Assessment of beef sensory attributes and physicochemical characteristics: A comparative study of intermediate versus normal ultimate pH striploin cuts

Iliani Patinho, Cecylyana Leite Cavalcante, Erick Saldaña, Mohammed Gagaoua, Jorge Behrens, Carmen Contreras-Castillo

Assessment of beef sensory attributes and physicochemical characteristics: A comparative study of intermediate *versus* normal ultimate pH striploin cuts

Iliani Patinho [a*], Cecylyana Leite Cavalcante [a], Erick Saldaña [b], Mohammed Gagaoua [c], Jorge H. Behrens [d], Carmen J. Contreras-Castillo [a*]

[a] Department of Agri-food Industry, Food and Nutrition, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, SP 13418-900, Brazil

[b] Sensory analysis and consumer study group, Escuela Profesional de Ingeniería Agroindustrial, Universidad Nacional de Moquegua, Prolongación Calle Ancash s/n, Moquegua, 18001, Peru.

[c] PEGASE, INRAE, Institut Agro, 35590 Saint-Gilles, France

[d] Department of Food Science and Nutrition, School of Food Engineering, University of Campinas, SP, Brazil

[*]Corresponding authors email: ilianipatinho@usp.br; ccastill@usp.br

Abstract

The quality of beef, defined by key attributes such as texture, flavour, and post-cooking texture, is shaped by various intrinsic and extrinsic factors. This study conducted a detailed examination of Nellore beef, dividing it into two categories based on ultimate pH (pHu) levels: intermediate (pHu ≥ 5.8) and normal (pHu < 5.6). A comprehensive approach was taken, involving twenty trained assessors who applied the Optimised Descriptive Profile (ODP) method to evaluate grilled striploin steak samples. In parallel, consumer preferences were measured through a hedonic test and a Check-all-that-apply (CATA) task, involving 135 participants. The ODP results revealed that the intermediate pHu samples were significantly juicier ($P < 0.05$) compared to the normal pHu group. The CATA analysis highlighted differences in both intermediate and normal pHu beef, especially in juiciness, a crucial factor for consumer satisfaction. Notably, variations in deoxymyoglobin content linked to ageing were observed, with higher levels on the 3rd day compared to the 28th day, especially in the intermediate pHu samples ($P < 0.05$). Moreover, colour-related aspects such as L^*, b^*, chroma, and oxymyoglobin were significantly influenced ($P < 0.05$) by both the pHu category and ageing duration. Regarding consumer acceptance, the study found no significant difference in perception between the intermediate and normal pHu groups ($P > 0.05$). These findings illuminate the complex interaction between pHu levels, sensory characteristics, and consumer preferences in beef quality, offering valuable insights for both the industry and research community.

Keywords: Beef quality, *Longissimus thoracis et lumborum,* Nellore, Consumer acceptability, Sensory evaluation.

1. Introduction

Animal protein, particularly bovine meat, is a fundamental part of the human diet, especially in Western countries where beef is a major source of protein. Consumer preferences for beef vary across different markets and are influenced by diverse factors including (i) cultural and psychological aspects of consumers (Troy & Kerry, 2010), (ii) the sensory properties of the products (Saldaña et al., 2020a), and (iii) marketing considerations such as price, regulation, quality standards, and distribution (Font-i-Furnols & Guerrero, 2014).

Texture is a key sensory attribute of beef, offering a range of mouthfeel experiences during consumption, such as tenderness, juiciness, and chewiness (Zhu et al., 2021). The aroma and flavour are also critical, arising from chemical compounds like amino acids, peptides, organic acids, and adenine nucleotide metabolism by-products (Spanier et al., 1997). The Maillard reaction and lipid degradation in meat generate various volatile compounds, contributing to the distinctive aromas (Kosowska et al., 2017). Recent research suggests that the chemical properties of muscle fibres (type I and II) vary and significantly influence the formation of volatiles and beef flavour (Li et al., 2023).

The sensory quality of beef is largely determined by its ultimate pH (pHu), which is linked to the animal's ability to store glycogen in muscles and the differing amounts of mitochondria in muscle fibres (Picard & Gagaoua, 2020; Poleti et al., 2018). Variations in pHu between intermediate and normal beef can result from factors like animal diet, exercise, pre-slaughter stress, and processing conditions, all impacting the final quality, particularly in terms of colour, tenderness, and flavour (Gagaoua et al., 2021a; Loudon et al., 2018; Ponnampalam et al., 2017). This presents a challenge for food scientists and the industry in aligning consumer perception with variations in beef pHu (Warner et al., 2021).

The meat industry aims to consistently produce high-quality cuts while reducing consumer dissatisfaction (Gagaoua & Picard, 2020). Various sensory evaluation methods have been deployed, including both analytical (trained panels) and holistic (consumer) approaches (Saldaña et al., 2021). According to Aaslyng et al. (2014), a product's sensory profile can be established using the Optimised Descriptive Profile (ODP) by a trained panel, where product attributes are selected and agreed upon by the assessors (Murray et al., 2001). Conversely, affective methods rely on consumer reactions, such as hedonic measures, and can be augmented with techniques like Check-all-that-apply (CATA) questions, a versatile tool for capturing perceptions, feelings, and attitudes (Jaeger et al., 2015).

Considering the prominence of beef in Brazilian diets and the preference for this animal protein, this study aimed to develop a comprehensive sensory profile and deepen our understanding of the acceptance and perception of beef cuts within two pHu ranges: intermediate and normal. This innovative study combined the expertise of trained assessors for consistent data, alongside the views of 135 regular beef consumers. Additionally, instrumental measurements taken at 3, 14, and 28 days *post-mortem* were analysed. The hypothesis was that individual reactions to sensory and physicochemical properties of beef could predict consumer perception of beef quality, providing valuable insights to the meat industry about consumer expectations and purchasing decisions.

2. Materials and Methods

All procedures and protocols used in this study complied with the Institutional Animal Care and Use Committee Guidelines (protocol 2019/22) and were approved by the committees of the Luiz de Queiroz College of Agriculture at the University of São Paulo. All participants in the sensory tests provided informed consent, in accordance with the form approved by the ethics committee, under CAAE number: 56217222.2.0000.5395.

2.1 Animals, treatments, and sampling

Animals were sourced from a commercial meat slaughterhouse under federal inspection, and they were part of a larger experiment involving 104 animals. The normal and intermediate pHu frequencies stood at 76.92% and 16.35%, respectively. From this pool, ten carcasses of Nellore (*Bos Indicus*) bulls, categorised as intermediate (pHu $\geq$ 5.8; n = 5) and normal (pHu < 5.6; n = 5) beef, were selected. The bulls, aged between 18 and 35 months, had 2 to 6 permanent incisor teeth and a hot carcass weight of 303 ± 40 kg. Beef samples (~1 g) were excised from the right side of the *Longissimus thoracis* (LT) muscle between the 10^{th} and 11^{th} ribs, aging after 3 days *post-mortem*, for pHu determination. Each animal's LT muscle was sheared perpendicular to the natural muscle fibres, into five steaks (2.0 cm thick), vacuum-packed, and stored at -18 °C for subsequent sensory analysis. For instrumental analysis, the *Longissimus thoracis et lumborum* (striploin cut) muscles were sheared between the 3^{rd} and 6^{th} lumbar vertebrae, portioned into steaks (2.0 cm thick), vacuum-packed, and refrigerated in a chamber (Danic, Model C-EC/U) at 0±2.0 °C without light exposure during the aging period of 3, 14, and 28 days *post-mortem*.

2.2 Sensory analyses

Sensory evaluations were conducted on LT steaks 3 days *post-mortem*, utilising the methodology outlined by Patinho et al. (2019, 2021). For data collection, the Compusense Cloud platform (Compusense Inc., Guelph, Canada) was employed, with the information being recorded on Android tablets (Samsung Galaxy Tab E T560).

2.3 Sample preparation

Sensory evaluations of LT steaks, cut into 2.0 cm thick steaks, were carried out 3 days after slaughter. The steaks were grilled on an electric griddle at 200 °C (SSE50, EDANCA, São Paulo, Brazil) until the internal temperature reached 71 °C, monitored using a puncture thermometer. The steaks were flipped every 60 seconds during grilling, a process that lasted about 5 minutes to achieve the desired internal temperature. Post-cooking, each steak was cut into 1 cm^3 cubes, as advised by Gomes et al. (2014) and based on preliminary tests. In consumer testing, the steaks were seasoned with salt (1.7% w/w) before grilling (Martins et al., 2021). The samples, approximately 5g each, were then wrapped in aluminium foil, kept warm in an electrically heated display at around 50 °C for 20 minutes, and served to assessors in plastic dishes marked with three-digit codes. Assessors were instructed to cleanse their palates with water between samples.

2.4 Optimised Descriptive Profile

The ODP involved candidates including undergraduate and postgraduate students, staff, and faculty from the Department of Agri-food Industry, Food and Nutrition at the University of São Paulo (ESALQ/USP). These individuals were selected for their experience with meat product sensory evaluations, availability, dietary or health constraints, and non-smoking status. The ODP, conducted with 20 trained assessors (age 18-65; comprising 57% women, 39% men, and 4% non-binary individuals; all regular beef consumers), spanned six independent one-hour sessions. In the initial session, the ODP methodology was introduced, and a list of attributes from prior studies (Martins et al., 2021; Yang et al., 2021; Gomes et al., 2014) was discussed, leading to the consensus on sixteen descriptors. The second session was dedicated to finalising the attribute order, evaluation techniques, reference samples, and intensity terms defined as "low" or "high". The third session focused on training the assessors in using the Compusense Cloud and the 9-cm unstructured linear scale to measure attribute intensity in each sample. The final three sessions involved the actual evaluations, with each assessor analysing two beef samples per session, one from the intermediate and the other from the normal pHu range, presented monadically following the Latin Square design of Williams (Wakeling & MacFie, 1995).

2.5 Consumer testing

For consumer testing, 135 regular beef consumers (age 18-64; 56% women, 43% men, and 1% non-binary; 90% with higher education; beef consumption varied from daily to once a week) from the University of Campinas campus rated the samples' overall liking on a 9-point hedonic scale. The scale ranged from (1) "extremely disliked" to (9) "extremely liked". Participants then used a Check-all-that-apply (CATA) task to identify sensory descriptors for each sample. After tasting both samples, they were asked to describe the sensory profile of an ideal beef using the same CATA task, based on the previous study by Martins et al. (2021).

2.6 Quality parameters of beef 3 days post-mortem: pHu, composition, drip loss and texture analysis

2.6.1 pHu determination

Muscle pHu was determined according to the method by Bendall (1973). Briefly, the LT muscle samples were taken 3 days *post-mortem* and homogenised using an Ultra Turrax (IKA, model T18 basic, Wilmington, NC, USA) with a buffer containing 5mM sodium iodoacetate and 150mM KCl (pH 7.0) at a 1:8 ratio (w/v). The muscle homogenates were centrifuged using an Eppendorf centrifuge (model 5810R, Hamburg, Germany) at 13,000×g for 5 minutes at room temperature, equilibrated to 25 °C, and measured using a digital pH meter (Lucadema, model LUCA-210, São José do Rio Preto, Brazil) with automatic temperature compensation and a glass penetration electrode.

2.6.2 Proximate composition

The moisture content, crude protein, ash, and total lipids of muscle samples, taken 3 days *post-mortem*, were evaluated (**Table 2**). The moisture content was determined by oven-drying samples at 105 °C until a constant weight was achieved (Association of Official Analytical Chemists [AOAC], 1995). Total nitrogen was measured using the Kjeldahl method (Nx6.25) (ISO 1871:2009) to ascertain the crude protein content, while ash content was determined by calcining organic matter at 550 °C (ISO 936:1998). Total lipids were extracted with hexane using the Soxhlet method (AOAC, 1995). The results were calculated on a wet basis and expressed as a percentage.

2.6.3 Drip loss

Drip loss in refrigerated striploin steaks was determined 3 days *post-mortem*. Approximately 50 g of rectangular muscle (30 mm thickness, 60 mm length, 25 mm width) was cut in the direction of the fibre, weighed, placed inside a net, and suspended into air-filled polyethene plastic. After 48 hours at 0±4.0 °C, all samples were weighed again, and the drip loss percentage was calculated according to the method outlined by Honikel (1998), with adaptations from Torres Filho et al. (2017), using the following equation:

$$\textit{Drip loss}\ (\%) = [(\text{Initial weight} - \text{Final weight})/\text{initial weight}] \times 100 \quad (1)$$

2.6.4 Texture profile analysis (TPA)

Before conducting the texture profile analysis (TPA), the *Longissimus thoracis* (LT) muscle samples, taken 3 days *post-mortem* and initially frozen at -18 °C, were thawed at a temperature of 2-4 °C for 24 hours. These were then cooked on an electric griddle until reaching an internal temperature of 71 °C (Martins et al., 2021). The TPA was carried out using a TA-XT texturometer (Stable Micro Systems, Godalming, United Kingdom) equipped with a P-35 probe (long shaft, regular base), adhering to the methods outlined by Jia et al. (2022). For the analysis, the samples were shaped into cylinders using a circular stainless-steel cutter. Each sample yielded five to seven cylinders, each 3.0 cm in diameter. These were then tested at room temperature under specific conditions: a 5.0-second interval between the first and second compressions, a crosshead speed of 1.0 mm/s, a trigger force of 5 g, and a working distance set at 50% strain. The TPA, defined as the peak force needed to compress the sample, mimics the mechanical process of chewing, thus providing data on texture characteristics such as hardness (measured in Newtons), springiness, cohesiveness, chewiness, and gumminess. The average values from five replicates were used for statistical analysis, with the results for hardness specifically expressed in Newtons.

2.7 Quality parameters of beef at 3, 14, and 28 days post-mortem: colour, water-holding capacity, cooking losses and shear force

2.7.1 Instrumental colour

The instrumental colour measurement of the refrigerated striploin steaks was carried out at 3, 14, and 28 days *post-mortem* using a MiniScan XE Plus spectrophotometer® (HunterLab Associates, Virginia, USA). The spectrophotometer was calibrated with a white ceramic plate adjusted to Y = 93.7, x = 0.3160, and y = 0.3323, using an 8 mm diameter measurement area, a 10° observation angle, and A10 illumination. CIELAB coordinates *L** (lightness), *a** (redness), and *b** (yellowness) were obtained by taking 5 readings at random locations on the steak surface without connective tissue, after allowing 30 minutes of oxygenation at 4 °C. Chroma (C*), hue angle (h*), and the percentages of metmyoglobin, deoxymyoglobin, and oxymyoglobin were calculated using equations defined by American Meat Science Association [AMSA] (2012). The reflectance ratio at 630 nm and 580 nm (R630/580) was measured to estimate the stability of meat colour, where a higher ratio indicates lower metmyoglobin accumulation on the beef surface and thus greater colour stability (AMSA, 2012; Canto et al., 2016).

2.7.2 Water-holding capacity (WHC)

The water-holding capacity (WHC) of the refrigerated striploin steaks at 3, 14, and 28 days *post-mortem* was determined using the method described by Grau and Hamm (1953), modified by Hoffmann et al. (1982). Meat samples (0.5 g) were weighed on filter paper and pressed with a 5 kg weight for 5 minutes. The WHC result was expressed as a percentage based on the difference between the final and initial weights of the sample.

2.7.3 Cooking losses

Cooking losses for refrigerated striploin steaks at 3, 14, and 28 days *post-mortem* were cooked on an electric plate (model SSE50, EDANCA, São Paulo, Brazil) at 200 °C until they reached an internal temperature of 71 °C. The cooking loss was calculated by subtracting the sample's weight after cooking ($weight_2$) from its weight before cooking ($weight_1$) (Anne et al., 2022), and converting the result to a percentage using the following equation:

$$\textit{Cooking loss}\ (\%) = \frac{weight_1\ (g) - weight_2\ (g)\ x\ 100}{weight_1\ (g)} \quad (2)$$

2.7.4 Shear force

The Warner–Bratzler shear force of the refrigerated striploin steaks at 3, 14, and 28 days *post-mortem* was determined according to Shackelford et al. (1991), with modifications. Briefly, the LT steaks were cooked on an electric plate (model SSE50, EDANCA, São Paulo, Brazil) at 200 °C until they reached an internal temperature of 71 °C (Martins et al., 2021). After cooking, the steaks were refrigerated at 0±2.0 °C for 24 h. Using a drill and leaker, between five and seven sections (1.27 cm in diameter) were extracted by cutting parallel to the muscle fibres. Each section was sheared perpendicular to the fibre orientation using a Warner-Bratzler shear blade (HDP/BS) attached to a TA-XT texturometer (Stable Micro Systems, Godalming, United Kingdom) calibrated for a pre-test speed of 2 mm/s, test speed of 2 mm/s, and post-test speed of 10 mm/s. The device was programmed to travel 30 mm at the end of the three phases. The maximum force peak recorded during the analysis represented the shear force in Newtons (N) for hardness. The highest and lowest measurements were excluded, and the mean of five sections was used for statistical analysis.

2.8 Data analysis

Statistical analyses were carried out using XLSTAT (Student 2023.1.2.1406) and R project version 4.3.1 (R core team, 2023).

2.8.1 Optimised Descriptive Profile

The Optimised Descriptive Profile (ODP) data were examined univariately using a Student's t-test to identify potential sensory differences between the pHu ranges at a 5% significance level in R software. Principal Component Analysis was conducted on the correlation matrix from a multivariate standpoint in XLSTAT.

2.8.2 Consumer testing

Hedonic data from the meat acceptance tests were analysed using ANOVA, with pHu range as a fixed factor and consumers as a random factor, treating both samples and consumers as sources of variation. The frequency of terms by pH range from the Check-All-That-Apply (CATA) data was compiled and then analysed using Correspondence Analysis (CA). The correlation between liking and sensory attributes was explored through Principal Coordinate Analysis, as recommended by Saldaña et al. (2020b). Additionally, a penalty analysis was conducted to calculate the impact on liking when sensory attributes were marked or unmarked as ideal, following the method of Selani et al. (2022). All these analyses were performed using XLSTAT.

2.8.3 Quality parameters of beef 3 days post-mortem

For the instrumental analyses, data related to pH, drip loss, and texture profile analysis (TPA) of the two different pHu ranges of steaks were compared using a Student's t-test in R software to identify significant differences.

2.8.4 Quality parameters of beef at 3, 14, and 28 days post-mortem

Instrumental data assessing colour, water-holding capacity, cooking losses, and shear force considered two pHu ranges and three refrigeration periods as sources of variation. ANOVA was then performed at a 5% significance level, with pHu range as a fixed factor and animal as a random factor, using R software. A Principal Component Analysis (PCA) was conducted with combinations of pHu and storage time as individuals (rows) and instrumental data as variables (columns), using the Pearson correlation matrix in XLSTAT.

3. Results

3.1 Optimised Descriptive Profile

The study revealed significant differences in the sensory profile only regarding juiciness (**Table 3**). Specifically, the panel found steaks with intermediate pHu to be juicier ($P<0.05$) than those with normal pHu. When considering the overall impact of pHu on beef quality, other sensory characteristics appeared similar. Vector size was used to analyse the positive and/or negative relationships between the 16 sensory characteristics and intermediate and normal pHu. Notably, the flavour attributes of roast beef, fat, connective tissue, and blood odour were prominent in steaks within the intermediate pH range. Conversely, the surface brown colour, chewiness, and hardness were more intense in steaks from the normal pHu range (**Fig. 1A;B**).

3.2 Consumer testing

The relationship between striploin steak samples from intermediate and normal pHu ranges and the sensory descriptors used by consumers in the CATA task is represented by the first two dimensions of the Correspondence Analysis (CA), which captures 100% of the original information (**Fig. 2A**). Steaks treated with intermediate pHu were characterised by the attributes of blood odour, hardness, fibrousness, surface brown colour, and a meaty beef flavour. In contrast, the sample with a normal pHu was described as having apparent opacity, blood flavour, dryness, and an internal brown colour. Significantly, none of the samples were near the ideal sensory profile, indicating substantial room for improvement in order to satisfy consumers' ideal sensory preferences. It is worth noting that samples with intermediate and normal pHu were perceived similarly, with no significant difference in terms of acceptance ($P > 0.05$) and overall liking scores of 6.93 and 6.63, respectively (data not shown). Overall liking was associated with the attributes of overall juiciness, fat flavour, roast beef aroma, roast beef flavour, overall tenderness, chewiness, and saltiness (**Fig. 2B**), suggesting that these attributes are instrumental in driving consumer liking. Furthermore, it was identified that the characteristics of chewiness, saltiness, overall tenderness, juiciness, aroma, and roast beef flavour were the main drivers of preference for the optimal beef sample (**Fig. 2A**).

A penalty-lift analysis pinpointed attributes positively and negatively associated with overall liking. Positively influencing acceptance were roast beef flavour, internal red colour, juiciness, chewiness, tenderness, and roast beef aroma (**Fig. 3**). Conversely, hardness, fibrousness, dryness, and apparent opacity were inversely related, indicating their detrimental effect on beef acceptability.

3.3 Quality parameters of beef 3 days post-mortem

The results demonstrated that the differences between intermediate and normal pHu did not significantly affect the drip loss or any texture parameters of the beef (**Table 4**).

3.4 Quality parameters of beef at 3, 14, and 28 days post-mortem

According to **Table 5**, there was a significant ($P<0.05$) impact of pHu and ageing time on the L^*, b^* *parameters*, specifically on chroma, oxymyoglobin, and deoxymyoglobin. Only the ageing time had an effect on the a^*, hue, surface colour stability, metmyoglobin, and water-holding capacity; without interaction between the two variables (pHu x ageing time). However, only in the pHu ranges was a difference seen for shear force. Compared to meat with a normal pHu, meat with an intermediate pHu had a lower shear force value.

Lastly, data collected for both intermediate and normal pHu samples during the ageing period was subjected to Principal Component Analysis (PCA). 93.21% of the variation in the total could be explained by the first two principal components, according to this analysis. The first dimension was primarily formed by the intermediate pHu (70.96%), whereas the second dimension was primarily formed by the normal pHu (22.25%) (**Fig. 4**). There were noticeable variations in quality metrics between the two pHu ranges. In relation to lightness (L*), redness (a*), yellowness (b*), chroma, hue, oxymyoglobin, metmyoglobin, and shear force for normal pHu, the intermediate pHu was positively correlated with deoxymyoglobin, cooking losses, water-holding capacity, and surface colour stability.

4. Discussion

4.1 Optimised Descriptive Profile

Amongst the range of sensory characteristics examined, pHu notably influenced only the juiciness of meat. Meat juiciness, defined as the sensation of moisture released during chewing, is a vital aspect of meat texture. While initial tenderness sensations diminish rapidly, the experience of juiciness persists in various intensities throughout mastication. This study found that meat samples with intermediate pHu were juicier ($P < 0.05$) than those with normal pHu (Table 3), supporting the findings of Grayson et al. (2016) and mirroring results from Yang et al. (2021) for high pHu (dark-cutting) meat.

A higher water-holding capacity (WHC), associated with greater juiciness, was noted in meat with intermediate pHu (**Table 5**). Although there wasn't a significant statistical difference in WHC across pHu ranges, a positive correlation with the intermediate pHu group was evident (**Fig. 4**). This phenomenon can be attributed to the isoelectric point (IP) of myofibrillar proteins, like myosin, near pH 5.4, where myofilaments and myofilament spaces have zero net charge. As explained by Zhang et al. (2022), a pH shift above this point results in myofibrillar proteins having a higher net charge, thus increasing myofilament distances and improving WHC. This seems to be the case with intermediate pHu muscle. Conversely, a rapid pH decline leading to low pHu is linked to reduced WHC, likely due to increased surface hydrophobicity and decreased solubility of myofibrillar proteins, impacting the myofilament structure and surface charges (Huff-Lonergan & Lonergan, 2005; Sharedeh et al., 2015). Additionally, a decrease in pH affects the redox stability of myoglobin, accelerating the oxidation of ferrous myoglobin to ferric, increasing surface moisture, and altering the appearance of *post-mortem* muscle (Faustman & Suman, 2017). These findings for low/normal pHu are consistent with our study's observations.

Significantly, an improvement in water-holding capacity was noted during extended ageing (**Table 5**). Changes in muscle structure during this period, such as proteolysis of myofibrillar and cytoskeletal proteins and alterations in collagen, affect ion-protein interactions, thereby increasing the space available for water retention (della Malva et al., 2021).

The perception of fat and roast beef flavours appears positively influenced by the higher lipid content in intermediate pHu (**Table 2**). The rich flavour of meat is the result of complex interactions among various ingredients during thermal processing (Aaslyng et al., 2014; Troy & Kerry, 2010), likely explaining the noted preference for roast beef flavour in intermediate pHu samples. The differences in fatty acid profiles between pHu ranges significantly impact the cooking process and the formation of aroma and flavour-active compounds (Kerth & Miller, 2015). O'Quinn et al. (2012) observed a correlation between meat flavour and fat content, suggesting that higher fat content in steaks enhances meat flavour intensity. In summary, **Table 3** indicates that all sensory panel evaluators consistently rated the same attributes - taste, colour, and aroma - despite the inherent challenges in achieving a consensus on these aspects.

4.2 Consumer testing

Our study's findings, supported by trained sensory panel evaluations and consumer CATA task results (Ares & Jaeger, 2023), are significant. Both panels characterized normal pHu steaks as dry, and intermediate pHu steaks as tough (**Table 3**; **Fig. 2A**). This aligns with Miller et al. (2001), who noted that flavour and juiciness significantly influence consumer satisfaction as steak toughness increases (**Fig. 2B**). Key determinants for consumer approval in beef are tenderness, juiciness, and flavour (Duarte et al., 2022). The primary attributes influencing consumer preferences in our study include roast beef aroma, flavour, chewiness, salty taste, overall tenderness, and juiciness (**Fig. 2A**). This aligns with Liu et al. (2022), who highlighted juiciness as a vital factor in meat quality, with the American Meat Science Association (AMSA) stating it accounts for 10% of consumer approval variation (Watson et al., 2008). Future studies should focus on juiciness, especially in under-researched regions like Brazil.

Concerning overall acceptance (**Fig. 2B**), previous research indicated a preference for tender and juicy beef (Corcoran et al., 2023; Gomes et al., 2014). Lyford et al. (2010) and Malheiros et al. (2022) suggested consumers are willing to pay more for tender steaks. Zebu beef, compared to *Taurus* cattle, often has lower tenderness and marbling, potentially due to feeding regimes and the rate of muscle pH decline (Antonelo et al., 2022). Our findings affirm tenderness as a desirable quality in beef. Gomes et al. (2014) noted that high cooking temperatures, such as grilling, can enhance meat's appearance, flavour, and aroma. However, cooking meat above 70 °C typically results in drier and tougher meat compared to lower temperatures (60 °C) (Obuz & Dikeman, 2003). The Nellore breed (*Bos taurus indicus*) and the internal temperature used for cooking (71 °C) in our study, recommended by Brazilian restaurants and AMSA, might have influenced the lack of perceived tenderness in both pHu ranges **(Fig. 2A**).

Meat colour perception involves more than just brown or red (Corlett et al., 2021; Hopkins, 1996). Factors influencing myoglobin's thermal stability and internal colour of cooked meat include the protein's redox state (Suman et al., 2016). Higher muscle pH helps maintain myoglobin in the reduced ferrous state, offering protection against denaturation (Faustman & Suman 2017; Hunt et al., 1999). Additionally, the brown colour on cooked meats' outer surfaces, observed in the intermediate pHu group (**Fig. 2A**), involves Maillard reaction pigments, which are separate from myoglobin pigments (Faustman & Suman 2017). The Maillard reaction, a heat-induced reaction between reducing sugars and proteins, occurs more slowly at low pH (Feiner, 2006). Higher pH may increase non-protonated amino acids in meat, enhancing reactivity in the Maillard reaction (Madruga & Mottram, 1995). High pHu contributes to this reaction, leading to more pigments that affect the external colour of beef.

Our study also shows that taste and aroma are major drivers of consumption and satisfaction in cooked beef. Previous studies highlight the importance of identifying common sensory attributes that satisfy consumer preferences and promote regular beef consumption. The meat industry should focus on efficient technological solutions that balance large-scale production with defined quality parameters, catering to Brazilian consumers' demands for affordable, high-quality products meeting hygienic, nutritional, and ethical standards.

The penalty-lift analysis suggests flavour and aroma significantly impact perceived sensory quality, supporting the idea that juiciness enhances liking (**Fig. 3**). These factors collectively increase overall liking, as beef taste and juiciness are perceived early in mastication (Djekic et al., 2022), chewing affects juiciness perception (Aaslyng et al., 2003), and meat flavour and aroma are released during this process (Watanabe et al., 2019). Research indicates a negative correlation between beef pH and hardness (Troy & Kerry, 2010), leading to increased sensory hardness and dryness and decreased liking.

4.3 Parameters of beef quality during ageing (3, 14, and 28 days post-mortem)

Fresh meat colour intensity is shaped by various intrinsic and extrinsic factors during muscle-to-meat conversion (Gagaoua et al., 2018). The pH value plays a pivotal role in meat colour development, primarily by affecting oxygen consumption and the reducing activity of myoglobin (Ramanathan & Mancini, 2018).

Important colour parameters like L^*, a^*, b^*, chroma, hue, oxymyoglobin, and metmyoglobin were significantly influenced ($P < 0.05$) by the normal pHu class and ageing period (3, 14, and 28 days) (**Fig. 4**). pHu values explain variations in L^* values, with higher L^* values observed in steaks with normal pHu (**Table 5**). Increased pH levels lead proteins to retain more water, causing muscle fibres to swell and reduce the distance between them, resulting in a darker muscle surface due to decreased light scattering and increased light absorption by myoglobin (Wu et al., 2020). On day three, steaks showed higher L^* values, but after 14 and 28 days, the values were similar (**Table 5**). This supports Canto et al. (2016), who suggested variations in L^* values might be due to higher myoglobin content on day three, as myoglobin concentration in beef negatively correlates with surface luminosity. Studies also observed higher L^* values in *Longissimus thoracis et lumborum* on day 3 during cold storage (Jeong et al., 2009; Hwang et al., 2010).

No significant difference was found between pHu ranges in terms of redness (a^*), suggesting either no effect of higher pH on colour stability or an insufficient pH increase to cause additional darkening. However, the highest values ($P < 0.05$) at 14 and 28 days of ageing occurred during the ageing period (**Table 5**). According to **Fig. 4**, low a^* values were linked to high deoxymyoglobin content 3 days *post-mortem* in intermediate pHu beef, while high a^* values were directly associated with oxymyoglobin content in beef with normal pHu (Wu et al., 2020). Previous studies reported increased a^* values in *longissimus* steaks during cold storage (Canto et al., 2016; Kim et al., 2009) due to limited oxygen availability for myoglobin in the early *post-mortem* period (Li et al., 2020). The increase of *a** during ageing may suggest increased colour stability and lessened susceptibility to ageing-induced discolouration (della Malva et al., 2021). Stable-coloured *longissimus* steaks exhibited a surfeit of glycolytic enzymes contributing to enhanced colour stability (Suman et al., 2023). This may also elucidate the elevated oxymyoglobin content in the 14 and 28 day *post-mortem* samples.

The *a** value has been suggested as a simple and reliable indicator to predict consumer acceptability of beef colour (Wang et al., 2020). According to Holman et al. (2017), meat colour is considered acceptable when *a** values are equal to or greater than 14.5. In our study, beef samples from both pH ranges at 3, 14, and 28 days displayed *a** values above 14.5, indicating likely consumer acceptance. Similarly, the yellowness values (*b**) followed a similar pattern as the *a** values, with higher values ($P < 0.05$) at 14 and 28 days, aligning with the results of Canto et al. (2016), Kim et al. (2009), and McKenna et al. (2005).

Chroma and hue angles, derived from *a** and *b** values, are influenced by myoglobin content and mitochondrial functions (Li et al., 2020). In our study, elevated chroma values at 14 and 28 days associated with an increase in oxymyoglobin and a decrease in metmyoglobin, consistent with a prior study by Kannan et al. (2001). The hue angle demonstrated a positive association with oxymyoglobin at 14 and 28 days and a negative association with metmyoglobin at 3 days, which corresponds to the study by Lindahl et al. (2001).

Surface colour stability (R630/580) showed a significant difference related to ageing time ($P<0.05$), with lower values on day 3 compared to days 14 and 28. This observation of surface colour stability is consistent with our findings on *a** values and supports a previous study that noticed higher R630/580 values in *Longissimus lumborum* muscles during retail exposure on days 5 and 9 (Joseph et al., 2012). McKenna et al. (2005) noted that muscles with robust colour stability, like the *Longissimus lumborum*, tend to have higher *b** values during storage, which aligns with our results. Also, the presence of sarcoplasmic proteins, acting as antioxidants to inhibit lipid and myoglobin oxidation, may contribute to the enhanced colour stability *in longissimus* steaks (Suman et al., 2023).

In our study, the relative content of deoxymyoglobin was influenced by both the pHu range and ageing time ($P < 0.05$). Steaks with an intermediate pHu had higher deoxymyoglobin content on day 3 than after 28 days of ageing (**Table 5**). This aligns with findings by Li et al. (2020), indicating that meat with limited glycolysis and higher pH impacts the presence of deoxymyoglobin and oxymyoglobin formation by promoting a higher rate of mitochondrial respiration (Gagaoua et al., 2021b). Elevated muscle pH increases mitochondrial respiration capacity, leading to more oxygen consumption by the mitochondria, which in turn causes muscle darkening by reducing myoglobin's oxygen (Kiyimba et al., 2022). Several pathways influence the activity of metmyoglobin reductase (MRA), all relying on NADH, a crucial electron donor. Mitacek et al. (2019) highlighted NADH's key role in reducing metmyoglobin in *post-mortem* muscle. These insights help explain variations in colour stability between beef with normal and intermediate pHu.

No significant difference in cooking loss was observed during ageing (**Table 5**), though the intermediate pHu group showed higher cooking loss values. This suggests that beef muscles with more moisture undergo greater cooking losses (Jeremiah et al., 2003). Higher fat content in meat corresponds to less bound water and protein (**Table 2**), impacting cooking losses (Ueda et al., 2007). Protein denaturation during cooking, particularly at temperatures above 70 °C, contributes to meat weight loss (Purslow et al., 2016). Our findings agree with Wicklund et al. (2005) and Berger et al. (2018), who reported no significant differences in cooking loss over varying ageing periods and ageing methods, respectively.

Shear force results aligned with Gomes et al. (2014), showing a negative correlation between normal pHu beef and shear force, as opposed to intermediate pHu beef, which exhibited lower values. However, our study found no significant interaction between pHu and storage period for shear force values. Liu et al. (2022) suggest that higher pH isn't always the sole factor in reduced meat quality. Pre-slaughter stress, even if the final carcass pH is acceptable (Ferguson & Warner, 2008), can increase shear force values (Gruber et al., 2010). Heat treatment can also raise meat shear force, as it causes muscle fibre disruption due to the thermal denaturation of actin and sarcoplasmic proteins at around 70 to 80 °C (Christensen et al., 2000). Various factors, including breed, species, diet, animal age, pH rate, and myofibrillar protein breakdown, significantly affect meat tenderization

(Ponnampalam et al., 2017). These factors also influence shear force results. The role of μ-calpain in breaking down larger proteins like titin and nebulin early *post-mortem* is thought to delay meat tenderization in normal pHu meat (Lomiwes et al., 2014).

From a sensory science perspective, tenderness isn't just about the force needed to chew but also involves mouthfeel sensations from moisture and fat. Our study, comparing the pHu group to texture profile at 3 days *post-mortem* and shear force at 3, 14, and 28 days *post-mortem*, found these differences to be non-significant. This is in line with Powell et al. (2011), who found no impact of shear force on consumer ratings across seven muscles. Liu et al. (2022) argue that the lack of significant correlation between shear force and perceived tenderness indicates these are distinct aspects.

5. Conclusion

This study provides valuable insights into assessing beef quality attributes and consumer perceptions based on variations in beef muscle pHu levels. We observed that beef with an intermediate pHu showed increased juiciness compared to typical pHu beef. This heightened juiciness is likely due to increased water-holding capacity. These findings align with consumer preference assessments that identified steaks with intermediate pHu as tougher and those with normal pHu as drier. Consumer feedback indicates that attributes like tenderness and juiciness significantly influence overall beef appreciation. Other important factors include roast beef flavour, chewability, saltiness, and juiciness. The study also showed that steaks with intermediate pHu had higher deoxymyoglobin concentrations on the third day compared to the twenty-eighth day of ageing. Instrumental colour measurements, such as L^*, b^*, chroma, and oxymyoglobin, were significantly affected by both pHu category and ageing duration (3, 14, and 28 days). This research enhances our understanding of pHu variations in beef muscle and their impact on sensory attributes and consumer preferences. Further studies with larger sample sizes are essential to support and expand these findings, aligning with consumer expectations for premium-quality beef.

Acknowledgements

This work was supported by the São Paulo Research Foundation FAPESP (grant # 2017/26667-2; 2019/26026-2).

Declarations of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Aaslyng, M. D., Bejerholm, C., Ertbjerg, P., Bertram, H. C., & Andersen, H. J. (2003). Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. *Food Quality and Preference, 14(4)*, 277-288. https://doi.org/10.1016/S0950-3293(02)00086-1

Aaslyng, M. D., Meinert, L., Bejerholm, C., & Warner, R. (2014). Sensory assessment of meat. In Encyclopedia of Meat Sciences (pp. 272-279). Elsevier Inc. https://doi.org/10.1016/B978-0-323-85125-1.00042-9

AMSA. (2012). Guidelines for meat color measurement. American Meat Science Association. http://www.meatscience.org/publications-resources/printedpublications/amsa-meat-color-measurement-guidelines.

AMSA American Meat Science Association (2016). Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of fresh meat (2 ed, version 1.02). American Meat Science Association, Champaign, IL, USA.

Anne, D., Thierry, A., Keisuke, S., & Michiyo, M. (2022). Transformation of highly marbled meats under various cooking processes. Meat Science, 189, Article 108810. https://doi.org/10.1016/j.meatsci.2022.108810

Antonelo, D. S., Gómez, J. F., Silva, S. L., Beline, M., Zhang, X., Wang, Y., Pavan, B., Koulicoff, L. A., Rosa, A. F., Goulart, R. S., Li, S., Gerrard, D. E., Suman, S. P., Wes Schilling, M., & Balieiro, J. C. (2022). Proteome basis for the biological variations in color and tenderness of *longissimus thoracis* muscle from beef cattle differing in growth rate and feeding regime. *Food Research International*, *153*, Article 110947. https://doi.org/10.1016/j.foodres.2022.110947

AOAC. (1995). Official Methods of Analysis. Association of Official Analytical Chemists International. Washington D.C.

Ares, G., & Jaeger, S. R. (2023). Check-all-that-apply (CATA) questions with consumers in practice: Experimental considerations and impact on outcome. In Rapid sensory profiling techniques (pp. 257-280). Woodhead Publishing. https://doi.org/10.1016/B978-0-12-821936-2.00013-3

Bejerholm, C., Tørngren, M. A., & Aaslyng, M. D. (2014). Cooking of meat. In M., & C.

(Eds.), Encyclopedia of meat sciences (pp. 370-376) (2nd ed.). Oxford: Academic Press.

Bendall, J. R. (1973). *Postmortem* changes in muscle. The structure and function of muscle, 2(Part 1), 243-309.

Berger, J., Kim, Y. H. B., Legako, J. F., Martini, S., Lee, J., Ebner, P., & Zuelly, S. M. S. (2018). Dry-aging improves meat quality attributes of grass-fed beef loins. *Meat Science, 145*, 285-291. https://doi.org/10.1016/j.meatsci.2018.07.004

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

Canto, A. C. V. C. S., Costa-Lima, B. R. C., Suman, S. P., Monteiro, M. L. G., Viana, F. M., Salim, A. P. A. A., Mahesh, N. N., Silva, T. J. P., & Conte-Junior, C. A. (2016). Color attributes and oxidative stability of *longissimus lumborum* and *psoas major* muscles from Nellore bulls. *Meat Science, 121*, 19-26. https://doi.org/10.1016/j.meatsci.2016.05.015

Christensen, M., Purslow, P. P., & Larsen, L. M. (2000). The effect of cooking temperature on mechanical properties of whole meat, single muscle fibres and perimysial connective tissue. *Meat Science, 55(3),* 301-307. https://doi.org/10.1016/S0309-1740(99)00157-6

Corcoran, L. C., Schlich, P., Moloney, A. P., O'Riordan, E., Millar, K., Botinestean, C., Gallagher, E., O'Sullivan, M. G., & Crofton, E. C. (2023). Comparing consumer liking of beef from three feeding systems using a combination of traditional and temporal liking sensory methods. *Food Research International, 168,* Article 112747. https://doi.org/10.1016/j.foodres.2023.112747

Corlett, M. T., Pethick, D. W., Kelman, K. R., Jacob, R. H., & Gardner, G. E. (2021). Consumer perceptions of meat redness were strongly influenced by storage and display times. *Foods, 10(3),* Article 540. https://doi.org/10.3390/foods10030540

Della Malva, A., Gagaoua, M., Santillo, A., De Palo, P., Sevi, A., & Albenzio, M. (2022). First insights about the underlying mechanisms of Martina Franca donkey meat tenderization during aging: A proteomic approach. *Meat Science, 193,* Article 108925. https://doi.org/10.1016/j.meatsci.2022.108925

Djekic, I. V., Ilic, J. G., Sołowiej, B. G., Djekić, R. I., & Tomasevic, I. B. (2022). Application of Food Mechanics and Oral Processing in Modelling First Bite of Grilled Meat. *Journal of Food Quality, 2022*, Article ID 9176628. https://doi.org/10.1155/2022/9176628

Duarte, T. L., Bolkenov, B., Klopatek, S. C., Oltjen, J. W., King, D. A., Shackelford, S.

D., Wheeler, T. L., & Yang, X. (2022). Evaluating the Shelf Life and Sensory Properties of Beef Steaks from Cattle Raised on Different Grass Feeding Systems in the Western United States. *Foods, 11(14),* Article 2141. https://doi.org/10.3390/foods11142141

Faustman, C., & Suman, S. P. (2017). The Eating Quality of Meat: I—Color. Lawrie´s Meat Science (Eight Edition), 329-356. https://doi.org/10.1016/B978-0-08-100694-8.00011-X

Ferguson, D. M., & Warner, R. D. (2008). Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? *Meat Science, 80(1),* 12-19. https://doi.org/10.1016/j.meatsci.2008.05.004

Feiner, G. (2006). Definitions of terms used in meat science. *Meat Products Handbook,* 46-71. https://doi.org/https://doi.org/10.1533/9781845691721.1.46

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

Gagaoua, M., Micol, D., Picard, B., Terlouw, C. E. M., Moloney, A. P., Juin, H., Meteau, K., Scollan, N., Richardson, I., & Hocquette, J-F. (2016). Inter-laboratory assessment by trained panelists from France and the United Kingdom of beef cooked at two different end-point temperatures. *Meat Science, 122,* 90-96. https://doi.org/10.1016/j.meatsci.2016.07.026

Gagaoua, M., Picard, B., & Monteils, V. (2018). Associations among animal, carcass, muscle characteristics, and fresh meat color traits in Charolais cattle. *Meat Science, 140,* 145-156. https://doi.org/10.1016/j.meatsci.2018.03.004

Gagaoua, M., Terlouw, E. M. C., Mullen, A. M., Franco, D., Warner, R. D., Lorenzo, J. M., Purslow, P. P., Gerrard, D., Hopkins, D. L., Troy, D., & Picard, B. (2021a). Molecular signatures of beef tenderness: Underlying mechanisms based on integromics of protein biomarkers from multi-platform proteomics studies. *Meat Science, 172,* Article 108311. https://doi.org/10.1016/j. meatsci.2020.108311

Gagaoua, M., Warner, R. D., Purslow, P., Ramanathan, R., Mullen, A. M., López-Pedrouso, M.,… & Terlouw, E. M. C. (2021b). Dark-cutting beef: A brief review and an integromics meta-analysis at the proteome level to decipher the underlying pathways. *Meat Science, 181*, Article 108611. https://doi.org/10.1016/j.meatsci.2021.108611

Gomes, C. L., Pflanzer, S. B., Cruz, A. G., De Felício, P. E., & Bolini, H. M. A. (2014). Sensory descriptive profiling and consumer preferences of beef strip loin steaks. *Food Research International*, *59,* 76-84. https://doi.org/10.1016/j.foodres.2014.01.061

Grau, R., & Hamm, R. (1953). Eine einfache methode zur bestimmung der wasserbindung im muskel. *Naturwissenschaften, 40(1),* 29-30.

Grayson, A. L., Shackelford, S. D., King, D. A., McKeith, R. O., Miller, R. K., & Wheeler, T. L. (2016). The effects of degree of dark cutting on tenderness and sensory attributes of beef. *Journal of Animal Science, 94(6),* 2583-2591. https://doi.org/10.2527/jas.2016-0388

Gruber, S. L., Tatum, J. D., Engle, T. E., Chapman, P. L., Belk, K. E., & Smith, G. C. (2010). Relationships of behavioral and physiological symptoms of preslaughter stress to beef *longissimus* muscle tenderness. *Journal of Animal Science, 88(3),* 1148-1159. https://doi.org/10.2527/jas.2009-2183

Hoffmann, H., Hamm, R., & Bluchel, E. (1982). Neus uber die bestimung der wasserbinding des nut hielf filter paper premethods. *Fleishwirtchaft, 62(1),* 87-94.

Holman, B. W., van de Ven, R. J., Mao, Y., Coombs, C. E., & Hopkins, D. L. (2017). Using instrumental (CIE and reflectance) measures to predict consumers' acceptance of beef colour. *Meat Science, 127,* 57-62. https://doi.org/10.1016/j.meatsci.2017.01.005

Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat science, 49(4),* 447-457. https://doi.org/10.1016/S0309-1740(98)00034-5

Hopkins, D. L. (1996). Assessment of lamb meat colour. *Meat Focus International*, *5*(11), 400-401.

Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science, 71(1),* 194-204. https://doi.org/10.1016/j.meatsci.2005.04.022

Hunt, M. C., Sørhelm, O., & Slinde, E. (1999). Color and heat denaturation of myoglobin forms in ground beef. *Journal of Food Science, 64(5),* 847-851. https://doi.org/10.1111/j.1365-2621.1999.tb15925.x

Hwang, Y-H., Kim, G-D., Jeong, J-Y., Hur, S-J., & Joo, S-T. (2010). The relationship between muscle fiber characteristics and meat quality traits of highly marbled Hanwoo (Korean native cattle) steers. *Meat Science, 86(2),* 456-461. https://doi.org/10.1016/j.meatsci.2010.05.034

Jaeger, S. R., Beresford, M. K., Paisley, A. G., Antúnez, L., Vidal, L., Cadena, R. S., Giménez, A., & Ares, G. (2015). Check-all-that-apply (CATA) questions for sensory product characterization by consumers: Investigations into the number of terms used in CATA questions. *Food Quality and Preference, 42,* 154-164. https://doi.org/10.1016/j.foodqual.2015.02.003

Jeong, J. Y., Hur, S. J., Yang, H. S., Moon, S. H., Hwang, Y. H., Park, G. B., & Joo, S. T. (2009). Discoloration Characteristics of 3 Major Muscles From Cattle During Cold Storage. *Journal of Food Science, 74(1),* C1-C5. https://doi.org/10.1111/j.1750-3841.2008.00983.x

Jeremiah, L., Dugan, M., Aalhus, J., & Gibson, L. (2003). Assessment of the chemical and cooking properties of the major beef muscles and muscle groups. Meat Science, 65(3), 985-992. https://doi.org/10.1016/S0309-1740(02)00308-X

Jia, W., Wang, X., Zhang, R., Shi, Q., & Shi, L. (2022). Irradiation role on meat quality induced dynamic molecular transformation: From nutrition to texture. *Food Reviews International, 1,* 1-23. https://doi.org/10.1080/87559129.2022.2026377

Joseph, P., Suman, S. P., Rentfrow, G., Li, S., & Beach, C. M. (2012). Proteomics of muscle-specific beef color stability. *Journal of Agricultural and Food Chemistry, 60(12),* 3196-3203. https://doi.org/10.1021/jf204188v

Kannan, G., Kouakou, B., & Gelaye, S. (2001). Color changes reflecting myoglobin and lipid oxidation in chevon cuts during refrigerated display. *Small Ruminant Research, 42(1),* 67-74. https://doi.org/10.1016/S0921-4488(01)00232-2

Kerth, C. R., & R. K. Miller. 2015. Beef flavor: A review from chemistry to consumer. *Journal of the Science of Food and Agriculture, 95,* 2783-2798. https://doi.org/10.1002/jsfa.7204

King, N. J., & Whyte, R. (2006). Does it look cooked? A review of factors that influence cooked meat color. *Journal of food science, 71(4),* R31-R40. https://doi.org/10.1111/j.1750-3841.2006.00029.x

Kim, Y. H., Keeton, J. T., Smith, S. B., Berghman, L. R., & Savell, J. W. (2009). Role of lactate dehydrogenase in metmyoglobin reduction and color stability of different bovine muscles. *Meat Science, 83(3),* 376-382. https://doi.org/10.1016/j.meatsci.2009.06.009

Kiyimba, F., Hartson, S. D., Rogers, J., Vanoverbeke, D. L., Mafi, G. G., & Ramanathan, R. (2022). Dark-cutting beef mitochondrial proteomic signatures reveal increased biogenesis proteins and bioenergetics capabilities. *Journal of Proteomics, 265,* Article 104637. https://doi.org/10.1016/j.jprot.2022.104637

Kosowska, M., Majcher, M. A., & Fortuna, T. (2017). Volatile compounds in meat and meat products. *Food Science and Technology (Brazil), 37(1),* 1-7. https://doi.org/10.1590/1678-457X.08416

Li, C., Wang, D., Xu, W., Gao, F., & Zhou, G. (2013). Effect of final cooked temperature on tenderness, protein solubility and microstructure of duck breast muscle. *LWT - Food*

Science and Technology, 51(1), 266-274. https://doi.org/10.1016/j.lwt.2012.10.003

Li, X., Zhang, D., Ijaz, M., Tian, G., Chen, J., & Du, M. (2020). Colour characteristics of beef *longissimus thoracis* during early 72 h postmortem. *Meat Science*, *170*, 108245. https://doi.org/10.1016/j.meatsci.2020.108245

Li, Z., Ha, M., Frank, D., Hastie, M., & Warner, R. D. (2023). Muscle fibre type composition influences the formation of odour-active volatiles in beef. *Food Research International*, *165*, Article 112468. https://doi.org/10.1016/j.foodres.2023.112468

Lindahl, G., Lundström, K., & Tornberg, E. (2001). Contribution of pigment content, myoglobin forms and internal reflectance to the colour of pork loin and ham from pure breed pigs. *Meat Science, 59(2)*, 141-151. https://doi.org/10.1016/S0309-1740(01)00064-X

Liu, J., Ellies-Oury, M. P., Stoyanchev, T., & Hocquette, J. F. (2022). Consumer perception of beef quality and how to control, improve and predict it? Focus on eating quality. *Foods, 11(12),* Article 1732. https://doi.org/10.3390/foods11121732

Lomiwes, D., Farouk, M. M., Wu, G., & Young, O. A. (2014). The development of meat tenderness is likely to be compartmentalised by ultimate pH. *Meat Science, 96(1),* 646-651. https://doi.org/10.1016/j.meatsci.2013.08.022

Loudon, K. M. W., Lean, I. J., Pethick, D. W., Gardner, G. E., Grubb, L. J., Evans, A. C., & McGilchrist, P. (2018). On farm factors increasing dark cutting in pasture finished beef cattle. *Meat science, 144*, 110-117. https://doi.org/10.1016/j.meatsci.2018.06.011

Lyford, C., Thompson, J., Polkinghorne, R., Miller, M., Nishimura, T., Neath, K., Allen, P., & Belasco, E. (2010). Is willingness to pay (WTP) for beef quality grades affected by consumer demographics and meat consumption preferences? *Australasian Agribusiness Review, 18,* 1-17.

Madruga, M. S., & Mottram, D. S. (1995). The effect of pH on the formation of maillard-derived aroma volatiles using a cooked meat system. *Journal of the Science of Food and Agriculture, 68(3),* 305-310. https://doi.org/10.1002/jsfa.2740680308

Malheiros, B. A., Spers, E. E., Silva, H. M. R. D., & Contreras Castillo, C. J. (2022). Southeast Brazilian Consumers' Involvement and Willingness to Pay for Quality Cues in Fresh and Cooked Beef. *Journal of Food Products Marketing, 28(6),* 276-293. https://doi.org/10.1080/10454446.2022.2129539

Martins, M. M., Saldaña, E., Teixeira, A. C. B., Selani, M. M., & Contreras-Castillo, C. J. (2021). Going beyond sensory and hedonic aspects: A Brazilian study of emotions evoked by beef in different contexts. *Meat Science, 180,* Article 108536. https://doi.org/10.1016/j.meatsci.2021.108536

McKenna, D., Mies, P., Baird, B., Pfeiffer, K., Ellebracht, J., & Savell, J. (2005). Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles. *Meat Science, 70(4),* 665-682. https://doi.org/10.1016/j.meatsci.2005.02.016

Miller, M. F., Carr, M. A., Ramsey, C. B., Crockett, K. L., & Hoover, L. C. (2001). Consumer thresholds for establishing the value of beef tenderness. *Journal of animal science, 79(12),* 3062-3068. https://doi.org/10.2527/2001.79123062x

Mitacek, R. M., Ke, Y., Prenni, J. E., Jadeja, R., VanOverbeke, D. L., Mafi, G. G., & Ramanathan, R. (2019). Mitochondrial degeneration, depletion of NADH, and oxidative stress Decrease color stability of wet-aged beef Longissimus steaks. *Journal of Food Science, 84(1),* 38-50. https://doi.org/10.1111/1750-3841.14396

Murray, J. M., Delahunty, C. M., & Baxter, I. A. (2001). Descriptive sensory analysis: past, present and future. *Food research international, 34(6),* 461-471. https://doi.org/10.1016/S0963-9969(01)00070-9

Obuz, E., & Dikeman, M. E. (2003). Effects of cooking beef muscles from frozen or thawed states on cooking traits and palatability. *Meat Science, 65(3),* 993–997. https://doi.org/10.1016/S0309-1740(02)00314-5

O'Quinn, T. G., Brooks, J. C., Polkinghorne, R. J., Garmyn, A. J., Johnson, B. J., Starkey, J. D., Rathmann, R. J., & Miller, M. F. (2012). Consumer assessment of beef strip loin steaks of varying fat levels. *Journal of Animal Science, 90(2),* 626-634. https://doi.org/10.2527/jas.2011-4282

Patinho, I., Saldaña, E., Selani, M. M., Camargo, A. C., Merlo, T. C., Menegali, B. S., Silva, A. P. S., & Contreras-Castillo, C. J. (2019). Use of Agaricus bisporus mushroom in beef burgers: Antioxidant, flavor enhancer and fat replacing potential. *Food Production, Processing and Nutrition, 1,* 1-15. https://doi.org/10.1186/s43014-019-0006-3

Patinho, I., Selani, M. M., Saldaña, E., Bortoluzzi, A. C. T., Rios-Mera, J. D., Silva, C. M., Kushida, M. M., & Contreras-Castillo, C. J. (2021). Agaricus bisporus mushroom as partial fat replacer improves the sensory quality maintaining the instrumental characteristics of beef burger. *Meat science, 172,* Article 108307. https://doi.org/10.1016/j.meatsci.2020.108307

Picard, B., & Gagaoua, M. (2020). Muscle fiber properties in cattle and their relationships with meat qualities: An overview. *Journal of Agricultural and Food Chemistry, 68(22),* 6021-6039. https://doi.org/10.1021/acs.jafc.0c02086

Poleti, M. D., Moncau, C. T., Silva-Vignato, B., Rosa, A. F., Lobo, A. R., Cataldi, T. R., Negrão, J. A., Silva, S. L., Eler, J. P., & Balieiro, J. C. C. (2018). Label-free quantitative proteomic analysis reveals muscle contraction and metabolism proteins linked to ultimate pH in bovine skeletal muscle. *Meat science, 145*, 209-219. https://doi.org/10.1016/j.meatsci.2018.06.041

Ponnampalam, E. N., Hopkins, D. L., Bruce, H., Li, D., Baldi, G., & Bekhit, A. E. (2017). Causes and contributing factors to "dark cutting" meat: Current trends and future directions: A review. *Comprehensive Reviews in Food Science and Food Safety, 16(3),* 400-430. https://doi.org/10.1111/1541-4337.12258

Powell, L., Nicholson, K. L., Huerta-Montauti, D., Miller, R. K., & Savell, J. W. (2011). Constraints on establishing threshold levels for Warner–Bratzler shear-force values based on consumer sensory ratings for seven beef muscles. *Animal production science, 51(10),* 959-966. https://doi.org/10.1071/AN10267

Purslow, P., Oiseth, S., Hughes, J., & Warner, R. (2016). The structural basis of cooking loss in beef: Variations with temperature and ageing. *Food Research International, 89*, 739-748. https://doi.org/10.1016/j.foodres.2016.09.010

Ramanathan, R. & Mancini, R. A. (2018). Role of Mitochondria in Beef Color: A Review, *Meat and Muscle Biology 2(1),* 309-320. https://doi.org/10.22175/mmb2018.05.0013

Ramanathan, R., Kiyimba, F., Gonzalez, J., Mafi, G., & DeSilva, U. (2020). Impact of Up and Down regulation of Metabolites and Mitochondrial Content on pH and Color of the *Longissimus Muscle* from Normal-pH and Dark-Cutting Beef. *Journal of Agricultural and Food Chemistry, 68*, 7194-7203. https://doi.org/10.1021/acs.jafc.0c01884

Saldaña, E., Martins, M. M., Behrens, J. H., Valentin, D., Selani, M. M., & Contreras-Castillo, C. J. (2020a). Looking at non-sensory factors underlying consumers' perception of smoked bacon. *Meat science, 163*, Article 108072. https://doi.org/10.1016/j.meatsci.2020.108072

Saldaña, E., Serrano-León, J., Selani, M. M., & Contreras-Castillo, C. J. (2020b). Sensory and hedonic impact of the replacement of synthetic antioxidant for pink pepper residue extract in chicken burger. *Journal of food science and technology, 57*, 617-627. https://doi.org/10.1007/s13197-019-04093-x

Saldaña, E., Merlo, T. C., Patinho, I., Rios-Mera, J. D., Contreras-Castillo, C. J., & Selani, M. M. (2021). Use of sensory science for the development of healthier processed meat products: A critical opinion. *Current opinion in food science, 40*, 13-19.

https://doi.org/10.1016/j.cofs.2020.04.012

Selani, M. M., Ramos, P. H. B., Patinho, I., França, F., dos Santos Harada-Padermo, S., Contreras-Castillo, C. J., & Saldaña, E. (2022). Consumer's perception and expected liking of labels of burgers with sodium reduction and addition of mushroom flavor enhancer. *Meat Science, 185*, Article 108720. https://doi.org/10.1016/j.meatsci.2021.108720

Shackelford, S. D., M. Koohmaraie, M. F. Miller, J. D. Crouse, and J. O. Reagan. 1991. An evaluation of tenderness of the *longissimus* muscle of Angus by Hereford versus Brahman crossbred heifers. *Journal of Animal Science, 69*, 171-177. https://doi.org/10.2527/1991.691171x

Sharedeh, D., Gatellier, P., Astruc, T., & Daudin, J. D. (2015). Effects of pH and NaCl levels in a beef marinade on physicochemical states of lipids and proteins and on tissue microstructure. *Meat Science, 110*, 24-31. https://doi.org/10.1016/j.meatsci.2015.07.004

Spanier, A. M., Flores, M., McMillin, K. W., & Bidner, T. D. (1997). The effect of post-mortem aging on meat flavor quality in Brangus beef. Correlation of treatments, sensory, instrumental and chemical descriptors. *Food Chemistry, 59(4)*, 531-538. https://doi.org/10.1016/S0308-8146(97)00003-4

Suman, S. P., Nair, M. N., Joseph, P., & Hunt, M. C. (2016). Factors influencing internal color of cooked meats. *Meat Science, 120*, 133-144. https://doi.org/10.1016/j.meatsci.2016.04.006

Suman, S. P., Wang, Y., Gagaoua, M., Kiyimba, F., & Ramanathan, R. (2023). Proteomic approaches to characterize biochemistry of fresh beef color. *Journal of Proteomics, 281*, Article 104893. https://doi.org/10.1016/j.jprot.2023.104893

Torres Filho, R. D. A., Cazedey, H. P., Fontes, P. R., Ramos, A. D. L. S., & Ramos, E. M. (2017). Drip loss assessment by different analytical methods and their relationships with pork quality classification. *Journal of Food Quality, 2017*, Article ID 9170768. https://doi.org/10.1155/2017/9170768

Troy, D. J., & Kerry, J. P. (2010). Consumer perception and the role of science in the meat industry. *Meat science, 86(1)*, 214-226. https://doi.org/10.1016/j.meatsci.2010.05.009

Ueda, Y., Watanabe, A., Higuchi, M., Shingu, H., Kushibiki, S., & Shinoda, M. (2007). Effects of intramuscular fat deposition on the beef traits of Japanese Black steers (Wagyu). *Animal Science Journal, 78(2)*, 189-194. https://doi.org/10.1111/j.1740-0929.2007.00424.x

Wakeling, I. N., & MacFie, H. J. (1995). Designing consumer trials balanced for first and higher orders of carry-over effect when only a subset of k samples from t may be tested. *Food Quality and Preference, 6(4)*, 299-308. https://doi.org/10.1016/0950-3293(95)00032-1

Wang, F., Holman, B. W., Zhang, Y., Luo, X., Mao, Y., & Hopkins, D. L. (2020). Investigation of colour requirements of frozen beef rolls by Chinese consumers for hot pot. *Meat Science, 162,* Article 108038. https://doi.org/10.1016/j.meatsci.2019.108038

Warner, R. & Miller, R. & Ha, M. & Wheeler, T. L. & Dunshea, F. & Li, X. & Vaskoska, R. & Purslow, P. (2021). Meat Tenderness: Underlying Mechanisms, Instrumental Measurement, and Sensory Assessment. *Meat and Muscle Biology 4(2)*, 1-25. https://doi.org/10.22175/mmb.10489

Watanabe, G., Motoyama, M., Orita, K., Takita, K., Aonuma, T., Nakajima, I., Tajima, A., Abe, A., & Sasaki, K. (2019). Assessment of the dynamics of sensory perception of Wagyu beef strip loin prepared with different cooking methods and fattening periods using the temporal dominance of sensations. *Food Science & Nutrition, 7(11)*, 3538-3548. https://doi.org/10.1002/fsn3.1205

Watson, R., Polkinghorne, R., & Thompson, J. M. (2008). Development of the Meat Standards Australia (MSA) prediction model for beef palatability. *Australian Journal of Experimental Agriculture, 48(11)*, 1368-1379. https://doi.org/10.1071/EA07184

Wicklund, S. E., Homco-Ryan, C., Ryan, K. J., Mckeith, F. K., Mcfarlane, B. J., & Brewer, M. S. (2005). Aging and Enhancement Effects on Quality Characteristics of Beef Strip Steaks. *Journal of Food Science, 70(3)*, S242-S248. https://doi.org/10.1111/j.1365-2621.2005.tb07164.x

Wu, S., Han, J., Liang, R., Dong, P., Zhu, L., Hopkins, D. L., Zhang, Y., & Luo, X. (2020). Investigation of muscle-specific beef color stability at different ultimate pHs. *Asian-Australasian Journal of Animal Sciences, 33(12)*, 1999-2007. https://doi.org/10.5713/ajas.19.0943

Yang, X., Wang, J., Holman, B. W., Liang, R., Chen, X., Luo, X., Zhu, L., Hopkins, D. L., & Zhang, Y. (2021). Investigation of the physicochemical, bacteriological, and sensory quality of beef steaks held under modified atmosphere packaging and representative of different ultimate pH values. *Meat Science, 174*, Article 108416. https://doi.org/10.1016/j.meatsci.2020.108416

Zhang, Y., Kim, Y., Puolanne, E., & Ertbjerg, P. (2022). Role of freezing-induced myofibrillar protein denaturation in the generation of thaw loss: A review. *Meat Science,* *190*, Article 108841. https://doi.org/10.1016/j.meatsci.2022.108841

Zhu, Y., Gagaoua, M., Mullen, A. M., Viala, D., Rai, D. K., Kelly, A. L., Sheehan, D., & Hamill, R. M. (2021). Shotgun proteomics for the preliminary identification of biomarkers of beef sensory tenderness, juiciness and chewiness from plasma and muscle of young Limousin-sired bulls. Meat science, 176, Article 108488. https://doi.org/10.1016/j.meatsci.2021.108488

Zimoch, J., & Gullett, E. A. (1997). Temporal aspects of perception of juiciness and tenderness of beef. *Food Quality and Preference*, *8(3)*, 203-211. https://doi.org/10.1016/S0950-3293(96)00049-3

Table 1. Attributes, definitions, assessment techniques, and scale endpoints used in the Optimised Sensory Profile of beef.

*Attribute	Definition	Technique	Scale endpoints
Connective tissue presence	Visual presence of white-coloured gelatinous matter	The assessor should be seated at a 90° angle and can approach the sample without touching the plate, at a minimum distance of ~40 cm	Lowest: beef knuckle Highest: beef shin
Apparent opacity	Opacity outside colour (opaque)	The assessor should be seated at a 90° angle and can approach the sample without touching the plate, at a minimum distance of ~40 cm	Lowest: roasted Chester™ Highest: grilled broiler breast fillet
Surface brown colour	Brown pigmentation on meat surface, characteristic of cooked beef	The assessor should be seated at a 90° angle and can approach the sample without touching the plate, at a minimum distance of ~40 cm	Lowest: boiled beef Highest: grilled beef
Internal colour	Internal colour of beef, ranging from red to brown	The assessor should be seated at a 90° angle and can approach the sample without touching the plate, at a minimum distance of ~40 cm	Lowest: rare grilled beef Highest: well-done grilled beef
Roast beef aroma	Characteristic aroma of roast beef	Raise the sample container to the nose and perform up to 3 long inhalations	Lowest: meat cooked in water Highest: meat baked in the oven
Blood aroma	Characteristic aroma of raw beef	Raise the sample container to the nose and perform up to 3 long inhalations	Lowest: medium steak Highest: raw beef
Overall tenderness	Little force required for chewing	Chewing with molar teeth until sample is swallowed	Lowest: grilled beef outside flat Highest: grilled beef tenderloin
Overall juiciness	Release of liquid during chewing	Chewing with molar teeth until sample is swallowed	Lowest: grilled beef knuckle Highest: Grilled sirloin cap steak
Hardness	Force required to achieve certain product deformation	Chewing with molar teeth until sample is swallowed	Lowest: medium rump steak (63 °C) Highest: well-done outside flat steak (79 °C)
Dryness	No liquid release during chewing	Chewing with molar teeth until sample is swallowed	Lowest: rare sirloin cap steak (60 °C) Highest: cooked pork chop (79 °C)
Fibrousness		Place the sample in the mouth and generate a perception within 7 chews	

*Attribute	Definition	Technique	Scale endpoints
	Presence of fibres that persists throughout chewing		Lowest: grilled tenderloin steak Highest: grilled outside flat steak
Chewiness	The number of chews required for the product to be ready for swallowing	Place the sample in the mouth and generate a perception within 7 chews	Lowest: medium tenderloin (63 °C) Highest: well-done pork chop (79 °C)
Roast beef flavour	Characteristic roast meat flavour	Chewing with molar teeth until sample is swallowed	Lowest: Boiled beef Highest: baked beef
Meaty beef flavour	Characteristic beef flavour	Chewing with molar teeth until sample is swallowed	Lowest: Boiled beef Highest: grilled beef
Fat flavour	Perception of fat in the mouth during chewing	Place the sample in the mouth and generate a perception within 7 chews	Lowest: cooked chicken breast fillet (79 ºC) Highest: cooked beef rib (69 ºC)
Blood flavour	Flavour associated with blood in cooked meat products; closely related to a metallic flavour	Place the sample in the mouth and generate a perception within 7 chews	Lowest: well-done sirloin cap steak (82 °C) Highest: rare sirloin steak (55 °C)

* For each attribute, a 9-cm unstructured linear intensity scale was utilised, with intensity levels ranging from 'Lowest' at 0 to 'Highest' at 9, anchored at both ends of the scale

Table 2. Proximate composition of intermediate and normal pHu beef 3 days *post-mortem*

Treatment	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Intermediate pHu	74.95	20.89	2.67	1.03
Normal pHu	73.49	24.02	1.40	1.06

Proximate composition was determined by method (AOAC, 1995) and (ISO 1871:2009; 936:1998).

Table 3 – Mean ratings* of the sensory attributes evaluated in the intermediate and normal pHu beef 3 days *post-mortem.* Measurements on a 9-cm unstructured linear scale (0 = none/Lowest; 9= Highest)

Sensory attributes	Intermediate pHu	Normal pHu	**t-value**	***P*-value****
Connective tissue presence	1.90±2.21	1.62±2.21	0.68	0.50
Apparent opacity	2.36±2.31	3.02±2.31	-1.57	0.12
Surface brown colour	7.18±1.57	7.44±1.57	-0.90	0.37
Internal colour	6.35±1.98	6.22±1.98	0.36	0.72
Roast beef aroma	6.92±2.29	7.10±2.29	-0.43	0.67
Blood aroma	0.97±1.46	0.81±1.46	0.60	0.55
Overall tenderness	4.19±2.88	4.06±2.88	0.23	0.82
Overall juiciness	**5.88±2.50**	**4.48±2.50**	**3.18**	**0.00**
Hardness	4.35±2.94	4.21±2.94	0.27	0.79
Dryness	2.35±2.35	3.02±2.35	-1.56	0.12
Fibrousness	3.66±2.80	3.64±2.80	0.03	0.97
Chewiness	5.42±2.85	5.36±2.85	0.12	0.91
Roast beef flavour	6.92±2.26	6.89±2.26	0.06	0.95
Meaty beef flavour	7.95±1.30	7.87±1.30	0.34	0.73
Fat flavour	1.76±2.02	1.78±2.02	-0.04	0.97
Blood flavour	1.71±1.71	1.21±1.71	1.64	0.10

* *Measurements on a 9-cm unstructured linear scale (0 = none/Lowest; 9= Highest)*

**Sensory attributes with a significant (P < 0.05) effect on responses are marked in bold. Results are expressed as mean ± standard deviation.

Table 4. Quality parameters of intermediate and normal pHu beef 3 days *post-mortem*

Parameters	Intermediate pHu	Normal pHu	***P*-value***
Ultimate pH	**5.89±0.04**	**5.58±0.04**	**0.01**
Drip loss (%)	2.12±0.40	3.32±0.40	0.10
TPA			
Hardness (N)	62.19±7.01	81.94±7.01	0.12
Springiness	0.01±0.00	0.01±0.00	0.66
Cohesiveness	0.01±0.00	0.01±0.00	0.19
Chewiness	21.83±3.65	32.08±3.65	0.12
Gumminess	34.13±3.96	48.73±3.96	0.06

The parameters which have a significant (P<0.05) effect on responses are marked in bold. Results are expressed as mean ± standard error of the mean. TPA, texture profile analysis. N = Newtons.

Table 5. Effect of ultimate pH (pHu) and aging days on quality parameters of intermediate and normal pHu beef at 3, 14, and 28 days *post-mortem*

Parameters	*L**	*a**	*b**	Chroma	Hue	Surface colour stability	%OMb	%MMb	%DMb	%WHC	%Cooking losses	Shear force (N)
Source of variation												
Intermediate pHu	38.50[b]	25.81[a]	18.12[b]	31.55[b]	34.70[a]	5.94[a]	68.22[b]	17.17[a]	14.51[a]	34.22[a]	28.43[a]	85.19[b]
Normal pHu	41.90[a]	28.06[a]	20.60[a]	34.87[a]	35.61[a]	6.00[a]	73.38[a]	17.92[a]	8.66[b]	32.74[a]	26.00[a]	102.43[a]
SEM	0.44	0.74	0.76	1.05	0.39	0.31	1.27	0.65	1.21	0.77	1.14	5.34
Aging days	***L****	***a****	***b****	**Chroma**	**Hue**	**Surface colour stability**	**%OMb**	**%MMb**	**%DMb**	**%WHC**	**%Cooking losses**	**Shear force (N)**
3	42.13[a]	21.83[b]	13.55[b]	25.71[b]	31.59[b]	3.64[b]	61.78[b]	23.06[a]	15.16[a]	30.92[b]	25.10[a]	104.89[a]
14	38.60[b]	28.79[a]	21.63[a]	36.03[a]	36.75[a]	6.99[a]	73.09[a]	14.05[b]	12.68[a]	33.86[ab]	29.44[a]	92.67[a]
28	39.87[b]	30.17[a]	22.90[a]	37.89[a]	37.13[a]	7.28[a]	77.53[a]	15.52[b]	6.91[b]	35.65[a]	27.10[a]	83.86[a]
SEM	0.53	0.91	0.93	1.28	0.48	0.38	1.55	0.79	1.48	0.95	1.40	6.54

Different letters in the same column indicate significant differences ($P < 0.05$) according to the Tukey's test. SEM: Standard error of the mean. Luminosity (*L**), Redness (*a**), Yellowness (*b**), Chroma and Hue. OMb: oxymyoglobin, MMb: metmyoglobin. DMb: deoxymyoglobin. Surface colour stability, Water-holding capacity (WHC), Cooking losses and Shear force (N = Newtons).

Fig. 1. Principal Component Analysis of ODP data from intermediate and normal pHu beef at 3 days *post-mortem*. **A)** Individual data points. **B)** Variables plots.

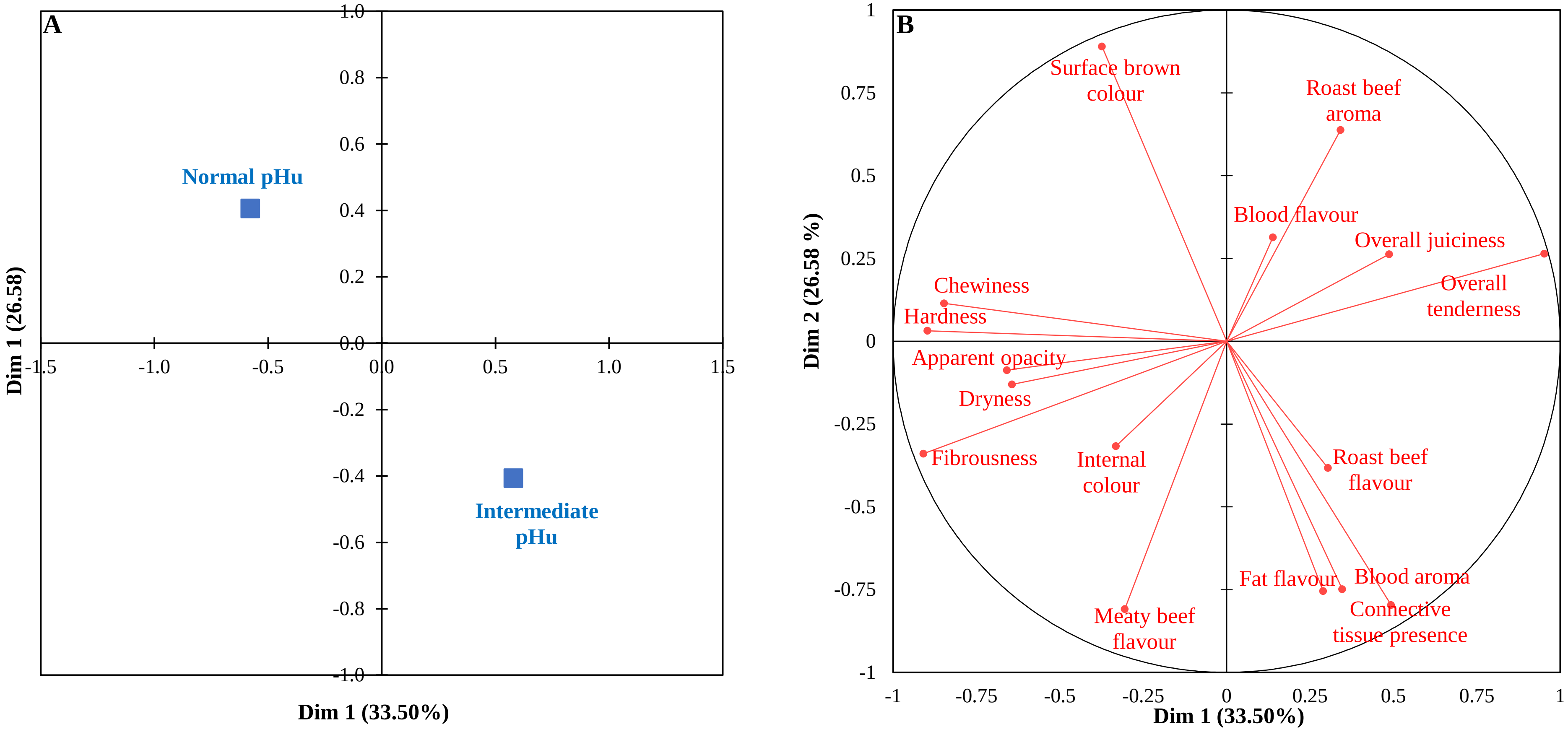


Fig. 2. Correspondence and Principal Coordinate Analysis of Sensory Descriptors. A) Correspondence Analysis of sensory descriptors for intermediate and normal pHu beef at 3 days post-mortem, depicted in the first two dimensions derived from PCA of the Check-All-That-Apply (CATA) questions and the ideal sample. **B)** Representation of the relationship between overall liking and the sensory descriptors by Principal Coordinate Analysis.

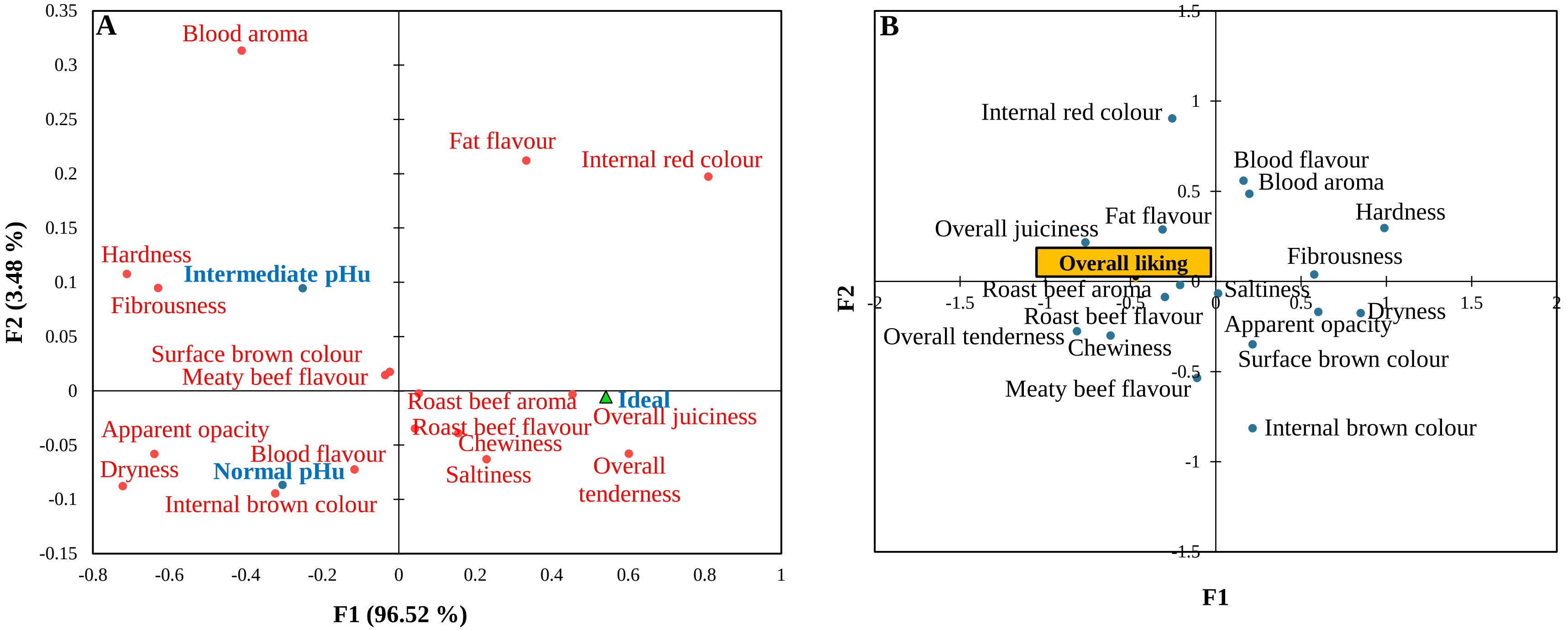


Fig. 3. Penalty-Lift Analysis on Sensory Attributes and Overall Liking Scores. Penalty-lift analysis showing the impact of sensory attributes on the mean overall liking scores for beef samples with intermediate and normal pHu, 3 days post-mortem.

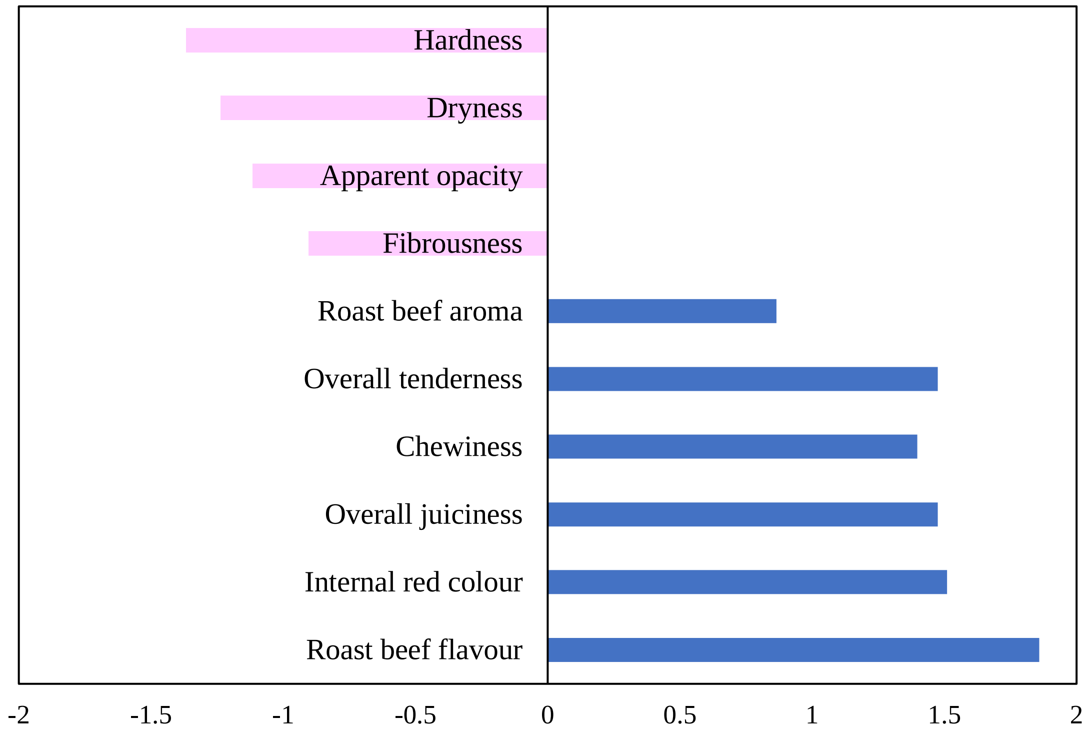
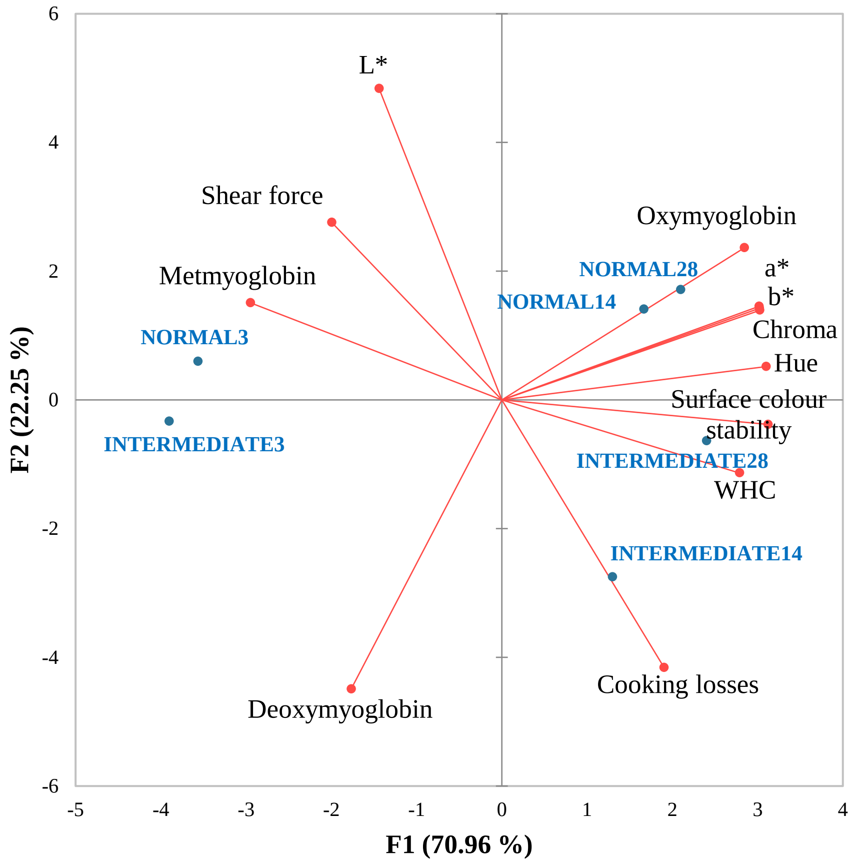


Fig. 4. Principal Component Analysis of Instrumental Quality Parameters in Beef Samples. Principal Component Analysis of various instrumental quality parameters measured in beef samples with intermediate and normal pHu at 3, 14, and 28 days post-mortem. The parameters evaluated include luminosity (L*), redness (a*), yellowness (b*), chroma, hue angle, and concentrations of oxymyoglobin, metmyoglobin, and deoxymyoglobin, alongside assessments of surface color stability, water holding capacity (WHC), cooking losses, and shear force.



Highlights

- Sensory analysis of Nellore beef comparing normal and intermediate pHu
- Intermediate pHu beef scored higher for juiciness according to trained panellists
- Consumers could not differentiate between intermediate and normal pHu beef
- Higher deoxymyoglobin on day 3 than on day 28 in intermediate pHu beef
- Beef colour is influenced by pHu and ageing: L^*, b^*, Chroma, and Oxymyoglobin