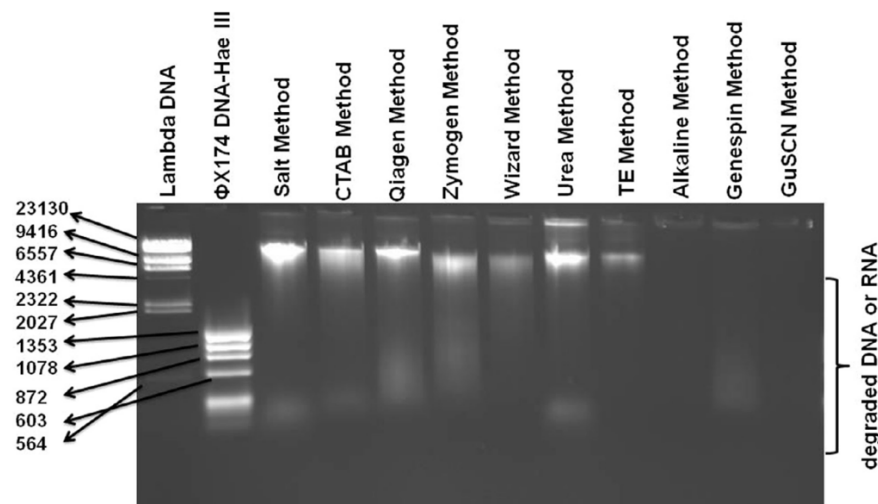


Figure 1.3 Comparison of different genomic DNA isolation methods analyzed by gel electrophoresis and visualized under UV light (0.8% agarose) (Yalçinkaya et al., 2017).

Additionally, Smith et al., (2024) evaluated the effects of the spoilage microflora in beef and the effects of color stability. Within this study *longissimus lumborum* (color stable) and *psoas major* (color liable) muscles were displayed in an aerobic retail condition where trained sensory panels, metmyoglobin reducing activity (**MRA**), and 16S ribosomal RNA (**rRNA**)

amplicon sequencing was performed. Visual panels were utilized an 8-point scale with 1 = extremely bright, cherry red, and 8 = extremely dark red. Discoloration was also evaluated by trained sensory panelists on a scale of 0% to 100% (Smith et al., 2024). As a result of rRNA sequencing Smith et al., (2024) found no differences in microbiome between muscles, however, they did find a shift in microbiome across display day (Smith et al., 2024). While this study proved there are not differences in spoilage microflora between muscles with different color stability, Smith et al., (2024) failed to evaluate the consumer opinion in conjunction with the spoilage microflora and more research remains in the area of ground muscle (Smith et al., 2024).

Impact of different packaging types on spoilage

Microbial growth and color are significantly influenced by the type of packaging used as well as display and storage conditions. However, consumers' purchasing decisions are primarily affected by the package type, as it conveys the perceived quality of the product on the shelf (Carpenter et al., 2001). There are three primary methods for packaging fresh meats: aerobic, vacuum, and modified atmosphere packaging (**MAP**). Within MAP, different gas concentrations have been formulated such as: high-oxygen MAP (80% O₂, 20% CO₂), low-oxygen MAP (30% CO₂, 70% N₂) and low-oxygen carbon monoxide MAP (30% CO₂, 0.4% CO, and 69.6% N₂) (McMillin, 2017). The most common retail packaging is an aerobic package type using polyvinyl chloride film (**PVC**), which promotes the expected bright, cherry-red color. However, prolonged exposure to oxygen causes OMb to quickly convert to MMb, leading to a color change (Rogers et al., 2014). In traditional PVC overwrap packages, the color is highly susceptible to change as oxygen penetrates the surface. Over time, the perceived bright-cherry red color transitions to brown due to MMb formation through autooxidation (Mancini et al., 2022). Although aerobic meat packages initially produce a bright, cherry-red color by allowing myoglobin and oxygen to

bind rapidly, the reducing capacity is quickly exhausted, resulting in earlier production of MMb, higher spoilage rates, and discoloration (McMillin, 2017).

Vacuum packaging, on the other hand, allows for slower oxidation rates due to the absence of oxygen. Without oxygen, DMb becomes the dominant form of myoglobin, making vacuum-packaged meat appear less appealing to consumers at retail due to its purplish color. To address this, MAP has been developed, allowing PVC packages to remain in a controlled environment for extended periods before retail display (Mize and Kelly, 2004).

MAP modifies the package environment to reduce bacterial growth and spoilage, often used in "motherbags" containing multiple retail packages. Motherbags are not opened until ready for shelf placement. The gases used in MAP include nitrogen, carbon dioxide, oxygen, and small amounts of carbon monoxide, the latter approved by the Food and Drug Administration to be up to 0.4% of the gas mixture since 2002 (Brooks et al., 2007; Cornforth and Hunt, 2008; Hearn et al., 2021). MAP can decrease the growth of *Listeria sp.*, *Salmonella sp.*, and other microorganisms, aiding in color preservation and extending the shelf life and consumer appeal of fresh meat (Stratakos, 2011). However, in the United States, all retail packages must be removed from a controlled environment including CO before being placed on the retail shelves (Boeckman, 2006).

Hunt et al. (2004) evaluated the effects of PVC and MAP packaging on ground beef and four different muscles (Hunt et al., 2004). The muscles compared in the study were *longissimus dorsi*, inside *semimembranosus*, outside *semimembranosus*, and *psoas major*. They compared PVC-wrapped packages that had been previously stored in MAP with CO to packages without prior MAP exposure, sampling microbial counts at the beginning and end of the display period (Hunt et al., 2004). Steaks stored in MAP were stored for 7, 21, and 35 days while ground beef in

MAP was stored for 7, 14, and 21 days. In addition to microbial sampling, Hunt et al. (2004) also completed sensory panels to evaluate the visual appearance and odor of the samples (Hunt et al., 2004). The study found ground beef with acceptable color had less than 6 logs of microbial growth, while packages with unacceptable color had greater than 7 logs of microbial growth. However, microbial growth was only sampled at the beginning and end of the study, not throughout the 7-day shelf life, showing little correlation between visual color scores and aerobic plate counts (Hunt et al., 2004). As shown in Figure 1.4, there was little to no correlation between the APC log CFU/g and the visual color scores of samples. Despite these limitations, the study concluded that MAP provides ground beef with acceptable color for longer periods, thus extending its shelf life in the retail case (Hunt et al., 2004).

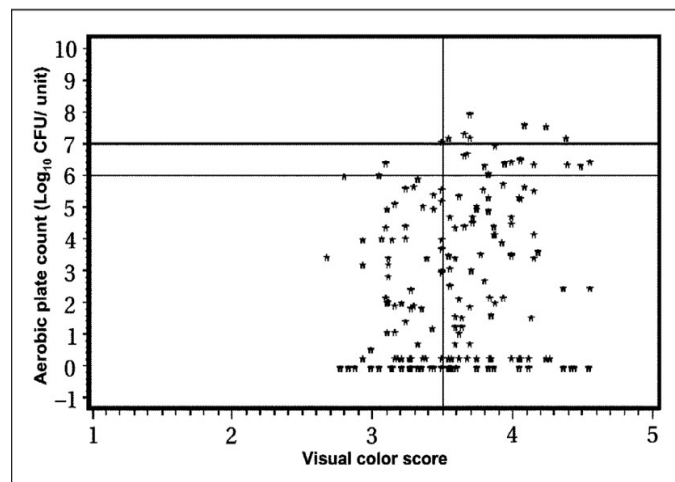


Figure 1.4 Scatter plot of data collected from Hunt et al. (2004) depicting the relationship of visual color score (x-axis) and aerobic plate count (y-axis) (Hunt et al., 2004).

Once an oxygen-permeable package is exposed to high amounts of oxygen, autooxidation occurs, causing shifts in ligand binding to myoglobin. The industry has conducted many packaging studies to evaluate how to preserve the bright cherry-red color, extending the time products remain appealing in the retail case. Rogers et al. (2014) studied ground beef packages,

testing five different packaging types in 0-2°C and 10°C environments. They concluded ground beef packages tend to discolor at different rates depending on the package type and storage temperature (Rogers et al., 2014). Regardless of the package type, temperature abuse accelerated oxidation and microbial growth rates (Rogers et al., 2014). Colder temperatures tend to slow discoloration by inhibiting organisms competing for oxygen, such as mitochondria and microorganisms (Ramanathan et al., 2020)

Conclusion

Despite ground beef being a popular choice among consumers, much of the industry's research focuses on whole muscle cuts rather than ground products. This oversight is significant, as meat spoilage is a major contributor to the high percentage of global food waste. The spoilage mechanisms in ground beef are complex and not yet fully understood. Consequently, this study aims to establish the thresholds for various spoilage factors, providing a comprehensive definition of beef spoilage from both consumer and industry perspectives. By achieving these objectives, we hope to offer the meat industry valuable insights for better product management, ultimately increasing the shelf life of beef and aligning it with consumer expectations of freshness and quality.

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Chapter 2 - A Fresh Look at Ground Beef Spoilage

Introduction

The versatility, cost-effectiveness, and convenience of ground beef has resulted in a recent increase in popularity among consumers, setting ground beef as a valuable segment of the beef sector (Speer et al., 2015). The United States is the world's largest consumer of beef, with total pounds of fresh ground beef sold increasing by 2.5% in 2024 (NCBA, 2024a). Additionally, in 2023, ground beef sales accounted for 50.6% of all retail beef sales in terms of pounds sold in the United States (NCBA, 2024b), providing additional evidence of the significance of ground beef and its economic value to the beef industry.

Spoilage can be defined as “a dynamic process and is affected by the intrinsic and extrinsic attributes of meat” such as chemical composition, water activity, animal husbandry, initial microbiota, and storage rendering it unfit/unappealing for human consumption (Yang and McMullen, 2024). With such a vast array of factors affecting the spoilage process of meat products, it is challenging to determine the cause and point of spoilage within a meat product (Gill, 1983). Thus, numerous assessments are used to evaluate the spoilage of meat products, but microbial evaluation is among the most common. Current and past research has focused on the microbial aspect of spoilage (Butler et al., 1953; Hunt et al., 2004; Smith et al., 2024) with failure to link the causation of product deterioration at a certain time point. There are no current studies that evaluate the consumer opinion in conjunction with microbial analysis. Spoilage is assessed by consumers every time they make the decision to purchase a product from the retail case based on the appearance leading to high amounts of product discard (Ramanathan, 2022; Lybarger et al., 2023).

Meat color is the number one purchasing motivator influencing consumers buying beef in the retail case (Lybarger et al., 2023). Red meat color relies on the protein myoglobin and the rate of oxidation resulting in metmyoglobin (**MMb**). MMb is the myoglobin form responsible for the discolored brown color (Mancini and Hunt, 2005). Once the myoglobin protein has fully oxidized, the product will remain in the brown discolored state and consumers' purchase intent will drastically decrease (Lybarger et al., 2023). As a result, the economic impact from the amount of discolored meat discarded annually in the United States is estimated at about \$3.73 billion equating to approximately 780,000 head of beef animals annually (Ramanathan, 2022).

While previous research has determined consumers will reject discolored product in the retail case, (Hood and Riordan, 1973; Holman et al., 2016; Holman et al., 2017; Lybarger et al., 2023) the complexity of spoilage in conjunction with the consumer opinion is limited. In 1953, a study conducted using inoculated strip steaks by (Butler et al., 1953) established log 7 colony forming units (**CFU**)/g as the threshold to determine when meat is considered spoiled. Since then, this threshold has been used as a benchmark for industry when considering beef shelf-life and spoilage. However, few subsequent studies have evaluated whether this proposed threshold corresponds with consumer assessment of spoilage, despite the widespread acceptance and use. Moreover, literature evaluating consumer perceptions of ground beef spoilage are lacking, especially those that have attempted to pair consumer perceptions of spoilage with objective measures of product quality.

Therefore, the objective of this study was to determine the point at which ground beef reaches spoilage as determined by consumers relative to the microbiological, lipid oxidation, color measurements, and the meat microbiome.

Materials and Methods

The procedures for the use of human subjects in the sensory panel evaluations in this study was approved by the Kansas State University (KSU) Institutional Review Board (IRB 7440.9; December 2023).

Sample Collection

This study utilized 256 454-g ground beef (80% lean) packages obtained from a commercial case-ready facility located in the Midwest. Prior to display, samples were transported to the Kansas State University (**KSU**) meat laboratory under refrigerated temperatures (2 – 4°C). Four individual ground beef packages were stored in each tri-gas flushed mother bag (69.6% N, 30% CO₂, 0.4% CO), and stored under 2 – 4°C in the absence of light. For each mother bag, paired packages were randomly designated a display date (0, 2, 4, 6, 8, 10, 12, and 14d). One sample from each pair was designated for color and taste analysis, while the other paired sample was designated for sensory evaluation of smell and touch as well as microbial, lipid oxidation, objective color, and microbiome analysis.

On the designated display date, samples were placed into a designated section (A-I) in three coffin-style cases (model DMF8; Tyler Refrigeration Corp., Niles, MI) at 2 – 4°C under continuous fluorescent lights (32 W Del-Warm White 3,000 K; Phillips Lighting Company, Somerset, NJ) averaging $2,143 \pm 113$ lx emission case-wide. The cases were programmed to defrost once per day in the morning to avoid any fogging of packages during evaluation times. Samples were placed in the case at the same time every other day and rotated every 24 h.

Trained sensory panel evaluation

Trained sensory panelists evaluated color, smell, touch, and taste of all samples. Sensory panelists were trained according to the American Meat Science Association (**AMSA**) meat color measurement and sensory evaluation guidelines (American Meat Science Association, 2016;

King et al., 2023). Sensory panelists were trained with 100 – point line scales visually anchored with two end points for all analyses. All panelists were also subjected to the Farnsworth – Munsell 100 Hue Color Vision Test (Munsell Color X-Rite, Grand Rapids, MI) to screen for color blindness. The trained panelists were asked to record their answers on electronic tablets (Fire HD 8; Amazon, Seattle, WA) utilizing a digital survey (Qualtrics Software, Provo, UT).

For visual panels, panelists evaluated discoloration and redness of samples using methods and anchors outlined by Lybarger et al. (2023). During training sessions, panelists were trained using samples varying in discoloration and redness that represented the entire scale. On evaluation days, panelists were given an hour time period to evaluate samples. A minimum of 8 trained sensory panelists evaluated 32 samples for visual, touch, and odor evaluation on each day. Moreover, 8 trained sensory panelists were fed 8 samples per panel session, one from each display period (0, 2, 4, 6, 8, 10, 12, 14d).

For touch sensory panels, approximately two 2.54 x 2.54 cm pieces of the ground beef loaves were placed in small mason jars and stored at 2 – 4°C. One mason jar was allocated for touch, and the other was allocated to odor. Panelists were trained in multiple sessions using samples that represented a range of display periods and variations in touch characteristics on a 100 - point continuous line scale with two anchors and a “non-applicable” option. 0 represented a characteristic beef texture while 100 represented a non-characteristic beef texture. Panelists were given a multiple-choice question asking, “If the touch is non-characteristic, what does the sample feel like?”. The answer choices provided were slimy, dry, tacky, or an option to type in their own answer. These samples were evaluated in individual sensory booths under low-intensity (<107.64 lumens) red incandescent lights to mitigate bias related to visual appearance.

Once trained sensory panelists touched all samples, they were asked to evaluate the odor of the samples. Panelists were trained on odor using verbally anchored 100-point continuous line scales in conjunction with the touch training sessions. The line scale consisted of two anchors where 0 represented no off-odor present and 100 represented an extreme off-odor present with a “non-applicable” option. Sensory panelists were asked to describe the odor with a multiple-choice question stating, “If there is off-odor present, what does it smell like?”. The answers provided were rancid, sour, oxidized or the option to type in their own answer. Different samples were utilized for touch and odor evaluation under the same red lighting conditions.

For taste, trained sensory panelists were familiarized with scales and trained in 6 sessions in the weeks immediately prior to evaluation using the methods and anchors described by Davis et al. (2021). Prior to cooking, samples were thawed 24 h in advance at 2°C – 4°C and split in half creating 113.3g portions and then pressed into 1.2 cm thick patties using a handheld patty maker. Patties were cooked to an end peak temperature of 82.2°C on a Cuisinart Griddler Deluxe clam-shell style grill (Stamford, Connecticut, USA) set to a surface temperature of 177°C. To reach the optimum end point temperature, patties were pulled from the grill at 79.4°C assessed by a Beckman Industrial Doric 205 thermocouple thermometer (Brea, California, USA). Patties were cut into 8 equal wedges and served to trained panelists in a random order. A representative ground beef sample was used at the beginning of each panel to allow for calibration and prevention of panelist drift. Each sample was evaluated for juiciness, tenderness, texture, beef flavor intensity, and off-flavor intensity. Trained sensory panelists evaluated samples in individual sensory booths in the same red-light conditions when evaluating touch and odor. Water, apple slices, and unsalted crackers were provided as pallet cleansers along with an expectorant cup and napkin.

Consumer sensory panel evaluation

Consumer sensory panelists ($N = 128$ / panel type) were recruited from Manhattan, KS, and neighboring communities and monetarily compensated for their participation. Consumers were asked to visually evaluate samples in the retail case, as well as smell and touch samples in sensory booths under the same red-light conditions as the trained sensory panelists to prevent any bias against the color of the samples for these evaluations. For all sensory evaluation, each consumer evaluated 8 samples, one from each day of display (0, 2, 4, 6, 8, 10, 12, and 14d) in a random order. A continuous 100 – point line scale was provided to assess the desirability of the sample as described by Lybarger et al. (2023) and Davis et al. (2021). Consumers were asked a yes/no question if they would purchase the package based on the appearance, touch, odor, and taste, and a yes/no question if they would consider the package spoiled based upon the appearance, touch, odor, or taste.

Consumers evaluated touch and odor samples in separate mason jars, which also differed from the trained sensory panel samples. In sensory booths under red-light conditions, consumers were given the mason jars without the lids on and asked to touch the samples by gently feeling the surface. Consumers were handed an additional 8 mason jars with the lids on to assess the odor and asked to place the lid back on the sample before passing it to the next consumer. Samples were split into 113.3g portions, vacuum packaged, and froze immediately following visual, touch, and odor sensory evaluation in -20°C .

Consumer taste evaluation was conducted several months later. The same thawing and cooking protocol was followed as outlined for trained sensory panel analysis. Consumers evaluated samples under fluorescent lighting in a large lecture-style classroom. Four sessions of 24 consumers and two sessions of 16 consumers were conducted within the same week. Each panel lasted approximately 1h. In addition to their electronic ballot, each consumer was supplied

with water, apple juice, and unsalted crackers to cleanse their pallets as well as an expectorant cup and napkin.

Objective color measurements

Before each trained sensory panel and approximately 30-minutes after being placed in the retail cases, instrumental color was measured on each sample. Commission Internationale de l'Eclairage (CIE) L^* , a^* , and b^* values were collected using a Hunter Lab Miniscan spectrophotometer (Illuminant A, 2.54 cm aperture, 10° observer, Hunter Lab Associates Laboratory, Reston, VA) using methods outlined in the AMSA Color Guidelines (King et al., 2023). Three separate scans were taken on the surface of the paired ground beef sample designated for visual evaluation and the values were averaged. Moreover, spectral data were recorded to calculate hue angle, chroma, percent oxymyoglobin (**OMb**) and percent MMb according to the AMSA Color Guidelines (King et al., 2023).

pH

On each evaluation day, the 2 d0 samples designated for lab analysis were measured for pH utilizing a Mettler Toledo pH meter (Columbus, OH) calibrated according to manufacturer's specification previously outlined by Hammond et al., (2022). Five g of each sample, in duplicate, previously froze in liquid nitrogen and powdered, was weighed into a 100 mL beaker. Fifty mL of MQ water was then added to each beaker and the sample was mechanically homogenized for 20 seconds at 10,000 rpm (Homogenizer 850; Fisher Scientific International, Hampton, NH). The pH of the sample was read and recorded.

Microbiological

Microbiological analysis was conducted on all four evaluation days with Aerobic Plate Counts (**APC**), *Enterobacteriaceae* plate counts (**EB**), and *Escherichia coli* (***E. coli***) Coliform plate counts (**ECC**). Samples from 0, 2, 4, 6, 8, 10, 12, and 14d were evaluated. Once the paired

sample designated for lab analysis was brought to the laboratory, 25g of the sample was weighed with sample collected all four corners and the center of the package, being sure to include samples from the surface of the product. Individual sterilized scoopulas were used for each sample. The 25g sample was combined with 225mL of peptone water (**PW**) and stomached for 60 seconds (Stomacher 400, Seward, Bohemia, NY). Serial dilutions were produced for each sample using PW and duplicate 3M Petrifilm™ Aerobic Count Plates (**AC**), 3M Petrifilm™ EB, and 3M Petrifilm™ ECC were plated with 1 mL of each dilution. AC Petrifilms™ were incubated (Incubator F Air 6.3CF, VWR, Radnor, PA) at $35 \pm 1^\circ\text{C}$ for 48 h and EB Petrifilms were incubated at $30 \pm 1^\circ\text{C}$ for 24 h. AC and EB Petrifilms™ were evaluated following the incubation period per the manufacturer's guidelines. Furthermore, ECC Petrifilms™ were incubated at $30 \pm 1^\circ\text{C}$ for 24 h then evaluated for *E. coli* coliforms according to the manufacturer's protocol.

Lipid oxidation

Lipid oxidation was determined using the Thiobarbituric acid reactive substances (**TBARS**) assay following the procedures outlined in Ahn et al. (1998) and similar to previous procedures done at KSU (Dahmer et al., 2022; Beyer et al., 2024). In summary, approximately 0.2 grams of a previously powdered sample was weighed and added to 2 mL bead tubes with 1.4 mL of Thiobarbituric acid/trichloroacetic acid (TBA/TCA) and 0.1 mL butylated hydroxytoluene (**BHT**), homogenized (D2400 Homogenizer, Benchmark Scientific, Edison, NJ), and then centrifuged at $3,000 \times g$ for 5 min. Supernatant was removed from the sample tube and placed in a separate tube that was then covered with aluminum foil and incubated for 30 min at 70°C . Samples were chilled in an ice bath for 5 min and placed into a 96-well plate along with 0.2 mL of MDA concentration standards and read using a spectrophotometer and 532 nm. The final

MDA concentration was calculated and expressed as μM malonaldehyde using the standard curve developed.

DNA extraction and genome sequencing

A microbial DNA sample was extracted from each package of ground beef using Qiagen™ DNeasy Mericon Food standard protocol (Qiagen, Germantown, MD). Two g samples of ground beef was combined with a food lysis buffer and proteinase K solution, vortexed, then incubated (MaxQ Shaker, ThermoFisher Scientific, Waltham, MA) for 1 h with constant shaking at 100 rpm at 60°C. Samples were cooled on ice for 5min and centrifuged at 2,500 x g for 5min. Supernatant was removed from the sample tubes, combined with chloroform, vortexed, and centrifuged at 14,000 x g for 15-min. The upper aqueous phase of the sample was removed and added to a buffer PB and mixed thoroughly. The sample was placed into a QIAquick spin column and centrifuged at 17,900 x g for 1min. An AW2 buffer was added to the spin column and centrifuged with the previous settings described. After each centrifuge, the flow-through was discarded. The spin column was placed in a new 2 mL tube, an EB buffer was added for the final centrifuge at 17,900 x g for 1min and the flow-through was not discarded. Samples were stored at -80°C until further analysis.

Three negative controls (i.e., no samples added to lysis tube) were included at the DNA extraction step to detect potential contaminants in the kits. Additionally, three positive controls (ZymoBIOMICS Microbial Community Standard, Zymo Research, Irvine, CA) were included in the DNA extraction step to verify the extraction efficiency. DNA concentration was quantified with High Sensitivity double strand DNA kit (Thermo Fisher Scientific) using the Qubit 2.0 Fluorometer (Thermo Fisher Scientific) and sent for amplification of the 16S V4 region, library preparation, and sequencing to Novogene Co. (Sacramento, CA). Quality control of amplicons was conducted utilizing an Agilent 5400 automatic capillary electrophoresis system (Agilent

Technologies, Palo Alto, CA). PCR products were pooled in equimolar concentration, end-repaired, A-tailed, and ligated with Illumina adapters. The quality of the pooled library was then quantified using qPCR. After quality control, libraries were sequenced on an Illumina platform (Nocaseq6000 SP_PE250) to produce 250 bp paired end reads. The resulting sequences were analyzed using DADA2 (v. 1.30.0) with quality filtering (Q2), truncation set to 220 bp, and the default overlap for sequence merging.

Compositional data analysis framework, Gloor et al. (2016); Gloor et al. (2017) was used in R (v 4.3.2) to characterize the bacterial microbiota composition of ground beef samples from eight different display periods. All ASVs with “0” count values were assigned a small non-zero value using the Count Zero Multiplicative Method using the R package zCompositions (v 1.5.0.4) Palarea-Albaladejo and Martín-Fernández (2015) prior to applying a center-log ratio (CLR) transformation. To determine whether there was a significant effect of the day of display on the microbiota composition, Aitchison distances were calculated using CLR-transformed data, and Pairwise Permutational Multivariate Analysis of Variance (PERMANOVA) was used with a linear model that included the effects day of display using the R package pairwiseAdonis v0.4.1, Martinez (2017), with 999 permutations. Specifically, the composition of microbiota of samples on display for 0, 4, 6, and 14d. These days were selected, as consumer purchase intent and spoilage rating predictors suggested samples began to drastically change after d4 and completely inverted by d14. *P*-values were corrected for multiple comparisons using the Bonferroni correction. Principal component analysis was performed on CLR-transformed data to visualize differences in bacterial microbiota composition. The first two principal components were plotted to visualize similarity of samples in terms of microbiota composition and relative abundances of

genera were plotted in a bar plot to visualize predominant bacteria. Graphs were plotted in R using ggplot2 (v 3.5.1).

Statistical analysis

The statistical analyses were performed using the procedures of SAS (SAS Inst. Inc., Cary, NC). PROC GLIMMIX was used with treatment comparisons considered significant with an α of 0.05. Paired ground beef samples served as the experimental unit with data analyzed as a completely random design with day of display treatment as the fixed effect. Using PROC LOGISTIC, logistic regression models were calculated for the probability of a sample being identified as “would purchase” and “spoiled” by consumer sensory panelists. PROC CORR was used to determine Pearson correlation coefficients for sensory and objective measurements. The Kenward-Roger adjustment was used in all analyses.

Results

Demographics

Demographic information for the consumer panelists who participated in visual, touch, and odor sensory panels, as well as taste panels is located in Table 2.1. Both groups of consumers were the same size ($N = 128$). For visual, touch, and odor panels, consumers were split between 55.2% males and 45.8% females, whereas the split between males and females was equal (50%) for consumer taste sensory panels. The majority of both groups of consumers were single (69.6% and 54.3%) and reported they had a household size of 1 or 2 people (70.4% and 62.2%). The majority of both groups was made up of Caucasian ethnicity (93.2% and 78.0%). The majority of the first group of consumers reported an annual income less than 50,000 USD (51.2%) while the second group reported an annual income greater than 50,000 USD (52.7%). Both groups of consumers were educated with some college, a college degree, or a post college degree (81.6% and 80.3%) with most consumers under the age of 40 (69.6% and 58.2%).

Moreover, the majority of the consumers reported they consumed beef 1- 6 times per week (69.6% and 78.8%) and purchased beef in the retail setting 1-6 times per month (70.4% and 76.4%).

pH

The average pH of the ground beef product was 5.95. A summary of the overall mean, minimums, maximums, and variation for all independent variables evaluated is provided in Table 2.2.

Objective color measurement analysis

The least-squares means from objective color measurements are presented in Table 2.3. L^* , a^* , and b^* values were all the highest for d0 samples and decreased ($P < 0.05$) as the day of display increased. However, a^* values increased ($P < 0.05$) from d12 to d14 of display. Similarly, percent MMb increased ($P < 0.05$) from d0 to d12, but decreased ($P < 0.05$) from d12 to d14, where there was also an increase ($P < 0.05$) in percent OMb present.

Consumer and trained sensory analysis

Table 2.4 shows the least-squares means for trained sensory panels and consumer ratings of the appearance, touch, odor, and taste of retail ground beef samples. For all sensory traits excluding taste, d0 samples were rated the highest ($P < 0.05$) by consumers, whereas all samples were rated similar ($P < 0.05$) for taste except for d14 samples which were rated the lowest ($P < 0.05$). Consumer purchase intent across all days of display is shown in Figure 2.1 in relation to a^* (A), trained redness scores (B), trained discoloration scores (C), and percent MMb (D). Consumer purchase intent was the most affected by the appearance of the samples with the steepest decline in consumer purchase intent occurring from d4 to d8 of display where there was a decrease ($P < 0.05$) of 81.72%. The a^* values and trained redness scores were closely associated with consumer purchase intent. Conversely, trained sensory panel discoloration scores

and MMb percentages had an inverse relationship with the consumer purchase intent, as expected. The consumer purchase intent of ground beef never dropped below 50%, even after d8 of display based on the consumer taste ratings. Trained sensory panelists did not detect a difference ($P > 0.05$) in beef flavor or off flavor among the days of display. A summary of consumer purchase intent across all days of display for all organoleptic traits measured is presented in Table 2.5.

Table 2.5 also shows a summary of consumer spoilage ratings for the measured organoleptic traits across all days of display. When compared to consumer purchase intent, an inverse relationship was observed (Figure 2.2) for consumer spoilage ratings. Figure 2.2 depicts the consumer spoilage ratings across all days of display for appearance, touch, odor, and taste compared to a^* (a), trained redness scores (B), trained discoloration scores (C), and percent MMb (D). The largest increase ($P < 0.05$; 78.23%) in consumer spoilage ratings occurred between d4 and d8 based on the appearance. Trained discoloration scores and percent MMb strongly aligned with the consumer spoilage ratings, whereas a^* and trained redness scores were inverse. For both odor and taste, there was less than a 50 and 20% chance, respectively, consumers would rate the samples as spoiled across all days of display.

Microbiological analysis

Results encompassing the microbiological analyses of APC, EB and ECC relative to the day of display are presented in Table 2.2. Figure 2.3 shows the consumer purchase intent for all evaluated organoleptic traits compared to APC, EB, and ECC counts. While APC counts started at an average of 6.50 log CFU/g on d0, consumers were 97.75% likely to purchase the product. There was only a 3.07% chance of a consumer rating a sample as spoiled on d0 (Figure 2.4). EB and ECC counts were lower than APC counts, with both starting at 3.24 and 1.12 log CFU/g and steadily increased ($P < 0.05$) over the 14-d display period. Between d4 and d6 of display, the

greatest change in consumer purchase intent (81.72%) and spoilage rating (78.23%) occurred based on the appearance while APC counts only increased ($P < 0.05$) 0.06 log CFU/g on average. On d14, APC, EB and ECC counts reached 9.48, 6.49, and 5.25 log CFU/g and consumers were still 55.65% likely to purchase and only 17.94% likely to rate the product as spoiled based on the taste. While the appearance was the most likely to deter the consumer from the sample, the purchase intent and spoilage ratings based upon the taste of the samples did not decrease as severely.

Lipid oxidation analysis

Table 2.2 presents the average lipid oxidation measured by TBARs for retail ground beef across all days of display. As expected, there was a steady increase ($P < 0.05$) in oxidation throughout storage, with d 0 samples averaging 0.22 MDA/kg of ground beef and d 14 samples averaging 0.43 MDA/kg of ground beef.

Logistic regression equations

Table 2.6 and 2.7 show the logistic regression equations generated using the objective measurements to predict consumer purchase intent and the likelihood of a consumer spoilage classification of retail ground beef as spoiled based upon the appearance, odor, touch, and taste of the product. Overall, the models showed all objective measurements were predictors ($P < 0.05$) of consumer purchase intent based upon appearance, with a^* values (Figure 2.5), percent MMB (Figure 2.6), trained sensory panel redness and discoloration scores (Figure 2.7) all having R^2 values of 0.8 or higher. Models demonstrating APC, EB, and ECC thresholds relative to the consumer purchase intent and spoilage ratings based upon appearance can be found in Figure 2.8 and 2.9. Although all objective measurements were predictors ($P < 0.05$), the R^2 values predicting the consumer purchase intent and spoilage classification were much weaker ($R^2 < 0.41$; $R^2 < 0.36$) for odor and touch sensory. Logistic regression equations generated to predict

purchase intent and spoilage classification based on the taste of the product were the weakest models. When using taste to predict purchase intent, the only strong predictor ($P < 0.05$) was the consumers' own flavor scores, but still only accounted for a small percentage (27%) of variation within the model. Only two objective measurements were able to predict consumer spoilage classification relative to taste ($P < 0.05$); APC counts and consumers' flavor scores. Nonetheless, these models proved to be very weak predictors only accounting for 4% and 16% of the variation when predicting spoilage classification.

Specific thresholds for purchase intent and spoilage classification using appearance, odor, touch, and taste as predictors were generated from the logistic regression models. Threshold a^* values of 21.5, 24.74, 27.96, and 30.16 correspond with a 50, 75, 90, and 95% chance a consumer would purchase the product. Percent MMb values of 37.25, 30.80, 24.35, and 19.95% corresponded to a 50, 75, 90 and 95% chance of consumer purchase. Additionally, trained panel sensory discoloration scores showed threshold values of 38.7, 23, and 7.3% corresponding to 50, 75, and 90% chance of consumer purchase intent. A complete representation of all objective measures and threshold values relative to appearance, touch, odor, and taste when predicting consumer purchase intent are shown in Table 2.8. Consumers indicated willingness to purchase ground beef with lower APC values (Figure 2.4) with values of 7.25, 6.7, 6.1, and 5.75 log CFU/g relative to 50, 75, 90, and 95%.

When predicting the odds of a consumer classifying the product as spoiled based upon the appearance, a^* values of 30.14, 27.15, 22.75, and 18.36 were associated with 5, 10, 25, and 50% likelihood of consumer spoilage classification. Moreover, 20.55, 26.8, 35.95, and 45.10% MMb and 1.55, 16.5, 38.45, and 60.40% trained sensory discoloration corresponded 5, 10, 25, and 50% spoilage classification. Consumers were more likely to classify ground beef as spoiled

with higher APC counts with values of 5.30, 5.90, 6.80, and 7.70 corresponding to 5, 10, 25, and 50% spoilage classification. All threshold values for objective measures for appearance, odor, touch, and taste when predicting the likelihood of consumer spoilage classification are shown in Table 2.8.

Pearson correlation coefficients

Pearson correlation coefficients shown in Table 2.9 were calculated using objective measures and the consumer liking responses. Objective color measurements including a^* , percent MMb, chroma, hue angle, trained redness, and trained discoloration, had among the highest correlation coefficients ($P < 0.05$) when compared to consumer appearance scores ($r > 0.91$; $r < -0.91$). Trained discoloration and trained redness were closely related ($P < 0.05$) to the consumer's opinion of appearance of ground beef ($r = 0.96$; $r = -0.96$). Of the microbiological assays, APC counts had a higher association with consumer appearance, touch, and odor ratings than objective color measurements. Nonetheless, consumer taste scores had the lowest correlation coefficient ($r = -0.09$; $P > 0.05$) relative to APC counts.

Consumer touch scores were moderately correlated to objective measurements, with a moderate ($P < 0.05$) correlation with EB counts ($r = -0.53$). Likewise, consumer odor scores had the strongest ($P < 0.05$) correlation with EB counts ($r = -0.56$). However, consumers' taste ratings showed no association ($P > 0.05$) with all objective measures aside from trained beef flavor ratings ($r < 0.22$; $r > -0.15$). Measurements such as TBARS showed a correlation ($P < 0.05$) to most measurements, excluding trained touch and trained taste measurements; however, the associations were relatively weak ($r < 0.46$; $r > -0.43$).

Microbiome analysis

Statistical differences in the microbiota composition between days 0, 4, 6, and 14 were assessed at the amplicon sequence variant (ASV) level for samples collected in the first and

second week of the experiment. Microbiota composition on samples analyzed at d0 did not differ ($P > 0.05$) compared to that on samples at 4, 6, and 14d. However, there was a difference ($P < 0.05$) in microbiota composition of week 1 samples on d4 compared to d14. This is consistent with the decrease in consumer purchase intent and increase in spoilage rating following d4 of display. Due to refined ASV sequencing, minimal differences in the amplified sequences were detected. This may suggest that while there was no overall change in the population, a shift within the population occurred. Figure 2.10 illustrated the relative abundance of ASV sequencing by day of display during weeks 1 and 2. Samples from both weeks indicated *Lactobacillus* and some *Pseudomonas* species were predominant during the first 4 days of display but shifts to primarily different species of *Pseudomonas*. Figure 2.11 illustrates the relative abundance by genus level according to day of display during weeks 1 and 2. Between 0d and 4d *Lactobacillus* was the most abundant genus, with small amounts of *Carnobacterium* present. Throughout the display period, a more apparent shift is shown in genus abundance between d4 and d8, with *Pseudomonas* becoming the predominant genus in samples up to d14. The extraction results should be taken into consideration as the positive control samples proved to excel in *Bacilis*, *Salmonella*, *Escherichia-Shigella*, *Pseudomonas* extraction, and proved to be inadequate in determining *Listeria*, *Staphylococcus*, and *Lactobacillus fermentum*.

Discussion

Ground beef spoilage

Red meat provides the ideal conditions for bacteria growth with its unending supply of nutrients and high water activity. Ground beef has an even higher occurrence of bacterial contamination due to the grind process (Yang and McMullen, 2024). Avoiding increased

bacterial contamination in ground fresh meat products is nearly impossible, therefore spoilage is inevitable (Nychas et al., 2008).

As previously stated, meat spoilage is a complex interplay between color, lipid oxidation, and microbiology. Past and present studies have evaluated color and microbial growth as the main measurement of spoilage (Butler et al., 1953; Hunt et al., 2004; Hanlon et al., 2021; Smith et al., 2024) with a failure to fully identify causation and time frame of the spoilage process. Therefore, the goal of this study was to evaluate multiple objective measures of spoilage and their relationship with consumer acceptability and willingness to purchase. With the use of microbial enumeration, a bacterial threshold of 7-log CFU/g was identified in past literature as a critical threshold for spoilage detection (Butler et al., 1953). The study analyzed the days of storage, color fading, and microbial growth of the steak samples. Correlation coefficients were determined between the index of fading and $\log_{10}$ bacterial count of +0.807 (Butler et al., 1953). With the most change in color fading and percent MMb present in the inoculated steaks at the time of 7-log CFU/g of bacteria, the threshold of spoilage was set, but this proposed threshold has never extensively been reevaluated in subsequent literature (Butler et al., 1953). The Butler et al. (1953) study's lack of subjective and objective organoleptic analysis and use of inoculum fail to encompass critical components used to measure spoilage.

A number of studies have evaluated the color changes under different conditions in conjunction with microbial growth. A previous study evaluated the effects of mother bag storage time, gas concentration, and temperature abuse on ground beef and compared color and odor using objective measurements to the microbial enumeration during a simulated retail display period (Hunt et al., 2004). Their results did not indicate a high correlation between APC counts or trained odor scores with trained color scores (Hunt et al., 2004). While this study contained

trained sensory evaluation, the consumer aspect of the current work was still lacking in their design. In this current study, there was a weak correlation between trained and consumer sensory evaluation and microbial enumeration with all Pearson correlation coefficients below 0.67.

Determining spoilage with organoleptic measurements

Ultimately, the shelf-life of a product is defined by the consumer opinion. Although a product may be safe for consumption, consumer rejection results in product discard. Previous studies have shown the appearance of the product is not the only factor for consumers, but traits such as odor are also very important when determining spoilage in food products such as milk and other dairy or processed products (Lynch et al., 1986; Hunt et al., 2004; Lu et al., 2013). In recent years, a study was conducted to evaluate consumer opinions on the appearance of ground beef in the retail case using two different methods. In phase I, consumers evaluated ground beef in the case from multiple days of display, while in phase II consumers evaluated ground beef samples from only one day of display (Lybarger et al., 2023). While the study generated very strong models for predicting the consumer opinion of color, the study did not evaluate any other objective measurements to gain an understanding of other intrinsic factors compensated in the discoloration of the ground beef (Lybarger et al., 2023). Similar to Lybarger et al. (2023), the visual appearance of the product in the current study was the strongest predictor for consumer purchase probability and spoilage classification probability. While consumers refused to purchase samples due to their appearance and rated them as spoiled, they were not as severely impacted by the touch and odor of the sample and even less influenced by the taste.

The consumer opinion shift for the appearance occurred from d4 to d8 of display. During this same time frame, objective color measurements of a^* , trained redness scores, trained discoloration scores, and percent MMB followed suit. Conversely, microbial counts (APC, EB, and ECC), while steadily increased, did not appear to be the most critical factor in deeming the

product as spoiled to the consumer, with APC counts beginning at nearly 7 log CFU/g formerly identified as the spoilage threshold. Although drastic changes in microbial counts did not occur, the shift of the ground beef microbiome proved a transition from anaerobic species (*Lactobacillus*) to aerobic species (*Pseudomonas*). While both *Lactobacillus* and *Pseudomonas* are psychrophilic organisms, *Lactobacillus* is a gram-positive bacteria capable of fermenting sugars into lactic acid (Laranjo et al., 2019). Thus, *Lactobacillus* is commonly used in fermented foods such as yogurt, cheese, and fermented meats (Laranjo et al., 2019). LAB producing bacteria's increase the safety of fermented meat products by means of rapid matrix acidification and production of antimicrobial substances (Laranjo et al., 2019). In the current study, when the microbiome was predominantly *Lactobacillus* species within the early display period, *Pseudomonas* were likely inhibited by the LAB producing bacteria.

Pseudomonas, a gram-negative bacterium, is commonly labeled as the “spoilage bacteria” causing slime formation after a period of time (Nychas et al., 2007). *Pseudomonas* can diversify into multiple food spoilage species such as *P. fluorescens*, *P. putida*, *P. fragi*, *P. lundensis*, and *P. weihenstephanensis* in ground beef products (Hilgarth et al., 2019; Kolbeck et al., 2021). *Pseudomonas* contribution to meat spoilage is high due to the microbe's proteolytic and lipolytic enzymes which initiate organoleptic changes to the product once released into the cell (Kolbeck et al., 2021). In the current study, as ground beef was stored and displayed in the retail setting for extended period of time, the microbiome shifted to predominantly *Pseudomonas*.

Previous studies have shown the type of packaging type does not have an effect on the microbiome but with increased storage time APC counts will also increase (Hunt et al., 2004; Hanlon et al., 2021). DNA samples from week 1 and week 2 were compared to acknowledge the

microbiome had an extended storage period in low oxygen which may have had an impact on the microbiome dynamics for week 2 samples. Based upon this, we felt it was appropriate to evaluate the weeks separately to account for the influence. Although there was a significant difference between week 1 and week 2 at the ASV level, the same trend held true at the genus level. The samples from week 2 had greater amounts of *Lactobacillus* species growth early in the display period after being stored in the motherbag for an extra week. A higher percentage of *Lactococcus* and other *Pseudomonas* species were detected in week 2, late in the display period. During this shift in microbiome, consumer purchase intent probability increased, and the spoilage rating probability decreased. The next noticeable shift occurred from d12 to d14 of display when it appeared the ground beef samples turned back to a red color after fully reaching the MMb state. Additionally, the a^* values for d14 samples were similar to those values recorded for those samples from d6 of display. Previous literature describes this color as a red derivative caused by microbes such as *Staphylococcus* strains and *Pseudomonas* (Morita et al., 1996). However, there was no detection in the meat microbiome of *staphylococcus* in this study, though *Pseudomonas* was the primary microbe present at this time, potentially causing the observed re-reddening of the product. Although, myoglobin plays a crucial role in the change in color of red meat products, lipids are also vitally important (Wang et al., 2021).

While lipids are known to deteriorate the product quicker, they are also essential for palatability due to their role of flavor formation in beef products (Kerth and Miller, 2015; Wang et al., 2021). The shift in color from OMb to MMb is most likely accounted for by lipid oxidation (Gray et al., 1996; Domínguez et al., 2019). TBARS results increased over time, but there was little to no correlation as to when consumers or trained sensory panelists detected oxidation in the aroma or taste of the product. With samples being 80% lean, 20% fat, the lipid oxidation

amounts would be expected to be high, especially with an exaggerated display period in the retail case. Irwin and Hedges (2004) highlights samples high in fat could be problematic when utilizing the TBARS method where turbidity can be a problem. Notably, the recovery of MDA using this method may be incomplete (Irwin and Hedges, 2004). The method of TBARS has been revisited in recent years determining the absorbances of the wavelengths were readable with only a 540nm plate rather than the use of and incubator/shaker (Kerth and Rowe, 2016). A study evaluating the relationship between lipid content in ground beef patties and the rate of discoloration reached an average of 1.05 mg MDA/kg for TBAR analysis of 80/20 ground beef (Wang et al., 2021). The lipid content only explained 28 and 48% of the variation when predicting the trained discoloration percentage and a^* values (Wang et al., 2021).

In previous studies evaluating the *longissimus lumborum* muscle, TBARS values only reached 0.2 mg MDA/kg with no display treatment and reached up to 0.5 mg MDA/kg after being stored in a high oxygen MAP environment and a 6d display period (English et al., 2016; Decker et al., 2024). Ground beef is naturally more susceptible to higher oxidation rates where previous ground beef studies reported 0.96 mg MDA/kg after a 42d subprimal age period and 24h retail display period (Garner et al., 2014). Comparatively, the current study showed oxidation only as high as 0.43mg MDA/kg following a 14d display period. Due to lack the lack of consistency in TBARS measurements across studies, other methods to determine the lipid oxidation rates of fresh meat should be explored to determine a more specific result of the lipid oxidation occurring.

Although odor, touch, and taste, were also used to generate prediction models in this study, the models were much weaker than those generated by the appearance of the samples. Surprisingly, the weakest of the models was those generated from the consumer's opinion of the

taste. From d0 to d14 of display, there was only a 9.27% difference in the probability a consumer would purchase the sample, and an 8.32% difference in the chance consumers rated a sample as spoiled based upon the taste of the sample. Trained panelists also found no differences in the off flavors from ground beef displayed for extended periods of time. From this we can determine, consumers are the most likely to say a ground beef product is spoiled based upon the color of the product. Consumers did detect a difference in the odor of our ground beef samples, however the prediction models generated were not as strong as those generated by the appearance of the samples. Consumers were 46.47% less likely to purchase the product on d14 as compared to d0 and 42.19% more likely to rate a sample as spoiled on d14 compared to d0 of display based upon the odor.

Conclusion

Overall, spoilage is determined by the consumers' opinion based upon the intrinsic and extrinsic factors taking place within the ground beef product. The visual appearance of the red meat product proved to be the best predictor based upon objective measurements and the consumers' opinion of spoilage. These results indicated that the most relied upon indicator of spoilage to consumers is the appearance of the product. Although an apparent shift in the microbiome occurred, there was no evidence that microbial growth caused the shift in color from red to brown. Thus, future research must be conducted to demonstrate a more indicative measurement of lipid oxidation. Additionally, future research may include the inoculation of specific microbial species to determine the overall effect of spoilage on ground beef color.

Table 2.1 Demographic characteristics of consumer panelists from visual, touch, and odor evaluation ($N = 128$) and taste evaluation ($N = 128$)

Characteristic	Response	Percentage of consumers (visual, touch, odor)	Percentage of consumers (taste)
Gender	Male	55.2	49.6
	Female	44.8	50.4
Household size	1 person	36.0	23.6
	2 people	34.4	38.6
	3 people	8.0	11.8
	4 people	8.8	0.0
	5 people	5.6	9.4
	6 people	6.4	6.3
	> 6 people	0.8	10.2
Marital status	Married	30.4	45.7
	Single	69.6	54.3
Age	< 20	15.2	16.5
	20-29	46.4	29.1
	30-39	8.0	12.6
	40-49	0.8	14.2
	50-59	12.0	11.0
	> 60	17.6	16.5
Ethnic origin	African American	0.0	3.9
	Asian	0.0	10.2
	Caucasian/white	93.2	78.0
	Latino	5.1	2.4
	Mixed race	0.8	1.6
	Native American	0.8	1.6
	Other	0.8	2.4
Household income level	< \$25,000	38.4	27.6
	\$25,000-\$34,999	8.0	7.9
	\$35,000-\$49,999	4.8	11.8
	\$50,000-\$74,999	11.2	11.8
	\$75,000-\$99,999	17.6	9.4
	\$100,000-\$149,999	11.2	19.7
	\$150,000-\$199,999	4.0	6.3
	> \$199,999	4.8	5.5
Education level	Non-high school graduate	1.6	0.8
	High school graduate	16.8	18.9
	Some college/technical school	53.6	36.2
	College graduate	19.2	24.4
	Post-college graduate	8.8	19.7
Weekly beef consumption	0 times	0	0.8
	1-3 times	36.0	44.9
	4-6 times	33.6	33.9
	7-9 times	12.0	7.9
	10 or more times	18.4	12.6
Monthly retail beef purchase	0 times	15.2	10.2
	1-3 times	42.4	38.6
	4-6 times	28.0	37.8
	7-9 times	6.4	7.9
	10 or more times	8.0	5.5

Table 2.2 Summary statistics for independent variable evaluated in the study for retail ground beef

Measurement	Mean	Minimum	Maximum	Standard Deviation
<i>L</i> *	53.8	48.4	58.8	2.4
<i>a</i> *	19.6	8.8	34.2	8.3
<i>b</i> *	19.9	15.4	25.9	3.1
Metmyoglobin ¹¹	44.1	21.3	68.1	17.0
Deoxymyoglobin ¹¹	1.7	-6.4	11.3	3.9
Oxymyoglobin ¹¹	54.3	30.6	74.7	15.3
Chroma ¹¹	28.2	17.9	42.9	7.9
Hue Angle ¹¹	0.83	0.6	1.1	0.2
APC ¹²	7.7	5.4	10.6	1.1
EB ¹³	4.7	0.0	7.6	1.4
ECC ¹⁴	3.2	0.0	6.7	1.9
TBARS	0.31	0.14	0.76	0.1
Trained sensory panel redness score ¹	41.9	0.0	100.0	35.7
Trained sensory panel discoloration score ²	53.2	0.0	100.0	40.7
Trained sensory panel odor score ³	35.6	8.7	90.6	17.9
Trained sensory panel touch score ⁴	33.0	9.7	59.8	9.6
Trained sensory panel juiciness ⁵	63.0	55.3	69.4	2.8
Trained sensory tenderness ⁶	64.3	57.0	70.1	2.6
Trained sensory texture ⁷	65.6	55.5	71.5	2.8
Trained sensory beef flavor ⁸	36.4	30.7	39.7	1.6
Trained sensory off flavor ⁹	17.5	0.0	49.0	12.3
Consumer appearance score ¹⁰	44.0	3.1	95.5	31.9
Consumer touch score ¹⁰	56.7	18.4	82.5	12.6
Consumer odor score ¹⁰	44.5	3.0	74.3	14.5
Consumer taste score ¹⁰	62.7	27.4	85.1	9.5

¹Sensory scores: 0 = extremely dark red; 100 = bright, cherry red

²Sensory scores: 0 = no visible discoloration; 100 = extreme discoloration

³Sensory scores: 0 = no odor present; 100 = extreme odor present

⁴Sensory scores: 0 = characteristic beef touch; 100 = non-characteristic beef touch

⁵Sensory scores: 0 = extremely dry; 100 = extremely juicy

⁶Sensory scores: 0 = extremely tough; 100 = extremely tender

⁷Sensory scores: 0 = extremely soft; 100 = extremely firm

⁸Sensory scores: 0 = extremely bland; 100 = extremely intense

⁹Sensory scores: 0 = no off flavor; 100 = extreme off flavor

¹⁰Sensory scores: 0 = extremely dislike; 100 = extremely like

¹¹Calculated utilizing the equations presented in the AMSA Meat Color Guidelines (AMSA, 2012)

¹²Aerobic plate counts

¹³*Enterbactereaceae*

¹⁴*E. coli* coliform counts

Table 2.3 Least-squares means for objective color, microbiological, and TBARs evaluation across all days of display

Day of Display	<i>L</i> *	<i>a</i> *	<i>b</i> *	Met ^{2,3}	Oxy ^{2,4}	Chroma ²	Hue Angle ²	APC ⁵	EB ⁶	ECC ⁷	TBARS ⁸
0	57.3 ^a	33.1 ^a	25.0 ^a	22.2 ^f	68.8 ^b	41.5 ^a	0.65 ^f	6.5 ^g	3.2 ^e	1.1 ^d	0.22 ^e
2	55.3 ^b	28.3 ^b	23.0 ^b	24.6 ^f	73.0 ^a	36.4 ^b	0.68 ^{ef}	6.7 ^{fg}	3.7 ^{de}	2.7 ^c	0.27 ^{de}
4	54.8 ^{bc}	24.8 ^c	21.3 ^c	28.9 ^c	68.4 ^b	32.7 ^c	0.71 ^c	7.0 ^{ef}	4.0 ^d	2.6 ^c	0.27 ^{cde}
6	53.9 ^{cd}	17.7 ^d	18.1 ^c	43.1 ^d	53.7 ^c	25.4 ^c	0.81 ^d	7.1 ^c	4.3 ^d	3.5 ^{bc}	0.30 ^{bcd}
8	52.8 ^{de}	11.5 ^{ef}	16.6 ^f	60.4 ^b	37.5 ^c	20.2 ^g	0.97 ^b	7.6 ^d	5.0 ^c	2.9 ^c	0.31 ^{bcd}
10	53.3 ^{de}	10.0 ^f	16.6 ^f	65.0 ^a	34.6 ^c	19.4 ^g	1.0 ^a	8.3 ^c	5.2 ^{bc}	3.0 ^c	0.34 ^{bc}
12	52.3 ^e	13.1 ^e	18.2 ^c	59.2 ^b	42.2 ^d	22.5 ^f	0.96 ^b	8.8 ^b	5.8 ^b	4.1 ^b	0.36 ^{ab}
14	51.0 ^f	18.1 ^d	20.3 ^d	49.2 ^c	55.7 ^c	27.3 ^d	0.85 ^c	9.5 ^a	6.5 ^a	5.3 ^a	0.43 ^a
SEM ¹	0.40	0.67	0.30	1.5	1.5	0.64	0.02	0.14	0.24	0.38	0.03
<i>P</i> Value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

¹Standard error (largest) of least-squares means. SEM = standard error of the mean

²Calculated utilizing the equations presented in the AMSA Meat Color Guidelines (AMSA, 2012)

³Metmyoglobin

⁴Oxymyoglobin

⁵Aerobic Plate Counts

⁶*Enterobacteriaceae*

⁷*E.coli* coliforms

⁸TBARS were measured in mg MDA/kg

^{a-f}Least-squares means within the same column without a common superscript differ ($P < 0.05$)

Table 2.4 Least-squares means for trained sensory panels scores for color, odor, touch and taste, and consumer ratings across all days of display

Day of Display	Redness ²	Discoloration ³	Odor ⁴	Touch ⁵	Juiciness ⁶	Tenderness ⁷	Texture ⁸	Beef Flavor ⁹	Off-Flavor ¹⁰	Consumer Appearance ¹¹	Consumer Touch ¹¹	Consumer Odor ¹¹	Consumer Taste ¹¹
0	98.1 ^a	1.9 ^f	52.6 ^a	35.3 ^{bc}	64.2 ^a	66.2 ^a	63.7 ^d	36.7	16.3	88.6 ^a	66.4 ^a	54.4 ^a	62.0 ^a
2	85.7 ^b	5.7 ^{ef}	22.1 ^{de}	32.00 ^{bcd}	63.7 ^{ab}	66.2 ^a	64.4 ^{cd}	36.4	16.1	82.8 ^a	66.8 ^a	53.2 ^a	65.0 ^a
4	69.9 ^c	11.7 ^c	21.6 ^c	27.6 ^d	62.8 ^{abc}	64.6 ^b	65.6 ^{bc}	37.0	12.9	73.9 ^b	62.2 ^{ab}	51.8 ^a	62.9 ^a
6	33.9 ^d	50.8 ^d	26.7 ^{cde}	27.7 ^d	62.4 ^{bc}	63.9 ^{bcd}	66.2 ^{ab}	36.2	14.2	40.4 ^c	59.0 ^b	48.0 ^{ab}	63.5 ^a
8	9.5 ^{fg}	94.9 ^{ab}	30.2 ^{cd}	31.6 ^{cd}	62.2 ^{bc}	62.9 ^{de}	67.00 ^{ab}	36.7	15.6	15.0 ^c	52.8 ^c	41.2 ^{bc}	62.3 ^a
10	7.4 ^g	98.2 ^a	31.4 ^c	28.9 ^d	61.3 ^c	62.6 ^c	67.4 ^a	36.4	19.0	11.4 ^c	51.9 ^c	41.6 ^{bc}	64.3 ^a
12	14.0 ^{ef}	89.3 ^b	41.4 ^b	37.4 ^b	63.0 ^{ab}	63.3 ^{cde}	66.4 ^{ab}	36.3	20.8	16.5 ^c	49.9 ^c	39.4 ^c	65.9 ^a
14	17.0 ^e	73.0 ^c	59.1 ^a	43.4 ^a	64.3 ^a	64.5 ^{bc}	64.2 ^{cd}	35.9	25.2	23.6 ^d	44.4 ^d	26.7 ^d	55.9 ^b
SEM ¹	2.1	2.9	3.1	2.1	0.7	0.6	0.6	0.4	3.1	2.3	2.6	3.0	2.4
P Value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.36	0.08	< 0.01	< 0.01	< 0.01	< 0.01

¹Standard error (largest) of least-squares means. SEM = standard error of the mean

²Sensory scores: 0 = extremely dark red; 100 = bright, cherry red

³Sensory scores: 0 = no visible discoloration; 100 = extreme discoloration

⁴Sensory scores: 0 = no odor present; 100 = extreme odor present

⁵Sensory scores: 0 = characteristic beef touch; 100 = non-characteristic beef touch

⁶Sensory scores: 0 = extremely dry; 100 = extremely juicy

⁷Sensory scores: extremely tough; 100 = extremely tender

⁸Sensory scores: 0 = extremely soft; 100 = extremely firm

⁹Sensory scores: 0 = extremely bland; 100 = extremely intense

¹⁰Sensory scores: 0 = no off flavor; 100 = extreme off flavor

¹¹Sensory scores: 0 = extremely dislike; 100 = extremely like

^{a-d}Least-squares means within the same column without a common superscript differ ($P < 0.05$)

Table 2.5 Consumer purchase intent and spoilage ratings of ground beef samples for appearance, touch, odor, and taste across all days of display

Day of Display	Consumer Appearance ²	Consumer Touch ²	Consumer Odor ²	Consumer Taste ²
Purchase Probability				
0	97.8 ^a	78.0 ^{ab}	66.8 ^a	64.9
2	96.2 ^a	85.9 ^a	64.4 ^{ab}	68.6
4	88.6 ^b	78.8 ^{ab}	58.8 ^{ab}	72.8
6	31.5 ^c	73.2 ^{bc}	52.4 ^{bc}	69.8
8	6.8 ^d	63.6 ^{cd}	40.3 ^{cd}	68.1
10	3.0 ^d	55.5 ^{de}	37.9 ^d	70.1
12	3.8 ^d	54.7 ^{de}	36.3 ^d	74.1
14	6.8 ^d	46.7 ^e	20.4 ^e	55.7
SEM ¹	5.1	4.9	4.9	5.2
<i>P</i> Value	< 0.01	< 0.01	< 0.01	0.10
Spoilage Probability				
0	3.1 ^d	20.3 ^{bc}	23.2 ^{de}	9.6 ^{ab}
2	3.1 ^d	10.0 ^d	19.2 ^e	5.2 ^{bc}
4	3.1 ^d	13.2 ^{cd}	27.2 ^{de}	3.5 ^c
6	45.6 ^c	20.3 ^{bc}	31.2 ^{cd}	6.7 ^{bc}
8	81.3 ^b	24.2 ^{ab}	44.2 ^b	9.4 ^{abc}
10	90.8 ^a	33.9 ^a	39.3 ^{bc}	11.0 ^{ab}
12	80.5 ^b	28.2 ^{ab}	46.7 ^b	9.4 ^{abc}
14	78.9 ^b	33.9 ^a	65.4 ^a	17.9 ^a
SEM ¹	3.9	4.8	5.2	3.9
<i>P</i> Value	< 0.01	< 0.01	< 0.01	0.01

¹Standard error (largest) of least-squares means. SEM = standard error of the mean

²Sensory scores: 0 = extremely dislike; 100 = extremely like

^{a-d}Least-squares means within the same column without a common superscript differ ($P < 0.05$)

Table 2.6 Logistic regression equations for predicting consumer purchase intent based upon visual appearance, touch, odor, and taste of retail ground beef

Measurement	Intercept	Slope	Max – rescaled R ²	P – value	C – statistic ¹	% Correct ²
Visual						
<i>L</i> [*]	-39.1	0.72	0.48	< 0.01	0.84	84.3
<i>a</i> [*]	-7.3	0.34	0.80	< 0.01	0.94	93.8
<i>b</i> [*]	-14.7	0.71	0.68	< 0.01	0.91	90.5
Metmyoglobin ³	6.3	-0.17	0.81	< 0.01	0.94	94.3
Oxymyoglobin ³	-10.7	0.18	0.75	< 0.01	0.94	93.4
Chroma ³	-10.2	0.34	0.78	< 0.01	0.93	93.2
Hue Angle ³	15.2	-19.7	0.77	< 0.01	0.94	94.0
APC ⁴	14.4	-2.0	0.59	< 0.01	0.89	88.5
EB ⁵	5.5	-1.3	0.50	< 0.01	0.86	86.1
ECC ⁶	0.9	-0.41	0.17	< 0.01	0.72	71.0
Trained panel sensory redness score ⁷	-3.8	0.08	0.86	< 0.01	0.94	94.2
Trained panel sensory discoloration score ⁸	2.7	-0.07	0.83	< 0.01	0.95	94.4
Consumer appearance score ¹²	-4.9	0.10	0.89	< 0.01	0.96	96.1
TBARS	2.1	-7.9	0.20	< 0.01	0.76	74.5
Touch						
APC ⁴	3.9	-0.41	0.11	< 0.01	0.64	63.6
EB ⁵	2.3	-0.33	0.10	< 0.01	0.63	62.6
ECC ⁶	1.3	-0.17	0.05	< 0.01	0.60	58.6
Trained touch score ⁹	1.6	-0.03	0.03	< 0.01	0.56	55.9
Consumer touch score ¹²	-3.5	0.08	0.36	< 0.01	0.73	72.7
TBARS	1.5	-2.5	0.04	< 0.01	0.59	57.5
Odor						
APC ⁴	3.8	-0.50	0.17	< 0.01	0.65	64.9
EB ⁵	1.6	-0.35	0.13	< 0.01	0.64	63.6
ECC ⁶	0.54	-0.21	0.08	< 0.01	0.62	60.2
Trained off-odor score ¹⁰	0.45	-0.02	0.04	< 0.01	0.57	57.0
Consumer odor score ¹²	-3.2	0.07	0.40	< 0.01	0.73	73.0
TBARS	0.79	-2.9	0.06	< 0.01	0.62	60.0
Taste						
APC ⁴	1.2	-0.06	< 0.01	0.30	0.52	52.0
EB ⁵	0.83	-0.20	< 0.01	0.68	0.51	50.3
ECC ⁶	0.82	-0.03	< 0.01	0.48	0.51	49.1
Trained off-flavor score ¹¹	0.62	0.01	< 0.01	0.21	0.53	50.2
Consumer flavor score ¹²	-4.0	0.08	0.27	< 0.01	0.68	67.9
TBARS	0.77	-0.13	< 0.01	0.83	0.50	50.0

¹Measure of goodness of fit for binary outcomes in a logistic regression model, ranging from 0-1

²Percentage of correctly classified events and nonevents by the model

³Calculated utilizing the equations presented in the AMSA Meat Color Guidelines (AMSA, 2012)

⁴Aerobic plate counts

⁵*Enterobacteriaceae*

⁶*E. coli* coliform counts

⁷Sensory scores: 0 = extremely dark red; 100 = bright, cherry red

⁸Sensory scores: 0 = no visible discoloration; 100 = extreme discoloration

⁹Sensory scores: 0 = characteristic beef touch; 100 = non-characteristic beef touch

¹⁰Sensory scores: 0 = no odor present; 100 = extreme odor present

¹¹Sensory scores: 0 = no off flavor; 100 = extreme off flavor

¹²Sensory scores: 0 = extremely dislike; 100 = extremely like

Table 2.7 Logistic regression equations for predicting consumer spoilage rating based upon visual appearance, touch, odor, and taste of retail ground beef

Measurement	Intercept	Slope	Max – rescaled R ²	P - value	C – statistic ¹	% Correct ²
Visual						
<i>L</i> [*]	27.2	-0.51	0.36	< 0.01	0.79	78.3
<i>a</i> [*]	4.6	-0.25	0.70	< 0.01	0.89	88.9
<i>b</i> [*]	10.8	-0.55	0.58	< 0.01	0.86	85.3
Metmyoglobin ³	-5.4	0.12	0.73	< 0.01	0.90	89.3
Oxymyoglobin ³	6.4	-0.12	0.66	< 0.01	0.89	88.7
Chroma ³	6.9	-0.26	0.68	< 0.01	0.88	88.1
Hue Angle ³	-10.6	12.7	0.67	< 0.01	0.89	88.8
APC ⁴	-9.5	1.2	0.46	< 0.01	0.83	82.9
EB ⁵	-4.6	0.95	0.40	< 0.01	0.81	80.8
ECC ⁶	-1.1	0.32	0.13	< 0.01	0.69	67.3
Trained panel sensory redness score ⁷	2.3	-0.07	0.77	< 0.01	0.89	88.9
Trained panel sensory discoloration score ⁸	-3.0	0.05	0.76	< 0.01	0.90	89.4
Consumer appearance score ¹²	3.0	-0.08	0.82	< 0.01	0.92	91.5
TBARS	-1.9	5.9	0.15	< 0.01	0.72	70.5
Touch						
APC ⁴	-3.1	0.25	0.04	< 0.01	0.59	58.9
EB ⁵	-2.1	0.20	0.03	< 0.01	0.59	58.7
ECC ⁶	-1.5	0.10	0.02	< 0.01	0.57	55.1
Trained touch score ⁹	-2.1	0.03	0.03	< 0.01	0.57	56.7
Consumer touch score ¹²	2.1	-0.06	0.25	< 0.01	0.70	69.7
TBARS	-1.7	1.7	0.02	< 0.01	0.56	54.5
Odor						
APC ⁴	-4.4	0.50	0.17	< 0.01	0.66	65.9
EB ⁵	-2.4	0.38	0.14	< 0.01	0.65	64.5
ECC ⁶	-1.3	0.25	0.10	< 0.01	0.63	61.9
Trained off-odor score ¹⁰	-1.3	0.02	0.08	< 0.01	0.60	59.5
Consumer odor score ¹²	2.6	-0.07	0.41	< 0.01	0.75	74.2
TBARS	-1.3	2.6	0.05	< 0.01	0.60	58.6
Taste						
APC ⁴	-4.7	0.31	0.04	< 0.01	0.60	59.8
EB ⁵	-3.3	0.22	0.03	0.01	0.59	58.8
ECC ⁶	-2.7	0.13	0.02	0.03	0.56	54.8
Trained off-flavor score ¹¹	-2.7	0.02	0.03	0.01	0.58	55.6
Consumer flavor score ¹²	1.8	-0.07	0.16	< 0.01	0.66	65.8
TBARS	-2.4	0.56	< 0.01	0.54	0.52	51.9

¹Measure of goodness of fit for binary outcomes in a logistic regression model, ranging from 0-1

²Percentage of correctly classified events and nonevents by the model

³Calculated utilizing the equations presented in the AMSA Meat Color Guidelines (AMSA, 2012)

⁴Aerobic plate counts

⁵*Enterobacteriaceae*

⁶*E. coli* coliform counts

⁷Sensory scores: 0 = extremely dark red; 100 = bright, cherry red

⁸Sensory scores: 0 = no visible discoloration; 100 = extreme discoloration

⁹Sensory scores: 0 = characteristic beef touch; 100 = non-characteristic beef touch

¹⁰Sensory scores: 0 = no odor present; 100 = extreme odor present

¹¹Sensory scores: 0 = no off flavor; 100 = extreme off flavor

¹²Sensory scores: 0 = extremely dislike; 100 = extremely like

Table 2.8 50, 75, 90, and 95% likeliness thresholds of appearance measures for consumer purchase intent and 5, 10, 25, and 50% likeliness of consumer spoilage rating of retail ground beef

Measurement	Purchase Probability				Spoilage Probability			
	50%	75%	90%	95%	5%	10%	25%	50%
<i>L</i> *	54.4	55.9					55.4	53.2
<i>a</i> *	21.5	24.7	28.0	30.2	30.1	27.2	22.3	18.4
<i>b</i> *	20.7	22.3	23.8	24.9	24.9	23.9	21.6	19.6
Metmyoglobin ¹	37.3	30.8	24.4	19.0	20.6	26.8	36.0	45.1
Oxymyoglobin ¹	59.5	65.7	71.8	75.9	77.0	71.5	62.3	53.2
TBARS	0.3							0.5
Trained sensory redness score ²	60.2	73.9	87.6	96.0	74.8	64.1	48.4	32.7
Trained sensory discoloration score ³	38.7	23.0	7.3		1.6	16.5	38.5	60.4
Trained odor score ⁴	22.5						9.6	64.5
Trained touch score ⁵	51.6	15.0					34.1	
Consumer appearance score ⁶	48.5	59.5	70.5	77.0	74.7	65.3	51.6	37.9
Consumer odor score ⁶	45.6	61.3	77.0	87.7	79.1	68.4	52.7	37.0
Consumer touch score ⁶	43.15	56.9	70.6	80.0	84.6	72.1	53.8	35.5

¹ Calculated utilizing the equations presented in the AMSA Meat Color Measurement Guidelines (AMSA, 2012)

² Sensory scores: 0 = extremely dark red, 100 = bright cherry red

³ Sensory scores: 0 = no visible discoloration, 100 = complete discoloration

⁴ Sensory scores: 0 = no off odor, 100 = extreme off odor

⁵ Sensory scores: 0 = characteristic beef texture, 100 = non-characteristic beef texture

⁶ Sensory scores: 0 = extremely dislike, 100 = extremely like

Table 2.9 Pearson correlation coefficients for objective color, microbiological, TBARS, trained sensory panel, and consumer measurements

Measurement	<i>L</i> [*]	<i>a</i> [*]	<i>b</i> [*]	Met ¹¹	Oxy ¹¹	Chroma ¹¹	Hue Angle ¹¹	APC ¹²	EB ¹³	ECC ¹⁴
<i>a</i> [*]	0.53**									
<i>b</i> [*]	0.40**	0.96**								
Metmyoglobin ¹¹	-0.56**	-0.97**	-0.88**							
Oxymyoglobin ¹¹	0.45**	0.94**	0.88**	-0.98**						
Chroma ¹¹	0.51**	0.99**	0.98**	-0.95**	0.92**					
Hue Angle ¹¹	-0.48**	-0.96**	-0.87**	0.99**	-0.97**	-0.94**				
APC ¹²	-0.62**	-0.53**	-0.39**	0.57**	-0.44**	-0.50**	0.52**			
EB ¹³	-0.57**	-0.51**	-0.39**	0.58**	-0.43**	-0.49**	0.50**	0.80**		
ECC ¹⁴	-0.49**	-0.26**	-0.21*	0.24**	-0.13	-0.22*	0.18*	0.54**	0.51**	
Trained redness ¹	0.69**	0.95**	0.87**	-0.95**	0.88**	-0.13**	-0.90**	-0.67**	-0.63**	-0.38**
Trained discoloration ²	-0.60**	-0.95**	-0.86**	0.98**	-0.94**	0.11**	0.96**	0.62**	0.57**	0.30**
Trained odor ³	-0.21*	0.09	0.18*	0.03	< 0.01	-0.08	-0.03	0.40**	0.30**	0.17*
Trained touch ⁴	-0.22*	0.05	0.13	0.03	0.02	-0.20	-0.01	0.27**	0.21*	0.24**
Trained juiciness ⁵	0.002	0.26	0.30**	-0.22*	0.24*	0.27**	-0.23**	-0.01	-0.06	-0.20*
Trained tenderness ⁶	0.25*	0.50**	0.50**	-0.46**	0.45*	0.50**	-0.45**	-0.21*	-0.25**	-0.19*
Trained texture ⁷	-0.16	-0.42**	-0.43**	0.39**	-0.42**	-0.43**	0.40**	0.10	0.09	0.09
Trained beef flavor ⁸	0.08	0.03	0.02	-0.04	0.02	0.03	-0.02	-0.13	-0.17	-0.22*
Trained off flavor ⁹	-0.14	-0.05	0.001	0.09	-0.04	-0.04	0.07	0.29**	0.24**	0.18*
Consumer appearance ¹⁰	0.63**	0.93**	0.85**	-0.94**	0.89**	0.91**	-0.91**	-0.67**	-0.65**	-0.37**
Consumer touch ¹⁰	0.47**	0.43**	0.36**	-0.46**	0.38**	0.42**	-0.41**	-0.53**	-0.52**	-0.39**
Consumer odor ¹⁰	0.48**	0.36**	0.28**	-0.38**	0.30**	0.34*	-0.34**	-0.56**	-0.51**	-0.44**
Consumer taste ¹⁰	0.22	-0.09	-0.10	0.06	-0.09	-0.09	0.12	-0.09	-0.04	-0.15
TBARS	-0.37**	-0.34**	-0.28**	0.34**	-0.26**	-0.33**	0.30**	0.46**	0.40**	0.44**

¹Sensory scores: 0 = extremely dark red; 100 = bright, cherry red

²Sensory scores: 0 = no visible discoloration; 100 = extreme discoloration

³Sensory scores: 0 = no odor present; 100 = extreme odor present

⁴Sensory scores: 0 = characteristic beef touch; 100 = non-characteristic beef touch

⁵Sensory scores: 0 = extremely dry; 100 = extremely juicy

⁶Sensory scores: 0 = extremely tough; 100 = extremely tender

⁷Sensory scores: 0 = extremely soft; 100 = extremely firm

⁸Sensory scores: 0 = extremely bland; 100 = extremely intense

⁹Sensory scores: 0 = no off flavor; 100 = extreme off flavor

¹⁰Sensory scores: 0 = extremely dislike; 100 = extremely like

¹¹Calculated utilizing the equations presented in the AMSA Meat Color Guidelines (AMSA, 2012)

¹²Aerobic plate counts

¹³*Enterobacteriaceae*

¹⁴*E. coli* coliform counts

Table 2.10 (Cont.) Pearson correlation coefficients for objective color, microbiological, TBARS, trained sensory panel, and consumer measurements

	Trained redness ¹	Trained discoloration ²	Trained odor ³	Trained touch ⁴	Trained juiciness ⁵	Trained tenderness ⁶	Trained texture ⁷	Trained beef flavor ⁸	Trained off flavor ⁹	Consumer appearance ¹⁰	Consumer touch ¹⁰	Consumer odor ¹⁰	Consumer taste ¹⁰
Trained discoloration ²	-0.96**												
Trained odor ³	-0.09	0.11											
Trained touch ⁴	-0.08	0.08	0.36**										
Trained juiciness ⁵	-0.02	0.07	0.27**	0.06									
Trained tenderness ⁶	-0.22*	0.24**	0.43**	0.28**	0.59**								
Trained texture ⁷	0.08	-0.12	-0.28**	-0.27**	-0.36**	-0.38**							
Trained beef flavor ⁸	-0.13	0.10	0.06	-0.13	0.14	0.13	0.02						
Trained off flavor ⁹	0.25	-0.21*	-0.09	0.05	-0.07	-0.07	-0.14	-0.14					
Consumer appearance ¹⁰	0.96**	-0.96**	-0.10	-0.11	0.20*	0.45**	-0.35**	0.10	-0.12				
Consumer touch ¹⁰	0.50**	-0.48**	-0.15	-0.32**	0.12	0.29**	-0.01	0.07	-0.16	0.51**			
Consumer odor ¹⁰	0.45**	-0.42**	-0.33**	-0.25**	< 0.01	0.14	0.03	0.10	-0.14	0.45**	0.61**		
Consumer taste ¹⁰	-0.11	0.11	-0.11	-0.10	-0.09	-0.03	0.12	0.34**	0.05	-0.00	0.04	0.18	
TBARS	-0.43**	0.37**	0.27**	0.14	< 0.01	-0.04	0.08	-0.07	0.14	-0.40**	-0.35**	-0.41**	-0.03

¹Sensory scores: 0 = extremely dark red; 100 = bright, cherry red

²Sensory scores: 0 = no visible discoloration; 100 = extreme discoloration

³Sensory scores: 0 = no odor present; 100 = extreme odor present

⁴Sensory scores: 0 = characteristic beef touch; 100 = non-characteristic beef touch

⁵Sensory scores: 0 = extremely dry; 100 = extremely juicy

⁶Sensory scores: 0 = extremely tough; 100 = extremely tender

⁷Sensory scores: 0 = extremely soft; 100 = extremely firm

⁸Sensory scores: 0 = extremely bland; 100 = extremely intense

⁹Sensory scores: 0 = no off flavor; 100 = extreme off flavor

¹⁰Sensory scores: 0 = extremely dislike; 100 = extremely like

Figure 2.1 Percentage of samples classified by consumers as “would purchase” based upon appearance, touch, odor, and taste ratings across all days of display compared to (A) a^* , (B) trained redness scores, (C) trained discoloration scores, and (D) percent metmyoglobin

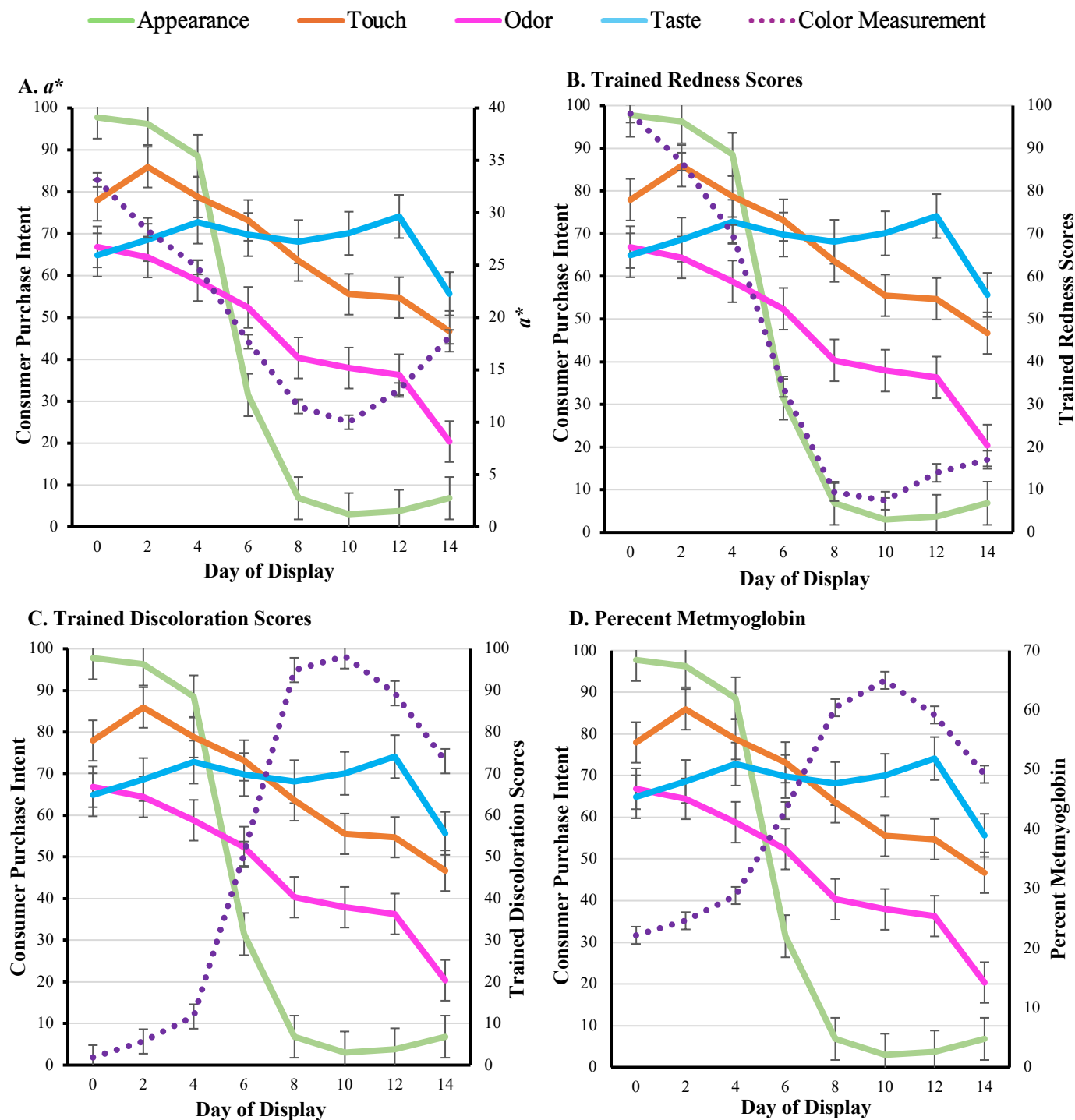


Figure 2.2 Percentage of samples classified by consumers as “spoiled” based upon appearance, touch, odor, and taste ratings across all days of display compared to (A) a^* , (B) trained redness scores, (C) trained discoloration scores, and (D) percent metmyoglobin

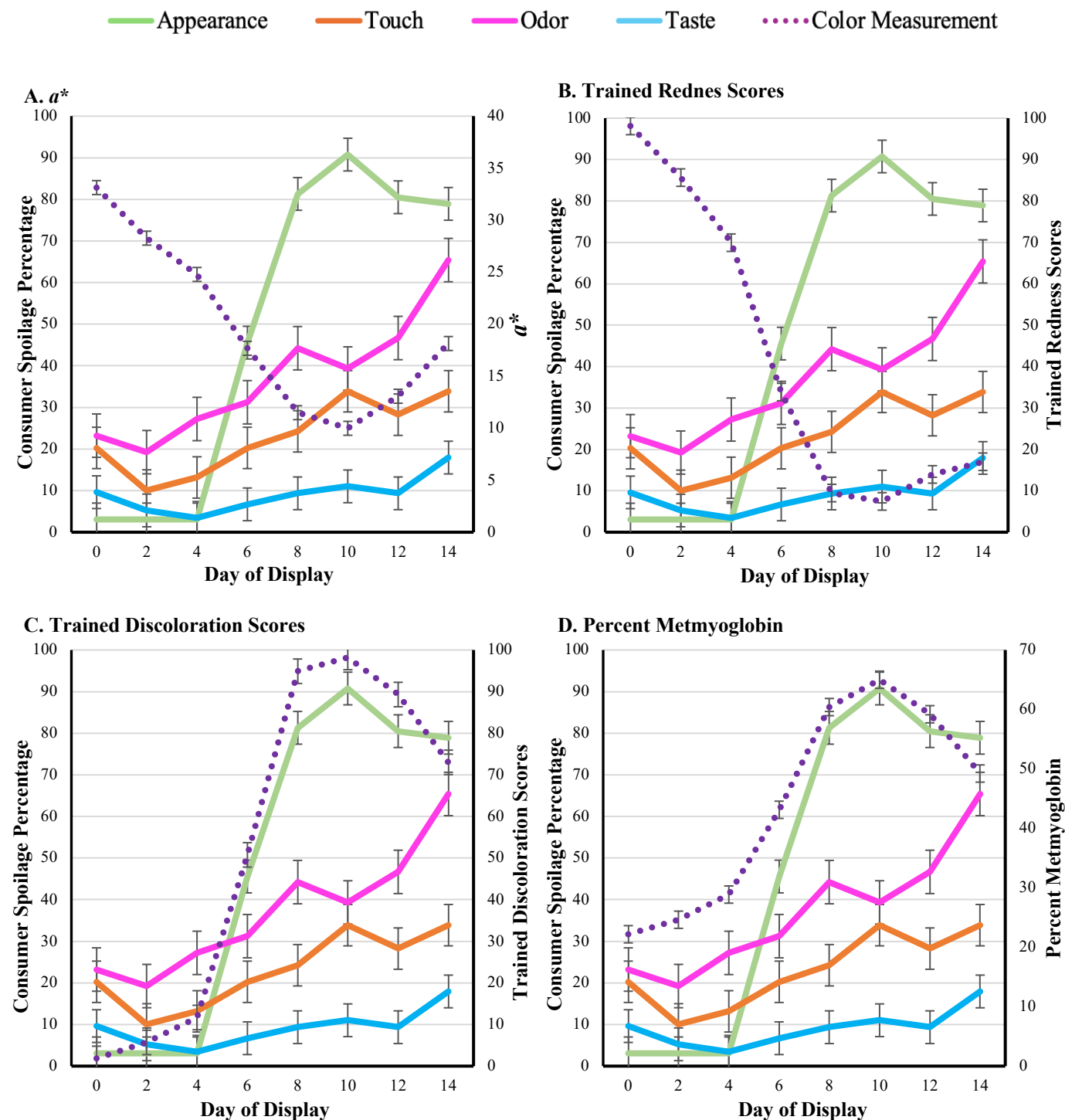


Figure 2.3 Consumer purchase intent based upon appearance, touch, odor, and taste ratings across all days of display compared to (A) APC, (B) EB, and (C) ECC enumeration

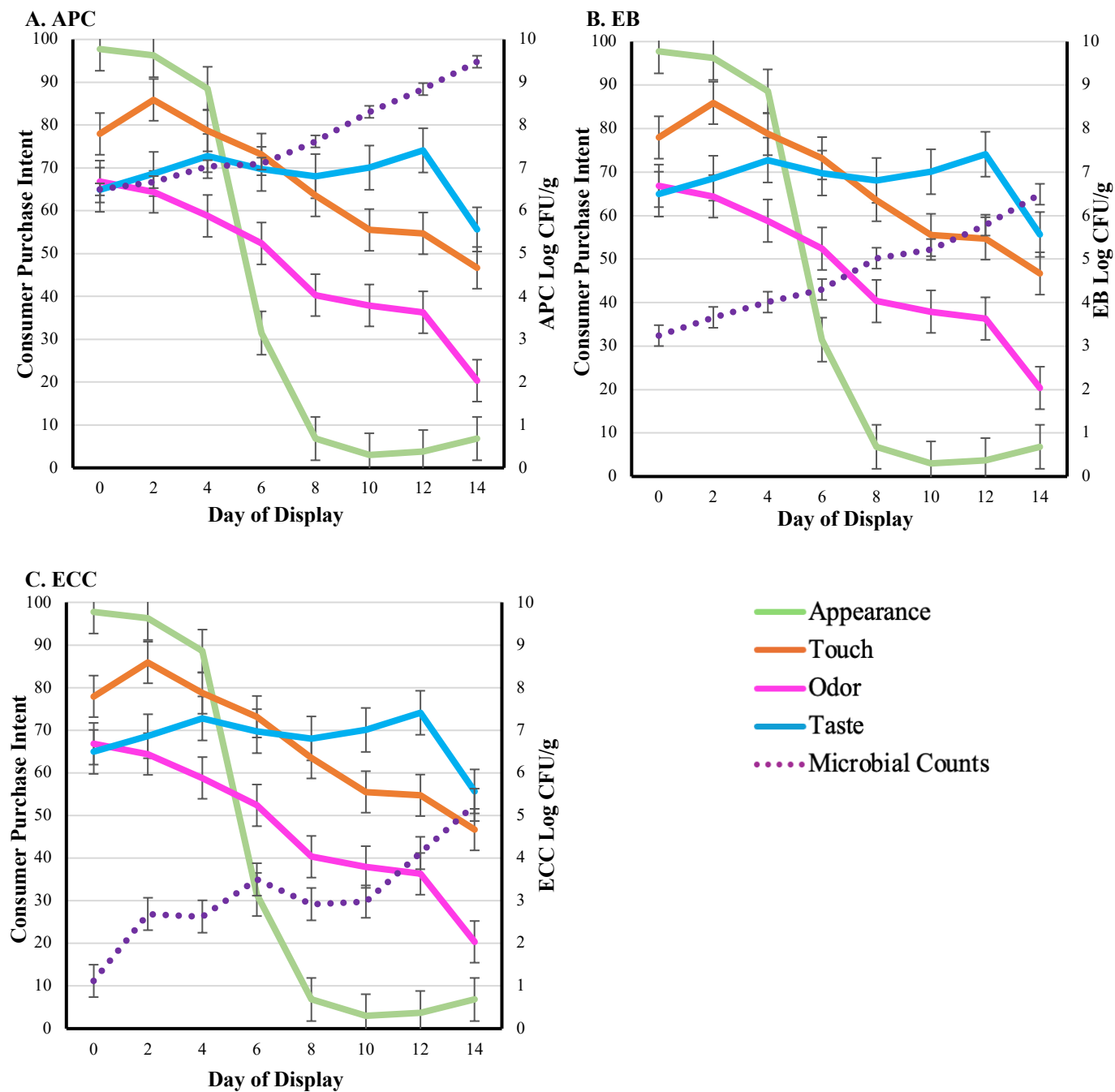


Figure 2.4 Consumer spoilage rating based upon appearance, touch, odor, and taste ratings across all days of display compared to (A) APC, (B) EB, and (C) ECC enumeration

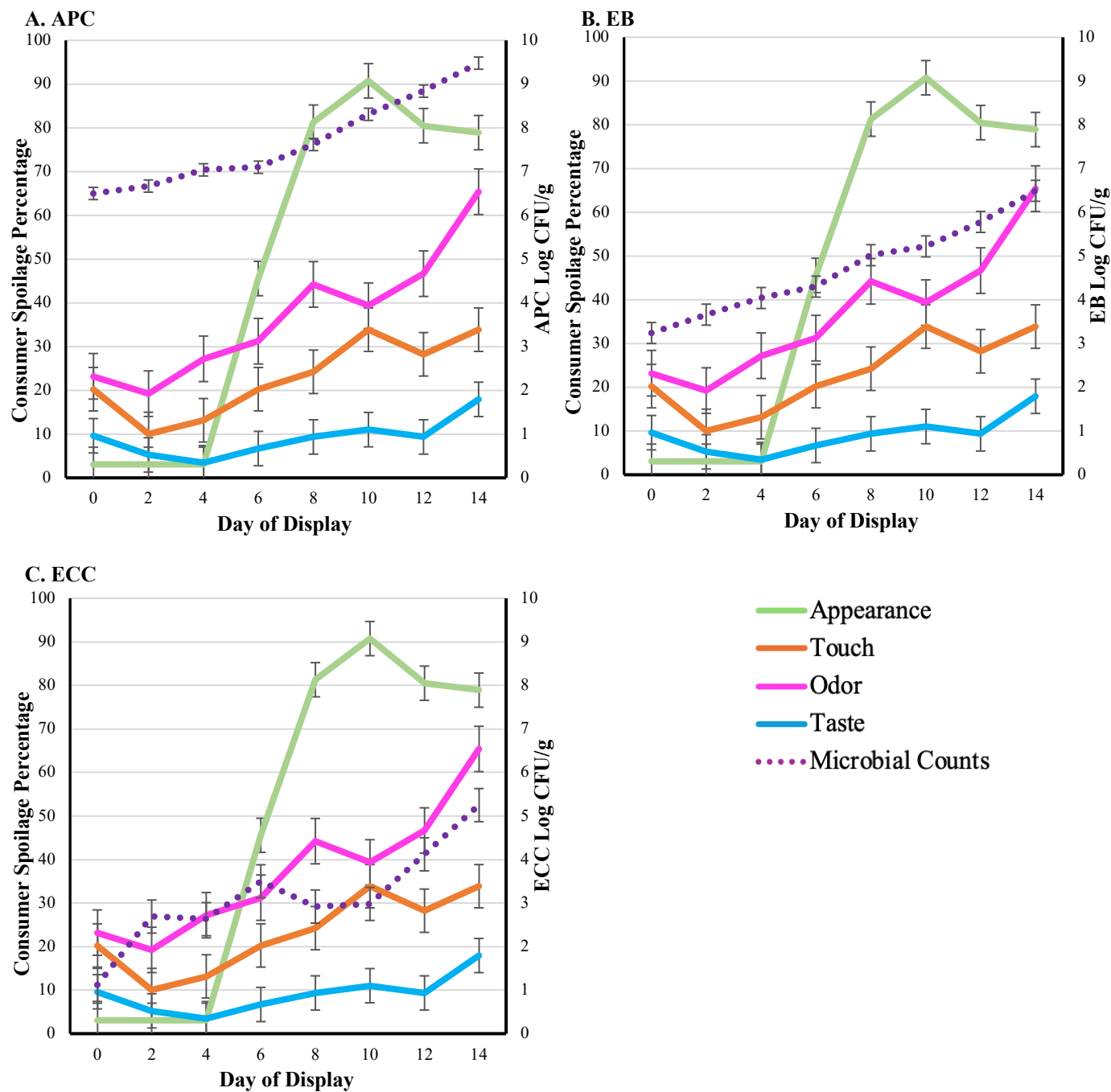


Figure 2.5 Logistic regression for consumer likeliness to purchase retail ground beef or classify as spoiled according to a^* values and consumer appearance ratings

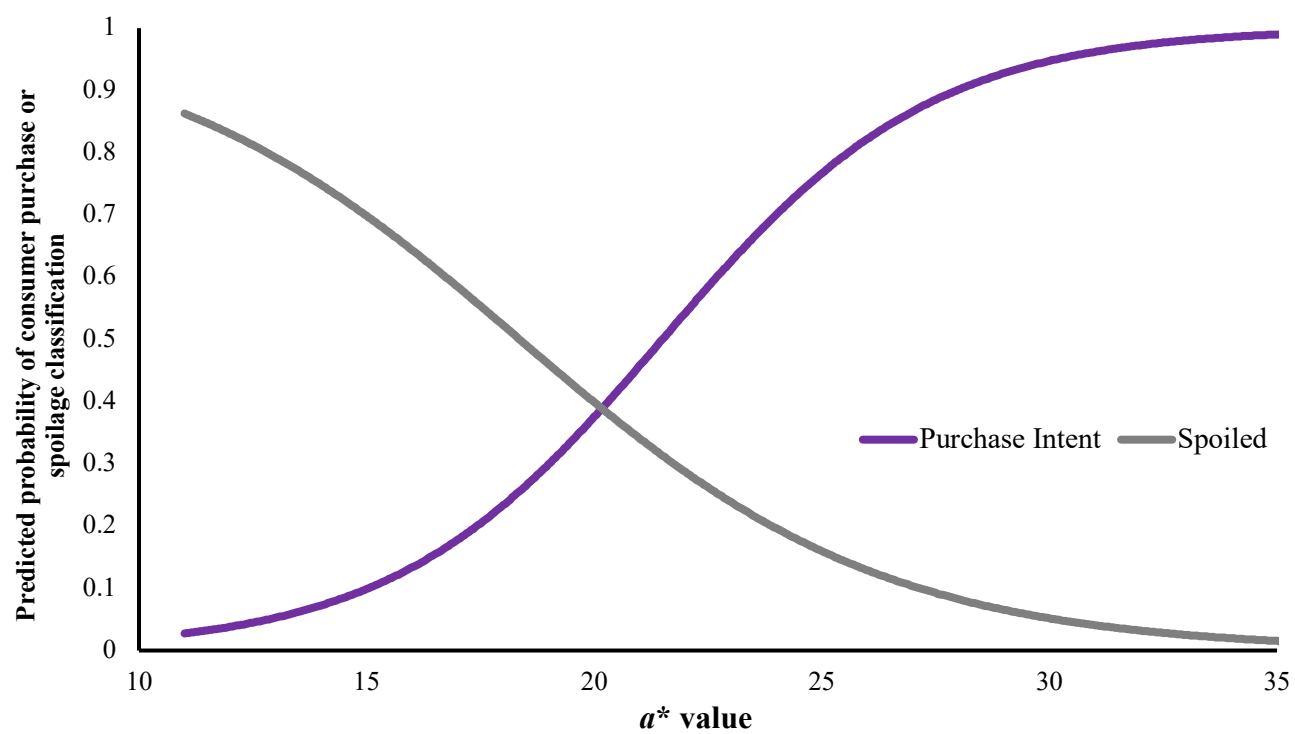


Figure 2.6 Logistic regression for consumer likeliness to purchase retail ground beef or classify as spoiled according to metmyoglobin percentage and consumer appearance ratings

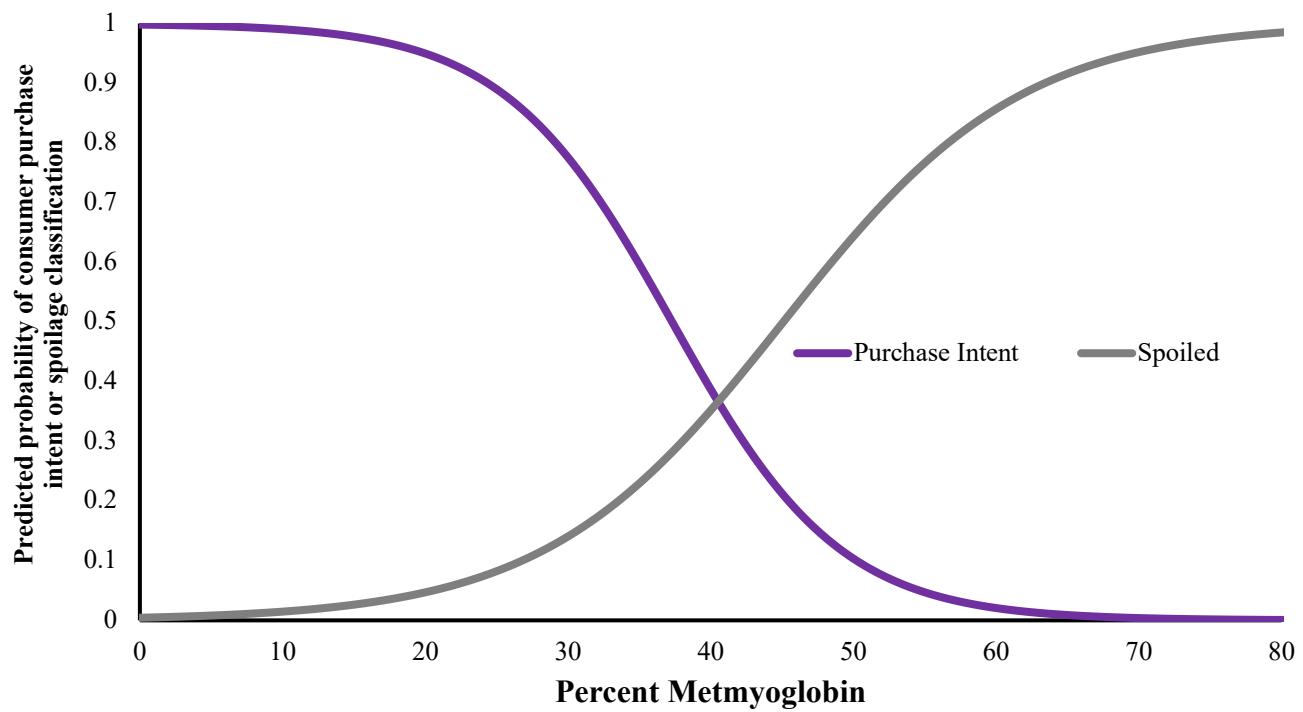


Figure 2.7 Logistic regression for consumer likeliness to purchase retail ground beef or classify as spoiled according to trained discoloration scores and consumer appearance ratings

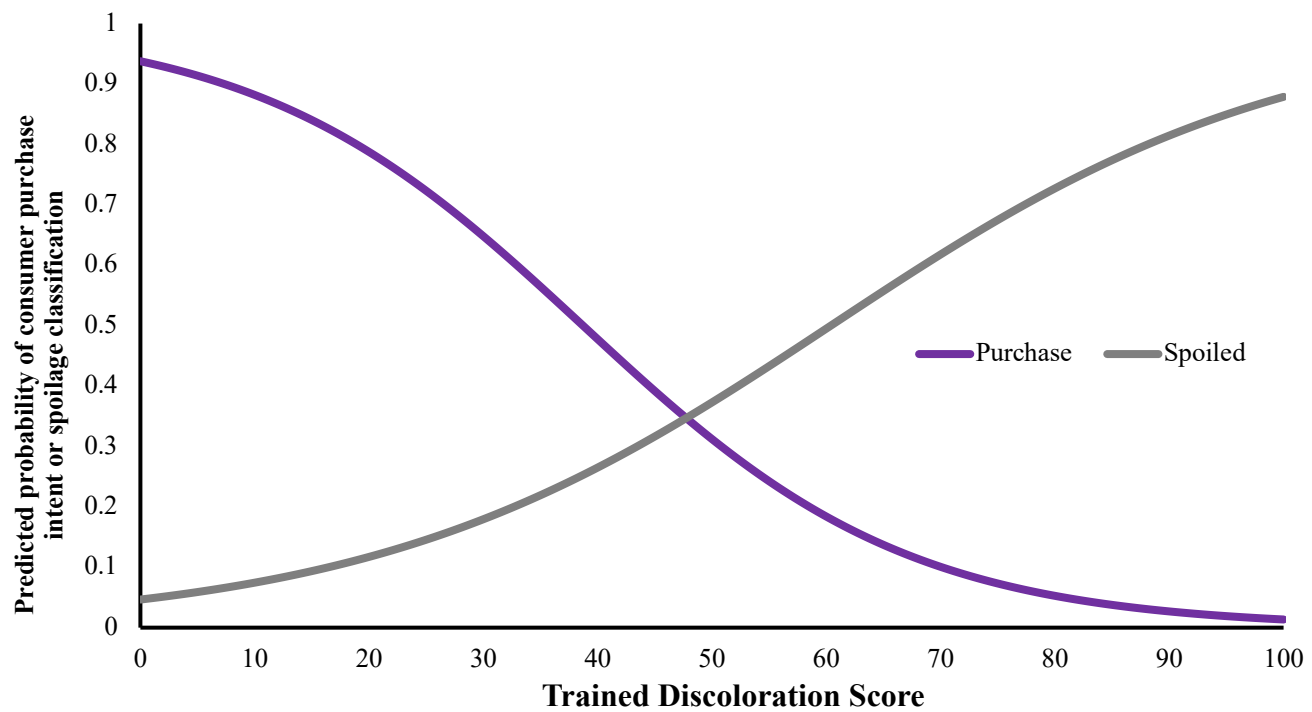


Figure 2.8 Logistic regression for consumer likeliness to purchase retail ground beef according to APC, EB, and ECC enumeration and consumer appearance ratings

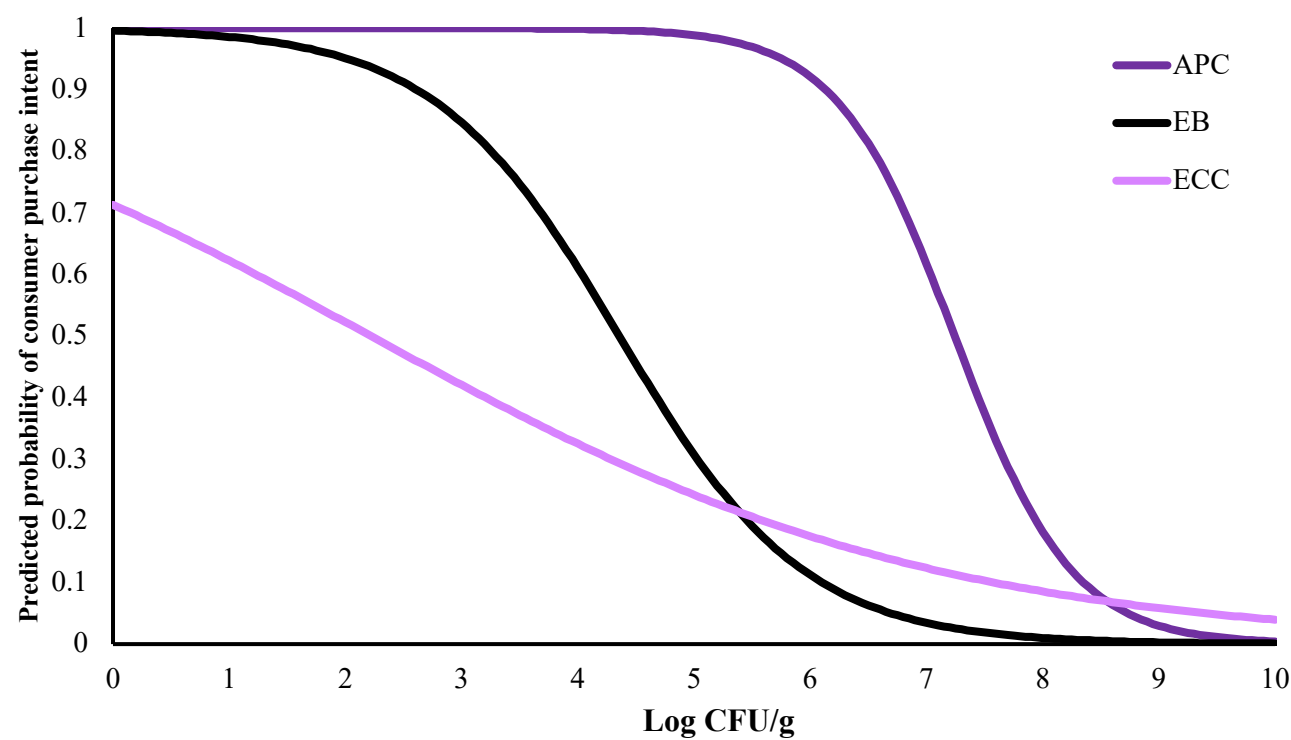


Figure 2.9 Logistic regression for consumer likeliness to classify retail ground beef as spoiled according to APC, EB, and ECC enumeration and consumer appearance ratings

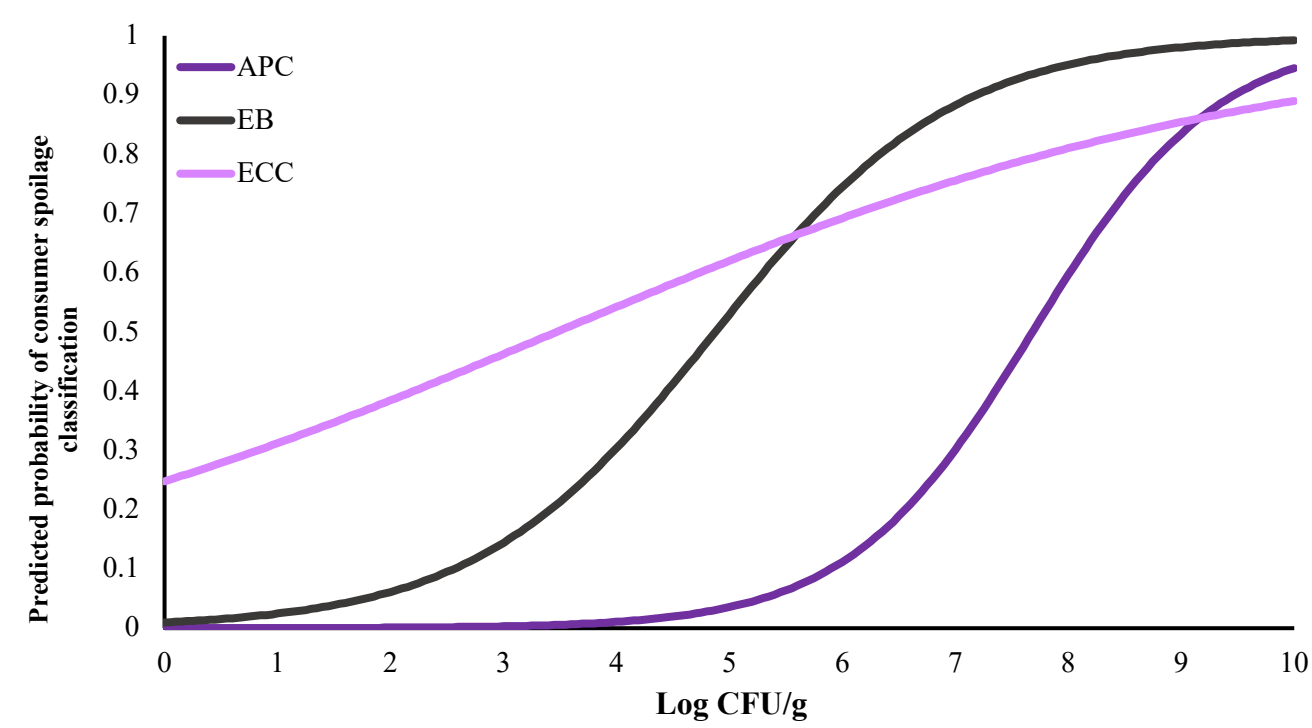


Figure 2.10 Relative abundance at the ASV level by day of display for week 1(A) and week 2 (B)

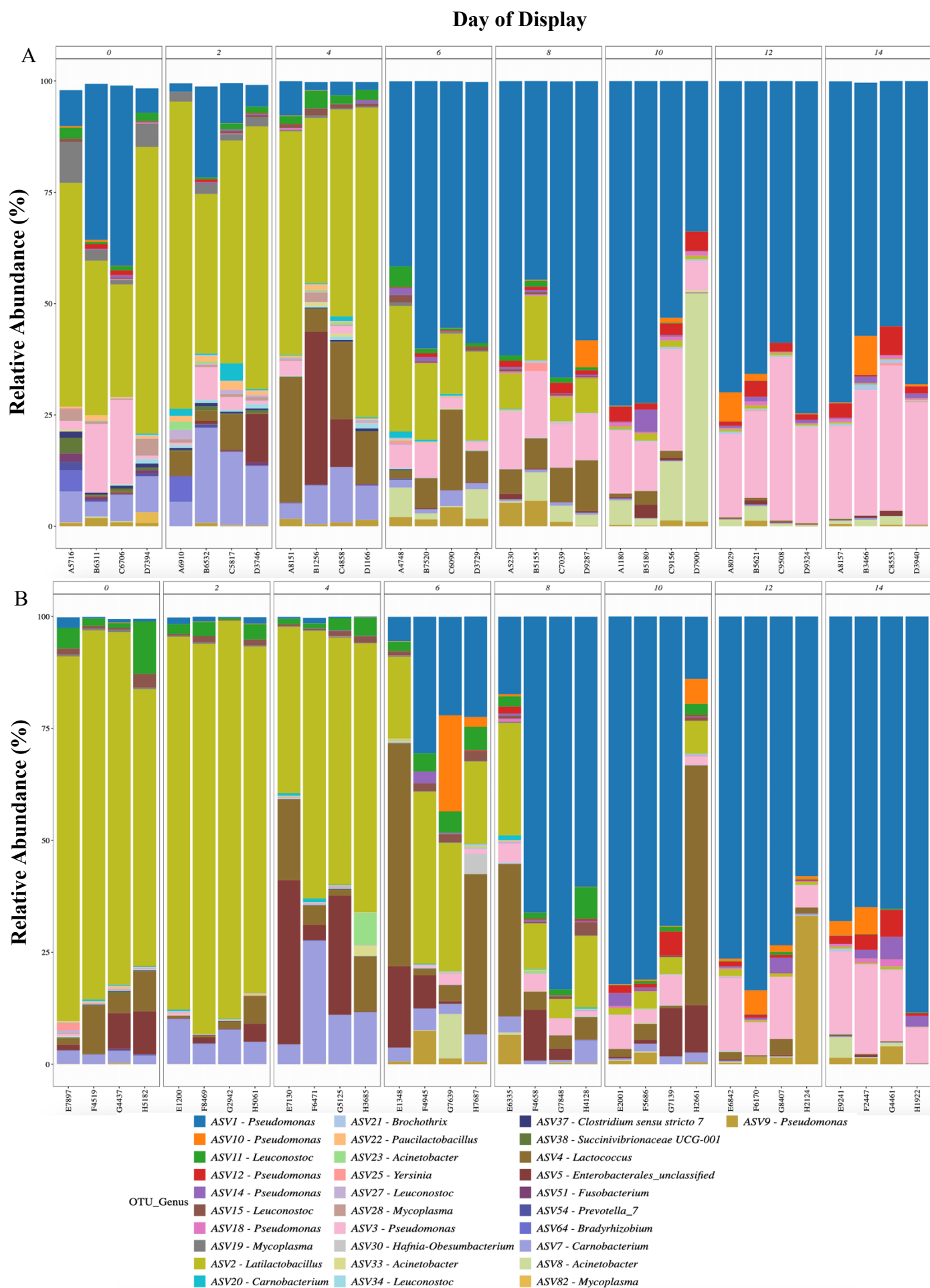
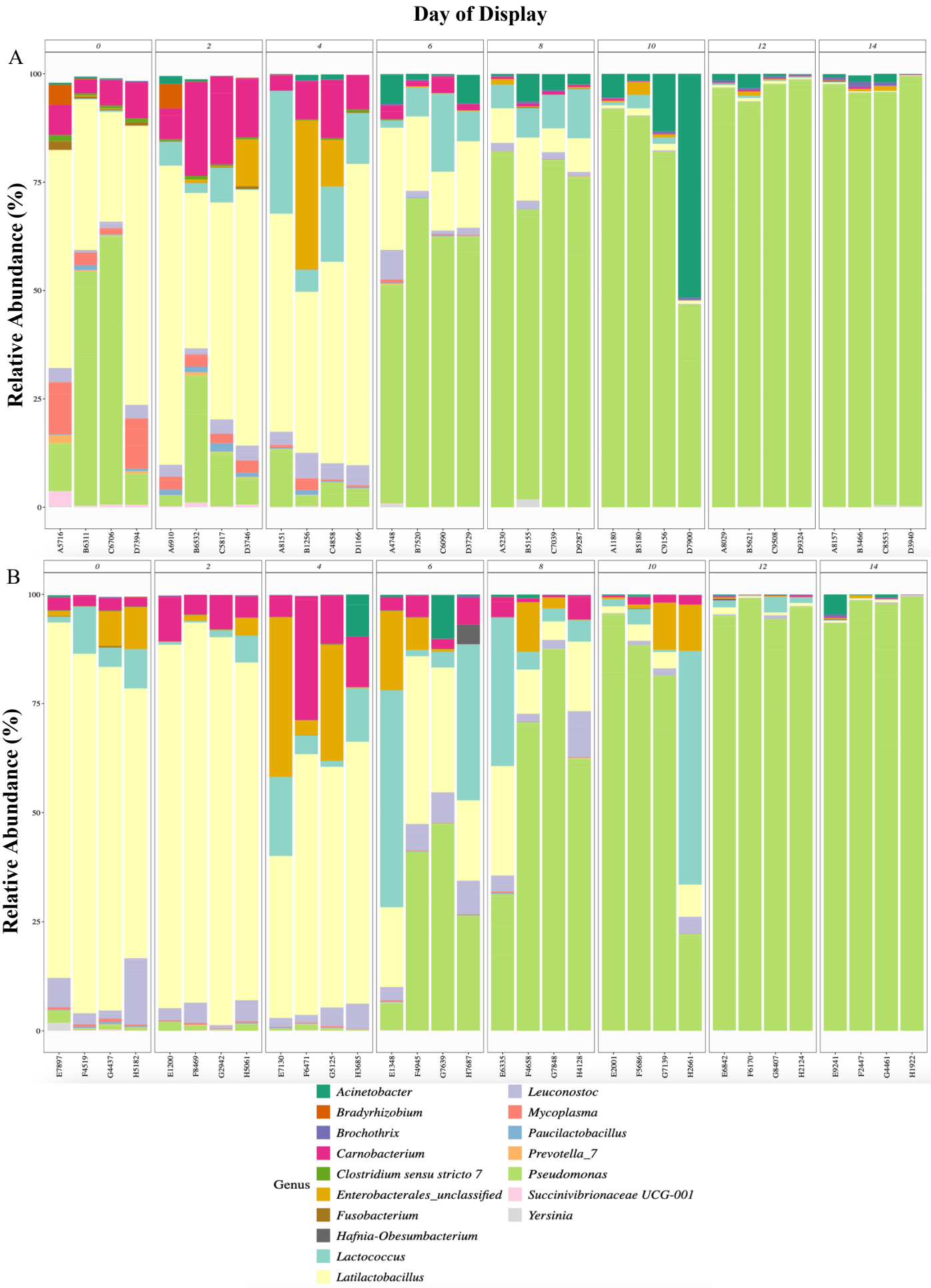


Figure 2.11 Relative abundance of genera by day of display for week 1 (A) and week 2 (B)



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Appendix A - Multivariate Logistic Regression Equation

Appendix Table A.1. Multivariate logistic regression equations for predicting consumer sensory panel purchase intent and spoilage ratings of retail ground beef

Measurement	Estimate	Max Rescaled R^2	P – Value	C – Statistic ¹	% Correct ²
Appearance					
APC ⁴	0.55	0.76	< 0.01	0.90	90.0
Hue Angle ³	-14.46	0.77	< 0.01	0.90	90.0
Metmyoglobin ³	0.20	0.72	< 0.01	0.90	90.0
b^*	-0.23	0.78	< 0.01	0.90	90.0
Touch					
APC ⁴	0.25	0.04	< 0.01	0.59	58.9
Odor					
APC ⁴	0.11	0.17	< 0.01	0.67	66.3
ECC ⁵	0.41	0.18	0.01	0.67	66.3

¹Measure of goodness of fit for binary outcomes in a logistic regression model, ranging from 0-1

²Percentage of correctly classified events and nonevents by the model

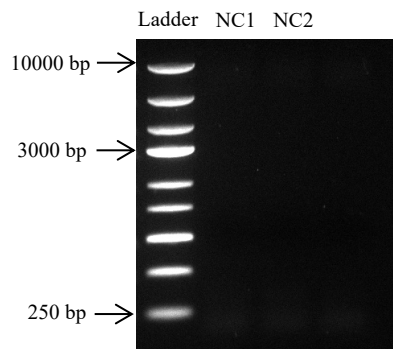
³Calculated utilizing the equations presented in the AMSA Meat Color Guidelines (AMSA, 2012)

⁴Aerobic plate counts

⁵*E. coli* coliform counts

Appendix B - DNA Extraction Negative Control Stain

Appendix Figure B.1.



Appendix C - Sensory Panel Evaluation Forms

Informed Consent Statement

1. I volunteer to participate in research involving Sensory Evaluation of Meat. This research will be conducted by personnel in the Department of Animal Sciences and Industry at Kansas State University.

2. I fully understand the purpose of the research is for the evaluation of beef steaks, pork chops, lamb chops, goat meat, poultry meat, ground meat, and processed meat products from the previously mentioned species for the sensory traits of tenderness, juiciness, flavor intensity, connective tissue amount, off flavor presence, odor, and color and sensory evaluation will last approximately one hour.

3. I understand that there are minimal risks associated with participating and that those risks are related to possible food allergies. All meat products will be USDA inspected and all ingredients are GRAS (generally accepted as safe) by FDA.

4. I understand that my performance as an individual will be treated as research data and will in no way be associated with me for other than identification purposes, thereby assuring confidentiality of my performance and responses.

5. My participation in this study is purely voluntary; I understand that my refusal to participate will involve no penalty or loss of benefits to which I am otherwise entitled and that I may discontinue participation at any time without penalty or loss of benefits to which I am otherwise entitled.

6. If I have any questions concerning my rights as a research subject, injuries or emergencies resulting from my participation, I understand that I can contact the Committee on Research Involving Human Subjects, 203 Fairchild Hall, Kansas State University, Manhattan, KS 66506, at (785) 532-3224.

7. If I have questions about the rationale or method of the study, I understand that I may contact, Dr. Travis O'Quinn, 247 Weber Hall, Kansas State University, Manhattan, KS 66506, at (785) 532-3469 or Sally Stroda, 107 Weber Hall, at 785-532-1273.

I have read the Subject Orientation and Test Procedure statement and signed this informed consent statement, this _____ day of _____, _____.

Printed name

Signature

Consumer Sensory Panel Ballot – Appearance



Visual Panel 1

Please tell us a little about yourself.

Panelist Initials

Gender

☐ Male

☐ Female

Age

☐ Under 20

☐ 20 to 29 years old

☐ 30 to 39 years old

☐ 40 to 49 years old

☐ 50 to 59 years old

☐ over 60

Ethnic Origin

☐ African American

☐ Asian

☐ Caucasian/White

☐ Latino

☐ Native American

☐ Other

☐ Mixed Race

Marital Status

☐ Single

☐ Married

Household Size

☐ 1 person

☐ 2 People

☐ 3 People

☐ 4 People

☐ 5 People

☐ 6 People

☐ > 6 People

Annual Household Income

☐ < \$25,000

☐ \$25,000 – \$34,999

☐ \$35,000 – \$49,999

☐ \$50,000 – \$74,999

☐ \$75,000 – \$99,999

☐ \$100,000 – \$149,999

☐ \$150,000 – \$199,999

☐ > \$199,999

Highest Level of Education Completed

☐ Non-High School Graduate

☐ High School Graduate

☐ Some College / Technical School

☐ College Graduate

☐ Post-College Graduate

How many times a month do you purchase beef from a retail store?

0 2 3 5 6 8 9 11 12 14 15

None



How many times a week do you consume beef?

0 3 6 9 12 15 18 21

None



Are you color blind?

☐ Yes

☐ No

Sample Number

☐ 5402

Based upon the **visual appearance** of the sample, how would you describe the package?

Extremely Dislike 0 10 20 30 40 50 60 70 80 90 100 Neither like or dislike Extremely Like

Overall Appearance



Based on the **visual appearance**, would you purchase this package?

☐ Yes

☐ No

Based on the **visual appearance**, would you consider this package spoiled?

☐ Yes

☐ No

Consumer Sensory Panel Ballot – Touch and Odor

KANSAS STATE
UNIVERSITY

Sample Number

☐ 0000

Based upon the **touch**, how would you describe the sample?

Extremely Dislike 20 30 40 Neither like or dislike 60 70 80 90 100 Extremely Like

Overall Appearance



Based upon the **touch**, would you purchase this sample?

☐ Yes

☐ No

Based upon the **touch**, would you consider this sample spoiled?

☐ Yes

☐ No

Sample Number

☐ 0000

Based upon the **odor**, how would you describe the sample?

Extremely Dislike Neither like or dislike Extremely Like
0 10 20 30 40 50 60 70 80 90 100

Overall Appearance



Based upon the **odor**, would you purchase this sample?

☐ Yes

☐ No

Based upon the **odor**, would you consider this sample spoiled?

☐ Yes

☐ No

Consumer Sensory Panel Ballot – Taste

KANSAS STATE
UNIVERSITY

Sample Number

☐ 7962

Based upon the **taste** of the sample, how would you describe the sample?

Extremely Dislike 0 10 20 30 40 50 60 70 80 90 100 Neither like or dislike Extremely Like

Overall Appearance



Based on the **taste**, would you purchase this sample?

☐ Yes

☐ No

Based on the **taste**, would you consider this sample spoiled?

☐ Yes

☐ No

Trained Sensory Panel Ballot – Appearance

KANSAS STATE
UNIVERSITY

Name

Date

Time

Sample

Total Redness

Extremely Dark Red 0 10 20 30 40 50 60 70 80 90 100 Bright cherry-red

Redness

☐ Not Applicable



Total Discoloration

0% discoloration 0 10 20 30 40 50 60 70 80 90 100 100% discoloration

Discoloration

☐ Not Applicable



Trained Sensory Panel Ballot – Touch and Odor

Sample

☐ 4720

Touch

Characteristic

0 10 20 30 40 50 60 70 80 90 100

Non-characteristic

Touch

☐ Not Applicable



If the **touch** is non-characteristic, what does the sample feel like?

☐ Slimy

☐ Dry

☐ Tacky

☐ Other

Sample

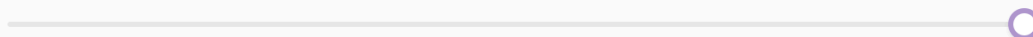
☐ 4051

Off-Odor Presence

No odor
0 10 20 30 40 50 60 70 80 Extreme off-odor
90 100

Off-Odor

☐ Not Applicable



If there is **off-odor** present, what does it smell like?

☐ Rancid

☐ Sour

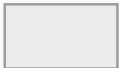
☐ Oxidized

☐ Other

Trained Sensory Panel Ballot - Taste



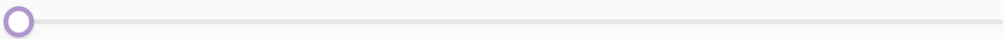
Sample



Juiciness

Extremely dry 0 10 20 30 40 Neither dry nor juicy 50 60 70 80 90 100 Extremely juicy

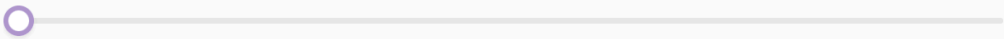
Juiciness



Tenderness

Extremely tough 0 10 20 30 40 Neither tough nor tender 50 60 70 80 90 100 Extremely tender

Tenderness



Texture

Extremely Soft Neither soft nor firm Extremely firm
0 10 20 30 40 50 60 70 80 90 100

Texture



Beef-Flavor Intensity

Extremely bland Extremely intense
0 10 20 30 40 50 60 70 80 90 100

Beef-Flavor



Off-flavor

No off-flavor Extreme off-flavor
0 10 20 30 40 50 60 70 80 90 100

Off-flavor

☐ Not Applicable



If off-flavor is present, please describe.