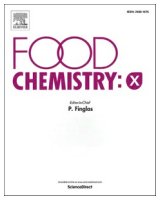
Food Chemistry: X 24 (2024) 101951

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Effects of different slaughtering methods on the biochemical characteristics and quality changes of tilapia (*Oreochromis niloticus*) during cold storage

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ARTICLE INFO

Keywords:
Tilapia
Slaughter methods
Apoptosis
Fish
Quality

ABSTRACT

Inappropriate slaughter methods can lead to differences in fish quality. In the past few years, few studies have focused on the effects of different slaughter methods on the postmortem effects of tilapia, especially the cold storage of tilapia after slaughter. The aim of this study was to investigate the effects of different slaughter methods on the biochemical characteristics and quality changes of tilapia during cold storage. In terms of blood and plasma parameters, the CS sample had lower levels of lactate dehydrogenase (LDH), cortisol (COR), and glucose (GLU) than the other samples. The results of K-value and FAAs showed that CS and ASCN groups were beneficial to prolong the freshness life of tilapia during cold storage. The texture properties of CS group were better. In summary, group CS is more conducive to prolonging the fresh life of refrigerated tilapia and is a recommended method of slaughter.

1. Introduction

Today, fish slaughtering methods are of major concern to consumers (Nima et al., 2024). The method of slaughter is one of the most important factors among the key points of aquaculture. Inappropriate slaughter can cause strong stress response in fish, leading to changes in osmoregulation, enzyme activity, metabolism and blood composition (Wang et al., 2021). This leads to undesirable consequences such as early massive water loss, rigor mortis, lipid oxidation and severe protein denaturation, which seriously affect fish quality (Wang et al., 2021). In Italy, the most applied stunning killing methods were “asphyxia in ice/thermal shock” and “electric in water bath”, followed by “percussion”,“asphyxia in air” and “electric dry system” (Clemente et al., 2023). However, cranial spiking (CS) around the medulla oblongata (hindbrain region) is one of the most extensively utilized methods in Japan and is considered effective in terms of freshness and meat quality (Mochizuki & Sato, 1994). Carbon dioxide (CO2) anesthesia has the advantages of lower residual toxicity compared with other anesthetic methods. Gräns et al. (Gräns et al., 2016) found that plasma cortisol levels were halved under CO2 anesthesia compared to electrical stimulation of Arctic char (*Salvelinus alpinus*). Aside from carbon dioxide, a mixture of nitrogen in various proportions has also been used. Poli et al. (Poli et al., 2005) used a mixture of gases (60–70 % N_2 and 40–30 % CO_2) to reduce the time required to stun bass.

Tilapia (*Oreochromis niloticus*) is the richest freshwater fish species in the world and dominates the fisheries market. It is widely recognized that inappropriate slaughtering methods can lead to differences in the quality of fish (Rucinque et al., 2023). Stress is a non-specific response of fish to external or internal stressors that can lead to disturbed physiological hemostasis and reduced meat quality (L. T. Zhang et al., 2021). Dong et al. (Dong, Zhang, et al., 2023) found that croaker asphyxiated asphyxiated with food grade CO_2 had higher antioxidant enzyme activity, more stable MP structure and better gel properties than the group asphyxiated with 40 % CO_2 + 30 % N_2 + 30 % O_2 during the cold storage process.

In the last few years, few studies have focused on the effects of different slaughter methods on the postmortem effects on tilapia, particularly regarding post-slaughter cold storage of tilapia. Therefore, the effect of different slaughtering methods on the postmortem quality of tilapia deserves further study. The purpose of this study was to

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https://doi.org/10.1016/j.fochx.2024.101951
Received 12 September 2024; Received in revised form 30 October 2024; Accepted 30 October 2024
Available online 2 November 2024

examine the impact of head trauma, spinal cord piercing, low temperature death, CO_2 anesthesia and 30 %CO_2 + 70 %N_2 asphyxia on postmortem stress, apoptosis and microbial diversity of tilapia.

2. Material and methods

2.1. Sample preparation

A total of 80 tilapias (*Oreochromis mossambicus*) were obtained from a aquatic food market in Shanghai, China (Lingang New Town), with a body length of 30 ± 5 cm and a weight of 750 ± 20 g. Live fish were transferred in oxygenated polyethylene containers and transported to the laboratory within 30 min. In order to recover from the stress that may occur during transportation, the fish were transiently housed in a 500 L tank and fasted for 24 h. The transient conditions were temperature, salinity, and dissolved oxygen of 20–22 °C, 16 ‰, and 4–6 mg/L, respectively. Fish of the same size and respiration rate were divided into five groups. They were stunned by trained laboratory members of the slaughter laboratory (Fig. 1).

PC (n = 16): cranial euthanasia by percussion.

CS (n = 16): spinal cord crushing euthanasia.

IW (n = 16): euthanasia of fish by immersion in a 1:1 ice-water bath at 3 ± 1 °C.

ASC (n = 16): fish experienced asphyxiation-induced loss of consciousness in a saturated CO_2 environment.

ASCN (n = 16): fish experienced asphyxiation-induced loss of consciousness in a saturated 30 % CO_2 + 70 % N_2 environment.

The criteria for unconsciousness in fish are loss of balance, cessation of eye movement, and lack of response to external stimuli (Dong, Zhang, et al., 2023). Subsequently, the dorsal muscles of the unconscious tilapia were removed and washed with deionized water to remove blood. The samples were then individually wrapped in 0.2 mm PE polyethylene bags (Zhejiang Province, China) and stored at 4 °C. Measurements were taken at 0, 12th, 24th, 36th, 48th, 96th, 144th and 192nd h, respectively.

2.2. Blood and plasma parameters

Blood samples were taken from the tail vein. The sample was allowed to stand at 4 °C for 12 h before being centrifuged at 4 °C for 20 min at 4050 ×g. The acquired blood serum was stored at −80 °C for further index measurements.

The levels of LDH, COR, and GLU were assessed using designated commercial assay kits sourced from Nanjing Jiancheng Bioengineering Institute in China. LDH was determined by microplate method. 20 μL of the supernatant was determined for each group.The distilled water, pyruvate standard solution, test supernatant, matrix buffer, and coenzyme were added in sequence according to the instructions and then incubated at 37 °C for 15 min. This was followed by incubation with phenylhydrazine at 37 °C for 15 min and then mixed with NaOH standard solution for 5 min at room temperature. The absorbance of the sample was measured at 440 nm with a microplate reader (Tecan Spark, Groedig, Austria). COR test was performed by enzyme-linked immunosorbent assay and absorbance was measured at 450 nm. GLU content was determined by utilizing the glucose oxidase reaction and the hydrogen peroxide peroxidation reaction, with absorbance measured at 515 nm.

2.3. Apoptosis

Apoptosis was determined by 4′, 6-diaminidine 2-phenylindole (DAPI staining). Paraffin sections were made from tissue preserved in 4 % formaldehyde solution. After deparaffinization and rehydration, the sections were stained with DAPI staining reagent and incubated at room temperature for 10 min. The slide was placed in PBS (pH = 7.4) and washed by shaking on the decolorizing table for 3 times, 5 min each time. The sections were sealed with anti-fluorescence quenching sealer. Sections were viewed under a fluorescence microscope (Nikon, Tokyo, Japan) and images were captured. The nuclei appeared white after image processing. Specifies that the magnification of the image is 2.5 × .

2.4. Microbiology

About 5 g of fish was homogenized with 45 mL of sterilized normal

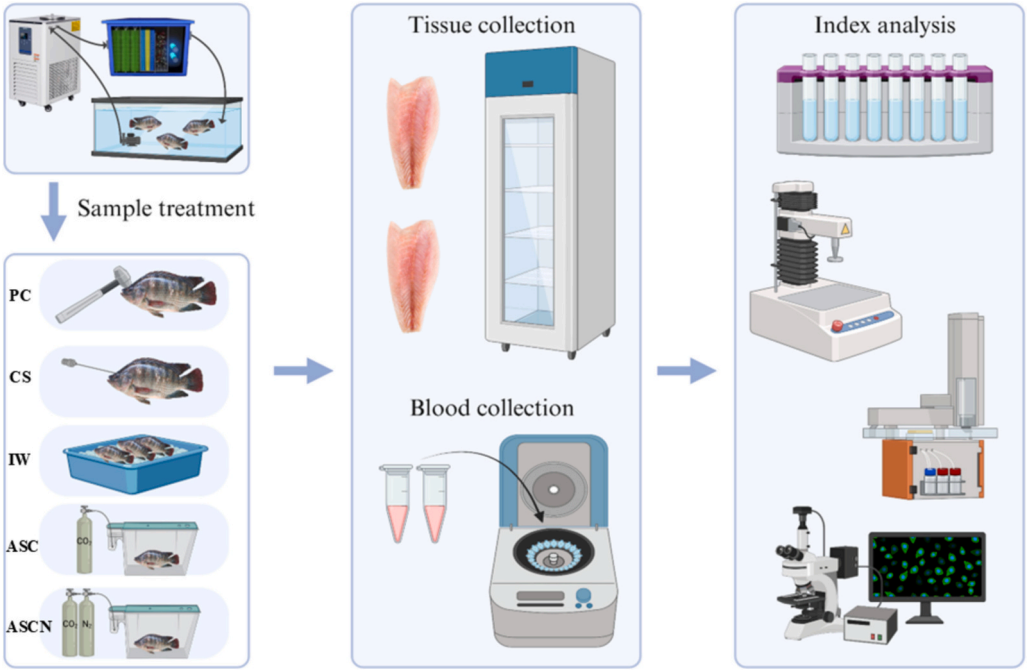

Fig. 1. Schematic diagram showing the experimental design.

saline. 0.5 mL of the above homogenized solution was continued to make a 10-fold dilution with sterilized saline and then continued to dilute to the desired concentration. The plate count agar from Hopebiol was prepared according to the instructions. 100 μL of the diluted sample had been spread evenly on the plate counting agar medium. The plates were incubated at 30 °C for 48 h and the total viable count (TVC) counts were recorded. When TVC exceeds 7 log CFU/g, deterioration of fish meat begins to occur (Tolba et al., 2023).

2.5. Centrifugal loss

2 *g* of tilapia dorsal muscle was wrapped in 2 layers of filter paper and centrifuged for 10 min (5980 ×*g*, 4 °C). The filter paper was manufactured by Fushun Civil Affairs Filter Paper Factory, located in Shuncheng District, Fushun City, Liaoning Province, China. The pore size was 30–50 μm. Filtration rate is 35–70 s. Centrifugation losses were calculated using the following formula:

$$\text{Centrifugal loss}(\%) = (W_b - W_a)/W_b \times 100\%$$

W_b:the initial weight of the sample.
W_a: the weight of the sample after centrifuging

2.6. Total volatile basic nitrogen (TVB-N)

The contents of TVB-N were quantified utilizing a Kjeltec analyzer (FOSS 8400, Denmark), with the results expressed in terms of mg N/100 g.

2.7. Texture profile analysis (TPA)

The hardness, springiness, chewiness and resilience of samples were studied in the experiment. The samples were chopped into 20 mm × 20 mm × 20 mm pieces, and the texture properties were examined in five copies using a texture analyzer (Stable Micro Systems, Ltd., Godalming, Surrey, UK), which used a P/5 probe. Test speed was 1 mm/s, compression interval was 5 s, compression degree was 50 %, and relaxation time was 5 s.

2.8. K-value and related compounds

ATP-related compounds were measured by HPLC (Waters e2695, USA) according to the method of Cen et al. (Cen et al., 2021). The K value was calculated as follows:

$$K\ values = (Hx + HxR)/(ATP + ADP + AMP + IMP + Hx + HxR) \times 100\%$$

2.9. pH

The pH was measured using a pH meter (PB-10, Sartorius, Germany). The pH meter was calibrated using an acidic calibration solution (pH 4.00 ± 0.01) and an alkaline calibration solution (pH 9.18 ± 0.01). Measurements were carried out after calibration.

2.10. Free amino acids (FAAs)

The measurement of FAAs followed the method described by Dong et al. (Dong, Niu, et al., 2023).

2.11. Statistical analysis

The experiments have been carried out for 3 repeats. All results are expressed as mean ± standard deviation ($n \geq 3$). Data were analyzed by statistical ANOVA using SPSS 22.0. Data were plotted by Origin 2018.

3. Results and discussion

3.1. Blood and plasma parameters

COR, a physiological hormone, expedites the mobilization of glucose in fish by enhancing both hepatic glycolysis and gluconeogenesis processes. This swift metabolic adaptation ensures the rapid availability of energy to the body. The COR concentration in fish blood swiftly reflects stress intensity, acting as a reliable stress indicator for fish (Liu et al., 2021). The COR concentration varied significantly among fish subjected to various slaughter methods. The COR content in the IW sample was markedly elevated compared to other samples ($P < 0.05$). Oliveira et al. (de Oliveira et al., 2015) noted that blood COR levels in Nile tilapia were higher in ice water than under CO2 anesthesia, which is similar to what was found in this study. The accumulation of COR may be due to the prolonged lethal time of IW samples. Zampacavallo et al. (Zampacavallo et al., 2003) also found that COR levels were positively correlated with longer time to death. This is because the CS sample had the shortest time to death and its COR level was significantly lower ($p < 0.05$) than the other samples. This can be seen as a good slaughtering method for tilapia.

In general, COR accelerates GLU mobilization in fish through liver glycolysis and gluconeogenesis, thereby providing energy for its own rapid response to stress (Veit et al., 2017). Upon experiencing stress, fish release hormones, leading to an elevation in blood sugar levels to sustain vital bodily functions. Additionally, lactic acid accumulation occurs, triggering anaerobic respiration. Changes in pressure of fish and dissolved oxygen in water during slaughter can cause changes in GLU metabolism (Zhang et al., 2023). The GLU level in sample IW was significantly higher ($p > 0.05$) than the other sample, which was like the findings of Liu et al. (Liu et al., 2021). The GLU levels in the CS sample were significantly lower ($p < 0.05$) than the other samples, suggesting that lower COR levels may be beneficial in slowing down glucose degradation in tilapia.

LDH is an enzyme responsible for facilitating the conversion of pyruvate into lactic acid during the metabolic process of glucose breakdown (Hatami et al., 2019). Muscular trauma and the promotion of anaerobic glycolysis both contribute to a notable enhancement in LDH activity (Santos et al., 2018). The LDH activity of CS samples was markedly less than that of other samples (Table 1). This may be due to the rapid death caused by the pounding of the fish's spinal cord, resulting in a shorter anaerobic metabolic cycle, and low stress in the fish's heart tissue. Due to the rapid death caused by pounding the spinal cord of the fish, the resulting LDH is not released into the blood in large quantities. The LDH activity were higher in PC, IW, ASC and ASCN samples. This may be due to intense muscle contraction activity that produces anaerobic metabolism, leading to changes in carbohydrate metabolism (Venturini et al., 2018). Oliveira et al. (Oliveira et al., 2021) revealed that LDH activity was higher in tilapia under low temperature conditions. This is similar to the results of this study.

Table 1
Changes of COR (ng/L), GLU (nmol/L) and LDH (U/L) of tilapia by different slaughter methods (PC: cranial euthanasia by percussion; CS: spinal cord crushing euthanasia; IW: euthanasia of fish by immersion in a 1:1 ice-water bath at 3 ± 1 °C; ASC: fish experienced asphyxiation-induced loss of consciousness in a saturated CO_2 environment; ASCN: fish experienced asphyxiation-induced loss of consciousness in a saturated 30 % CO_2 + 70 % N_2 environment). The letter from "a" to "c" are used to describe the significance of differences between the samples ($p < 0.05$).

	COR	GLU	LDH
PC	182.12 ± 8.42^{b}	4.83 ± 0.15^{b}	321.10 ± 1.45^{a}
CS	126.57 ± 9.95^{a}	3.51 ± 0.32^{a}	299.13 ± 3.90^{a}
IW	208.93 ± 7.68^{c}	5.47 ± 0.25^{c}	446.57 ± 7.70^{c}
ASC	176.87 ± 6.81^{b}	4.57 ± 0.21^{b}	359.90 ± 5.05^{b}
ASCN	179.20 ± 4.35^{b}	4.67 ± 0.15^{b}	310.13 ± 1.60^{a}

3.2. Apoptosis

Apoptosis is a genetically encoded form of cell death that encompasses a sequence of intricate biochemical reactions, ultimately culminating in a multitude of cellular alterations (Aslantürk & Çelik, 2013). Subsequent to animal slaughter, muscle cells encounter hypoxia-ischaemia, leading to an inevitable progression towards apoptosis. Therefore, it is of great significance to evaluate flesh quality based on the changes in the apoptotic process (Fuente-García et al., 2021). DAPI staining results showed that the nuclear chromatin of apoptotic cells was concentrated, and fluorescence was enhanced. After 12 h of cold storage, CS samples showed obvious apoptosis, condensed chromatin, and enhanced fluorescence compared with PC samples (Fig. 2). ASCN samples showed weaker nuclei fluorescence and significantly attenuated fluorescence. The results showed that 30 % CO_2 + 70 % N_2 asphyxiation death had an attenuating effect on the antioxidant damage of tilapia flesh cells (×200). Due to apoptosis, the gaps between muscle cells increase after death. This may be due to water loss during storage. Apoptosis, an important biochemical change after fish slaughter, also regulates protein hydrolysis. Oxidative stress triggers the activation of mitochondrial apoptosis pathways, plays a role in modulating apoptotic factors within fish muscle, and ultimately contributes to the breakdown of structural proteins (X. Li et al., 2022). The mechanism underlying textural oxidation in fish is intricate, particularly with regards to the alterations in myofibrillar proteins and the intricate regulation of mitochondrial apoptotic factors (X. Li et al., 2022).

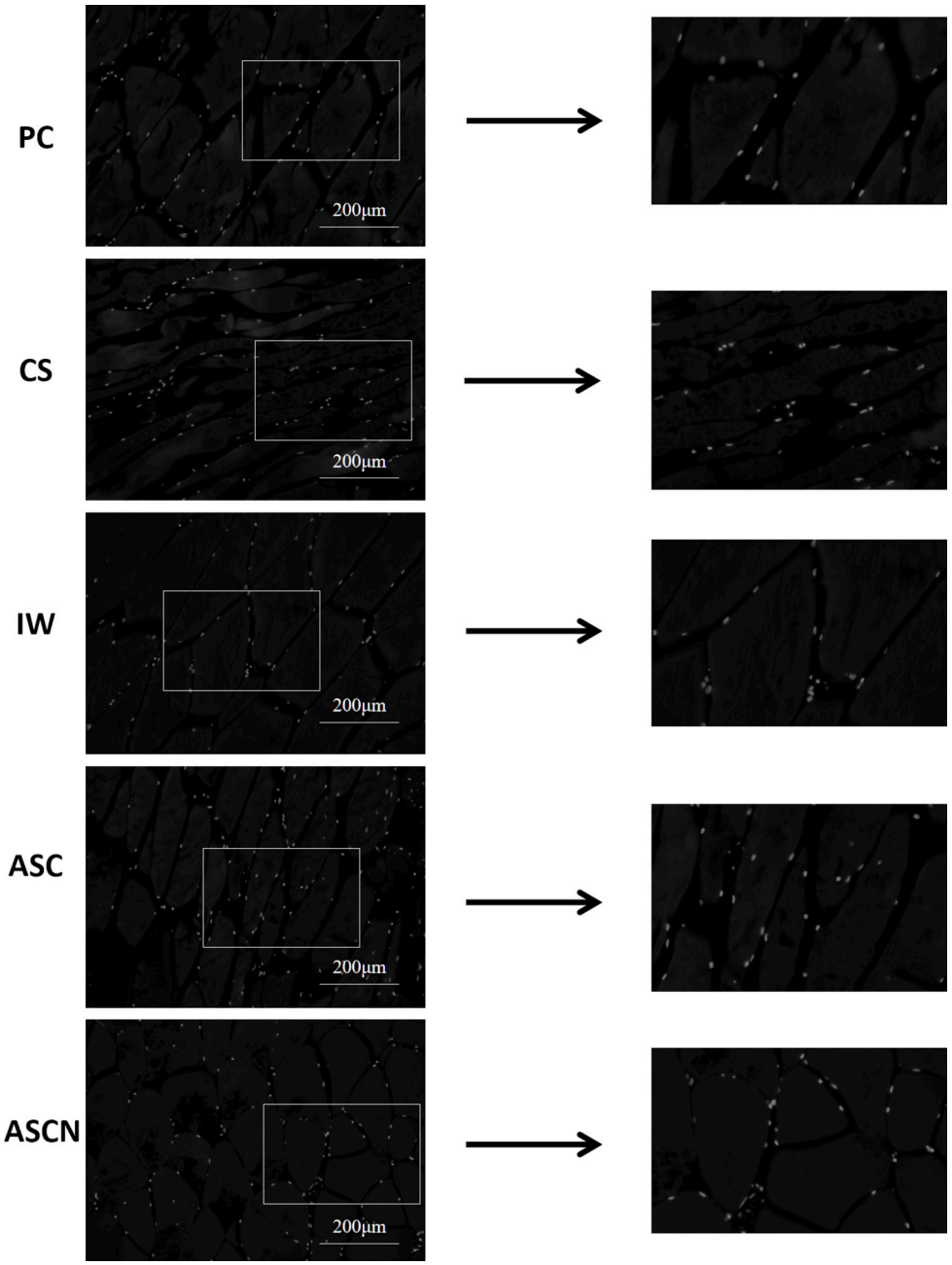

Fig. 2. Changes in DAPI tilapia slaughtered with different treatments (PC: cranial euthanasia by percussion; CS: spinal cord crushing euthanasia; IW: euthanasia of fish by immersion in a 1:1 ice-water bath at 3 ± 1 °C; ASC: fish experienced asphyxiation-induced loss of consciousness in a saturated CO_2 environment; ASCN: fish experienced asphyxiation-induced loss of consciousness in a saturated 30 % CO_2 + 70 % N_2 environment) at 12 h.

3.3. Microbiological analysis

Microbiological contamination has the potential to deteriorate the quality of fresh meat, curtail its shelf life, and consequently lead to economic losses as well as potential health risks (Jouki & Khazaei, 2011). The microbiological quality of meat is influenced by a multitude of factors, including the animal's physiological condition at the time of slaughter and the dissemination of contaminants during the slaughter and processing procedures. The microbiological counts of tilapia preserved in 4 °C after treatment according to different slaughter methods are shown in Fig. 3 (a). The TVC was 2.1 log CFU/g at the beginning, reflecting the tilapia with good quality. In general, an increase in the growth of all microorganisms with storage time was observed in tilapia samples slaughtered by the five different slaughtering methods. However, the ice water slurry slaughter method resulted in a more pronounced increase. This heightened activity prior to death could potentially conditions for the growth of contaminating microflora. All samples exceeded the 7 log CFU/g limit on 144 h and spoilage began to occur. Tilapia treated with ASCN had lower TVC than the other samples throughout the storage period. At the later stage of storage, tilapia in sample IW had significantly ($p < 0.05$) higher TVC than the other samples. This could be attributed to the fact that this treatment resulted in blood penetration into the fish flesh at the time of slaughter. Nakyinsige et al. (Nakyinsige et al., 2014) found that the residual blood volume within the carcass post-bleeding is a pivotal factor influencing contamination levels, thereby exacerbating the extent of deterioration. The nutritional richness of blood, coupled with conducive temperature, pH levels, water activity, and relative humidity, collectively impact the extent of deterioration in meat. For example, glucose, a substrate preferentially used by many microorganisms for growth in meat, is readily available in the bloodstream.

3.4. Analysis of centrifugal loss

Centrifugal loss mainly reflects the ability of fish to retain its own water. During storage, as the muscle tissue and protein structure change under the action of microorganisms and enzymes, the water-holding capacity of fish will also change accordingly (Zhang & Xie, 2019). Therefore, the centrifugal loss can reflect the quality of fish to a certain extent. As can be seen from Fig. 3 (c), the centrifugal loss of fish showed an overall increasing trend with the prolongation of storage time after different slaughtering methods of tilapia. At the beginning of storage,

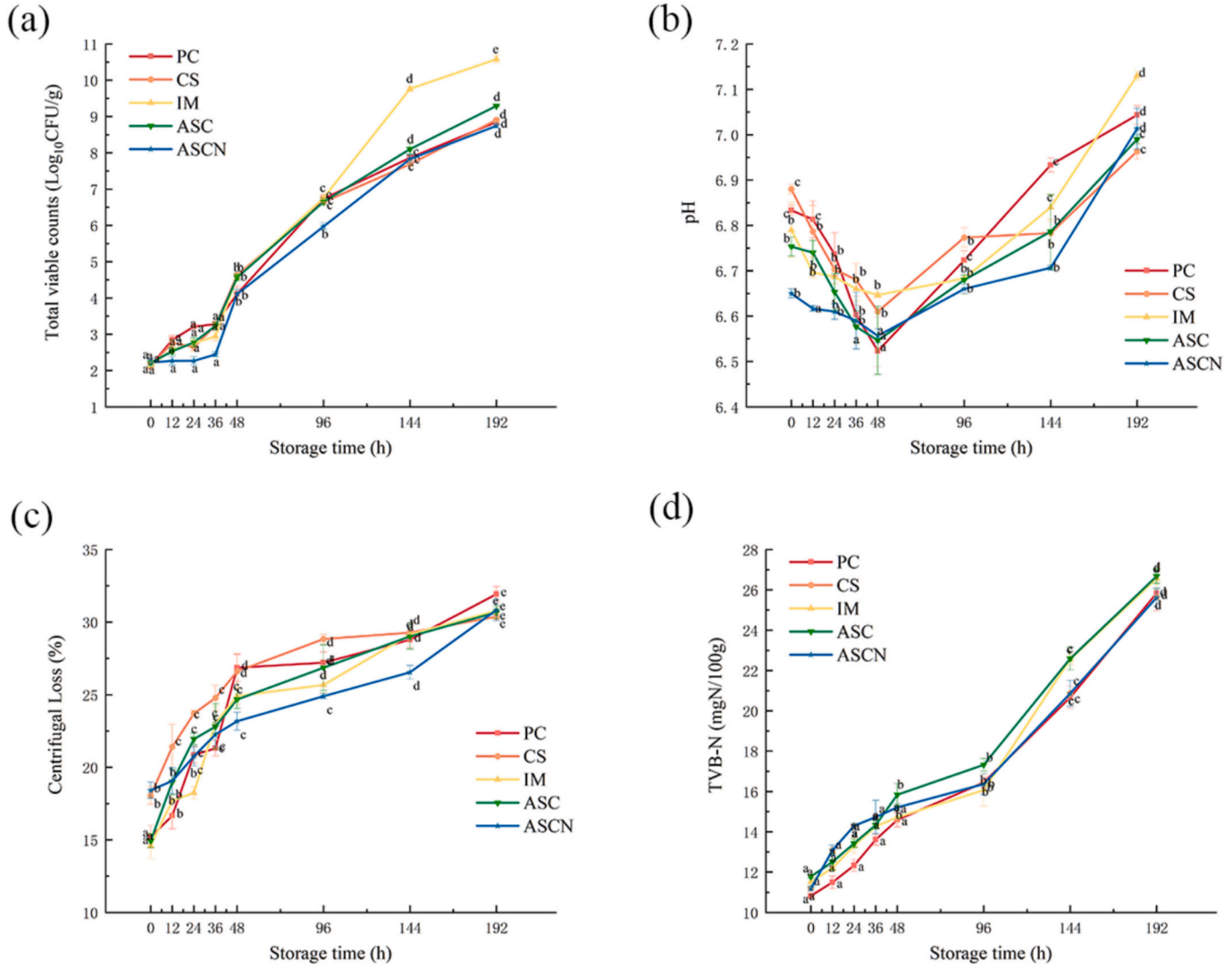

Fig. 3. Changes in TVC (a), pH (b), centrifugal loss (c) and TVB-N (d) of tilapia preserved at 4 °C after different slaughter treatments (PC: cranial euthanasia by percussion; CS: spinal cord crushing euthanasia; IW: euthanasia of fish by immersion in a 1:1 ice-water bath at 3 ± 1 °C; ASC: fish experienced asphyxiation-induced loss of consciousness in a saturated CO_2 environment; ASCN: fish experienced asphyxiation-induced loss of consciousness in a saturated 30 % CO_2 + 70 % N_2 environment). The letter from "a" to "e" are used to describe the significance of differences between the samples ($p < 0.05$).

the centrifugal loss of IW and ASCN samples were 18.07 % and 18.42 %, respectively. It was significantly higher ($p < 0.05$) than the other treatment samples. It is possible that these two slaughtering methods better retained the moisture content of the fish. With the prolongation of storage time, the protein and fat of the fish began to decompose under the action of microorganisms and enzymes, the muscle tissue structure was destroyed, and the water-holding capacity of the fish decreased, which resulted in the increase of centrifugal loss. The centrifugal losses of all ASCN samples were at a low level after 48 h, which shows that this treatment can effectively avoid the moisture loss of tilapia during cold storage. Lund et al. (Lund et al., 2011) showed that the water retention capacity of muscle is influenced by a number of factors such as pH, postmortem protein oxidation, protein hydrolysis activity of meat tenderizing enzymes, and cross-linking of myofibrillar proteins. As the muscle stiffens, the space to hold water decreases, causing fluid to flow into the spaces between myofibrils, where water is more easily lost. Degradation of the limited cellular scaffolding proteins causes enhanced cellular contraction, ultimately leading to water loss.

3.5. Analysis of TVB-N

TVB-N is a commonly employed quality indicator for fish, and its elevation is primarily attributed to the activity of spoilage bacteria and endogenous enzymes. The breakdown of amino acids in fish muscle by bacterial catabolism leads to the accumulation of compounds like trimethylamine, dimethylamine, monoethylamine, ammonia, and other volatile bases. This accumulation occurs in a curvilinear or linear fashion as the fish spoils, contributing to the development of characteristic undesirable flavors. The initial TVB-N values in PC, CS, IW, ASC, ASCN samples were 10.82, 11.19, 11.52, 11.78, 11.19 mg N/100 g, respectively (Fig. 4 (d)). This indicates that the freshness of the slaughtered tilapia samples was satisfactory. The initial TVB-N values of the five lethal methods were not significantly different ($P > 0.05$), and the TVB-N values changed slowly in the first 48 h during storage, indicating that refrigeration can effectively inhibit the decomposition of proteins by microorganisms and enzymes. All five different lethal methods significantly affected the TVB-N values of tilapia at earlier storage, and all of them increased with the storage time ($P < 0.05$). It can be seen that the protein was gradually decomposed and the degree of deterioration was gradually deepened during the storage at 4 °C. The

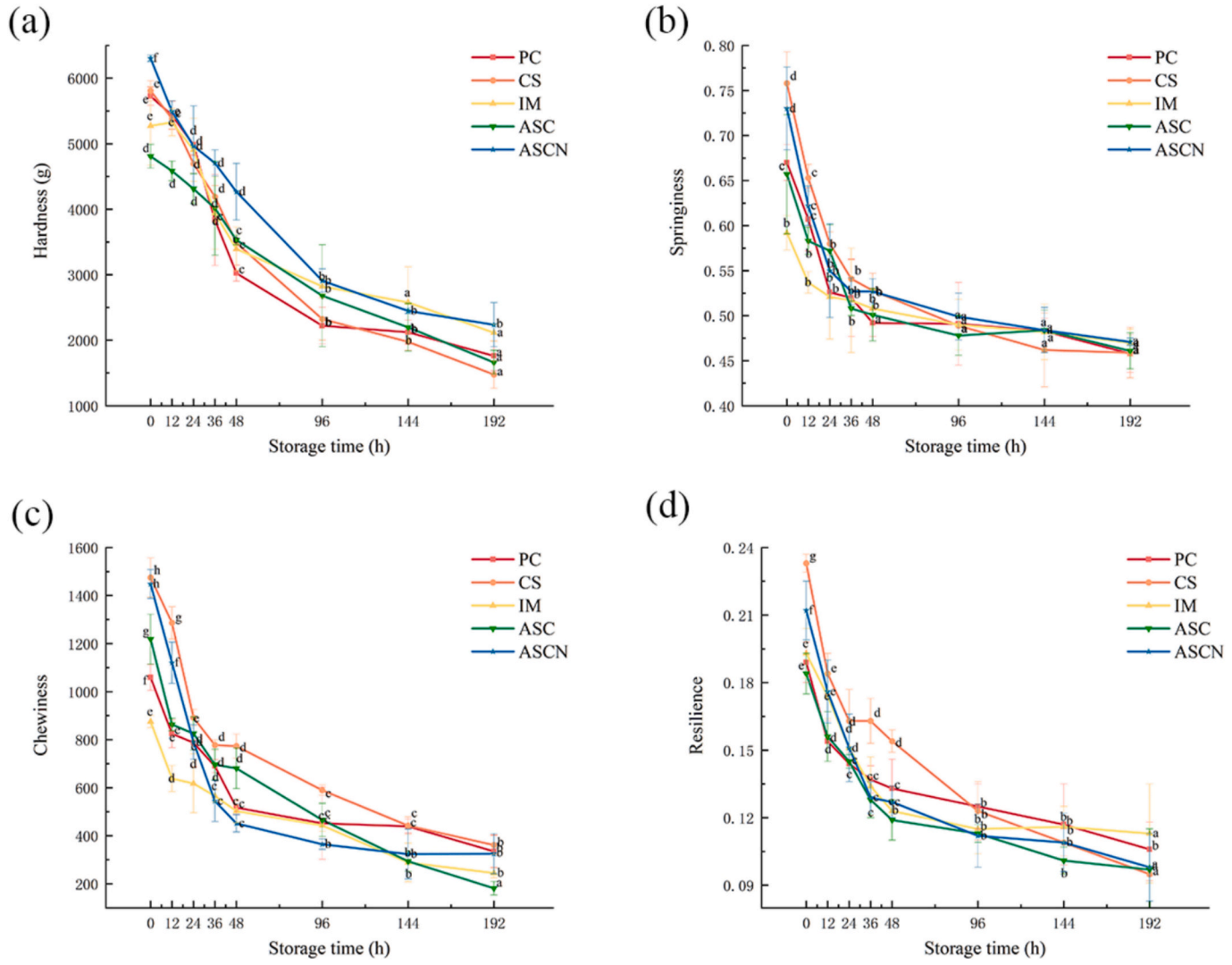

Fig. 4. Changes in hardness (a), springinesschewiness (b), chewiness (c), and resilience (d) of tilapia preserved at 4 °C after different slaughter treatments (PC: cranial euthanasia by percussion; CS: spinal cord crushing euthanasia; IW: euthanasia of fish by immersion in a 1:1 ice-water bath at 3 ± 1 °C; ASC: fish experienced asphyxiation-induced loss of consciousness in a saturated CO_2 environment; ASCN: fish experienced asphyxiation-induced loss of consciousness in a saturated 30 % CO_2 + 70 % N_2 environment). The letter from "a" to "h" are used to describe the significance of differences between the samples ($p < 0.05$).

late increase in TVB-N can be attributed to bacterial decay, endogenous enzyme activity, and subsequent accumulation of ammonia, monoethylamine, dimethylamine, trimethylamine, and other volatile bases. At the ending of storage, the TVB − N contents of the PC, CS, IW, ASC, ASCN were 25.83, 25.62, 26.56, 26.68 and 25.62 mg N/100 g, respectively. The TVB-N content of sample ASCN was at a low level throughout the storage period, indicating that tilapia from sample ASCN maintained a high level of freshness during cold storage. This may be due to the fact that tilapia has been in a packaging environment, which is conducive to maintaining freshness, reducing microbial contamination and inhibiting enzyme activity, and therefore the TVB-N value will slowly increase during the refrigeration process.

3.6. Analysis of TPA

TPA is a method that helps to evaluate the mechanical properties of food products, by applying a controlled deformation to the sample and recording the force vs time curve. Strength refers to the force required to compress the sample to a specified deformation, while springiness is the capacity of a sample to recover its former state after distortion (Weiqing et al., 2024). The texture of the fish depends on many factors such as protein, fat content, and muscle shape. Generally, the texture of fresh fish is firm, moist, and slightly springy. The initial springiness of IW (6.15 × 103 g) was significantly lower ($P < 0.05$) than other group, indicating that the slaughtering method had an effect on the postmortem springiness of fish (Fig. 4 (b)). Upon the death of the fish, certain autolytic enzymes and microbial activities become activated, resulting in a reduction of muscle elasticity and softness (X. P. Li et al., 2011). At 0 h, the hardness of PC, CS, IW, ASC, and ASCN groups were 5.7 × 10^3, 5.8 × 10^3, 5.3 × 10^3, 4.8 × 10^3, and 6.3 × 10^3, respectively. With the increase of storage time, the hardness of all samples decreased significantly ($p < 0.05$), especially in the early storage period. This may be due to the increase of interstitial space and adhesion of myofibrils and separation of myofilm (Roy et al., 2012). The ASCN sample had the highest stiffness at 0 h, which could be attributed to their more intense stress response at slaughter, leading to muscle contraction and a tighter myogenic fibre structure. The hardness decreased to a lesser extent during storage. Textural softening of fish during storage is mainly attributed to protein deterioration under the action of endogenous histone proteases and exogenous proteases (Zarandona et al., 2021). Chewiness is the energy required to chew a solid sample into a stable state for swallowing and is numerically expressed as the ratio of the

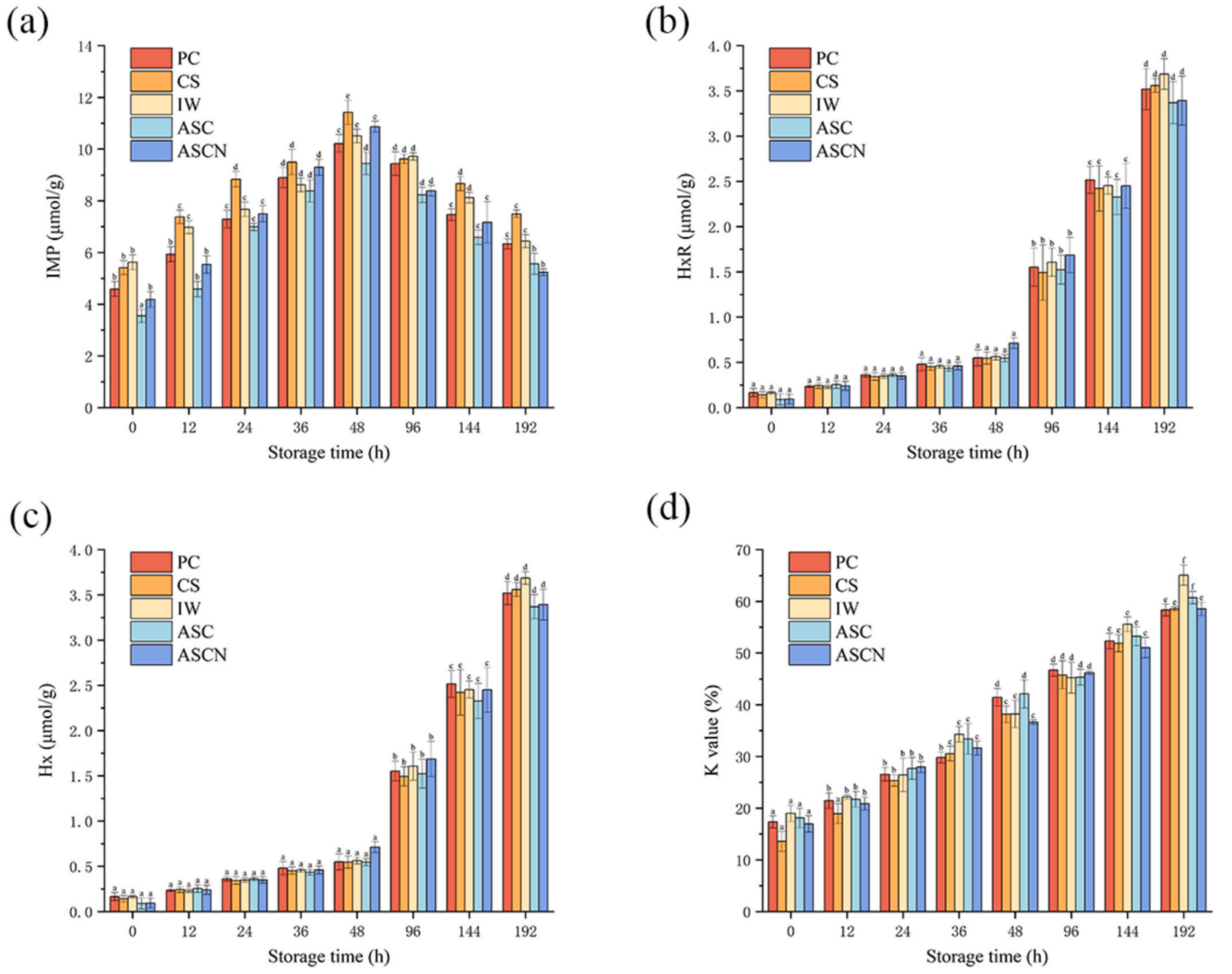

Fig. 5. Changes in IMP (a), HxR (b), Hx (c), and K value (d) of tilapia preserved at 4 °C after different slaughter treatments (PC: cranial euthanasia by percussion; CS: spinal cord crushing euthanasia; IW: euthanasia of fish by immersion in a 1:1 ice-water bath at 3 ± 1 °C; ASC: fish experienced asphyxiation-induced loss of consciousness in a saturated CO_2 environment; ASCN: fish experienced asphyxiation-induced loss of consciousness in a saturated 30 % CO_2 + 70 % N_2 environment). The letter from "a" to "d" are used to describe the significance of differences between the samples ($p < 0.05$).

Table 2
FAAs content (mg/100 g) of Tilapia during storage with different slaughter methods (PC: cranial euthanasia by percussion; CS: spinal cord crushing euthanasia; IW: euthanasia of fish by immersion in a 1:1 ice-water bath at 3 ± 1 °C; ASC: fish experienced asphyxiation-induced loss of consciousness in a saturated CO_2 environment; ASCN: fish experienced asphyxiation-induced loss of consciousness in a saturated 30 % CO_2 + 70 % N_2 environment).

	Asp	Thr	Ser	Glu	Gly	Ala	Cys	Val	Met
PC-0 h	6.01 ± 0.51	11.96 ± 0.2	8.6 ± 0.09	13.2 ± 0.22	112.6 ± 2.69	39.72 ± 1.11	0.82 ± 0.08	6.64 ± 0.18	3.81 ± 0.13
PC-96 h	2.38 ± 0.04	10.16 ± 0.19	2.86 ± 0.04	12.32 ± 0.25	128.48 ± 1.52	56.07 ± 0.83	0.79 ± 0.09	7.11 ± 0.18	3.49 ± 0.11
PC-192 h	2.24 ± 0.1	2.16 ± 0.15	0.58 ± 0.06	15.22 ± 0.45	120.08 ± 1.56	64.28 ± 1.29	5.64 ± 0.92	11.01 ± 0.77	7.86 ± 0.58
CS-0 h	6.07 ± 0.47	20.9 ± 0.4	10.25 ± 0.19	13.53 ± 0.24	110.55 ± 0.69	55.8 ± 0.39	0.81 ± 0.06	7.69 ± 0.11	3.73 ± 0.02
CS-96 h	2.93 ± 0.02	12.68 ± 0.1	4.65 ± 0.02	11.5 ± 0.1	116.23 ± 0.71	43.94 ± 0.3	0.72 ± 0.05	6.73 ± 0.07	3.3 ± 0.02
CS-192 h	1.92 ± 0.04	5.1 ± 0.09	0.37 ± 0.03	11.17 ± 0.21	96.91 ± 0.54	52.81 ± 0.61	2.65 ± 0.36	7.71 ± 0.19	4.69 ± 0.11
IW-0 h	6.65 ± 0.24	17.6 ± 0.37	8.16 ± 0.22	8.84 ± 0.49	129.51 ± 0.71	48.46 ± 0.86	1.31 ± 0.05	8.28 ± 1.17	4.74 ± 1.24
IW-96 h	2.47 ± 0.2	10.49 ± 0.06	3.51 ± 0.12	16.53 ± 0.28	120.13 ± 1.52	56.1 ± 0.61	0.71 ± 0.17	6.5 ± 0.03	3.12 ± 0.07
IW-192 h	2.24 ± 0.03	4.38 ± 0.08	0.38 ± 0.02	15.48 ± 0.35	112.04 ± 1.54	61.96 ± 0.96	3.18 ± 0.17	8.55 ± 0.42	5.13 ± 0.49
ASC-0 h	8.18 ± 0.19	16.87 ± 0.39	9.59 ± 0.21	17.16 ± 0.05	103.77 ± 2.42	65.42 ± 1.33	3.64 ± 0.64	9.62 ± 0.09	6.45 ± 0.24
ASC-96 h	3.69 ± 0.1	13.39 ± 0.35	3.27 ± 0.07	18.5 ± 0.43	153 ± 2.93	50.25 ± 1.15	0.17 ± 0.01	7.38 ± 0.21	3.69 ± 0.12
ASC-192 h	2.28 ± 0.18	7.52 ± 0.04	0.51 ± 0.1	15.05 ± 0.41	139.09 ± 2.86	62.4 ± 1.48	1.17 ± 0.01	7.05 ± 0.2	3.66 ± 0.04
ASCN-0 h	6.03 ± 0.05	21.33 ± 0.55	10.43 ± 0.21	15.22 ± 0.45	120.08 ± 1.56	64.28 ± 1.29	5.64 ± 0.92	11.01 ± 0.77	7.86 ± 0.58
ASCN-96 h	4.12 ± 0.09	16.96 ± 0.44	6.68 ± 0.25	19.14 ± 0.32	145.73 ± 3.93	58.09 ± 1.72	0.99 ± 0.22	6.83 ± 0.1	3.35 ± 0.08
ASCN-192 h	2.24 ± 0.1	2.16 ± 0.15	0.58 ± 0.06	21.41 ± 0.61	72.29 ± 1.61	64.46 ± 1.64	0.85 ± 0.07	7.25 ± 0.14	3.75 ± 0.06
	Ile	Leu	Tyr	Phe	Lys	His	Arg	Pro	
PC-0 h	4.72 ± 0.18	8.09 ± 0.3	4.45 ± 0.2	4.6 ± 0.2	13.83 ± 0.62	14.28 ± 0.62	3.8 ± 0.2	6.97 ± 0.03	
PC-96 h	4.54 ± 0.13	7.8 ± 0.2	4.26 ± 0.14	3.95 ± 0.16	17.63 ± 0.47	13.69 ± 0.39	4.75 ± 0.2	6.61 ± 1.06	
PC-192 h	6.6 ± 0.53	10.5 ± 0.67	8.71 ± 0.61	6.55 ± 0.72	22.41 ± 0.69	19.05 ± 0.47	0.54 ± 0.01	9.65 ± 0.24	
CS-0 h	5.22 ± 0.03	9.34 ± 0.04	4.19 ± 0.03	4.49 ± 0.02	29.02 ± 0.16	21.49 ± 0.12	5.9 ± 0.01	7.38 ± 0.84	
CS-96 h	4.26 ± 0.13	7.39 ± 0.02	4.03 ± 0.01	3.83 ± 0.06	22.22 ± 0.26	16.61 ± 0.15	4.36 ± 0.04	5.15 ± 0.01	
CS-192 h	4.44 ± 0.04	7.65 ± 0.01	3.21 ± 0.03	4.63 ± 0.06	11.83 ± 0.16	12.58 ± 0.2	0.26 ± 0.04	7.42 ± 0.13	
IW-0 h	6.56 ± 0.64	10.73 ± 0.45	4.76 ± 0.27	4.72 ± 0.19	16.15 ± 0.29	12.44 ± 0.23	4.72 ± 0.14	6.51 ± 1.43	
IW-96 h	3.85 ± 0.06	6.65 ± 0.03	3.7 ± 0.01	3.31 ± 0.02	9.82 ± 0.14	12.95 ± 0.21	2.36 ± 0.02	6.28 ± 0.93	
IW-192 h	5.09 ± 0.16	8.5 ± 0.23	5.27 ± 0.15	6.3 ± 0.17	14.39 ± 0.4	17.07 ± 0.47	0.74 ± 0.02	9.61 ± 0.17	
ASC-0 h	5.96 ± 0.08	9.87 ± 0.1	6.78 ± 0.15	4.12 ± 0.18	25.01 ± 0.83	20.65 ± 0.68	1.18 ± 0.04	8.63 ± 0.31	
ASC-96 h	5.85 ± 0.21	9.82 ± 0.26	4.63 ± 0.14	3.68 ± 0.12	15.18 ± 0.41	10.67 ± 0.32	3.84 ± 0.15	6.04 ± 10.93	
ASC-192 h	4.32 ± 0.09	7.69 ± 0.18	4 ± 0.09	5.91 ± 0.11	23.46 ± 0.78	18.15 ± 0.6	4.6 ± 0.17	16.16 ± 11.23	
ASCN-0 h	6.6 ± 0.53	10.5 ± 0.67	8.71 ± 0.61	4.55 ± 0.92	22.41 ± 0.69	19.05 ± 0.47	0.54 ± 0.01	9.65 ± 0.24	
ASCN-96 h	4.35 ± 0.27	7.54 ± 0.35	3.81 ± 0.16	3.83 ± 0.14	14.37 ± 0.62	16.55 ± 0.69	4.08 ± 0.19	7.37 ± 0.26	
ASCN-192 h	4.67 ± 0.14	7.91 ± 0.23	3.99 ± 0.08	4.12 ± 0.09	27.06 ± 0.81	19.89 ± 0.56	6.15 ± 0.21	7.16 ± 0.92	

Abbreviations: glycine, Gly; arginine, Arg; leucine, Leu; valine, Val; alanine, Ala; serine, Ser; proline, Pro; isoleucine, Ile; phenylalanine, Phe; lysine, Lys; threonine, Thr; methionine, Met; histidine, His; tyrosine acid, Tyr; aspartic acid, Asp; cysteine, Cys; glutamic acid, Glu.

work done in the second compression divided by the work done in the first compression (Manju et al., 2007). A high value of this parameter implies good elasticity and chewability of the fish, thus reflecting good textural properties. The chewability of all the samples showed a decreasing trend as the storage time increased. According to Fig. 4, it can be seen that the CS sample maintained a higher level during the storage period compared to all other treatment samples. This suggests that CS-treated tilapia samples are less prone to fracture and mechanical damage. This shows that the treatment CS can better maintain the textural structure of tilapia.

3.7. Analysis of K-value and related compounds

ATP degradation is among the major physiochemical changes in postmortem muscle of fish. Measuring the combined nucleotide degradation compound concentrations is an effective method for ascertaining freshness. Therefore, K-values and related compounds are extensively employed as indicators of freshness. IMP was the most significant contributor to the freshness of fish, but it was further degraded to HxR and Hx, leading to a decrease in freshness and deterioration of flavour (Wang et al., 2024). As can be seen from Fig. 5 (a), the IMP content showed a tendency of increasing and then decreasing during the cold storage process. It indicates that the freshness of tilapia began to decrease after 48 h, which was caused by the combined effect of endogenous and exogenous enzymes (Huang et al., 2017). The decrease of IMP caused the gradual loss of tilapia freshness. Also, accumulated HxR and Hx, as well as some amino acids and peptides, may contribute to the bitter taste of meat. Autolytic decomposition of nucleotides produces Hx, but bacteria also form Hx (Wang, Lin, Lu, Afrin, Tian, Hirai, et al., 2024). During storage, the HxR and Hx contents of tilapia in the CS and ASCN samples were remarkably different from other samples, suggesting that the CS and ASCN samples had a significant effect on the deterioration of tilapia, in agreement with the TVB-N results. Fish products with a K-value of less than 20 % are considered extremely fresh, K-values of less than 60 % are considered moderately fresh, and K-values of more than 60 % are considered un-fresh (Shen et al., 2015). The variation of K-value of tilapia during refrigerated storage are shown in Fig. 5 (d). K-value of all samples at 0 h after slaughter were less than 20 %. With the prolongation of the cold storage time, the K-value of all five samples of samples were increasing, indicating a gradual decrease in freshness. The end of storage at 192 h, only sample IW had a k value greater than 60 %.

3.8. Analysis of pH

After slaughter, the glycogen in the flesh continues to metabolize, allowing lactic acid to accumulate, resulting in a drop in pH. Therefore, pH is an important indicator that can reflect the quality of fish. As can be seen from Fig. 3 (b), the pH of tilapia showed a tendency of decreasing and then increasing during the storage after the five slaughtering methods. This is because after the death of fish, under the condition of anaerobic, glycogen and adenosine triphosphate (ATP) in the fish body undergo fermentation, producing lactic acid, phosphoric acid and so on, so that the pH of the fish decreases and the acidity increases (Weiqing et al., 2024). As the rate of ATP depletion and anaerobic glycolytic reactions increased, pH decreased to a minimum at 48 h post-mortem in all samples. With the prolongation of storage time, glycogen and ATP were completely consumed and acid production ended. Hydrolytic enzymes and microorganisms in the fish gradually break down the proteins and amino acids of the fish, producing some low molecular alkaline substances (Chu et al., 2023). This leads to an increase in the pH value of the fish, accompanied by the development of a rancid odor (Shen et al.,

2015).The initial pH of CS was 6.88, which was significantly higher than the other samples ($p < 0.05$). The large increase in pH during the later stages of storage may be due to bacterial contamination. This is because the metabolic activity of bacteria breaks down nitrogen compounds to form compounds such as ammonia and trimethylamine, which increase pH (Ran et al., 2011). The two slaughtering methods, CS and ASCN samples, were effective in delaying the degradation of glycogen and ATP, as well as inhibiting the catabolism of fish proteins by microorganisms and endogenous enzymes in fish.

3.9. Analysis of FAAs

FAAs play an important part in flavour exploitation of aquatic products by imparting various taste attributes such as freshness, sweetness and bitterness. The flavour properties exhibited by FAAs are essentially dependent on the structure of their functional groups and the presence of specific side chains (denoted as R groups) in the molecular composition (Mingyu et al., 2023). Most D-amino acids are predominantly sweet; the shorter side chains Met, Gly, Thr, Ala and Ser are predominantly sweet and umami; and the larger and longer side chains Tyr, Phe, Ile, Val and Leu are predominantly bitter (Ma, Yang, Qiu, Mei, & Xie, 2021). The 17 detected FAAs were quantified as shown in Table 2. Throughout the refrigeration period, both the content of free amino acids and the total free amino acid content in all samples exhibited a consistent upward trend. It is the decomposition of proteins and peptides by proteolytic enzymes that leads to an increase in FAA, while the decrease in FAA content is a result of the interaction of these amino acids with other compounds (Chu et al., 2023). The most enriched FAAs in tilapia samples were Gly, followed by Ala and Lys. Ozden (Özden, 2005) found that glycine, glutamic acid, aspartic acid and alanine play an active role in providing fresh sweetness to aquatic products. Phe content was significantly lower in all samples of tilapia treated with CS and ASCN compared to PC sample. These results suggest that the reduction of flavour-enhancing amino acids and the accumulation of flavour-reducing amino acids influence the changes in tilapia flavour. The use of CS treatments can effectively delay this process during storage, thus maintaining the high food value of tilapia.

4. Conclusion

The results showed that different slaughtering methods had a greater effect on the biochemical characteristics and preservation of tilapia. Spinal cord crushing euthanasia shortened the time to death for tilapia. Tilapia treated by this method showed better texture, flavour and freshness after 192 h of refrigeration. Therefore, spinal cord crushing euthanasia method has great potential in the aquatic processing industry.

Funding

This work was supported by National Key Research and Development Program of China (2023YFD2401402) and Shanghai Agricultural Science and Technology Innovation Program (2023-02-08-00-12-F04614).

CRediT authorship contribution statement

Xinrui Yang: Writing – original draft, Software, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Jun Mei:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Jing Xie:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration 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.

Data availability

The authors do not have permission to share data.

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