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MICROBIAL OCCURRENCE AT DIFFERENT FREEZING DURATION IN SPECKLED SHRIMP, *METAPENAEUS MONOCEROS* (FABRICIUS, 1798) FROM LOCAL MARKETS OF DHAKA CITY

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Abstract

A study was carried out at four different (0, 1, 7 and 30 days) freezing durations in regard to the microbiological incidence in speckled shrimp (*Metapenaeus monoceros*) from three different local markets - Ananda bazaar, New Market and Polashi bazaar of Dhaka city. The study unveiled that Ananda bazaar samples were more or less contaminated than those of others. The microbial load varied with market sources as total bacteria count (TBC), total coliform count (TCC) and faecal coliform count (FCC) were found in all the storage periods crossing the acceptable limit. Except for *E. coli* (EC), all the bacterial counts were significantly different ($p < 0.05$) in 7 and 30 days freezing durations. A significant difference ($p < 0.05$) was found in all freezing durations only for EC counts. After 30 days storage duration for all counts showed 10^1 cfu/g reduction of microbial load from every initial count. The *Salmonella-Shigella* (SS) and *Vibrio* spp. were identified in all the samples of the three markets. In case of biochemical composition, an inverse relation was found in moisture - protein and moisture - fat where the moisture content increased after 30 days freezing duration but protein and lipid contents decreased with time. This study revealed that shrimp available in local markets of Dhaka city was highly ingested with pathogenic bacteria which indicate the unhygienic condition of the market premises.

Introduction

Bangladesh is an agro-based riverine country where fisheries sub-sector contributes about 63% in domestic animal protein consumption(1). But fish food safety concerning with animal proteins derived from fish and fishery products creates a high risk commodity regarding pathogenic/microbial contaminations, adulterants and toxicity, infirmity and death(2). Besides, bacteriological quality is directly related to the spoilage of fish and becomes the cause of outbreak of food poisoning(3). On the other hand, seafood is also known to have been responsible for a significant percentage of foodborne

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diseases[4]. Besides, the changing life style pattern has an impact on market demand since consumers accept products in terms of both quality and safety. So food safety is a burning question for the consumers all around the world.

Shrimps, exported principally to USA, Japan and Europe from Bangladesh[1], is threatened for low quality of processed shrimp products due to small size, bacterial load or presence of chemical residue levels[5]. Raw or frozen shrimp are deteriorated by improper handling and responsible for causing foodborne diseases[6] and help in diseases transmission. Fishes are very much susceptible to be contaminated with different bacteria by their environment[7] where contamination chance is much higher than on board. It results a series of complicated changes in the dead fish mainly by enzymes and bacteria that start spoilage in the fish as soon as they are harvested[8]. Moreover, there is a great chance for fish to be contaminated during transportation from the catch points to the markets[4]. So, the bacterial flora in fish might reflect the hygienic condition of market and potential indicator of contamination[9]. Low quality frozen shrimps and fish are related to improper processing and poor hygienic conditions, may lose their exportability due to the presence of harmful types of bacteria[2,3]. Therefore, quality of exportable frozen shrimps is of major concern to fish processors and public health authorities.

The objective of this study was to estimate the harmful microbial load as well as nutritional quality in locally available speckled shrimp in Dhaka city under three storage durations with a view to assuring the safety and to provide future approaches for quality of marketed shrimps and create awareness among the customers.

Materials and Methods

Sample collection and identification: Fresh speckled shrimp (*Metapenaeus monoceros*, locally known as horina chingri) samples were collected from three different local retail markets - Ananda bazaar, New Market and Polashi bazaar of Dhaka city in between January to July, 2011. The fish samples were collected by special sterile ziplock bags to avoid further contamination and transported in an insulated box[10] to the laboratory. Fish identification was done according to Shafi and Quddus[11].

Sample preparation: The samples were processed and used within 2 hours of collection. The whole fish body was considered and thawed at room temperature to assess the microbial load following the method of APHA[12]. The samples (25 g) were separately homogenized with normal saline solution and prepared using 1 - 0.1 ml of the treated samples.

Microbial analysis: Spread plate method was used to enumerate total bacterial count (TBC) using nutrient agar (NA) medium and serially diluted samples were pour-plated on MacConkey and membrane faecal coliform (mFC) agar for total coliform count (TC) and faecal coliform count (FC), respectively[13]. The plates were incubated for 24 hrs at 35 - 37^{0}C for TBC and at 44.5^{0}C for 24 hrs for TC and FC. Further confirmation was done by

growing in eosin methylene blue (EMB) agar and calculated as cfu/g. A loopful of growth from the broth was streaked on *Salmonella-Shigella* agar (SSA), incubated for 48 hrs at 35 - 37^{0}C. Xylose lysine deoxycholate (XLD) agar also used for the selective isolation of *Shigella* and *Salmonella* spp. Diluted homogenates were pour-plated on thiosulphate citrate bile sucrose (TCBS) agar to enumerate *Vibrio* spp. All cultured pathogens were identified by biochemical tests.

Biochemical tests: Biochemical tests were done according to the manual for general bacteriology of the American Society of Microbiology[14]. Oxidase, catalase, carbohydrate fermentation/utilization, Kligler's iron agar (KIA), indole production, methyl red (MR), Voges-Proskauer (VP), citrate utilization, nitrate reduction, motility indole urea (MIU), and salt tolerance (3, 5, 10 and 15% NaCl) tests were done to identify the bacteria[15].

Proximate composition analysis: Proximate analysis such as moisture, protein, lipid and ash were carried out following the methods of AOAC[16].

Statistical analysis: Statistical analysis was performed with the SPSS software package (version 11.5) following Tukey's HSD post hoc for the multiple comparisons.

Results and Discussion

The results of microbial quality analysis conducted at four different freezing durations (0, 1 7, 30 days) of *M. monoceros* samples collected from three different local markets of Dhaka city shown in Table 1. The results confirm that microbial load was more or less higher in Ananda bazaar samples than the others.

Total bacterial count: The highest TBC ($6.5 \pm 0.12 \times 10^7$ cfu/g) was found in normal condition (0 day) of Ananda bazaar sample and the lowest load ($5.3 \pm 0.15 \times 10^6$ cfu/g) was recorded after 30 days storage in New Market sample (Table 1). No significant difference ($p < 0.05$) was found in TBC of the samples between 0 day and 1 day storage for all local markets. But the TBC were significantly different from each other in 1, 7 and 30 days freezing duration, where 30 days storage showed 10^1 cfu/g reduction of bacterial count from initial stage.

In this study, the TBC in speckled shrimp in all storage durations was found to cross the acceptable limit (10^5 cfu/g) recommended by International Commission on Microbiological Specifications for Foods (ICMSF)[17]. The present findings are supported by several scientists' workings on marketed shrimp. Nilla *et al*.[2,5] recorded TBC ranged between $4.2 \pm 0.45 \times 10^6$ and $1.3 \pm 0.25 \times 10^8$ cfu/g in Indian white shrimp (*Penaeus indicus*) from local markets of Dhaka city and in case of culture source, the highest TBC was found to be $5.83 \pm 0.12 \times 10^7$ cfu/g in aquacultured *P. indicus* from Satkhira[6]. Nayem *et al*.[18] estimated total viable bacterial count (TVBC) to vary between 6.4×10^4 and 7.0×10^6 cfu/g in case of freshwater giant prawn (*Macrobrachium rosenbergii*) sold in local markets. On the contrary, Yousuf *et al*.[19] measured TBC to be 1.2×10^3 cfu/g in black tiger

shrimp (*P. monodon*) and 4.5 × 10^3 cfu/g in *M. rosenbergii* from local market which were within the acceptable limit.

As the whole shrimp was considered for microbial analysis, it might be the reason for higher TBC occurrence[2]. Rather the bacteriological quality of Ananda bazaar shrimp was found comparatively poor than that of other local markets; this might be because of harmful microorganisms contamination from the source of water, poor hygiene and sanitation condition of the market premises and also from catching vessels[2,3,20]. In addition, most of the microbes can multiply within short time, when favorable condition comes back[21]. Moreover, the bacterial load seems to be high due to the reduced immunity of shrimps as the shrimp farms in Bangladesh were recently attacked by virus diseases[2].

Total coliform count: Table 1 represents that the highest TC load was 4.4 ± 0.24 × 10^4 cfu/g in Ananda bazaar sample at normal condition (day 0) and the lowest load was 3.1 ± 0.22 × 10^3 cfu/g in Polashi bazaar sample after 30 days freezing storage. A significant difference ($p < 0.05$) was found between 7 and 30 days freezing duration for all market sources except day 0 and day 1, where 30 days storage showed 10^1 cfu/g reduction of TC count from initial day.

In all the market samples the TC was found exceeding the ICMSF[17] suggested limit (10^2 cfu/g) and revealed the poor condition of the shrimp supplied in the markets. In different studies, Nilla *et al*.[2,5] reported highest TC in *P. indicus* from Dhaka city retail markets and shrimp farms of Khulna[6] exceeding the acceptable limit. Similar results were found by Nayem *et al*.[18] for *M. rosenbergii* sold in local markets and Yousuf *et al*.[19] for *P. monodon* and *M. rosenbergii* collected from local markets. The presence of TC in shrimp samples indicates the holding temperature[2] as well as contamination during handling processing and selling in the markets. It is reported that sewage contagion in the market as a contamination source of fish[2,3]. The water that is used for washing or icing of shrimp in the landing center and markets may also act as the source of contamination[22].

Faecal coliform count and E. coli: The highest FC and EC were found in Ananda bazaar sample at 0 day as 2.6 ± 0.10 × 10^4 and 7.1 ± 0.12 × 10^4 cfu/g, respectively whereas the lowest FC and EC were found, respectively 1.5 ± 0.12 × 10^2 and 2.3 ± 0.28 × 10^2 cfu/g in Polashi bazaar sample after 30 days freezing (Table 1). No significant difference ($p < 0.05$) was found in FC of the samples between day 0 and day 1 storage for all the markets whereas in 7 and 30 days freezing duration, they were significantly different from each other. The EC count for all the markets showed a significant difference ($p < 0.05$) among the storage durations. For both counts, 30 days storage showed 10^1 cfu/g reduction from initial stage (Table 1).

Table 1. Microbial density (mean ± SEM) of total bacteria, total coliform count, fecal coliform and *E. coli*, *Salmonella-Shigella* and *Vibrio* spp. detected at different freezing duration in *Metapenaeus monoceros* sampled from different local markets of Dhaka city.

Storage Duration	Density (CFU/g)					
	TBC	TCC	FC	EC	SS	*Vibrio* spp.
Ananda bazar						
0 day	$6.5 \pm 0.12 \times 10^{7a}$	$4.4 \pm 0.24 \times 10^{4a}$	$2.6 \pm 0.10 \times 10^{4a}$	$7.1 \pm 0.12 \times 10^{4a}$	$4.6 \pm 0.17 \times 10^{4a}$	$7.8 \pm 0.07 \times 10^{4a}$
1 "	$6.3 \pm 0.14 \times 10^{7a}$	$4.0 \pm 0.13 \times 10^{4a}$	$1.9 \pm 0.15 \times 10^{4ab}$	$6.3 \pm 0.17 \times 10^{4b}$	$4.3 \pm 0.19 \times 10^{4ab}$	$7.6 \pm 0.09 \times 10^{4a}$
7 "	$5.4 \pm 0.09 \times 10^{7b}$	$3.2 \pm 0.12 \times 10^{4b}$	$1.7 \pm 0.13 \times 10^{4b}$	$5.6 \pm 0.15 \times 10^{4c}$	$4.1 \pm 0.11 \times 10^{4b}$	$6.5 \pm 0.09 \times 10^{4b}$
30 "	$5.7 \pm 0.28 \times 10^{6c}$	$3.4 \pm 0.34 \times 10^{3c}$	$1.5 \pm 0.14 \times 10^{3c}$	$6.2 \pm 0.20 \times 10^{3d}$	$4.1 \pm 0.10 \times 10^{3c}$	$6.2 \pm 0.20 \times 10^{3c}$
New market						
0 day	$7.1 \pm 0.09 \times 10^{7a}$	$4.0 \pm 0.28 \times 10^{4a}$	$2.1 \pm 0.23 \times 10^{3a}$	$7.1 \pm 0.17 \times 10^{3a}$	$4.7 \pm 0.23 \times 10^{4a}$	$6.7 \pm 0.12 \times 10^{4a}$
1 "	$6.8 \pm 0.14 \times 10^{7a}$	$3.5 \pm 0.32 \times 10^{4ab}$	$1.6 \pm 0.17 \times 10^{3ab}$	$6.2 \pm 0.15 \times 10^{3b}$	$4.4 \pm 0.22 \times 10^{4a}$	$6.5 \pm 0.17 \times 10^{4a}$
7 "	$6.0 \pm 0.12 \times 10^{7b}$	$3.1 \pm 0.45 \times 10^{4b}$	$1.2 \pm 0.07 \times 10^{3b}$	$5.3 \pm 0.13 \times 10^{3c}$	$3.6 \pm 0.22 \times 10^{4b}$	$5.6 \pm 0.21 \times 10^{4b}$
30 "	$5.3 \pm 0.15 \times 10^{6c}$	$3.3 \pm 0.37 \times 10^{3c}$	$1.7 \pm 0.05 \times 10^{2c}$	$6.1 \pm 0.21 \times 10^{2d}$	$2.7 \pm 0.19 \times 10^{3c}$	$5.3 \pm 0.27 \times 10^{3c}$
Polashi bazar						
0 day	$6.7 \pm 0.11 \times 10^{7a}$	$4.2 \pm 0.14 \times 10^{4a}$	$2.4 \pm 0.17 \times 10^{3a}$	$2.4 \pm 0.30 \times 10^{3a}$	$7.5 \pm 0.20 \times 10^{3a}$	$6.1 \pm 0.28 \times 10^{4a}$
1 "	$6.5 \pm 0.17 \times 10^{7a}$	$3.8 \pm 0.23 \times 10^{4a}$	$2.2 \pm 0.05 \times 10^{3a}$	$1.9 \pm 0.06 \times 10^{3b}$	$7.2 \pm 0.19 \times 10^{3a}$	$5.8 \pm 0.31 \times 10^{4a}$
7 "	$5.7 \pm 0.18 \times 10^{7b}$	$2.4 \pm 0.30 \times 10^{4b}$	$1.5 \pm 0.05 \times 10^{3b}$	$1.1 \pm 0.05 \times 10^{3c}$	$5.9 \pm 0.22 \times 10^{3b}$	$5.0 \pm 0.36 \times 10^{4b}$
30 "	$5.9 \pm 0.18 \times 10^{6c}$	$3.1 \pm 0.22 \times 10^{3c}$	$1.5 \pm 0.12 \times 10^{2c}$	$2.3 \pm 0.28 \times 10^{2d}$	$6.2 \pm 0.38 \times 10^{2c}$	$5.8 \pm 0.37 \times 10^{3c}$

Values within a column with same superscript letters are not significantly different (ANOVA, HSD, $p < 0.05$).

Table 2. Biochemical characteristics of the selected strains of *Metapenaeus monoceros* sampled from different local markets of Dhaka city.

Isolate No.	Medium	Biochemical characterization													Name of the Genera
		TSI			Citrate	MIU		Indole (PW)	The methyl red and Voges-Proskauer tests	Oxidase	Catalase	Nitrate	Glucose	Lactose	Sucrose	
		Slant	Butt	H_2S		Motility	Urea									
1	XLD	Alkaline	Acid	+	+	+	-	-	-	-	-	+	Acid -gas	-	-	*Salmonella*
2	XLD	"	"	_	+	-	-	-	-	-	+	+	Acid, no gas	-	-	*Shigella* spp.
3	SSA	"	"	+	+	-	-	-	-	-	-	+	Acid -gas	-	-	*Salmonella* spp.
4	MAC	Acid	"	-	+	+	+	-	+	-	-	+	Acid -gas	+	+	*Klebsiella* spp.
5	MAC	Alkaline	"	-	-	+	-	+	+	-	+	+	Acid -gas	-	-	*E. coli*
6	EMB	"	"	-	-	+	-	+	+	-	+	+	Acid -gas	-	-	*E. coli*
7	EMB	Acid	"	-	+	+	+	-	+	-	-	+	Acid -gas	+	+	*Enterobacter*
8	mFC	"	"	-	-	+	-	+	+	-	+	+	Acid -gas	+	-	*E. coli*
9	TCBS	Alkaline	Alkaline	-	-	+	-	-	-	+	+	+	Acid -gas	-	+	*Vibrio* spp.

In similar work, Nilla *et al*.[2,5] reported highest FC and EC in *P. indicus* from Dhaka city local markets and shrimp farms of Khulna[6] exceeding the acceptable limit. Nayem *et al*.[18] estimated FC in 42% samples of *M. rosenbergii* sold in local markets. Since the bacteria are found in high concentrations, the water could be linked to human sewage contamination[2,3]. Center for Disease Control and Prevention (CDC)[23] proved that FC is as more accurate indication of animal or human waste than the TC because the FC origins are more specific than that of the TC.

Since, all of the speckled shrimp samples were exceeding the limit 1.0×10^2 cfu/g according to ICMSF[17], further identification was carried out to investigate the presence of other harmful and pathogenic microorganisms.

Salmonella-Shigella: The SS was identified in all samples of three markets ranged between 10^2 and 10^4 cfu/g for shrimps also exceed ICMSF[17] suggested acceptable limit 1.0×10^2 cfu/g. The highest ($4.6 \pm 0.17 \times 10^4$ cfu/g) and the lowest intensity ($6.2 \pm 0.38 \times 10^2$ cfu/g) of SS were found in New Market and Polashi bazaar sample, respectively (Table 1). A significant difference ($p < 0.05$) was found between 7 and 30 days freezing duration for all markets, where 30 days storage showed 10^1 cfu/g reduction from initial day. Table 2 shows the brief colony characteristics of all the enteric bacteria identified including their biochemical properties.

Nilla *et al*.[2,5] found SS abundance in 67% of studied local marketed *P. indicus* from Dhaka city exceeding the acceptable limit whereas in case of culture source, highest $2.70 \pm 0.10 \times 10^4$ cfu/g was recorded in farmed shrimp samples from Khulna[6]. Besides, Nayem *et al*.[18] estimated SS in 50% of *M. rosenbergii* from local markets. Yousuf *et al*.[19] found SS 0.2×10^2 cfu/g in *P. monodon* and 1.2×10^3 cfu/g in *M. rosenbergii* from local market.

The marketed shrimp may contain frequently the SS as they come directly from the shrimp farms. The SS in marketed shrimp mainly initiate from the environment rather than from poor standards of hygiene and sanitation[2,3] though external contamination may also act as source of these bacteria in shrimp[24]. Future outbreak of pathogenic diseases through cross-contamination via kitchen utensils or by handling[24] might be by unwashed hands of infected food handlers[2,3].

Vibrio spp.: All the samples from three local markets were found containing *Vibrio* spp. which could be a cause of infection to the consumers. The highest load was found in Ananda bazaar sample ($7.8 \pm 0.07 \times 10^4$ cfu/g) at day 0 and the lowest density $5.3 \pm 0.27 \times 10^3$ cfu/g in New Market samples after 30 days freezing duration as shown in Table 1. A significant difference ($p < 0.05$) was found between 7 and 30 days freezing duration for all market sources, where 30 days freezing storage showed 10^1 cfu/g reduction in count from initial stage (Table 1).

Nilla *et al*.[2,5] found *Vibrio* spp. in 50% of examined *P. indicus* samples from Dhaka city local markets ranged between $0.7 \pm 0.0 \times 10^2$ and $2.2 \pm 0.25 \times 10^3$ cfu/g and the highest abundance was $1.59 \pm 0.04 \times 10^5$ cfu/g for cultured *P. indicus* from Satkhira[6]. Nayem

et al.[18] estimated 3.6 × 10^2 cfu/g *Vibrio* spp. (58% incidence) in local marketed *M. rosenbergii*. Yousuf *et al*.[19] reported 44% occurrence of *Vibrio* spp. in *P. monodon* and *M. rosenbergii* from local markets.

It is very significant to find *Vibrio* spp. in shrimp samples after 30 days of freezing because normally *Vibrio* cannot survive in frozen condition due to the absence of moisture[25]. The inadequate freezing condition, power interruption and the presence of moisture during freezing[2,3] might be the probable reasons for survival of *Vibrio* spp. in all market samples.

Proximate composition: Table 3 represents the decrease trends of moisture, protein and lipid contents in *M. monoceros* samples from three different local markets where the ash contents were increased after 30 days freezing period. A significant difference ($p < 0.05$) was found between the storage durations for all nutrient contents of three local markets (Table 3). An inverse relation was found in this study between moisture and protein, moisture and fat that the moisture content was raised after 30 days freezing storage but protein and lipid contents were decreased with storage duration.

Table 3. Nutritional composition (mean ± SEM) of *Metapenaeus monoceros* sampled from different local markets of Dhaka city at initial and final stages of study.

Source	Freezing duration	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Ananda bazar	0 day	77.91 ± 1.17^a	18.18 ± 0.48^a	2.72 ± 0.32^a	1.18 ± 0.14^b
	30 "	79.82 ± 1.54^b	16.24 ± 0.32^b	1.46 ± 0.23^b	2.47 ± 0.22^a
New market	0 "	77.43 ± 1.12^a	18.41 ± 0.54^a	2.84 ± 0.42^a	1.31 ± 0.14^b
	30 "	79.32 ± 1.36^b	16.32 ± 0.37^b	1.63 ± 0.38^b	2.72 ± 0.28^a
Polashi bazar	0 "	76.94 ± 1.06^a	18.66 ± 0.62^a	2.95 ± 0.55^a	1.44 ± 0.22^b
	30 "	78.76 ± 1.14^b	16.58 ± 0.45^b	1.76 ± 0.45^b	2.89 ± 0.35^a

Different superscript letters within columns show significant difference between storage durations (ANOVA, HSD, $p < 0.05$).

The body composition of fish seems to be varied from species to species and even within the same species from one individual to another[26]. Several different studies along with the present study are more or less in agreement with the general rule formulated by Stansby[26]. Protein is the most prominent biochemical component of crustaceans from egg to adult and is strikingly dominant in younger phases. Nurullah *et al*.[27] reported that the average moisture content of fish increased during freezing, whereas protein, lipid and ash reduced in fresh condition. The present findings are strongly supported by these results. The composition varies with season, size, stages of maturity, temperature and deviation may occur due to natural feeding habits and availability of feed, fasting duration during spawning and migration[28].

This study demonstrated that the quality of horina chingri (*M. monoceros*) available in local markets of Dhaka city were not at satisfactory level for consumption. The presence

of high bacterial load in the shrimp samples indicated the faecal contamination from water sources, rough handling and poor hygienic practices by fishermen and market dwellers as well as the unhygienic conditions and poor sanitation facilities of the market premises. The higher microbial profusion in shrimp signified the possibility of future outbreak of pathogenic diseases through ingestion including chances of cross contamination via kitchen utensils or handling. Besides, the products are preserved with poor quality ice through rough handling for long period. To overcome this situation, it is necessary to follow the code of practice concerning handling, icing and hygiene measures. Moreover, speckled shrimp is a good source of protein and can be consumed after freezing for a long time. So, regular monitoring of local fish markets as well as consumers' awareness on food safety should be increased and the retailers should be trained to secure public health as well as considerable economic costs.

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