# Bacterial Contamination in Raw Shucked Oysters in Shucking Houses and Retail Shops in Chon Buri Province, Thailand

Chaweewun\*,\*\*,\*\*\*\*\* Intarakul Suwanna Panutrakul\*,\*\*\*,\*\*\*\*\*\*
Sirichom Thungkao\*,\*\*\*\*,\*\*\*\*\*

#### **ABSTRACT**

Oysters may be cross-contaminated with bacteria from an unsanitary shucking process and sale. Hence the aims of this study were to compare bacterial contamination in samples of shucked oysters from aseptic shucking, in shucking houses, and in retail shops in Chon Buri Province, and to investigate bacterial contamination in samples from surface areas of equipment, the fresh water used in the shucking process, and oysters processed for sale. Analysis of variance was performed after a logarithmic transformation of bacterial counts was conducted. The results revealed that counts of total bacteria, Staphylococcus aureus, and fecal coliforms in aseptically shucked oysters, were significantly lower than those same measurements in freshly-shucked oysters (p < 0.01, = 0.01, < 0.01, respectively) and in packed-shucked oysters (p < 0.01, < 0.01, < 0.01, respectively). About 90-100% of the shucking equipment was contaminated with total bacterial counts higher than acceptable limits, both before and during use. Fresh water exceeded the standards for total bacteria and coliforms in all samples, both before and after washing. The 40% of unwashed and 50% of washed shucked oysters exceeded the standard for total bacteria.

The results indicated that bacterial contamination in these samples may be a consequence of unsanitary cleaning, storage, and handling of equipment and fresh water, including improper temperature controls during oyster processing and sale.

Key words: Bacterial contamination, Oyster, Food safety

J Public Health 2011; 41(2): 184-198.

 $Correspondence: \ \ Lecturer\ Chawewun\ \ Intarakul, Faculty\ of\ Public\ Health, Burapha\ University.\ ChonBuri 20131, Thail and Chawewun\ \ Ch$ 

<sup>\*</sup> Faculty of Public Health, Burapha University

<sup>\*\*</sup> Graduate School Program (Environmental Science Program), Burapha University

<sup>\*\*\*</sup> Department of Aquatic Science, Faculty of Science, Burapha University

<sup>\*\*\*\*</sup> Department of Microbiology, Faculty of Science, Burapha University

<sup>\*\*\*\*\*</sup> Center of Excellence on Environmental Health, Toxicology and Management of Chemicals, Faculty of Science, Mahidol University

#### Introduction

Oysters are one of the most popular types of seafood among Thais, and are often consumed raw or partly cooked. There are two main types of oyster cultures in Thailand and those are the large oyster (Crassostrea spp.), and the rock or small oyster (Saccostrea spp.). The main production area of the large oyster is southern Thailand, while the main culture area of the rock oyster is eastern Thailand, especially in Chon Buri Province<sup>1</sup>. Links between gastroenteritis outbreaks and raw oyster consumption has been well established. This is due mainly to the feeding behavior of oysters. The oyster is a filter-feeding organism; it pumps surrounding water through its gills and filters suspended material from the water, including microorganisms such as phytoplankton and bacteria. Some pathogenic microbes may also be picked up and accumulated in the oyster's tissue<sup>2-3</sup>.

Consumption of the large oyster (*Crassostrea* spp.) is often done immediately after shucking. Since rock oysters are small (6-7 cm in length) and difficult to shuck, most rock oysters sold in markets are shucked and packed with some fresh water and placed in plastic bags or boxes. Discarded shells are placed on the floor, and sometimes covered with a plastic sheet. Oysters are shucked with a knife, and oyster flesh is scraped from the shell into a container that contains fresh water<sup>4</sup>. Oyster flesh may sit in this container for an hour or more, depending on the amount of shells shucked. The shucked oysters are then washed with fresh water, drained in a colander and packed into plastic bags with fresh water.

The whole shucking process, including packaging, is often done without temperature controls, therefore a number of pathogenic bacteria on and in the oysters may increase due to the use of contaminated equipment and contaminated fresh water used during processing. Poor hygiene in the work environment is also an important issue<sup>4</sup>. Moreover, storage conditions for packed-shucked oysters may also promote the proliferation of pathogenic bacteria in the oysters. Hence, consuming uncooked oysters may pose human health risks including diarrhea and food poisoning.

The aims of this study were: (1) to compare bacterial contamination in raw rock oysters, shucked in an aseptic way, with those shucked in shucking houses and those packed-shucked oysters sold in retail shops in Chon Buri Province, Thailand; (2) to determine the level of bacterial contamination of equipment surfaces used in the shucking process; and (3) to compare the bacterial contamination in oysters after having been shucked and washed, with those in fresh water both before and after washing.

#### **Materials and Methods**

#### 2.1 Experiment 1

Whole-shell rock oysters (shucked aseptically in the laboratory) and oysters freshly-shucked were purchased from ten shucking houses. Packed-shucked oysters were purchased from twenty retail shops in Chon Buri Province. Samples were collected in triplicate from each location, giving a total of 30 whole-shell rock oyster samples (about four to six oysters in one sample), 30 freshly-shucked oyster samples, and 60 packed-shucked

oyster samples. The purchased oyster samples were placed in a cooler box and transported to the laboratory immediately. In the laboratory, the wholeshell rock oysters were shucked aseptically after cleaning the shells and then placed in sterile bags containing sterile fresh water. Samples from these three sources were refrigerated while waiting to be analyzed.

#### 2.2 Experiment 2

The surface-area sampling of shucking equipment (shucking knives, gloves, containers, and colanders) used a swab contact metho<sup>5-6</sup>. Sterile cotton swabs were used in swabbing, and 0.1% peptone water was used as the rinse solution. Shucking equipment, except for containers, was swabbed both before and during use. Containers were swabbed only before use because food-contact areas were full of water during use. Swab samples were transported to the laboratory in a cooler box.

#### 2.3 Experiment 3

Oysters processed for sale in containers were sampled after the shucking and washing steps and then collected in sterile plastic bags without fresh water. Fresh water used to process the oysters and was sampled both before and after the washing (soaking step). Samples were collected in sterile 100 ml-wide-mouth bottles. One set of oyster and fresh water samples was collected from each of the ten shucking houses. One of the locations used both tap water and well water. A second set of water samples was collected accordingly. The samples were placed in a cooler box and transported to the laboratory immediately.

#### 2.4 Analytical techniques

- 2.4.1 Aerobic mesophilic bacterial (total bacterial) counts (cfu/g or ml) were enumerated by the Aerobic Plate Count method<sup>7</sup>.
- 2.4.2 Fecal coliform counts (MPN/g) were enumerated by the three-tube-series MPN method<sup>8</sup>.
- 2.4.3 *S. aureus* counts (cfu/g) were enumerated by the direct plate count method using Baird-Parker-egg-yolk tellurite agar <sup>9</sup>. The culture with typical *S. aureus* was purified on new Baird-Parker-egg-yolk tellurite agar, and then *S. aureus* counts were identified with the API Staph test strip (BioMe'rieux, Marcy l'Etoile, France).
- 2.4.4 Coliform and *E. coli* counts (MPN/g or ml) in samples of oysters after shucking and after washing, and surface swabs of shucking equipment, were enumerated by the three-tube-series MPN method, and *E. coli* was confirmed by the IMViC test<sup>8</sup>. Coliform and *E. coli* counts (MPN/100 ml) in samples of fresh water, prior to washing and after soaking the oysters, were enumerated by the five-one-one-tube-series MPN method, and *E. coli* was confirmed by IMViC test<sup>8,10</sup>.

#### 2.5 Data analysis

To facilitate statistical analyses of quantitative data obtained by direct plating, and by the three-tube-series MPN method, half the lowest detection limit for S. aureus (50 cfu/g) and for the MPN value (1.5 MPN/g or ml or 15 MPN/piece) were substituted when levels were below the limit of detection (< 100 cfu/g for S. aureus, and < 3 MPN/g or ml for coliforms, fecal coliforms, and E.  $coli)^{11}$ . When the value of the

lowest detection limit of the five-one-one-tubeseries MPN method was zero, this data (with some zero values) was transformed to  $\log(x+1)$  for analysis of variance. Analysis of variance was performed after logarithmic ( $\log_{10}$ ) transformation of bacterial counts (data dispersed to approach the normal distribution). Box and whisker plots were made using the  $\log_{10}$ —transformed values.

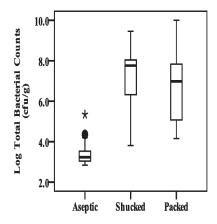
#### Results

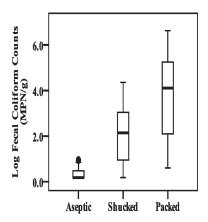
#### 3.1 Experiment 1

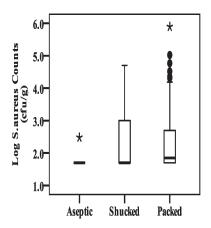
Samples of aseptically-shucked oysters, freshly-shucked oysters, and packed-shucked oysters were contaminated with the mean  $\pm$  SD of log total bacteria counts (TBC) at  $3.38 \pm 0.52$ ,  $7.11 \pm 1.55$ ,  $6.67 \pm 1.52$  cfu/g, with the mean  $\pm$  SD of log *S. aureus* counts at  $1.73 \pm 0.14$ ,  $2.34 \pm 1.07$ ,  $2.40 \pm 1.00$  cfu/g, and with the mean  $\pm$  SD of log fecal coliform counts at  $0.33 \pm 0.27$ ,  $2.16 \pm 1.31$ ,  $3.72 \pm 1.81$  MPN/g, respectively. Log counts of total bacteria, *S. aureus*, and fecal coliforms of aseptically shucked rock oysters were significantly lower than of freshly-shucked oysters (p < 0.01, = 0.01, < 0.01, respectively) and of packed-shucked oysters (p < 0.01, < 0.01, < 0.01, < 0.01, respectively) (Figure 1).

Log counts of total bacteria and *S. aureus* in freshly-shucked oysters and packed-shucked oysters were not significantly different, while log

fecal coliform counts in packed-shucked oysters were significantly greater than in freshly-shucked oysters (p < 0.01). Total bacteria were found in 100% of the samples, from all three sources of shucked oysters. However, none of the aseptically shucked oyster samples had TBCs higher than the microbiological quality criterion (< 1 × 10<sup>6</sup> cfu/ g)12, whereas 80% and 70% of the freshly-shucked rock oyster and packed-shucked oyster samples had TBCs higher than the criterion, respectively (Tables 1 and 2). The occurrences of fecal coliforms were found to increase from 26.7% in the aseptically shucked oysters to 93.3% in the freshly-shucked rock oysters and 100% in packed-shucked oysters. However, all of the aseptically shucked oyster samples with positive fecal coliform counts had levels below the microbiological quality criterion  $(< 20 \text{ MPN/g})^{12}$ , whereas 70% and 86.7% of the samples of freshly-shucked rock oysters and packedshucked oysters had levels above the criterion, respectively. The occurrences of S. aureus counts were found to increase from 3.3% in aseptically shucked oyster samples to 33.3% in freshly-shucked rock oyster samples and 51.7% in packed-shucked oysters (Table 1). The criterion for S. aureus (< 100 cfu/g)<sup>12</sup> was exceeded in 3.3% of aseptically shucked oysters, 30% of freshly-shucked oysters, and 50% of packed-shucked oysters.







**Figure 1** Box plots of log counts of total bacteria, fecal coliforms and *S. aureus* in samples of whole-shell rock oysters shucked aseptically in the laboratory, freshly-shucked rock oysters from the shucking houses and packed-shucked oysters

- represents median value
- represents outlier value (value between 1.5 and 3 IQR)
- \* represents extreme value (value more than 3 IQR)

**Table 1** Percent of occurrences (number of samples found/total number of samples) of total bacteria, fecal coliforms and *S. aureus* in whole-shell rock oysters shucked aseptically in the laboratory, freshly-shucked rock oysters from shucking houses, and packed-shucked oysters.

Contaminants	Aseptic-shucked	Freshly-shucked	Packed-shucked	
	oysters	oysters	oysters	
Total bacteria	100 (30/30)	100 (30/30)	100 (60/60)	
Fecal coliforms	26.7 (8/30)	93.3 (28/30)	100 (60/60)	
S. aureus	3.3 (1/30)	33.3 (10/30)	51.7 (31/60)	

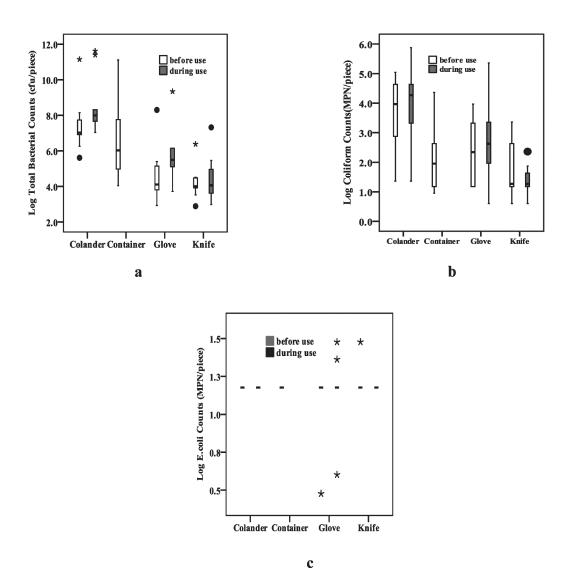
**Table 2** Percent of samples with levels of total bacteria, fecal coliforms and *S. aureus* higher than the microbiological quality criteria (number of samples not complying with the criterion/total samples) in whole-shell rock oysters shucked aseptically in the laboratory, freshly-shucked rock oysters from shucking houses and packed-shucked oysters.

Contaminants	Aseptic-shucked	Freshly-shucked	Packed-shucked	
	oysters	oysters	oysters	
Total bacteria	0 (0/30)	80 (24/30)	70 (42/60)	
Fecal coliforms	0 (0/30)	70 (21/30)	86.7 (52/60)	
S. aureus	3.3 (1/30)	30 (9/30)	50 (30/60)	

<sup>\*</sup> Microbiological criteria in shucked oysters: TBC < 1 × 10<sup>6</sup> cfu/g, fecal coliforms < 20 MPN/g, *S. aureus* < 100 cfu/g, (the Notification of the Department of Medical Sciences, Ministry of Public Health, Thailand, B.E. 2536)

#### 3.2 Experiment 2

The aim of this experiment was to determine and to compare the levels of bacterial contamination on the surfaces of shucking equipment before and during use. There were four main types of equipment used during the shucking process: a shucking knife, a glove, a container, and a colander.



**Figure 2** Box plots of log counts of total bacteria (a), coliforms (b), *E. coli* (c) in samples of colanders, containers, gloves, and knive

- represents median value
- represents outlier value (value between 1.5 and 3 IQR)
- \* represents extreme value (value more than 3 IQR)

Samples from colanders, gloves, and shucking knives were contaminated with the mean ± SD of log TBCs (before and during use) at  $7.42 \pm 1.49$  and  $8.58 \pm 1.58$ ,  $4.63 \pm 1.49$  and  $5.74 \pm 1.46$ ,  $4.20 \pm 0.90$  and  $4.40 \pm 1.31$  cfu/ piece; with the mean  $\pm$  SD of log coliform counts (before and during use) at  $3.68 \pm 1.13$  and  $3.89 \pm$ 1.35,  $2.37 \pm 1.05$  and  $2.85 \pm 1.56$ ,  $1.71 \pm 0.96$ and  $1.42 \pm 0.48$  MPN/piece; and with the mean  $\pm$ SD of log *E. coli* counts (before and during use) at  $1.18 \pm 0.00$  and  $1.18 \pm 0.00$ ,  $1.11 \pm 0.22$ and  $1.11 \pm 0.29$ ,  $1.21 \pm 0.10$  and  $1.18 \pm 0.00$ MPN/piece, respectively. Samples from containers before use were contaminated with the mean ± SD of log TBCs at  $6.45 \pm 2.07$  cfu/piece, of log coliform counts at 2.09 ± 1.06 MPN/piece, and of log.

*E. coli* counts at 1.18  $\pm$  0.00 MPN/piece, respectively. TBCs were found on the surfaces of all shucking equipment, both before and during use (Table 3). All swab samples (100%) of beforeand during-use colanders, during-use gloves, and before-use containers had TBCs higher than the criterion ( $< 1 \times 10^3$  cfu/piece)<sup>12</sup>. Similarly, 90% of samples from before- and during-use shucking knives, and before-use gloves had TBCs higher than the criterion (Table 3). Log TBCs were highest on surfaces of colanders, followed by containers, gloves, and knives (Figure 2a). Log TBCs of

shucking equipment during use was significantly greater than before use (p = 0.04). Log TBCs of colanders were significantly greater than that of gloves (p < 0.01) and knives (p < 0.01); also, log TBCs of containers were significantly greater than of knives (p = 0.01). However, log TBCs between colanders and containers, containers and gloves, gloves and knives, were not significantly different.

The occurrences of coliforms on the surfaces of shucking equipment (before and during use) were 70-100%, and 60-100%, respectively (Table 3). Log coliform counts were highest on samples from colanders, followed by gloves, containers, and shucking knives (Figure 2b). Log coliform counts from shucking equipment before and during use were not significantly different. However, log coliform counts from colanders were significantly greater than from gloves (p = 0.02), containers (p < 0.01), and knives (p < 0.01); also, coliform counts from gloves were significantly greater than from knives (p = 0.04). However, coliform counts between gloves and containers, and between containers and knives; were not significantly different. E. coli counts were found only on the surfaces of before-use shucking knives, and beforeand during-use gloves, with occurrences of 10%, 10%, and 40%, respectively (Table 3).

**Table 3** Percentage of occurrences (number of samples found/total number of samples) of total bacteria, coliforms, and *E. coli* in samples of colanders, containers, gloves, and shucking knives before and during use.

Contaminants	Colanders		Containers	Gloves		Colanders	
	before	during	before	before	during	before	during
Total bacteria	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)
	100 (10/10) <sup>a</sup>	100 (10/10) <sup>a</sup>	100 (10/10) <sup>a</sup>	90 (9/10) <sup>a</sup>	100 (10/10) <sup>a</sup>	90 (9/10) <sup>a</sup>	90 (9/10) <sup>a</sup>
Coliforms	100 (10/10)	100 (10/10)	80 (8/10)	70 (7/10)	100 (10/10)	70 (7/10)	60 (6/10)
E. coli	0 (0/10)	0 (0/10)	0 (0/10)	10 (1/10)	40 (4/10)	10 (1/10)	0 (0/10)

<sup>&</sup>lt;sup>a</sup> Percentage of samples with levels of total bacteria higher than the microbiological quality criteria (number of samples not complying with the criterion/total number of samples).

The criterion of total bacteria / piece of food contact equipment, utensils, and hands of food handlers  $< 1 \times 10^3$  cfu / piece (the Notification of the Department of Medical Sciences, Ministry of Public Health, Thailand, B.E. 2536)

#### 3.3 Experiment 3

The aim of this experiment was to study the trend of cross-contamination that may result from fresh water being used for soaking and washing shucked oysters at the shucking houses. Based on interviews with rock oyster shuckers, eight shucking houses used tap water for processing oysters, one used only well water from a private well, and another used both tap water and well water. Samples of shucked oysters before and after washing, and fresh water before and after washing, were contaminated with the mean  $\pm$  SD of log TBCs at  $5.41 \pm 1.28$  and  $6.01 \pm 1.77$  cfu/g, 5.08 $\pm$  1.34 and 6.84  $\pm$  2.30 cfu/ml; of log coliform counts at  $2.79 \pm 1.15$  and  $3.14 \pm 1.32$  MPN/ g,  $3.85 \pm 1.09$  and  $4.74 \pm 1.03$  MPN/100 ml; of log E. coli counts at  $0.18 \pm 0.00$  and 0.18 $\pm$  0.00 MPN/g, 0.21  $\pm$  0.71 and 0.09  $\pm$  0.20 MPN/100 ml, respectively. Occurrences of total bacteria and coliforms were 100% for all of fresh

water samples and all of shucked oyster samples, both before and after washing. Fresh water samples exceeded the standards for total bacteria ( $\leq 500$  colonies/ml) and coliforms (< 2.2 MPN/100 ml)<sup>13</sup> in all samples, both before and after washing, while 40% of unwashed and 50% of washed shucked oyster samples exceeded the standard for total bacteria ( $<1 \times 10^6$  cfu/g)<sup>12</sup>. Occurrences of *E. coli* in before- and after-use water samples were 9.09% and 18.18%, respectively; the samples that tested positive for *E. coli* thus exceeded the criterion (*E. coli* not found)<sup>13</sup>. *E. coli* was not found in any of the samples of oysters, either before or after washing.

#### Discussion

TBC (mean  $\pm$  SD) of aseptically- shucked rock oyster samples in this present study (3.38  $\times$  0.52 log cfu/g) was lower than in the previous study by Gannarong and Sopakul

 $(7.1 \times 10^5 \pm 1.7 \times 10^5, 4.0 \pm 10^5 \pm 1.3 \times 10^5,$ and  $1.3 \times 10^5 \pm 4.6 \times 10^4$  cfu/g of asepticallyshucked oyster samples — C. belcheri, C. lugubris, and S. commercialis, respectively)<sup>14</sup>. On the other hand, the range of TBCs in freshly-shucked oyster samples in this study (3.81-9.46 log cfu/g) was higher than in handled samples (the bivalve Pinctada imbricata shucked by the salesman)  $(30-2.8 \times 10^5)$ cfu/g) studied by De Bastardo and Aristizabal<sup>15</sup>. In Trinidad and Tobago, Laloo, et al. found that the mean TBC (± SD) of raw oysters from vendors ranged from  $1.0 \times 10^7 \pm 4.3 \times 10^7$  to  $1.4 \times$  $10^8 \pm 6.4 \times 10^8$  cfu/g<sup>16</sup> whereas in this study TBCs in packed-shucked oyster samples were in the range of 4.15-10.05 (mean  $\pm$  SD =  $6.67 \pm 1.52$ ) log cfu/g. Similar TBCs, the range of S. aureus counts in the freshly-shucked oyster samples at shucking houses of this present study (1.70 - 4.70 log cfu/g) was higher than in raw oyster samples shucked by the salesman  $(2.3 \times 10 - 4.6 \times 10^2 \text{ MPN/g})^{15}$ . The previous study by Utrarachkij and colleagues found that the range of fecal coliform counts in aseptically- shucked raw oysters (0-540 MPN/g)<sup>17</sup> was higher than in this present study (0.18-0.95 log MPN/g). While the range of fecal coliform counts in oysters shucked by the salesman  $(4-4.6 \times 10^2)$ MPN/g)<sup>15</sup> was less than in freshly-shucked oysters from the oyster shucking houses in this study (0.18-4.36 log MPN/g).

Log counts of total bacteria, fecal coliforms and *S. aureus* of aseptically shucked rock oysters were distinctly lower than of freshly-shucked rock oysters and packed-shucked oysters (Figure 1). This study indicates that the cleanliness of equipment and sanitary handling practices in the aseptic oyster-

shucking process will help protect shucked oysters against bacterial contamination. Furthermore, these study results were consistent with the previous report by De Bastardo and Aristizabal<sup>15</sup> that unhandled oyster samples (samples shucked aseptically in the laboratory) had counts of total bacteria, fecal coliforms and S. aureus lower than handled oyster samples (oysters shucked by the salesman). This study suggests that cross-contamination of total bacteria, fecal coliforms and S. aureus of freshly-shucked oysters may be caused by contaminated equipment, before and during use (Table 3), and fresh water, before and after the washing step (experiment 3). Occurrences (%) of fecal coliforms and S. aureus in packed-shucked oysters were higher than in freshly-shucked oysters, and more of the packed shucked oysters exceeded the criteria for fecal coliforms and S. aureus. Higher counts in packed-shucked oyster samples may come from many sources of cross-contamination and improper temperature control of the product. First, packed-shucked oysters at retail shops may have been transferred to smaller containers for sale, and later pooled into larger containers and kept on ice overnight. As a result, there were opportunities for cross-contamination from food handlers, surfaces of equipment, and water replaced in the packages of oysters. An estimated 30-50% of the population are nasal and throat carriers of

*S. aureus*, and 15% are skin carriers. Skin lesions, e.g. boils and infection of cuts and burns are often caused by the organisms. Even small amounts of pus associated with these conditions can contain many millions of *S. aureus* cells<sup>18</sup>. Second, shucking of whole-shell rock oysters at

all ten shucking houses in this study was conducted at ambient temperature, and rock oysters were shucked into containers with fresh water. However, food safety guidelines for processing oysters state that ice should added to the water, that the containers be made of stainless steel, and that the shucked product be kept at a maximum temperature of 4.4°C<sup>19</sup>. Furthermore, most of the packs of shucked oysters on sale were kept at ambient temperature for a longer time than freshly-shucked oysters, allowing for more bacterial growth. The range from 5 to 63°C (41 to 145°F) is called the Danger Zone because the hazard of bacterial growth is great within this range<sup>20</sup>.

Aerobic Plate Count (total bacteria) can be used to evaluate sanitary conditions of equipment and utensils. This can be done during processing to monitor buildup and after sanitation to gauge its effectiveness<sup>21</sup>. The high counts of total bacteria on shucking equipment may result from many possible causes. First, water used for cleaning the shucking equipment was likely contaminated, even before use, as was the case with water used for cleaning the oysters (experiment 3). Second, improper handling of oysters was observed at shucking houses. None of the ten shucking houses cleaned the whole-shell rock oysters before shucking, exposing the surfaces of equipment to soil or other material that had adhered to the oyster shells. Also, most of shucking houses did the shucking on floors, where soil, dust, or standing water could be spattered into containers with the shucked oysters. A great diversity of microorganisms inhabit soil. including pathogenic microorganisms<sup>22</sup>. Third, observations were made that during pauses in

shucking, equipment was often laid on unclean surfaces such as on piles of rock oysters or unclean table tops. Finally, observations were made that shucking equipment and water used for soaking oysters were rarely replaced during use, allowing for the accumulation of dirt and bacterial pathogens, and allowing for further cross-contamination. Most shucking equipment (60-100%) was contaminated with coliforms, and some was contaminated with E. coli. Coliforms can be present in feces of humans and warm-blooded animals and birds. Some can be present in soil, water, and plants. E. coli is present in the lower intestinal tract of humans and warm-blooded animals and birds. Its presence is considered an indication of direct or indirect fecal contamination<sup>23</sup>. Coliforms and E. coli found on the surfaces of shucking equipment in this study indicates unsanitary conditions (improper cleaning, storage, and handling of equipment). Shuckers may not properly wash their hands after using the toilet; thus, they could be a source of bacterial contamination and facilatators of E. coli crosscontamination of shucking knives and gloves.

All samples of shucked oysters, before and after washing, were contaminated with total bacteria at levels higher than the criterion; also, all samples of rinse water, before and after use, were contaminated with total bacteria and coliforms at levels higher than the standards. Certainly, samples of water taken before use in washing should be clean and should meet the microbiological criteria. Bacterial contamination in these samples may come from many causes. First; fresh water tanks of some shucking houses were not covered with lids, or had lids with holes or cracks. Second, utensils

(possibly unclean) were sometimes used to dip into the fresh water tanks. Third, fresh water tanks may not have been cleaned often. Not surprisingly, water that had been used for soaking oysters had counts of total bacteria and coliforms higher than water before use. Soil (and microorganisms) accumulates while shucking and soaking rock oysters, and the water may be rarely replaced. The washed oyster samples should have had bacteria counts less than the unwashed oyster samples, but in fact the opposite was true. This was probably due to cross-contamination from unclean water (results in experiment 3). The occurrences of E. coli counts, which were found in some of the water samples (both before and after use), may have resulted from cross-contamination by shucking knives or gloves (Table 3), or from contaminated well water. Based on interviews with rock oyster shuckers, some shucking houses used water from wells which may be unclean and contaminated by sewage.

The results of this study indicated contamination with high counts of indicator bacteria (total bacteria, coliforms, *E. coli*) on the surfaces of shucking equipment, in water used in shucking processes, and in oyster flesh processed for sale. Total bacteria, *S. aureus*, and fecal coliforms were also found in freshly-shucked rock oysters, and packed-shucked oysters. The study identified possible sources of contamination, including improper cleaning, storage and handling of shucking equipment, inadequately-cleaned water tank or unsanitary condition of water tank, unsanitary handling of rock oysters during shucking and improper temperature control during processing,

sale and storage. These problem areas should be addressed in any effort to improve the safety of rock oysters produced for consumption.

### Acknowledgements

This research was supported in part by Split Program of Burapha University and Grant encouragement of Center of Excellence on Environmental Health, Toxicology and Management of Chemicals, Faculty of Science, Mahidol University, Bangkok, Thailand.

#### References

- Brohmanonda P, Mutarsint K, Chongpeepien T, Amornjaruchit S. Oyster culture in Thailand. In: McCoy EW, Chongpeepien T, eds. Bivalve mullusc culture research, Thailand. ICLARM Technical Reports 19. 170P. Department of Fisheries Bangkok, Thailand. International Center for Living Aquatic Resources Management, Manila, Philippines and Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH, Eschborn, Federal Republic of Germany, 1988: 31-9.
- Cook DW, Burkhardt W, Ill, Depaola A, McCarthy SA, Calci, KR. Molluscan shellfish: oysters, mussels, & clams. In: Downes FP, Ito K, eds. Compendium of Methods for the Microbiological Examination of Foods. 4<sup>th</sup> ed. Washington, DC: American Public Health Association, 2001: 507-10.
- Banwart GJ. Basic food microbiology. 2<sup>nd</sup> ed. New York: Van Nostrand Reinhold, 1989.
- Panutrakul S, Thungkao S, Senanan W, Khaoseejan T, Waiprib Y, Chalermwat K.

Sanitation guidelines for reduction of pathogenic bacteria contamination in shucked oysters. Chon Buri: Department of Aquatic Science, Faculty of Science, Burapha University, 2007.

- The Department of Medical Sciences, Ministry of Public Health. Usage manual for 21 types of food test serials: Technology transfer to local organization and community. Bangkok: Yonggich, 2003.
- Evancho GM, Sveum WH, Moberg LJ, Frank JF. Microbiological monitoring of the food processing environment. In: Downes FP,. Ito K eds. Compendium of Methods for the Microbiological Examination of Foods. 4<sup>th</sup> ed. Washington, DC: American Public Health Association, 2001: 27-29.
- Bacteriological Analytical Manual (BAM)
   [Online] 2001. Aerobic plate count. Available
   at http://www.cfsan.fda.gov/~ebam/bam-3.html,
   accessed December 24, 2004.
- Kornacki JL, Johnson JL. Enterobacteriaceae, coliform, and Escherichia coli as quality and safety indicators. In: Downes FP, Ito K, eds. Compendium of Methods for the Microbiological Examination of Foods. 4<sup>th</sup> ed. Washington, DC: American Public Health Association, 2001:69-77.
- Lancette GA, Bennett RW. Staphylococcus aureus and staphylococcal enterotoxins. In:
   Downes FP,. Ito K eds. Compendium of Methods for the Microbiological Examination of Foods. 4<sup>th</sup> ed. Washington, DC: American Public Health Association, 2001;387-91.
- Loengsakul S. Handbook of food microbiological laboratory practice. Bangkok: Chaijaroen, 1997: 49-51.

- 11. Parveen S, Hettiarachchi KA, Bowers JC, Jones JL, Tamplin ML, McKay R, et al. Seasonal distribution of total and pathogenic *Vibrio parahaemolyticus* in Chesapeake Bay oysters and waters. Int J Food Microbiol 2008; 128: 354-61.
- 12. The Notification of the Department of Medical Sciences, Ministry of Public Health, Thailand, B.E. 2536. Microbiological quality criteria for food, utensils and food handlers.
- 13. The Notification of the Ministry of Industry No.332, B.E. 2521. Industrial product criteria for potable water. In: Environmental Engineering Association of Thailand, Summation of Environmental Law for Practitioner. Bangkok: Mitrnara, 2001: 6-32.
- Gannarong M, Sopakul C. Comparison of growth and bacterial contamination among Crassostrea belcheri, Crassostrea lugubris and Sassostrea commercialis, culture in Ban Don Bay, Surat Thani. J Fisheries 2000; 53: 565-71.
- 15. De Bastardo LBV, Aristizabal LE. Microbiological quality of the bivalve *Pinctada imbricata* commercialized in Cumana, Venezuela. Food Technology, Acta Cientifica Venezolana 2001; 52: 55-61.
- 16. Laloo S, Rampersad FS, Borde AL, Maharaj K, Sookhai L, Teelucksingh JD, et al. Bacteriological quality of raw oysters in Trinidad and the attitudes, knowledge and perceptions of the public about its consumption. Int J Food Microbiol 2000; 54: 99-107.
- Utrarachkij F, Intalapaporn K, Kumkrong K, Kittigul L. Microbiological quality of raw oysters from local markets in Bangkok. J Public Health 2006; 36: 112-22.

- Garbutt J. Essentials of food microbiology.
   London: Arnold, a member of the Hodder Headline Group, 1997: 168-9.
- Otwell WS, Garrido VM. Total quality assurance (TQA) and hazard analysis critical control point (HACCP): Manual for oyster production and processing (pp.15-20). University of Florida: Florida Sea Grant College Program, 1995.
- Roday S. Hygiene and sanitation in food industry. New Delhi: Tata McGrow-Hill, 1999: 80-81.

- 21. Morton RD. Aerobic plate count. In: Downes FP, Ito K eds. Compendium of Methods for the Microbiological Examination of Foods. 4<sup>th</sup> ed. Washington, DC: American Public Health Association, 2001: 507-10.
- Pelczar MJ, Chan ECS, Krieg NR. Microbiology: concepts and applications. New York,
   U.S.A.: McGraw-Hill, 1993: 772-3.
- Ray B, Bhunia A. Fundamental food microbiology. 4<sup>th</sup> ed. New York: CRC, 2008: 351-2.

# การปนเปื้อนแบคทีเรียในหอยนางรมที่แกะในร้านค้าที่แกะหอยนางรม และหอยนางรมในร้านค้าปลีกในจังหวัดชลบุรี ประเทศไทย

ฉวีวรรณ อินทรกุล\*<sup>,\*\*,\*\*\*\*\*</sup> สุวรรณา ภาณุตระกูล<sup>\*,\*\*\*,\*\*\*\*\*</sup> ศิริโฉม ทุ่งเก้า<sup>\*,\*\*\*\*,\*\*\*\*</sup>

## าเทคัดย่อ

หอยนางรมอาจปนเปื้อนด้วยแบคทีเรียจากการแกะและขายที่ไม่ถูกสุขลักษณะ ดังนั้นวัตถุประสงค์ ของการศึกษานี้เพื่อเปรียบเทียบการปนเปื้อนแบคทีเรียในตัวอย่างหอยนางรมจากการแกะแบบปลอดเชื้อ ร้านค้า ที่แกะหอยนางรม และร้านค้าปลีกในจังหวัดชลบุรี และเพื่อสอบสวนการปนเปื้อนแบคทีเรียในตัวอย่างจาก พื้นผิวของอุปกรณ์ น้ำจืดที่ใช้ในการแกะหอยนางรม และหอยนางรมที่แกะสำหรับขาย วิเคราะห์ความแปรปรวน จากจำนวนแบคทีเรียที่แปลงค่าเป็นลื่อกการิทึมแล้ว ผลการศึกษาพบว่าจำนวนแบคทีเรียทั้งหมด สแตฟฟิลโล คือกลัส ออเรียส และฟิคอล โคลิฟอร์มในหอยนางรมที่แกะด้วยวิธีปลอดเชื้อมีค่าน้อยกว่าในหอยนางรม ที่แกะใหม่ อย่างมีนัยสำคัญทางสถิติ p < 0.01, = 0.01, < 0.01, ตามลำดับ) และในหอยนางรมที่บรรจุใน หีบห่อ (p < 0.01, < 0.01, < 0.01, < 0.01, ตามลำดับ) อุปกรณ์ก่อนและระหว่างใช้ประมาณร้อยละ 90-100 ปนเปื้อน ด้วยแบคทีเรียทั้งหมดเกินเกณฑ์มาตรฐาน น้ำจืดทั้งหมดก่อนและหลังการใช้ล้างมีแบคทีเรียทั้งหมด และ ฟิคอล โคลิฟอร์มเกินเกณฑ์มาตรฐาน หอยนางรมก่อนล้างร้อยละ 40 และหอยนางรมหลังล้างร้อยละ 50 มี แบคทีเรียทั้งหมดเกินมาตรฐาน

ผลการศึกษาชี้ให้เห็นว่าการปนเปื้อนแบคทีเรียในตัวอย่างอาจเป็นผลจากการล้าง การเก็บและการ ใช้อุปกรณ์ น้ำจืดที่ไม่ถูกสุขลักษณะ และการควบคุมอุณหภูมิที่ไม่ถูกต้องในระหว่างการแกะและขายหอยนางรม คำสำคัญ: การปนเปื้อนแบคทีเรีย, หอยนางรม, ความปลอดภัยอาหาร

วารสารสาธารณสุขศาสตร์ 2554; 41(2): 184-198.

<sup>\*</sup> คณะสาธารณสุขศาสตร์ มหาวิทยาลัยบูรพา

<sup>\*\*</sup> บัณฑิตวิทยาลัย มหาวิทยาลัยบูรพา

<sup>\*\*\*</sup> ภาควิชาวาริชศาสตร์ คณะวิทยาศาสตร์ มหาวิทยาลัยบูรพา

<sup>\*\*\*\*</sup> ภาควิชาจุลชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยบุรพา

<sup>\*\*\*\*\*</sup> ศูนย์ความเป็นเลิศด้านอนามัยสิ่งแวคล้อม พิษวิทยาและการบริหารจัดการสารเคมี คณะวิทยาศาสตร์ มหาวิทยาลันมหิดล