

**A preliminary study on microbiological and chemical analysis in shrimp species (*Penaeus monodon*) (black tiger) for export**

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The aim of this study was to perform microbiological analysis and chemical analysis for the quality assurance of different shrimp species for export. Food safety and quality play an important role for consumers. As frozen shrimp is the most promising sea food product exported by Myanmar a preliminary study on the microbiological analysis was carried out on (*Penaeus monodon*) (black tiger) according to the International Commission of Microbiological Specification (ICMSF) for food. Total plate count for *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Salmonella*, *Shigella*, *Escherichia coli*, *Staphylococcus aureus* was determined. Simultaneously, coliforms and faecal coliforms were also determined. Sixteen amino acids in protein, total amino acids and free amino acids in *P. monodon* (black tiger) were measured by reverse phase high performance liquid chromatography (HPLC), Model 10 AXL spectrofluometric detector (Shimadzu, Duisburg, Germany) using ortho-phthaldialdehyde (OPA) precolumn derivatization method. Determination of some heavy metals was carried out by atomic absorption spectrophotometer (AAS). It was found that total plate counts and coliform counts were reduced from  $10^6$  CFU/g to  $10^3$  CFU/g in shrimp species and faecal coliform, *E. coli*, *S. aureus*, *Salmonella*, *Shigella*, *V. cholerae* and *V. parahaemolyticus* were not found in all tested samples. All 16 amino acids were found to be present in shrimp samples with high concentrations of glycine, arginine, alanine and lysine. The heavy metals such as arsenic, mercury, copper, iron, phosphorus, and zinc were within the acceptable range.

## INTRODUCTION

Food safety and quality are of crucial importance to prevent the health of consumers as well as to fulfill the health sanitary requirement. Myanmar produced and exported 19477 metric tons of shrimp in 2001, earning US dollar 104.23 million. Among many bacterial organisms, *Vibrio* species are commonly found in Vietnam, Indonesia, Bangladesh and Japan. Determination of some heavy metals in shrimp for export is also important for human health [1]. Due to the habitat and different environmental status, the quality status of prawns need to be elucidated.

The aims of this study were to establish modern standardized methods with regard to the quality assurance of prawns and shrimps and to assist in controlling of food safety.

## MATERIALS AND METHODS

### *Samples*

Local shrimp species were collected from the Ministry of Livestock and Fisheries and from various export companies. Each of the 50 samples for export were examined according to the International Commission of Microbiological Specification for Food (ICMSF) for microbiological analysis and for determination of chemical constituents [2].

### Total plate count and microbiological analysis

Ten grams of frozen whole shrimp samples were weighed in sterile stomacher bags and macerated with 90 ml of peptone. After serial dilutions, the total plate counts were determined. Pure colonies were identified by biochemical tests [3, 4].

### Chemical analysis

#### Determination of aminoacids

It was done using the HPLC (Model LC10, Shimadzu). Samples were separated on an OPA-3 Reverse phase column (Grom, Herrenberg, Germany) equipped with gradient controller (Model RF 10A, Shimadzu, Japan). The total hydrolysis of the products was done at 220nm wavelength and Orthophthaldialdehyde (OPA) precolumn derivatization were detected by a spectrofluometer (Model RF 10AXL) [5-8].

#### Determination of heavy metals

Determination of some heavy metals was carried out by atomic absorption spectrophotometry (AAS) using the Perkin Elmer AA analyst 800 (USA) and GBC 932AA analyst (Australia) .

## RESULTS AND DISCUSSION

In this study, it was found that the total plate count and coliform count of the export samples were reduced from  $10^6$  CFU/g to  $10^3$  CFU/g when water and ice were used for washing and preservation compared to the raw samples of shrimps (Table 1, 2).

Table 1. Total bacterial count from *P. monodon* (Black Tiger), (headless and peeled and devined )

Bacterial count (per gram)	<i>P. monodon</i> (Black Tiger) n=50		Washing & preservation n=50		
	Headless %	Peeled & devined %	Bacterial count CFU/sq cm	Water %	Ice %
$10^3$	86	76	1-20	80	78
$10^4$	8	14	21-100	14	20
$10^5$	4	8	101-500	6	4
$10^6$	2	2			

*Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, *Shigella*, *Vibrio cholerae* and *Vibrio parahaemolyticus* were not isolated in all tested samples.

Table 2. Distribution of coliforms from *P. monodon* (Black Tiger)

Colliform count (MPN/g)	<i>P. monodon</i> (Black Tiger) (n=50)	
	Headless %	Peeled & devined %
<3	70	66
3-8	24	22
9-24	6	12

Table 3. Concentration of amino acids ( $\mu\text{mol/g}$ ) in *P. monodon* (Black Tiger) from Sittway region

Sr No.	Amino acid	Total amino acid	Free amino acid (protein)	Bound amino acid (literature value)	Total amino acid (Reference value)
1	Aspartic acid	128.06 $\pm$ 10.0	74.16 $\pm$ 8.50	35.41 $\pm$ 7.00	129-163*
2	Glutamic acid	169.50 $\pm$ 9.00	101.41 $\pm$ 7.90	56.50 $\pm$ 6.20	170-199**
3	Serine	55.39 $\pm$ 3.20	39.40 $\pm$ 2.80	14.91 $\pm$ 1.00	64-79*
4	Histidine	27.23 $\pm$ 2.50	16.94 $\pm$ 2.10	9.19 $\pm$ 3.00	19.3-77*
5	Glycine	571.99 $\pm$ 1.16	534.66 $\pm$ 1.21	37.94 $\pm$ 2.00	139-152**
6	Theonine	75.03 $\pm$ 4.00	61.65 $\pm$ 1.50	12.37 $\pm$ 1.10	56-74**
7	Arginine	195.50 $\pm$ 3.10	181.20 $\pm$ 2.10	15.20 $\pm$ 1.26	64.7-95.7*
8	Alanine	179.65 $\pm$ 2.20	143.05 $\pm$ 2.30	29.95 $\pm$ 1.51	107-123**
9	Tyrosine	35.46 $\pm$ 2.50	25.56 $\pm$ 1.70	9.81 $\pm$ 2.05	5-32**
10	Valine	70.05 $\pm$ 1.12	40.38 $\pm$ 1.10	25.60 $\pm$ 2.01	64-92*
11	Methionine	47.64 $\pm$ 1.35	16.43 $\pm$ 1.40	30.89 $\pm$ 3.30	31-94*
12	Tryptophan	18.62 $\pm$ 2.80	14.71 $\pm$ 1.70	3.57 $\pm$ 4.00	8-14**
13	Phenylalanine	38.60 $\pm$ 3.10	22.56 $\pm$ 2.90	15.08 $\pm$ 2.20	39-45*
14	Isoleucine	40.16 $\pm$ 3.20	19.60 $\pm$ 3.00	20.91 $\pm$ 3.10	52.5-80**
15	Leucine	80.79 $\pm$ 1.70	32.50 $\pm$ 3.10	48.20 $\pm$ 2.10	104-115.5*
16	Lysine	145.90 $\pm$ 1.90	80.63 $\pm$ 4.10	62.29 $\pm$ 3.50	86-150*

Method: OPG precolumn derivatization with HPLC

\*Reference No.1

\*\*Reference No.10

*Salmonella* contamination of frozen shrimp is of great concern, but in this study *Salmonella* and *Shigella* were not detected in water and ice used for washing and preservation. It was found that the bacteria load of whole shrimp (shell on) was higher compared to the headless shrimp.

Similarly, headless shrimp samples had higher bacteria load than the peeled and undevined shrimp. Thus, the bacterial load of processed shrimp was dependent on the method of processing as well as on environmental factors of the processing plant, especially, the quality of water and ice used during the processing steps.

In this study, the adaptation of appropriate sanitation and hygienic principles during the shrimp processing could significantly improve the microbiological quality of the frozen shrimps. All 16 amino acids were found to be present in shrimp sample with high concentrations of glycine, arginine, alanine and lysine in the marine species (*P. monodon*, Black Tiger). Among them, glycine and arginine were the major contributors to the total free amino acid.

It was found that the more the free amino acid, the less the protein bound amino acid are present in shrimp samples (Table 1, 2). As shrimp comes from aquatic environment, it may be contaminated with metals from the water where they live [9].

In this study, it was found that the content of arsenic was within the range of (1.5-0.5) ppm and mercury (0.004-0.3) ppm, copper (0.4-0.6) ppm, iron (0.34-5.0) ppm, phosphorus (0.3-0.6) ppm, zinc (0.1-0.5) ppm respectively when analysed with the atomic absorption spectrophotometer (Perkin Elmer AA analyst 800 and GBC 932AA analyst, Australia). Thus, the concentration of these elements was within the acceptable range of internationally recommended standard reference values (Table 3, 4).

Table 4. Concentration of amino acids ( $\mu\text{mol/g}$ ) in *P. monodon* (Black Tiger) from Delta region

SrNo	Amino acid	Total amino acid	Free amino acid (protein)	Bound amino acid (literature value)	Total amino acid (Reference value)
1	Aspartic acid	145.93 $\pm 8.50$	105.56 $\pm 7.10$	40.371 $\pm 6.00$	129-163*
2	Glutamic acid	169.79 $\pm 7.90$	112.76 $\pm 6.20$	56.99 $\pm 5.50$	170-199**
3	Serine	66.59 $\pm 1.30$	48.64 $\pm 1.20$	7.951 $\pm 1.50$	64-79*
4	Histidine	23.29 $\pm 1.70$	15.43 $\pm 2.20$	7.81 $\pm 2.00$	19.3-77*
5	Glycine	494.75 $\pm 1.60$	451.73 $\pm 1.50$	34.02 $\pm 1.20$	139-152**
6	Theonine	68.19 $\pm 2.10$	56.95 $\pm 2.00$	11.24 $\pm 1.50$	56-74**
7	Arginine	135.85 $\pm 2.20$	125.29 $\pm 1.50$	10.55 $\pm 1.10$	64.7-95.7*
8	Alanine	150.10 $\pm 3.00$	115.01 $\pm 2.20$	25.12 $\pm 2.00$	107-123**
9	Tyrosine	33.77 $\pm 1.20$	24.33 $\pm 1.50$	10.44 $\pm 1.90$	5-32**
10	Valine	67.71 $\pm 1.80$	42.95 $\pm 3.00$	24.77 $\pm 2.00$	64-92*
11	Methionine	39.42 $\pm 1.60$	13.86 $\pm 2.20$	25.66 $\pm 1.20$	31-94*
12	Tryptophan	16.61 $\pm 2.00$	13.40 $\pm 1.60$	3.19 $\pm 1.00$	8-14**
13	Phenylalanine	41.92 $\pm 1.10$	25.55 $\pm 2.00$	16.39 $\pm 1.10$	39-45*
14	Iso-leucine	48.48 $\pm 1.50$	22.95 $\pm 1.30$	25.23 $\pm 1.60$	52.5-80**
15	Leucine	92.89 $\pm 1.20$	38.55 $\pm 1.20$	54.64 $\pm 1.40$	104-115.5*
16	Lysine	145.90 $\pm 2.00$	85.20 $\pm 2.10$	63.48 $\pm 2.00$	86-150*

Method: OPG precolumn derivatization with HPLC

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