



## Evaluation of portable, low-cost techniques for discriminating the DFD (dark, firm, dry) condition in beef

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### ABSTRACT

This study evaluated the effectiveness of portable, low-cost techniques, specifically colorimetry and visible spectroscopy for discriminating dark, firm, and dry (DFD) beef carcasses during processing in a certified slaughterhouse. Portable, low-cost devices were used for real-time assessments. A total of 523 chilled carcasses were analyzed, of which 449 were classified as normal and 74 as DFD, based on pH measurements ( $\geq 5.8$  for DFD). Colorimetric variables ( $L^*$ ,  $a^*$ ,  $b^*$ , chroma, and hue) were consistently higher in normal carcasses compared to DFD. Supervised classification models were used to predict DFD status using the evaluated detection techniques. Given the class imbalance, the Synthetic Minority Over-sampling Technique (SMOTE) was applied, significantly improving the performance of machine learning models. Portable spectroscopy showed superior performance, especially when combined with preprocessing techniques such as multiplicative scatter correction and second-order derivatives, achieving 96.77 % sensitivity and 98.06 % specificity with the Random Forest model. Although colorimetry proved effective, spectroscopy yielded greater reliability for real-time DFD detection. These findings highlight the potential of these techniques as cost-effective alternatives to traditional methods, supporting their applicability in rapid and objective meat quality assessments under industrial conditions.

### 1. Introduction

Dark, firm, and dry (DFD) beef is a meat quality defect that occurs in cattle subjected to pre-slaughter stress (Ponnampalam et al., 2017), altering final muscle pH and structure (Clinquart et al., 2022), and resulting in a darker meat color (Mahmood et al., 2016). This effect is mainly driven by the elevated ultimate pH, which sustains mitochondrial oxygen consumption and limits myoglobin oxygenation (Ramanathan & Mancini, 2018). Simultaneously, the increased water-holding capacity swells the myofibrils, reduces light scattering, and lowers surface reflectance, producing the characteristic dark appearance of DFD beef (Ramanathan et al., 2020).

The relationship between pH and meat color is critical for early detection of this condition (Abril et al., 2001). Although there is no global consensus on the threshold values for DFD classification (Holman & Hopkins, 2019; Ponnampalam et al., 2017), pH measurements taken

between 24- and 48-h postmortem are commonly used as an indicator, with  $\text{pH} \geq 5.8$  generally accepted as characteristic of DFD meat (Carrasco-García et al., 2020).

This condition negatively impacts the global meat industry by reducing the commercial value of the product (Ferreira et al., 2024). Consumers tend to prefer bright red meat over darker shades, which lowers the market demand for DFD products (Leighton et al., 2023). Therefore, the development of rapid and non-invasive methods to detect this anomaly is essential to maintain competitiveness in the global meat market (Ferreira et al., 2024).

Several methods are available for evaluating meat color, including visual assessment, color reference charts, and instrumental tools (Prieto et al., 2014). Due to their speed, precision, and repeatability, optical instruments such as colorimeters and spectrophotometers are increasingly adopted (Holman et al., 2015). However, conventional optical devices are often large, less portable, expensive, and prone to variability

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due to factors such as calibration, illuminant type, aperture size, and viewing angle (Tomasevic et al., 2021).

Spectroscopy is considered one of the most promising non-invasive techniques for assessing commercial meat attributes, due to its versatility in capturing data across different regions of the electromagnetic spectrum. Nevertheless, challenges such as data noise and redundancy can limit its effectiveness (Khaled et al., 2021). Therefore, spectral preprocessing is crucial to remove irrelevant information and improve signal quality, contributing to greater repeatability and accuracy in analyses (Stevens & Ramírez-López, 2024).

In this context, machine learning (ML) algorithms are valuable tools for meat quality evaluation because they can handle high-dimensional and collinear data such as spectral and colorimetric measurements. Their flexibility allows capturing complex relationships beyond traditional linear models, which makes them suitable for objective and rapid classification tasks in meat science (Dixit et al., 2021).

Previous studies have demonstrated the capacity of spectroscopy (Prieto et al., 2014; Tejerina et al., 2022) and colorimetry (Holman & Hopkins, 2019) combined with machine learning approaches to discriminate DFD carcasses. Extensive research on dark-cutting beef has been carried out in countries such as the United States, Canada, and Australia, predominantly under intensive or feedlot-based production systems (Holman & Hopkins, 2019; McGilchrist et al., 2012; Ponnampalam et al., 2017; Prieto et al., 2018). In contrast, evidence from tropical, pasture-based systems remains scarce. In Colombia, where DFD prevalence has been reported at levels exceeding 48 % (Patiño et al., 2019; Romero Peñuela et al., 2017), slaughter plants still rely almost exclusively on pH measurements, with little adoption of rapid instrumental methods. The economic limitations of local industries to implement advanced technologies such as high-resolution cameras or hyperspectral systems further underscore the relevance of exploring accessible alternatives. This study therefore evaluates DFD beef discrimination under real-life slaughterhouse conditions in Colombia using low-cost handheld devices, combined with data balancing and machine learning approaches rarely applied in tropical environments with pasture-fed cattle, but with significant potential for cost-effective, online grading of carcasses.

The objective of this study was to evaluate spectroscopy and colorimetry techniques for DFD beef discrimination using low-cost, rapid, and non-invasive portable devices under processing line conditions in slaughterhouses.

## 2. Materials and methods

### 2.1. Data collection

This study was conducted in a slaughterhouse certified by sanitary authorities, located on the northern coast of Colombia, with ethical approval granted by the Ethics Committee of the University of Antioquia (Approval No. 150, February 7, 2023).

A convenience sampling was carried out over 12 months, evaluating 523 chilled carcasses from 43 cattle lots. The animals included different sexes and breeds (zebu and crossbreeds), were over 24 months of age, and were mainly pasture-fed under cow-calf, fattening, and dual-purpose systems (UPRA – Unidad de Planificación Rural Agropecuaria, 2021). Evaluations were performed 36 h postmortem, including instrumental measurements of color, spectral capture, and pH on the internal surface of the *longissimus thoracis* muscle, from carcasses refrigerated between 2 and 4 °C, after being pre-chilled in rooms with controlled air circulation and relative humidity of 85–95 %.

Color, spectral, and pH assessments were performed using a NIX Pro 2 colorimeter (Nix Sensor Ltd., Ontario, CAN), a NIX Spectro 2 spectrophotometer (Nix Sensor Ltd., Ontario, CAN), and a HI981036 meat pH meter with a built-in sensor for temperature compensation (Hanna Instruments, Romania), respectively. Both the colorimeter and spectrophotometer were configured with a 15.0 mm aperture, 45/

0° measurement geometry, D65 illuminant, and a 10° standard observer (Holman et al., 2021). The pH meter was calibrated on-site following the manufacturer's guidelines, using pH 4.01 and 7.01 buffer solutions at the start of the measurements and after every 20 samples (Hanna Instruments).

After 36 h of carcass chilling, the left half-carcass was cut between the fifth and sixth ribs, exposing the *longissimus* muscle to oxygen for 45 min before measurements. The procedure followed the methodology described by Holman et al. (2018). For each sample, five readings were taken with the colorimeter, five with the spectrophotometer, and one pH reading with the pH meter (Fig. 1). Color ( $L^*$ ,  $a^*$ ,  $b^*$ ) and spectral data were automatically averaged by the devices and stored in CSV format, while pH values were manually recorded.

### 2.2. Data processing

Data analyses were conducted using R Project software (version 2023.12.1), employing libraries such as Prospectr, ggplot2, doBy, reshape2, caret, lattice, textshape, performanceEstimation, and pls. Supervised classification models were implemented to generate predictions based on previously trained models (Villalba, 2018).

#### 2.2.1. Database construction

Two datasets were constructed: one for color values and another for spectral data. The color dataset included five independent variables ( $L^*$ ,  $a^*$ ,  $b^*$ , hue angle, and chroma) with a total of 523 observations. Each observation consisted of the average of five readings from each sample. Hue angle was calculated as the arctangent of  $b^*/a^*$ , while chroma (saturation index) was calculated as the square root of the sum of squares of  $a^*$  and  $b^*$  ( $\text{chroma} = \sqrt{a^{*2} + b^{*2}}$ ) (King et al., 2023). The spectral dataset included 31 independent variables (wavelengths from 400 to 700 nm, at 10 nm intervals) and the same number of observations. In both datasets, pH was used as the dependent variable to classify samples into two categories: normal carcass (pH 5.23–5.79) and DFD carcass (pH  $\geq$  5.8) (Dixit et al., 2021; Holman & Hopkins, 2019; Ponnampalam et al., 2017). Only 14 out of 523 carcasses (2.7 %) showed values below 5.3. Although such low ultimate pH values are rare, they are considered plausible given the strict on-site calibration (pH 4.01 and 7.01 every 20 samples) and automatic temperature compensation performed during data collection (King et al., 2023).

#### 2.2.2. Dataset balancing

Of the 523 observations, 449 were classified as normal and 74 as DFD, revealing a class imbalance. To address this issue, the Synthetic Minority Over-sampling Technique (SMOTE) was applied (Chawla et al., 2002), which generates synthetic data through random linear interpolation among nearest neighbors. This procedure produced a balanced dataset with 518 observations per class. Compared to models trained on the imbalanced dataset, the use of SMOTE increased the sensitivity to minority classes and improved the overall AUC by +0.28 points, supporting its suitability for this study.

#### 2.2.3. Data transformation

Both the original and balanced datasets underwent spectral preprocessing, including Savitzky-Golay filtering, multiplicative scatter correction (MSC), standard normal variate (SNV), and first- and second-order derivatives. These techniques were used to improve spectral signal quality by reducing noise and redundancy (Stevens & Ramírez-López, 2024).

#### 2.2.4. Dataset partitioning

Transformed datasets were split into training (70 %) and validation (30 %) subsets using both random partitioning and the Kennard-Stone algorithm. The createDataPartition function from the Caret package and the kenStone function from the Prospectr package were used for these procedures.



**Fig. 1.** Portable devices used for data acquisition: Nix Pro 2 and Nix Spectro 2 spectrophotometers (left and center) for colorimetry and visible spectroscopy, and Hanna Foodcare pH meter (right) for ultimate pH measurement in beef carcasses.

### 2.2.5. Statistical analysis

Group comparisons for color variables ( $L^*$ ,  $a^*$ ,  $b^*$ , chroma, hue angle) were performed on the observed (unbalanced) dataset using two-tailed Welch's  $t$ -tests with a significance threshold of  $\alpha = 0.01$ . SMOTE-generated samples were not included in inferential tests.

### 2.2.6. Construction and evaluation of supervised classification models

Supervised classification models were developed, including Partial Least Squares Discriminant Analysis (PLS-DA),  $k$ -nearest neighbors (KNN), Support Vector Machines (SVM), and Random Forest (RF).  $K$ -fold cross-validation (10 folds, repeated 10 times) was applied to improve model robustness.

Hyperparameters assessed included: The number of randomly selected variables at each split (mtry) in RF; number of neighbors ( $k$ ) in KNN; the regularization parameter ( $C$ ) in SVM; and the number of components in PLS-DA.

Model performance was evaluated using confusion matrices, sensitivity, specificity, and the area under the receiver operating characteristic curve (AUC-ROC), using the pROC package in R.

## 3. Results

### 3.1. Characterization of the DFD condition

Table 1 presents descriptive statistics for the colorimetric variables obtained from the observed samples, both before and after the generation of synthetic data using the SMOTE technique. A total of 449 carcasses were classified as normal and 74 as DFD, resulting in a 13.9 % prevalence of the DFD condition. In the unbalanced dataset, normal beef showed a lower final pH ( $-0.34$ ) and higher of  $L^*$  ( $+1.94$ ),  $a^*$  ( $+1.40$ ),  $b^*$  ( $+1.19$ ), chroma ( $+1.79$ ), and hue ( $+1.40$ ) values compared to DFD beef. All differences were significant according to Welch's  $t$ -tests ( $p < 0.01$ ).

After balancing the dataset with SMOTE, the same trend was preserved. Normal beef maintained lower pH ( $-0.44$ ) and higher values of  $L^*$  ( $+2.03$ ),  $a^*$  ( $+1.33$ ),  $b^*$  ( $+1.17$ ), chroma ( $+1.73$ ), and hue ( $+1.47$ )

compared to DFD beef ( $p < 0.01$ ). These results confirm that the colorimetric differences between DFD and normal samples are robust, regardless of class balancing, and emphasize the darker and less vivid appearance of DFD meat.

Fig. 2 shows the average reflectance spectral behavior for samples classified as DFD and normal in both imbalanced and balanced datasets generated using the SMOTE algorithm.

The application of data balancing did not affect the overall shape of the spectral curves, as both balanced and imbalanced datasets showed highly similar reflectance patterns (Fig. 2). However, subsequent spectral pre-processing (MSC combined with second derivative transformation) modified the data structure by reducing baseline effects and scatter, thereby enhancing the visibility of key peaks. After transformation, divergence trends between normal and DFD spectra became noticeable around 450 nm, while clearer and more consistent differences appeared beyond 600 nm (Fig. 3). These regions were also confirmed by VIP scores from the Random Forest model as the most relevant contributors to class discrimination.

### 3.2. Performance of classification models – Colorimetry

Table 2 summarizes the performance of the classification models applied to colorimetry data. Results are presented for both training and validation sets and include area under the ROC curve (AUC), sensitivity, and specificity.

The centered and scaled  $K$ -nearest neighbors (KNN-CS) model achieved perfect classification in the training set (AUC: 1.0; sensitivity and specificity: 100 %). However, in the validation set, AUC dropped to 0.8766, with both sensitivity and specificity values at 87.66 %. The Random Forest (RF) model also showed strong performance with an AUC of 1.0 in training and 0.8572 in validation, reaching 85.06 % sensitivity and 86.36 % specificity.

### 3.3. Performance of classification models – Spectroscopy

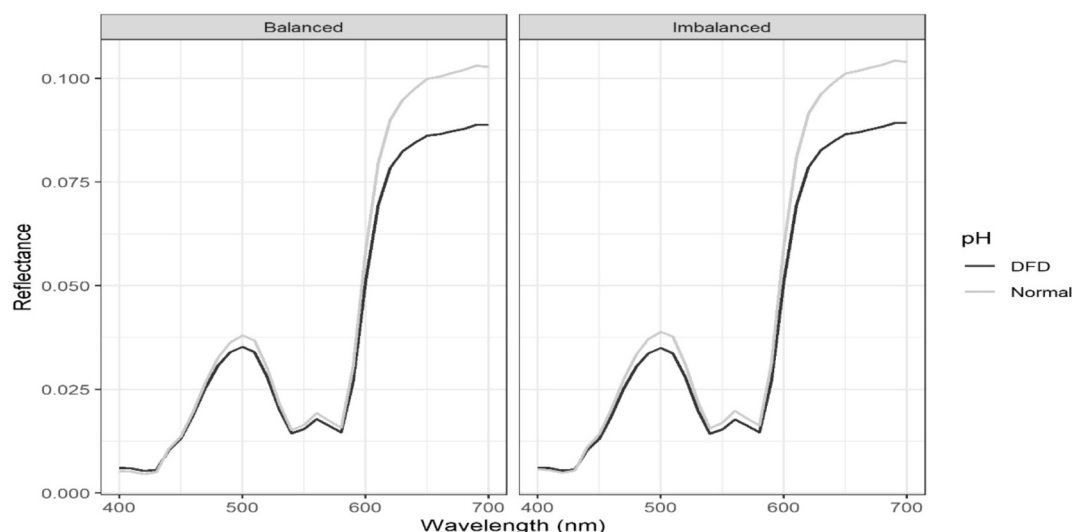
Table 3 summarizes the classification performance of models applied

**Table 1**

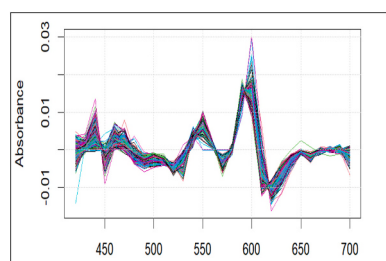
Descriptive statistics (mean  $\pm$  standard deviation) of pH and colorimetric values for normal and DFD beef (*longissimus thoracis*).

| Type of dataset          | Condition   | n    | pH (36 h)       | $L^*$            | $a^*$            | $b^*$            | Chroma           | Hue              |
|--------------------------|-------------|------|-----------------|------------------|------------------|------------------|------------------|------------------|
| Unbalanced dataset       | DFD         | 74   | $5.86 \pm 0.15$ | $28.30 \pm 3.82$ | $18.13 \pm 3.90$ | $8.93 \pm 2.70$  | $20.24 \pm 4.60$ | $25.84 \pm 3.38$ |
|                          | Normal      | 449  | $5.52 \pm 0.12$ | $30.25 \pm 3.51$ | $19.53 \pm 3.36$ | $10.11 \pm 2.25$ | $22.03 \pm 3.83$ | $27.24 \pm 3.68$ |
|                          | Difference  | +375 | $-0.34$         | $+1.94$          | $+1.40$          | $+1.19$          | $+1.79$          | $+1.40$          |
|                          | t-Statistic | –    | –               | 4.03 (90)        | 2.86 (87)        | 3.52 (86)        | 3.11 (86)        | 3.20 (98)        |
|                          | p-Value     | –    | –               | 0.0001           | 0.0054           | 0.0007           | 0.0026           | 0.0018           |
|                          | Average     | 523  | $5.58 \pm 0.20$ | $29.98 \pm 3.62$ | $19.34 \pm 3.36$ | $9.95 \pm 2.25$  | $21.79 \pm 3.83$ | $27.05 \pm 3.68$ |
|                          | DFD         | 518  | $5.96 \pm 0.14$ | $28.37 \pm 3.50$ | $18.13 \pm 3.64$ | $9.00 \pm 2.56$  | $20.27 \pm 4.31$ | $26.03 \pm 3.19$ |
|                          | Normal      | 518  | $5.52 \pm 0.25$ | $30.40 \pm 3.48$ | $19.46 \pm 3.36$ | $10.17 \pm 2.28$ | $22.00 \pm 3.83$ | $27.50 \pm 3.66$ |
| Balanced dataset - SMOTE | Difference  | 0    | $-0.44$         | $+2.03$          | $+1.33$          | $+1.17$          | $+1.73$          | $+1.47$          |
|                          | Average     | 1016 | $5.74 \pm 0.12$ | $29.38 \pm 3.63$ | $18.80 \pm 3.56$ | $9.59 \pm 2.49$  | $21.14 \pm 4.17$ | $26.77 \pm 3.51$ |

**Note:** DFD: dark, firm, dry; n: sample size;  $L^*$ : lightness;  $a^*$ : red index;  $b^*$ : yellow index. Group comparisons were assessed using two-tailed Welch's  $t$ -tests ( $\alpha = 0.01$ ).



**Fig. 2.** Mean reflectance spectra (400–700 nm) of normal and DFD (Dark, Firm, Dry) beef samples in both original (imbalanced) and SMOTE-balanced datasets. Spectral differences between classes become more evident beyond 450 nm, with normal carcasses showing consistently higher reflectance values.



**Fig. 3.** Spectral absorbance profiles (400–700 nm) of normal and DFD beef samples after spectral pre-processing using multiplicative scatter correction (MSC) combined with second derivative transformation. Data transformation reduced baseline effects and scatter, enhancing peak visibility and revealing divergence trends between classes, particularly around 450 nm and more clearly beyond 600 nm.

to spectroscopy data, considering different partition methods, data transformations, and hyperparameters.

In the balanced dataset with random partitioning, the RF model achieved excellent performance, with an AUC of 0.9849 in training, sensitivity of 94.21 %, and specificity of 92.45 %. In the validation set, the RF model maintained a high performance with an AUC of 0.9677, sensitivity of 96.77 %, and specificity of 98.06 %.

The KNN model, using first derivative transformation, showed an

AUC of 0.9472 in training and 0.9097 in validation. Sensitivity reached 93.55 % in training and 95.48 % in validation, while specificity was 79.35 % and 86.45 %, respectively. The support vector machine (SVM) model, with multiplicative scatter correction and second derivative transformation, achieved an AUC of 0.9102 in training and 0.8774 in validation. Sensitivity reached 88.56 % and 92.26 %, and specificity was 83.09 % and 83.23 %, respectively ( $C = 100$ ).

The partial least squares (PLS) model using second derivative pre-processing and the balanced dataset reached an AUC of 0.9221 in training and 0.8613 in validation, with sensitivity values of 86.08 % and 90.32 %, and specificity values of 82.04 % and 81.94 %.

Models with Kennard-Stone (KS) partitioning showed lower performance compared to those using random partitioning. For instance, the RF model with second derivative transformation reached an AUC of 0.8553 in training, but validation sensitivity decreased to 51.27 %, although specificity remained high at 94.62 %.

The PLS model trained on the imbalanced dataset with second derivative preprocessing showed an AUC of 0.8852 in training and 0.7689 in validation, with 57.91 % sensitivity and 92.06 % specificity. KNN and SVM models also showed more modest results, with validation AUC values of 0.7626 and 0.7561, respectively.

Overall, models trained on imbalanced datasets performed worse. For example, the KNN model with KS partitioning and first derivative-gap 10 preprocessing reached an AUC of 0.7766 in training and 0.6836 in validation, with sensitivity of 32.47 % and specificity of 96.91

**Table 2**

Performance of classification models for DFD beef based on colorimetry.

| Model    | Type of dataset | Data partition | Data transformation | Training |             |             | Validation |             |             |
|----------|-----------------|----------------|---------------------|----------|-------------|-------------|------------|-------------|-------------|
|          |                 |                |                     | ROC      | Sensitivity | Specificity | ROC        | Sensitivity | Specificity |
| KNN - CS | Balanced        | Random         | Untransformed       | 1        | 1           | 1           | 0.8766     | 0.8766      | 0.8766      |
| RF       | Balanced        | Random         | Untransformed       | 1        | 1           | 1           | 0.8572     | 0.8506      | 0.8636      |
| RF       | Balanced        | Random         | SNV                 | 1        | 1           | 1           | 0.8444     | 0.8571      | 0.8312      |
| KNN - CS | Balanced        | Random         | SNV                 | 1        | 1           | 1           | 0.8216     | 0.8312      | 0.8117      |
| RF       | Balanced        | Random         | d1                  | 1        | 1           | 1           | 0.8187     | 0.7987      | 0.8377      |
| KNN - CS | Balanced        | Random         | SG                  | 1        | 1           | 1           | 0.802      | 0.7468      | 0.8506      |
| KNN - CS | Balanced        | Random         | d2                  | 1        | 1           | 1           | 0.7955     | 0.7987      | 0.7922      |
| RF       | Balanced        | Random         | d2                  | 1        | 1           | 1           | 0.7924     | 0.7792      | 0.8052      |
| RF       | Balanced        | Random         | SNVd2               | 1        | 1           | 1           | 0.7828     | 0.7987      | 0.7662      |
| KNN - CS | Balanced        | Random         | SNVd2               | 1        | 1           | 1           | 0.7825     | 0.7792      | 0.7857      |
| RF       | Balanced        | Random         | SG                  | 1        | 1           | 1           | 0.7662     | 0.7078      | 0.8182      |

**Note:** KNN-CS: KNN with centering and scaling; SNV: standard normal variate; d1: first derivative; d2: second derivative; SNVd2: standard normal variate with derivative; SG: Savitzky-Golay filter; ROC: receiver operating characteristic curve.

**Table 3**

Performance of classification models for DFD beef based on visible spectroscopy.

| Model | Type of dataset | Data partition | Data transformation | Hyperparameter | Hyperparameter range | Training |             |             | Validation |             |             |
|-------|-----------------|----------------|---------------------|----------------|----------------------|----------|-------------|-------------|------------|-------------|-------------|
|       |                 |                |                     |                |                      | ROC      | Sensitivity | Specificity | ROC        | Sensitivity | Specificity |
| RF    | Balanced        | Random         | mscd2               | Mtry           | 3–1500               | 0.9849   | 0.9421      | 0.9245      | 0.9677     | 0.9677      | 0.9806      |
| KNN   | Balanced        | Random         | d1                  | k              | 3                    | 0.9472   | 0.9355      | 0.7935      | 0.9097     | 0.9548      | 0.8645      |
| MVS   | Balanced        | Random         | mscd2               | C              | 100                  | 0.9102   | 0.8856      | 0.8309      | 0.8774     | 0.9226      | 0.8323      |
| PLS   | Balanced        | Random         | d2                  | Components     | 15                   | 0.9221   | 0.8608      | 0.8204      | 0.8613     | 0.9032      | 0.8194      |
| RF    | Balanced        | KS             | d2                  | Mtry           | 7–500                | 0.8553   | 0.5127      | 0.9462      | 0.8290     | 0.6581      | 1.0000      |
| PLS   | Balanced        | KS             | d2                  | Components     | 15                   | 0.8852   | 0.5791      | 0.9206      | 0.7689     | 0.5788      | 0.9590      |
| KNN   | Balanced        | KS             | d2                  | k              | 4                    | 0.7854   | 0.4496      | 0.9105      | 0.7626     | 0.6400      | 0.8852      |
| MVS   | Balanced        | KS             | d2                  | C              | 1000                 | 0.8621   | 0.3669      | 0.9414      | 0.7561     | 0.5290      | 0.9832      |
| KNN   | Imbalanced      | KS             | d1–10               | k              | 12                   | 0.7766   | 0.3247      | 0.9691      | 0.6836     | 0.3889      | 0.9783      |
| RF    | Imbalanced      | KS             | Untransformed       | Mtry           | 2–500                | 0.7087   | 0.3120      | 0.9601      | 0.6763     | 0.3889      | 0.9638      |
| RF    | Imbalanced      | Random         | d2                  | Mtry           | 5–500                | 0.8352   | 0.2347      | 0.9753      | 0.6326     | 0.2727      | 0.9925      |
| MVS   | Imbalanced      | Random         | SNVd2               | C              | 1                    | 0.8349   | 0.1773      | 0.9804      | 0.6326     | 0.2727      | 0.9925      |
| KNN   | Imbalanced      | Random         | d1_10               | k              | 13                   | 0.7644   | 0.3473      | 0.9753      | 0.6289     | 0.2727      | 0.9851      |
| PLS   | Imbalanced      | Random         | SNVd2               | Components     | 15                   | 0.8441   | 0.2740      | 0.9759      | 0.6136     | 0.2273      | 1.0000      |
| MVS   | Imbalanced      | KS             | SG                  | C              | 500                  | 0.8111   | 0.1180      | 0.9929      | 0.6111     | 0.2222      | 1.0000      |
| PLS   | Imbalanced      | KS             | d2                  | Components     | 15                   | 0.8379   | 0.2260      | 0.9845      | 0.5560     | 0.1111      | 1.0000      |

**Note:** mscd2: multiplicative scatter correction with second derivative, d1: first derivative, d2: second derivative, d1–10: first derivative with a gap of 10, SNVd2: standard normal variate with second derivative, SG: Savitzky-Golay filter, Mtry: number of variables randomly selected at each tree split, k: neighbors, C: regularization parameter.

%). The RF model without data transformation and with KS partitioning yielded an AUC of 0.7087 in training and 0.6763 in validation, with sensitivity of 31.20 % and specificity of 96.01 %. SVM and PLS models also performed poorly, with AUC values below 0.8000 in the imbalanced datasets.

#### 4. Discussion

The novelty of this study lies not in the techniques themselves, which are well established in meat science, but in their application under tropical, pasture-based production systems in Colombia, using portable, low-cost devices directly in slaughterhouse conditions. Furthermore, the integration of data balancing (SMOTE) with machine learning approaches provides a novel framework rarely applied in DFD detection, reinforcing the potential of these methods as cost-effective tools for industrial meat quality assessment in developing countries.

In colorimetry, higher  $L^*$ ,  $a^*$ ,  $b^*$ , chroma, and hue values were observed in carcasses classified as normal compared to DFD samples. These findings are consistent with previous studies by Ijaz et al. (2020), Ribeiro et al. (2021), and McKeith et al. (2016).

Although the observed variations in  $L^*$  ( $\approx 2$  units) and  $a^*$  ( $\approx 1$  unit) may seem small, they fall within the range of visual perceptibility. In the CIE Lab system, a total color difference ( $\Delta E$ ) above 2.0 is generally noticeable to consumers, while values above 3.0 are clearly distinguishable (Pathare et al., 2013; King et al., 2023). In our dataset, combined differences across  $L^*$ ,  $a^*$ , and  $b^*$  yielded  $\Delta E$  values close to 2.5, suggesting that DFD samples would indeed appear darker and less vivid to the human eye. Moreover, evidence from our parallel consumer study indicates that even subtle reductions in redness and lightness become relevant when ultimate pH exceeds 5.8, reinforcing the practical impact of these findings.

While the classification of DFD beef in this study was based on ultimate pH ( $\geq 5.8$ ), the average colorimetric values observed also provide indicative ranges for distinguishing darker carcasses. Normal beef showed mean values of  $L^* = 30.25$ ,  $a^* = 19.53$ , and  $b^* = 10.11$ , whereas DFD carcasses averaged  $L^* = 28.30$ ,  $a^* = 18.13$ , and  $b^* = 8.93$ . These differences indicate that carcasses with  $L^*$  values below  $\approx 29$  and  $a^*$  values below  $\approx 18.5$  tend to fall within the DFD category. Although these thresholds should be interpreted cautiously and validated in consumer-based studies, they offer preliminary benchmarks to complement pH-based classification.

The lower colorimetric values in DFD carcasses may be attributed to the muscle color, which, beyond fiber type, also depends on the degree

of myoglobin oxygenation (Gagaoua, 2022). This oxygenation is limited by mitochondrial activity, which itself depends on pH and postmortem time (England et al., 2016). According to Ramanathan et al. (2020), there is competition for available oxygen among mitochondria, myoglobin, and certain enzymes. When pH is below 5.7, mitochondrial activity is minimal, and myoglobin can remain oxygenated. However, above this threshold, mitochondrial activation leads to a predominance of deoxymyoglobin (Ramanathan et al., 2020). Additionally, higher pH increases the negative charge in the muscle matrix and intra-/interfibrillar spaces, leading to greater water retention, increased light absorption, and reduced reflectance (Ramanathan et al., 2020; Hughes et al., 2020), which results in the darker appearance characteristic of DFD meat.

However, the colorimetric values obtained in this study for normal meat ( $L^* = 30.25$ ;  $a^* = 19.53$ ;  $b^* = 10.11$ ) and DFD meat ( $L^* = 28.30$ ;  $a^* = 18.13$ ;  $b^* = 8.93$ ) were lower than those reported by McKeith et al. (2016), who found  $L^*$ ,  $a^*$ , and  $b^*$  values of 42.8, 30.4, and 22.4 for normal meat and 34.8, 22.4, and 14.1 for DFD meat. Ijaz et al. (2020) also reported higher  $L^*$  values (40.82 for normal and 38.26 for DFD), although their  $a^*$  and  $b^*$  values were lower than those found in our study.

Similarly, Ribeiro et al. (2021) reported  $L^*$  values of 45.19 for normal meat and 38.87 for DFD meat, also higher than our findings. However, their  $a^*$  and  $b^*$  values for normal meat (18.23 and 7.89, respectively) were similar to those obtained in this study, while values for DFD meat were considerably lower (8.97 and 2.95).

As highlighted by King et al. (2023), meat color is affected by several animal-related factors such as species, genetics, and nutritional status. Additionally, instrumental factors like device type, illuminant, observer angle, aperture size, standardization, sample thickness, and sample characteristics also contribute to color variability (Tomasevic et al., 2021). These differences likely explain the variation between studies and our results.

Further variability may arise from differences in equipment and measurement timing. For example, Ripoll et al. (2012) reported an  $L^*$  value of 39.87 in normal meat using a Minolta CM-2600d colorimeter with D65 illuminant, 10° observer, and 8 mm aperture. Holman and Hopkins (2019), using a device similar to ours under the same settings, reported  $L^*$  values of 40 measured at 24 h postmortem—12 h earlier than our study—on carcasses from castrated animals. Our samples came from animals with varied production systems, sex, and age, which are known modifiers of meat color (Clinquart et al., 2022).

Iraola Jerez et al. (2021), in similar tropical conditions, reported  $L^*$

values of 35.16 at 24 h postmortem in normal meat, supporting the hypothesis that tropical, pasture-based systems yield less bright meat color (Apaoblaza et al., 2020). Other studies suggest that color is influenced by mitochondrial concentration, muscle fiber type, and oxidative metabolism (Han et al., 2024; Ramanathan et al., 2020; Suman et al., 2023), which in turn are shaped by prenatal development, post-natal growth, fasting, and physical activity (Picard & Gagaoua, 2020). These factors are especially relevant under extensive tropical production systems, where seasonal drought, forage scarcity, and delayed slaughter age often necessitate the use of zebu crossbreeding to enhance hybrid vigor (Huerta-Leidenz et al., 2023).

Regarding  $a^*$  values (red intensity), our results (19.53 for normal; 18.13 for DFD) were slightly lower than those reported by Holman and Hopkins (2019), who found average  $a^*$  values of 23.9 (Hunter device) and 21.1 (NIX device). Their samples, however, were measured on 3–4 cm steaks, whereas ours were taken directly on carcasses on the processing line. Sample thickness and size may influence color measurements (King et al., 2023). Ribeiro et al. (2021) and Iraola Jerez et al. (2021) reported lower  $a^*$  values than ours in both normal and DFD meat. King et al. (2023) notes that colorimetric values vary by illuminant;  $a^*$  values range between 30 and 40 under illuminant A and 20–30 under D65, suggesting that  $a^*$  is also influenced by observer angle and sample pH.

Regarding  $b^*$  values (yellow intensity), our results (10.11 for normal; 8.93 for DFD) were also lower than those reported by Holman and Hopkins (2019), but higher than those of Iraola Jerez et al. (2021) and Ribeiro et al. (2021). Differences may relate to intramuscular and subcutaneous fat content, which varies in color due to age and pasture-based feeding (Clinguaret al., 2022; Dunne et al., 2009). Fat deposits and instrument positioning may also influence  $b^*$  values (Holman & Hopkins, 2019).

In this study, meat color was evaluated using the CIELab scale ( $L^*$ ,  $a^*$ ,  $b^*$ ), which reflects brightness, red intensity, and yellow intensity, respectively (Mancini & Hunt, 2005), and these parameters are associated with wavelengths in the visible range (400–700 nm), particularly  $a^*$ , which is linked to hemopigments like myoglobin and its oxidation states (Cozzolino & Murray, 2004).

The DFD class showed a 6.15:1 imbalance compared to normal samples. Class imbalance is known to affect model performance (Kaur et al., 2019; Wang et al., 2021). In this context, SMOTE (Chawla et al., 2002) was applied to mitigate this effect. SMOTE has been widely used in fields like health, finance, engineering, and biology to improve classification tasks (Werner de Vargas et al., 2023). Its application in meat quality studies is rare, particularly for DFD detection (Lee et al., 2015; Shahinfar et al., 2020; Shin et al., 2021; Prakash et al., 2021), though in all cases it improved model performance (AUC, precision, or sensitivity >90 %), as observed here.

In this study, SMOTE improved AUC by 0.28 points compared to models using imbalanced data. Balanced datasets enhance model sensitivity and specificity. The impact of imbalance was also seen in Tejerina et al. (2022), where imbalanced PLS-DA and SIMCA models reached 100 % sensitivity but only 19 % specificity.

For colorimetry, the best classification results were obtained with KNN and RF models without data transformation, with AUC between 0.8572 and 0.8766, considered acceptable (Martínez Pérez & Pérez Martín, 2023). Portable colorimeters have been reported as efficient tools for meat quality evaluation (Holman & Hopkins, 2019; Wei et al., 2021; Schelkopf et al., 2021; Dang et al., 2021). Holman and Hopkins (2019) showed that low-cost colorimetry devices could objectively discriminate between dark and normal cuts using a chroma threshold of 30.5 (sensitivity: 0.91; specificity: 0.86). The portability and affordability of the device used in this study make it suitable for industrial applications (Hodgen, 2016; Holman et al., 2018; Schelkopf et al., 2021; Ladegourdie et al., 2023).

Spectroscopy, long considered a rapid and precise tool for assessing meat quality, showed better results than colorimetry, likely due to the

greater amount of information collected by spectrophotometers (Prieto et al., 2014). Good predictive capacity was observed in PLS (D2), SVM (MSC + D2), and KNN (D1) models, with AUC values of 0.8613, 0.8774, and 0.9097, respectively. However, RF (MSC + D2) performed best (AUC: 0.9697; sensitivity: 0.9677; specificity: 0.9806). Preprocessing methods like first/s derivatives and MSC improved signal quality by reducing baseline and additive effects (Marison et al., 2013; López Martínez & López de Alba, 2019; Palencia & Martínez, 2017).

The performance of the different machine learning algorithms in this study reflects their intrinsic characteristics. Specifically, PLS-DA, while useful for reducing dimensionality, showed moderate predictive capacity, likely due to its assumption of linear relationships among predictors (Barker & Rayens, 2003; Tejerina et al., 2022). KNN achieved competitive results with colorimetry, confirming its strength as a simple, non-parametric classifier that performs well when clusters are clearly separated (Altman, 1992). In meat science, approaches related to nearest-neighbor classification have also been explored within broader ML frameworks for quality assessment (Gagaoua, 2022; Hashem et al., 2025). SVM, particularly with kernel transformations, effectively captured non-linear boundaries but showed reduced validation accuracy, suggesting higher sensitivity to class imbalance and noise (Cortes & Vapnik, 1995). This observation is consistent with reports that SVM performance can deteriorate under imbalanced conditions depending on kernel type parameterization (Hsu et al., 2003). Similar challenges have been documented in food science contexts using SVM (Dixit et al., 2021; Shahinfar et al., 2020). By contrast, Random Forest consistently provided the best performance across transformations, which can be attributed to its ensemble learning structure, robustness to collinearity, and ability to internally estimate error (Breiman, 2001; Liaw & Wiener, 2002; Díaz-Uriarte & Alvarez de Andrés, 2006). Recent evidence in meat science reinforces these strengths, as Nisbet et al. (2024) reported that Random Forest achieved competitive accuracy in predicting carcass traits such as weight, conformation, and fat class from 3D imaging data, highlighting its stability when handling highly correlated and multidimensional datasets. The results therefore indicate that while several ML methods are applicable, ensemble-based models may offer greater reliability for DFD detection under industrial conditions.

Unlike our study (visible range only), Prieto et al. (2014) used VIS-NIRS (400–2500 nm) and found improved DFD classification by combining information on myoglobin redox states (visible) and water/O-H bonds and glycolytic potential (NIRS). This comparison highlights the potential of extending portable visible spectroscopy approaches towards wider spectral ranges, while confirming the feasibility of low-cost devices for real-time DFD detection in tropical beef production systems.

## 5. Conclusions

Although the prevalence of DFD condition in the slaughterhouse was relatively low, it highlights an opportunity for improvement in both the factors influencing this meat quality anomaly and the methods for real-time detection.

Visible spectroscopy and colorimetry, evaluated under processing line conditions in slaughter plants, emerge as cost-effective and accessible alternatives to conventional equipment. This is key to implementing rapid and objective methods for DFD detection in beef.

Visible spectroscopy, combined with advanced preprocessing techniques such as multiplicative scatter correction (MSC) and second-order derivative (D2), along with data balancing using SMOTE, proved to be the most accurate and reliable tool for real-time DFD discrimination when using a Random Forest algorithm. Although portable colorimetry was less accurate, it presents considerable potential for improvement through the application of appropriate statistical techniques and classification models, making it a promising option for efficient classification.

Overall, this work provides novel evidence by applying portable, rapid, non-invasive, and low-cost devices in tropical pasture-fed

production systems, combined with data balancing and machine learning, thus extending the applicability of these methods beyond the contexts in which they have traditionally been evaluated.

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## Ethical statement

This study was approved by the ethics committee for animal experimentation at the University of Antioquia under approval number 150, dated February 7, 2023.

## CRediT authorship contribution statement

**Leonardo Hernández-Hernández:** Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Liliana Mahecha-Ledesma:** Writing – review & editing, Supervision, Project administration. **Joaquín Angulo-Arizala:** Writing – review & editing, Supervision, Methodology. **Wilson Barragán-Hernández:** Writing – review & editing, Methodology, Formal analysis, Data curation.

## Declaration of competing interest

The authors declare that there are no financial or personal conflicts of interest that could have influenced the preparation of this manuscript.

## Data availability

Data will be made available on request.

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