

## Determining the Spoilage Threshold for Ground Beef Using Microbial, Color, and Oxidation Measures

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### Abstract

The objective of this study was to determine the point at which ground beef becomes spoiled relative to microbiological, lipid oxidation, and color measurements. One lb ground beef packages from a case-ready facility were stored at 36 – 40°F in the absence of light until displayed in three coffin-style cases under fluorescent lighting. The packages were assigned to one of eight display periods (0, 2, 4, 6, 8, 10, 12, and 14 days). Samples were evaluated by consumers for visual appearance, touch, and odor liking; as well as evaluated for discoloration, redness, off-odor presence, and characteristic beef texture by trained sensory panelists. Additionally, objective measurements of aerobic plate counts (APC), *Enterobacteriaceae* plate counts (EB), and *Escherichia coli* (*E. coli*) coliform plate counts (ECC) for microbiology were obtained along with thiobarbituric acid reactive substances (TBARS) for lipid oxidation and  $L^*$  (lightness),  $a^*$  (redness), and  $b^*$  (yellowness) values for color. Logistic regression models were generated to identify purchase intent thresholds and consumer spoilage classification based on the objective measures. As expected, consumer appearance liking was the strongest predictor compared to the microbiological assays, explaining 81% of the variation when predicting consumer purchase intent. Logistic models for APC ( $R^2 = 0.59$ ;  $P < 0.05$ ) identified values of 7.3, 6.7, 6.1, and 5.8 log colony forming units (CFU)/g representing 50, 75, 90, and 95% likelihood a consumer would purchase the product. Additionally, APC values ( $R^2 = 0.46$ ;  $P < 0.05$ ) of 5.3, 5.9, 6.8, and 7.7 log CFU/g relating to 5, 10, 25, and 50% likelihood a consumer would consider a product spoiled. The EB and ECC models also showed the strongest relationships corresponded to appearance liking, but not as strong as the APC predictors.

### Introduction

Spoilage can be defined and measured in a number of ways. Assays to evaluate microbial growth, lipid oxidation, and color can all be used to determine spoilage. However, spoilage is ultimately defined as the end of the product shelf-life and “the perception of a state of spoilage is, therefore, essentially a subjective evaluation which will vary with consumer expectations” (Gill, 1983). It’s essential to study the effects of spoilage relevant to industry due to the millions of pounds of product discarded on an annual basis. Approximately 23% of the meat produced in the world is wasted, with spoilage playing a large role (Karwowska et al., 2021). Nonetheless, the annual loss for animals wasted in the beef industry is 780,000 animals in conjunction with \$3.73 billion lost due to

discoloration that makes beef fall short of consumer expectations. The purpose of this study was to establish thresholds of spoilage for ground beef using multiple objective measures of color, microbial growth, and lipid oxidation to gain valuable insight for the beef industry for better product management. The goal is to increase the shelf-life of ground beef by identifying consumer expectations of freshness and quality.

## Experimental Procedures

This study used one lb ground beef packages (80% lean) sourced from a commercial case-ready ground beef facility. The packages were stored at 36 to 40°F in gas-flushed mother bags (Tri gas composition: 69.6% nitrogen, 30% carbon dioxide, and 0.4% carbon monoxide) without exposure to light at the Kansas State University Meat Laboratory. Each mother bag contained four individual packages. Within each mother bag, two pairs of packages were randomly assigned to specific display days (0, 2, 4, 6, 8, 10, 12, and 14 days). One package per pair was designated for color and visual analysis, while the other was used for sensory evaluation of smell and touch. In addition to consumer sensory panels, objective measurements were taken, including trained sensory panel assessments, as well as microbiological, lipid oxidation, and  $L^*$  (lightness),  $a^*$  (redness), and  $b^*$  (yellowness) values using a Hunter Lab Miniscan spectrophotometer (Illuminant A, 2.54 cm aperture, 10° observer; Hunter Lab Associates Laboratory, Reston, VA). Spectral data were collected to determine the percentage of myoglobin present, following the methods outlined in the American Meat Science Association (AMSA) color guidelines (AMSA, 2012). Microbiological analysis was conducted using aerobic plate counts (APC), *Enterobacteriaceae* plate counts (EB), and *Escherichia coli* (*E. coli*) coliform plate counts (ECC). From each ground beef sample, a 0.9 oz portion was combined with 7.6 oz of peptone water (PW) and stomached for 60 seconds (Stomacher 400, Seward, Bohemia, NY). On duplicate 3M Petrifilm for each APC, EB, and ECC, serial dilutions of 1 ml were plated using PW. Petrifilms were incubated for their respective times and colonies were counted for data collection. Additionally, lipid oxidation was measured using the thiobarbituric acid reactive substances (TBARS) assay following procedures outlined in Ahn et al. (1998) and similar procedures followed in other Kansas State University studies (Beyer et al., 2024). The samples ( $n = 128$ ) were placed in three coffin-style display cases (model DMF8; Tyler Refrigeration Corp., Niles, MI) maintained at 36 to 40°F under continuous fluorescent lighting (32 W Del-Warm White 3,000 K; Phillips Lighting Company, Somerset, NJ) for their entire assigned display period. Samples were introduced to the display cases every other day, starting 14 days before evaluation, with day 0 samples placed on display last. The samples were added at the same time daily and rotated within the cases, which were set to defrost once each morning to prevent package fogging during evaluations.

Consumer sensory panelists ( $n = 128$ ) were asked to rate the visual appearance of eight samples (one from each display period) using a 100-point continuous line scale. Additionally, they were asked (yes/no) if they would purchase the sample and (yes/no) if they thought the sample was spoiled. Consumers were also asked to rate the touch and odor of samples on 100-point continuous line scales with the same (yes/no) questions above after evaluating each trait.

Similar to the consumers, trained sensory panelists were asked to evaluate percent discoloration, redness, touch, and odor. Panelists were trained with multiple samples of varying characteristics anchored to the scales. Additionally, panelists were trained according to the AMSA meat color measurement guidelines (AMSA, 2012). Discolor-

ation and redness of the samples were evaluated with a 100-continuous line scale with anchors at 0 (0% discoloration and extremely dark red) and 100 (100% discoloration and bright, cherry-red). On similar 100-point continuous line scales, sensory panelists evaluated touch and odor with anchors at 0 (characteristic beef texture and no odor present) and 100 (non-characteristic beef texture and extreme off odor present). Both consumer and trained sensory panelists evaluated touch and odor samples under red lights to avoid bias toward the color of the sample. All panelists recorded their answers using electronic tablets (Model 5709 HP Stream 7, Hewlett-Packard, Palo Alto, CA) using a digital survey (Qualtrics Software, Provo, UT). Data were analyzed using logistic regression models to identify the points at which consumers determined the product to be spoiled based on visual, touch, and odor characteristics.

## Results and Discussion

Overall, consumer sensory panelists rated samples in the case for 0 and 2 days higher ( $P < 0.05$ ) for all three attributes evaluated (color, touch, and odor liking) than any samples in the case for 8, 10, 12 and 14 days.

### *Logistic regressions predicting consumer purchase intent*

Predictors of consumer purchase intent were generated utilizing logistic regression models, and common threshold values (50, 75, 90, and 95%) were identified in regard to the objective measurements. Different models were generated for all microbiological measurements (APC, EB, and ECC) corresponding to the consumer visual appearance liking as shown in Figure 1. A model with an  $R^2$  of 0.59 ( $P < 0.05$ ) was generated for APC, with values of 7.3, 6.7, 6.1, and 5.8 log colony forming units (CFU)/g corresponding to 50, 75, 90, and 95% likelihood of the consumer purchasing the product. Additionally, values for EB of 4.4, 3.5, 2.6, and 2.0 log CFU/g were associated with 50, 75, 90, and 95% likelihood of purchase intent, with the model ( $P < 0.05$ ) explaining 49% of the variation in purchase intent. An ECC level of 2.2 log CFU/g corresponded to a 50% chance a consumer would purchase the product ( $R^2 = 0.17$ ;  $P < 0.05$ ). The same logistic techniques were used to generate predictor models for consumer purchase intent in regard to the consumer odor liking as shown in Figure 2. When evaluating the odor of the product, APC values of 7.5 and 5.3 log CFU/g corresponded to a 50 and 75% chance a consumer would purchase the product ( $R^2 = 0.17$ ;  $P < 0.05$ ). The EB and ECC models were significant ( $P < 0.05$ ), but only explained 12 and 8% of the variation in purchase intent. Logistic regression models for TBARS were also significant ( $P < 0.05$ ), but again only explained a low (6%) amount of variation in consumer purchase intent. Likewise, models were created to predict purchase intent based on consumer touch liking as shown in Figure 3. Although models were significant ( $P < 0.05$ ), the strongest predictor variable was APC, but again that only explained 11% of the variation for consumer purchase intent.

### *Logistic regressions predicting consumer likeliness to classify a sample as spoiled*

Common thresholds (5, 10, 25, and 50%) were generated using logistic regression models to predict the likelihood of consumers classifying a sample as spoiled based on the objective measurements evaluated. Shown in Figures 4, 5, and 6, three models were generated from all microbial assays (APC, EB, and ECC) for consumer appearance ratings to predict spoilage classification. The APC model ( $R^2 = 0.46$ ;  $P < 0.05$ ) generated values of 5.3, 5.9, 6.8, and 7.7 log CFU/g corresponding to a 5, 10, 25, and 50%

likelihood consumers would classify a sample as spoiled. The EB and ECC assays also had significant ( $P < 0.05$ ) models; however, they only accounted for 46 and 12% of the variation when predicting consumer spoilage classification. In addition, thresholds were determined for spoilage classification with consumer odor liking and touch liking scores. Although models for APC, EB, ECC, and TBARS were significant ( $P < 0.05$ ), APC explained the most variation, but still only a relatively small (17%) amount when predicting spoilage. Similarly, APC regression models explained the most variation in consumer spoilage assessment based on touch but only explained 10% of the variation.

## Implications

Overall, logistic regression models demonstrated relationships between the objective and microbial measures evaluated and consumer willingness to purchase and assessment of spoilage; however, many of the measures explained only a minimal amount of variation in the consumer responses, providing evidence that spoilage determination is likely not dependent upon these variables.

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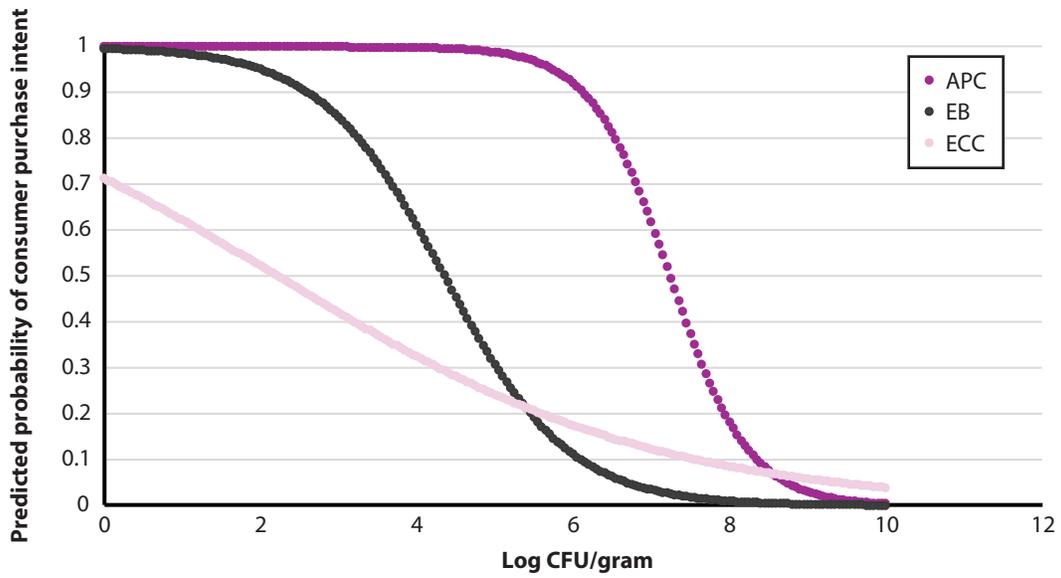


Figure 1. Threshold values for consumer likeliness to purchase 80% lean ground beef according to aerobic plate counts (APC), *Enterobacteriaceae* plate counts (EB), and *Escherichia coli* coliform plate counts (ECC) based on appearance.

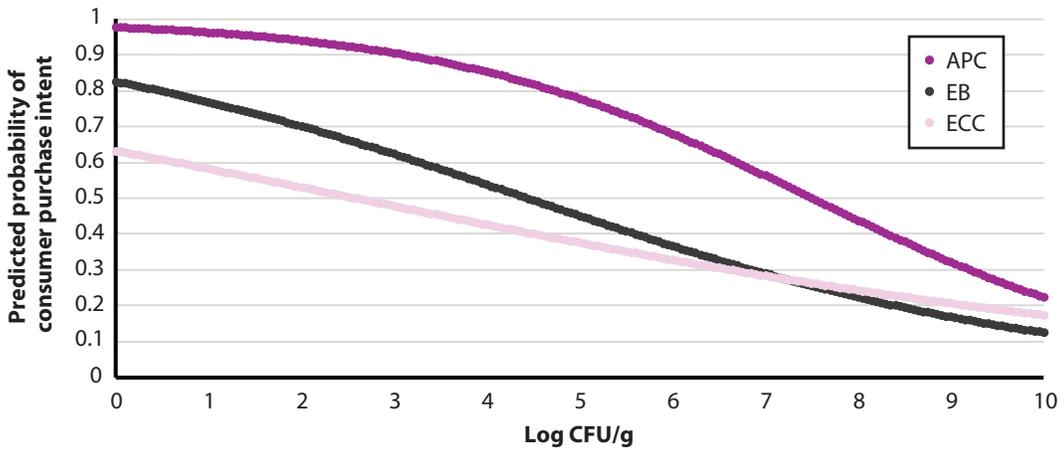


Figure 2. Threshold values for consumer likeliness to purchase 80% lean ground beef according to APC, EB, and ECC based on odor.

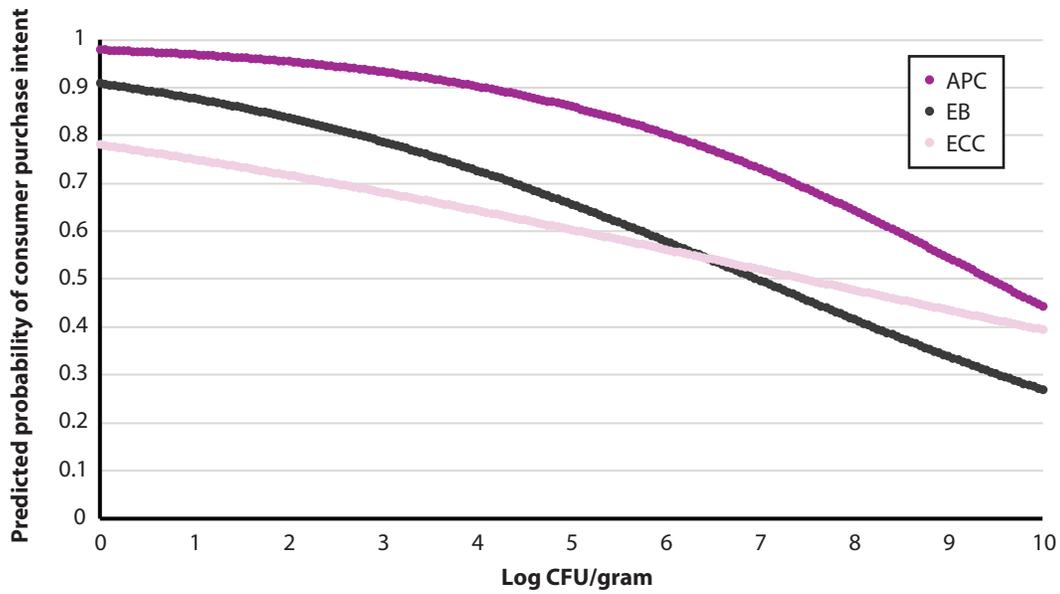


Figure 3. Threshold values for consumer likeliness to purchase 80% lean ground beef according to APC, EB, ECC based on touch.

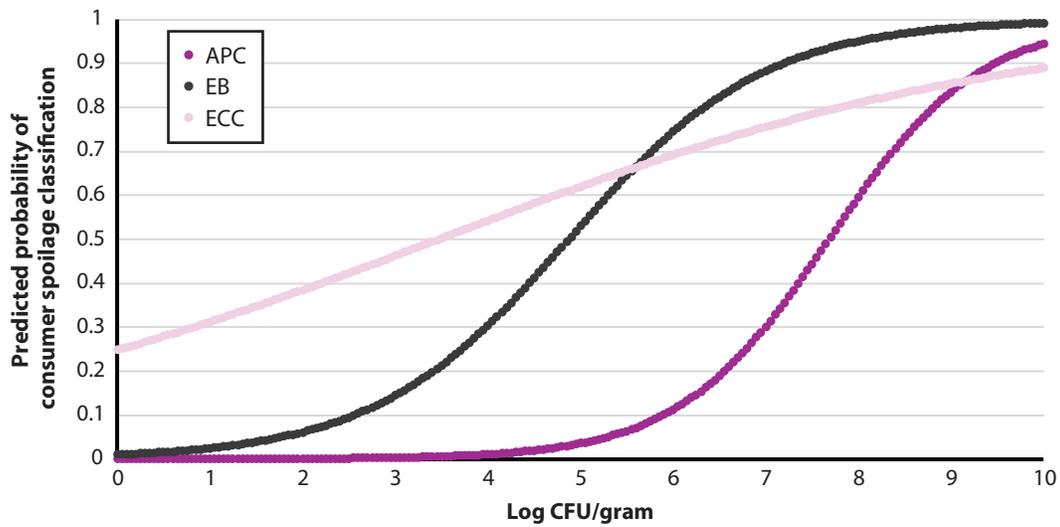


Figure 4. Threshold values for consumer likeliness to classify 80% lean ground beef spoiled according APC, EB, and ECC based on appearance.

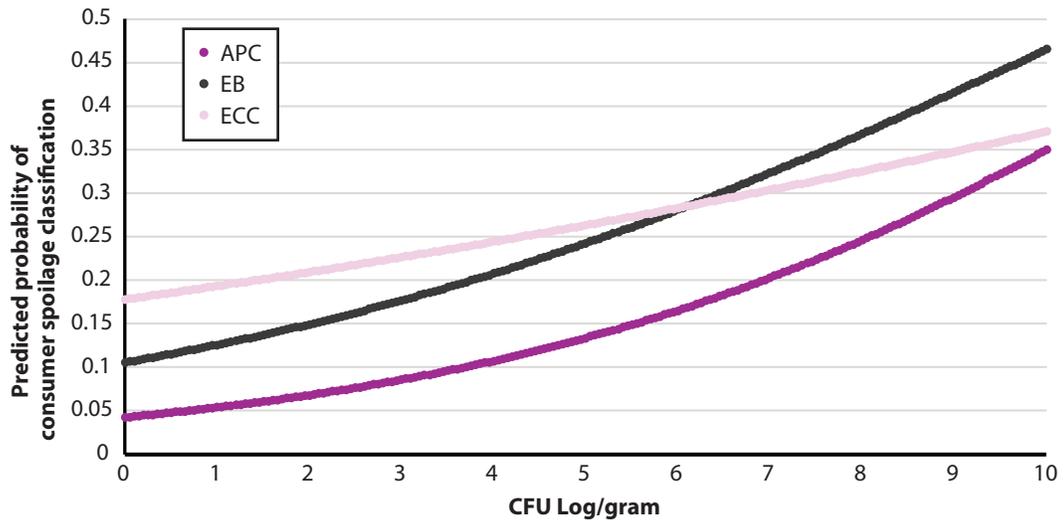


Figure 5. Threshold values for consumer likeliness to classify 80% lean ground beef spoiled according to APC, EB, and ECC based on touch.

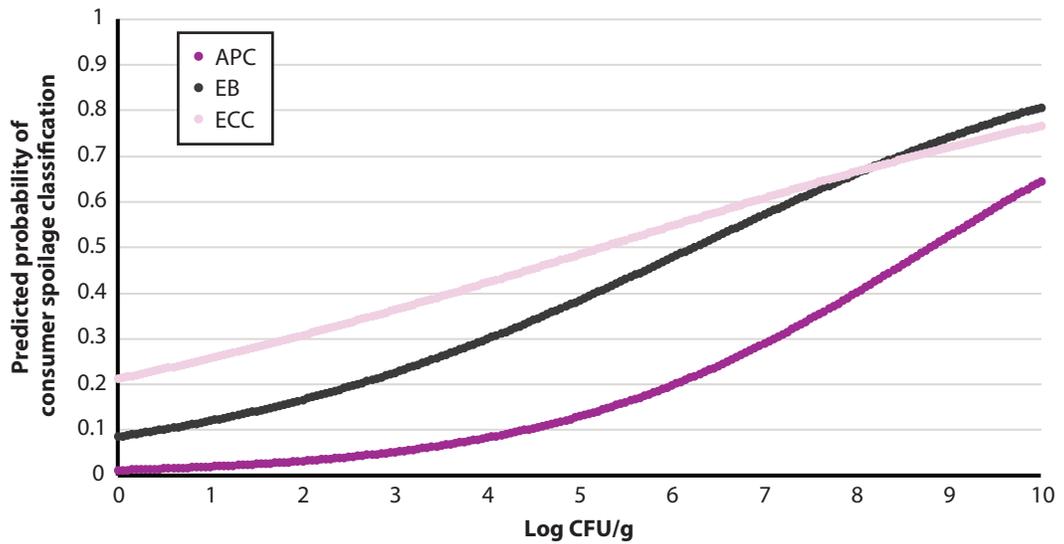


Figure 6. Threshold values for consumer likeliness to classify 80% lean ground beef spoiled according to APC, EB, and ECC based on odor.