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Effects of various alternative stunning techniques on welfare indicators and meat quality of Nile tilapia

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Introduction: Humane slaughter practices are increasingly recognized as essential for improving fish welfare and maintaining product quality in aquaculture systems. In tilapia production, inappropriate stunning and killing methods can induce severe stress responses, leading to compromised welfare, accelerated rigor mortis, and reduced fillet quality. Despite the availability of multiple stunning techniques, comparative evidence linking welfare indicators to postmortem biochemical changes and fillet quality remains limited. This study aimed to evaluate and compare the effects of six commonly used stunning methods on welfare outcomes and flesh quality in Nile tilapia.

Materials and methods: A total of 54 Nile tilapia (*Oreochromis niloticus*) were randomly allocated to six slaughter treatments (n = 9 per group): priest stunning, bolt pistol, ikijime, live freezing, anesthetic overdose, and asphyxiation. Fish welfare was assessed immediately post-stunning using behavioral response (BR) scores and morphological damage (MD) indices. Postmortem physiological and biochemical parameters—including muscle pH, lactate concentration, ATP levels, and rigor mortis index—were analyzed over storage time. Fillet quality was evaluated based on texture, color, proximate composition, and water drip loss.

Results: Anesthetic overdose and bolt pistol stunning produced the most favorable welfare and quality outcomes. Both methods resulted in immediate loss of consciousness (BR = 0), minimal morphological damage, and significantly lower muscle lactate concentrations (3.0 ± 0.5 mmol/L) compared with other treatments. Asphyxiation demonstrated the poorest welfare performance, characterized by prolonged behavioral responses (BR = 1), elevated stress biomarkers, rapid rigor mortis onset, and inferior fillet texture, color stability, and storage potential. Live freezing and priest stunning showed relatively acceptable behavioral welfare indicators; however, these methods did not confer advantages in stress reduction or fillet quality parameters. The effectiveness of ikijime varied considerably, largely depending on operator skill and consistency.

Discussion: The findings demonstrate a strong association between rapid induction of unconsciousness, reduced physiological stress, and improved fillet quality in tilapia. Anesthetic overdose and bolt pistol stunning consistently outperformed other methods across welfare, biochemical, and quality metrics, highlighting their suitability as humane slaughter techniques. In contrast,

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asphyxiation remains unacceptable from both welfare and product quality perspectives. Although some traditional methods may appear behaviorally acceptable, their limited benefits on physiological stress and flesh quality reduce their practical value. Overall, these results support the adoption of anesthetic overdose or bolt pistol stunning in commercial tilapia production. Pilot implementation with industry stakeholders is recommended to facilitate ethical compliance, improve product value, and enhance consumer confidence.

KEYWORDS

aquaculture, fillet quality, stunning techniques, tilapia, welfare indicators

1 Introduction

Animal welfare has emerged as an important component of sustainable aquaculture, with increasing global recognition of its impact on both product quality and consumer confidence (World Organisation for Animal Health, 2024). As awareness surrounding the physiological and behavioral needs of fish expands, welfare-based management practices are being increasingly adopted across various aquaculture systems. These practices are designed not only to minimize stress but also to optimize growth performance and meat quality. Comprehensive welfare assessments have already been established in several high-value species, such as Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), and pikeperch (*Sander lucioperca*) (Stien et al., 2013; Noble et al., 2018; Tschirren et al., 2021). In Nile tilapia (*Oreochromis niloticus*), a species widely farmed in tropical regions, welfare evaluations have been integrated in all stages of production—from broodstock handling through hatchery and grow-out phases, transportation, and slaughter (Pedrazzani et al., 2020; Pedrazzani et al., 2023). As the final step in the production cycle, slaughter-stage welfare assessments are integral to ensure that prior welfare gains are not undermined at harvest and offer practical guidance for selecting stunning methods that align with both animal ethics and product integrity.

Among the stages of the tilapia production cycle, pre-slaughter handling and stunning procedures represent critical points where fish are prone to cumulative physical and psychological stressors. Such stressors can compromise animal welfare and disrupt physiological homeostasis, including the release of stress hormones, impairment of osmoregulation, and a shift to anaerobic metabolism, which collectively alter muscle biochemistry and reduced meat quality (Rucinque et al., 2023; Mercogliano et al., 2024). For example, high pre-slaughter stocking densities combined with asphyxiation exacerbate respiratory distress in Nile tilapia, which may lead to muscle degradation and increased cooking weight loss (Prestes Dos Santos et al., 2024). Similarly, ineffective stunning accelerates the onset of rigor mortis, thereby shortening shelf life due to increased bacterial growth, lipid oxidation, and enzymatic degradation (Poli et al., 2005; Filho et al., 2014). Demand for live fish in domestic markets in producing countries can exacerbate poor welfare outcomes associated with slaughter of tilapias. Current slaughter practices in both traditional wet markets and supermarkets include asphyxiation, ice slurry immersion, or percussive stunning—are frequently implemented without standardized protocols or adequate training. These methods have been criticized for causing unnecessary suffering and compromising fillet quality in other piscine species (Morzel et al., 2003; Van De Vis et al., 2003). As a result, interest in humane and efficient stunning techniques that induce immediate and irreversible unconsciousness, preserve product quality and ensure the economic sustainability of aquaculture production are required.

Currently, a range of alternative stunning techniques has been explored to improve tilapia welfare during slaughter. Percussive stunning using a pneumatic bolt pistol, for example, delivers a high-pressure impact to the cranial region, effectively induce rapid and irreversible unconsciousness (Brijs et al., 2024; Sundell et al., 2024). Another method, ikijime is a traditional Japanese technique involving the insertion of a spike into the brain and brainstem, causing rapid insensibility prior to exsanguination (Hjelmstedt et al., 2024; Wang et al., 2024). This technique is particularly valued for its ability to preserve freshness and fillet quality for high value species destined for luxury culinary markets (Poli et al., 2005). In addition, the use of anesthetic overdose—such as clove oil had been advocated as a humane method that minimizes stress responses and ensures loss of consciousness prior to slaughter (López-Cánovas et al., 2020; Terto et al., 2024; Espinoza-Ramos et al., 2025). Despite their promise, these alternative stunning methods have not been systematically compared to conventional practices, a step that is essential to generate evidence-based recommendations for humane slaughter. Accordingly, this study therefore, aims to compare three stunning techniques; bolt pistol stunning, ikijime, and anesthetic overdose with conventional practices commonly used in local fish wet markets, including priest stunning, asphyxiation and live freezing. Evaluating their impacts on key indicators of fish welfare and fillet quality will support the refinement of stunning protocols and guide future welfare standards.

2 Materials and methods

2.1 Fish source and holding conditions

Fifty-four Nile tilapia (*Oreochromis niloticus*) with a mean body weight of 768 ± 162 g and a total length of 32.29 ± 2.30 cm were obtained from a commercial farm in Chachoengsao, Thailand. Fish were harvested from the earthen pond using a seine net in the early morning, and the operation was completed within approximately 10 min. Then, they were transported in oxygenated water and arrived at the animal facility at the Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand within 2 h to minimize transportation-related stress. Upon arrival, fish were housed in 150-L aerated glass tanks with controlled water conditions: temperature 25.10 ± 0.38 °C; pH 8.16 ± 0.07; dissolved oxygen 6.46 ± 0.41 mg/L, non-ionized ammonia 0.03 ± 0.01 mg/L; and nitrite 0.13 ± 0.06 mg/L. Two fish were maintained per tank, separated by an opaque divider. All fish were acclimated for 24 h prior to the experiment.

2.2 Fish stunning and slaughtering procedures

All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Kasetsart University under the protocol number ACKU67-VET-094. On the day of the experiment, each fish were carefully removed from the tanks using a knotless fish net and subjected to one of six stunning methods: priest stunning, bolt pistol, ikijime, live freezing, anesthetic overdose, or asphyxiation (Figure 1). The procedures were performed by the same operator who had prior hands-on experience with percussive stunning and *ikijime* through routine work on a commercial tilapia farm, which helped ensure procedural consistency and reduce the risk of operational errors. Each stunning methods were performed as follows:

1. Percussive stunning (wooden priest): Fish were placed on a wet, flat surface, and struck three times with a wooden priest, approximately 1–2 cm posterior to the eyes (Figure 1A). Additional strikes were applied if the fish were still conscious.
2. Percussive stunning (bolt pistol): A Zephyr F captive bolt pistol (Bock industries, Philipsburg, PA, USA) equipped with a 24 mm plastic stunning cylinder was used at a pressure of 800 kPa. Fish were held vertically, and the bolt was discharged at the cranium region between the eyes (Sundell et al., 2024) (Figure 1B).
3. Ikijime: Fish were positioned laterally and immobilized at the midsection while a stainless steel ikijime spike was inserted at the intersection of the lateral line and dorsolateral edge of the operculum (Wang et al., 2024) (Figure 1C). The spike was manipulated for approximately 3 sec. An additional insertion was made if reflexes persisted (Figure 1C).
4. Live Freezing: Fish were placed in sealed plastic bags and transferred to a −20 °C freezer for 1 h (Liu et al., 2023) (Figure 1D).

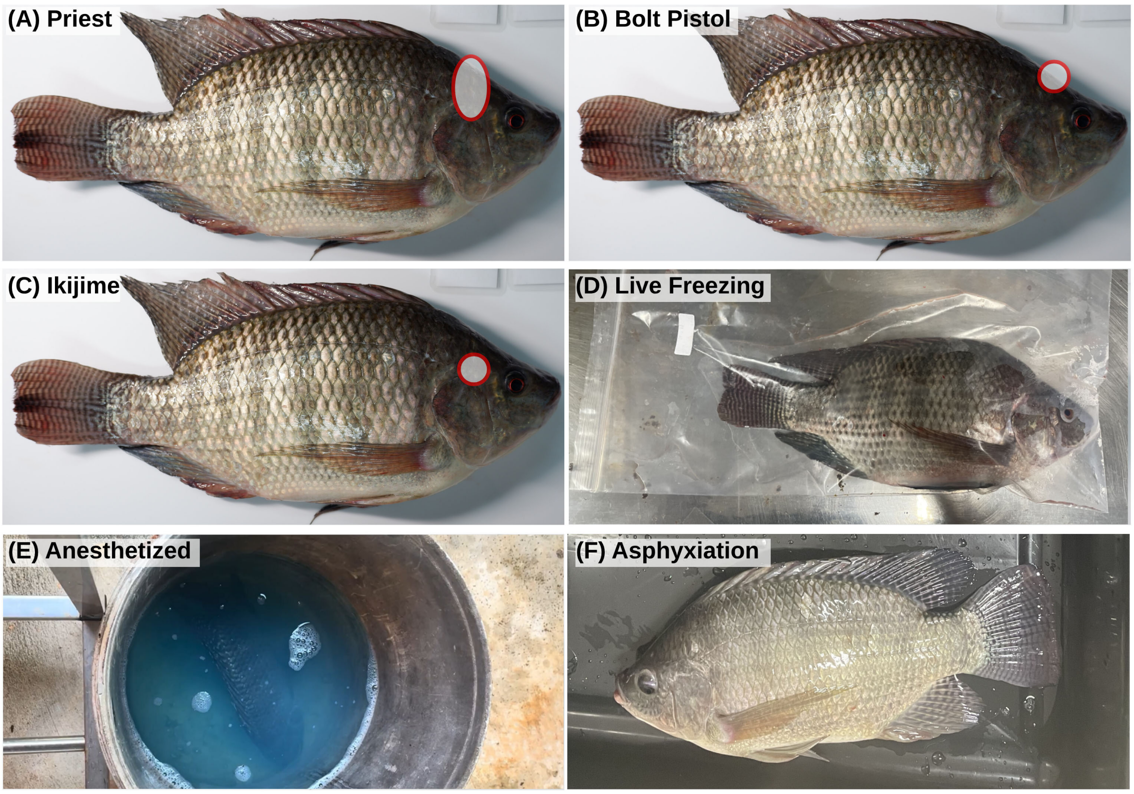

FIGURE 1
Application of six stunning methods in Nile tilapia, **(A)** Percussive stunning using a wooden priest, delivered at 1–2 cm posterior to the eyes **(B)** Bolt pistol stunning at the cranium, targeted between the eyes **(C)** Ikijime via spike insertion at the intersection of the lateral line and the dorsal edge of the operculum **(D)** Fish packed in plastic bags for live freezing at −20 °C **(E)** Immersion in 400 ppm eugenol solution for anesthetic overdose and **(F)** Fish placed on a dry tray for air asphyxiation.

TABLE 1 **Behavioral response (BR) indicators used to assess consciousness in tilapia following stunning [adapted from Morzel and van de Vis, 2003 (Morzel et al., 2003)].**

Indicators	Score	Observations
Flopping/Attempt to escape	0	No response
	1	Weak movements
	2	Strong flopping/attempt to escape
Breathing and opercular movements	0	No opercular movement
	1	Irregular or slow opercular movement
	2	Normal opercular movement
Eye movement	0	No response, no eye roll
	1	Slow movement of the eye
	2	Normal eye movement
Response to tail pinching	0	No response
	1	Sluggish escape attempt
	2	Clear escape attempt

5. Anesthetic overdose: Fish were immersed in a 400-ppm eugenol solution (Aquanes®, Better Pharma, Bangkok, Thailand) prepared in 10 L of water for 5 min (Terto et al., 2024) (Figure 1E).
6. Asphyxiation: Fish were placed on a dry plastic tray (dimensions: 9.80 × 50.5 × 38.7 cm^3) at 25 °C for 3 h and gently secured in position using a fitted foam holder (Figure 1F). Reflexes returned upon re-immersion in water; therefore, fish were euthanized with a 400-ppm eugenol overdose for 5 min.

2.3 Behavior and morphological analyses

Fish welfare was evaluated immediately following stunning based on behavioral responses and morphological damage. Repeated reflex checks were conducted within 20 seconds, and additional stunning was applied if any sign of consciousness remained. Behavioral indicators induced flopping or escape attempts, opercular and eye movements, and response to tail pinching, adapted from the assessment guideline in turbot (Morzel et al., 2003). Each parameter was scored on a three-point scale (0–2) as described in Table 1. Morphological damage was assessed externally including skin, scales, eyes, jaw, operculum, fins, gills, and spine, using a three-point scale (Table 2) adopted from Pedrazzini et al., 2023 (Pedrazzani et al., 2023). The relative score indicators were calculated for behavioral response (BR) and morphological damage (MD) using the following formula (Lertwanakarn et al., 2023; Pedrazzani et al., 2023):

$$\text{Relative Score Index} = \frac{(1 \times n_1) + (2 \times n_2)}{(n_0 + n_1 + n_2) \times 2}$$

where n_0, n_1, and n_2 represent the number of fish assigned scores 0, 1, and 2, respectively.

TABLE 2 **Morphological damage (MD) indicators for assessing physical damage in tilapia post-slaughter [adapted from Pedrazzini et al., 2023 (Pedrazzani et al., 2023)].**

Indicators	Score	Observation
Skin and Scales	0	No visible damage; intact scales
	1	Minor scratches; scale loss affecting 10% of the body; small area of hemorrhage; pinhole from ikijime
	2	Severe wounds (>10% of body affected); deep lacerations; extensive hemorrhage; torn skin; or skull fractures
Eyes	0	No visible damage; clear and intact cornea
	1	Minor hemorrhage; slight eye indentation; superficial scratches without affecting the lens
	2	Severe hemorrhage; ruptured eye; deformation of the eyeball
Jaw	0	No damage; intact jaw structure
	1	Mild jaw displacement; minor abrasions; slight misalignment
	2	Severe jaw deformation; fractures; jaw misalignment affecting mouth closure
Operculum	0	No damage; intact gill cover
	1	Mild operculum displacement; minor hemorrhage; small tears
	2	Severe operculum damage; tears; complete detachment exposing gills
Fins	0	No fin damage; intact structure
	1	Minor fin splits or small tears affecting<10% of the fin area
	2	Severe fin damage; large tears (>10% of fin area); fin base injuries; fin loss
Gills	0	No visible damage; normal coloration
	1	Mild injury, including slight splitting of gill filaments, small areas of hemorrhage, or pale gill coloration
	2	Severe gill damage; extensive hemorrhage; detachment of filaments; visible tissue loss
Spine	0	No damage; normal body posture
	1	Mild spinal deviation; slight bending; partial loss of rigidity
	2	Severe spinal damage; complete deformity; loss of structural integrity affecting body posture

2.4 Fillet quality assessment

Following behavioral and morphological evaluations, 36 fish were allocated for fillet quality analysis, while an additional 18 fish were used for rigor index assessments (Supplementary Figure 1). Both left and right fillets were excised and assigned to specific quality parameters (Supplementary Figure 2). The anterior dorsal portion of the left fillet was immediately processed for biochemical analysis of muscle pH, lactic acid, and ATP concentrations (Zampacavallo et al., 2014; Secci et al., 2018). The remaining tissue from the left fillet was stored at 4 °C for proximate composition analysis. The right fillet was divided into two sections: the dorsal portion was used for texture profile analysis (TPA) as described by (Filho et al., 2014), while the ventral portion was placed on stainless steel trays ($36 \times 28.5 \times 6.5$ cm^3), covered with plastic wrap, and stored at 4 °C until further analysis for evaluation of water drip loss and muscle color.

2.4.1 Biochemical analysis

Muscle pH, lactic acid concentration, and ATP levels were analyzed in the anterior dorsal muscle of the left fillet at 0, 3, 6, and 24 h post-slaughter (hps) with slight modification from Ismail et al., 2023 (Ismail et al., 2023). Briefly, muscle samples (7 g) were homogenized in 18 mL of distilled water using a VELP OV5 homogenizer (VELP, Usmate Velate, Italy) for 30 sec. The homogenate was centrifuged at 461 ×*g* for 2 min at 4 °C (Eppendorf Centrifuge 5430R, Enfield, CT, USA), and the supernatant was collected. Muscle pH was measured using a SevenCompact S220 pH Benchtop Meter (Mettler Toledo, Zürich, Switzerland). Lactic acid concentration was determined using an automated lactate meter (Eaglenos, Nanjing, China), with 10 µL of the supernatant applied to a test strip.

ATP levels were quantified using the CellTiter-Glo® Luminescent cell viability assay (Promega, Madison, WI, USA). A 1 g of muscle samples was homogenized in 500 µL of 0.3 M perchloric acid using a PowerMasher II homogenizer (Nippi, Tokyo, Japan), followed by the addition of an another 500 µL of 0.3 M perchloric acid as previously described (Saraiva et al., 2024). The mixture was vortexed for 20 sec using a Vortex-Genie 2 (Scientific Industries, Bohemia, NY, USA), centrifuged at 461 ×*g* for 1 min at 4 °C, and the supernatant was stored at −20 °C until analysis. ATP concentrations were measured using a Synergy H1 microplate reader (Biotek, Charlotte, VT, USA) at 37 °C for 5 sec, and luminescence data were analyzed using Gen5 software (Biotek, Charlotte, VT, USA). The ATP concentration was extrapolated from a standard curve (1–100 µM, Sigma, St. Louis, MO, USA).

2.4.2 Physical analysis

The ventral portion of the right fillet was gently blotted to remove excess moisture, maintained in 4 °C fridge and weighed to assess water drip loss (DL) and muscle color at 0, 1, 3 and 7 days post-slaughter (dps), following (Secci et al., 2018; Ismail et al., 2023). DL was calculated as the percentage of weight loss relative to the initial weight. Muscle color was evaluated using a WR10–8 colorimeter (Shenzhen Wave Optoelectronics Technology, Shenzhen, China) at the posterior end of the visceral surface (Morzel et al., 2003), and expressed as CIELab color space values: lightness (L*), redness (a*), and yellowness (b*). Triplicate measurements were recorded at each time point and location.

Texture profile analysis (TPA) was conducted on the dorsal region of the right fillet at 1, 3, and 7 dps, as described by (Filho et al., 2014; Ismail et al., 2023). Bone-free muscle samples at the size of $1 \times 1 \times 1$ cm^3 were analyzed using a TA-XT plus texture analyzer (Stable Micro Systems, Godalming, UK) equipped with a 50-N load cell and a cylindrical probe (P/50, diameter: 50 mm). The testing parameters included a pre-test speed of 1 mm s^{-1}, test speed of 2 mm s^{-1}, post-test speed of 2 mm s^{-1}, and a compression strain of 60%. Each sample was tested in six replicates to determine hardness (N), adhesiveness (N·s), springiness (cm), cohesiveness, gumminess (N) and chewiness (N·cm), with average values recorded.

2.4.3 Proximate composition analysis

Proximate composition was determined on left fillets at 1 dps using (Areekijseree et al., 2006) standard methods. Each analysis was performed in six replicates, with results expressed as percentages of fresh matter (%FM) for moisture, crude protein, crude fat, and ash.

2.5 Rigor index measurement

Eighteen fish ($n = 3$ per treatment) were assessed for rigor mortis progression following the method of (Diouf and Rioux, 1999; Filho et al., 2014; Islami et al., 2014). After slaughter, fish were placed on a horizontal surface with the caudal fin extending over the edge of a flat surface. Rigor index was recorded at 0, 3, 6, 24, and 48 hps, and calculated using the formula: $[(D_0 - D)/D] \times 100$; where D_0 is the initial vertical distance from the caudal fin to the surface, and D is the distance measured at each time point.

2.6 Statistical analysis

Descriptive statistics were used to summarize fish welfare indicators (BR and MD scores). Inferential statistical analyses and graphical representations were performed using Prism 9.0 (GraphPad Software, San Diego, CA, USA). Differences in muscle quality parameters across stunning methods and time points were analyzed using repeated measures two-way ANOVA, followed by Bonferroni's *post hoc* test for multiple comparisons. Statistical significance was set at $p < 0.05$.

3 Results

3.1 Welfare assessment of tilapia subjected to different stunning methods

Welfare outcomes following all stunning methods were evaluated based on behavioral response (BR) and morphological damage (MD), as summarized in Tables 3, 4. Among all methods, bolt pistol and anesthetic overdose demonstrated the most effective stunning techniques, with all fish exhibiting complete insensibility (BR = 0), and significantly better than asphyxiation ($p < 0.05$). Live freezing also showed high efficacy (BR = 0.028), with only one out of nine fish (11.1%) showing mild flopping and a weak response to tail pinching (score 1). In contrast, percussive stunning with a wooden priest was less effective (BR = 0.056), as two fish (22.2%) demonstrated mild flopping (score 1) and one fish (11.1%) retained slow opercular movement accompanied by a weak tail pinch response (score 1). Similarly, ikijime demonstrated limited

TABLE 3 Proportion of tilapia showing different levels of behavioral responses following six stunning by methods (n = 9).

Stunning methods	Score*	Behavioral responses (%)				Behavior response score (BR)
		Flopping	Opercular movements	Eye movement	Tail pinch response	
Priest	0	77.8	88.9	100	88.9	0.056^{ab}
	1	22.2	11.1	–	11.1	
	2	–	–	–	–	
Bolt pistol	0	100	100	100	100	0.000^{a}
	1	–	–	–	–	
	2	–	–	–	–	
Ikijime	0	44.4	77.8	66.7	44.4	0.222^{ab}
	1	55.6	22.2	22.2	55.6	
	2	–	–	11.1	–	
Live freezing	0	88.9	100	100	88.9	0.028^{a}
	1	11.1	–	–	11.1	
	2	–	–	–	–	
Anesthetic overdose	0	100	100	100	100	0.000^{a}
	1	–	–	–	–	
	2	–	–	–	–	
Asphyxiation	0	–	–	–	–	1.000^{b}
	1	–	–	–	–	
	2	100	100	100	100	

*Scoring system: 0 = No response, 1 = Weak response, and 2 = Normal response.
Different letters indicate statistically significant differences among groups, as determined by the Kruskal–Wallis test for ordinal data.

efficacy (BR = 0.222), with five fish (55.6%) responding to tail pinching and showing weak flopping (score 1), two fish (22.2%) had slow opercular and eye movements (score 1), and one fish (11.1%) maintaining normal eye movement (score 2). Asphyxiation was the least effective method (BR = 1.0), with all fish (100%) remaining fully conscious after 3 hours of the procedure.

The extent of morphological damage also varied across stunning methods (Table 4; Figure 2). Anesthetic overdose resulted in the least damage (MD = 0.024), with only three fish (33.3%) showing minor eye hemorrhages (score 1). Fish subjected to live freezing showed a good MD score at 0.111, with six out of nine fish (66.7%) showing minor eye indentation (score 1). Specifically, three fish (33.3%) developed slight ice formation on the gills and minor fin splits in three fish (33.3%), and mild bruising on the body or operculum in one fish (11.1%). Asphyxiation produced generalized tissue damage (MD = 0.175), including petechiae on skin and fins (77.8%), minor eye hemorrhages (66.7%), opercular injuries (11.1%), and pale gills (11.1%). In contrast, ikijime caused localized but pronounced injuries at the spiking site (MD = 0.230), with skin tears observed in seven fish (77.8%) and puncture wounds in two fish (22.2%). Additional lesions included minor eye hemorrhages (88.9%), opercular injuries (33.3%), and torn opercular (11.1%). Bolt pistol stunning results in moderate head damage (MD = 0.159), with scale loss in seven fish (77.8%), and skull fractures in two fish (22.2%). Minor eye hemorrhages were also observed in eight fish (88.9%), and one fish (11.1%) showed a small opercular injury. Nevertheless, percussive stunning with a wooden priest caused the most extensive physical damage (MD = 0.333), and significantly higher than overdose anesthesia and live freezing ($p < 0.05$). All fish in this group (100%) had eye indentation (score 1), while most displayed scale loss (88.9%) and detachment of gill filaments (score 2). Additional injuries included fin tears or hemorrhages in four fish (44.4%), opercular bruising in three fish (33.3%), and a torn operculum in one fish (11.1%).

3.2 Pre-slaughtering stress profiles of the stunned fish

3.2.1 Biochemical indicators in fillet

Biochemical parameters, including muscle pH, lactic acid concentration, and adenosine triphosphate (ATP) levels showed similar overall trends across all stunning methods (Figure 3). Initially, fillet pH values did not differ significantly among groups ($p > 0.05$) and showed a consistent decline over the 24-h period (Figure 3A). By 3 hours post-slaughter (hps); however, exhibited the lowest pH (6.66 ± 0.11), significantly lower than priest stunned

TABLE 4 Proportion of tilapia showing different levels of morphological damage following six stunning methods (n = 9).

Stunning methods	Score*	Morphological damages (%)							Morphological damage score (MD)
		Skin/Scale	Eyes	Jaw	Operculum	Fins	Gills	Spine	
Priest	0	11.1	–	100 – –	55.6	55.6	11.1	100	0.333^{a}
	1	88.9	100	–	33.3	44.4	–	–	
	2	–	–	–	11.1	–	88.9	–	
Bolt pistol	0	–	11.1	100	88.9	100	100	100	0.159^{ab}
	1	77.8	88.9	–	11.1	–	–	–	
	2	22.2	–	–	–	–	–	–	
Ikijime	0	–	11.1	100	55.6	100	100	100	0.230^{ab}
	1	22.2	88.9	–	33.3	–	–	–	
	2	77.8	–	–	11.1	–	–	–	
Live freezing	0	88.9	33.3	100	88.9	66.7	66.7	100	0.111^{b}
	1	11.1	66.7	–	11.1	33.3	33.3	–	
	2	–	–	–	–	–	–	–	
Anesthetic overdose	0	100	66.7	100	100	100	100	100	0.024^{b}
	1	–	33.3	–	–	–	–	–	
	2	–	–	–	–	–	–	–	
Asphyxiation	0	22.2	33.3	100	88.9	22.2	88.9	100	0.175^{ab}
	1	77.8	66.7	–	11.1	77.8	11.1	–	
	2	–	–	–	–	–	–	–	

*Scoring system: 0 = No damage, 1 = Scratch or Mild injury, 2 = Severe damage.
Different letters indicate statistically significant differences among groups, as determined by the Kruskal–Wallis test for ordinal data.

(6.88 ± 0.08, $p = 0.0014$), anesthetic overdose (6.86 ± 0.08, $p = 0.0047$) and live freezing (6.83 ± 0.07, $p = 0.0191$). This pattern persisted at 6 hps, with asphyxiated fish maintaining the lowest pH (6.56 ± 0.11), which was significantly below that of the priest stunned fish (6.73 ± 0.13, $p = 0.0406$). By 24 hps, pH values converged across all groups.

Lactic acid accumulation was inversely correlated with muscle pH ($p < 0.0001$; $r^2 = 0.82$), increasing progressively over 24 hps (Figure 3B). At 0 hps, asphyxiated fish had the highest lactate concentration (12.32 ± 3.32 mmol L^{-1}), which was significantly higher than live-frozen fish (7.68 ± 2.54 mmol L^{-1}, $p < 0.05$). This trend continued at 3 hps, with lactic acid levels in asphyxiated fish (14.87 ± 2.71 mmol L^{-1}), significantly higher than those in live-freezing (10.95 ± 1.35 mmol L^{-1}, $p < 0.05$) and anesthetic-overdosed fish (10.57 ± 2.04 mmol L^{-1}, $p < 0.05$). By 6 hps, ikijime-stunned fish showed the highest lactic acid levels (18.28 ± 2.42 mmol L^{-1}), which was significantly higher than anesthetic-overdosed fish (13.95 ± 1.90 mmol L^{-1}, $p = 0.0249$). At 24 hps, lactic acid concentration were similar across all groups, paralleling the pH trend.

ATP concentration showed a positive correlation with pH ($p < 0.0001$; $r^2 = 0.3911$), remaining stable up to 3 hps and declining markedly by 24 hps in all groups (Figure 3C). ATP differences among treatments were generally not significant ($p > 0.05$), except for 24 hps, where priest stunned fish retained the highest ATP levels (10.29 ± 6.34 nmol g^{-1}), which is significantly higher than live-frozen fish (3.21 ± 3.15 nmol g^{-1}, $p = 0.0187$).

3.2.2 Rigor index in fish subjected to six stunning methods

Rigor index patterns were broadly similar across all stunning methods, showing rapid increase after slaughter, peaking within 6 hps, and then gradually declining until 360 hps (Figure 4). Notably, live-frozen fish showed higher rigor indices at 3 dps (40.72% ± 1.32%) and 6 dps (66.17% ± 16.71%) compared to other groups. Likewise, asphyxiated fish had elevated rigor indices at 6 dps (76.95% ± 17.64%). By 24 hps, all groups had reached full rigor (98%–100%), with a significant increase in rigor index observed across all stunning methods ($p < 0.0001$).

After being fully rigid, the onset of rigor resolution in fillets was mildly different among stunning methods. Asphyxiated fish were the first to enter rigor resolution, beginning at 96 hps (97.78% ± 1.50%), with values declining steadily thereafter and remaining the lowest throughout the study. By 168 hps, rigor resolution was observed in fish subjected to live freezing (96.73% ± 1.04%), priest stunning (97.65% ± 2.28%), and anesthetic overdose (97.86% ± 2.15%). Ikijime-stunned fish entered rigor resolution later, at 216 hps (96.75% ± 1.79%), while bolt pistol stunning produced the slowest onset, beginning at 264 hps (92.91% ± 3.49%). By the end of the experiment, rigor resolution had occurred across all groups. Fish subjected to asphyxiation showed the lowest final rigor index (54.11% ± 6.55%), which was significantly lower than those stunned by priest stunning (78.51%

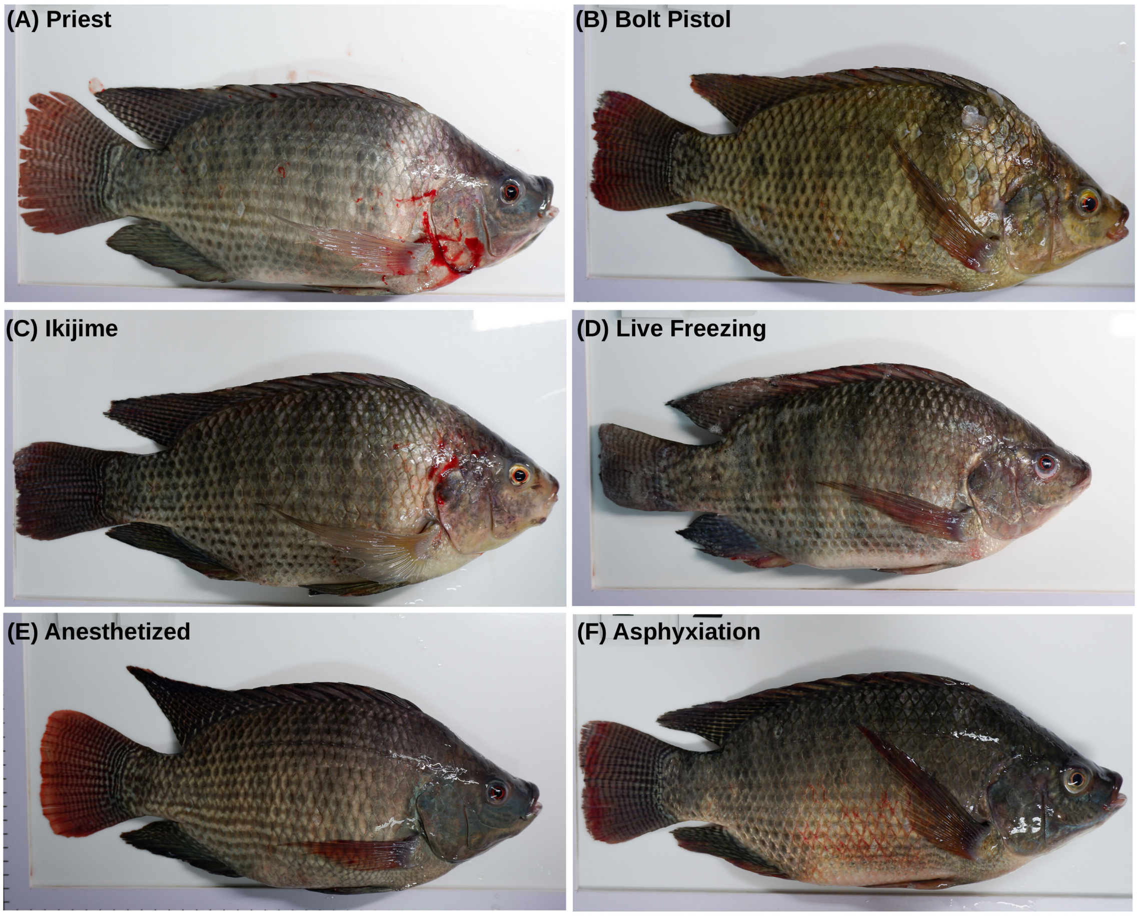

FIGURE 2
External appearances of Nile tilapia subjected to six stunning methods: **(A)** Percussive stunning with a wooden priest **(B)** Percussive stunning with a bolt pistol **(C)** Ikijime, **(D)** Live freezing **(E)** Anesthetized overdose **(F)** Asphyxiation.

± 5.20%; *p* = 0.0071), bolt pistol (73.75% ± 6.37%; *p* = 0.0312), and anesthetic overdose (72.40% ± 10.74%; *p* = 0.0476).

3.3 Assessment of product quality across stunning methods

3.3.1 Texture profile analysis

Fillet texture was evaluated at 1, 3, and 7 dps based on hardness, cohesiveness, springiness, adhesiveness, gumminess, and chewiness parameters (Table 5). At 1 dps, hardness values were comparable among groups, ranging from 23.07 to 27.63 N. By 3 dps, muscle hardness reduced in all groups, although no significant differences were detected. At 7 dps, fillets from asphyxiated fish exhibited the lowest hardness (15.24 ± 3.58 N), which was significantly lower than those from ikijime (23.14 ± 3.59 N, *p* = 0.0083), and priest-stunned fish (22.20 ± 6.46 N, *p* = 0.0244).

While there were no significant differences of cohesiveness, springiness and adhesiveness values across all groups at any time points, gumminess and chewiness followed patterns similar to the hardness. At 1 and 3 dps, both parameters were comparable across groups, with gumminess ranging from 3.01 ± 0.56 N to 6.28 ± 1.27 N, and chewiness from 1.12 ± 0.26 N·cm to 2.36 ± 1.00 N·cm, respectively. By 7 dps, fillets from asphyxiated fish had the lowest gumminess (3.18 ± 0.69 N), significantly lower than those from ikijime fish (6.26 ± 1.00 N, *p* = 0.0022). Similarly, chewiness was lowest in fillets from asphyxiated fish (1.33 ± 0.68 N·cm), which was significantly below that of the ikijime group (2.60 ± 0.77 N·cm, *p* = 0.0197).

3.3.2 Muscle color

Fillet color was determined at 0, 1, 3, and 7 dps using lightness (L*), redness (a*), and yellowness (b*) (Table 6). At 0 dps, no significant differences in lightness were observed among stunning methods. Fillets from live-frozen fish showed the highest redness (5.13 ± 0.63), whereas those from priest-stunned fish exhibited the highest yellowness (−6.53 ± 3.24). Beyond this initial variation, no significant differences in color parameters were detected among groups at 1, 3, or 7 dps. Supplementary Figure 2 illustrates temporal changes in CIELAB color values across stunning methods.

3.3.3 Water drip loss

Water drip loss (DL) in fillets followed a similar trend across all stunning methods, increasing progressively during storage (Figure 5). At 1 dps, DL values ranged from 1.89%–2.76% in all groups, rising slightly by 3 dps to 4.15%–4.77%. By 7 dps, a

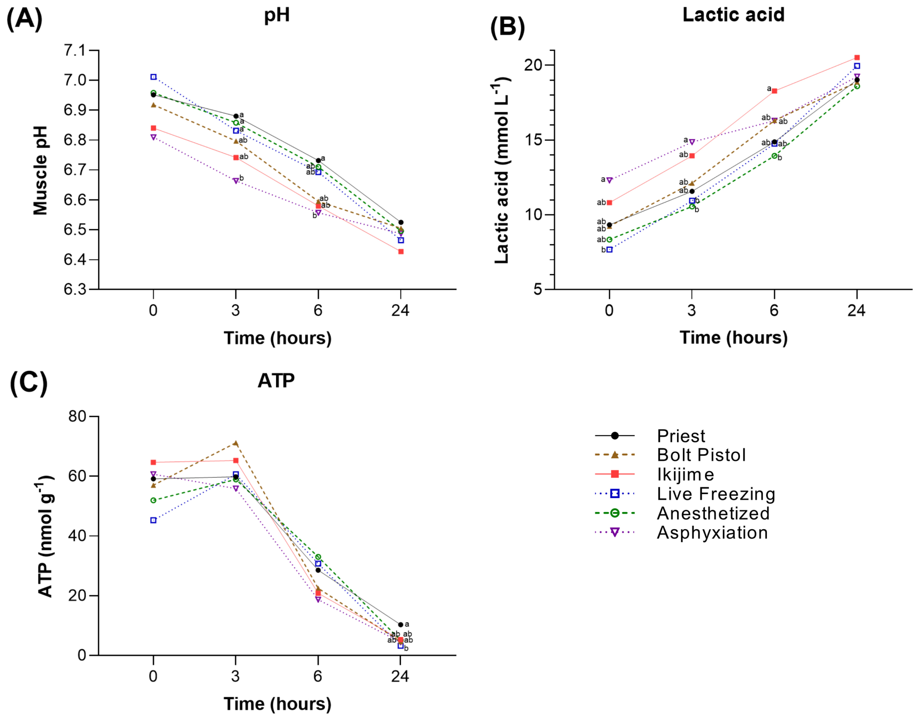

FIGURE 3
Biochemical profiles of Nile tilapia fillets (n = 6) subjected to six different stunning methods, measured at 0, 3, 6 and 24 hours post-slaughter: percussive stunning with a wooden priest, ikijime, bolt pistol stunning, anesthetic overdose, live freezing, and asphyxiation. **(A)** Muscle pH, **(B)** Lactic acid concentration **(C)** Adenosine triphosphate (ATP) levels. Different letters indicate significant differences among stunning methods ($p < 0.05$).

significant increase in DL was observed in all groups except the anesthetic-overdose treaetment ($p < 0.05$). Nevertheless, no significant differences in DL were detected among stunning methods at any storage time ($p > 0.05$).

3.3.4 Proximate analysis

The proximate composition of tilapia fillets did not differ significantly among the six stunning methods (Supplementary Table 1). Overall, fillets contained 75.25%–75.82% moisture and 24.18%–24.75% dry matter. Macronutrient composition was consistent across groups, with crude protein ranging from 19.38%–19.76%, crude fat from 3.53%–3.97%, and ash from 1.05%–1.09% on a fresh matter basis.

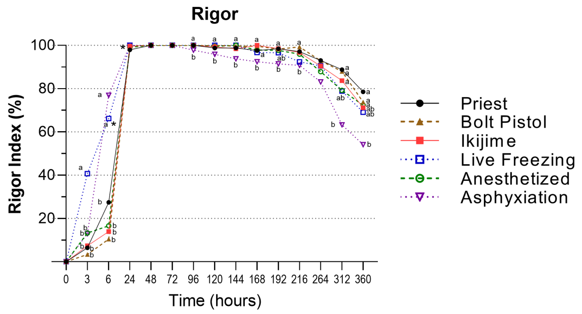

FIGURE 4
Rigor mortis index (%) of Nile tilapia (n = 3) subjected to six stunning methods over a 15-day period. Different letters indicate significant differences ($p < 0.05$) among stunning methods.

4 Discussion

Inhumane slaughter methods are well documented to induce severe stress in animals, violating welfare principles and compromising meat quality. In aquaculture, ensuring humane killing is critical to reduce stress and strengthen consumer confidence in product quality. This study provides a comprehensive evaluation of fish welfare between the current stunning methods used in the markets, such as asphyxiation, priest stunning and live freezing, with other humane techniques including anesthetic overdose, bolt pistol and ikijime. Among these, asphyxiation demonstrated the least humane method, with all fish remaining fully responsive for up to three hours after removal from water. This prolonged oxygen deprivation leads to anaerobic respiration, which resulted in the ATP depletion and lactate accumulation (Prestes Dos Santos et al., 2024). These pre-slaughtered stress biomarkers are associated with accelerated rigor mortis onset (Skjervold et al., 2001; Morzel et al., 2003; Poli et al., 2005), as well as rapid rigor resolution, suggesting that this prolonged suffocation can accelerate muscle autolysis and enzymatic degradation (Singh and Benjakul, 2018). As a result, fillets from asphyxiated fish initially exhibited highest hardness, gumminess, and chewiness at 1 dps due to the rigor (Skjervold et al., 2001) and later declined by 7 dps, likely due to the myofibrillar protein denaturation and collagen fiber breakdown (Gómez-guillén et al., 2000; Morzel et al., 2003; Singh and Benjakul, 2018). Due to limitations in achieving irreversible unconsciousness using asphyxiation alone in tilapia, the interpretation of biochemical and fillet quality outcomes may reflect combined effects of

TABLE 5 Texture profile analysis of tilapia fillets (*n* = 6) subjected to six stunning methods, measured at 1, 3 and 7 days post-slaughter (dps).

Texture profiles	Time (dps)	Priest	Ikijime	Bolt pistol	Anesthetic overdose	Live freezing	Asphyxiation
Hardness (N)	1	23.07 ± 5.42	24.68 ± 2.90	24.02 ± 6.68	24.36 ± 5.34	23.61 ± 1.40	27.63 ± 4.42
	3	15.42 ± 1.73	15.82 ± 4.34	18.16 ± 4.47	14.83 ± 1.45	17.52 ± 3.44	16.98 ± 1.91
	7	22.20 ± 6.46^{a}	23.14 ± 3.59^{a}	21.04 ± 2.28^{ab}	20.97 ± 5.21^{ab}	21.46 ± 7.02^{a}	15.24 ± 3.58^{b}
Adhesiveness (N·s)	1	−19.64 ± 6.95^{b}	−16.28 ± 3.48^{ab}	−12.56 ± 3.06^{a}	−15.28 ± 3.42^{ab}	−18.72 ± 3.22^{b}	−19.01 ± 4.60^{b}
	3	−15.15 ± 3.47	−15.53 ± 2.54	−16.35 ± 2.48	−14.94 ± 2.31	−18.90 ± 3.77	−16.68 ± 2.02
	7	−17.65 ± 7.30	−17.73 ± 2.26	−16.58 ± 2.07	−17.53 ± 3.60	−15.50 ± 2.87	−14.38 ± 1.87
Springiness (cm)	1	0.39 ± 0.10	0.34 ± 0.07	0.39 ± 0.08	0.37 ± 0.08	0.39 ± 0.09	0.37 ± 0.08
	3	0.41 ± 0.03	0.37 ± 0.04	0.39 ± 0.02	0.37 ± 0.04	0.42 ± 0.06	0.37 ± 0.04
	7	0.41 ± 0.07	0.41 ± 0.06	0.42 ± 0.06	0.38 ± 0.05	0.37 ± 0.02	0.38 ± 0.07
Cohesiveness	1	0.21 ± 0.04	0.20 ± 0.03	0.20 ± 0.04	0.20 ± 0.02	0.20 ± 0.02	0.23 ± 0.04
	3	0.22 ± 0.01	0.19 ± 0.03	0.23 ± 0.03	0.20 ± 0.02	0.22 ± 0.02	0.20 ± 0.03
	7	0.23 ± 0.03^{ab}	0.27 ± 0.05^{a}	0.20 ± 0.02^{b}	0.23 ± 0.04^{ab}	0.24 ± 0.03^{ab}	0.22 ± 0.03^{b}
Gumminess (N)	1	4.86 ± 1.94	5.11 ± 1.26	5.00 ± 2.08	4.93 ± 1.56	4.80 ± 0.78	6.28 ± 1.27
	3	3.45 ± 0.50	3.14 ± 1.22	4.24 ± 1.74	3.01 ± 0.56	3.92 ± 1.04	3.44 ± 0.75
	7	5.34 ± 2.37^{ab}	6.26 ± 1.00^{a}	4.19 ± 0.71^{ab}	5.07 ± 2.15^{ab}	5.37 ± 2.30^{ab}	3.40 ± 1.19^{b}
Chewiness (N·cm)	1	2.00 ± 1.39	1.81 ± 0.80	1.98 ± 0.99	1.88 ± 1.03	1.90 ± 0.84	2.36 ± 1.00
	3	1.42 ± 0.24	1.21 ± 0.56	1.65 ± 0.71	1.12 ± 0.26	1.61 ± 0.36	1.28 ± 0.30
	7	2.27 ± 1.39^{ab}	2.60 ± 0.77^{a}	1.78 ± 0.46^{ab}	2.04 ± 1.22^{ab}	2.05 ± 0.97^{ab}	1.33 ± 0.68^{b}

Data are presented as mean ± S.D. Different superscript letters within the same row indicate significant difference stunning methods ($p < 0.05$).

asphyxiation and anesthetic exposure. Nevertheless, these findings emphasize that asphyxiation is not only an inhumane stunning method, but also significantly compromises tilapia fillet quality and storage time.

In contrast, anesthetic overdose provided effective stunning results as all fish completely losing consciousness within 5 minutes, consistent with previous findings (López-Cánovas et al., 2020). Consequently, the fillet exhibited consistently higher pH levels and lower lactic acid concentrations at all post-mortem time points, which reflect minimal pre-slaughter stress responses due to the reduction in muscular activity (Robb and Kestin, 2002). Despite the anesthetic solution may influence the fillet color, the fillet texture by 3 dps had the highest adhesiveness and the lowest chewiness among all stunning methods, suggesting that limiting muscle activity may contribute to improving flesh texture by minimizing protein denaturation and preserving muscle integrity (Singh and Benjakul, 2018). Additionally, the preservation of higher pH levels may have slowed proteolytic enzyme activity, delaying post-mortem muscle degradation and rigor resolution (Cheret et al., 2007). Rigor mortis analysis was performed using a limited sample size (*n* = 3 per treatment), which may reduce statistical power and the ability to detect subtle differences among stunning methods. This reflects ethical considerations and the labor-intensive nature of repeated postmortem measurements. Therefore, the results should be interpreted as indicative of relative postmortem dynamics rather than precise quantitative estimates, and future studies with larger

TABLE 6 Fillet color parameters (L*, a*, b*) of tilapia (*n* = 6) subjected to six stunning methods, measured at 0, 1, 3 and 7 days post-slaughter (dps).

Fillet color parameters	Time (dps)	Priest	Ikijime	Bolt Pistol	Anesthetic overdose	Live freezing	Asphyxiation
Lightness (L*)	1	44.76 ± 4.56	45.88 ± 3.04	45.21 ± 1.20	45.42 ± 1.18	45.24 ± 1.40	45.38 ± 1.21
	3	44.31 ± 4.21	45.82 ± 3.30	43.82 ± 2.38	44.29 ± 2.84	44.65 ± 2.33	44.53 ± 1.93
	7	42.29 ± 5.08	46.09 ± 3.42	42.90 ± 1.45	44.53 ± 3.30	47.20 ± 4.65	46.01 ± 3.64
Redness (a*)	1	5.10 ± 0.65	5.55 ± 0.39	5.89 ± 0.32	5.63 ± 0.47	5.78 ± 0.62	5.77 ± 0.51
	3	5.31 ± 0.72	5.32 ± 0.43	5.31 ± 0.54	5.34 ± 0.57	5.45 ± 0.57	5.57 ± 0.41
	7	5.12 ± 0.64	5.22 ± 0.68	5.06 ± 0.37	5.52 ± 0.66	5.09 ± 0.66	5.13 ± 0.56
Yellowness (b*)	1	−10.91 ± 1.65	−12.71 ± 2.27	−12.78 ± 0.60	−12.38 ± 0.56	−12.57 ± 1.24	−11.94 ± 2.01
	3	−10.88 ± 2.51	−12.85 ± 2.28	−12.63 ± 1.02	−13.75 ± 1.35	−12.74 ± 1.72	−11.98 ± 2.62
	7	−11.77 ± 1.94	−13.70 ± 1.20	−12.92 ± 0.62	−14.12 ± 1.89	−13.91 ± 2.34	−12.71 ± 2.23

Values are represented as mean ± SD. Different superscript letters within the same row indicate significant difference stunning methods ($p< 0.05$).

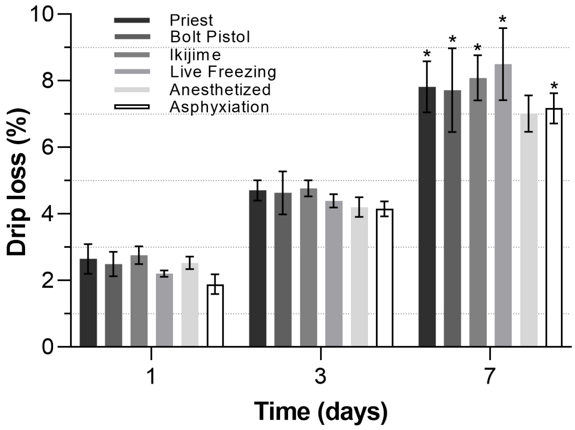

FIGURE 5
Water drip loss (%) in tilapia fillets (*n* = 6) subjected to six stunning methods, measured at 0, 1, 3 and 7 days post-slaughter. Values are expressed as mean ± S.D. An asterisk (*) indicates a significant difference ($p < 0.05$) compared with the preceding time point.

sample sizes are warranted. Despite its clear welfare benefits, the potential presence of anesthetic residues in fish tissues with no withdrawal period raises concerns regarding food safety (Food and Drug Administration, 2007; Jia et al., 2025), limiting its practical application in commercial aquaculture. These findings highlight the need to develop alternative humane stunning techniques that provide similar welfare benefits while complying with food safety regulations.

Percussive stunning using a priest is considered humane when applied correctly, as it immediately induces unconsciousness while minimizing pre-slaughter stress (Robb and Kestin, 2002; Poli et al., 2004). However, its effectiveness is highly dependent on the accuracy and intensity of the applied force, as insufficient impact can result in incomplete stunning, prolonging consciousness and suffering (Poli et al., 2005), while may cause extensive damage that compromises both welfare and meat quality. In this study, fish stunned using a priest showed significantly higher muscle pH at 3, 6, and 24 hps, and ATP levels at 24 hps compared to other stunning methods. These remaining ATP and pH inhibit proteolytic enzyme activity and delay protein degradation (Cheret et al., 2007), results in the slowest rigor resolution observed at 360 hps. Nevertheless, these fillets exhibited the lowest initial adhesiveness and more yellowish color than other methods, which may be attributed to oxidative changes in muscle pigments post-mortem (Sriket et al., 2023). While the fillet quality did not show any significant alterations, priest stunning offers advantages in terms of efficiency, cost-effectiveness, and ease of implementation.

Percussive stunning using a bolt pistol was identified as an effective method for promoting fish welfare, as it induces immediate unconsciousness through the brain destruction (Sundell et al., 2024). Compared with priest stunning, this bolt pistol method demonstrated greater consistency in stunning efficacy as the physical damage was localized to the skull which effectively disrupts brain function and ensures insensibility until death (Poli et al., 2005; Sundell et al., 2024). However, pre-slaughter stress indicators suggested that fish subjected to bolt pistol stunning experienced moderate physiological stress, likely due to the pre-handling stress, which accelerates ATP depletion, increases lactate accumulation, and potentially influences postmortem muscle changes (Erikson et al., 1999; Thomas et al., 1999). However, the resolution of rigor mortis in this group was slower than in other methods, which affected fillet texture and the product storage time (Cheret et al., 2007; Singh and Benjakul, 2018). The highest adhesiveness and lowest chewiness found from the fish in this group, indicates muscle contractions and tissue damage caused by post-stun convulsions, may affect the consumer favorable (Poli et al., 2005). Therefore, precise positioning and careful handling protocols before stunning may contribute to moderate pre-slaughter stress are required to optimize both welfare outcomes and product quality.

Live freezing was effective stunning method in terms of stunning efficiency, while it is relatively reliable in rendering fish unconscious; however, a significant drawback is the prolonged time required for complete loss of consciousness. During the initial freezing phase, fish may experience substantial distress due to the gradual decline in temperature before the sedative effects induced by the coldness (Lambooij et al., 2002; Morzel et al., 2003; Zampacavallo et al., 2014). In this study, behavioral observations during freezing could not be assessed due to equipment constraints, which limited our ability to evaluate welfare outcomes inside the freezer. Future studies incorporating real-time behavioral monitoring, such as video recording or temperature-linked behavioral assessment, are needed to more accurately evaluate welfare outcomes during live freezing. Nevertheless, despite the high stress imposed on fish, biochemical indicators immediate after slaughtered showed the most favorable values, which was explained by that the exposure to cold temperature could temporarily slow postmortem acidification (Lee et al., 1998; Skjervold et al., 2001; Zampacavallo et al., 2014). However, once the temperature returned to ambient levels, biochemical changes occurred rapidly and subsequently hastens rigor formation and resolution. As a result, fillets from live-frozen fish demonstrated the highest springiness, which may be attributed to the retained muscle elasticity from slower biochemical degradation (Qiao et al., 2022). Conversely, the lower adhesiveness suggests that freezing-induced cellular damage may have compromised muscle protein structure, thereby reducing protein-water interactions (Qiao et al., 2022). Remarkably, fillet color revealed a greater redness immediately after slaughter by 1 dps, possibly due the redistribution of blood from visceral organs to muscle tissues and the accumulation of myoglobin (Digre et al., 2011; Ismail et al., 2023). Increased muscle redness is often associated with increased pre-slaughter stress and is generally undesirable in consumer markets (Morzel and van de Vis, 2002). Thus, while live freezing may yield acceptable short-term fillet quality, its prolonged induction of unconsciousness and associated stress responses raise serious welfare concerns and the product storage time.

The ikijime method is widely recognized as a humane stunning technique that ensures both high welfare standards and superior product quality (Poli et al., 2005; Diggles, 2015). However, in our study, the effectiveness of this technique is highly dependent on the skill and accuracy of the operator. When performed correctly, ikijime induces rapid and irreversible brain death, minimizing suffering and preserving meat quality (Islami et al., 2014). Poli et al., 2005 (Poli et al., 2005) and Robb et al., 2000 (Robb et al., 2000) noted that improper execution can lead to incomplete stunning, deep puncture wounds, and prolonged consciousness, causing unnecessary distress. Furthermore, Poli et al., 2004 (Poli et al., 2004) observed that fish subjected to ikijime exhibited higher stress levels than those stunned using percussive methods, suggesting that the handling and precise positioning required for this technique contribute to pre-slaughter distress and should be considered if the technique is applied to practice. In this study, fish stunned using ikijime exhibited a relatively low muscle pH, accompanied with ATP depletion and lactate accumulation, which indicate substantial pre-slaughter stress. Nevertheless, fish stunned with ikijime demonstrated significantly highest cohesiveness, gumminess, and chewiness at 7 dps compared to other methods. This outcome may be attributed to that ikijime destroys the brainstem and prevents excessive muscle convulsions at death (Robb and Kestin, 2002). Additionally, fillet color analysis revealed the lowest redness and higher lightness immediately after slaughter. This alteration is attributed to significant blood loss from the technique which reduces myoglobin content in the muscle (Ismail et al., 2023). Thereby, this lighter-colored fillets are often preferred in consumer markets, particularly for white-fleshed fish such as tilapia (Filho et al., 2014). Despite these advantages, the success of ikijime in Nile tilapia has never been reported and should be further optimized to achieve both high welfare standards and quality products in aquaculture.

5 Conclusion

The findings of this study demonstrate the influence of stunning methods on both welfare outcomes and fillet quality in Nile tilapia. Among the six approaches evaluated, anesthetic overdose and bolt pistol stunning were the most effective, rapidly inducing unconsciousness and minimizing welfare compromise. Conversely, asphyxiation proved to be the least humane, causing severe distress and pronounced biochemical and textural deterioration. Physiological stress markers, including muscle pH, lactic acid, and ATP, further highlighted how stunning techniques shape postmortem metabolism, rigor mortis progression, and meat integrity. Ikijime offered advantages in exsanguination and fillet texture but its reliance on operator skill limits its large-scale applicability. Live freezing and priest stunning produced intermediate results: while the former raised ethical concerns due to prolonged distress, the latter demonstrated variability depending on operator proficiency. Overall, the findings reinforce the need to implement humane stunning practices in tilapia aquaculture to optimize both welfare and meat quality. Future research should aim to refine existing approaches, develop cost-effective and user-friendly devices, and explore automation to improve welfare standards and ensure high-quality fish products in commercial production.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was approved by Institutional Animal Care and Use Committee of Kasetsart University under the protocol number ACKU67-VET-094. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

AH: Conceptualization, Funding acquisition, Investigation, Methodology, Formal analysis, Resources, Visualization, Writing – original draft. JY: Investigation, Methodology, Writing – original draft, Data curation. NY: Data curation, Investigation, Methodology, Writing – original draft. PS: Investigation, Writing – review & editing. WS: Writing – review & editing, Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation. TL: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing, Investigation, Methodology.

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Conflict of interest

Author AH was employed by company Somsak Farm.

The remaining author(s) declared that this work was conducted in theabsence of any commercial or financial relationships that could beconstrued as a potential conflict of interest.

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