



**INTERNATIONAL CONFERENCE ON
FOOD SCIENCE AND TECHNOLOGY**

**THE CHALLENGE OF
UNIVERSAL FOOD QUALITY AND
SAFETY REGIME**

**THEATRE ROOM,
3RD FLOOR OF
THOMAS AQUINAS BUILDING,
SOEGIJAPRANATA
CATHOLIC UNIVERSITY,
SEMARANG, INDONESIA**

**ON:
THURSDAY AND FRIDAY,
31 JULY AND 1 AUGUST 2008**

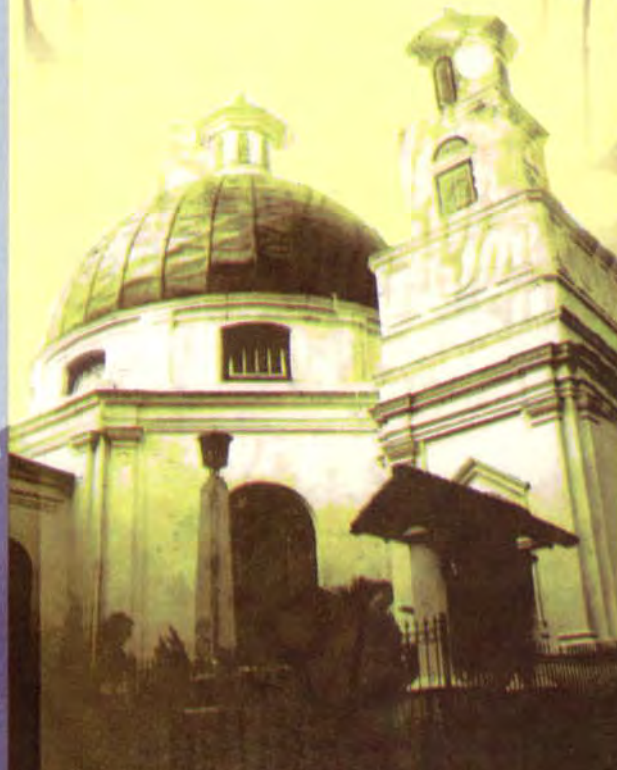
**PROCEEDING
BOOK**

ISBN : 978-979-1268-36-3



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PREFACE

Food quality and safety is nowadays not just the main concern in every stage of food chain, but is likely a new stage in food culture. Food scientist, food technologists, food industry, as well as business communities and the governmental bodies are challenged not just to care of, but to guard the new era of man-kind culture on food. For this the faculty of Agricultural Technology, Soegijapranata Catholic University invites food communities all over the world to share their ideas, research findings as well as opinions in the International Conference on Food Science and Technology, to welcome “The Challenge of Universal Food Quality and Safety Regime”.

The conference successfully gathering about 100 papers, presented by more than 7 countries in two days. This proceeding brings together these papers, organized in two presentation schemes :

A. Oral, covering of 6 topics :

1. Food Supply Chain Diversity
2. Food Processing and Engineering
3. Food Microbiology and Biotechnology
4. Food Marketing and Quality Management
5. Nutritional and Functional Food
6. Food Safety and Quality

B. Poster, followed by 34 papers

In order to response the newest trend on food quality and safety regime, the conference also organized 6 plenary presentation focusing on “The Challenge of Universal Food Quality and Safety Regime”. The organizing committee is grateful to all honorable speakers, participants and sponsors, for joining this gathering and for their valuable contribution on the conference.

Semarang, August 2008

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AN INVESTIGATION OF MICROBIOLOGICAL QUALITY OF TIGER PRAWN, COCKLE, AND SQUID FROM TRADITIONAL MARKET AND SUPERMARKET IN SURABAYA

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ABSTRACT

Prawn, cockle, and squid are marine products which contain high protein and moisture. Therefore, these commodities have high risk of growth of spoilage and pathogenic bacteria. *Salmonella* is a pathogenic bacteria which was frequently found in seafood. According to the Indonesian National Standard, microbiological quality of fresh seafood are total bacteria of 10^7 cfu/g and negative for *Salmonella*. The objective of this research is to investigate the microbiological quality of tiger prawn, cockle and squid collected from a traditional market and a supermarket in Surabaya. Samples were microbiologically tested: Standard Plate Count for enumeration of total bacteria and conventional method for *Salmonella* detection comprising of selective media Bismuth Sulphite Agar and *Salmonella-Shigella* Agar. Then, isolate of *Salmonella* was identified microscopically and biochemically. The total bacteria of tiger prawn, cockle, and squid obtained from traditional market were 6.6×10^6 ; 2.1×10^8 and 1.6×10^7 cfu/g, respectively, whereas those from supermarket were 2.6×10^5 ; 2.6×10^7 and 2.0×10^6 cfu/g, respectively. The result were significant (t test). This indicated that those products in the supermarket were microbiologically better than those in traditional market. In all samples were found *Salmonella*; they were 30 isolates in which 2 of them were belong to *Salmonella typhi*, 7 isolates were *Salmonella paratyphi* A, 5 isolates were *Salmonella enteridis*, but 16 colonies were unidentified. In conclusion, the tiger prawn, cockle, and squid were not complied with Indonesian National Standard. It is needed better handling and sanitation.

Keywords: *microbiological quality, total-bacteria, Salmonella, tiger prawn, cockle, squid, traditional-market, supermarket*

INTRODUCTION

Tiger prawn, cockle, and squid are high perishable food since its high content of protein, other nitrogenous constituents such as free amino acids; volatile nitrogen bases like ammonia and trimethylamine; creatine; taurine; betaines; uric acid; anserine; carnosine and histamine; and moisture (Jay, 2000). Those products are rapidly spoiled microbiologically and high risk of growth of pathogenic bacteria. The bacterial biota of freshly seafood, reflect the quality of waters from which those products are taken, and contamination from the deck, handlers, and washing waters.

The bacterial biota of freshly caught seafood should be expected to reflect the waters from which these products are caught, and contamination from the deck, handlers, and washing waters. The bacteria that make up the biota of seafood are *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Bacillus*, *Corynebacterium*, *Enterobacter*, *Escherichia*, *Flavobacterium*, *Lactobacillus*, *Listeria*,

Microbacterium, *Moraxella*, *Photobacterium*, *Pseudomonas*, *Psychrobacter*, *Shewanella*, *Vibrio*, *Weissella* (Jay, 2000). Some spoilage and pathogenic bacteria species commonly found in prawn are *Achromobacter*, *Alcaligenes*, *Pseudomonas*, *Micrococcus*, *Proteus*, *Escherichia coli*, *Salmonella*, *Staphylococcus aureus* and *Listeria* (Hadiwiyoto, 1993; Michalowski, 2006). Microbiological standard of fresh seafood in Indonesia are total bacteria not more than 10^7 cfu/g, Coliform not more than 4×10^2 MPN/g, *Salmonella* negative, *Staphylococcus aureus* not more than 5×10^3 cfu/g and *Vibrio cholerae* negative (The National Agency of Drug and Food Control, 1989). Among the Gram negative rods that cause foodborne gastroenteritis, the most important are members of the genus *Salmonella*.

Salmonellas are small, Gram negative, non-sporing rods, usually motile with peritrichus flagella, facultative anaerobes, biochemically characterized by their ability to ferment glucose with the production of acid and gas, and their inability to attack lactose and sucrose. Their optimum growth temperature, as with most poisoning bacteria, is about 38°C, relatively heat sensitive being killed at 60°C in 15-20 min and they fail to grow below about 7 or 8 °C. *Salmonellas* are pathogenic bacteria induce the illness (Salmonellosis) by their death following multiplication in the host's gut and their subsequent lysis with the release of potent endotoxin. This endotoxin forms part of the membrane of the cell and is primarily responsible for the clinical symptoms. Enterotoxin produced within the human intestine may well have an important role in the disease. The principle symptoms of salmonellosis are nausea, abdominal pain, drowsiness, diarrhea and a moderate fever, dehydration may occur resulting in great thirst. This organism can invade the blood stream and thus cause a septicemia and in the more extreme cases the patient may go into a coma (Forsythe and Hayes, 1998). They are widely distributed in nature, the primary habitat is intestinal track of animals such as farm animals, reptiles, human, birds and occasionally insects. Therefore meat from these sources may be contaminated with these bacteria (Jay, 2000; Forsythe and Hayes, 1998). *Salmonellas* were found in tropical seafood such as shrimp, clams, finfish, oyster (Hatha *et al.*, 2003), mollusks and also in seawater (Martinez-Urtaza *et al.*, 2004).

Tiger prawn, cockle, and squid are seafood commonly found both at traditional market and supermarket. For generations, Indonesians have bought their food at traditional markets. Traditional market is a place where seller and buyer meet for direct transaction. Prices are not marked on items at traditional markets. Therefore, the fine art of bargaining is taken to new levels as housewives and household help try to get the cheapest prices possible. Going to the traditional market is a daily activity for Indonesian women or their household help. Most traditional markets are owned by the local government. Local governments in Indonesia usually have an Office of Market Management, or Dinas Pasar, which manages traditional markets. In Surabaya, there are 81 traditional markets which owned and operated by PD Pasar Kota Surabaya (<http://www.surabaya.go.id/perdagangan.php#pasar>, 2008).

Supermarket is a self-service store offering a wide variety of food and household merchandise, organized into departments. It is larger in size and has a wider selection than a traditional grocery store and it is smaller than a hypermarket. In supermarket, seller and buyer do not meet for transaction. Prices are marked on items in supermarket. In Indonesia, supermarkets are all privately owned and their permits are issued by the central government's Department of Trade. Local governments usually have no authority to refuse permits issued by the central government, although some require supermarkets to apply for local permit (Suryadarma *et al.*, 2007).

The objective of this research is to investigate the microbiological quality of tiger prawn, cockle and squid collected from a traditional market and a supermarket in Surabaya.

MATERIALS AND METHODS

Samples

Tiger prawn, cockle and squid were collected from a traditional market and a supermarket in Surabaya. Those products were aseptically packed in sterile Polyethylene plastic bag, then brought (in cool condition) to the laboratory to be analyzed of the total bacteria and *Salmonella*.

Media and Reagents

Plate Count Agar (MERCK 1.05463), Buffer Pepton Water (MERCK 1.07228), Selenite Cystine Broth (MERCK 1.07709), Bismuth Sulphite Agar (MERCK 1.05418), *Salmonella-Shigella* Agar (MERCK 1.07667), Pepton Broth (MERCK 1.07224), Methyl Red-Voges Proskauer, Simon Citrate (MERCK 2501), Kliger Iron Agar (MERCK 3913), Semi Solid Sucrose, Lysine Iron Agar (MERCK 11640), Motility Indole Ornithine, Nutrient Agar (MERCK 1.05450), Nutrient Broth (MERCK 1.05443), Gram staining reagents (Crystal Violet, Iodine, Alcohol-Acetone, Safranin Gram stain), KOH 40%, Methyl Red, α -naphthol.

Sampling and Sample Preparation

25 g of sample were aseptically weighed and blended with 225 mL of sterile Buffer Phosphate for enumeration of total bacteria, whereas for *Salmonella* detection, 25 g of sample were aseptically weighed and blended with 225 mL of Buffer Pepton Water.

Enumeration of Total Bacteria

Aseptically weigh 25 g of sample into sterile blending container. Add 225 ml sterile Phosphate buffer and blend 5 min. Transfer homogenized mixture aseptically to sterile wide-mouth jar (500 ml). Using separate sterile pipets, prepare decimal dilutions of 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} and 10^{-8} of sample homogenate by transferring 10 ml of previous dilution to 90 ml of phosphate buffer. Pipet 1 ml of each dilution into separate, duplicate, appropriately marked petri dishes. Add 12-15 ml plate count agar (cooled to $50 \pm 1^\circ\text{C}$) to each plate within 5 min of original dilution and also for negative control plate. Mix immediately sample dilutions and agar medium thoroughly and uniformly by alternate rotation and back-and-forth motion of plates on flat level surface. Let agar solidify. Incubate promptly for 24 h at 37°C . Count the colonies which growth on the medium (Bacteriological Analytical Manual, USDA).

Salmonella Detection, Isolation and Identification

Pre-enrichment

Aseptically weigh 25 g of sample into sterile blending container. Add 225 ml sterile Buffer Peptone Water and blend 5 min. Aseptically transfer homogenized mixture to sterile wide-mouth jar (500 ml) and let stand 60 ± 5 min at room temperature with jar securely capped, then incubated 24 hours at 37°C .

Enrichment

Gently shake incubated sample, 10 mL of the sample was inoculated to 90 mL of Selenite Cystine Broth, then incubated 24 hours at 37°C .

Detection and Isolation

Gently shake incubated sample, streak 0.1 mL of enriched sample on Bismuth Sulphite Agar (BSA) and *Salmonella-Shigella* Agar (SSA), then incubate plates 24 hours at 37°C . Examine plates for presence of colonies that may be *Salmonella*. Typical *Salmonella* colonies are brown, gray, or black colonies; sometimes they have a metallic sheen. If typical colonies are present on the BSA and SSA after 24 ± 2 h incubation, then pick 1 or more colonies for examining

microscopically. Morphological of colony and microscopic characteristic were compared to the positive control cultures (Bacteriological Analytical Manual, USFDA).

Identification

All cultures that give similar characteristics should be retained as potential *Salmonella* isolates and submitted for biochemical test. Typical colony as suspected *Salmonella* was purified to obtain the pure culture prior to biochemical test. A loopful of colony was streaked on Nutrient Agar then incubated plates 24 hours at 37°C. Perform the biochemical tests on cultures suspected *Salmonella*. Biochemical tests consist of 8 reaction tests i.e. indole, Methyl Red, Voges-Proskauer, Simon Citrate, Kliger Iron Agar, Semi Solid Sucrose, Lysine Iron Agar and Motility Indole Ornithine. The biochemical test result was compared to the positive control cultures and references.

Statistical Analysis

The data of total bacteria of each sample collected from different market was analyzed by t test with α 5%.

RESULTS AND DISCUSSION

Total Bacteria of Tiger Prawn, Cockle and Squid

The total bacteria of tiger prawn, cockle and squid collected from traditional market and supermarket in Surabaya shown in Table 1. Total bacteria of those products, in exception of tiger prawn in both markets and squid in supermarket, were higher than total bacteria of Indonesian National Standard, which was 7.00 log₁₀ cfu/g. In a study of 597 of fresh seafood collected from retail stores the aerobic plate count from 4.89 to 8.43 log₁₀ cfu/g (Jay, 2000).

Table 1. Total Bacteria of Tiger Prawn, Cockle and Squid Collected from Traditional Market and Supermarket in Surabaya

Sample	Total Bacteria (log ₁₀ cfu/g)	
	Traditional Market	Supermarket
Tiger Prawn	6.82	5.41
Cockle	8.32	7.41
Squid	7.20	6.30

Total bacteria of cockle, in both markets, were higher than of squid and of tiger prawn. The chief differences of total bacteria in those products are the quality of the waters from which those products are caught, their chemical composition, the way which they are handled, the quality of washing water and other factors. Molluscan shellfish (cockle and squid) are generally differs in their chemical composition from crustacean shellfish (tiger prawn) in having a significant content of carbohydrate material and quantity of nitrogen in their flesh. The carbohydrate is largely in the form of glycogen. Molluscan meats contain high levels of nitrogen bases particularly free arginine, aspartic, and glutamic acid, as much as do other shellfish. Liew *et al* (1998) found 4.89 log cfu/g of *Vibrio* spp. in non-heat-treated cockles and the predominant species were *V. parahaemolyticus* and *V. alginolyticus*. Squid and *Vibrio fischeri* have unique cooperative association. The squid has developed a specialized light-emitting organ to house the bacteria within animal's mantel cavity (McCann *et al.*, 2003).

Total bacteria of each sample in traditional market were differ significantly from those in supermarket (t test), indicated that those products in supermarket were microbiologically better than those in traditional market. Generally, control of the quality of foods supplied to the traditional markets were worse than in the supermarkets since supermarkets are managed professionally by private. Quality of washing water; hygiene and temperature of room and or facilities where the

product storage and display; hygiene of employees were affected on the total bacteria of the products.

***Salmonella* Isolation and Identification of Tiger Prawn, Cockle and Squid**

Colonies suspect *Salmonella* were found on Tiger prawn, cockle and squid collected from both markets. The colonies were isolated on both selective media i.e. Bismuth Sulphite Agar and *Salmonella-Shigella* Agar. Each of colonies then was coded like shown in Table 2.

Table 2. Colonies Suspect *Salmonella* Isolated from Tiger Prawn, Cockle and Squid in Traditional Market and Supermarket

Sample	Traditional Market	Supermarket
Tiger Prawn	UTS1	USS1
	UTS2	USS2
	UTS3	USB1
	UTS4	USB2
	UTB1	
	UTB2	
Cockle	KTS1	KSS1
	KTS2	KSS2
	KTB1	KSS3
	KTB2	KSB1
		KSB2
Squid	CTS1	CSS1
	CTS2	CSS2
	CTS3	CSS3
	CTS4	CSB1
	CTB1	CSB2
	CTB2	

Note: Code U=Tiger Prawn; K=Cockle; C=Squid; T=Traditional Market; S=Supermarket; S=*Salmonella-Shigella* Agar; B=Bismuth Sulphite Agar; UTS1=colony suspected *Salmonella* isolated from Tiger Prawn of Traditional Market using *Salmonella-Shigella* Agar, number 1.

All samples were contaminated by *Salmonella* that indicated those products were not in complied with the microbiological quality of Indonesian National Standard, which is negative for *Salmonella*. The source of *Salmonella* contaminated those products might be were waters where the products taken and or washing water. Other researchers also found *Salmonella* in 43% of 60 clam samples, 0.1% of 1264 frozen tiger prawn samples (Fraiser and Koburger, 1984; Hatha *et al.*, 2003).

Thirty isolates of *Salmonella* from the samples then were identified by biochemical test. Two isolates were classified to *Salmonella typhi*, 7 isolates were *Salmonella paratyphi* A, 5 isolates were *Salmonella enteridis*, but 16 colonies were unidentified. The identification results of all isolates were shown in Table 3.

Table 3. Identification of *Salmonella* Isolated from Tiger Prawn, Cockle and Squid in Traditional Market and Supermarket

Colony Code	Result of Identification*
UTS1	Unidentified
UTS2	Classified to <i>Salmonella typhi</i>
UTS3	Classified to <i>Salmonella paratyphi</i> A
UTS4	Unidentified

UTB1	Classified to <i>Salmonella enteridis</i>
UTB2	Unidentified
USS1	Classified to <i>Salmonella typhi</i>
USS2	Unidentified
USB1	Classified to <i>Salmonella enteridis</i>
USB2	Classified to <i>Salmonella enteridis</i>
KTS1	Classified to <i>Salmonella paratyphi A</i>
KTS2	Unidentified
KTB1	Unidentified
KTB2	Unidentified
KSS1	Classified to <i>Salmonella paratyphi A</i>
KSS2	Classified to <i>Salmonella paratyphi A</i>
KSS3	Unidentified
KSB1	Unidentified
KSB2	Unidentified
CTS1	Unidentified
CTS2	Classified to <i>Salmonella paratyphi A</i>
CTS3	Unidentified
CTS4	Classified to <i>Salmonella enteridis</i>
CTB1	Unidentified
CTB2	Classified to <i>Salmonella enteridis</i>
CSS1	Unidentified
CSS2	Unidentified
CSS3	Unidentified
CSB1	Classified to <i>Salmonella paratyphi A</i>
CSB2	Unidentified

Other reports showed that the most frequently *Salmonella* isolated from cockle were classified to *Salmonella seftenberg*, *Salmonella typhimurium*, *Salmonella agona* (FDA, 2005), and *Salmonella typhi* (Trihendrokesowo *et al.*, 1989). Katsuno *et al* (2005) found that squid contaminated which was caused outbreak were contaminated by *Salmonella oranienburg*. Hatha *et al* (2003) also found that 846 samples of prawn were contaminated by *Salmonella typhimurium*.

CONCLUSIONS

The total bacteria of cockle, in both traditional market and supermarket, were higher than of squid and of tiger prawn. Total bacteria of those products obtained from traditional market were higher than those from supermarket. This indicated that those products in the supermarket were microbiologically better than those in traditional market. In all samples were found *Salmonella*; they were 30 isolates in which 2 of them were belong to *Salmonella typhi*, 7 isolates were *Salmonella paratyphi A*, 5 isolates were *Salmonella enteridis*, but 16 colonies were unidentified. In conclusion, the tiger prawn, cockle and squid were not complied with Indonesian National Standard.

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