



# Physicochemical Characteristics of Marinated Bali Cattle Beef: Effects of Organic Acid Type, Coloring Agents, and Marination Time

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**Abstract** | Bali cattle beef is known for its unique flavour but is often considered tough and visually inconsistent, limiting its appeal for *ready-to-cook* products. This study evaluated whether traditional Indonesian marinade components can improve its pre-cooking quality. A  $3 \times 3 \times 2$  full-factorial experiment was conducted using three organic acids: tamarind (TA), lime juice (LJ), and apple vinegar (AV); three colouring treatments: turmeric (TU), sodium nitrite (NI,  $50 \text{ mg kg}^{-1}$ ), and their combination (TN); and two marination times (12 h, 24 h). All main factors significantly affected tenderness; AV  $\times$  NI  $\times$  24 h produced the lowest shear force (mean:  $0.578 \pm 0.087 \text{ kg f}$ ), while TA increased toughness after 24 h. pH was affected by acid type and its interaction with time; TA caused a marked rise at 24 h, unlike LJ and AV. TBA values (mean:  $0.153 \pm 0.329$ ) were lowest in TA  $\times$  TN  $\times$  12 h. Colour attributes were strongly modulated by treatment combinations. LJ increased lightness, while TA darkened the surface. The TA  $\times$  TN  $\times$  24 h treatment yielded the highest redness ( $a^* \approx 10.8$ ), yellowness ( $b^* \approx 19.6$ ), and chroma, suggesting synergistic stabilisation between curcuminoids and nitrite. No adverse effects on pH or tenderness were observed due to colouring agents. These findings support tailored marination to improve tenderness and visual appeal of Bali beef, with future work needed on microbial stability and consumer acceptance.

**Keywords** | Bali cattle, Marinades, Organic acids, beef, Color attributes

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## INTRODUCTION

Local beef in Indonesia, Bali cattle (*Bos Javanicus*) is widely recognized for its comparatively firmer bite and deeper coloration, traits that stem from the animals' slow growth on forage of modest nutritional quality and from minimal concentrate supplementation (Sofyan *et al.*, 2021; Sriyani *et al.*, 2022; Tahuk *et al.*, 2020). Such intrinsic attributes, while valued in certain traditional dishes, tend to restrict

the meat's suitability for quick, high-heat preparations that are gaining popularity with modern consumers pressed for time and captivated by global culinary trends (Ježek *et al.*, 2019; Malak, 2024). Conventional moist-heat techniques long simmering or boiling have long served to mitigate toughness (García-Segovia *et al.*, 2007), yet these methods are increasingly perceived as labor-intensive and energy-demanding. Consequently, attention is shifting toward natural marination as a means of tenderization that

also introduces desirable flavours while resonating with contemporary expectations for sustainability and “clean-label” food processing (Gómez *et al.*, 2020; Okpala *et al.*, 2023).

Over the past decade, natural marinades have emerged as credible, health-forward substitutes for synthetic tenderizers and preservatives, aligning with a broader consumer quest for minimally processed proteins (Aminzare *et al.*, 2019). Besides conferring flavour complexity, these bio-based solutions demonstrably improve textural properties by accelerating proteolysis, solubilising collagen, and promoting myofibrillar disruption (Serdaroglu *et al.*, 2024). Proteolytic enzymes, bromelain from pineapple, papain from papaya, ficin from figs and small organic acids sourced from vinegar, citrus fruits, or tamarind disrupt the structural integrity of muscle, lower pH, and foster connective-tissue breakdown (Arganosa and Marriott, 1989; Latoch *et al.*, 2023; Lewis and Purslow, 1991; Vişan *et al.*, 2021). These mechanisms collectively accelerate tenderization without prolonged thermal input, thereby dovetailing with quicker cooking formats.

In Indonesian cuisine, tamarind (Patriani *et al.*, 2024), turmeric (Febrianta, 2023), and lime juice (Malelak and Jelantik, 2022) stand out as traditional marinades with unique properties for improving meat quality. Tamarind is rich in tartaric acid (Azad, 2018), while lime juice contains citric acid (Penniston *et al.*, 2008), and apple vinegar is characterized by its acetic acid content (El-Abdali *et al.*, 2023); contribute to collagen degradation and provide distinctive flavors (Ke *et al.*, 2009; Latoch *et al.*, 2023). Turmeric, known for its bioactive compound curcumin, has antioxidant and anti-inflammatory properties (El-Saadony *et al.*, 2023) and aids enzymatic breakdown of muscle proteins (Mohd Azmi *et al.*, 2023). These ingredients, when used in combination, offer an accessible, natural method to improve the texture, flavor, and sensory appeal of Bali cattle beef, making it more suitable for fast cooking methods such as grilling or frying.

Despite their widespread use in traditional cooking, marinated Bali cattle beef slices represent an innovative presentation method intended to enhance the convenience and consumer acceptability of Bali beef. The scientific evaluation of natural marinades in enhancing the physicochemical and sensory properties of Bali cattle beef remains limited. This study addresses this gap by examining the effects of tamarind, turmeric, and lime juice, combined with varying marination times and natural coloring agents, on the pre-cooking quality and sensory attributes of fermented Bali cattle beef slices.

## EXPERIMENTAL DESIGN AND MARINATION PROCEDURE SAMPLE PREPARATION

Fifteen Bali cattle bulls ( $\approx 30$  months; mean live weight  $\approx 300$  kg) were harvested at a government-licensed abattoir in Makassar, South Sulawesi, Indonesia. Slaughter followed traditional halal procedures and complied with national animal-welfare regulations as well as institutional ethical guidelines for pre-slaughter handling and exsanguination (Abdullah *et al.*, 2019; Chao, 2022; Farouk *et al.*, 2014). Immediately post-mortem, the *Longissimus dorsi* muscles from both left and right sides were excised ( $\approx 40$  cm each), yielding  $\approx 6$  kg of boneless meat per animal. The muscles were placed in insulated cool boxes with ice packs and transported to the laboratory within 2 h, maintaining a core temperature of 0–4 °C (Abdullah *et al.*, 2019).

In the laboratory, external connective tissue and visible fat were carefully removed to ensure uniformity of texture and minimise analytical variability (Farouk *et al.*, 2014; Lewis and Purslow, 1991). The trimmed muscles were partially frozen at  $-5$  °C to facilitate precise slicing, then cut perpendicular to the fibre direction into 0.5 cm-thick slices ( $\approx 20$  g each) using a calibrated meat slicer ( $\pm 0.1$  mm accuracy). Subsequently, the samples were assigned to 18 thin-walled plastic containers, each representing a distinct treatment combination according to a  $3 \times 3 \times 2$  factorial design. The marination factors were: Organic acids: tamarind (TA), lime juice (LJ), and apple vinegar (AV); Coloring agents: turmeric (TU, 1%), sodium nitrite (NI, 50 mg  $\text{NaNO}_2/\text{kg}$  sample), or a combination of both (TN). All marination agents were freshly sourced from local traditional markets in Makassar.

## MARINATING PROCESS

Each meat slice was treated with a marination solution prepared using 3% (w/w) organic acids and coloring agents, which included turmeric (1%), sodium nitrite (50 mg  $\text{NaNO}_2/\text{kg}$  sample), or a combination of turmeric and sodium nitrite. The solutions were prepared fresh for each treatment, ensuring consistent composition and quality. The marination solution was evenly distributed over the surface of the meat slices, ensuring complete coverage. Samples were placed in sealed, thin-walled plastic containers and stored at 4°C for either 12 or 24 hours, depending on the treatment duration. Containers were labelled to track specific treatment combinations and stored under controlled conditions to prevent cross-contamination. Following the marination period, the samples were removed from their containers and frozen at  $-18^\circ\text{C}$  to halt enzymatic activity and preserve quality until subsequent physicochemical and sensory evaluations. Throughout the process, care was taken to standardize variables, including

the volume of the marination solution, container size, and storage environment, to ensure reproducibility.

Each 20 g slice was immersed in a freshly prepared marinade containing 3 % (w/w, relative to meat weight) of the designated organic acid tamarind (TA), lime juice (LJ), or apple vinegar (AV) and one of three colouring treatments: turmeric powder (TU, 1 %), sodium nitrite (NI, 50 mg NaNO<sub>2</sub> kg<sup>-1</sup>), or their combination (TN). The marinade (1 mL g<sup>-1</sup> meat) was gently massaged onto the surface to achieve complete coverage, after which slices were placed individually in thin-walled, food-grade plastic containers, sealed, and held at 4 °C for either 12 h or 24 h according to the experimental schedule. Containers were clearly labelled to identify acid source, colouring agent, and marination time, and were stored on a dedicated shelf to avoid cross-contamination. At the end of the designated period, slices were blotted dry, packed into moisture-proof bags, and frozen at -18 °C to arrest enzymatic reactions pending physicochemical and sensory analyses. All ancillary variables marinade volume, container dimensions, headspace, and storage temperature were standardised to maximise reproducibility across treatments.

## PHYSICOCHEMICAL CHARACTERISTICS EVALUATION

### SHEAR FORCE

Tenderness was quantified as peak shear force using a MIRINZ Tenderometer (AgResearch MIRINZ, Hamilton, New Zealand) following (Farouk *et al.*, 2009; Hopkins *et al.*, 2010) with minor modifications. After each marination regime and the prescribed pre-cooking step, slices were equilibrated to 20 ± 1°C and trimmed to uniform rectangles (≈ 40 mm × 10 mm × 5 mm) so that fibres ran parallel to the long axis. Specimens were sheared perpendicular to the fibres with a Warner-Bratzler V-blade moving at 200 mm min<sup>-1</sup>. The 500 N load cell was zero-balanced and verified against certified weights before every analytical batch. For each treatment combination, three slices were tested, and three shear readings were taken from spatially separate positions on every slice, yielding nine observations per replicate. Their arithmetic mean expressed in Newtons (N; 1 kgf= 9.81 N) served as the replicate value. Strict control over sample geometry and orientation ensured that any variation in peak force reflected treatment effects rather than dimensional heterogeneity.

### PH DETERMINATION

The pH of marinated meat was measured using a digital pH meter with a flat-tip electrode for food analysis. The device was calibrated with pH 4.0 and 7.0 buffers before use. Each sample was homogenized, and the electrode was immersed until the reading stabilized. Measurements were taken in triplicate at room temperature, and the mean value was used for analysis.

## THIOBARBITURIC ACID

Lipid oxidation was measured using the TBA method, with results expressed as mg malondialdehyde (MDA) per kg of sample. A 3 g portion of meat was homogenized with 50 mL distilled water, mixed with 2.5 mL HCl and 47.5 mL distilled water, and distilled to collect 50 mL of distillate. A 5 mL aliquot was reacted with 5 mL of 0.02 M TBA in 90% glacial acetic acid and incubated at 75 °C for 35 minutes. After cooling, absorbance was read at 528 nm using a spectrophotometer. All measurements were performed in triplicate, and mean values were used for analysis.

## COLOR ATTRIBUTES (L\* A\* B\* AND CHROMA)

The color of the meat samples was evaluated following the Hunter color system, which measures L\* (lightness), a\* (redness), and b\* (yellowness), as outlined in the American Meat Science Association Guidelines for Meat Color Measurement (King *et al.*, 2023). The L\* value represents lightness, ranging from 0 (black) to 100 (white), while a\* measures chromaticity, with positive values (+a\*) indicating redness and negative values (-a\*) indicating greenness. Similarly, b\* values quantify yellowness (+b\*) or blueness (-b\*). A portable TES-135 digital colormeter was used to measure surface color. The device was calibrated with a standard white tile before use, and measurements were taken at three randomly selected points on each sample. The average L\*, a\*, and b\* values were recorded for analysis. Chroma, representing color intensity, was calculated using the formula: Chroma = (a\*<sup>2</sup> + b\*<sup>2</sup>)<sup>1/2</sup>. All measurements were conducted under consistent lighting conditions, and the calculated values were used for statistical analysis.

## STATISTICAL ANALYSIS

A completely randomized design with a 3×3×2 factorial arrangement was used, comprising 15 replications (n= 15) per treatment. The three factors included: (1) Organic Acid (TA, LJ, AV), (2) Coloring Agent (TU, NIT, TN), and (3) Marination Time (12 h, 24 h). Physicochemical data were analyzed using one-way ANOVA to assess the main and interaction effects of the treatment factors. Where significant effects (p < 0.05) were found, Tukey's HSD test was used for pairwise comparisons. ANOVA assumptions were checked using the Shapiro-Wilk test for normality and Levene's test for homogeneity of variance. If assumptions were violated, data were transformed or non-parametric methods were applied. All analyses were conducted using IBM SPSS Statistics Version 29.

## RESULTS AND DISCUSSION

### SHEAR FORCE

The study recorded an average shear-force value of 0.578 ± 0.087 KgF. All three main factors (type of organic acid,

**Table 1:** Summary of three-way interaction effects (organic acid × coloring agent × marination time) on physicochemical, color, and sensory attributes.

| Parameter               | Highest combination | Value  | Lowest combination | Value  | SEM   | p value |
|-------------------------|---------------------|--------|--------------------|--------|-------|---------|
| <b>Physicochemical</b>  |                     |        |                    |        |       |         |
| Shear force             | TA × NI × 12h       | 0.685  | AV × NI × 24h      | 0.434  | 0.027 | 0.922   |
| pH                      | TA × TU × 24h       | 5.530  | LJ × TU × 12h      | 4.783  | 0.125 | 0.756   |
| TBA                     | LJ × TN × 24h       | 37.140 | TA × NI × 24h      | 15.457 | 2.418 | 0.002*  |
| <b>Color attributes</b> |                     |        |                    |        |       |         |
| L*                      | LJ × TU × 12h       | 39.932 | LJ × TU × 12h      | 32.128 | 1.495 | 0.242   |
| a*                      | LJ × TN × 12h       | 11.972 | AV × NI × 12h      | 7.632  | 0.919 | 0.004*  |
| b*                      | TA × TU × 12h       | 21.493 | LJ × NI × 12h      | 9.647  | 1.141 | 0.335   |
| Chroma                  | TA × TU × 12h       | 23.283 | AV × NI × 12h      | 13.091 | 0.98  | 0.497   |

The highest and lowest values for each parameter are presented as Least Square Means (LQ-means), along with their corresponding treatment combinations, SEM, and p-values for interaction effects. \*p-values indicate statistically significant interactions ( $p < 0.05$ ). Abbreviations: TA = Tamarind, LJ = Lime Juice, AV= Apple Vinegar, TU= Turmeric, NI = Nitrite, TN = Turmeric-Nitrite.

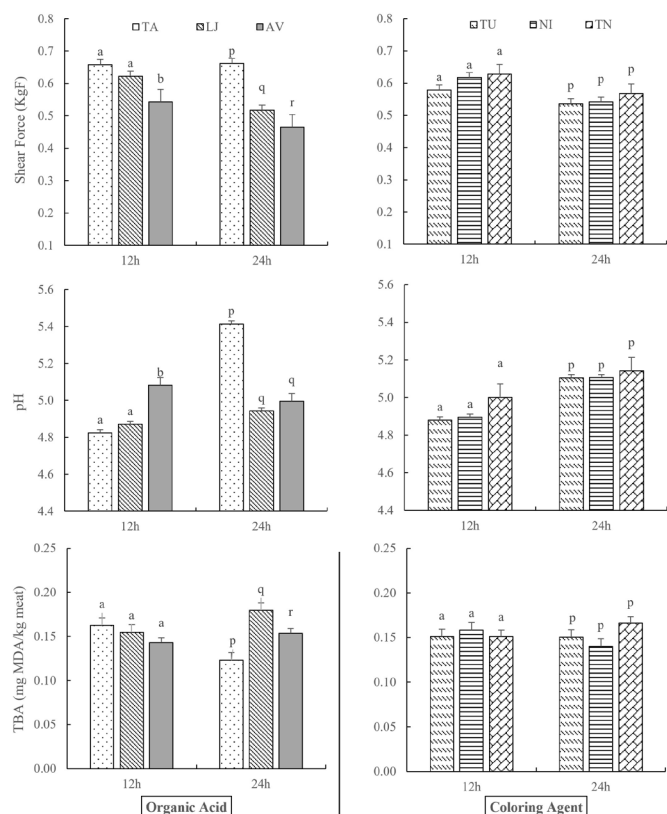
**Table 2:** The main effects of organic acid, coloring agents, and marination times on physicochemical properties of marinated meat samples.

| Treatment               | Physicochemical     |                    |                       | Color Attributes     |                     |                     |                     |
|-------------------------|---------------------|--------------------|-----------------------|----------------------|---------------------|---------------------|---------------------|
|                         | Shear force (KgF)   | pH                 | TBA (mg MDA/ kg meat) | L*                   | a*                  | b*                  | Chroma              |
| <b>Organic acid</b>     |                     |                    |                       |                      |                     |                     |                     |
| Tamarind TA             | 0.66 <sup>a</sup>   | 5.119 <sup>a</sup> | 0.035 <sup>a</sup>    | 34.696 <sup>a</sup>  | 10.084 <sup>a</sup> | 16.310 <sup>a</sup> | 19.443 <sup>a</sup> |
| Lime Juice LJ           | 0.57 <sup>b</sup>   | 4.907 <sup>b</sup> | 0.033 <sup>ab</sup>   | 38.178 <sup>b</sup>  | 10.129 <sup>a</sup> | 13.692 <sup>a</sup> | 17.209 <sup>b</sup> |
| Apple vinegar AV        | 0.504 <sup>c</sup>  | 5.038 <sup>a</sup> | 0.027 <sup>b</sup>    | 36.461 <sup>ab</sup> | 9.416 <sup>a</sup>  | 13.867 <sup>a</sup> | 16.917 <sup>b</sup> |
| SEM                     | 0.011               | 0.051              | 0.006                 | 0.601                | 0.375               | 0.466               | 0.400               |
| P <sub>Value</sub>      | <0.001              | 0.020              | 0.017                 | 0.001                | 0.333               | <0.001              | <0.001              |
| <b>Coloring agents</b>  |                     |                    |                       |                      |                     |                     |                     |
| Turmeric TU             | 0.557 <sup>a</sup>  | 4.993 <sup>a</sup> | 0.023 <sup>a</sup>    | 37.098 <sup>a</sup>  | 9.612 <sup>a</sup>  | 15.839 <sup>a</sup> | 18.666 <sup>a</sup> |
| Nitrite NI              | 0.579 <sup>ab</sup> | 5.001 <sup>a</sup> | 0.036 <sup>a</sup>    | 35.870 <sup>a</sup>  | 10.173 <sup>a</sup> | 11.139 <sup>b</sup> | 15.212 <sup>b</sup> |
| Turmeric-Nitrite TN     | 0.598 <sup>b</sup>  | 5.071 <sup>a</sup> | 0.038 <sup>a</sup>    | 36.367 <sup>a</sup>  | 9.843 <sup>a</sup>  | 16.893 <sup>a</sup> | 19.691 <sup>a</sup> |
| SEM                     | 0.011               | 0.051              | 0.006                 | 0.610                | 0.375               | 0.466               | 0.400               |
| P <sub>Value</sub>      | 0.045               | 0.508              | 0.504                 | 0.369                | 0.573               | <0.001              | <0.001              |
| <b>Marinating times</b> |                     |                    |                       |                      |                     |                     |                     |
| 12h                     | 0.608               | 4.926              | 0.024                 | 35.709               | 9.760               | 14.838              | 17.959              |
| 24h                     | 0.548               | 5.118              | 0.040                 | 37.181               | 9.992               | 14.408              | 17.754              |
| SEM                     | 0.009               | 0.042              | 0.005                 | 0.498                | 0.306               | 0.380               | 0.327               |
| P <sub>Value</sub>      | <0.001              | 0.002              | 0.855                 | 0.044                | 0.596               | 0.430               | 0.659               |

Values are presented as Least Square Means (LQ-means), with standard error of means (SEM) and p-values. Superscripts (a, b, c) within the same column indicate significant differences based on Tukey's post-hoc test ( $p < 0.05$ ). L\*: lightness, a\*: redness, b\*: yellowness.

coloring agent, and marination time) had a highly significant effect on shear force ( $p < 0.001$ ; Table 1). Regarding the acid treatments, beef marinated with tamarind extract (TA) showed a significantly higher shear force than samples treated with lime juice (LJ) or apple vinegar (AV) ( $p < 0.01$ ). For coloring agents, the turmeric-nitrite combination (TN) produced a higher shear-force value than turmeric alone (TU) ( $p < 0.05$ ), while its effect did not differ from that of nitrite alone (NI) ( $p > 0.05$ ).

Changes over time were treatment-specific: After 24 hours of marination, shear force increased in the TA group but decreased in both the LJ and AV groups (Figure 1). Overall, the lowest (i.e., most desirable) shear-force value was obtained with the AV × NI × 24 h treatment (Table 2). Nevertheless, the three-way interaction among acid type, coloring agent, and marination time was not statistically significant.



**Figure 1:** Least-squares means of physicochemical parameters showing the interaction between Acid Marination (AM: TA, LJ, AV), Coloring Agent (CA: TU, NI, TN), and Marination Time (MT: 12 h and 24 h). Different letters indicate statistically significant differences ( $p < 0.05$ ): a–b, differences among AM treatments at 12 h; p–q, differences among AM treatments at 24 h.

The significant variation in shear force across treatments indicates the pivotal role of acid type in modulating meat texture. Tamarind-treated (TA) samples exhibited higher shear force compared to those treated with lime juice (LJ) and apple vinegar (AV), suggesting a less pronounced tenderizing effect despite TA's high organic acid content. This finding contrasts with earlier studies that associated tartaric acid in tamarind with effective collagen solubilization and proteolysis (Latoch *et al.*, 2023; Serdaroglu *et al.*, 2024). A plausible explanation may lie in the acidic intensity or penetration dynamics of the tamarind extract, which may require longer exposure or enzymatic synergy to achieve full tenderization. Conversely, AV and LJ demonstrated greater reductions in shear force, particularly after 24 hours, aligning with reports that citric and acetic acids disrupt muscle structure more rapidly under chilled conditions (Lewis and Purslow, 1991).

## pH

Organic-acid treatment exerted a significant effect on muscle pH ( $p = 0.020$ ), yielding an overall mean of  $5.021 \pm 0.274$ . Among the acids tested, tamarind (TA) produced the highest pH, followed by apple-vinegar (AV) and

lime-juice (LJ) marinades. Marination time was likewise influential ( $p = 0.002$ ): samples marinated for 24 h displayed higher pH values than those marinated for 12 h (Table 1). Although no significant interaction emerged between organic acid and coloring agent, the acid  $\times$  time interaction was highly significant ( $p < 0.001$ ). Specifically, TA-treated samples experienced a pronounced pH rise after 24 h, whereas the pH of LJ- and AV-treated samples remained essentially unchanged over time (Figure 1). No significant three-way interaction (acid  $\times$  coloring agent  $\times$  time) was detected for pH.

Tamarind produced the highest post-marination values, suggesting its buffering capacity or limited acid diffusion compared to AV and LJ. The significant acid  $\times$  time interaction further underscores the dynamic equilibrium between acid penetration, muscle buffering, and enzymatic activity over time. Previous work has emphasized that meat pH is not only a function of acid concentration, but also of meat's intrinsic buffering systems and proteolytic balance (Bilen *et al.*, 2024; Yu *et al.*, 2016). These factors explain why the pH changed dynamically with the use of organic acids (Tong *et al.*, 2024). The increased pH in tamarind-treated samples after 24 hours may also indicate partial protein degradation and a reduced accumulation of hydrogen ions, a phenomenon noted in comparable acidulant-based systems. Although coloring agents did not show a significant direct effect on pH, their presence may still contribute to the biochemical environment that shapes color development and pigment stability. This aligns with previous findings suggesting that such additives influence surface reactions in muscle tissue, particularly through interactions involving pigments, antioxidants, and proteins that are sensitive to pH fluctuations (Patriani *et al.*, 2024; Zawani *et al.*, 2022).

## THIOBARBITURIC ACID (TBA)

Thiobarbituric-acid-reactive substances (TBA) averaged  $0.153 \pm 0.329$  mg MDA  $\text{kg}^{-1}$  and were significantly affected by the type of organic acid ( $p = 0.017$ ) as well as by its interaction with marination time ( $p = 0.001$ ) (Table 1). TA produced the lowest TBA values, followed in ascending order by AV and LJ. Although marination time alone did not alter lipid oxidation ( $p = 0.845$ ) (Figure 1), the organic acid  $\times$  time interaction revealed that TA combined with a 12-h marination minimized TBA, whereas LJ paired with 24 h generated the highest values (Table 2). Moreover, the triple combination of TA, the turmeric-nitrite colorant, and a 12-h MT further reduced TBA ( $p = 0.002$ ).

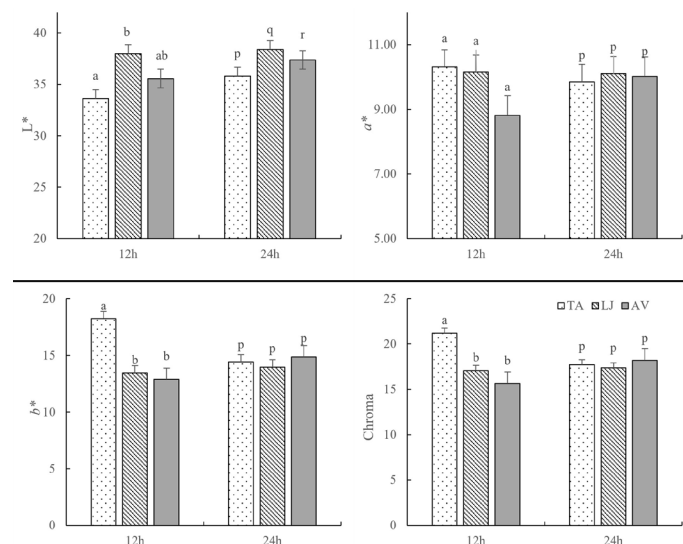
Lipid oxidation, as measured by TBA values, was lowest in tamarind-treated samples, particularly when combined with a 12-hour marination. This supports the hypothesis that tamarind's polyphenolic content contributes antioxidant capacity in addition to acidification effects

(Aminzare *et al.*, 2019). Notably, the combination of TA with turmeric-nitrite (TN) and shorter marination time yielded the lowest oxidative values, suggesting a synergistic antioxidant effect where nitrite stabilizes pigments and turmeric contributes curcumin-based free radical scavenging (Serdaroglu *et al.*, 2024; Waga *et al.*, 2017). The effectiveness of acidulants in minimizing lipid peroxidation has been shown to vary depending on the composition and origin of the vinegar used, with some types offering better oxidative control than others (Fenciglu *et al.*, 2022). Other ingredients commonly used in marinades, such as soy sauce and sugar, have also been reported to influence TBARS levels through their interactions with muscle proteins and their impact on water retention (Al-Dalali *et al.*, 2022). In this study, the high TBA values found in lime juice treatments, particularly after 24 hours, may be attributed to over-acidification and limited antioxidant content, which potentially accelerated oxidative reactions (Çelik *et al.*, 2025). Evidence from recent research indicates that the application of thermal-assisted pressure can also elevate TBARS depending on treatment severity, underscoring the importance of optimizing both composition and processing conditions in meat preparation (Silva Paz *et al.*, 2025). The significant three-way interaction observed in this study supports the notion that lipid oxidation is best controlled through the combined action of acid source, antioxidant agents, and optimal marination duration (Aydemir *et al.*, 2024). The use of hawthorn vinegar, for example, was shown to inhibit both lipid oxidation and advanced glycation end products when paired with proper exposure time, a finding consistent with the present results (Kılıç-Altun *et al.*, 2024).

### COLOR ATTRIBUTES (L\*, a\*, b\*, CHROMA)

Lightness (L\*), yellowness (b\*), and chroma were all significantly influenced by the type of organic acid and its interaction with marination time ( $p = 0.001$ ,  $p < 0.001$ , and  $p < 0.001$ , respectively; Table 1). LJ treatments yielded the highest L\* values, whereas TA produced markedly darker samples (Table 2). The combination of TA, the TN, and a 24-hr marination generated the most intense colour overall, with redness (a\*) of  $10.79 \pm 1.71$  and yellowness (b\*) of  $19.56 \pm 2.36$  (Figure 2). Although redness was not affected as a main effect ( $p = 0.333$ ; Table 1), a significant three-way interaction (acid  $\times$  colourant  $\times$  time;  $p = 0.004$ ) underscored the importance of treatment combinations, with TA + TN at 24 h producing the deepest red hue. Yellowness, by contrast, was strongly influenced by the organic acid, the colouring agent, and their interaction with marination time ( $p < 0.001$  and  $p = 0.032$ , respectively). The highest b\* values again occurred in the TA + TN, 24-h treatment (Table 2). Chroma, which reflects overall colour vividness, followed the same pattern: both the main effect of organic acid and its interaction with time were highly significant ( $p < 0.001$ ), and the TA + TN combination after

24 h produced the most saturated and vibrant colour (Table 2). When the effect of coloring agents was examined across time (Figure 3), TN showed a general increase in a, b\*, and chroma from 12 to 24 h, whereas TU and NI exhibited smaller changes over time. L\* values were relatively similar among treatments at 12 h, with minor divergence at 24 h. These patterns suggest that the influence of coloring agents on color attributes was dependent on marination duration.

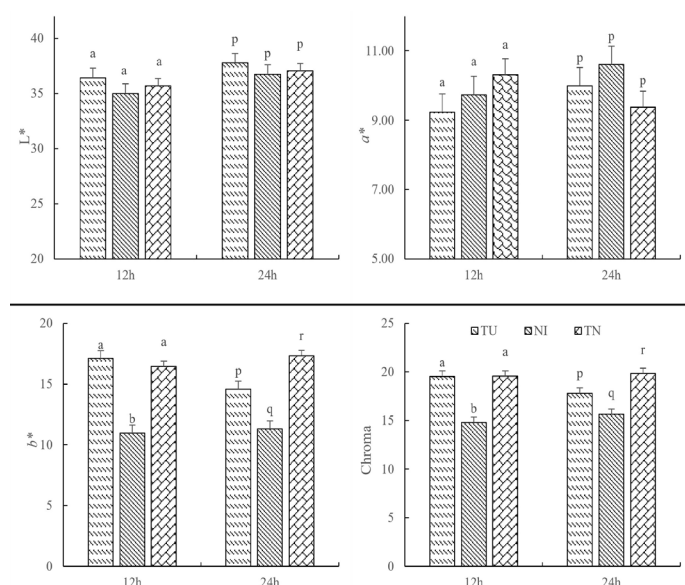


**Figure 2:** Least-squares means of color parameters showing the interaction between Acid Marination (AM: TA, LJ, AV) and Marination Time (MT: 12 h and 24 h). Different letters indicate statistically significant differences ( $p < 0.05$ ): a–b, differences among AM treatments at 12 h; p–q, differences among AM treatments at 24 h.

Color attributes were also markedly influenced by treatment combinations. Lime juice increased lightness (L\*), likely due to protein denaturation and water release under acidic conditions, which affect the refractive properties of muscle fibers (Al-Dalali *et al.*, 2022; Fenciglu *et al.*, 2022). In contrast, tamarind produced darker cuts, which may reflect either greater pigment preservation or limited structural breakdown compared to more aggressive acidulants (Çelik *et al.*, 2025; Silva-Paz *et al.*, 2025). This observation is consistent with the hypothesis that pigment stability is maintained when oxidation is suppressed and myofibrillar disruption is minimized (Serdaroglu *et al.*, 2024; Yu *et al.*, 2016).

The highest values of redness (a\*) and yellowness (b\*) were observed in samples treated with tamarind and the turmeric-nitrite combination, particularly after 24 hours of marination. This affirms the critical role of natural coloring agents, especially turmeric and nitrite, in enhancing surface pigmentation and visual appeal (Fenciglu *et al.*, 2022; Waga *et al.*, 2017). Turmeric is known to contribute intense yellow hues through its curcuminoid content, while nitrite stabilizes myoglobin-derived pigments

through the formation of nitrosomyoglobin complexes, thus intensifying red coloration in meat products (Beltrán-Cotta *et al.*, 2023; Serdaroglu *et al.*, 2024). The combination of these agents not only improves color saturation but also helps maintain color uniformity during processing and storage, supporting their synergistic role in chromatic stabilization (Aydemir *et al.*, 2024; Guo, 2024; Latoch *et al.*, 2023). Evidence from fruit- and herb-based marinades further indicates that phenolic-rich ingredients can enhance  $b^*$  values and cooperate with curing salts to sustain vivid coloration over time (Çelik *et al.*, 2025; Turp *et al.*, 2025). Although no significant three-way interactions were observed for most physicochemical parameters, the significant interactions in TBA and redness ( $a^*$ ) suggest the importance of combination strategies over single-factor optimizations. This reinforces recent calls for more integrative approaches in marination systems that combine acidulants, natural colorants, and appropriate holding times to achieve both quality enhancement and clean-label status (Aminzare *et al.*, 2019; Serdaroglu *et al.*, 2024).



**Figure 3:** Least-squares means of color attributes showing the interaction between Coloring Agent (CA: TU, NI, TN) and Marination Time (MT: 12 h and 24 h). Different letters indicate statistically significant differences ( $p < 0.05$ ): a–b, differences among CA treatments at 12 h; p–q, differences among CA treatments at 24 h.

Chroma values, which represent overall color vividness, were significantly elevated in the tamarind–turmeric–nitrite group, particularly at longer marination times, confirming that the combined use of organic acid, phenolic pigments, and curing salt can yield a highly saturated surface color (Beltrán-Cotta *et al.*, 2023; Huang *et al.*, 2025). This reflects the combined influence of acid-induced structural changes, antioxidant activity, and pigment binding, which together intensify hue saturation and minimize discoloration caused by oxidative degradation

(Aydemir *et al.*, 2024; Latoch *et al.*, 2023). These findings align with earlier studies showing that naturally derived marinades can outperform synthetic additives in color preservation, especially when formulated with bioactive-rich ingredients and applied under optimized conditions (Fencioglu *et al.*, 2022; Turp *et al.*, 2025).

Tamarind's moderate acid strength tenderised muscle while preserving a darker, stable colour, whereas the stronger denaturation induced by lime juice produced a brighter surface, indicating that formulators can tailor visual appearance by selecting acid type and marination time. When tamarind was paired with a turmeric–nitrite blend, redness ( $a^*$ ), yellowness ( $b^*$ ), and chroma were maximised, demonstrating that natural pigments and curing salts cooperate to fix vivid hues and allowing processors to lower nitrite levels by supplementing with polyphenol-rich spices. In addition, the same tamarind-based marinades yielded the lowest TBA values, confirming that the combined action of organic acids and phenolic antioxidants can curb lipid oxidation, a benefit also reported for hawthorn, unripe grape, and *Micromeria fruticosa* marinades, thereby extending shelf life without synthetic preservatives (Al-Dalali *et al.*, 2021; Kılıç Altun *et al.*, 2024; Turp *et al.*, 2025). These findings provide novel insight into the physicochemical modulation of Bali cattle beef via traditional and accessible marinade components. As natural marinades continue to attract interest in both artisanal and industrial contexts, the present study contributes to evidence-based formulation strategies that combine heritage ingredients with modern meat science principles.

## CONCLUSION

This study demonstrates that the physicochemical quality of Bali cattle beef can be deliberately directed through the judicious selection of organic acids, colouring agents, and marination time. Lime-juice marinades brightened surface colour and decreased shear force, whereas tamarind yielded darker cuts, suppressed lipid oxidation, and in combination with turmeric–nitrite generated the highest redness, yellowness, and chroma values. Apple-vinegar with nitrite and a 24 h holding period produced the lowest shear-force value, highlighting its potential for tender, ready-to-cook applications. Overall, the tamarind + turmeric–nitrite system at 12 h minimised TBA, while extending the hold to 24 h maximised visual appeal, underscoring time-dependent trade-offs between oxidative stability and colour saturation.

From an industrial standpoint, these results show that traditional Indonesian ingredients can match or surpass conventional additives in tenderness, oxidative stability, and colour retention, thereby supporting clean-label

strategies. Processors can reduce nitrite inclusion without sacrificing visual quality by pairing it with polyphenol-rich spices such as turmeric, and can tailor surface hue from bright retail cuts to premium dark roasts by switching between lime- and tamarind-based acids. Such flexible, evidence-based formulations provide a pathway to elevate the market value and consumer acceptance of Bali cattle beef while maintaining cultural authenticity.

The present work focused on pre-cooking physicochemical traits at fixed acid and spice concentrations; it did not assess microbial safety, sensory perception, or lower nitrite thresholds. Future research should therefore (i) validate consumer liking of the darker tamarind-based profiles, (ii) investigate microbial and shelf-life outcomes under commercial chilling, and (iii) explore synergistic technologies such as vacuum tumbling or ultrasound to shorten marination time while retaining quality. Addressing these aspects will further refine natural-marinade protocols and facilitate scale-up for both artisanal and industrial meat sectors.

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## NOVELTY STATEMENT

This study provides new empirical evidence on how combinations of organic acids, coloring agents, and marination time modify the physicochemical and color properties of Bali cattle beef, offering a scientific basis for natural, clean-label marination strategies.

## AUTHOR'S CONTRIBUTION

Conceptualization: HH. Data curation: NN, AII, KK. Formal analysis: H, AII. Methodology: NN, AMA. Software: AMA, MR. Validation: LAM. Investigation: MLR, H. Writing original draft: HH, AMA, LAM. Writing review and editing: HH, H.

## ETHICS APPROVAL

This study did not involve procedures requiring ethical approval.

## GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

Generative AI and AI-assisted tools were used in the preparation of this manuscript. Paperpal was employed solely for language editing and improving clarity of ex-

pression. Dimension.AI and SciScape were used to support the identification of relevant and up-to-date literature. All study design, data collection, statistical analysis, interpretation of results, and scientific conclusions were conducted independently by the authors.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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