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Bacteriological Characteristic of *Atrina pectinata* and *Ruditapes philippinarum* under Non-refrigerated and Refrigerated Storage Conditions

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ABSTRACT

In order to estimate the necessity of refrigerated storage of fresh seafood for short-term storage, and evaluate the effect of refrigerated storage on pen shell *Atrina pectinata* and clam *Ruditapes philippinarum* collected from Jang-su of Deukryang Bay and I-mok of Sunchen Bay in South Korea, the counts of coliform, *Escherichia coli* and total aerobic bacteria in *A. pectinata* and *R. philippinarum* under non-refrigerated (28±1℃) and refrigerated storage conditions (4±1℃) were determined. The results indicated that the storage at temperature of 4℃ possessed significant effects on inhibiting bacterial growth in live seafood. And refrigerated storage had different effect on *A. pectinata* and *R. philippinarum*. Different species and culture environments significantly influenced the initial and ultima bacteria counts. This study confirmed that refrigerated storage for short-term storage of live seafood was necessary, and indicated that the effect of refrigerated storage was influenced by comprehensive effectors.

Key words: *Atrina pectinata*, *Ruditapes philippinarum*, Coliform, *Escherichia coli*, Total aerobic bacteria, Refrigerated storage

INTRODUCTION

Bivalves are not only known as important sources of food for human, but also considered as potential vehicles and incubators of foodborne diseases. As filter-feeding animals, these invertebrates can amass high levels of microbial pathogens contained in aquaculture water within their internal tissues. And bivalves are usually farmed in coastal areas severely affected by human activities especially sewage dumpling. Outbreaks of bacterial pathogens are reported to be observed at the rate of 35% in shellfish including molluscan shellfish and crustaceae (Huss *et al.*, 2000). So they are related more than other marine animals to seafood-borne disease (Lipp and Rose, 1997).

The pen shell *A. pectinata* and clam *R. philippinarum* are commercial benthic species which

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are widely farmed in many Asian countries such as South Korea, China and Japan. Fresh *A. pectinata* and *R. philippinarum* are popular in South Korea, especially during early summer-fall period when the temperature can even reach 30℃. Currently the two species are growing in shellfish farms and are transported alive without water to retail fish stores and markets sold for immediate consumption.

During transportation, there is usually no any method of storage have been utilized to control the proliferation of pathogens and spoilage bacteria in them. In spite of the availability of modern transport facilities which remarkably shorten the time of transportation, the change of bacteriological quality during storage and distribution of them, especially at high temperature, is a problem worthy of our attention.

The bacteriological activities induce the loss of quality and subsequent spoilage which cause the rapid deterioration of the shellfish (Gram and Huss, 1996). Moreover, the proliferation of bacteria during storage and transportation increases the potential infection risk of pathogenic bacteria for humans. Determining all bacteria in seafood is so costly and waste of time that it is not necessary except there is special need. Instead, coliform bacteria usually are selected as indicators of pathogenic bacteria. The presence and concentration of coliform bacteria (total, fecal, or a specific species) can not only indicate that water and shellfish has been polluted with feces, but also reflect the contaminative condition of bacteria (EPA, 2000). They are defined as facultatively anaerobic, gram-negative, non-endospore forming, rod-shaped bacteria that ferment lactose to form gas within 48?h of being placed in lactose broth at 35℃ (Tortora *et al.*, 2001).

The loss of quality directly depends on the nature of fish species and storage conditions (Olafsdóttir *et al.*, 1997; Whittle *et al.*, 1990). After shellfish harvesting, storage conditions exert a strong effect on the quality of shellfish and thus on their commercial value (Ashie *et al.*, 1996). Several storage methods have been proposed to preserve the quality of shellfish, including storage in slurry ice (Rodríguez *et al.*, 2006), refrigerated seawater (Kraus, 1992) or the addition of chemicals (Hwang and Regenstein, 1995). For practical reasons, the refrigerated storage is often proposed to control the temperature during the course of storage, transportation, retail display, and home storage.

With the aim of having a better understanding about the necessity of applications of refrigerated storage, in this study we determined: (a) the level of coliform, E. coli and total aerobic bacteria; (b) the effect of refrigerated storage on different species and (c) the influence of culture environment on initial and ultima bacteria counts.

MATERIALS AND METHODS

Collection and storage of bivalve samples

The pen shell *A. pectinata* and clam *R. philippinarum* (Table 1) were collected from two offshore plants located in the Jang-su of Deukryang Bay and I-mok of Sunchen Bay adjacent to Yeosu (Chonnam, South Korea). After collection, the samples were respectively placed into incubators set at 4±1℃ and 28±1℃ immediately, and transported to laboratory within 2 hours. All samples were taken at July 5, 2007. At both sampling sites the water quality (temperature, salinity, pH, COD and the concentration of *E. coli* and coliform) was recorded (Table 2).

Table 1. Measurements of the *Atrina pectinata* and *Ruditapes philippinarum* in the experiments

Species	Sampling station	Shell length ±SD (mm)	Shell height ±SD (mm)	Shell width ±SD (mm)	Total weight ±SD (g)
A. pectinata	Jang-su	234.1±8.1	123.8±6.1	42.2±5.6	411.6±51.3
	I-mok	274.8±40.1	110.4±2.9	51.5±3.4	362.3±46.2
R. philippinarum	Jang-su	33.08±3.47	22.85±2.17	15.25±1.37	7.23±2.09
	I-mok	32.92±5.12	24.80±3.87	16.86±2.90	9.68±4.69

Table 2. Examination results of water quality in Jang-su and I-mok

Sampling site	Temperature (° C)	Salinity	pH	COD (mg/L)	*Escherichia coli* (MPN $10mL^{-1}$)	Fecal coliform (MPN $10mL^{-1}$)
Jang-su	23.3	32.9	8.05	1.20	2.0	⟨1.8
I-mok	22.7	32.9	8.01	1.48	4.0	⟨1.8

Microbiological analyse

Before and after 12 h storage, the level of coliform, *E. coli* and total aerobic bacteria in each sample which were collected from different sampling stations and stored under different conditions were determined. Only living bivalves were used for analysis. For each treatment, three subsamples (each of about 500 g of soft tissue) were used for the microbiological analyses. The bivalves were scrubbed free of dirt, washed in hypochlorite solution (20 mg L^{-1}), rinsed with sterile distilled water, and shucked with a sterile knife. Tissue and shell liquor samples were homogenized in a blender (Vernocchi *et al.*, 2007).

1) Enumeration of total aerobic bacteria (TPC)

To determine the TPC, plating was performed into plate count agar (PCA, BD 247940) from the prepared dilutions by spread plate method. After 48 h incubation at 35℃, the formed colonies under aerobic conditions were counted (Swanson *et al.*, 1992).

2) Enumeration of coliforms

The coliform was determined by the five tube most probable number (MPN) method. Lauryl tryptose broth (LTB, BD 224150) and 2% brillant green lactose bile broth (BGLB, BD 274000) were used for presumptive and confirmed tests for coliform, respectively. Results were evaluated according to the MPN tables (Harrigan and McCance, 1976; Temelli *et al.*, 2006).

3) Enumeration of *E. coli*

E. coli was determined by the MPN method. For this, gas positive LTB tubes were gently agitated and a loopful from this culture was transferred to EC (BD 231430) broth tubes. The tubes were incubated at 44.5℃ for 48 h and examined for gas formation. Gas positive cultures were streaked onto eosine methylene blue agar (EMBA, BD 211215) and were incubated at 35℃ for 24 h. Confirmation of *E. coli* was carried out by IMViC test and counts were determined in accordance to MPN table (Temelli *et al.*, 2006).

The relative increase rate of bacteria was esti-

mated through the equation:

$$R = \frac{BC_f - BC_i}{BC_i} \times 100\%$$

where:

R: Relative increase rate of bacteria (%)

BC_i: Initial bacteria content (coliform: MPN $100g^{-1}$; *E. coli*: MPN $100g^{-1}$; TPC: CFU g^{-1})

BC_f: Final bacterial content (coliform: MPN $100g^{-1}$; *E. coli*: MPN $100g^{-1}$; TPC: CFU g^{-1})

The inhibitory rate of bacteria was estimated through the equation:

$$IR = \frac{BC_N - BC_R}{BC_N} \times 100\%$$

where:

IR: Inhibitory rate of bacteria (%)

BC_N: Bacteria content in non-refrigerated stored bivalves (coliform: MPN $100g^{-1}$; *E. coli*: MPN $100g^{-1}$; TPC: CFU g^{-1})

BC_R: Bacterial content in refrigerated stored bivalves (coliform: MPN $100g^{-1}$; *E. coli*: MPN $100g^{-1}$; TPC: CFU g^{-1})

Statistical analyses

Data from the different species were subjected to one-way analysis of variance; Data from one species under non-refrigerated and refrigerated storages were subjected to Student' s T-test. Statistical comparison was based on 5 samples for each treatment. Significance of differences was defined as $P < 0.05$ in all cases. Statistics were performed using the statistical software SPSS for Windows.

RESULTS

The bacterial counts of *A. pectinata* and *R. philippinarum* collected from Jang-su and I-mok were expressed in Table 3. The results showed that the bacterial levels significantly influenced by different species and culture places. Whether in Jang-su or I-mok, the *R. philippinarum* showed higher bacterial levels than *A. pecinata*. And the higher bacteria concentrations were observed in samples collected from I-mok than those in Jang-su. The results indicated that the initial bacterial counts of various bivalves were significantly different, and obviously influenced by their culture environments.

After 12 h storage, the bacterial counts in samples under refrigerated storage were significantly lower than those under non-refrigerated storage regardless species and culture places (Table 4). The bacterial counts in *R. philippinarum* were significantly higher than those in *A. pectinata* collected from same culture places. And the higher bacteria concentrations were observed in samples collected from I-mok than those in Jang-su.

The bacteria increase rates were showed in table 5.

Table 3. Counts of Coliform (MPN $100g^{-1}$), *E. coli* (MPN $100g^{-1}$) and TPC (CFU g^{-1}) in *Atrina pectinata* and *Ruditapes philippinarum* collected from Jang-su and I-mok.

	Atrina pectinata		*Rudita pesphilippinarum*	
	Jang-su	I-mok	Jang-su	I-mok
Coliform (MPN $100g^{-1}$)	127±5.2a	170±6.1b	150±4.3c	281±7.7d
E. coli (MPN $100g^{-1}$)	13±1.1a	21±2.4b	36±2.1c	40±2.5c
TPC (CFU g^{-1})	228±13.1a	350±14.5b	352±12.5b	725±11.7c

Table 4. Counts of coliform (MPN $100g^{-1}$), *E. coli* (MPN $100g^{-1}$) and TPC (CFU g^{-1}) in *Atrina pectinata* and *Ruditapes philippinarum* collected from Jang-su and I-mok after 12 h refrigerated storage (R) and non-refrigerated storage (N).

	Atrina pectinata				*Ruditapes philippinarum*			
	Jang-su		I-mok		Jang-su		I-mok	
	N	R	N	R	N	R	N	R
Coliform (MPN g^{-1})	790±50a	170±35b	1,100±32c	230±68b	1,100±21c	200±12b	1,800±77d	490±27a
E. coli (MPN $100g^{-1}$)	93±5.1a	20±1.9b	110±5.1a	40±2.1c	140±8.7a	45±2.2c	170±8.9d	45±3.1c
TPC (CFU g^{-1})	1,250±55a	480±24b	1,870±63c	680±37d	1,700±51c	740±32d	3,620±67e	1,450±43c

Data with different letters within a same raw are significant different ($P<0.05$).

Table 5. Bacteria increase rates in *Atrina pectinata* and *Ruditapes philippinarum* collected from Jang-su and I-mok under refrigerated (R) and non-refrigerated storage conditions (N).

	Atrina pectinata				*Ruditapes philippinarum*			
	Jang-su		I-mok		Jang-su		I-mok	
	N	R	N	R	N	R	N	R
Coliform (%)	522.3±17.1a	33.1±2.4b	547.4±15.7a	35.2±1.8b	633.7±16.9c	33.3±2.8b	540.4±7.9a	74.1±3.8d
E. coli (%)	615.8±9.8a	53.8±3.1b	423.4±7.8c	90.8±2.2d	289.9±11.3e	25.7±1.7f	325.4±11.1e	13.2±1.1f
TPC (%)	448.2±17.1a	110.5±5.1b	434.3±13.8a	94.3±7.5b	382.9±11.3c	110.2±8.9b	399.4±13.2c	101.3±4.8b

Data with different letters within a same raw are significant different ($P<0.05$).

Table 6. Bacteria inhibitory rates in *Atrina pectinata* and *Ruditapes philippinarum* collected from Jang-su and I-mok under refrigerated (R) and non-refrigerated storage conditions (N).

	Atrina pectinata		*Ruditapes philippinarum*	
	Jang-su	I-mok	Jang-su	I-mok
Coliform (%)	78.5±3.1a	79.1±2.9a	81.8±3.8b	72.8±2.1c
E. coli (%)	78.4±2.7a	63.6±1.8b	67.9±2.4b	73.5±3.1b
TPC (%)	61.6±1.9a	63.7±2.4a	56.5±2.1b	59.9±2.6b

Data with different letters within a same raw are significant different ($P<0.05$).

The results showed that bacteria increase rates were significantly lower in the samples under refrigerated storage conditions. In general, the bacteria increase rates were not significantly different for the same species, even if they were collected from different culture places and under different storage conditions. But the bacteria increase rates in different species were significantly differed.

The bacteria inhibitory rates also showed the refrigerated storage effectively inhibited bacteria increase, and the refrigerated storage showed different effects for different species.

The results indicated that the storage at temperature of 4℃ possessed the effects on inhibiting bacterial growth, and the storage showed different effects for different species. The initial and ultima bacteria counts were significantly influenced by different species and culture environments.

DISCUSSION

Effect of refrigerated storage

The results indicated that the storage at temperature of 4℃ possessed the effects on inhibiting bacterial growth. Commonly, lower temperature can provide better prevent effect. But for the storage of whole unprocessed seafood, refrigerated storage at 4℃ may be a better choice, because previous study have indicated that at 4℃ some seafood can be stored for a relatively long period prior to death (Robson *et al.*, 2007).

Khan *et al.* (2005) reported that a short shelf life of Charles Arm mussel stored at 2℃ could be attributed to the relatively higher initial bacterial counts. Khan *et al.* (2005) also indicated that low cold temperature storage can maintain the initial microbial status of the product, but cannot improve it; therefore, for improve shelf life it is important to obtain *A. pectinata* and *R. philippinarum* with low initial bacterial counts. Storing *A. pectinata* and *R. philippinarum* at 4℃ may ameliorate the bacteria contamination.

Influence of different species on bacteria counts and effect of refrigerated storage

According to the results from this study, the bacterial levels in *R. philippinarum* samples were significantly higher than those in *A. pectinata* samples which were collected from a same culture place. This phenomenon may be attributed to the relatively higher filtration rates of *R. philippinarum* than those of *A. pectinata*. Generally speaking, the bivalves with higher filtration rate accumulate more bacteria in their bodies by filtering more water.

The results from this study showed that under refrigerated storage conditions the bacteria inhibitory rates in *R. philippinarum* was significantly higher than those in *A. pectinata*. Although the bacteria inhibitory rates in *R. philippinarum* were higher than those in *A. pectinata*, the bacterial levels in *R. philippinarum* samples were still higher than those in *A. pectinata* samples due to the higher initial bacterial levels in *R. philippinarum*.

The results confirmed that the effect of storage method was various on different species (Tzikas *et al.*, 2007; Robson *et al.*, 2007).

Influence of different culture environments on bacteria counts

The examination results of water quality showed that the concentration of COD and *E. coli* in I-mok were relatively higher than Jang-su. This can explain the observation that in the samples collected from I-mok with higher microbial concentration than samples collected from Jang-su. Some studies have indicated that the seawater can influence the microbial counts in samples through its nutrient concentration (Grimes *et al.*, 1986). So the variability of temperature and salinity as well as nutrient concentration in aquaculture water may influence the composition and abundance of bacteria in seafood (Refugio Castañeda Chávez *et al.*, 2005; Vernocchi *et al.*, 2007).

In conclusion, under refrigerated storage, low levels of coliform, *E. coli* and TPC were attained. The application of refrigerated storage to *A. pectinata* and *R. philippinarum* is advisable to achieve better quality maintenance during short-term storage and distribution. The effect of refrigerated storage differed on different seafood, and the culture environment significantly influenced the initial and ultima bacteria counts. So comprehensive factors should be considered when the refrigerated storage is applied.

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