

Shelf-life Extension of Refrigerated Soft Shell Mud Crab (*Scylla serrata* Forskal) by Ozone Water and Storage under Air and Modified Atmosphere Packaging

Nathapol Phapinyo^{1*}, Tawirat Chaowalit¹, Aiyakarn Tanpipattanakul¹,
Kijja Sooknet¹, Aekarat Kumthong¹, Wanchai Worawattanamateekul² and Jim P. Smith³

ABSTRACT

The effects of ozone water combined with modified atmosphere packaging on the refrigerated storage quality of soft shell mud crab at 4 ± 0.5 °C were investigated. Soft shell mud crab stored under modified atmosphere packaging and vacuum had lower microbial counts compared with that of mud crab stored in an aerobic package ($p < 0.05$). The odor and overall acceptability were acceptable for the soft shell mud crabs packaged under $80\%CO_2+20\%N_2$ and vacuum throughout the storage period of 11 days. Microbial changes in all samples were generally in agreement with sensory panel evaluations. The shelf life of soft shell mud crab soaked in 1.0 ppm for 20 min and packaged under $80\%CO_2+20\%N_2$ and vacuum was extended to at least 11 days at 4 ± 0.5 °C. The shelf life of soft shell mud crab soaked in water and aerobically packaged was 3 days.

Key words: shelf-life, soft shell mud crab, ozone water, vacuum packaging, modified atmosphere packaging

INTRODUCTION

Fresh crustaceans are an extremely perishable food compared with other food commodities. The quality of fresh products declines rapidly due to microbial growth and cross contamination from various sources (Graham, 1997). The food industry is currently in need of innovative processing technologies in order to meet consumers' demand for fresher and safer products. Due to the perishable nature of seafood, there is an obvious need for development of new

technologies and efficient preservation methods, to allow shelf life extension of these products. Besides traditional methods used to extend the shelf life of fresh fishery products involving rapid chilling and ice storage (Himmelbloom *et al.*, 1994), various methods involving the use of organic acids, natural antimicrobials (Gelman *et al.*, 2001), modified atmosphere packaging (Davis, 1993), high-pressure treatment (Hugas, *et al.* 1998) and ozonation have been proposed. Washing removes and reduces microbial loads. Normally, seafood and seafood products have been traditionally

¹ The Program of Food Science and Technology, Faculty of Science and Technology, Rajabhat Suan Dusit University, Bangkok 10300, Thailand.

² Department of Fishery Products, Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand.

³ Department of Food Science and Agricultural Chemistry, Macdonald Campus of McGill University, Montreal, Qc., Canada.

* Corresponding author, email: nphapinyo@yahoo.com

washed in chlorine solutions to reduce microbial levels. However, chlorine's relative ineffectiveness and health concerns regarding its by-products are leading to a search for alternative sanitizers (Xu, 1999). Ozone has been seen by many as a natural replacement for chlorine for washing produce (Graham, 1997; Kim *et al.*, 1999). The antimicrobial action of ozone is due to the strong oxidizing activity of either molecular ozone itself or its decomposition products which rapidly react with intracellular enzymes, nucleic material and components of their cell envelope, spore coats, or vital capsids (Khadre *et al.*, 2001). Since ozone is reduced down to oxygen, no residuals are formed and other by-products of ozone are considered less of a health risk to humans compared with those formed by chlorine treatment (Graham, 1997). Effects of ozone have been studied and documented on a wide variety of organisms, including Gram positive and Gram negative bacteria as well as spores and vegetative cells (Restaino *et al.*, 1995). Ozone has been used in several studies to decontaminate freshly caught fish (Goche and Cox, 1999), poultry products, meat and milk products (Sheldon and Brown, 1986).

Modified atmosphere packaging (MAP) offers multiple advantages to the fish processing industry and to the consumer. Various atmospheres have been examined (Silvertsvik *et al.*, 2002). Oxygen, nitrogen and carbon dioxide are the most usual gases used in MAP (Gimenez *et al.*, 2002). Oxygen causes oxidative rancidity in fatty fish, stimulates growth of aerobic bacteria and inhibits growth of anaerobic bacteria. Nitrogen delays oxidative rancidity and inhibits the growth of aerobic microorganisms by displacing the oxygen in packs (Church, 1998). Carbon dioxide is the major gas used in MAP due to its effectiveness in retarding microbial growth in refrigerated, perishable foods. It is especially effective against Gram negative spoilage microorganisms by extending lag phase and decreasing growth rate

during the log phase (Farber, 1991). The proper combination of gases in the headspace of food packs results in suppression of the microbial flora of perishable foods such as meat, fish and related products, developed under aerobic conditions and retention of their sensorial attributes (Davies, 1995). Since, carbon dioxide acts as an antimicrobial agent; it inhibits the growth of microorganisms during the logarithmic phase and extends the lag phase (Phillip, 1996). Statham (1984) explains that weak acids are known to have antimicrobial activity in their undissociated form, and carbonic acid is unique as a microbial inhibitor since at pH values near neutrality at least one half of the acid is in the undissociated form.

The objective of this initial study was to determine the effects of selected packaging / dipping solutions on the shelf life and keeping quality of the soft shell mud crab at 4 ± 0.5 °C.

MATERIALS AND METHODS

Preparation of soft shell mud crab

Fresh soft shell mud crabs (*Scylla serrata* Forskal) with an average weight of 200-220 g were obtained from Andaman Soft Shell Crab farm located in Ranong Province. The soft shell mud crabs were stunned by immersing in ice cold water (hypothermia) and delivered to the laboratory within 6 h of harvesting, using foamed polystyrene boxes containing dry ice. Soft shell crabs were then placed into a rectangular plexiglas tank (capacity = 50 l) containing 20 l of water with crushed ice (water/ice ratio, 2:1) to bring the water temperature down to 4 ± 0.5 °C for a control. From Figure 1 the samples were soaked with ozone water 1.0 ppm for 20 minutes. The ozone concentration was achieved by passing ozone gas through a tube and a gas sparger, generated by a model OZ-3020 ozonator (Triple Science, Thailand) operated at 60 Hz and 55.2 kPa oxygen pressure. Voltage was regulated to achieve ozone concentration. The concentration of aqueous

ozone, after 20 min was 1.0 mg/l. The ozone gas produced was dispersed through a fritted-glass diffuser into chilled water.

Packaging of samples

After treatment, all samples including the control were packaged in low density polyethylene/polyamide/polyethylene (LDPE/PA/LDPE) barrier pouches (3 soft shell mud crabs per pouch) 75 μm in thickness having an oxygen permeability of 52.2 ml/ m² day atm) and a water vapor permeability of 2.4 g/ m² day (T=25°C, RH=0%). One third of the bags were packed with atmosphere (AIR); one third were placed with

vacuum sealed (vacuum = 29 in Hg) using a Vacstar S220 vacuum sealer (Renown Technical Co.Ltd., Switzerland), and the other third were packed by air evacuated, and back flushed with CO₂ and N₂ reach 80% CO₂, 20% N₂ (MAP). All products were kept under refrigeration (4±0.5°C) (Figure 2) and monitored for physical, microbiological and sensorial changes until the shelf life of sample was deteriorated.

Physical analyses

Physical analysis comprised measurement of changes in headspace gas composition and pH until sample was deteriorated.

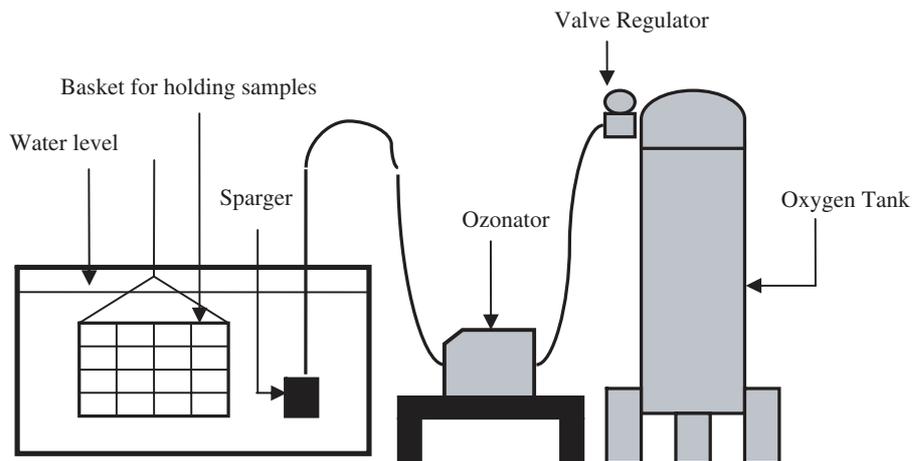


Figure 1 The schematic of ozonator for produce ozonated water.



Figure 2 Packaging conditions of soft shell mud crab under (a) air, (b) MAP (80%CO₂ and 20% N₂) and (c) vacuum condition kept at 4±0.5 °C.

Head space gas analysis

Samples were analyzed for head space gas composition using a previously calibrated Servomex portable O₂/CO₂ analyzer (Model 1450B3, Servomex Company Inc., Norwood, Massachusetts, USA). Gas samples were withdrawn from each bag, through a silicone septum affixed to the exterior of the bag, with a gas tight pressure-lock syringe and needle that was connected to the gas analyzer. Measurement was taken when gas samples were pumped through the analyzer.

pH

The pH value was recorded using a Jenway model 3020, pH meter. Soft shell mud crabs 5 g were thoroughly homogenized with 10 ml of distilled water and the homogenate used for pH determinations.

Microbiological analyses

Microbiological analyses were conducted on soaked/ packaged soft shell mud crab [i.e., total viable counts (TVC), psychrotrophic counts and lactic acid bacteria (LAB)]. The packaging film was cut aseptically by swabbing the outside of each package with 95% ethanol. An initial 10⁻¹ dilution was prepared by adding 25 g of soft shell mud crab to 225 ml of 0.1% sterile peptone water (Difco) in a stomacher (AES Laboratories) for 2 minutes. The series of decimal dilutions (10⁻² to 10⁻⁶) were made, using 0.1% peptone water as the diluents. Plate count agar (PCA) (Difco) was used for total viable counts and psychrotrophic bacteria whereas Lactobacilli MRS (Difco) was used for lactic acid bacteria. All microbiological analysis were spread on the surface of dry media. For total viable counts and lactic acid bacteria counts, all plates were incubated aerobically at 35 °C for 48 h. For psychrotrophic plate counts, plates were incubated aerobically at 4 °C for 10 days. Counts were expressed as log₁₀ cfu/g of sample which was obtained by multiplying count by the

reciprocal of the appropriate dilution.

Sensory evaluation

Sensory attributes of cooked soft shell mud crab were evaluated using a 7-member trained taste panel. Soft shell mud crab samples (approximately 180 g) were cooked individually by steaming for 5 min and immediately presented to the panelists (each panelist evaluated approximately 20 g of sample). The sensory descriptions of each attribute were developed and agreed upon by the trained panelists. Samples from an untreated control were evaluated as compared with treated samples for differences in taste and odor. Differences were measured using a modified method from a 9 point rating scale. The modified rating method consisted of appearance (9: fresh, 5: dull, 1: slimy) and odor (9: fresh, 5: marginal, 1: putrid). The experimental design was RCBD. Analysis of variance and Duncan's multiple range test were used to test for differences between means at the 5% significance level.

Statistical analysis

All statistical analysis were analyzed using of ANOVA, followed by DMRT to determine significant differences among means at $\alpha = 0.05$ level.

RESULTS AND DISCUSSION

Gas composition

Changes in headspace gas composition for the various packaging/ soaking treatments of soft shell mud crab are shown in Figure 3. For samples packaged in air and soaked in water or ozone water prior to packaging, headspace O₂ decreased to less than 1 % after 11 days with a concomitant increase in head space CO₂ to approximately 15-18 % (Figure 3a). This reduction in headspace O₂ and increase in CO₂ is mainly due to growth and metabolism of aerobic and facultative aerobic bacteria although some

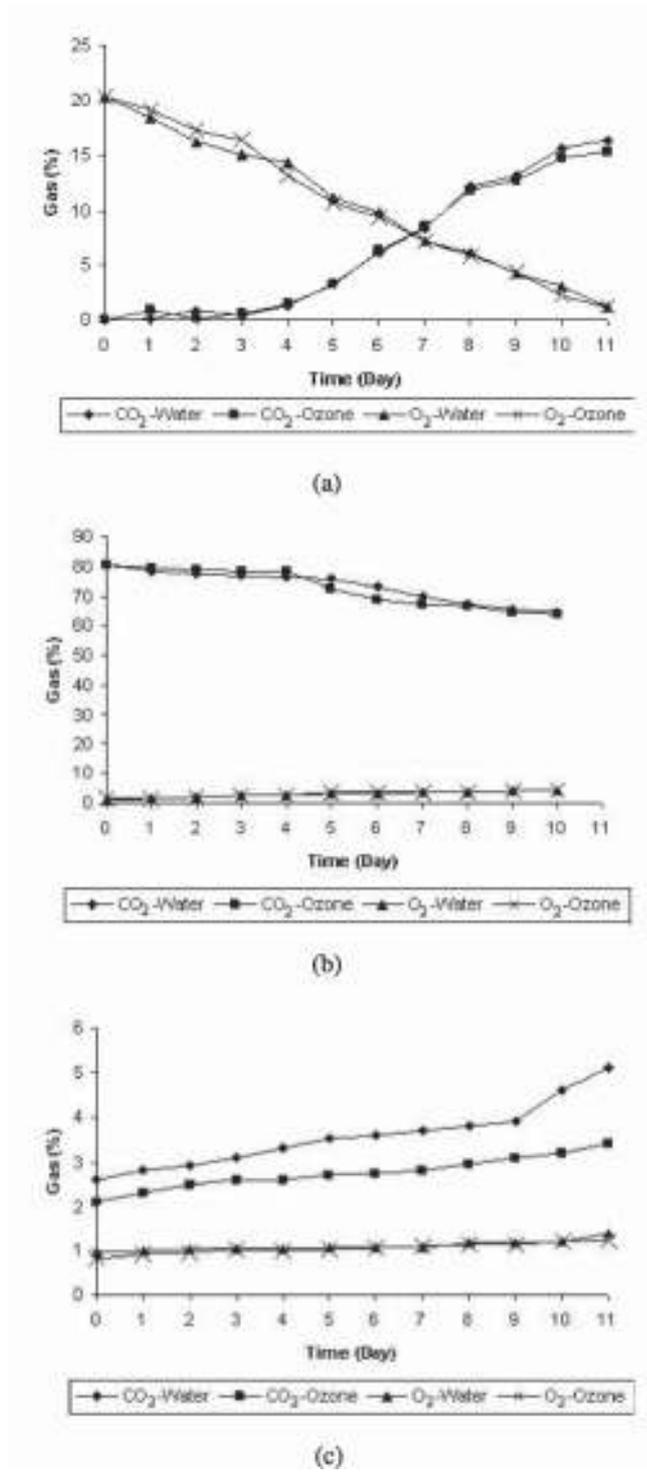


Figure 3 Changes in headspace gas composition for the various packaging/ dipping treatments of soft shell crab packaged under (a) air (b) MAP (80% CO₂ and 20% N₂) and (c) vacuum kept at 4±0.5 °C.

endogenous enzymes may contribute to a reduction in head space O_2 . Soaking soft shell mud crab in ozone water had little or no effect in controlling microbial growth in air packaged samples. In contrast, the headspace gas composition changed only slightly in gas packed/vacuum packaged samples due to the fact that concentrations of $CO_2 > 20\%$ or low O_2 concentration $< 1\%$ inhibit the growth and metabolism of aerobic spoilage microorganism of muscle foods (Smith *et al.*, 1991). From Figure 3b soft shell mud crab packaged in $CO_2: N_2$ (80: 20) gas mixture, headspace O_2 never increased beyond 1% throughout storage indicating the accuracy of gas flush and the integrity of the high O_2 barrier film used in this study. The slight decrease in head space CO_2 observed initially in the gas packaged samples is due to dissolution of CO_2 in the aqueous phase of soft shell mud crab at lower storage temperatures (Smith *et al.*, 1991). Changes in the headspace O_2 and CO_2 composition for vacuum packaged samples are shown in Figure 3c headspace O_2 remained at 1-2% throughout storage whereas headspace CO_2 increase from 2% to approximately 3-5% after 11 days due to its evacuation from intracellular respiring tissue and possible

production by facultative bacteria, particularly lactic acid bacteria during storage (Viana *et al.*, 2005). The trends observed for changes in headspace gas composition for packaging soft shell mud crab are similar to those observed in other muscle foods (Silvertsvik, 2002).

pH

In this study, the pH of all samples decreased only slightly from an initial pH of 7.2 to 6.96-6.34 after 11 days storage (Figure 4). The slight change of pH during the storage can be explained by the buffering effect of muscle proteins and release of amino acids as a result of proteolytic activity of aerobic and facultative aerobic spoilage microorganisms. At the end of 11 day storage at 4 ± 0.5 °C, pH of the water/vacuum packaged samples and ozone water/ air packaged sample were significantly less ($p < 0.05$) than all other packaging/ soaking treatments. The pH decrease can be explained by the dissolution of CO_2 in the soft shell mud crab tissue and production of lactic acid as a result of growth of lactic acid bacteria particularly in soft shell mud crab packaged under vacuum packaging conditions.

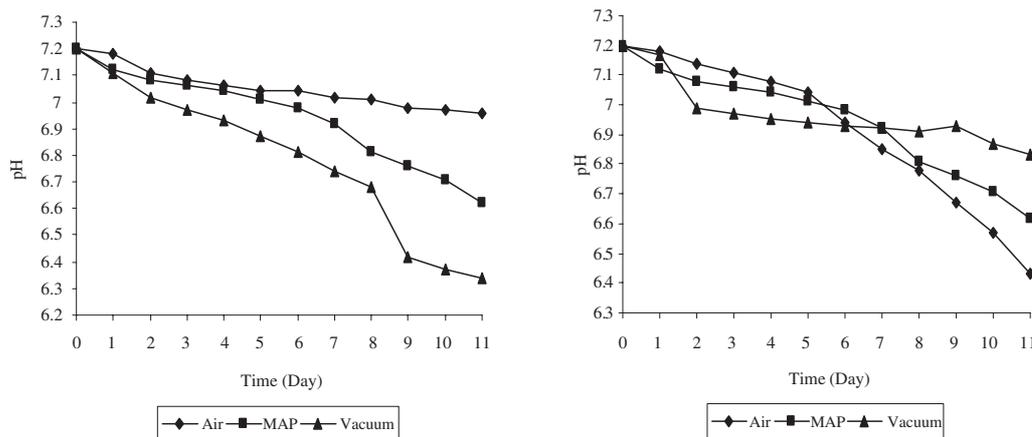


Figure 4 Changes of pH of soft shell mud crab dipped in water (a) and ozone water (b) and packaged in Air, MAP (80% CO_2 and 20% N_2) and vacuum kept at 4 ± 0.5 °C.

Microbiological analysis

The changes in total viable counts (TVC), psychrotrophic and lactic acid bacteria counts throughout the storage of refrigerated soft shell mud crab packed in air, 80% CO₂ + 20% N₂ and vacuum are presented in Figure 5. Initial TVC reached the value of 7 log cfu/g, which is considered as the upper acceptability limit of fresh food as defined by ICMSF (1986). From Figure 5a, TVC increased from approximately 3.41-3.46 log cfu/g (Day 0) to unacceptable only after 3 days for water soaking packaging in air treatments. Only soft shell mud crabs soaked in ozone water and gas packed or vacuum packaged were significantly different ($p < 0.05$) and acceptable TVC counts. The overall effect of ozone water soaking solution and MAP can extend the lag phases of bacterial growth and subsequently extend in shelf life. Also high CO₂ levels have residual bactericidal effects on the initial spoilage flora (Lannelongue *et al.*, 1982).

Changes in psychrotrophic bacteria counts for various treatments of soft shell mud crab are shown in Figure 5b. The growth of psychrotrophic bacteria counts were significantly high in soft shell mud crab soaked in water or ozone and air packed. A log reduction in psychrotrophic counts was obtained by soaking samples in ozone water prior to packaging in air. However, soaking soft shell mud crab in ozone water and packaging under CO₂: N₂ or vacuum packaging resulted in approximately a 3-4 log reduction in psychrotrophic count. This indicates that the combined use of ozone water soaking solutions and MAP has the potential to extend the shelf life of fresh soft shell mud crab by inhibiting *Pseudomonas* species, the main psychrotrophic spoilage bacteria of seafood or muscle foods (Lambert *et al.*, 1991).

Similar trends were observed for lactic acid bacteria count (Figure 5c). Lactic acid bacteria have been shown to become the predominant spoilage microorganism in vacuum packaged/ gas packaged muscle foods. They may also be the

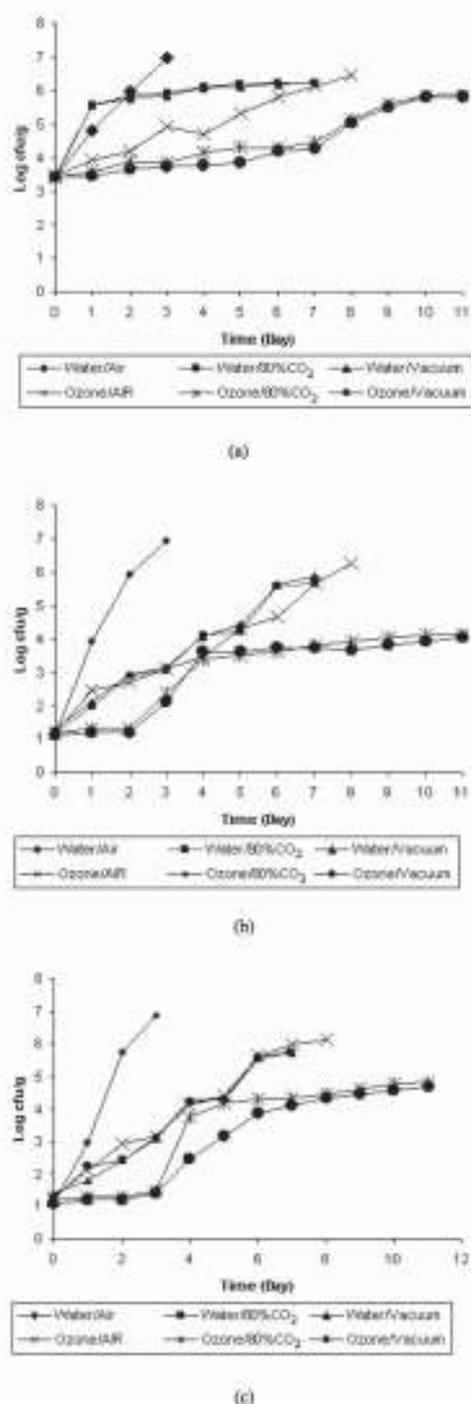


Figure 5 Changes in total viable counts (TVC) (a), psychrotrophic (b), lactic acid bacteria (c) on soft shell mud crab in different dipping/ packaged kept at 4 ± 0.5 °C.

dominant spoilage microorganism in air packaged fish (Lambert *et al.*, 1991). The low residual O₂ concentration and elevated CO₂ concentration favors the growth of lactic acid bacteria at refrigerated storage temperatures. Lactic acid bacteria reached spoilage levels (6.87 log cfu/g) within 3 days after soaking water and packaged in air, with the exception of ozone water/ CO₂: N₂ treatments.

Sensory analysis

Soft shell mud crabs were also evaluated simultaneously for odor and overall acceptability. A score of 6 was also regarded as the upper level of acceptability for both parameters. Soft shell mud crabs soaked only in water and air packaged had scores below 6 after 3 days. However, samples soaked in ozone water and packaged under MAP and vacuum had an acceptable odor/ overall acceptability scores for 11 days. Therefore the combined use of ozone water soaking solutions has the potential to retard microbial growth and metabolism as shown by the sensory index indicators of spoilage, and control off odors during storage at 4±0.5°C.

CONCLUSION

Combinations of ozone water with MAP and vacuum can extend the shelf life of soft shell mud crabs. Air-packing cannot extend the sensory shelf life of soft shell mud crab compared to chilled storage MAP and vacuum packaging. However, soaking the soft shell mud crabs with ozonated water 1.0 ppm for 20 min prior to packaging with MAP and vacuum resulted in a delay in microbiological and sensorial changes. Shelf life was extended to 11 days.

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