2011 2nd International Conference on Biotechnology and Food Science
IPCBEE vol.7 (2011) © (2011) IACSIT Press, Singapore

Improving the quality of cold storage black tiger prawn (*Penaeus monodon*) using ozonated water

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Abstract— **The ozonated water with total ozone concentration (TOC) 220-1130 mg/l or residual ozone concentration (ROC) less than 0.1-0.31 mg/l were evaluated against total bacteria and psychrotrophic bacteria. It was found that antimicrobial effect increased when the concentration of ozone increased for the cell suspension and on the shrimp sample. During storage at 4°C, the shrimps washed with water and ozonated water did not have sensory scores significantly different ($P \geq 0.05$) in color and firmness. The TVB and pH of the shrimp meat were significantly lower than the water washed. The ozonated water 1130 mg/l could reduce the number of bacteria on the shrimp and also retarded the growth of psychrotropic bacteria. The ozonated water could prolong the detectable bad odor for more than 4 days.**

Keywords*‐ *black tiger prawn, cold storage, quality, ozonated water

I. INTRODUCTION

The microbial safety of foods is increasingly concern especially raw food of animal origin. The attention is now being paid to the marine products, such as shrimp. Even though, shrimps were cooked before consumption, they were mostly not completely cooked or over cooked. The over cooking of these products affected the texture and palatability. It is therefore the producers necessary to find efficient methods of the removal of microorganisms from these raw products that do not adversely affect their organoleptic properties.

Ozone is 3 atoms of oxygen bound together instead of the normal 2 atoms. Ozonation has been used for years to disinfect water for drinking purpose in Europe [1]. Ozone destroys algae, viruses, bacteria, and fungi on contact and it breaks down harmful chemicals into simpler less damaging molecules. The biocidal effect of ozone is caused by its high oxidation potential, reacting with organic material up to 3,000 times faster than chlorine [2]. The bacterial cell surface has been suggested as the primary target of ozonation [3]. The kinetics of microbial inactivation by ozone in aqueous system was reported by Khadre et al. [4]. Ozone has been declared as a generally recognized as safe (GRAS) substance by an expert panel for use in food processing [5]. This affirmation encouraged broader use of this gas in food industry.

The use of ozone for food hygiene, ozone has been used for disinfecting recycled poultry chill water and disinfection of poultry carcasses [6]. Ozone destroyed the microorganisms with no significant lipid oxidation, off-flavor development or loss in carcass skin color. It has been seen by many as a replacement of chlorine for washing of produce [4,7]. Treating fruit and vegetable produces with ozone has been used to increase shelf-life [8]. Ozone reduced the microbial loads and did not cause oxidation of the volatile oils [9]. The affectivity of ozone depended on time of contact [10] and concentration [11].

Ozone has been successfully applied to many food products to reduce the microbial load without changes in the organoleptic properties. It could possibility to use in the sea food processing. This report was aimed to study the effect of ozone on the reduction of bacterial number on fresh shrimps and some qualities of shrimps during 4°C storage.

II. MATERIALS AND METHODS

The ozonated water was prepared by ozone generator model OZ 3050 (EBASE Corp., Thailand) with capacity of 2.5 g-ozone/hr. Various concentration of ozone in the ozonated water was accomplished by varying the production time at 20°C. Total ozone output (TOC) was analyzed by the iodometric method [12]. Residual ozone concentration (ROC) in sample water was determined using a reagent test kit (Spectroquant Merck, Merck Co., Germany) based on the N,N-diethyl-1,4 phenylenediammonium (DPD) spectrophotometric method [13].

A. *Effect of ozonated water on the survival of several bacteria*

The normal flora was cultured by inoculated the dilution water prepared for the total plate count into nutrient broth and incubated at 30°C. The enumeration of normal flora was done by the method of total plate count (TPC) described in [14]. The bacterial suspension was prepared and adjusted to reach the optical density (OD) of 0.25 when measure at the wavelength 550 nm, at which contained bacterial cells at the level of 9.0 $\log_{10}$ CFU/ml. One ml bacterial suspensions were mixed with 9 ml of 220, 450, 550, 930, 10206 and 1130 mg/liter ozonated water for 1 minute. The number of survivals was determined. The ozone activity was determined by the difference between number of bacteria of treated and untreated sample divided by untreated sample (death fraction: DF). The DF was calculated as equation 1.

$$DF = (N_0 - N)/N_0. \quad (1)$$

N_0 = initial number of bacteria express in $\log_{10}$ CFU
N = number of bacteria after treated express in $\log_{10}$ CFU

The 300 g of fresh shrimps from a market were washed with sterile water and left to dry in a biosafety cabinet. The bacterial suspension of Escherichia coli, Vibrio parahaemolyticus and Salmonella Anatum were prepared by culturing in Nutrient Broth (NB) for overnight and adjusting the optical density to 0.1 (OD_{625}) with sterile phosphate buffer solution (PBS) and then diluted to 1/10 of the adjusted solution. The dried shrimps were soaked in the prepared bacterial suspension for 5 minutes. The inoculated shrimps were washed with ozonated water at 0, 220, 450, 550, 930, 10206 and 1130 mg/liter (20^{o}C) for 1 minute. The ratio of shrimp to water of 1:3 (w/v) was applied. The reduction of bacteria was determined.

The experiments design was Completely Randomized Design (CRD), with 3 replications. The means were separated using Duncan's New Multiple range test [15]

B. The quality of ozone treated shrimps during 4^{o}C storage

Fresh shrimp samples were washed in water, ozonated water contained 220 mg/ml and ozonated water contained 1130 mg/ml under 20^{o}C. The ratio of shrimp to washing liquid was 1:3 (w/w). The washed shrimps were packed in a plastic bag and stored under 4^{o}C. The total volatile base (TVB), total count and psychotropic bacteria were determined. The organolyptic test of the stored shrimp was studied with 12 panelists to determine the texture, smell and color using descriptive test with 5 level score.

The experimental design for organoleptic studies was Randomized Completely Block Design (RCBD), with 2 replications. The means were separated using Duncan's New Multiple range test [15]

III. RESULTS AND DISCUSSION

A. Effect of ozonated water on the survival of several bacteria

The concentration and residue of ozone in 400 ml water when it was ozonated for 5, 10, 15, 20, 25, 30, 40, 50 and 60 min were shown in Figure 1. The TOC of the ozonated water reached the maximum of dissolving at 25 minutes of ozonation while the residue of the ozone was increased as the time for ozonation increased. The methodology that we applied for determination of ozone residue has sensitivity to determine at 0.1 mg/l and over. We were not able to detect the ozone residue in the water on the first 10 minute of ozonation. From observation, it was found that the amount

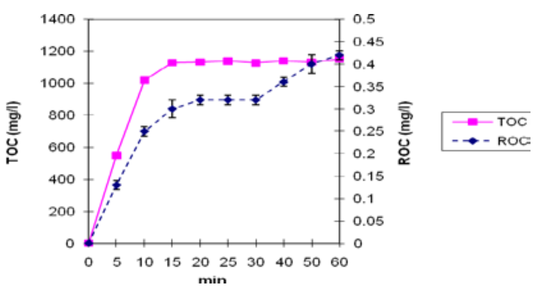


Figure 1. The concentration of ozone (TOC) and residue (ROC) in the ozonated water (400 ml) at various time of ozonation.

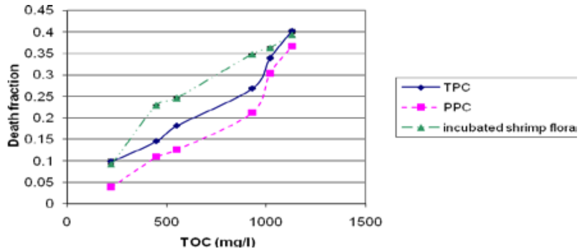


Figure 2. The activity of ozone at various concentrations (TOC) on bacteria in the suspension, incubated shrimp flora, and on shrimp samples (TPC and PPC) with 1 min contact time.

of water applied for ozonation also affected the concentration of TOC and ROC of the ozonated water. As we were not able to determine the ROC with the method we described above in the ozonated water at the TOC lower than 550 mg/l, only TOC would be reported in further study.

The contact time of ozonated water and microbes was fixed to 1 min followed the report of Restiano et al. [10] who described that the number of bacteria was rapidly reduced then started to level off after one minute (Figure 2). The initial bacterial number in the prepared suspension was about 9.0 $\log_{10}$ CFU/g. The higher ozone concentration in water increased the bactericidal ability. The same relationship of ozone concentration and biocidal activity showed on the shrimp samples. The activity of ozone which described as DF in the concentration range of 500 to 1000 mg/l on shrimp sample was lower than in the cell suspension. The ozone might reacted with organic materials, specially shrimp protein, which has been described in many reports [10,16,17, 18].

The ozone also had the biological activity on tested pathogenic bacteria on the shrimp samples (Figure 3) as on the shrimp flora. The different pathogenic bacteria differently responded to the effect of ozone concentration. *E. coli* and *V. parahaemolyticus* were more sensitive than *S.* Anatum. The 1130 mg/l ozonated water was able to reduce the number of both organism from 4.38 and 4.15 to2.49 and 2.27 log CFU/ml, DF of 0.43 and 0.45, respectively, with 1 min contact time.

This showed that ozonated water has ability to control the number of bacteria on the shrimp, both spoilage and pathogenic bacteria. The highest concentration of ozone

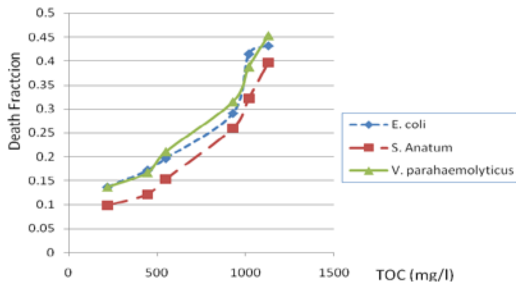


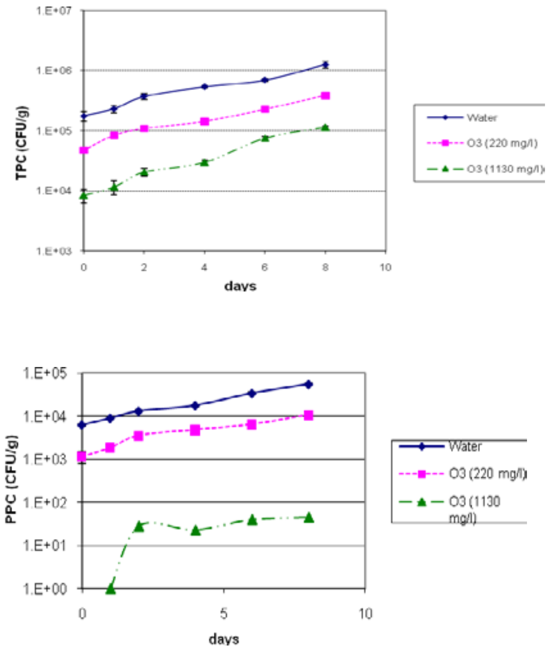
Figure 3. The activity of ozone at various concentration (TOC) on some food borne pathogenic bacteria on shrimp samples when contact for 1 min.

(1130 mg/l) and the lowest concentration (220 mg/l) were selected for the further study.

B. The quality of ozonated water treated shrimps during 4^oC storage

The number of total bacteria and psychotropic count on the shrimp samples were 2.9±0.73x10^5 and 6.87±1.69x10^{3}CFU/g, respectively. After washing with ozonated water, the number of bacteria in both groups was significantly lower than in the samples washed with water as shown in the Figure 4. However, the ozonated water did not inhibit or slow down the growth of the flora.

The washing of shrimp with ozonated water with 220 and 1130 mg/l reduced the number of bacteria for about 1 and 2 log_{10} CFU/g, which had DF equal to 0.11 and 0.24, respectively. The ozonated water (1130 mg/l) was able to eliminate most of the psychotrophic bacteria on shrimp and



(a)

(b)

Figure 4 Total plate count (a) and psychotropic plate count (b) of shrimp washed with solution contained various ozone concentration and stored under 4^{o}C

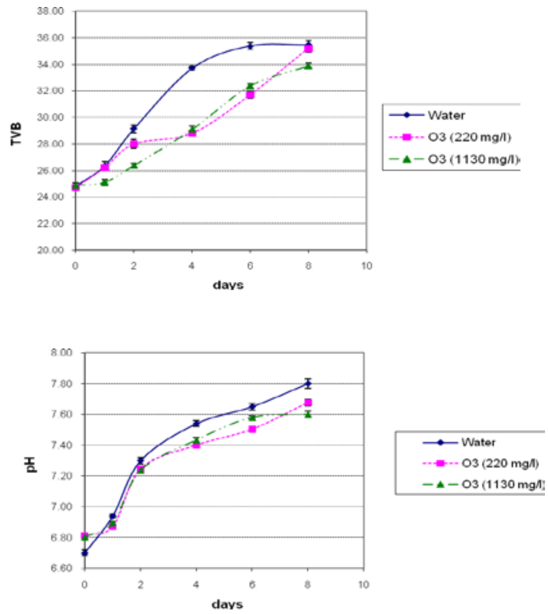
could pause growth of psychotrophic bacteria for 1 day. The TVB number (Figure 5a) also showed no significantly different of the zero and first day storage.

The enzymes from bacteria [19] and shrimp muscle [20,21]produce the hydrolytic products that cause changes in chemical and physical properties of shrimp. The reduction of bacterial number on shrimp can partially prolong the changes. The autolytic activity due to shrimp endogeneous enzymes still active and caused changes in shrimp muscle.

The volatile base is released as the deamination reaction of AMP (adenosine monophosphate) which is the activity of bacterial enzymes [22]. Fresh shrimp should have TVB (total volatile base) number lower than 30 mg/100g [23]. The increment of TVB number of the shrimp washed with distilled water was rapidly increased over 30 mg/100g after 2 day storage, while the TVB number of ozonated water washed shrimp pass 30 mg/100g after 4 day storage.

The increase of pH is the result from the increment of volatile base. The pH of shrimps washed with ozonated water was significantly lower than the shrimp washed with distilled water during the storage (Figure 5b). The reduction of bacterial number on the ozonated water washed shrimps could elucidate the different in TVB number of ozonated water and distilled water washed shrimps.

From observation, the shrimps washed with ozonated water did not show different on the appearance from those washed with distilled water. The ozonated water also did not affect the organoleptic evaluation of the shrimp on the firmness and color. There was no different on the score of the firmness and color of shrimps during 4^{o}C storage. The



(a)

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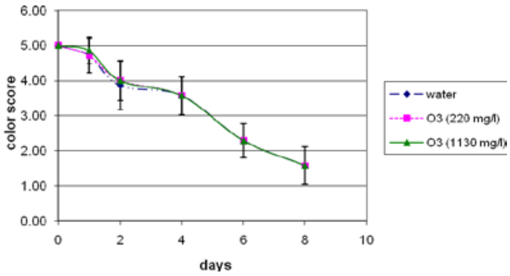
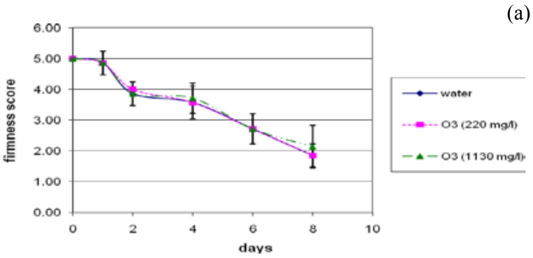
Figure 5. The TVB value (a) and pH (b) of the shrimp washed with various ozone concentration solution during stored under 4^{o}C



(a)



(b)

Figure 6. The color (a) and texture (b) scores of the shrimps washed with various concentration of ozone solution stored at 4°C. The scores of color were described as 1 = blackish head and tail with opaque meat; 2 = blakish head and parts of tail turn black; 3 = some parts of head turn black; 4 = parts of head turn grey and pale trunk color; 5 = color of fresh shrimp. The scores of texture were described as 1 = soft meat and shell; 2 = soft meat and firm shell; 3 = firm meat and shell; 4 = firm meat and hard shell; 5 = hard meat and shell

reduction of firmness of shrimp is caused by shrimp endogeneous enzymes, specially collagenolytic enzymes [24].

The bad smell of the shrimp also caused by the enzymatic hydrolyzation of protein which the sources of enzymes came from shrimp muscle and bacteria. The ozonated water has ability to reduce the number of bacteria on shrimps which resulted in the reduction of hydrolytic products. The ozonated water treated shrimp was significantly ($p \leq 0.05$) prolong the odor quality of the shrimps as shown in Fig 7. The distilled water washed shrimps were able to detect for bad smell on the fourth day of storage while the ozonated water washed shrimps were after 4 days of storage. The higher concentration (1130 mg/l) of ozone can prolong the odor quality better than lower concentration (220 mg/l).

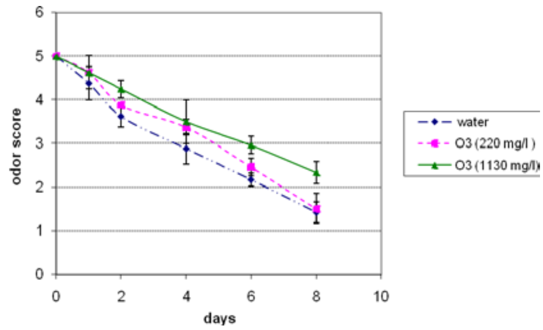


Figure 7. The odor scores of the shrimps washed with various concentration of ozone solution stored at 4°C. The odor scores were described as 1 = strong putrid smell; 2 = putrid smell; 3 = strong fishy smell; 4 = fishy smell; 5 = no fishy smell

IV. Conclusion

Washing shrimp with the ozonated water, 220 to 1130 mg/l (TOC) could reduce the number of bacteria on the shrimp. The concentration of ozone in the water 1130 mg/l was effectively pause the increment of psychotrophic bacteria. The ozonated water did not improve the quality of the shrimp on firmness and color, but delayed the changes in odor and improve the microbial quality of shrimp during the cold storage.

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