



# The effect of a novel photodynamic activation method mediated by curcumin on oyster shelf life and quality



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## ABSTRACT

In this paper, the effect of photodynamic method mediated by curcumin (PDT) on the shelf life and quality of pacific oysters during storage at  $5 \pm 1$  °C were analyzed. In our previous study we investigated the optimal treatment conditions of photodynamic method mediated by curcumin to sterilization were 10  $\mu$ M photosensitizer concentration and 5.4 J/cm<sup>2</sup> light energy density. Under these conditions, the effect of a novel photodynamic activation method mediated by curcumin on oyster shelf life and quality was researched. The total bacterial counts, TVB-N content and sensory analysis were used to evaluate the effects on oyster shelf life. The oyster shelf life was prolonged from 8 days to 12 days after photodynamic treatment and the oysters in the treatment group displayed notable odor retention, produced fewer odor corrupting substances when the control group oysters reached the end of their shelf life (day 8). Texture, free amino acid contents and fatty acid levels were applied to estimate the quality of the treated oysters. The texture had no significant change after treated with PDT. At the end of oyster shelf life, compared PDT group (PDT) with control group (control), total free amino acid contents (control: 234.30 mg/100 g, PDT: 813.02 mg/100 g) was higher and free fatty acid levels (control: 0.071 mEq/L, PDT: 0.0455 mEq/L) displayed lower in PDT group. This indicated that the treated oysters oxidized minimally, decayed slowly, decomposed fewer nutrients and had lower metabolic levels of spoilage microorganisms. PDT has a positive effect on prolonging oyster shelf life and its quality.

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## 1. Introduction

Oysters are the most abundant edible shellfish and are well known for their physiological activities, such as improving atherosclerosis, as well as their anti-inflammatory (Lee, Kim, & Han, 2013) and antioxidant effects (Umayaparvathi, Meenakshi, Vimalraj, et al., 2014). The oyster has a strong water filtering ability, which means to be easily enriched microbes in the culture process, so it has a short shelf life and small sales radius. Previous studies have demonstrated that microbial growth is one of mainly causes inducing seafood spoilage, which mainly refer to *proteobacteria*, *Pseudoalteromonas* and *Vibrio* (Madigan et al., 2014). Therefore, an effective oyster sterilization method which can implement the prolongation of oyster shelf life and expand the sales radius of fresh oysters is urgently needed in the oyster processing industry.

Current strategies for inactivating bacteria include heat treatment (Silva & Gibbs, 2012; Huang & Joseph, 2010), irradiation (Byun et al., 2000; Trivedi et al., 2007) high density carbon dioxide sterilization (Choi, Ryu, Lee, et al., 2008), and high pressure processing (HPP) (He, Adams, Farkas, & Morrissey, 2002; Houška et al., 2006; Tang et al., 2010). However, these treatments have their limitations, for instance,

higher temperatures can change the food structure or even induce a loss of flavor, color, sensory characteristics and nutritional substances (Silva, Hendrickx, Oliveira, & Tობback, 1992). Irradiation present a hazard to exposed workers and high pressure accelerate aerobic thermal degradation of unsaturated fatty acids (Kebede et al., 2013). High density carbon dioxide sterilization is limited by its treatment conditions (Hemmer, Drews, Laberge, & Matthews, 2007). Thus, a sort of new cold-sterilization technologies which are safe, efficient, practical, and preferably inexpensive for inactivating bacteria in fresh oysters are needed.

Photodynamic therapy (PDT) is a new cold-sterilization technology that involves the combination of non-toxic photosensitizers (PS) and oxygen to generate cytotoxic free radicals. It can irreversibly inactivate bacteria (Melo, 2014; Li et al., 2015; Brovko, 2010; Juzeniene, Peng, & Moan, 2008). Currently, PDT is used in a number of experimental cancer and dermatosis treatments (Roblero-Bartolón & Ramón-Gallegos, 2015; Kim, Jung, & Park, 2015), but there are few reports about its applications in the food industry. Curcumin, as the active ingredient, is isolated from the traditional Chinese herbal medicine - turmeric, the common source of curcumin, (Ghosh, Banerjee, & Sil, 2015) and it exhibits diverse biological activities, such as anti-oxidant (K et al., 2003), anti-inflammatory (Sinha, Sadhukhan, Saha, Pal, & Sil, 2015), anti-diabetes (Zhang, Fu, Gao, & Liu, 2013), anti-obesity (He et al., 2012), and anti-Alzheimer (Zhang

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et al., 2006) properties. More recently, curcumin (60  $\mu\text{M}$ ) has been studied as a potential photosensitizer of PDT (440–480 nm, LED) to kill the main gram-negative periodontal pathogens (Mahdi et al., 2015). With the wavelength of 470 nm at 3.6 J/cm<sup>2</sup> in PDT and 10  $\mu\text{M}$  curcumin, the norovirus was significantly inhibited by 1–3 log PFU/mL in oysters which has been reported by our group (Wu, Hou, Cao, et al., 2015). However, there is no work reported regarding its effect on oyster shelf life and quality.

Therefore, we evaluated the effect of photodynamic activation method mediated by curcumin on oyster shelf life and quality to explore whether it could be used as a non-thermal cold sterilization method for oysters.

## 2. Materials and methods

### 2.1. Photodynamic treatment

Curcumin (95% purity) (Ci Yuan Biotechnology Co., Ltd. Shanxi, China) was dissolved in edible alcohol (Food Grade, 95% ethanol; Tianjin Guayue Group Co., Ltd. Tianjin, China) at a concentration of 40 mM and kept in the dark at  $-20\text{ }^{\circ}\text{C}$  until used.

In oyster studies, 9 oysters per group bio-accumulated with a concentration of curcumin (10  $\mu\text{M}$ ) in an artificial seawater system (salinity 3.3%, the shellfish weight: the weight of the water = 1:4) for 3 h at about 10  $^{\circ}\text{C}$  as described in our previous report (Binbin, Juan, Chuanshan, et al., 2016). Artificial seawater was sterilized using UV irradiation for 1 h in advance. Care was taken to make sure that the nozzle of the oxygen tube was not directed toward the oysters. The oysters were then opened in sterile conditions and irradiated using blue light (470 nm) at 5.4 J/cm<sup>2</sup> (the optical power density was fixed at 0.06 W/cm<sup>2</sup> with an irradiation time of 90 s, LED blue light source was supplied by School of Chinese Medicine, the Chinese University of Hong Kong). The oysters used in this experiment were divided into 18 groups: neither blue light irradiation nor curcumin treatment (control, 9 groups) and blue light irradiation treatment plus curcumin (10  $\mu\text{M}$ ) (PDT, 9 groups). All samples were stored at  $5 \pm 1\text{ }^{\circ}\text{C}$  and detected every two days.

### 2.2. The calculation of oyster shelf life

#### 2.2.1. Total bacteria analysis

Conte et al. described that the microbiological analyses used to assess the oyster shelf life (Conte et al., 2009) and an aerobic plate colony count (APC) was used as a method of microbiological determination. In our study, 5 g oyster meat with 45 mL sterile water homogenized by food processor (type: JYL-C012, Joyoung Co., Ltd. China) and the homogenate was diluted with the proportion of 1:1000, 1:10000, 1:100000 before inoculated in LB agar medium (Beijing Land bridge Technology Co., Ltd. China). Aerobic plate count (APC) was determined by counting the number of colony-forming units after incubation at 37  $^{\circ}\text{C}$  for 48 h. Microbial counts were expressed as log<sub>10</sub> CFU/g.

#### 2.2.2. TVB-N analysis

The total volatile basic nitrogen (TVB-N) of the 9 samples was determined by the microdiffusion method as described by Goulas and Kontominas (Goulas & Kontominas, 2005). Results were expressed in mg N/100 g of oyster meat.

#### 2.2.3. Sensory analysis

9 samples in each group were evaluated by a 6-member trained panellists (one female and five males between 27 and 45 years old) according to ISO 8586-1 (Standard, B. O. I., 2009). Odor, color, mucus appearance, texture, pallium gill filaments and shell muscle were selected as the main quality attributes. The sensory evaluation was based on a five point scale to determine: Odor (5, extremely desirable; 1, extremely unacceptable/off-odours); color discoloration (5, no discoloration; 1, extreme discoloration); texture (5, firm; 1, very soft);

and overall acceptability (5, extremely desirable; 1, extremely unacceptable) of the samples. Shelf-life criteria assumed that rejection would occur when the sensory attributes declined below 2.5. The scoring guidelines are shown in Supplementary Table S1 (Cao, Xue, Liu, et al., 2009).

### 2.2.4. Flavor components analysis

An electronic nose (E-nose) (Fox4000, Alpha M.O.S co., LTD, France) was used to analyze the oyster flavor compounds. We detected the samples in control and PDT groups (0d, 8d), 9 samples per group and every group was detected three times. In the first stage, minced meat (10 g) was placed into a 150 mL glass flask and sealed with PE film. The flask was equilibrated at 40  $^{\circ}\text{C}$  for 5 min. In the second stage, the gaseous compounds from the headspace of the glass flask were pumped through the sensor array at a rate of 300 mL/min for 50 s. The inlet velocity was 1 L/min, and the testing time was 1 min.

### 2.3. The evaluation of oyster quality

#### 2.3.1. Texture analysis

Samples in PDT group or normal group were selected with the volume of 5 cm \* 3.5 cm \* 2 cm. 9 oysters in each group. Three replicates were tested for each time interval.

TMS-PRO texture analyzer (food technology corporation, USA) was used to detect indicators, including hardness, resilience, cohesiveness, viscoelasticity and chewiness. Around cylindrical stainless steel probe (PO.5) was used. The test conditions were set as follows: the maximum limit was 1000 N, the percentage of deformation was 20%, the inspection speed was set to 40 mm/min, and the initial deformation force was 0.01 N. Data were collected and processed by Texture Expert software.

#### 2.3.2. Free amino acid analyses

The concentration of free amino acids were detected by L-8900 amino acid analyzer (Hitachi, Japan) with ninhydrin reagent. The analytical column (60 mm  $\times$  4.6 mm i.d., 3  $\mu\text{m}$ ) and the guard column (5.0 mm  $\times$  4.0 mm i.d., 5  $\mu\text{m}$ ) consisted of polystyrene cross-linked by divinylbenzene, with sulfone groups as the active exchange sites. Minced meat (5 g, 9 samples) was diluted with distilled water to ten times volume. The mixture was then placed in a water (85–90  $^{\circ}\text{C}$ ) bath for 30 min and supernatant was obtained by centrifuging with the speed of 10,000 r/min for 10 min. The supernate was filtered by 0.22  $\mu\text{m}$  filter membrane after diluted with 0.01 M hydrochloric acid and detected by L-8900 amino acid analyzer.

#### 2.3.3. Total lipids analysis

The total lipids were extracted according to the method reported by Folch, Lees, and Stanley (1957). Appropriate volumes of chloroform and methanol (2:1, v/v) were mixed with 10 g (9 samples) of homogenized meat to a total of 50 mL, and the mixture was then placed in a water bath for 45 min at 37  $^{\circ}\text{C}$ . Ultrapure water was added into the collected filtrate to separate for 12 h. The supernatant was concentrated by rotary evaporation, and the concentrate was dissolved by petroleum ether, which was then transferred to a 50 mL volumetric flask and kept in constant volume. The total lipid were prepared by transmethylation with HCl/methanol at 1:5 by shaking at 90 for 3 h. The samples were analyzed by GC-MS. In order to quantify the fatty acid content, C15:0 (Sigma, co., LTD, USA), a standard fatty acid not present in rodents, was added to the lipid samples extracted from the organs.

Agilent 7820 gas chromatographic (Agilent co., LTD, USA) was equipped with a flame ionization detector (FID) and an Agilent INNOWAX capillary column (30 m  $\times$  0.32 mm  $\times$  0.25  $\mu\text{m}$ ). Sample split ratio is 25:1. The temperatures of the detector and the injector were kept at 250  $^{\circ}\text{C}$  and 230  $^{\circ}\text{C}$ , respectively. The column temperature was increase from 140  $^{\circ}\text{C}$  to 210  $^{\circ}\text{C}$  at a speed of 3  $^{\circ}\text{C}$  per minute and was maintained at 210  $^{\circ}\text{C}$  for 10 min.

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