International Journal of Food Microbiology 345 (2021) 109152

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Effects of the curcumin-mediated photodynamic inactivation on the quality of cooked oysters with *Vibrio parahaemolyticus* during storage at different temperature

Bowen Chen [a,b], Jiaming Huang [b], Yang Liu [a], Haiquan Liu [b,c], Yong Zhao [b,c], Jing Jing Wang [a,b,*]

[a] Department of Food Science, Foshan University, Foshan 528000, China
[b] College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China
[c] Laboratory of Quality & Safety Risk Assessment for Aquatic Products on Storage and Preservation (Shanghai), Ministry of Agriculture and Rural Affairs, Shanghai 201306, China

ARTICLE INFO

Keywords:
Photodynamic inactivation (PDI)
Oysters
Vibrio parahaemolyticus
Malondialdehyde (MDA)
Magnetic resonance imaging (MRI)
Storage quality

ABSTRACT

Photodynamic inactivation (PDI) is a promising method with multiple targets to inactivate bacteria on food using visible light. Inactivation potency of the curcumin-mediated blue light-emitting diode (LED) PDI against the pathogen *Vibrio parahaemolyticus* on cooked oysters and its effects on the storage quality were investigated by the microbiological, physical, chemical and histological methods during storage at 4 °C, 10 °C and 25 °C. Results showed that the PDI treatment obviously inhibited the recovery of *V. parahaemolyticus* on oysters during storage, and the maximal difference attained >1.0 Log_{10} CFU/g (> 90%) compared to control stored at 10 °C and 25 °C. Meanwhile, it displayed a potent ability ($p < 0.05$) to restrain the decrease of pH values, reduce the production of total volatile basic nitrogen (TVB-N), suppress the lipids oxidation, as well as retard the changes of color difference of the oysters. In addition, the PDI effectively maintained the integrity and initial attachments of muscle fibers, and hence decreased the loss of water in myofibrillar space and the texture softening of oysters during storage. On this basis, this study facilitates the understanding of the potency of bacterial inactivation and food preservation of PDI, and hence pave the way for its application in food industry.

1. Introduction

Oysters are bivalve mollusks and one of the most popular seafood with high nutritional value. Furthermore, the production and trade of oysters have shown an increasing trend in the past decade (FAO, 2020). Oysters are filter-feeding animals with abundant microbial flora including foodborne pathogens, but they are often freshly consumed. The pathogenic microorganisms are easily spread to consumers, generally causing diseases from mild gastroenteritis to life-threatening syndromes (Dewey-Mattia et al., 2018; He et al., 2002).

V. parahaemolyticus is considered to be a predominant pathogenic bacteria in seafood, especially in coastal countries and regions (Chen et al., 2020). It has been reported that the concentration of *V. parahaemolyticus* in oysters is higher than other marine products, therefore, consuming oysters is related to a high risk of *V. parahaemolyticus* infection (Ndraha et al., 2020). In addition, rapid spoilage of oysters caused by microbial floras shortens shelf life and gives a threat for their quality. Therefore, it is imperative to develop a method to effectively control the pathogenic bacteria on oysters with minimal impacts on their quality.

Compared to conventional sterilization technologies including heat processing, ultraviolet, pulsed electric fields, high density carbon dioxide sterilization, etc., photodynamic inactivation (PDI) is now recognized as a potent method to kill bacteria on food owing to its great safety to workers, environmental-friendly characteristic, minimally-processed virtue, low-energy consumption and low-cost inputs (Chen et al., 2020; Miao et al., 2019). For instance, Liu et al. (2016) reported that the PDI treatment decomposed fewer nutrients and prolonged the shelf life of pacific oysters, but greatly delayed the metabolic levels of spoilage microorganisms. Kim et al. (2017) found that 405 ± 5 nm LEDs could control *Salmonella* populations on illuminated cooked chicken under refrigeration conditions. Josewin et al. (2018) reported that the riboflavin-mediated PDI minimized the risk of *Listeria monocytogenes* associated with smoked salmon in food stores. Therefore, the PDI

* Corresponding author at: Department of Food Science, Foshan University, Foshan 528000, China.
E-mail address: w_j2010@126.com (J.J. Wang).

https://doi.org/10.1016/j.ijfoodmicro.2021.109152
Received 18 October 2020; Received in revised form 23 January 2021; Accepted 1 March 2021
Available online 10 March 2021

treatment is a valid technique to preserve the quality of seafood.

Although these studies investigated the efficiency of PDI on inactivating pathogenic microorganisms on food, it usually cannot completely inactivate food-borne pathogens and may cause sub-fatally injured cells, due to the food matrix providing shelters for microorganisms, protecting them from the irradiation and the fatty layers on meat surface having a protective potency (Ghate et al., 2019). After the food is moved to suitable environment, the injured cells will repair themselves and then proliferate (Wang et al., 2014). However, the recovery and proliferation of PDI-injured cells as well as the PDI-treated effects on food quality are barely reported.

Therefore, we selected the curcumin to construct potent PDI, because it is a natural product of turmeric powder, which was widely applied as food additives (Chen et al., 2020). Here, we studied the ability of curcumin-mediated PDI to inactivate *V. parahaemolyticus* on cooked oysters, and then monitored the recovery and proliferation of the PDI-treated cells as well as evaluated the quality changes of oysters during storage at different temperatures by investigating the microbiological, physical, chemical and histological changes. This study will provide systematic information to comprehend the effects of the PDI on seafood quality, and hence pave the way for its potential application in food industry.

2. Materials and methods

2.1. Bacterial strain and culture preparation

Four strains of *V. parahaemolyticus* (ATCC 17802, VPC17, VPC36 and VPC47 isolated from fecal specimens in our lab) were used. The strains were cultured in Tryptic Soy Broth (TSB) with glycerol (50%, *v*/v) at −80 °C. The bacteria were incubated in 9 mL of TSB supplemented with 3% (*w/v*) NaCl for 10–12 h at 37 °C (200 rpm). Cells were transferred to a 1.5 mL tube and centrifuged for 5 min at 4 °C (4000 g). Pelleted cells were resuspended in 0.85% physiological saline solution to obtain a suspension of ~8.0 Log_{10} CFU/mL.

2.2. Preparation of cooked oysters

Fresh oysters (each sample 10.0 ± 0.2 g) were bought from a local seafood market in Shanghai, China and transferred to −20 °C refrigerator before using. The thawed oysters were steamed at 100 °C for 10 min to kill the native microorganism (Soares et al., 2020). All samples were quickly moved into a biosafety safety cabinet, and then placed statically until its temperature naturally cooled to room temperature.

2.3. Photodynamic inactivation treatment

Each sample was inoculated with the cocktail of *V. parahaemolyticus* and curcumin (50, 60, 70, 75 and 100 μM) by gently spreading 100 μL of inoculum to oysters, and then air-dried for 20 min at 25 °C to allow for bacterial attachment. Curcumin (≥ 98.0% purity; Sigma, co., LTD, USA) was dissolved in ethanol (99.8% purity, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) to obtain the final concentration of 2.5 mM and then maintained in the dark at −20 °C before using. The injected *V. parahaemolyticus* on oysters was ~5.5 Log_{10} CFU/g.

The subsequent experiments included two parts: Firstly, the oysters were irradiated using blue LED light (10 W, 455–460 nm) with the power density of 5.20 mW/cm^2 and exposed to light for 0 min (0 J/cm^2), 30 min (9.36 J/cm^2) and 60 min (18.72 J/cm^2) and curcumin (50, 75 and 100 μM). After PDI treatment, the samples were homogenized with 40 mL 0.85% physiological saline solution for 2 min by a stomacher (BagMixer 400 VW, Inter-science, France), and then the supernatant was diluted to spread onto thiosulfate citrate bile salts sucrose agar (TCBS). All plates were incubated at 37 °C for 18 h and the bacteria cells were counted by Log_{10} CFU/g (Xie et al., 2012).

Secondly, the oysters were partitioned into 6 groups and each group contained 6 oysters: neither blue light irradiation nor curcumin treatment stored at 4 °C; neither blue light irradiation nor curcumin treatment stored at 10 °C; neither blue light irradiation nor curcumin treatment stored at 25 °C; blue light irradiation treatment plus curcumin (50 μM) stored at 4 °C; blue light irradiation treatment plus curcumin (50 μM) stored at 10 °C; blue light irradiation treatment plus curcumin (50 μM) stored at 25 °C. After treatment, all samples containing *V. parahaemolyticus* of ~3.5 Log_{10} CFU/g were stored for 10 days at 4 °C, 10 °C and 25 °C, then they were analyzed every second day by the microbiological, physical, chemical and histological methods. All experiments were conducted at least in triplicates.

2.4. Chemical analysis

pH was measured using 10.0 ± 0.2 g ground flesh of oysters. Briefly, the samples were added to 40 mL deionized water, and homogenized for 2 min at 25 °C. pH was determined by pH-meter (Metter-Toledo, Zurich, Switzerland) with a standard polymer pH electrode.

Total volatile basic nitrogen (TVB-N) was measured according to Lin et al. (2013). Oyster (10.0 ± 0.2 g) was homogenized with 40 mL 0.85% physiological saline solution for 5 min. 10 mL of oyster homogenate were mixed with 1.00 g magnesia (MgO) for subsequent use. TVB-N was measured by the UDK 159 analyzer unit (VELP, Italy), then the distilled components were absorbed by boric acid (20 g/L) and titrated with HCl (0.20 mol/L). TVB-N was calculated as mg TVB-N/100 g oyster meat.

The malondialdehyde (MDA) was analyzed by using MDA assay kits (Shanghai Zcibio Co., Ltd., China) by the manufacturer's instructions. The extracted solution was used to determine lipids oxidation by measuring the OD_{523nm} and OD_{600nm}. Then, the MDA values were calculated according to the formula in the manufacturer's instructions: MDA (nmol/g) =51.6 × (OD_{523nm} - OD_{600nm})/W (W: sample weight). All experiments were conducted in triplicate.

Free Fatty Acid (FFA) analysis was measured by using FFA assay kits (Nanjing Jiangcheng Bioengineering Institute, China). The extracted solution was employed to determine total lipids and all experiments were repeated in three times.

2.5. Physical analysis

Texture analysis. Texture profile analysis was conducted with Texture Analyzer (Stable Microsystems, Surrey, UK). Two successive compressions (each for 5 s elapse) were performed using an aluminum cylinder probe P/0.5S, and each compression compressed the original height to 50% at 1 mm/s speed test (5.0 × 2.0 × 1.0 cm). Primary parameters of the oyster including hardness, chewiness, and springiness were calculated from a force distance graph (Miao et al., 2019).

Color analysis. Color change of the oysters was monitored using a Chromatic meter NR20XE (Threenh Technology Co., Ltd., China). The color was represented by L*, a* and b* parameters, which separately represented luminosity, chromaticity on the green (−) to red (+) axis, and chromaticity on the blue (−) to yellow (+) axis (Peng et al., 2021). The browning of oysters was evaluated by L* parameter. The difference in total color difference (ΔE) was obtained according to the following equation: $\Delta E = [(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2]^{1/2}$. All experiments were conducted at least in triplicates.

Magnetic resonance imaging (MRI) analysis. An oyster flesh (10.0 g, 5.0 × 2.0 × 1.0 cm) was selected, and the surface juice was absorbed by a filter paper, and the vacuum packaging was carried out for MRI measurement. Two-dimensional (2D) MRI was also obtained by a low-field nuclear magnetic resonance analyzer (Niumag Analytical Instrument Corporation, China). The following scanning parameters were used: field of view = 100 mm × 100 mm, slice width = 1.6 mm, slice gap =1.6 mm, average = 2, read size = 256, phase size = 192. The echo time and repetition time were 50 ms and 1900 ms. The distribution of water molecules was obtained according to the changes of signal amplitude (Cheng et al., 2018).

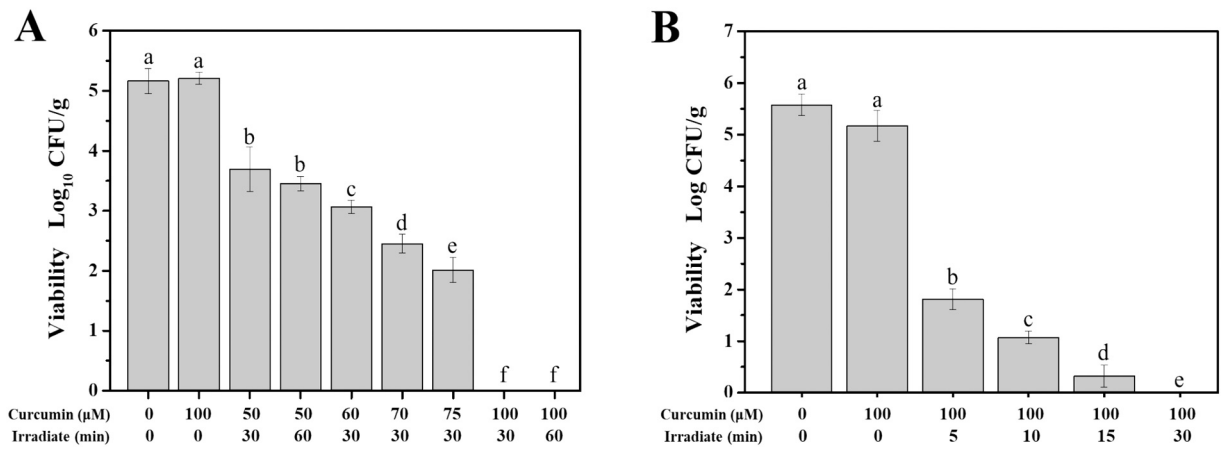

Fig. 1. Effects of the curcumin-medicated PDI on the viability of *V. parahaemolyticus* on cooked oysters. (A) different curcumin concentration under 9.36 J/cm^2 (30 min) and 18.72 J/cm^2 (60 min) irradiation; (B) different irradiation time with 100 μM curcumin. Different lowercase letters (a–f) indicate the significant differences ($p < 0.05$) between different treatment.

Histological analysis. The adductor tissues (1.0 × 1.0 × 1.0 cm) of oysters were cut and immediately fixed in Bouin's Fixative Solution (Phygene Scientific Co., Ltd., China) for 24 h. Subsequently, the tissue was dehydrated in series of ethanol solutions (75%, 80%, 85%, 90%, 95% and 100%), and placed into xylene and embedded in paraffin for 1–2 h, and then sliced with paraffin slicer (LEICARM2016). The tissue sections of 7 μm were acquired by the RM2235 microtome (Leica Microsystems CMS GmbH, Wetzlar, Germany), and then hematoxylin-eosin stained routinely. All samples were observed and photographed by an optical RM2235 microscope (Lin et al., 2013). All experiments were conducted at least in triplicates.

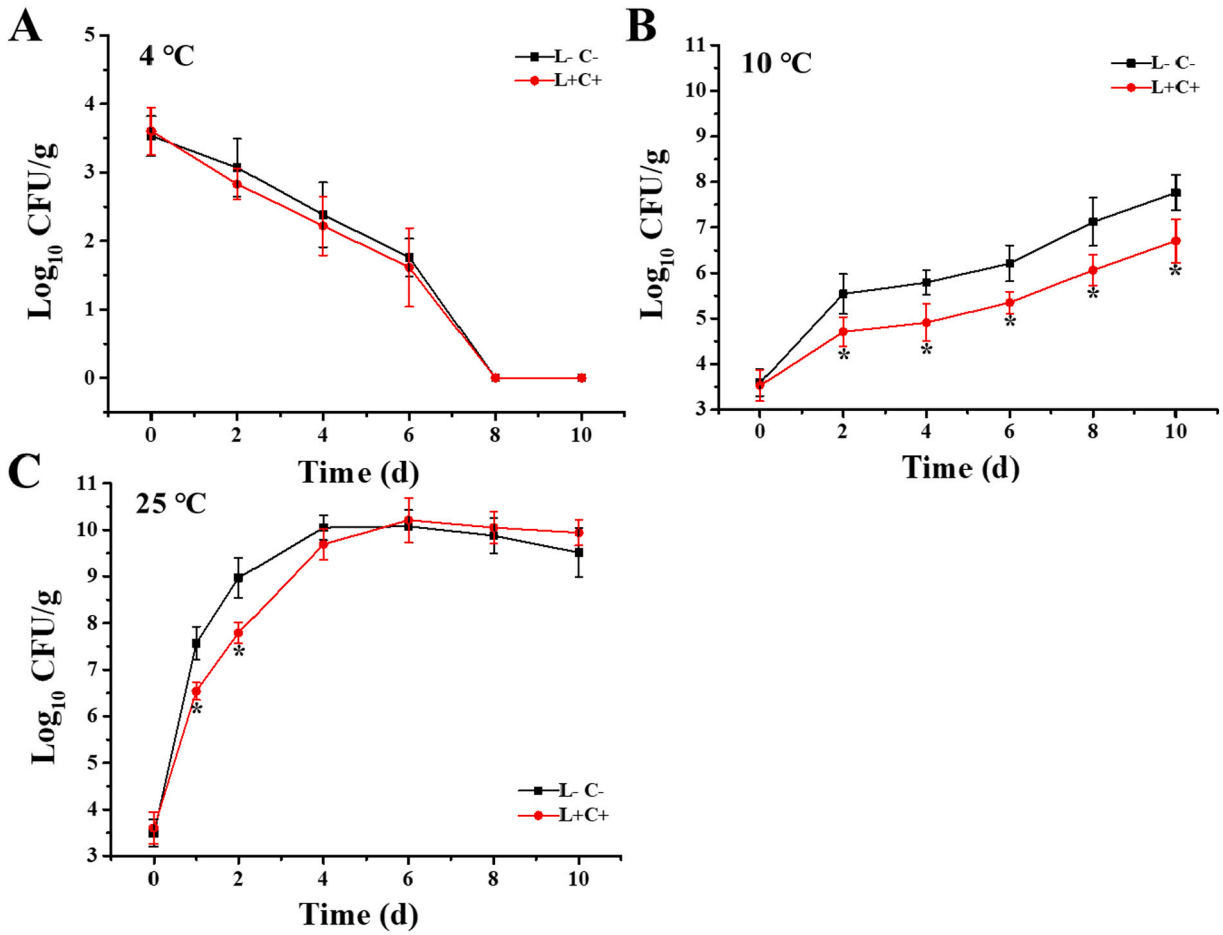

Fig. 2. Recovery and proliferation of *V. parahaemolyticus* on cooked oysters after the PDI treatment stored at 4 °C (A), 10 °C (B) and 25 °C (C).
* indicates the significant differences ($p < 0.05$) between the PDI-treated oysters with 30 min LED irradiation (9.36 J/cm^2) and 50 μM curcumin (L+ C+) and negative control oysters (L- C-).

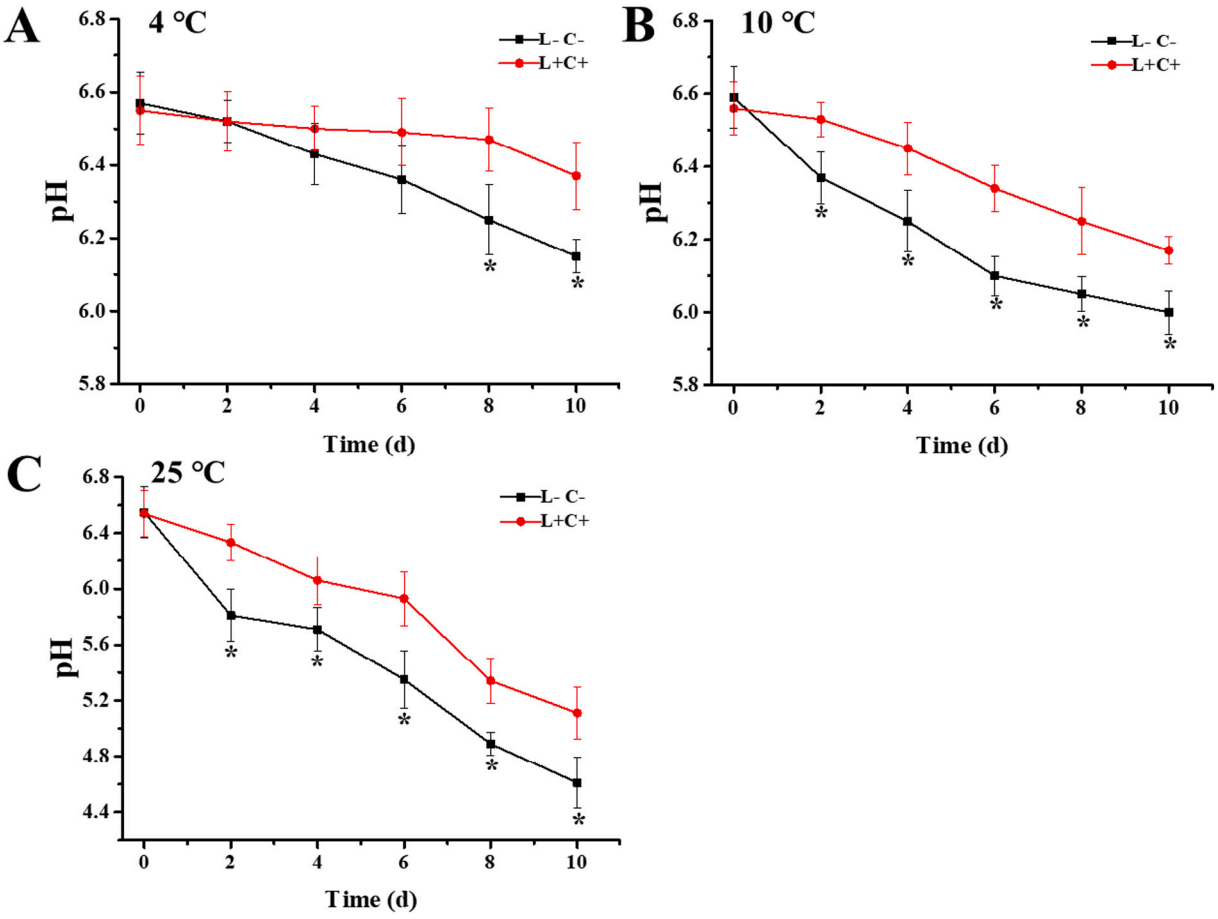

Fig. 3. Changes in the pH values of cooked oysters after the PDI treatment stored at 4 °C (A), 10 °C (B) and 25 °C (C).
* indicates the significant differences ($p < 0.05$) between the PDI-treated oysters with 30 min LED irradiation (9.36 J/cm^2) and 50 μM curcumin (L+ C+) and negative control oysters (L- C-).

2.6. Statistical analysis

All data were calculated as the mean ± standard deviation. One-way analysis of variance (ANOVA) was selected to test the value differences ($p < 0.05$) by SPSS 17.0 software.

3. Results and discussion

3.1. Antibacterial potency of the PDI against V. parahaemolyticus on cooked oysters

Fig. 1A shows effects of the curcumin concentration and irradiation dosage on the potency of PDI against *V. parahaemolyticus* on cooked oysters. The cells of negative control (L- C-) were ~5.2 Log$_{10}$ CFU/g, and obvious difference between the samples treated only with 100 μM curcumin (L- C+) and the control sample was not observed. After the PDI treatment with 9.36 J/cm^2 (30 min) irradiation and 50 μM curcumin, *V. parahaemolyticus* was evidently ($p < 0.05$) decreased to 3.7 Log$_{10}$ CFU/g. As the curcumin was increased to 100 μM, the PDI treatment with 9.36 J/cm^2 irradiation had an improved and concentration-dependent antibacterial results (Fig. 1A).

Fig. 1B shows the effects of the irradiation dosage of PDI on killing *V. parahaemolyticus* on cooked oysters. There was no apparent difference between the cells treated only with 100 μM curcumin (L- C+) and the negative control (L- C-). The PDI treatment with 1.56 J/cm^2 (5 min) irradiation and 100 μM curcumin obviously ($p < 0.05$) reduced *V. parahaemolyticus* to 1.8 Log$_{10}$ CFU/mL. As the irradiation dosage increased to 4.68 J/cm^2 (15 min), *V. parahaemolyticus* was further reduced. Furthermore, no visible colony was detectable on the selective TCBS plates under 9.36 J/cm^2 irradiation. On this basis, the PDI of *V. parahaemolyticus* on oysters displayed a curcumin concentration and irradiation dosage-dependent pattern. Overall, the association of curcumin (100 μM) and irradiation (30 min) were ideal PDI conditions for inducing undetectable *V. parahaemolyticus* on cooked oysters.

Our previous study has proved that *V. parahaemolyticus* was not visibly detected on TCBS plates after 1.14 J/cm^2 LED irradiation with 1.0 μM curcumin (Chen et al., 2020). However, the bactericidal effect of the PDI against *V. parahaemolyticus* on seafood was far weaker than that against planktonic bacteria in solution. Such phenomenon was highly attributed to the protective function of food matrix, which provided the crevices and fatty layers for microorganisms to hide themselves on the surface (Ghate et al., 2019). Kim et al. (2017) reported that the PDI treatment of 405 ± 5 nm LEDs decreased 0.9 Log CFU/cm^2 of the *Salmonella* populations on cooked chicken at dosage of 3.80 kJ/cm^2 (48 h) with endogenous photosensitizer porphyrin. However, relying on endogenous photosensitizers often presented much lower sterilization efficiency compared to exogenous photosensitizers (e.g. curcumin). In addition, Josewin et al. (2018) reported that the riboflavin-mediated PDI merely caused 1.2 log CFU/cm^2 reduction of L. *monocytogenes* on smoked salmon at a dosage of 2.4 kJ/cm^2 LED irradiation with 100 μM riboflavin. In fact, the riboflavin was a water-soluble exogenous photosensitizer (Huang et al., 2004), and it was less effective in adsorbing to seafood which was rich in liposoluble matter (e.g. fat, etc.), thus weakening the inactivation potency of PDI. On this basis, the liposoluble curcumin-medicated PDI would be a potent and promising antibacterial method to preserve the quality of seafood.

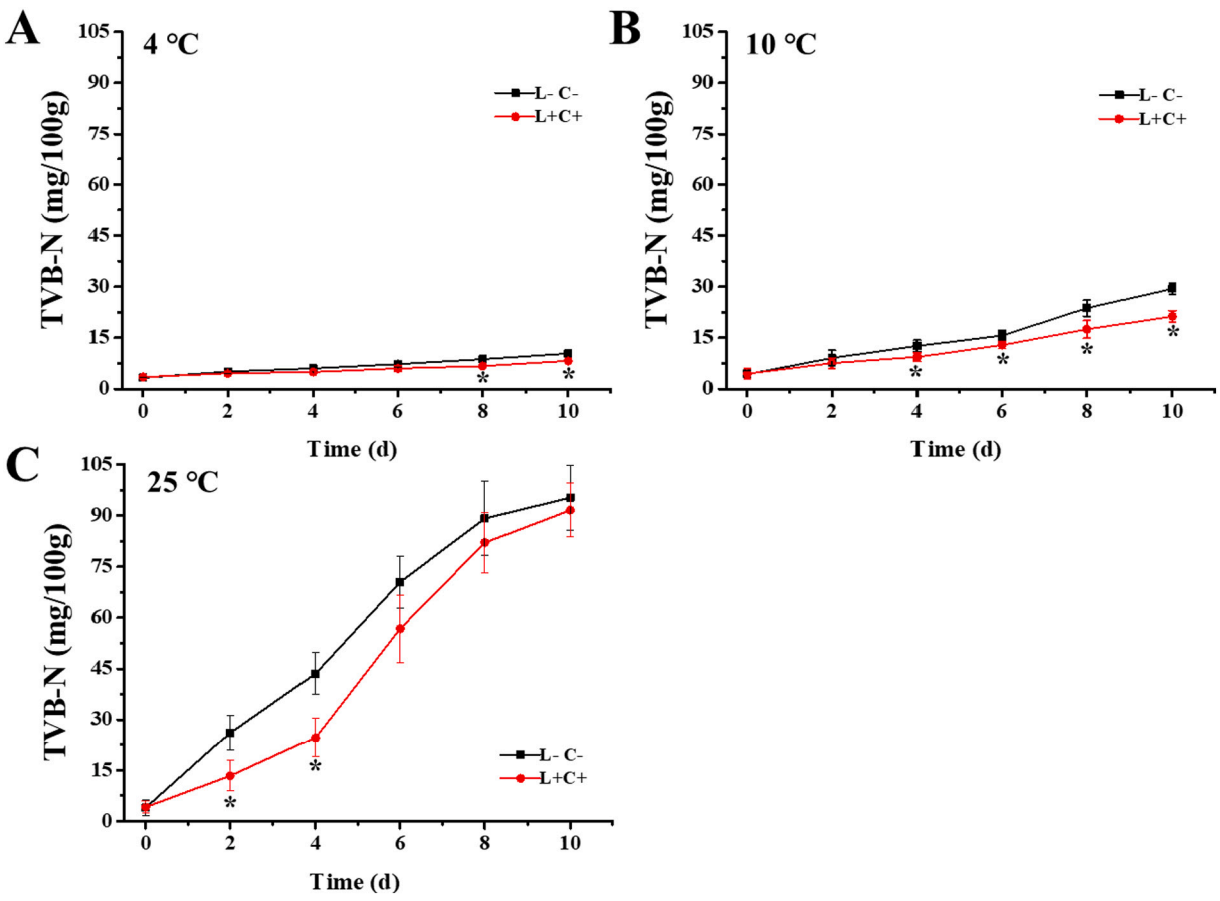

Fig. 4. Changes in the total volatile basic nitrogen (TVB-N) values of cooked oysters after the PDI treatment stored at 4 °C (A), 10 °C (B) and 25 °C (C). * indicates the significant differences ($p < 0.05$) between the PDI-treated oysters with 30 min LED irradiation (9.36 J/cm^2) and 50 μM curcumin (L+ C+) and negative control oysters (L- C-).

3.2. Recovery of *V. parahaemolyticus* on cooked oysters after the PDI treatment during storage

Fig. 2A-C shows the recovery and proliferation of the PDI-treated cells on oysters for 10 days under 4 °C, 10 °C and 25 °C. After the PDI treatment, the initial cells were determined to be ~3.5 Log_{10} CFU/g. Overall speaking, *V. parahaemolyticus* on oysters showed an obvious decrease during storge 10 days at 4 °C, regardless of the PDI-treated group (L+ C+) or negative control (L- C-) (Fig. 2A). The result was identical to our previous study, reporting that *V. parahaemolyticus* was sensitive to low temperature and generally inactivated at cool storage temperatures (e.g. 4 °C) (Wang et al., 2014). Fig. 2B shows the change of the PDI-treated cells on oysters for 10 days at 10 °C. For the negative control (L- C-), the *V. parahaemolyticus* cells on oysters increased from 3.5 Log_{10} CFU/g to 7.8 Log_{10} CFU/g. For the treated samples, the cells also presented an increase to 6.7 Log_{10} CFU/g. However, the growth of the PDI-treated cells was markedly ($p < 0.05$) lower than that of negative control. Therefore, the current study directly illustrated that PDI treatment obviously ($p < 0.05$) decreased the proliferation of *V. parahaemolyticus* on oysters during storage at 10 °C. Additionally, the evidence of increment of *V. parahaemolyticus* further confirms the guidelines of food refrigeration preservation proposing that a safe storage temperature was preferentially lower than 8 °C (U.S. Food and Drug Administration, 1997).

Compared with the samples stored at 4 °C and 10 °C (Fig. 2A–B), *V. parahaemolyticus* exhibited a sharp increase from ~3.5 Log_{10} CFU/g to ~10.0 Log_{10} CFU/g in both the PDI-treated group (L + C+) and negative control (L-C-) at 25 °C within initial 4 days. However, the PDI-treated cells displayed a significant lower growth than the negative control during the first 2 days (Fig. 2C). When the storge was further prolonged, no obvious difference in the cells was observed. Considering all above results, it was firmly believed that the PDI treatment indeed reduced the growth of harmful *V. parahaemolyticus* on oysters during storage.

Our previous study has revealed that the curcumin-mediated PDI could cause the damage and even rupture of cell structures, as well as the injury of intracellular proteins and DNA of *V. parahaemolyticus* (Chen et al., 2020). In this study, the PDI-treated *V. parahaemolyticus* showed obviously lower growth, which was highly due to the sublethal damage of cells caused by PDI according to the studies of Silva-Angulo et al. (2014) and Wang et al. (2014). Because the injured cells caused by PDI treatment needed more time to repair cell membrane, DNA, proteins, etc., and then adapt before they could multiply. For example, the injured cells of *V. parahaemolyticus* on oysters underwent long time of cellular repair to proliferate at unfavorable temperatures especially at 10 °C, resulting in a much lower growth of the populations compared to control samples (Fig. 2A). When the temperature was increased to the favorable 25 °C, the recovery and proliferation ability of the injured *V. parahaemolyticus* greatly increased, and relatively short time was needed to complete the cellular repair (48 h) (Wang et al., 2014). Therefore, the PDI treatment could slow down the growth of survival bacteria with appropriate storage temperatures, which decreased the risk of illness caused by the rapid multiplication of pathogens.

3.3. Effects of the PDI on the chemical changes of cooked oysters during storge

The pH changes of the PDI-treated oysters during the storage at 4 °C, 10 °C and 25 °C are illustrated in Fig. 3A–C. Generally, the pH of all

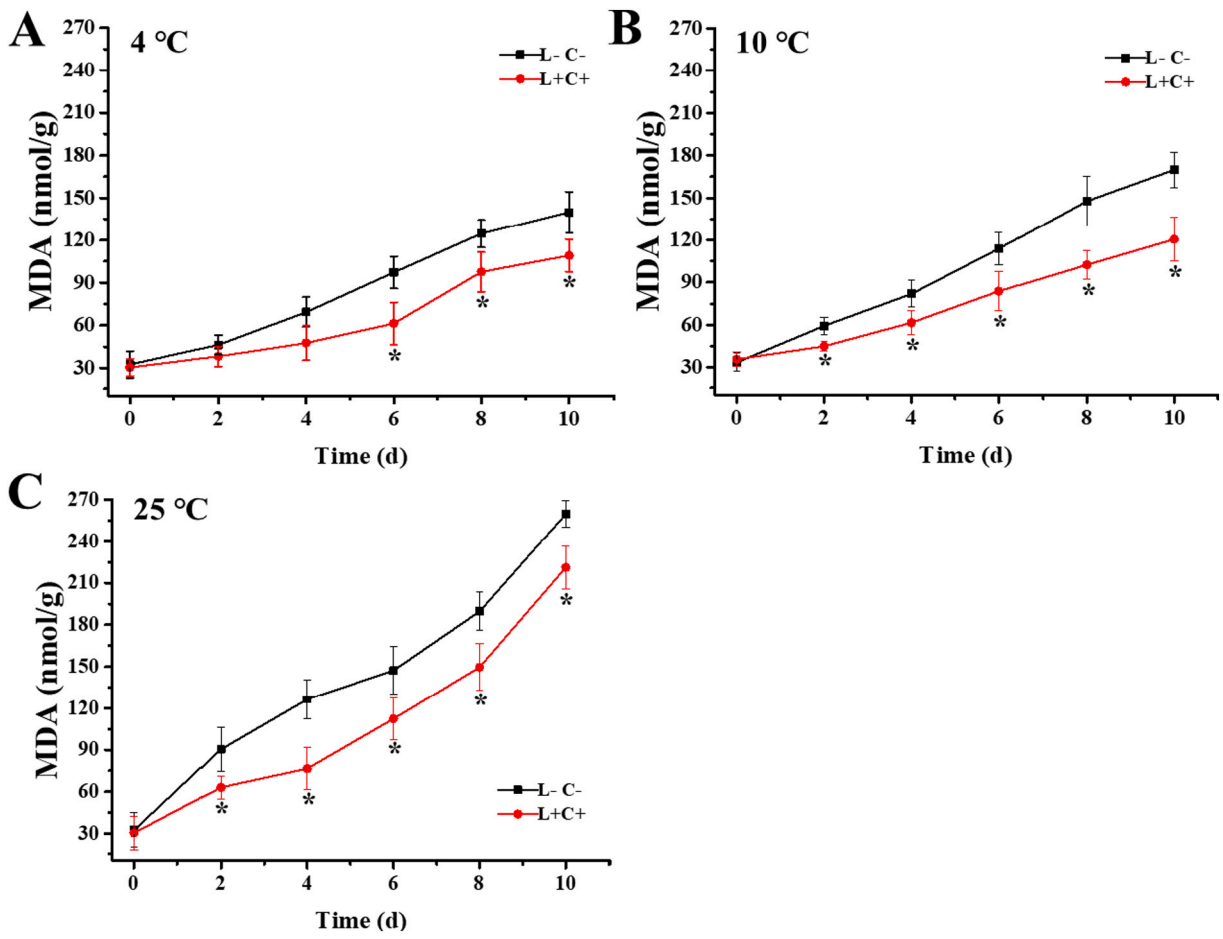

Fig. 5. Changes in the malondialdehyde (MDA) values of cooked oysters after the PDI treatment stored at 4 °C (A), 10 °C (B) and 25 °C (C).
* indicates the significant differences ($p < 0.05$) between the PDI-treated oysters with 30 min LED irradiation (9.36 J/cm^2) and 50 μM curcumin (L+ C+) and negative control oysters (L- C-).

samples slowly decreased with the storage prolonging. Such pH change trend of oysters has been reported in previous studies (Dewey-Mattia et al., 2018; He et al., 2002). Overall speaking, the pH of the PDI-treated oysters was higher than those of negative control (L-C-), especially, a significant difference was tested at 6th day under 4 °C storage (Fig. 3A). For the storage at 10 °C, the descending rate of pH of the PDI-treated samples was much lower than that of negative control (L-C-). Moreover, the pH was reduced from 6.53 (2nd day) to 6.17 (10th day), which was always higher than negative control from 6.37 (2nd day) to 6.0 (10th day) (Fig. 3B). After the temperature was increased to 25 °C, the pH values were further decreased to 5.11, and those of control samples were reduced to 4.61 during the storage (Fig. 3C).

Compared with most seafood, oysters had a richer carbohydrate content, especially in the form of glycogen. The putrefaction of oysters was usually the fermentation of glycogen generating lactic acid induced by the bacterial multiplication, the oxidation of lipids and the release of inorganic phosphate of ATP caused by ATPase, collectively leading to a gradual decrease of pH (Chen et al., 2020). Consequently, the decreased pH was often considered as a spoilage indicator of oyster and other molluscan shellfish (He et al., 2002). In this study, the PDI treatment displayed an apparent capability to restrain the pH changes of oysters, which was highly attributed to its inhibition against bacterial proliferation (Fig. 2) and anti-lipid oxidation ability (Fig. 5).

Hunter and Linden (1923) firstly used pH to determine the quality of oysters, and generally, oysters exhibited sour and putrid characteristics at pH ≤ 5.2 and was unacceptable. Additionally, Cook (1991) showed that oysters with liquor pH > 6.0 were accepted as "good", and pH ≤ 5.0 were recognized as an advanced stage of decomposition. On this basis, the PDI treatment possessed a good ability to preserve the quality of oysters with the chilling temperature (e.g. 4 °C and 10 °C), and also inhibited their degradation into the advanced stage at normal temperature (25 °C).

TVB-N is the products of nitrogenous substances produced by the action of bacteria and native proteases in marine products. A high TVB-N is unacceptable and accompanied by a disagreeable smell in the flesh (Kilincceker et al., 2009). Therefore, TVB-N is a frequently used quality indicator for fresh and chilled seafood. The changes in TVB-N values of oysters treated by PDI stored at 4 °C, 10 °C and 25 °C are shown in Fig. 4A–C. The total amount of TVB-N increased from 3.5 to 8.3 mg/100 g of the PDI-treated oysters, while TVB-N increased from 3.3 to 10.4 mg/100 g of negative control at 4 °C for 10 days storage (Fig. 4A). Furthermore, an obvious difference between the PDI-treated samples and negative control was observed at the 6th day of storage. At 10 °C storage, the TVB-N of the treated oysters increased from 4.5 to 21.3 mg/100 g, which was apparently lower than the negative control from the 4th day storage (Fig. 4B). While at 25 °C storage, the TVB-N values greatly increased and almost reached 100 mg/100 g regardless of the PDI-treated samples or negative control, but they were still lower than the negative control within initial 4 days (Fig. 4C). Therefore, the PDI treatment decreased the production of TVB-N by inactivating the bacteria and weakening the action of endogenous proteolytic enzymes (Songsaeng et al., 2010).

The TVB-N content of 25 mg/100 g of fish meat was widely recognized as the maximum value for the acceptable quality of aquatic

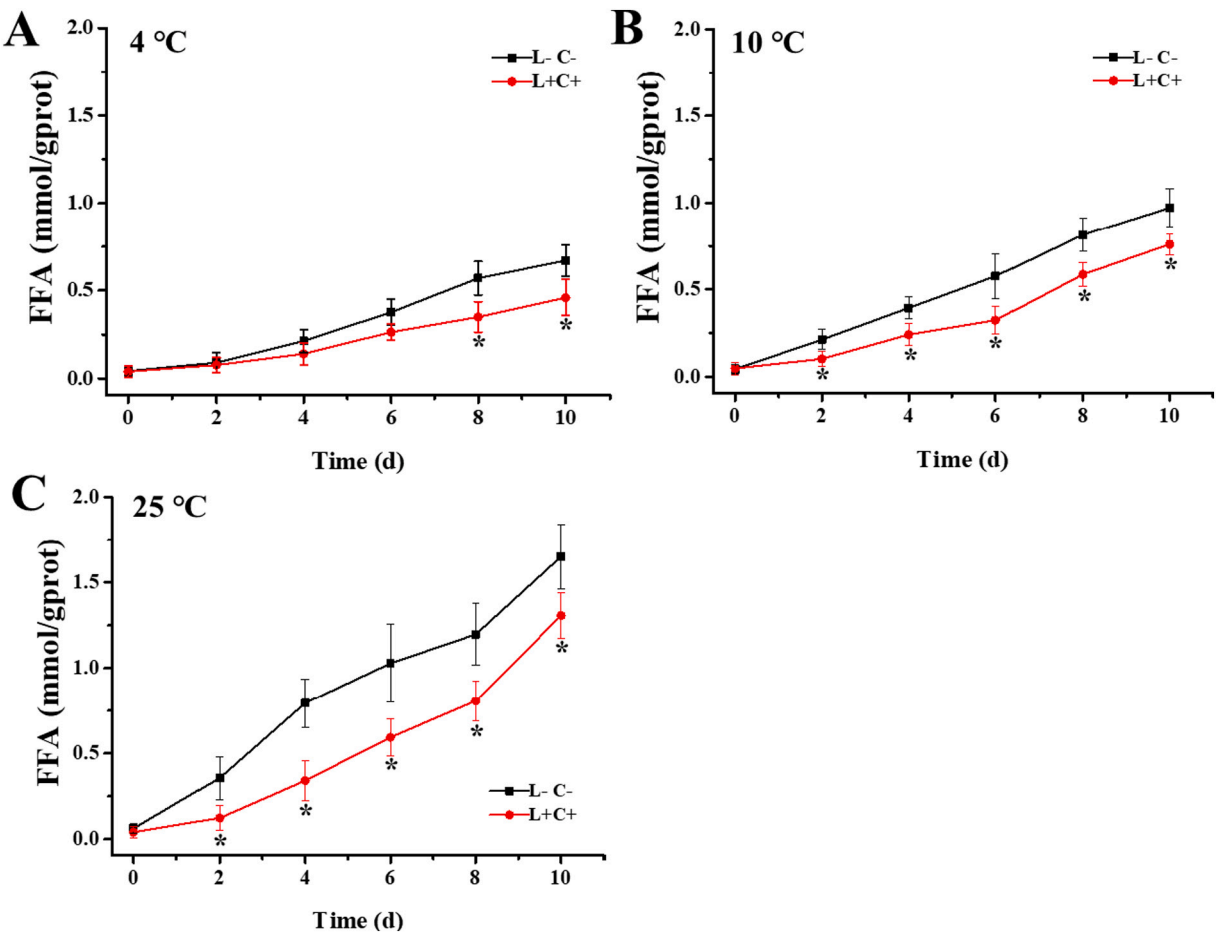

Fig. 6. Changes in the free fatty acid (FFA) values of cooked oysters after the PDI treatment stored at 4 °C (A), 10 °C (B) and 25 °C (C).
* indicates the significant differences ($p < 0.05$) between the PDI-treated oysters with 30 min LED irradiation (9.36 J/cm^2) and 50 μM curcumin (L+ C+) and negative control oysters (L- C-).

products (Kilincceker et al., 2009). In this study, the TVB-N values of oysters treated with PDI was well below the acceptable upper limit within 10 days storage at 4 °C. Meanwhile, the treated oysters were not over the limit (25 mg/100 g) at 10 °C, nevertheless, the negative control exceeded the acceptable limit at the 10th day storage. Likewise, the TVB-N of treated oysters was also below the acceptable limit during first 4 days at 25 °C, while the control samples were over the acceptable maximum. The results indicated that the PDI treatment extended the shelf life of oysters by suppressing the production of TVB-N.

Oxygen free radicals acted on unsaturated fatty acids of lipids to generate lipid peroxides, which was gradually decomposed into a series of complex compounds, including MDA (Tan et al., 2019). Therefore, the extent of lipid oxidation could be obtained by detecting the level of MDA. The changes of MDA values of oysters for 10 days storage at 4 °C, 10 °C and 25 °C are shown in Fig. 5A–C, and all samples presented an increased MDA values, which was similar to the results of Xuan et al. (2017). This may be attributed to the accumulation of the end-products of unsaturated fatty acid oxidation (Fan et al., 2008). In detail, the amount of MDA increased from 30.6 to 109.2 nmol/g for the PDI-treated oysters, while MDA increased from 32.5 to 139.8 nmol/g for the negative control at 4 °C for 10 days storage (Fig. 5A). Furthermore, a significant difference between the PDI-treated samples and negative control was observed from the 6th day storage. For the storage at 10 °C, the MDA of the treated oysters increased from 35.9 to 120.6 nmol/g, which was obviously lower than those of the negative control from the 2nd day storage (Fig. 5B). After the temperature was increased to 25 °C, the change trend of MDA was found to be similar with that stored at 10 °C (Fig. 5C). The results indicated that the PDI treatment effectively suppressed the lipids oxidation of oysters.

Free fatty acid (FFA) was generated by the degradation of triglycerides, and this process was hydrolysis. The FFA could be applied to characterize the degree of lipolysis, and hence evaluated the extent of lipid degeneration and freshness of seafood (Haghighi and Yazdanpanah, 2020). Fig. 6 showed that the amount of FFA increased significantly for the PDI-treated samples and negative control during the storage. FFA increased from 0.04 to 0.46 mmol/gprot for the PDI-treated oysters, while it increased to 0.67 mmol/gprot for the negative control at 4 °C for 10 days storage (Fig. 6A). Furthermore, an apparent difference between the PDI-treated samples and negative control was observed from the 8th day storage. At 10 °C, the treated FFA content increased from 0.11 (2nd day) to 0.76 mmol/gprot (10th day), which was always lower than the negative control from 0.21 (2nd day) to 0.97 mmol/gprot (10th day) (Fig. 6B). Likewise, the FFA content of the PDI-treated samples further increased to 1.31 mmol/gprot, which was still much lower than negative control in the whole storage at 25 °C (Fig. 6C). In conclusion, the PDI treatment possessed a potency to greatly inhibit the lipids hydrolysis.

The lipase and phospholipase induced lipid hydrolysis to produce FFAs, and these FFAs could be further oxidized to produce low molecular weight compounds which released unpleasant odor (de Aguiar Saldanha Pinheiro et al., 2020). Chaijan et al. (2006) reported that the lipids were hydrolyzed into FFAs by hydrolytic enzymes from internal organs of seafood. Furthermore, the lipids in aquatic products were more prone to be oxidated after directly exposure to the air (de Aguiar Saldanha Pinheiro et al., 2020). Previously, Liu et al. (2016) found that

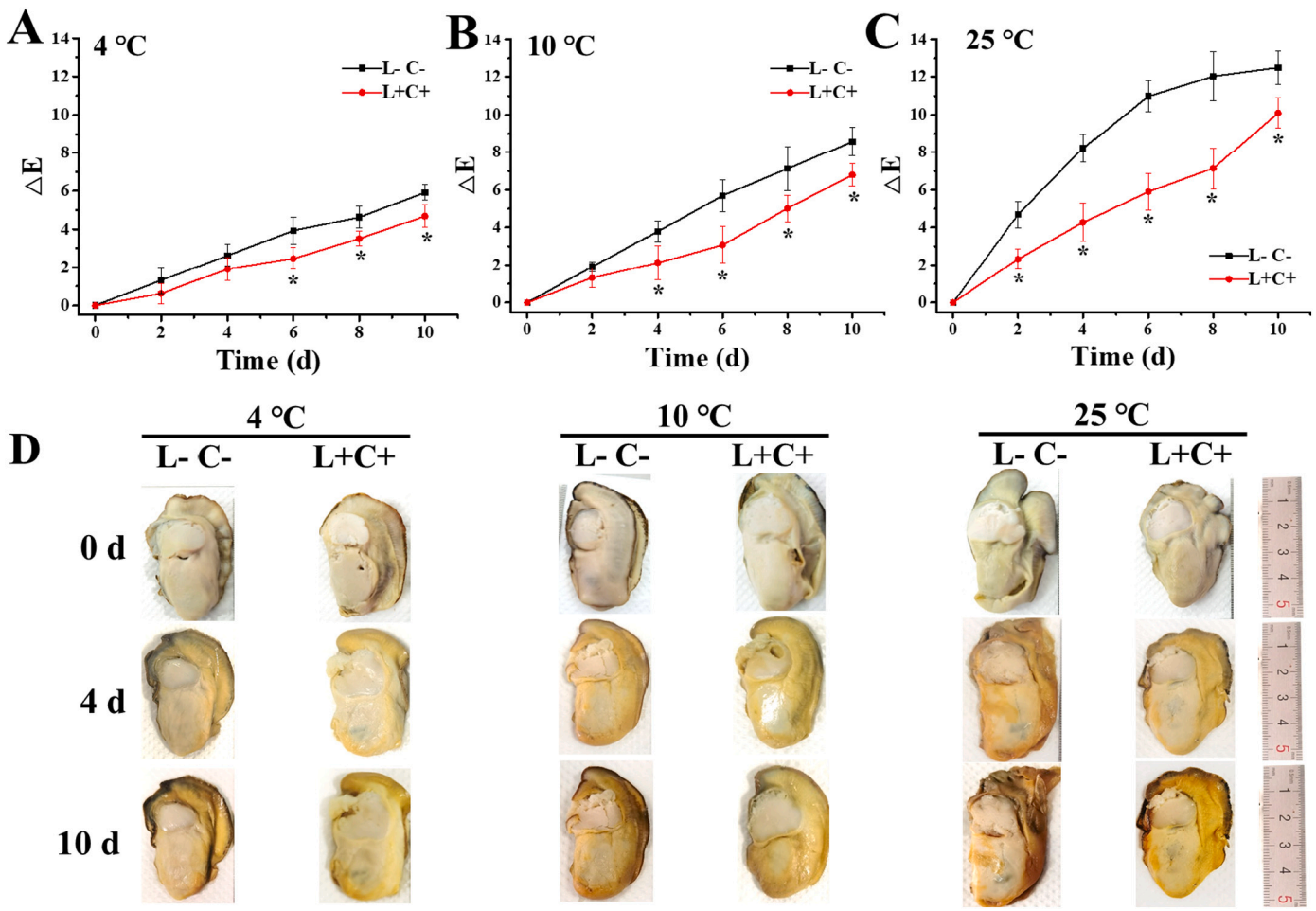

Fig. 7. Color changes of cooked oysters after the PDI treatment during storage for 10 days. (A–C) The changes of total color difference (ΔE) values; (D) Digital photos of the treated oysters during storage.
* indicates the significant differences ($p < 0.05$) between the PDI-treated oysters with 30 min LED irradiation (9.36 J/cm^2) and 50 μM curcumin (L+ C+) and negative control oysters (L- C-). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

PDI treatment could restrain the enzymatic activity of oyster. Therefore, it was inferred that the curcumin-mediated PDI might inhibit the endogenous enzymes to degrade the lipids. Meanwhile, it was well known that the PDI generated the reactive oxygen species (ROS) by consuming massive oxygen (Liu et al., 2016) which decreased O_2 molecules on the surface of oysters to prevent the lipids oxidation. In addition, the generated ROS had a very short half-life (Huang et al., 2020), therefore, they could cause cell death in a short time, but difficult to induce lipids oxidation during long storage. Therefore, it was concluded that the PDI could greatly maintain the quality of oysters by inhibiting the lipids degeneration to reduce the accumulation of MDA and FFA.

3.4. Effects of the PDI treatment on the physical changes of cooked oysters during storge

It is well known that color is a crucial factor determining the visual quality and the market value of seafood (Yadav et al., 2014). The changes of total color difference (ΔE) of the PDI-treated oysters during storage at 4 °C, 10 °C and 25 °C are expressed in Fig. 7. Generally, the ΔE values showed a gradual increase for all samples during the storage at 4 °C, 10 °C and 25 °C. The ΔE of oysters increased from 0 to 4.6 for the PDI-treated samples, while it increased from 0 to 5.9 for the negative control at 4 °C, and an obvious ($p < 0.05$) difference was determined from the 6th day at 4 °C (Fig. 7A). At 10 °C storage, the ΔE of the treated oysters increased from 0 to 6.8, which was lower than the negative control from 0 to 8.6 (Fig. 7B). While at 25 °C storage, the ΔE values greatly increased and almost reached 10 for the PDI-treated samples and negative control (Fig. 7C). Overall speaking, the ΔE of the PDI-treated oysters was obviously lower than those of negative control during storge. Therefore, the PDI treatment could retard the change of color to keep good appearance of oysters.

The ΔE describes the difference of the two samples in visual color "sphere", and generally its values within 0–1.5 indicate that color changes are too subtle to be recognized by naked eyes. However, the ΔE from 1.5 to 5.0 represent the visual color difference. When the ΔE exceeds 5.0, the color alteration is somewhat visible (Swer et al., 2019). On this basis, the color change of oysters could be visually observed between the PDI treatment and the negative control during storage at 4 °C, 10 °C and 25 °C (Fig. 7D). Kamble et al. (2020) and Peng et al. (2021) indicated that the changes of food color have been related to various factors like the lipid oxidation, enzymatic reaction, etc. This study revealed that the PDI effectively suppressed the oxidation of oyster lipids (Fig. 5). Therefore, the anti-lipids oxidation of the PDI might was beneficial for the maintenance of the sensory quality of oysters during storage.

Low-field nuclear magnetic resonance was employed to assess the freshness of aquatic products, and MRI as a complementary technology can permit the visual observations of spatial distribution of water molecules in food matrix (Marcone et al., 2013). Fig. 8 showed that the characteristics of water distribution in the PDI-treated oysters during 10 days storage at 4 °C, 10 °C and 25 °C. Generally, the red and blue color

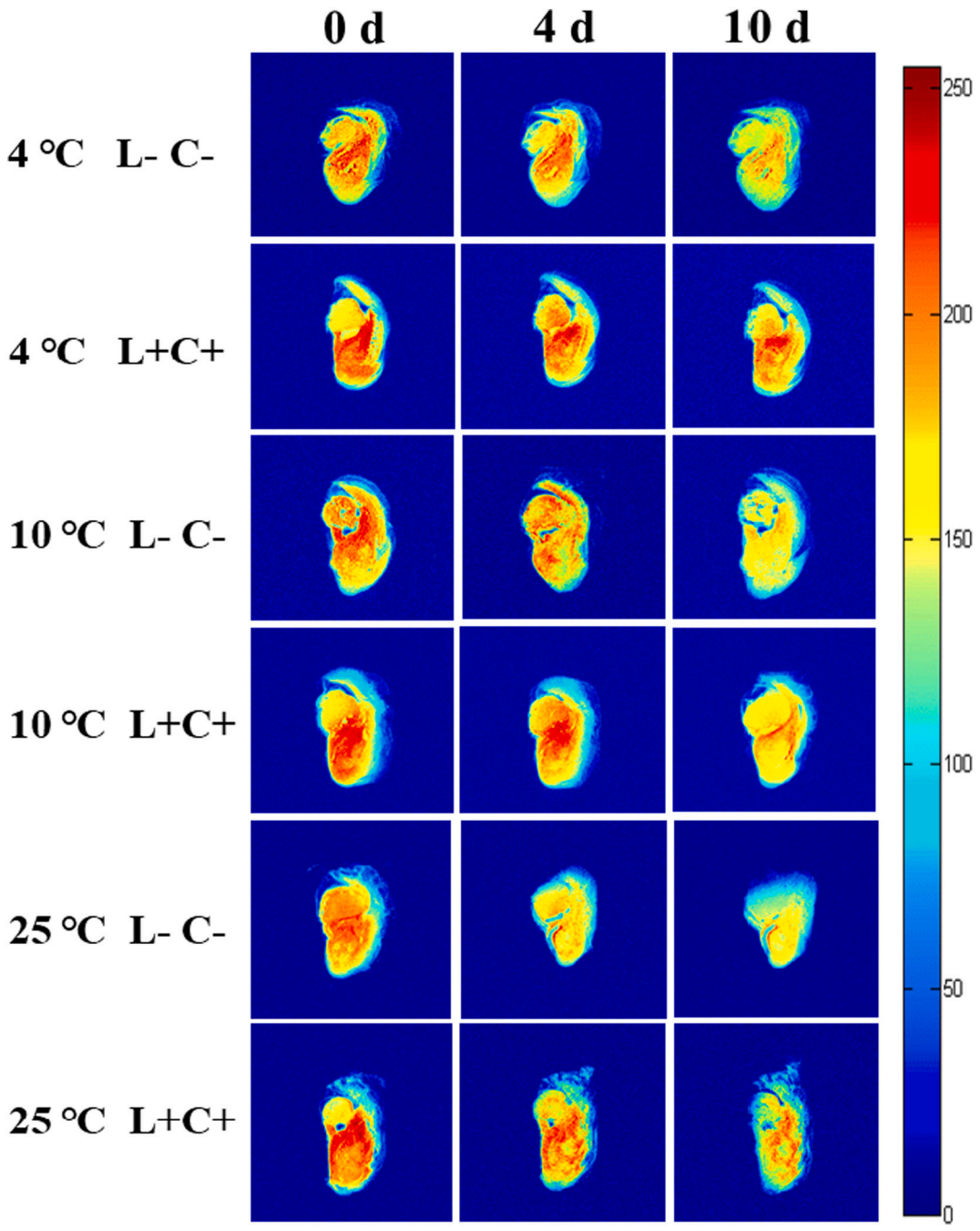

Fig. 8. Water distribution in cooked oysters after the PDI treatment during storage at 4 °C, 10 °C and 25 °C.

represent high and low proton density, respectively, in the pseudo-color images. In this study, the image brightness of all oysters shifted from red color to blue color, indicating the loss of water with the storage prolonging. However, all the PDI-treated samples were brighter than the negative control for all tested temperatures. These results proved that the PDI retarded the loss of water to maintain the freshness of oysters.

The texture characteristics played a crucial role in the quality of food during storage, such as hardness and springiness. Hardness was an important indicator for evaluating fish freshness, and the decreased hardness meant the softening of seafood (Cheng et al., 2014). Overall, the hardness values of the PDI-treated oyster presented a slightly ascendant tendency during storage at 4 °C (Fig. 9A). However, the PDI-treated oyster displayed a relatively higher ($p < 0.05$) hardness than negative control after the 6th day storage at 10 °C (Fig. 9B), although significant difference was not observed during initial storage. Additionally, the values were decreased from 328.5 g to 217.9 g for the PDI-treated oysters, which was evidently higher ($p < 0.05$) than the negative control (from 331.4 g to 182.8 g) during the whole storage at 25 °C (Fig. 9C).

Springiness referred to the ability of a body to deform and return to its original shape after removing external forces , which was also an important index to reflect the freshness of oysters (Xiong et al., 2015). The springiness showed a gradual decrease for the PDI-treated samples and negative control during the storage at 4 °C, 10 °C and 25 °C. However, the PDI-treated oyster showed perceptibly higher ($p < 0.05$) springiness from 8th day storage at 4 °C compared with the negative control (Fig. 9D). For the storage at 10 °C, the springiness of the treated oysters decreased from 3.4 to 1.3 mm, which was obviously higher than

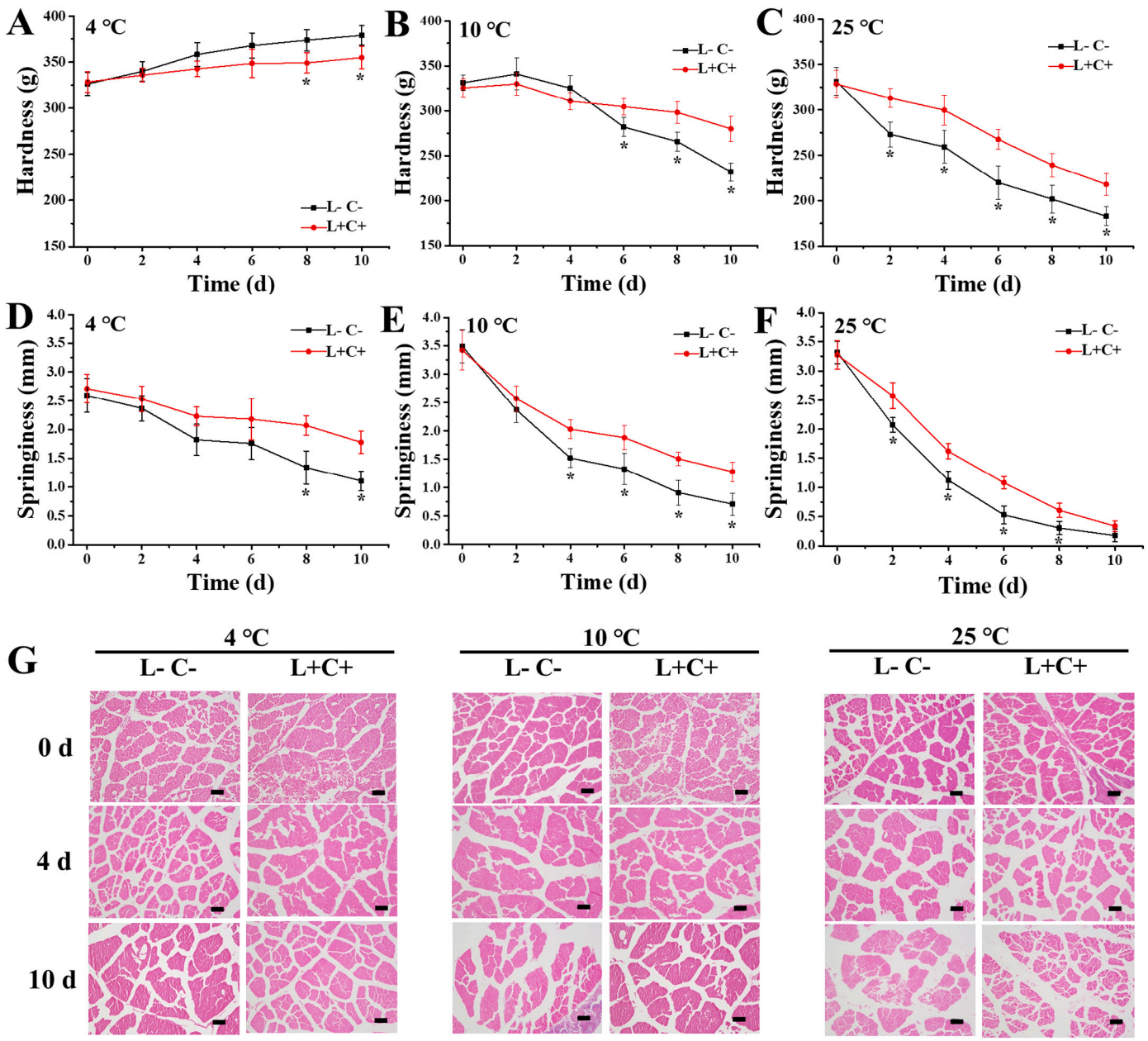

Fig. 9. Texture profile analysis (A–F) and histological section analysis (D) of cooked oysters after the PDI treatment during storage at 4 °C, 10 °C and 25 °C. * indicates the significant differences ($p < 0.05$) between the PDI-treated oysters with 30 min LED irradiation (9.36 J/cm^2) and 50 μM curcumin (L+ C+) and negative control oysters (L- C-).

those of the negative control from the 2nd day storage (Fig. 9E). Such phenomenon became much evident at 25 °C storage, because the PDI-treated samples always displayed higher ($p < 0.05$) springiness than the control samples during the storage (Fig. 9F). Therefore, the PDI treatment was effective in retarding the softening of oysters to retain good textural structure.

Fig. 9G showed the microscopic structures of muscle fibers in oysters during the storage at 4 °C, 10 °C and 25 °C. The strip fibers of oyster muscle were tightly arranged, and their structures were complete at day 0, and the average interstitial space of muscle fibers was measured as 30.7 μm. Obvious detachment of muscle fibers was observed at 4th day storage. At the 10th day, the muscle fibers showed serious detachments and a loss of integrity for both the PDI-treated samples and negative control, and the fiber gaps of the PDI-treated oysters increased to 39.7 μm (4 °C), 45.8 μm (10 °C) and 77.2 μm (25 °C), while those of the negative control increased to 48.9 μm (4 °C), 64.2 μm (10 °C) and 110.3 μm (25 °C). On this basis, the treated muscle fibers showed relatively tighter texture and smaller interstitial space than the negative control at all storage temperatures. Therefore, the PDI treatment could better preserve the quality of oyster muscle.

Lopez-Caballero et al. (2000) reported that the denaturation of myofibrillar fraction induced the increase of hardness, which led to aggregation and water loss during initial storage. However, as the temperature increasing, microbial growth and oxidation promotion accelerated the protein and lipid degradation rate of food, resulting in myofilament degradation and costameres distraction, thereby inducing the softening of muscle tissue and the decrease of hardness (Cheng et al., 2014; Lin et al., 2013). These facts greatly explained why the samples at 4 °C had a slightly changed hardness, but those at 10 °C and 25 °C presented an obviously decreased one. Therefore, the PDI treatment was efficient in maintaining the integrity of muscle fibers and their initial attachments to prolonging the freshness of oysters by reducing the

bacterial proliferation and lipid oxidation (Figs. 2 and 5).

4. Conclusions

The curcumin-mediated PDI efficiently inactivated *V. parahaemolyticus* on cooked oysters and greatly inhibited the recovery of injured cells in combination with suitable storage temperatures. In addition, it displayed the potent ability of restraining the decrease of pH, reducing the production of TVB-N, suppressing the lipids oxidation, and preventing the color change of the oysters. Moreover, the PDI treatment also possessed an excellent ability to maintain the integrity of muscle fibers to decrease the loss of water and the texture softening of oyster during storage. Therefore, the curcumin-mediated PDI is a highly effective technique in greatly preserving the storage quality and prolonging the shelf life of seafood.

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.

Acknowledgements

This research was supported by the National Key R&D Program of China (2018YFC1602205), the National Natural Science Foundation of China (31571917), Guangdong Basic and Applied Basic Research Foundation (2020A1515110960; 2020A1515110326), Shanghai Agriculture Applied Technology Development Program (T20170404), Luo Zhaorao Science and Technology Innovation Fund (A1-2004-20-201322).

References

Chaijan, M., Benjakul, S., Visessanguan, W., Faustman, C., 2006. Changes of lipids in sardine (Sardinella gibbosa) muscle during iced storage. Food Chem. 99, 83–91.
Chen, B., Huang, J., Li, H., Zeng, Q.-H., Wang, J.J., Liu, H., Pan, Y., Zhao, Y., 2020. Eradication of planktonic *Vibrio parahaemolyticus* and its sessile biofilm by curcumin-mediated photodynamic inactivation. Food Control 113.
Cheng, J.-H., Sun, D.-W., Han, Z., Zeng, X.-A., 2014. Texture and structure measurements and analyses for evaluation of fish and fillet freshness quality: a review. Compr. Rev. Food Sci. Food Saf. 13, 52–61.
Cheng, S., Zhang, T., Yao, L., Wang, X., Song, Y., Wang, H., Wang, H., Tan, M., 2018. Use of low-field-NMR and MRI to characterize water mobility and distribution in pacific oyster (Crassostrea gigas) during drying process. Dry. Technol. 36, 630–636.
Cook, D.W., 1991. Microbiology of bivalve molluscan shellfish. In: Ward, D.R., Hackney, C.R. (Eds.), Microbiology of Marine Food Products. Van Nostrand Reinhold, New York, pp. 19–39.
de Aguiar Saldanha Pinheiro, A.C., Tappi, S., Patrignani, F., Lanciotti, R., Romani, S., Rocculi, P., 2020. Effects of Novel Modified Atmosphere Packaging on Lipid Quality and Stability of Sardine (*Sardina pilchardus*) Fillets. Inter. J. Food Sci, Technol.
Dewey-Mattia, D., Manikonda, K., Hall, A.J., Wise, M.E., Crowe, S.J., 2018. Surveillance for foodborne disease outbreaks - United States, 2009-2015. MMWR Surveill. Summ. 67 (10), 1–11.
Fan, W., Chi, Y., Zhang, S., 2008. The use of a tea polyphenol dip to extend the shelf life of silver carp (Hypophthalmicthys molitrix) during storage in ice. Food Chem. 108, 148–153.
FAO. FAO Fisheries & Aquaculture - Fishery Statistical Collections - Global Aquaculture Production, http://www.fao.org/fishery/statistics/global-aquaculture-production/en Accessed: Oct, 9, 2020.
Ghate, V.S., Zhou, W., Yuk, H.-G., 2019. Perspectives and trends in the application of photodynamic inactivation for microbiological food safety. Compr. Rev. Food Sci. Food Saf. 18, 402–424.
Haghighi, M., Yazdanpanah, S., 2020. Chitosan-based coatings incorporated with cinnamon and tea extracts to extend the fish fillets shelf life: validation by FTIR spectroscopy technique. J. Food Quality 2020.
He, H., Adams, R.M., Farkas, D.F., Morrissey, M.T., 2002. Use of high-pressure processing for oyster shucking and shelf-life extension. J. Food Sci. 67, 640–645.
Huang, R., Choe, E., Min, D.B., 2004. Kinetics for singlet oxygen formation by riboflavin photosensitization and the reaction between riboflavin and singlet oxygen. J. Food Sci. 69, C726–C732.
Huang, J., Chen, B., Li, H., Zeng, Q.-H., Wang, J.J., Liu, H., Pan, Y., Zhao, Y., 2020. Enhanced antibacterial and antibiofilm functions of the curcumin-mediated photodynamic inactivation against *Listeria monocytogenes*. Food Control 108.
Hunter, A.C., Linden, B.A., 1923. An investigation of oyster spoilage. Am. Food J. 18, 538–540.
Josewin, S.W., Ghate, V., Kim, M.-J., Yuk, H.-G., 2018. Antibacterial effect of 460 nm light-emitting diode in combination with riboflavin against *Listeria monocytogenes* on smoked salmon. Food Control 84, 354–361.
Kamble, D.B., Singh, R., Kaur, B.P., Rani, S., 2020. Storage stability and shelf life prediction of multigrain pasta under different packaging material and storage conditions. J. Food Process. Pres. 44 (8).
Kilincceker, O., Dogan, I.S., Kucukoner, E., 2009. Effect of edible coatings on the quality of frozen fish fillets. Lwt-Food Sci. Technol. 42, 868–873.
Kim, M.-J., Adeline Ng, B.X., Zwe, Y.H., Yuk, H.-G., 2017. Photodynamic inactivation of *Salmonella* enterica Enteritidis by 405 ± 5-nm light-emitting diode and its application to control salmonellosis on cooked chicken. Food Control 82, 305–315.
Lin, T., Wang, J.J., Li, J.B., Liao, C., Pan, Y.J., Zhao, Y., 2013. Use of acidic electrolyzed water ice for preserving the quality of shrimp. J. Agric. Food Chem. 61, 8695–8702.
Liu, F., Li, Z., Cao, B., Wu, J., Wang, Y., Xue, Y., Xu, J., Xue, C., Tang, Q.J., 2016. The effect of a novel photodynamic activation method mediated by curcumin on oyster shelf life and quality. Food Res. Int. 87, 204–210.
Lopez-Caballero, M.E., Pe'rez-Mateos, M., Bonderi'as, A.J., Montero, P., 2000. Extension of shelf-life of prawns (*Panaeus japonicus*) by vacuum packaging and high-pressure treatment. J. Food Prot. 63, 1381–1388.
Marcone, M.F., Wang, S., Albabish, W., Nie, S., Somnarain, D., Hill, A., 2013. Diverse food-based applications of nuclear magnetic resonance (NMR) technology. Food Res. Int. 51, 729–747.
Miao, L., Walton, W.C., Wang, L., Li, L., Wang, Y., 2019. Characterization of polylactic acids-polyhydroxybutyrate based packaging film with fennel oil, and its application on oysters. Food Packag. Shelf Life 22.
Ndraha, N., Wong, H.-c., Hsiao, H.-I., 2020. Managing the risk of *Vibrio parahaemolyticus* infections associated with oyster consumption: a review. Compr. Rev. Food Sci. Food Saf. 19, 1187–1217.
Peng, Z., Wang, G., Zeng, Q.-H., Li, Y., Wu, Y., Liu, H., Wang, J.J., Zhao, Y., 2021. Synthesis, antioxidant and anti-tyrosinase activity of 1,2,4-triazole hydrazones as antibrowning agents. Food Chem. 341.
Silva-Angulo, A.B., Zanini, S.F., Rodrigo, D., Rosenthal, A., Martinez, A., 2014. Growth kinetics of Listeria innocua and *Listeria monocytogenes* under exposure to carvacrol and the occurrence of sublethal damage. Food Control 37, 336–342.
Soares, L.S., Flor Vieira, A.C., Fidler, F., Monteiro Fritz, A.R., Di Luccio, M., 2020. Pervaporation as an alternative for adding value to residues of oyster (Crassostrea gigas) processing. Sep. Purif. Technol. 232.
Songsaeng, S., Sophanodora, P., Kaewsrithong, J., Ohshima, T., 2010. Quality changes in oyster (Crassostrea belcheri) during frozen storage as affected by freezing and antioxidant. Food Chem. 123, 286–290.
Swer, T.L., Chauhan, K., Mukhim, C., Bashir, K., Kumar, A., 2019. Application of anthocyanins extracted from Sohiong (*Prunus nepalensis L.*) in food processing. Lwt-Food Sci. Technol. 114.
Tan, M., Li, P., Yu, W., Wang, J., Xie, J., 2019. Effects of glazing with preservatives on the quality changes of squid during frozen storage. Appl. Sci. l 9 (18).
U.S. Food and Drug Administration, 1997. Hazard Analysis and Critical Control Point Principles and Application Guidelines.
Wang, J.J., Sun, W.S., Jin, M.T., Liu, H.Q., Zhang, W., Sun, X.H., Pan, Y.J., Zhao, Y., 2014. Fate of *Vibrio parahaemolyticus* on shrimp after acidic electrolyzed water treatment. Int. J. Food Microbiol. 179, 50–56.
Xie, J., Sun, X.H., Pan, Y.J., Zhao, Y., 2012. Physicochemical properties and bactericidal activities of acidic electrolyzed water used or stored at different temperatures on shrimp. Food Res. Int. 47, 331–336.
Xiong, Z., Sun, D.-W., Dai, Q., Han, Z., Zeng, X.-A., Wang, L., 2015. Application of visible hyperspectral imaging for prediction of springiness of fresh chicken meat. Food Anal. Methods 8 (2), 380–391.
Xuan, X.-T., Fan, Y.-F., Ling, J.-G., Hu, Y.-Q., Liu, D.-H., Chen, S.-G., Ye, X.-Q., Ding, T., 2017. Preservation of squid by slightly acidic electrolyzed water ice. Food Control 73, 1483–1489.
Yadav, D.N., Sharma, M., Chikara, N., Anand, T., Bansal, S., 2014. Quality characteristics of vegetable-blended wheat–pearl millet composite pasta. Agric. Res. 3, 263–270.