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CONTENTS

DETERMINATION OF CONTAMINATION PROFILES OF HUMAN BACTERIAL PATHOGENS IN SHRIMP OBTAINED FROM JAVA, INDONESIA

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Abstract

Shrimp continues to be an important export commodity for Indonesia and contributed significantly to the country's revenue. However, shrimp exports have been frequently rejected by importing countries due to filth, *Salmonella* and insanitary conditions. This study was conducted to evaluate the profiles of bacterial contamination of ocean and aquaculture shrimp obtained from the area of West, Central and East Java; frozen shrimp and shrimp during industry production of frozen shrimp. The study indicated that both ocean and aquaculture shrimp obtained from the study area were heavily contaminated. On the average, shrimp obtained from West Java were more contaminated than those obtained from East and Central Java. The total bacterial counts were generally higher in ocean shrimp than those of aquaculture ones. *Salmonella* was present in two of 32 samples of ocean shrimp and in four of 32 samples of aquaculture shrimp obtained from the study area. *Vibrio cholerae* was not detected in shrimp from West Java, but was found in three out of 16 samples obtained from East and Central Java. *V. parahaemolyticus* was frequently identified in aquaculture shrimp but absent in fresh ocean shrimp. Studies on shrimp collected from six sampling points during frozen shrimp production revealed that processing will reduce the number of total bacterial, *E. coli*, and Staphylococal counts. However, the processing did not effectively reduce the incidence of *Salmonella* or *V. parahaemolyticus* when the raw material has been contaminated with the pathogens. Sizing and grading as well as arrangement of shrimp before freezing were considered as the critical points where bacteria should be controlled to inhibit growth and cross contamination with bacteria such as *Listeria.* Implementation of Good Agricultural Practices in production of raw shrimp as well as Hazard Analysis Critical Control Point at the line processing are expected to improve the quality of fresh and frozen shrimp.

1. INTRODUCTION

Shrimp is an important and expensive commodity in international trade. Indonesia, Mexico, Ecuador, China, Thailand, India, Vietnam, and the Philippines are the largest exporters of shrimp (Nickelson, 1992) [9]. In Indonesia the export volume of shrimp and fish has been decreasing within the past five years; however, they still contributed revenue to the amount of $1 048 423 975 in 2001 (Ministry of Industry and Trade, 2002) [7]. Shrimp from Indonesia is primarily exported as frozen headless shrimp to Japan and USA. However, shrimp from Indonesia is subject to automatic detention due to the risk of bacterial pathogens. The presence of filth and and *Salmonella* accounted for 90% of the rejection. Additionally, other factors such as environmental issues and antibiotic contamination may have caused the export to decrease.

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† Deceased, January 2000.

Shrimp for export are generally caught from the ocean and aquaculture ponds. Although most of the frozen shrimp processors are big industries using modern facilities and equipment and complying with the Good Manufacturing Practices, the fresh shrimp were generally caught by traditional fishermen with low knowledge in good handling practices and sanitation.

The objectives of the research were to determine (1) the bacterial contamination profiles of fresh shrimp collected from ocean and aquacultures of the northern coast of Java; (2) bacterial contamination profile of the existing frozen shrimp products available in Indonesian markets; and (3) the fate of bacterial contaminants in shrimp during in line processing in the preparation of frozen shrimp.

2. MATERIALS AND METHODS

2.1. Collection of shrimp samples

Samples of fresh ocean and aquaculture shrimp from the provinces of West, Central and East Java were obtained during the rainy season of 1998-1999, 2000, 2001, respectively. Ocean shrimp samples were collected from four catching place in West Java, i.e. Sukabumi (W1), Indramayu (W2), Jakarta (W3); and Tangerang (W4); two catching places in East Java, i.e. Tuban (E1) and Surabaya (E2), and two places in Central Java, i.e., Semarang (C1) and Pekalongan (C2). Aquaculture shrimp cultured in the pond were collected from aquaculture places of the three areae above, i.e. from Karawang (W5), Indramayu (W6), Tangerang (W8) and, Cirebon (W8), Tuban (E3), Surabaya (E4), Semarang (C3) and Pekalongan (C4).

Frozen shrimp were collected from five seafood processing industries, namely industries I1, I2, I3, I4 and I5 and from four different grocery stores, i.e. G1, G2, G3 and G4 located in West Java.

Shrimp samples for line processing study were obtained from an industry located in Java. Samples were collected at six sampling points during frozen shrimp processing. Samples were obtained (1) during receiving, (2) after head removal, (3) after sizing and grading (4) after final rinsing in water containing 30 ppm chlorine (5) after arrangement and water filling and (6) after freezing.

3. MICROBIOLOGICAL ANALYSIS

Black tiger shrimp samples were collected aseptically, heads were removed and the tails were analyzed promptly (frozen shrimp was thawed in refrigerator prior to analysis). Aquaculture water was also evaluated for its bacterial contents as supporting data. Each study was repeated two to five times. A composite sample of 450 g was collected and prepared for microbiological analysis. The analysis included the examination of total plate counts, *Staphylococcus*, coliform, *Enterobactericeae* and *E. coli* counts. Presence of *Salmonella, V. cholerae*, *V. parahamolyticus*, and *Listeria monocytogenes* (only for shrimp of line processing) was also determined.

The methods to quantify total microbes, staphylococci, coliform, *Enterobactericeae* and *E. coli* as well as procedures to detect the presence of bacterial pathogens were in accordance with AOAC (1995) [1]. Total plate counts determined in Plate Count agar (PCA), *Staphylococcus* counts in Baird Parker tellurite egg yolk Agar (BPA), coliform counts in Violet Red Bile Agar (VRBA), *Enterobactericeae* counts in VRBA + 1% glucose, and *E. coli* counts in Eosine Methylene Blue Agar (EMBA).

Procedures for detection of pathogenic bacteria in shrimp consisted of enrichment/selective enrichment, isolation and confirmation steps. The media for enrichment were Selenite Cystine Broth for *Salmonella*, Alkaline Peptone Water (APW) for *V cholerae* or APW + 3% NaCl buffer for *V. parahaemolyticus*, and Half Fraser Broth for *Listeria*. The selective media used to isolate the pathogenic bacteria were Hektoen, Bismuth Sulfite and XLD Agar for *Salmonella*, Thiosulfate Citrate Bile Salt Sucrose (TCBS) agar for *V.cholerae* and *V. parahaemolyticus*, and PALCAM Agar for *Listeria*. Confirmation of *Salmonella* was conducted in Triple Sugar Iron (TSI) Agar and Lysine Iron Agar (LIA) followed by API 20E test kit. Presence of *V. cholerae* and *V. parahaemolysis* was confirmed in TSI and SIM or SIM supplemented with 3% NaCl. Presence of *Listeria* was confirmed by growing the typical isolates from PALCAM in TSAYE Agar and tested for motility, Gram staining and catalase activity.

4. RESULTS AND DISCUSSION

4.1. Bacterial contamination profile of fresh shrimp

The study showed that the level of contamination of fresh shrimp varied between sampling sites. However, in general it was found that on average, shrimp from West Java contained more bacteria and was more frequently contaminated with human pathogens than shrimp from Central and East Java (Table1). The bacterial load ranged from (log_{10}CFU/g) 5.6 to 7.1, indicating a high level of contamination. The counts were similar to those obtained by Cann (1977) who reported a bacterial load between 10^5-10^7 per g of shrimp [3]. Most were spoilage bacteria such as those belonging to *Enterobacteriacea* which ranged from (log_{10}CFU/g) 5.6 to 6.8.

Table 1. BACTERIAL CONTAMINATION PROFILE OF FRESH OCEAN SHRIMP OBTAINED FROM JAVA

Bacteria	Log $_{10}$ CFU/g or + (present) and - (absent)							
	West Java				East Java		Central Java	
	W1	W2	W3	W4	E1	E2	C1	C2
Total Plate Count	7.1	5.9	6.2	6.1	5.7	5.6	5.7	6.3
Staphylococcus	5.3	4.4	4.8	4.5	3.3	3.2	3.1	4.1
Enterobacteriaceae	6.8	5.5	5.3	5.6	<1	4.0	2.5	4.2
Total Coliform	6.1	4.7	4.9	5	<1	3.4	2.1	3.8
E. coli	2.8	3.3	3.3	3.3	<1	3.7	2.4	3.8
Salmonella	+*	-	-	-	-	-	-	-
	(2/4)**	(0/4)	(0/4)	(0/4)	0/4	(0/4)	(0/4)	(0/4)
V. cholerae	-	-	-	-	-	-	-	+
	(0/4)	(0/4)	(0/4)	(0/4)	(0/4)	(0/4)	(0/4)	(1/4)
V. parahaemolyticus	-	-	-	-	-	-	-	-
	(0/4)	(0/4)	(0/4)	(0/4)	(0/4)	(0/4)	(0/4)	(0/4)

*) *S. paratyphi A*

**) Number in parenthesis is the number of positive isolates per number of samples.

The number of *E. coli* was low only in samples obtained from Surabaya, East Java (E1), but ranged from (log_{10}CFU/g) 2.4-3.8 in shrimp from other locations. Presence of these bacteria indicated fecal contamination as a result of poor sanitation. *Salmonella* was found in shrimp obtained from West Java with a frequency of 12.5% or 6.25% of overall samples. This number was lower than that in a report by Heinitz et al. (2000) who stated an isolation frequency of 7.2% of imported seafood during 1990-1998 in the USA and a 12.5% isolation rate of the pathogen from seafood imported from Central Pacific area (Australia, Cocos islands, Fiji, Indonesia, Malaysia, New Zealand, New Guinea and Singapore). *V. cholerae* was detected in one out of 32 samples, while *V. parahaemolyticus* was absent in all raw ocean shrimp samples. This result was different from Nascumento et al. (1998) who reported isolation of *V. parahaemolyticus* of ocean shrimp obtained from the coastal area of Maranhao state, Brazil [8].

Fresh shrimp obtained from ponds also had high bacterial contamination that varied in each location (Table 2). However the total bacterial counts of the aquaculture shrimp were generally lower, with the exception of shrimp from E1. Bacterial contamination of ocean shrimp may have come from poor handling during transport from the ocean. Some of the traditional fishermen do not have appropriate cold storage and may be equipped with insanitary containers. The number of *E. coli* in aquaculture shrimp was generally higher than that of ocean shrimp, although in E1 this was not the case.

Aquaculture shrimp from Central Java contained the lowest bacterial counts. However the *E. coli* contamination was low in shrimp obtained from East Java. *Salmonella* was isolated more frequently in aquaculture shrimp, especially those collected from West Java (12.5%). *V. cholerae* was frequently isolated from shrimp collected from East Java, while shrimp collected from all four locations in West Java contained *V. parahaemolyticus*. V. *cholerae* in crustacean has been reported as the vehicle for some cholera outbreak in the US (CDC, 1986) and Peru (CDC, 1991) [4, 5].

The level of contamination of aquaculture shrimp was a reflection of the contamination of water (Table 3). Most of the ponds were located near households that may be polluted with household waste. The highest level of *E. coli* in aquaculture shrimp obtained from Pekalongan, Central Java (C4) as shown in Table 2 was correlated with the high contamination of the water from the same pond (Table 3). A similar pattern was observed for *Salmonella* and *V. cholerae*. However, *V. parahaemolyticus* was not isolated from the pond water but was isolated from the shrimp obtained from East Java.

4.2. Microbiology of frozen shrimp available in the market

Bacterial contamination profiles of frozen shrimp available in the market are presented in Table 4. The frozen samples contained high bacterial counts, and none of the samples met the microbiological standards for frozen shrimp issued by many agencies. The Indonesian National Standard requires that frozen shrimp should contain less than 5×10^5 CFU/g (raw) or 2×10^5 CFU/g (cooked) for total plate counts, 10 CFU/g (raw) or negative (cooked) of *E. coli*, and no *Salmonella* in 25 g of sample. ICMSF states that frozen shrimp should contain less than 10^6 CFU/g total plate counts, 400 CFU/g coliforms, 25 CFU/g *E. coli*, and 100 CFU/g *Staphylococcus*. The USA states that good frozen shrimp should contain less than 4.0×10^5 CFU/g total plate counts, while those containing 4×10^5 to 1.9×10^6 CFU/g total plate counts are categorized as fair quality.

Table 2. BACTERIAL CONTAMINATION PROFILE OF FRESH AQUACULTURE SHRIMP OBTAINED FROM JAVA

Bacteria	Log $_{10}$ CFU/g or + (present) and - (absent)							
	West Java				East Java		Central Java	
	W1	W2	W3	W4	E1	E2	C1	C2
Total Plate Count	5.3	5.9	5.0	4.8	6.2	4.9	4.5	4.7
Staphylococcus	4.5	5.3	4.6	3.5	<1	2.9	3.3	3.4
Enterobacteriaceae	5.3	5.7	4.5	4.4	<1	4.0	3.4	2.6
Total Coliform	4.2	5.2	4.5	4.2	<1	3.2	3.4	2.5
E. coli	2.3	3.4	3.7	2.3	<1	2.7	2.8	4.0
Salmonella	+*	-	+*	+*	-	-	-	-
	(1/4)**	(0/4)	(1/4)	(2/4)	(0/4)	(0/4)	(0/4)	(0/4)
V. cholerae	-	-	-	-	-	+	+	+
	(0/4)	(0/4)	(0/4)	(0/4)	(0/4)	(2/4)	(1/4)	(2/4)
V. parahaemolyticus	+	+	+	+	-	+	-	-
	(2/4)	(2/4)	(1/4)	(2/4)	(0/4)	(1/4)	(0/4)	(0/4)

*) *S. paratyphi A*

**) Number in parenthesis is the number of positive isolates per number of samples

Table 3. MICROBIOLOGICAL QUALITY OF AQUACULTURE WATER*)

Bacteria	Log $_{10}$ CFU/g or + (present) and - (absent)			
	East Java		Central Java	
	E3	E4	C3	C4
Total Plate Count	6.2	4.9	4.5	4.7
Staphylococcus	<1	2.9	3.3	3.4
Enterobacteriaceae	<1	4.0	3.4	2.6
Total Coliform	<1	3.2	3.4	2.5
E. coli	<1	2.7	2.8	4.0
Salmonella	-	-	-	-
	(0/2)	(0/2)	(0/2)	(0/2)
V. cholerae	-	+	+	+
	(0/2)**	(1/2)	(1/2)	(1/2)
V. parahaemolyticus	-	-	-	-
	(0/2)	(0/2)	(0/2)	(0/2)

*) Ponds of West Java were not evaluated

**) Number in parenthesis is the number of positive isolates per number of samples

Analysis of bacterial contamination of frozen shrimp obtained from grocery stores suggested that the number of bacteria in sample G1 to G4 shrimp were similar to those obtained from the industry. The result suggested that frozen distribution/transportation between the industry and grocery stores was well maintained. In general the number of total microorganisms varied in shrimp obtained from the five industries and four grocery stores. *V. parahaemolyticus* was isolated from shrimp from G2 and G3. Frozen shrimp from G2 was also contaminated by *V. cholerae*. None of the samples contained *Salmonella*. This result suggests that frozen shrimp in the market did not contain pathogens as frequently as the fresh ones, because the pathogens may have been reduced by processing and frozen storage.

Table 4. BACTERIAL CONTAMINATION PROFILES OF FROZEN SHRIMP PRODUCTS AVAILABLE IN THE MARKET

	Log $_{10}$ CFU/g or + (present) and - (absent)								
Bacteria	Industries					Groceries			
	I1	I2	I3	I4	I5	G1	G2	G3	G4
Total Plate Count	5.4	5.6	6.0	6.0	5.8	7.0	6.0	6.4	6.4
Staphylococcus	2.8	3.8	4.7	5.0	3.4	4.3	3.8	3.9	3.9
Enterobacteriaceae	3.0	3.0	4.2	5.2	3.5	5.3	4.8	4.4	3.4
Total Coliform	2.4	2.7	3.6	4.0	2.5	4.7	4.7	3.9	3.9
E. coli	<1	1.8	2.2	2.7	1.8	3.5	2.2	2.7	2.7
Salmonella	-	-	+	-	-	-	-	-	-
	(0/4)*	(0/4)	(1/4)	(0/4)	(0/4)	(0/4)	(0/4)	(0/4)	(0/4)
V. cholerae	-	-	-	-	-	-	+	-	-
	(0/4)	(0/4)	(0/4)	(0/4)	(0/4)	(0/4)	(2/4)	(0/4)	(0/4)
V. parahaemolyticus	-	-	-	-	+	-	+	+	-
	(0/4)	(0/4)	(0/4)	(0/4)	(0/4)	(0/4)	(1/4)	(3/4)	(0/4)

*) Number in parenthesis is the number of positive isolates per number of samples

4.3. Fate of Bacterial Contaminants in Shrimp during Frozen Shrimp Processing

This study was conducted in the processing industry which processes aquaculture shrimp for frozen shrimp processing. Typical processing line of frozen shrimp in the industry consisted of (1) receiving, (2) first rinse in 100 ppm chlorinated water, (3) head removal, (4) second rinse in 30 ppm chlorinated water, (5) sizing and grading I, (6) sizing and grading II, (7) weighing, (8) final rinse, (9) arrangement and water filling, (10) freezing, (11) glazing, (12) metal detection, (13) packaging, and (14) storage.

Analysis of shrimp at six sampling points suggested that total bacterial contamination of the raw shrimp received by the processor was similar to those found in aquaculture shrimp as shown in Table 2. After second rinse, the bacterial population decreased due to the use of chlorine. After sizing and grading, however, the bacterial counts tended to increase, probably due to the inadequate control of time spent during this manual step. The bacterial counts also

increased during shrimp arrangement and water filling into trays for freezing which may have been caused by inadequate control of water temperature and lengthy time allocated during this process. However, after freezing, the bacterial counts decreased and thus resulted in products complying with the Indonesian standard for frozen shrimp. The average bacterial count of the finished products in this study was $\log_{10}$CFU/g 5.4 or 2.5 x 10^5 which was lower than the standard of 5x10^5. Although the raw material contained *E. coli* at levels up to $\log_{10}$CFU/g 2.9, processing decreased the count significantly.

Most of the products contained *Salmonella* which was present in the receiving materials and survived subsequent processing. Only one out of the five finisheded positive-evaluated was free of *Salmonella*. *V. cholerae* was absent in sampling points. *V. parahaemolyticus* was present in three of the five finished products. This bacteria was present in the raw material and survived freezing. The number of samples positive for *Listeria* increased during processing, suggesting contamination within the industrial facilities. *Listeria* has been reported as psychrotrophic organisms and was reported to be found in the food processing plant [2]. Listeria isolates from the study were not further identified, to determine if they were pathogenic. Contamination from pathogens other than *Listeria* did not occur during processing suggesting adequate hygiene practices. Results of this study are summarized in Table 5.

Table 5. BACTERIAL CONTAMINATION PROFILES DURING FROZEN SHRIMP PROCESSING

	Log CFU/g*			Present (+) or absent (-)*			
Steps during processing	Total Plate Count	*Staphylococcus*	*E. coli*	*Salmonella***	*V.cholerae*	*V.parahaemolyticus*	*Listeria spp.*
Receiving material	5.4	2.7	2.9	+ (5/5)	- (0/4)	+ (4/5)	+ (2/5)
After head removal	5.3	2.0	2.4	+ (5/5)	- (0/4)	+ (4/5)	+ (3/5)
After sizing and grading	6.7	2.1	<2	+ (5/5)	- (0/4)	+ (4/5)	+ (3/5)
After final rinse	5.6	1.3	0-<2	+ (5/5)	- (0/4)	+ (3/5)	+ (5/5)
After arrangement and water filling	5.6	1.4	2.3	+ (5/5)	- (0/4)	+ (3/5)	+ (5/5)
After packaging	5.4	1.3	0-<2	+ (4/5)	- (0/4)	+ (3/5)	+ (3/5)

*) Number in parenthesis is the number of positive isolates per number of samples
**) Confirmation using API 20E are incomplete

5. CONCLUSIONS

Ocean shrimp from Java had a higher bacterial contamination than did aquaculture shrimp. Shrimp samples obtained in the West Java contained more bacteria and were more frequently contaminated with *Salmonella* than those obtained from East and Central Java area. Several frozen shrimp products available in the Indonesian market did not meet the standard required by the Ministry of Industry and Trade for total plate counts, but only one out of 36 samples

tested was contaminated by *Salmonella*. This differed on shrimps sampled from in-line processing in industry. Although the total plate counts, *Staphylococcal* and *E. coli* counts met the standard requirement, *Salmonella* and *V. parahaemolyticus* were detected in the finished frozen product. The pathogens originated from the raw material and steps in the processing were not adequate to reduce the incidence of the pathogens. Control of processing temperature and chlorine concentration in the rinse water needs to be improved. Implementation of Good Agricultural Practices in production of raw shrimp as well as Hazard Analysis Critical Control Point at the line processing are expected to improve the quality of fresh and frozen shrimp.

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