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Relevance of cathepsins activity and texture in slightly acidic electrolyzed water-slurry iced mackerel (*Pneumatophorus japonicus*)

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ABSTRACT

The incorporation of SAEW and SI can effectively maintain the characteristics of texture in marine fish. This study aimed to investigate the relevance of cathepsin activity for texture and the establishment of a shelf-life model of mackerel (*Pneumatophorus japonicus*) stored at 4 °C. Before the cold storage, mackerel samples were exposed to flake ice (Control), slurry ice (SI), and slightly acidic electrolyzed water- slurry ice (SAEW-SI), respectively. Then the TVC, K-value, cathepsin activity, texture, and sensory attributes were investigated. The results showed that the TVC and K-value of samples in SAEW-SI group was significantly lower by approximately 1 log CFU/g and 17% than those in Control group ($P < 0.05$). Meanwhile, there was a tendency to first increase and then decrease on the activities of cathepsin B, D and L. Results of texture profile analysis (TPA) clarified that SAEW-SI can markedly suppress the decrease of hardness, springiness and chewiness ($P < 0.05$). During the experimental period, the highest sensory scores were obtained in SAEW-SI group. In addition, the heat map of correlation analysis suggested that texture attributes (hardness) were negatively correlated with cathepsin B (r = -0.66), cathepsin D (r = -0.49), and cathepsin L (r = -0.69), respectively. According to the principal component analysis (PCA) and analysis of linear regression, SAEW-SI treatment could effectively maintain mackerel quality and extend the estimated shelf-life of mackerel by at least 5 days compared to Control group. Therefore, SAEW-SI could be suggested as a novel strategy for cold-chain transportation in seafood industry.

1. Introduction

Mackerel (*Pneumatophorus japonicus*), a nutrient-rich fish species, can be served as an efficacious dietary source for human beings to obtain valuable polyunsaturated fatty acids (PUFA) (Apang et al., 2020). However, mackerels are prone to spoilage. As many studies have shown, the deterioration in the quality of aquatic products is caused by the changes in the activity of endogenous enzyme and the degradation of microorganisms (Lan et al., 2020; Zarandona et al., 2020). In terms of fish quality assessment, there were limited reports on the cathepsin activity, and the investigation mainly focused on microbial contamination. However, it was reported that cathespsins play an important role in the degradation of myofibrillar protein and the decrease of texture (Jiang et al., 2022). The strong autolytic capacity of endogenous proteases induces the hydrolysis of myofibrillar proteins, and thus leads to the disorganization of myofibrillar structure in postmortem muscle (Fidalgo, Delgadillo, & Jorge, 2020). These endogenous proteases, including lysosomal cathepsins, proteasomes, calpains, and caspases, were discovered in recent studies (Godiksen et al., 2009; Kumar et al., 2020; Saccà et al., 2016). Lysosomal cathepsins, especially cathepsin B, cathepsin D, and cathepsin L, are the main proteolytic enzymes that cause muscle degradation (Delbarre-Ladrat et al., 2006; Li et al., 2021). They are present in the form of zymogens originally and then convert into mature forms with a high level of enzymatic activity, which is liable

Abbreviations: ACC, Available chlorine concentration; ACP, Acid phosphatase; AMP, Adenosine monophosphate; ATP, Adenosine triphosphate; Hx, Hypoxanthine; HxR, Hypoxanthine riboside; IMP, Inosine monophosphate; ORP, Oxidation-reduction potential; PCA, Principle component analysis; TPA, Texture profile analysis; TVC, Total viable count; SAEW, Slightly acidic electrolyzed water; SI, Slurry ice.

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for proteins degradation and the formation of flavor (Hu et al., 2022). Therefore, the determination of cathepsin activity may have great significance for the quality evaluation of fish products.

Precooling is considered as an effective pretreatment method for preventing quality deterioration in the postharvest cold-chain of marine fish. Flake ice is a conventional ice commonly used for precooling marine fish. Nonetheless, this method have some drawbacks, such as long cooling times, insufficient chilling power, and causing physical damage to the fish (Lan et al., 2021). Slurry ice (SI) is an alternative cooling medium comprising ice microcrystals, liquid water, and one or more freezing-point depressants (Zhang et al., 2022). As an emerging chilling medium, it is gradually applied to the preservation of marine species. Numerous studies have shown that the comprehensive performance of SI is higher than that of flake ice (Ruhama et al., 2020). In fact, marine fish treated with SI had higher sensory scores and longer shelf-life than fish treated with flake ice (Liu, Lan, et al., 2021).

Slightly acidic electrolyzed water (SAEW) is recognized as a safe and pollution-free chemical disinfectant (Zhang, Zhang, Zhao, et al., 2019). SAEW can significantly slow down the rate of microbial proliferation owing to the high available chlorine concentration (ACC). In addition, the near-neutral pH value protects the related machines from erosion and effectively maintains the quality attributes of food, such as appearance, flavor and texture (Li, Ren, et al., 2017). The incorporation of SI and essential oils, ozone, or chitosan can prolong the shelf-life of aquatic products and maintain their biochemical, textural and microbial quality (Navarro-Segura et al., 2019; Zhang et al., 2022; Zhao et al., 2022). However, few studies have investigated the effects of SAEW-SI on the preservation of aquatic products, especially on endogenous proteases in postmortem muscle.

The purpose of this study was to evaluate the effects of cathepsin B, D, and L on the texture of mackerel, and the inhibitory mechanism of cathepsin activity was preliminarily elaborated. The relationship between cathepsins and texture was investigated through microbial, chemical and correlation analysis. Meanwhile, a shelf-life model was established to assess the performance of SAEW-SI on mackerel.

2. Materials and methods

2.1. Preparation of mackerel

Mackerel (*Pneumatophorus japonicus*) with an average weight of (250 ± 20) g and mean length of (28.0 ± 0.5) cm were purchased from a local market (Shanghai, China). Fresh samples were selected for the experiment and placed in a polyethylene foam box filled with flake ice and delivered to the laboratory within 30 min.

2.2. Preparation of slurry ice with different ice mediums

Flake ice was formed within 3 min after tap water was fed into the ice maker (Scotsman Ice, Italia). Slurry ice was obtained by running tap water (salinity: 3.3%) into an RF-1000-SP prototype (Nantong Ruiyou Trade Co., Ltd., Jiangsu, China). Similarly, slightly acidic electrolyzed water-slurry ice (SAEW-SI) was prepared after replacing tap water with slightly acidic electrolyzed water (salinity: 3.3%) (PG3.0, OSG Co., Ltd., Japan). The pH value of SAEW used in the present work was 6.12 ± 0.02, ACC and oxidation-reduction potential (ORP) was 60 mg/L and 1108 ± 2.31 mV respectively. The pH and ORP of electrolyzed water were promptly determined by pH and ORP meter, and ACC was measured by the ACC determination reagent (OSG Co., Ltd., Japan).

2.3. Handling of mackerel

Samples were divided into three groups randomly. Samples in the Control group were surrounded by flake ice. The other treated groups were surrounded by slurry ice (SI) and slightly acidic electrolyzed water-slurry ice (SAEW-SI), respectively. Temperature probes were prepared and placed in mackerels to monitor the internal temperature changes during storage. Thereafter, all samples were stored at 4 °C and all determinations were conducted in triplicate.

2.4. Microbiological analysis

The total viable count (TVC) of all samples was determined. Samples (10 g) cut from the anterior dorsal muscle were moved to a stomacher bag containing 90 mL of sterilized normal saline (0.1%) and homogenized for 60 s. 0.1 mL serial dilutions of fish homogenates were pipetted onto the surface of plate count agar (PCA, Base Bio-Tech, Hangzhou, China). After incubation at 30 ± 1 °C for 72 ± 3 h, the TVC of samples were measured according to the dilution multiple and the quantity of colony-forming units in plate.

2.5. K-value

The extraction and subsequent measurement of adenosine triphosphate (ATP) and its related substances refer to the method of Li, Zhang, et al. (2017) with slight modification. Briefly, 5 g of fish samples were homogenized with 10 mL 10% (v/v) perchloric acid (Sinopharm Chemical Reagent Co., Ltd., China) and centrifuged at 8000 ×*g* for 15 min to obtain the supernatant. The precipitate was mixed with 10 mL 5% (v/v) perchloric acid and centrifuged to collect the second supernatant. Then, the two supernatants were combined, adjusted to pH 6.5, and analyzed by high-performance liquid chromatography (HPLC) (LC-2010CHT, Shimadzu Corporation, Japan). The photo-diode array (PDA) detector and Waters C18 column (Waters, Milford, MA, USA) were the main equipment for analyzing target analytes. 98% potassium phosphate buffer (0.05 mol/L, pH 6.8) and 2% methanol were selected as the mobile phase of HPLC, and the flow rate was set to 1.0 mL/min. The target was separated by isocratic elution mode and detected at the UV wavelength of 254 nm. ATP and its related substances were qualitatively analyzed based on the retention time of standards and quantitatively analyzed according to the peak area of standards. Adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), hypoxanthine riboside (HxR), and hypoxanthine (Hx) were provided by Sigma-Aldrich (Shanghai) Trading Co., Ltd. Based on the method of Liu, Lan, et al. (2021), K-value was calculated according to the following formula:

$$K-value\ (\%) = \frac{HxR + Hx}{ATP + + ADP + AMP + IMP + HxR + Hx} \times 100$$

2.6. Cathepsins B, D and L activity

3 g of mackerel meat and 27 mL of ice-cold PBS buffer solution (0.01 M, pH7.4) were homogenized and centrifuged at 12,000 ×*g* at 4 °C for 20 min. The supernatant was filtered and stored at −20 °C for the determination of enzymatic activity. The activities of cathepsin B, D and L were measured using assay kits.

2.7. Texture profile analysis (TPA)

TPA was determined based on the modified method described by Lan et al. (2020). Samples were cut into fillets (2 cm × 2 cm × 1.5 cm) and compressed twice at ambient temperature (12–18 °C) using texture analyzer (SMS TA.XT plus, Stable Micro Systems Ltd., UK) equipped with a 50 mm cylindrical probe (P/50). The measurement speed was maintained at 1 mm/s, the compression interval was 5 s, and the compression ratio was 50%. All samples were determined in parallel for 6 times.

2.8. Sensory evaluation

The sensory evaluation of samples was performed throughout the

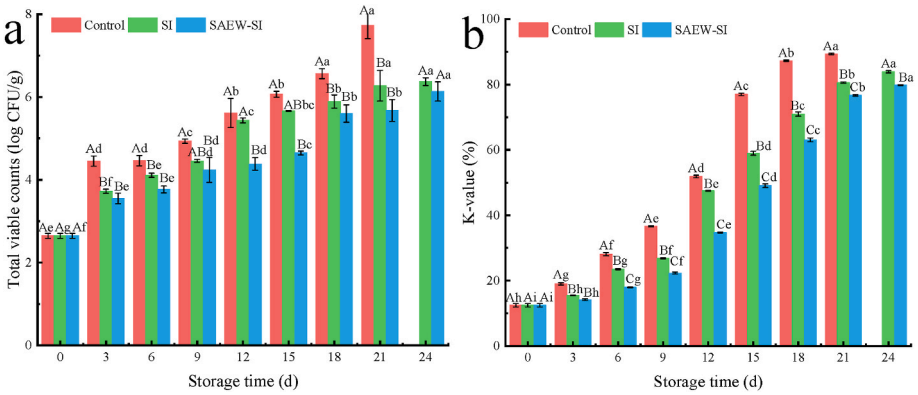

Fig. 1. Changes in TVC (a) and K-value (b) in mackerel during storage.

storage period, using 10 trained professionals with similar product evaluation experience. Fish samples were scored according to the color, texture, and odor, which were individually weighted 40%, 30%, and 30% of overall acceptability, respectively (Liu, Lan, et al., 2021). Results of sensory analysis were expressed using a 10-point Hedonic scale, ranging from 1 (unacceptable) to 10 (extremely like). Scores of less than 5 were assumed to be associated with rejection.

2.9. Statistical analysis

All data were mean ± standard deviation (SD) of three samples. Statistical analysis was performed through One-way analysis of variance using SPSS 20.0 software (IBM, Armonk, NY, USA). Significant differences were considered as $P < 0.05$ according to the Duncan's multiple range test. Principal component analysis (PCA) and correlation analysis were carried out using Origin 9.8 (Northampton, MA, USA).

3. Results and discussion

3.1. Microbiological analysis

The TVC can effectively reflect the degree of corruption (Azizi-Lalabadi et al., 2020). Typically, TVC at 7.0 log CFU/g was considered as the spoilage point for marine fish (Boonsiriwit et al., 2021). The initial TVC of samples was 2.65 log CFU/g, which indicated their freshness (Fig. 1a). The TVC of all samples increased significantly ($P < 0.05$) over time and remained below the acceptable limit during the first 18 days of storage. However, samples in the Control group experienced the fastest growth in TVC during storage and exceeded the acceptable limit at day 21, indicating samples in the Control group had severe microbial spoilage. Samples in the SI and SAEW-SI groups also experienced a rapid growth in TVC, but to a lesser extent ($P < 0.05$) compared to the Control group, with TVC reaching 6.37 and 6.14 log CFU/g at day 21, respectively. In general, SAEW-SI showed the best bacteriostatic effect during the whole storage period, in the following, SI and Control ($P < 0.05$). Chlorine compounds (HOCl, Cl_2, ^{-}OCl) in SAEW can destroy the cell wall and cell membrane of bacteria, resulting in the leakage of intracellular compounds (K^+, protein and DNA) that maintain bacterial life activities (Liu, Zhang, et al., 2021). In addition, the high cooling rate and fluidity of SI enable it to cover the surface of marine fish more completely, thereby showing a more obvious inhibitory effect on microbial reproduction (Wang et al., 2019).

3.2. K-value

K-value is a typical freshness index to evaluate the freshness of fish, which can reflect the degradation degree of ATP-related compounds (Yu et al., 2020). Fish with first-grade freshness have K-value < 20%, and those that are spoiled have K-value ≥ 60% (Chen et al., 2022). As described in Fig. 1b, there was no significant difference in K-value among groups in the first three days of storage ($P>0.05$). The K-value in all groups was close to 20% at day 3, which indicated that samples remained first-grade freshness. After that, the K-value of Control samples increased significantly ($P < 0.05$) to 77.03% at day 15, while the K-value of samples in SI and SAEW-SI groups were only 58.9% and 49.0%, respectively. These results were consistent with the changes in TVC. The bacteriostatic effect of SAEW-SI can affect the ability of microorganisms to secrete acid phosphatase (ACP), thus preventing the degradation of ATP and delaying the increase of K-value (He et al., 2022).

3.3. Changes in cathepsin activity

Cathepsins exist in lysosomes and function to maintain the homeostasis of cell metabolism by degrading heterophagous and autophagic substances (Stoka et al., 2016). Once the lysosomal membranes are disrupted, cathepsins can be released into the myofibrils, which accelerate the muscular degradation process (Lee et al., 2021). As shown in

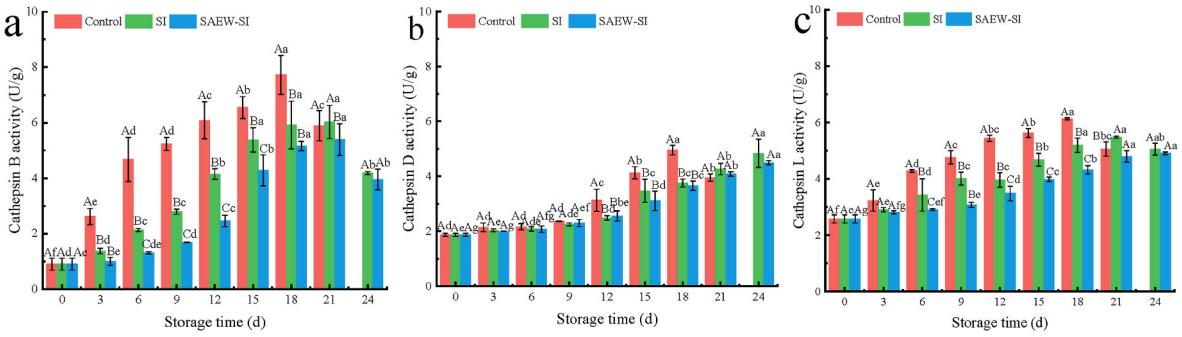

Fig. 2. The changes of cathepsin B (a), cathepsin D (b), and cathepsin L (c) activities in mackerel during storage.

Table 1
Changes in hardness, springiness, and chewiness in mackerel during storage.

Indexes	Group	Storage time (d)								
		0	3	6	9	12	15	18	21	24
Hardness (N)	Control	1704.04 ± 97.86^{Aa}	577.46 ± 44.78^{Ccd}	547.98 ± 35.59^{Bd}	690.08 ± 112.53^{Cc}	607.47 ± 43.62^{Ccd}	745.65 ± 23.42^{Cbc}	771.84 ± 38.79^{Bb}	522.14 ± 54.36^{Cd}	–
	SI	1704.04 ± 97.86^{Aa}	843.31 ± 159.09^{Bb}	626.35 ± 73.61^{Bcd}	805.17 ± 103.60^{Bb}	713.27 ± 23.96^{Bbc}	835.14 ± 104.89^{Bb}	817.27 ± 61.61^{Bb}	752.96 ± 21.44^{Bbc}	572.34 ± 35.80^{Bd}
	SAEW-SI	1704.04 ± 97.86^{Aa}	1097.09 ± 89.93^{Acde}	1288.44 ± 238.86^{Abc}	1354.32 ± 214.14^{Abd}	1068.70 ± 562.56^{Ade}	1144.32 ± 133.70^{Abce}	887.04 ± 48.25^{Af}	838.95 ± 59.29^{Af}	691.43 ± 20.15^{Af}
Springiness (mm)	Control	0.94 ± 0.02^{Aa}	0.84 ± 0.05^{Babc}	0.86 ± 0.10^{Aab}	0.79 ± 0.10^{Bbc}	0.75 ± 0.06^{Cbc}	0.79 ± 0.03^{Bbc}	0.87 ± 0.06^{Bab}	0.74 ± 0.03^{Bc}	–
	SI	0.94 ± 0.02^{Aa}	0.81 ± 0.05^{Bb}	0.84 ± 0.12^{Aab}	0.81 ± 0.03^{Bab}	0.88 ± 0.03^{Bab}	0.85 ± 0.02^{Bab}	0.87 ± 0.06^{Bab}	0.78 ± 0.03^{Bb}	0.86 ± 0.05^{Bab}
	SAEW-SI	0.94 ± 0.02^{Aab}	0.94 ± 0.03^{Aab}	0.82 ± 0.06^{Ac}	0.93 ± 0.07^{Aa}	0.95 ± 0.07^{Aab}	0.91 ± 0.04^{Aa}	0.96 ± 0.04^{Aab}	0.84 ± 0.07^{Abc}	0.92 ± 0.11^{Aa}
Chewiness (g)	Control	373.50 ± 3.63^{Aa}	139.21 ± 18.61^{Cd}	140.00 ± 23.76^{Cd}	167.80 ± 5.66^{Ccd}	165.15 ± 43.58^{Ccd}	205.30 ± 37.20^{Cbc}	193.85 ± 37.20 ^{Cbc}	234.71 ± 8.40^{Bb}	–
	SI	373.50 ± 3.63^{Aa}	210.09 ± 21.54^{Bcde}	196.49 ± 17.23^{Bde}	204.95 ± 14.84^{Bde}	183.28 ± 14.80^{Be}	271.80 ± 14.11^{Bb}	242.55 ± 22.70^{Bb}	276.32 ± 15.53^{Bb}	244.87 ± 61.39^{Bcd}
	SAEW-SI	373.50 ± 3.63^{Abc}	244.63 ± 56.61^{Ad}	373.07 ± 56.20^{Abc}	440.83 ± 11.88^{Aa}	421.74 ± 10.83^{Aab}	469.72 ± 36.65^{Aa}	382.20 ± 17.41^{Abc}	361.17 ± 11.33^{Ac}	383.36 ± 70.35^{Ad}

Note: The results are expressed as Means ± SD, different superscript lowercase letters represent significant differences within groups ($P < 0.05$), while different superscript uppercase letters represent significant differences between groups ($P < 0.05$).

Fig. 2, the activities of cathepsin B, D and L in the Control group increased first, then decreased, and reached the maximum at day 18. The transformation from inactive zymogens to activated cathepsins may be the reason for the initial enhancement of cathepsin activity (Zhang, Zhang, Jia, et al., 2019). The interaction between the myofibrillar fraction and the released cathepsins probably accounted for the subsequent decrease of cathepsin activity. Furthermore, compared to Control and SI groups, samples in SAEW-SI group showed a significant decrease in cathepsin activity ($P < 0.05$). The results demonstrated the effectiveness of SAEW-SI in reducing cathepsin activity, which is likely to play an important role in the maintenance of texture. On the one hand, due to its specific ORP and ACC, SAEW can inhibit the protease activity of aquatic products during storage (Yan et al., 2020). On the other hand, the high heat transfer capacity of SI leads to the formation of smaller ice crystals, which can weaken the mechanical damage of ice crystals to lysosomes and slow down the release of cathepsins (Wang et al., 2021). In addition, the activity of cathepsin B in fish samples was higher than that of cathepsin D and L, suggesting that cathepsin B might contribute more to the proteolysis of proteins and muscle softening.

3.4. Changes in TPA

The TPA parameters, such as hardness, springiness, and chewiness, were the considerable indicators to evaluate the texture of sample (Yan et al., 2020). The initial structure degree of food can be expressed by its hardness (Wee et al., 2018). As presented in Table 1, the hardness of Control group was dwindled most during storage, followed by SI and SAEW-SI treated samples ($P < 0.05$). Springiness represents the ability to recover the height of food after applying an external force, while chewiness represents the energy required to chew a solid food into a state that can be swallowed. (Liu et al., 2020; Yu et al., 2022). At the end of storage, the springiness and chewiness of all samples were significantly ($P < 0.05$) lower than the initial values, although they showed no obvious regularity during storage. Interestingly, samples in SAEW-SI group had the highest springiness and chewiness values at day 24, which were 0.92 nm and 383.36 g respectively ($P < 0.05$). Overall, SAEW-SI can better maintain TPA properties than SI and flake ice alone. The result was in coincidence with that of cathepsin activity. The mechanism of muscle softening has not been fully explained, but some researchers have emphasized the prominent role of lysosomal cathepsin activity (Li et al., 2022).

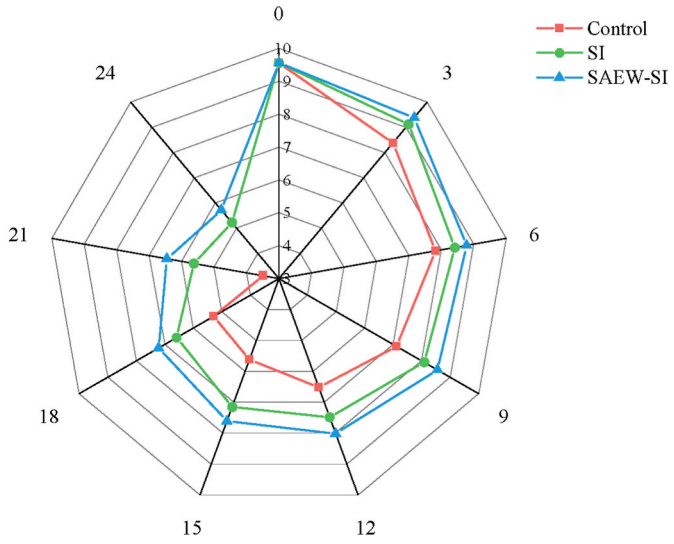

Fig. 3. Radar plot of sensory evaluation (mean scores) in mackerel during storage.

3.5. Sensory evaluation

Sensory analysis is an intuitive method to evaluate the properties of foods and materials (Cai et al., 2022). As described in Fig. 3, the sensory scores of all indicators (color, texture, odor, and overall acceptability score) were 9.55 at day 0, which means that mackerel was in good freshness and SAEW-SI treatment did not deteriorate the quality of mackerel. However, the sensory scores in all groups decreased gradually with the extension of storage. Among them, the sensory scores in Control group decreased most and approached the unacceptable threshold (5 points) at day 18 ($P < 0.05$). After 21 days of storage, samples in Control group showed the spoilage characteristics of fishy-smell and discoloration, and their scores dropped below the acceptable level. On the contrary, the sensory scores in SI and SAEW-SI groups were significantly higher than that in Control group ($P < 0.05$), which were 5.62 and 6.45, respectively. It is worth noting that the sensory scores of samples in SI and SAEW-SI groups were still higher than 5 points at the end of storage, and the samples treated with SAEW-SI had better sensory properties than SI. It was in coincidence with the above results, which indicated

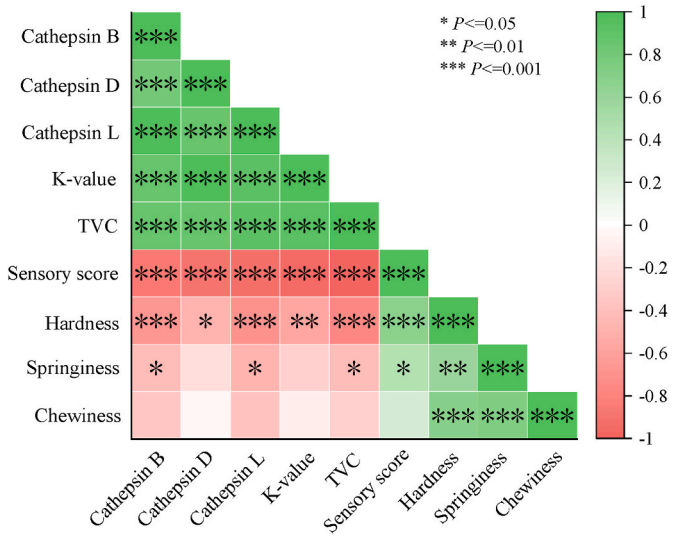

Fig. 4. The heat map of correlation analysis for indicators in mackerel.

that SAEW-SI is likely to delay the quality deterioration of mackerel by inhibiting biochemical reactions such as bacterial activity and oxidation reaction (Yu et al., 2020).

3.6. Correlations analysis

It is of great significance to analyze the correlation of all groups among indicators. Correlations analysis can reflect the relationship between variable indicators (Bu et al., 2022). As demonstrated in Fig. 4, a significant correlation with texture (hardness) was obtained for cathepsin B (r = -0.66), cathepsin D (r = -0.49), cathepsin L (r = -0.69) and TVC (r = -0.75; all $P < 0.05$). Thus, texture deterioration had a strong relationship with microorganism growth and cathepsin activity. It is reported that increasing the inhibitory activity of cathepsin B or cathepsin D could significantly improve the softening of fish (Zhang & Xie, 2020; Zhong et al., 2021). Furthermore, several reports have shown that cathepsin L is extremely active in the softening of muscle (Qiu et al., 2020; Shen et al., 2022). Moreover, strong significant correlations with sensory score were obtained for cathepsin B (r = -0.85), cathepsin D (r = -0.89), cathepsin L (r = -0.90) and TVC (r = -0.97; all $P < 0.05$), and the similar correlation with K-value were obtained for cathepsins and TVC. The texture attributes and quality of mackerel deteriorated along with the increase in cathepsins activities and TVC during storage, indicating that cathepsin B, D and L were likely to be involved in the quality deterioration of mackerel, thus leading to the texture softening of muscle.

In postmortem mackerel, the relationship between factors affecting texture was depicted in Fig. 5. At the beginning of storage, lysosomal destruction leads to the release of cathepsins, resulting in the hardness decrease of mackerel. Moreover, the proliferation of microorganisms decreased the pH, which further promoted the activities of cathepsins. With the extension of storage, microorganisms will release some extracellular enzymes and act on myofibrillar protein together with cathepsins. In contrast, SAEW-SI can effectively change this situation and maintain the hardness and sensory properties of mackerel. This is mainly due to three aspects: (1) the bactericidal action of chlorine compounds; (2) the effect of low temperature, derived from the high cooling rate of SI; (3) the tiny spherical particles of SAEW-SI insulate the oxygen and reduce the damage to lysosomes.

3.7. Principal component analysis (PCA) of indicators

PCA was used for linear combination to achieve dimensionality reduction reflecting data information through fewer comprehensive indexes. As described in Table 2, the first two principal components interpreted 90.2% of the total variance with 70.7% PC1 and 19.5% PC2, which suggested that the treatment has a strong correlation with the quality of samples during storage. The projection distribution of PC1 and PC2 on the two-dimensional plane was reflected in Fig. 6a. Texture attributes (hardness, springiness and chewiness) and sensory score were located in the negative side of PC1 axis, which indicated that they decreased with the storage time. An obtuse angle between hardness and cathepsins was observed, indicating that there was a negative correlation between hardness and cathepsins, which was consistent with the results of correlation analysis in the heat map. In addition, the spatial projection of samples showed a significant difference among different treatments as well as storage time (Fig. 6b). Overall, the dots in three

Table 2
Correlation of variables to factors in PCA based on factor loadings.

Variables	PC1 (70.7%)	PC2 (19.5%)
Cathepsin B	0.37	0.04
Cathepsin D	0.35	0.32
Cathepsin L	0.38	0.05
K-value	0.37	0.26
TVCs	0.38	0.09
Sensory Score	-0.38	-0.12
Hardness	-0.31	0.32
Springiness	-0.21	0.53
Chewiness	-0.17	0.65

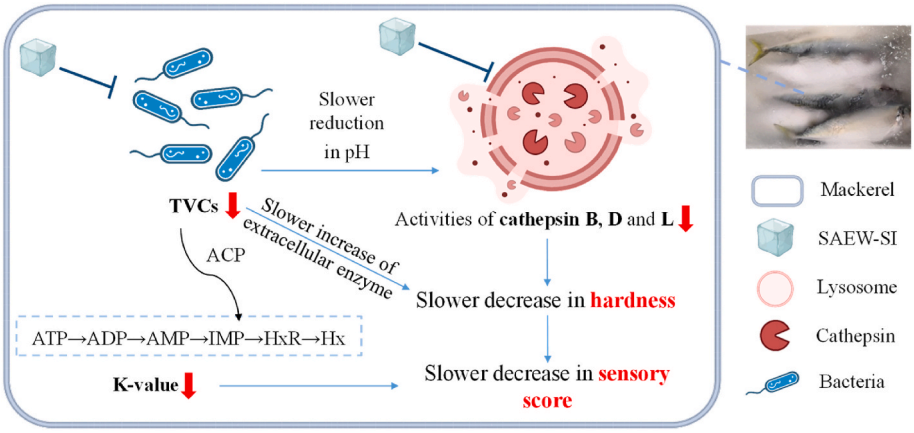

Fig. 5. Schematic representation of the effect of slightly acidic electrolyzed water-slurry ice (SAEW-SI) on the changes of cathepsins and microorganisms in mackerel (*Pneumatophorus japonicus*) during storage. The downward arrows indicate the decrease in parameter changes compared to the Control and SI groups.

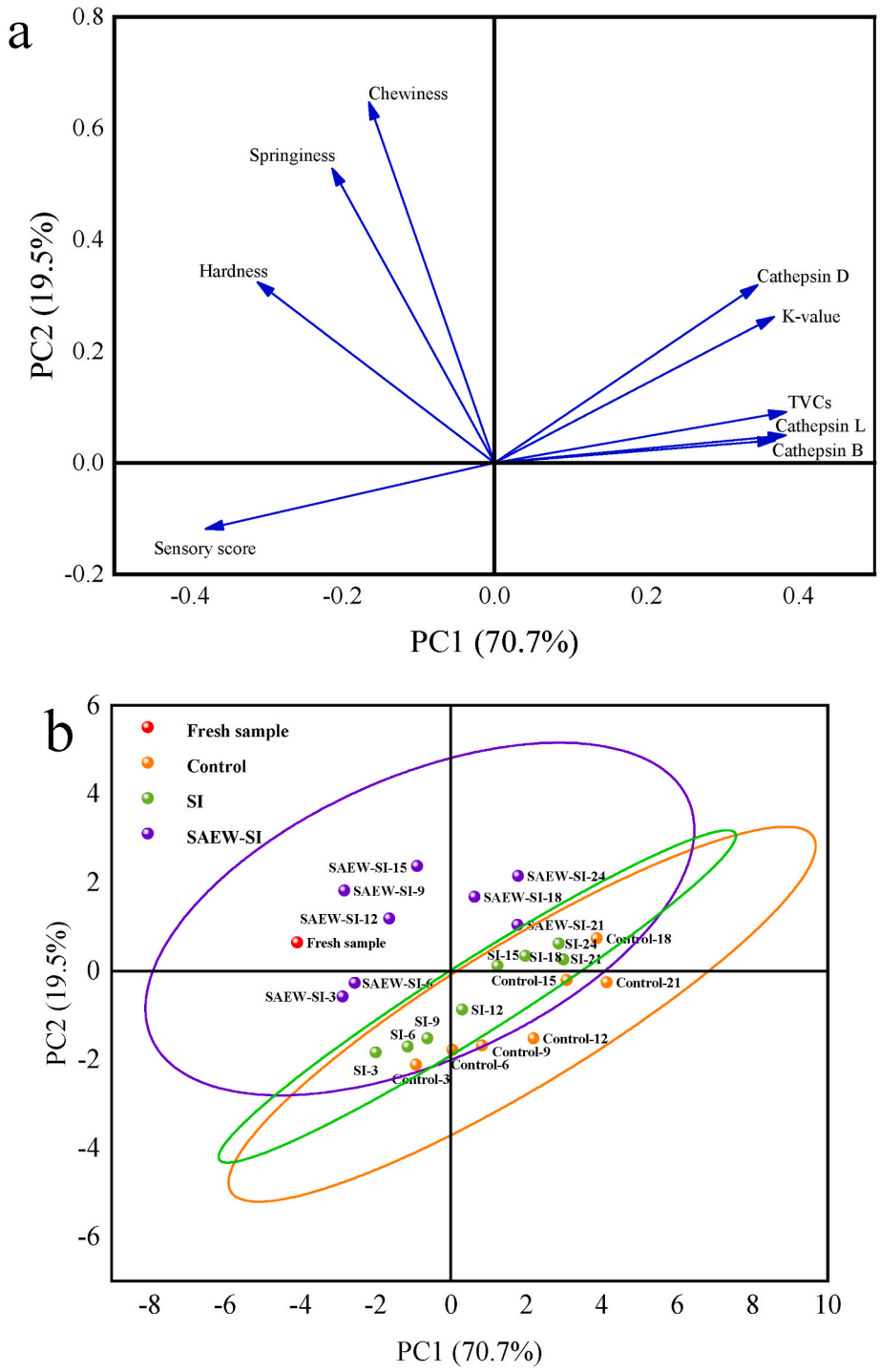

Fig. 6. Loadings (a) and scores (b) corresponding to the first two principal components (PC1 and PC2) in principal component analysis (PCA) of indicators of mackerel (*Pneumatophorus japonicus*) during storage.

groups increasingly moved away from the dot of fresh sample, suggesting that the quality of mackerel gradually deteriorated. For SAEW-SI treatment, the dots were located in a cluster (in purple circle) and close to the dot of fresh mackerel, indicating that samples in the SAEW-SI group remained their desired quality during the whole storage. SI group consisted of dots clustered in the green circle, which were not very far away from the dot of fresh sample, suggesting that the quality deterioration of samples in SI group was not serious. However, the Control samples (in orange circle) have undergone serious quality deterioration. In general, the dots of Control group were furthest from the dot of fresh sample, followed by the dots of SI and SAEW-SI group, suggesting that SAEW-SI was the most effective method to delay the quality deterioration of mackerel.

3.8. Linear regression model analysis

Linear regression curves were drawn to forecast the shelf-life of samples in each group. The regression analysis of shelf-life model in mackerel during storage at 4 °C was depicted in Fig. 7. The linear relationship among storage time and cathepsin B, D and L among groups

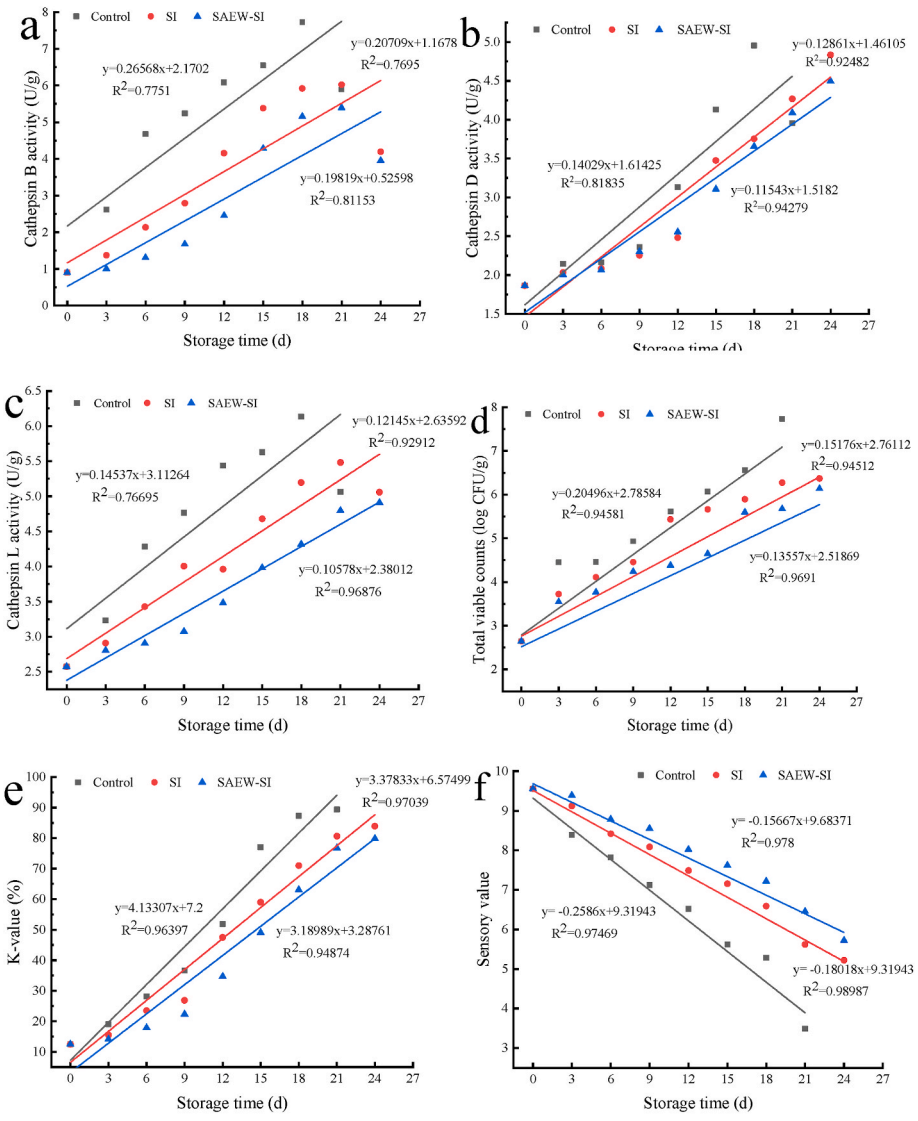

Fig. 7. The regression equation of cathepsin B (a), cathepsin D (b), cathepsin L (c) activities, TVC (d), K-value (e), and sensory score (f) in mackerel.

Table 3
Linear regression, coefficient of regression, and the estimation of shelf-life of mackerel (*Pneumatophorus japonicus*) during storage.

Indicators	Groups	Linear regression model	R^2	Estimated shelf-life (days)	Measured shelf-life (days)	Relative error (%)
Cathepsin B	Control	y = 0.26568x+2.1702	0.7751	cannot be measured for no threshold value		
	SI	y = 0.20709x+1.1678	0.7695			
	SAEW-SI	y = 0.19819x+0.52598	0.81153			
Cathepsin D	Control	y = 0.14029x+1.61425	0.81835			
	SI	y = 0.12861x+1.46105	0.92482			
	SAEW-SI	y = 0.11543x+1.5182	0.94279			
Cathepsin L	Control	y = 0.14537x+3.11264	0.76695			
	SI	y = 0.12145x+2.63592	0.92912			
	SAEW-SI	y = 0.10578x+2.38012	0.96876			
TVC	Control	y = 0.20496x+2.78584	0.9458	20.51	21	2.33
	SI	y = 0.15176x+2.76112	0.9451	27.93	24	16.37
	SAEW-SI	y = 0.13557x+2.51869	0.94874	33.05	24	37.71
K-value	Control	y = 4.13307x+7.2000	0.96397	12.78	15	14.8
	SI	y = 3.37833x+6.57499	0.97039	15.81	18	12.17
	SAEW-SI	y = 3.18989x+3.28761	0.94874	17.78	18	4.56
Sensory score	Control	y = −0.2586x+9.31943	0.97469	16.70	21	20.48
	SI	y = −0.18018x+9.31943	0.98987	23.97	24	0.13
	SAEW-SI	y = −0.15667x+9.68371	0.978	29.90	24	24.58

was shown in Fig. 7a, b, and c, respectively. Strong positive correlations among the storage time and TVC, K-value, sensory score were observed in all groups, respectively (Fig. 7d, e and 7f), which demonstrated that the freshness decreased with the extension of storage time. Meanwhile, the estimated shelf-life of mackerel can be calculated based on thresholds of some quality indicators (Table 3). The R^2 value of all regression models was very close to 1.000, indicating the reliability of the fit. In addition, most models have a relative error of less than 30%. These results showed that the predicted value of shelf-life was in good agreement with the actual value, which ensured the good generalization of shelf-life prediction.

The estimated shelf-life cannot be derived from cathepsin activities because there is no recommended threshold for cathepsin activities. However, the estimated shelf-life of SAEW-SI samples based on TVC and K-value prediction model was longer than that of Control samples by 12.54 and 5.00 days, respectively. Furthermore, according to the previous correlation analysis, sensory score was strongly correlated with TVC and K-value. It means that spoilage in mackerel samples could be predicted based on sensory evaluation (Nikzade et al., 2019). As shown in the regression equation of sensory score, the SAEW-SI treatment extended the shelf-life of mackerel for 13.20 additional days compared to the Control. Therefore, according to the regression equation of TVC, K-value and sensory score, the estimated shelf-life of SAEW-SI mackerel was longer than that of Control samples by at least 5.00 days.

4. Conclusions

The relevance of cathepsins activity for texture and the establishment of a shelf-life model of mackerel (*Pneumatophorus japonicus*) stored at 4 °C was investigated in the present study. A significant negative correlation with texture (hardness) was obtained for the activities of cathepsin B, D and L based on the heat map of correlation analysis. In comparison with Control, SI and SAEW-SI both had a positive effect on reducing the increase of K-value, inhibiting microbial growth, and maintaining the sensory quality of mackerel. Meanwhile, the combination of SAEW and slurry ice had obvious preservation performance. The estimated shelf-life of mackerel could extend for additional 5 days in comparison with Control samples according to the linear regression analysis. The information provided by this study should be of interest to seafood industry to form cold-chain logistics with SAEW-SI for the preservation of marine food products. Additionally, further studies are recommended to further investigate the specific antibacterial mode and cathepsin expression analysis to elucidate the preservative mechanism of SAEW.

Author contributions

Weiqing Lan: Conceptualization, Methodology, Writing- Reviewing and Editing.

Jiaxin Zhao: Formal analysis, Writing- Reviewing and Editing.

Lin Liu: Software, Formal analysis, Writing- Reviewing and Editing.

Jing Xie: Funding acquisition, Resources.

Declaration of competing interest

We declare that we have not represented a commercial or associative interest in a conflict of interest in connection with the work submitted.

Data availability

Data will be made available on request.

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