Food Control 35 (2014) 207–212

Contents lists available at SciVerse ScienceDirect

Food Control

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

# Effect of acidic electrolyzed water ice on quality of shrimp in dark condition

Jing Jing Wang [a,1], Ting Lin [a,1], Ji Bing Li [a], Chao Liao [a], Ying Jie Pan [a,b,c], Yong Zhao [a,b,c,*]

[a] College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China
[b] Laboratory of Quality & Safety Risk Assessment for Aquatic Products on Storage and Preservation (Shanghai), Ministry of Agriculture, Shanghai 201306, China
[c] Shanghai Engineering Research Center of Aquatic-Product Processing & Preservation, Shanghai 201306, China

ARTICLE INFO

*Article history:*
Received 31 March 2013
Received in revised form
24 June 2013
Accepted 2 July 2013

*Keywords:*
Acidic electrolyzed water ice
Preserving
Polyphenol oxidase (PPO)
PCR-DGGE

ABSTRACT

Electrolyzed water ice is a relatively new concept developed in food industry in recent years. The objective of this study was to investigate the effects of acidic electrolyzed water (AEW) ice, compared with tap water (TW) ice, on quality of shrimp (*Litopenaeus vannamei*) in dark condition. The chemical changes, microbiological changes and polyphenol oxidase (PPO) activity of shrimp stored in AEW ice or TW ice were measured periodically. The results showed that AEW ice significantly ($p < 0.05$) inhibited the changes of pH, the formation of total volatile basic nitrogen (TVBN), and the proliferation of total bacteria counts in shrimp. The diversity of bacterial flora in shrimp stored in AEW ice was greatly reduced according to the Shannon index and the average similarity coefficient based on PCR-DGGE method. Additionally, AEW ice could serve as a potential substance to inhibit PPO activity in shrimp. Based on above analysis, AEW ice is a valid post-harvest treatment for preserving the quality of seafood in dark condition.



## 1. Introduction

Shrimp are one of the most important fishery products in the South and South-eastern parts of Asia and is also the leading seafood consumed in many countries over the world due to their delicacy (Nilesh & Soottawat, 2012; Xu, Wang, Sun, Liu, & Li, 2013). However, this high value crustacean is very perishable associated with microbiological, chemical, and physical changes during post-mortem storage (Nirmal & Benjakul, 2010). Simultaneously, shrimp are also known to be carriers of pathogenic microorganisms, such as *Listeria monocytogenes*, *Vibrio parahaemolyticus* (Liu, Duan, & Su, 2006; McCarthy, 1997; Xie, Sun, Pan, & Zhao, 2012a; Xie, Sun, Pan, & Zhao, 2012b). Therefore, there is an obvious need for the development of new technologies and efficient preservation methods to meet the consumers' demand for seafood safety and high quality aquatic products (Aslí, Ahmet, Tuncay, & Mehmet, 2012).

In terms of preventing the rapid proliferation of bacteria and preserving the freshness of shrimp, the tap water (TW) ice is typically used to maintain the quality of shrimp (Koseki, Fujiwara, & Itoh, 2002). However, the bacteria in shrimp can't be inactivated generally in TW ice. Once shrimp are removed from TW ice and exposed to temperature-abused environments before consumption, the bacteria can multiply and cause spoilage (Feliciano, Lee, Lopes, & Pascall, 2010; Phuvasate & Su, 2010). Thus, if ice made with sanitized water is used to store the shrimp, it not only has the advantages of TW ice but also the potential to be bactericidal to the microorganisms (Feliciano et al., 2010).

Acidic electrolyzed water (AEW) ice has been demonstrated to have bactericidal activity. Koseki et al. (2002) reported that populations of aerobic bacteria in lettuce were reduced when packed in AEW ice. Koseki et al. (2004) also showed that AEW ice could significantly reduce *L. monocytogenes*, *Escherichia coli* O157:H7 with the increasing concentration of $Cl_2$ generated from AEW ice. Kim et al. (2006) showed that AEW ice significantly retarded the growth of aerobic and psychrotrophic bacteria on fish. Phuvasate and Su (2010) studied the efficacy of AEW ice in reducing histamine-producing bacteria (*Enterobacter aerogenes*, *Enterobacter cloacae* et al.) on fish skin. Moreover, the results from the study of our group demonstrated that the maintenance of ACC and bactericidal activity of AEW could be achieved with AEW ice. Consequently, AEW ice has the potential use of keeping freshness of products by solid ice and sanitization of products by melted AEW (Xie et al., 2012b).

* Corresponding author. College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China.
*E-mail address:* yzhao@shou.edu.cn (Y. Zhao).
[1] The first two authors contributed equally to this work.

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http://dx.doi.org/10.1016/j.foodcont.2013.07.005

All the above studies about AEW ice were conducted under light condition. However, storage conditions can affect chemical and physical properties of AEW (Hsu & Kao, 2004; Huang, Hung, Hsu, Huang, & Hwang, 2008). When stored under an open, agitated and diffused light condition, AEW had the highest chlorine loss rate. Moreover, under open condition chlorine loss through evaporation followed first-order kinetics (Huang et al., 2008). Therefore, the objective of this study was to investigate the effects of acidic electrolyzed water ice, compared with tap water ice, on the quality changes of shrimp (*Litopenaeus vannamei*) including the chemical changes, microbiological changes and polyphenol oxidase (PPO) activity in dark condition.

## 2. Materials and methods

### 2.1. Preparation of AEW ice

AEW was prepared with electrolysis of 0.1% sodium chloride solution at a certain time using strongly acidic electrolyzed water generator (FW-200, AMANO, Japan). The pH and ORP were determined using a pH/ORP meter (model pH 430, Corning Inc., NY). The ACC in AEW was determined by a colorimetric method using a digital chlorine test kit (RC-2Z, Kasahara Chemical Instruments Corp., Saitama, Japan). Each 2 L AEW was poured into sealed plastic bag and frozen at −20 °C for 24 h immediately after production. The obtained AEW ice was crushed using a hammer before treatment. TW ice was generated as a treatment control. The pH, ACC and ORP were measured after melting AEW ice and TW ice in a sealed bag in a 70 °C water bath completely. All measurements were carried out in triplicate.

### 2.2. Shrimp samples preparation and storage treatment conditions

Shrimp with an average weight of 10 ± 1 g were purchased from a local supermarket in Shanghai, PR China. The shrimp were kept alive and transported to laboratory. Upon arrival, all shrimp were washed with tap water before treatment.

The AEW ice and TW ice were poured into a sterile stainless steel tray with 2 blocks (72 × 48 × 9.5 cm). All shrimp samples were divided into 2 parts randomly and then treated with AEW ice and TW ice, respectively. Shrimp were placed onto the AEW ice and TW ice, and then the surface of shrimp was covered with AEW ice and TW ice. Subsequently, the sterile stainless steel tray was placed in a clean room without light, and stored for 6 days at 18 ± 3 °C. AEW ice and TW ice were renewed every 12 h and the chemical, microbiological changes and polyphenol oxidase (PPO) activity in shrimp were measured periodically. All measurements were carried out in triplicate.

### 2.3. pH and total volatile basic nitrogen (TVBN) analysis

pH value were measured according to the method of López-Caballero, Martinez-Alvarez, Gomez-Guillen and Montero (2007) with a slight modification. Shrimp (10 ± 1 g) were homogenized with 90 ml deionised water for 2 min and the homogenate was kept at room temperature for 5 min. Measurement was performed using a pH-meter (Mettler-Toledo, Switzerland). Total volatile basic nitrogen (TVBN) was determined using the method of Malle and Poumeyrol (1989). TVBN contents were expressed as mg TVBN/100 g shrimp meat.

### 2.4. Microbial analysis by traditional plate count enumeration

Microbial analysis was performed by the spreading plate method. On each day, shrimp samples (10 ± 1 g) under AEW ice and TW ice storage were collected aseptically, and then separately homogenized with 90 ml of sterile 0.85% physiological saline solution for 2 min in a filtered stomacher bag using a stomacher (BagMixer400 VW, Interscience, France). Subsequently, the homogenate was serially diluted ten-fold with 0.85% physiological saline solution, and 0.1 ml of each dilution was spread on plate count agar. After incubated at 37 °C for 24 h, the total bacteria counts were counted.

### 2.5. Bacterial diversity analysis using PCR-DGGE method

PCR-DGGE was performed to analyze the changes of the variety of bacteria on shrimp. DNA was extracted using Biospin Bacteria Genomic DNA Extraction Kit (BioFlux, Bioer Technology Co.,Ltd), and PCR reactions were performed based on V3 variable region. Primers V3-2 (5′-ATTA CCGCGGCTGCTGG-3′) and V3-3 incorporated a 40-bp GC clamp (5′-CGCCCGCCGCGCGCGGCGGGCGGGG CGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3′) were used to 16SrRNA gene amplification of the bacteria in shrimp samples (Jensen, Ovreas, Daae, & Torsvik, 1998). PCR amplification was performed in 20 μl reaction mixture, which contained 10 μl of Premix Ex Taq (Takara, Japan), 8 μl of dd$H_2O$, 0.5 μl of each primer and 1 μl of DNA template. PCR program was conducted in Hybaid PCR Express Thermal Cycler (Ashford, Middlesex) with the following parameters: initial denaturation at 95 °C for 3 min; 25 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 30 s; final extension at 72 °C for 5 min. The amplified products were separated with 1% (m/v) agarose gel electrophoresis and visualized under UV light.

The 200 bp PCR fragments were separated using DGGE, performed with the BioRad DCode™ Universal Mutation Detection System (BioRad Laboratories,USA). The PCR products were applied to 8% (m/v) polyacrylamide gels in 1× TAE buffer, with a gradient of between 40% and 60%. Electrophoresis was performed at 60 V for 16 h at a constant temperature of 60 °C. The DNA was stained with SYBR green I and visualized under UV light.

Scanned images of the DGGE gels were analyzed with Image Lab (Bio-rad, USA). Shannon index was calculated by DGGE banding pattern analysis. The Shannon index of bacterial diversity, $H'$, was obtained using the function: $H' = -P_i \log P_i$, where $P_i$ is the importance probability of the bands in a gel lane (Eichner, Erb, Timmis, & Wagner-Döbler, 1999). It was calculated as $P_i = n_i/N$, where $n_i$ is the height of a peak and $N$ is the sum of all the peak heights of the bands in the densitometric profile (Ogino, Koshikawa, Nakahara, & Uchiyama, 2001).

### 2.6. Polyphenol oxidase (PPO) activity assay

Enzymes were extracted based on the method described by Zhou, Li, Yan & Xie (2011). Briefly, 20 g of shrimp flesh were homogenized with 50 ml of 0.067 M phosphate buffer (pH 7.2) and followed by filter. The filtrate was centrifuged at 12,000 *g* for 30 min and the supernatant was used for further assays. PPO activity assay was conducted according to the method of Li, Li and Lin (2003) with a slight modification. Phenoloxidase activity was assayed by mixing 0.4 ml of the crude extracts with 0.2 ml 0.5 mol/L of each catechol and L-proline dissolved in 2.2 ml 0.067 M sodium phosphate buffer (pH 7.2). PPO activity was measured at $OD_{530}$ after 37 °C water bath for 10 min. All measurements were performed in triplicate. By definition, the specific activity of PPO was defined as the amount of enzyme that caused a change of 0.01 AU per min per volume.

### 2.7. Statistical analysis

Values were expresses as the mean ± standard deviation (SD). Statistical analysis was performed using SPSS statistical package