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Reduction of Bacterial Pathogens in *Penaeus monodon* and *Macrobrachium rosenbergii* Using Several Chemical Interventions

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Abstract: A study was undertaken to determine the most effective element among the commonly used antimicrobial agents, especially in the body muscle of black tiger shrimp (*Penaeus monodon*) and giant freshwater prawn (*Macrobrachium rosenbergii*) on the basis of different chemical interventions. Using 10 ppm calcium hypochlorite, the microbial load has been reduced more significantly ($p>0.001$) than trisodium phosphate, lactic acid, oxalic acid, sodium carbonate, potassium bicarbonate and acetic acid and as result the total plate count in the *Penaeus monodon* and *Macrobrachium rosenbergii* samples reduced by on average of 3.83 (99.53%) and 3.24 (95.5%) $\log_{10}$CFU/g, respectively. Moreover, the aforementioned compound was also found to be very effective against *Escherichia coli* and *Vibrio* spp i.e., the count of *E. coli* and *Vibrio* spp. reduced to nil or nearly nil.

Key words: Reduction of bacteria · Chemical interventions · Black tiger shrimp (*Penaeus monodon*) · Giant freshwater prawn (*Macrobrachium rosenbergii*)

INTRODUCTION

Increased export demand (12.4%) for shrimp (*Penaeus monodon*) and prawn (*Macrobrachium rosenbergii*) together with high economic returns i.e. 2.9% of total export earning has resulted in the involvement of different water bodies in Bangladesh is being augmented [1]. Freshly harvested aquaculture products, particularly those from tropical regions, may harbour pathogenic bacteria which form part of the natural micro-flora of the water body. In response to the public health concerns regarding the microbiological safety of raw shellfish, the contemporary food microbiologists face challenges on the effectiveness of mitigation strategies.

Emergence or re-emergence of serious diseases such as typhoid, bacillary dysentery, cholera, undulant fever, tuberculosis, listeriosis and hepatitis is a growing concern both in humans and food-animals in the predisposed populations [2]. Fish-borne pathogens can grow at a temperature range from –4 to 50°C [3]. Hepatitis viruses and *Yersinia enterocolitica* are major oyster pathogens [4]. Some of the pathogens found on fish are of marine origin, for example, *Vibrio vulnificus, V. parahaemolyticus* and *V. cholerae*, while others are from sewage, such as *Salmonella* spp. and *Campylobacter* spp. [5-7].

For improved mitigation measures to reduce risks several chemicals, salt, low temperature, heat and irradiation processes have been used, which leads to the undissociated form of the molecules during the inhibition activities [8]. Lactic acid, oxalic acid and acetic acid are commonly used in the meat industry to reduce the microbial loads in the carcasses [9,10]. Calcium hypochlorite, trisodium phosphate, sodium carbonate and potassium bicarbonate have been used as an effective sanitizer in controlling the growth of *Salmonella* spp. and *E. coli* [11]. *S. aureus* [12]. *Camplybacter, L. monocytogenes* and *psychrotrophs* [13].

The objectives of this study were to evaluate the intervention of chemicals e.g. calcium hypochlorite, trisodium phosphate, lactic acid, oxalic acid, sodium carbonate, potassium bicarbonate and acetic acid to determine the most effective chemical treatment in reducing bacterial pathogens.

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MATERIALS AND METHODS

To find out the efficacy of different chemical interventions to reduce the microbial load especially on the locally available shrimp and prawn species in the present study samples were obtained from five representative sources of Bangladesh such as tiger shrimp (*Penaeus monodon*) from Cox's Bazar and Satkhira; giant water prawn (*Macrobrachium rosenbergii*) from Noakahli; from local markets and departmental chain shops in the Dhaka city, which has been collected from December, 2005 to April, 2006. Among all the samples, some were collected instantly from the gher (earthen pond) and the others from the shopkeepers without having proper storage conditions. To avoid further contamination or spoilage, during transportation from the source to laboratory, samples were carried by special sterile bags packed in insulated box with ice to maintain the temperature around 4 to 6°c. The samples were stored at -80°C at the laboratory until use.

At first samples were deheaded and peeled and then it was rinsed thoroughly with sterile distilled water. Alongside the microbial evaluation of the sterile distilled water washing, the washed samples were also assessed for bacterial growth by mutilating, macerating and smashing into the distilled water. Dilution and plating by spread plate method were carried out soon after sampling. Approximate 15 ml of plate count agar (PCA), MacConkey agar, membrane fecal coliforms (mFC) agar, eosin methylene blue (EMB) agar, thiosulphate citrate bile salt sucrose agar, xylose lysine deoxycholate (XLD) agar has been melted and brought to 45°C was poured into the sterile Petri plates and different initiatives were taken for equal distribution of the media. After solidifying and inoculation, plates were inverted and placed in incubator at 37°C for 48 hours for evidence of growth. Total sixteen samples have been analyzed in terms of aerobic plate count (APC), enterobacteriaceae counts and *Vibrio* spp. counts. Gradually, these enumerated samples were chemically intervened using different commonly used antimicrobial compounds such as 10 ppm calcium hypochlorite, oxalic acid, sodium carbonate, acetic acid, lactic acid, trisodium phosphate and potassium bicarbonate. The samples have been washed 1 min with vigorously shaking by the aforementioned chemicals. In this way each treatment has been repeated four times.

RESULTS AND DISCUSSION

Bacterial load associated with raw shrimp and prawn samples had been investigated. Typically, pH values between 6.0 and 7.5 are used in sanitizer solutions to minimize corrosion of equipment and maximum solubility in water is observed near 4°C. Organic acids are commonly used as antimicrobial acidulates to food preservation either by direct addition or through microbiological fermentation [14]. Since many pathogens generally cannot grow at pH values much below 4.5, acidification may prevent microbial proliferation. Various high pH cleaners containing potassium bicarbonate, sodium carbonate and/or sodium orthophenylphenate (with/without surfactants) reduced populations of *E. coli* on shrimp muscle surfaces Pao *et al*., [15]. The high pH of typical alkaline wash solutions and concerns about environmental discharge of phosphates may be limiting factors for use of certain alkaline compounds. The results are summarized in Table 1 and 2..

At the initial stage, the enumeration of the total plate counts was found, in case of shrimp ranged from 3.31 to 5.65 $\log_{10}$ CFU/g, while prawn possessed range from 3.03 to 5.08 $\log_{10}$ CFU/g. Moreover, the total coliforms count ranged between 2.73 and 5.93 $\log_{10}$ CFU/g for the shrimp and for the prawn 2.57 and 4.64 $\log_{10}$ CFU/g. In addition 43.75% and 38.52% *Vibrio* spp. were isolated from the shrimp and prawn samples, respectively.

In these studies after chemical intervention, when shrimp and prawn samples were dipped in the calcium hypochlorite solution, remarkable reduction of *E. coli* and aerobic plate count as compared to the control has been recorded. Calcium hypochlorite at a concentration of 10 ppm reduced aerobic total counts in shrimp and prawn on average 3.83 (99.53%) and 3.24 (95.5%) $\log_{10}$ CFU/g, respectively (Table 1). Using the same treatment the count of *E. coli* and *Vibrio* spp. reduced to nil or nearly nil (Table 2). The reduction of *E. coli* and aerobic plate count (APC) using 10 ppm calcium hypochlorite was statistically significant at $p>0.001$ level. The least significant differences (LSD) of means were 0.095 for *E. coli* and 0.15 for aerobic plate count. Similar experiments showed that treatment of raw brown shrimp (*P. aztecus*) with 50 ppm calcium hypochlorite for 5 min reduced the population of *L. monocytogenes* by 1.7 and 1.2 $\log_{10}$ CFU/g, respectively [16]. Pao and Davis [17] showed that populations of *E. coli* inoculated onto yellow perch (*Perca flavescens*) fillets were reduced by more than

Table 1: Initial and after chemical intervention, total aerobic plate count (log_{10} CFU/g) of collected *Penaeus monodon* and *Macrobrachium rosenbergii* samples from different sources

		Total aerobic plate count (log_{10} CFU/g)							
			Chemical intervention						
Sample		Initial	CH	TSP	LA	OA	SC	PB	AA
Macrobrachium	A	2.31	0.01	0.34	1.49	1.70	0.57	0.86	0.15
rosenbergii	B	3.77	0.02	0.56	2.42	2.77	0.92	1.41	0.25
	C_1	3.65	0.11	0.54	2.35	2.68	0.89	1.37	0.24
	D_1	5.65	0.12	0.84	3.36	4.15	1.38	2.13	0.37
	AR(amount)		3.83	4.12	1.37	0.88	2.90	2.40	3.59
	AR (%)		(99.5%)	(85.2%)	(35.8%)	(26.6%)	(75.53%)	(62.6%)	(93.35%)
Penaeus	C_2	3.08	0.14	0.73	2.29	1.43	1.06	1.46	0.31
monodon	D_2	5.08	0.23	1.2	3.78	2.37	1.75	2.41	0.82
	E	2.03	0.09	0.48	1.51	0.95	0.70	0.96	0.33
	AR(amount)		3.24	2.59	0.87	1.81	2.23	1.79	2.91
	AR (%)		(95.5%)	(76.42%)	(25.6%)	(53.45%)	(65.6%)	(52.62%)	(83.93%)

CH (calcium hypochlorite), TSP (trisodium phosphate), LA (lactic acid), OA (oxalic acid), SC (sodium carbonate), PB (potassium bicarbonate), AA (acetic acid); Samples A from Cox's Bazar; B from Satkhira; C_1, C_2 and D_1, D_2 from local markets and departmental chain shops in Dhaka city, respectively; E from Noakhali of Bangladesh.; AR (average reduction) of total plate count (log_{10} CFU/g and %) after wash with different chemicals.

Table 2: Initial and after chemical intervention, Coliform and *Vibrio* spp. count (log_{10} CFU/g) of collected shrimp and prawn samples from different sources

		Coliform count (log_{10}CFU/g)								*Vibrio* spp. count (log_{10} CFU/g)	
			Chemical intervention								
Sample		Initial	CH	TSP	LA	OA	SC	PB	AA	Initial	ACI
Macrobrachium	A	1.73	-	-	-	-	-	-	-	0.01	Nil
rosenbergii	B	1.72	-	-	0.5	0.24	-	1.04	-	0.02	Nil
	C_1	2.66	0.01	-	1.6	1.05	0.57	1.17	-	0.11	Nil
	D_1	4.93	-	0.03	1.65	1.15	0.75	2.13	0.03	0.12	Nil
Penaeus	C_2	2.71	0.02	-	0.05	1.43	0.17	1.64	0.13	0.14	Nil
monodon	D_2	3.64	-	0.02	1.25	2.37	0.05	0.96	-	0.23	Nil
	E	1.7	-	-	-	0.95	-	-	-	0.09	Nil

CH (calcium hypochlorite), TSP (trisodium phosphate), LA (lactic acid), OA (oxalic acid), SC (sodium carbonate), PB (potassium bicarbonate), AA (acetic acid); ACI (after chemical intervention); Samples A from Cox's Bazar; B from Satkhira; C_1, C_2 and D_1, D_2 from local markets and departmental chain shops in Dhaka city, respectively; E from Noakhali of Bangladesh

2 log CFU/cm^2 after immersion in 100 ppm chlorine at 30°C for 3 min. Treatment of *M. rosenbergii* with 80 ppm hypochlorite significantly ($p<0.05$) reduced the surface microbial populations compared to water washed controls [18].

Treatment with 10 ppm trisodium phosphate caused reduction of bacterial load (APC) in shrimp and prawn on average of 4.12(85.2%) and 2.59 (76.42%) log_{10} CFU/g, respectively (Table 1), However, the same treatment caused complete elimination of *E. coli* and *Vibrio* spp.

(Table 2). The reduction of *E. coli* and aerobic plate count was statistically significant at p>0.001 level. The highest effect of trisodium phosphate may be due to its high pH (pH 10), which affects the cell wall and the adherence of bacteria. Trisodium phosphate may also repress enzyme synthesis and inhibit enzyme activity of bacteria [19]. The least significant difference of means was 0.095 for *E. coli* and 0.15 for aerobic plate count. Similar results have also been found in previous studies. Wang *et al.* [20] studied the effects of 10 ppm trisodium phosphate spraying on *S. typhimurium*. They found that the reduction was 1.5-2.3 logCFU/g. Aerobic bacteria were reduced by 1.21 log CFU/g when marine shrimp *Metapenaeus dobsoni* were treated by dipping in 8 ppm trisodium phosphate [21]. Lillard [22] found that the reduction of *S. typhimurium* on *P. monodon* was 2 log CFU/g when chicken skin was treated with 10 ppm trisodium phosphate concentration. Kim *et al*. [23] reported that the treatment of *Lates calcarifer* by dipping them in 10 ppm trisodium phosphate solution reduced the bacteria by1.6 to 1.8 log CFU/g.

The reduction of *E. coli* from samples dipped in 10 ppm lactic acid ranged between 0.05 and 1.65 log CFU/g (Table 2). No *Vibrio* spp. was detected after the treatment. The aerobic plate count was reduced significantly i.e. 1.37 (35.8%) and 0.87 (25.6%) log CFU/g, from shrimp and prawn respectively (Table 1). The effectiveness of lactic acid may be due to the metabolic inhibition by the undissociated acid molecules [24]. The least significant difference of means was 0.095 for *E. coli* and 0.15 for aerobic plate count. Ramirez *et al.* [25] reported that treatment of crab *Calappa lophos* in 8 ppm lactic acid caused a reduction of *E. coli* and aerobic plate count by 1.6 and 1.6 logCFU /g, respectively. Also, a 2.5 log reduction was observed when the calf carcass surface was treated with 10 ppm lactic acid [26]. Davidson and Juneja, [27] observed that the treatment with 1-2 ppm lactic acid reduced aerobic plate count by log 0.3-2.7 log CFU/g on *M. rosenbergii*; which were washed with 5 ppm lactic acid and aerobic plate count were reduced by 0.76 log CFU/cm^2 [28].

The reduction of aerobic plate count from shrimp and prawn was 0.88 (26.6%) and 1.81 (53.5%) log CFU/g, respectively using 10 ppm oxalic acid solution ((Table 1). The reduction of *E. coli* from the samples dipped in 10 ppm oxalic acid ranged from 0.24 - 2.37 log CFU/g (Table 2). No *Vibrio* spp. was detected from the samples. The reduction of *E. coli* and aerobic plate count in 10 ppm oxalic acid solutions was found to be significantly different from control samples. Treatment with oxalic acid in the form of lemon juice has been shown to reduce populations of *S. typhi* inoculated onto *P. monodon* [29]. When peeled *P. monodon* were dipped in 1.2 ppm oxalic acid for 10 sec, the populations of *S. typhimurium, Shigella sonnei, Yersinia enterocolytica, E. coli, Pseudomonas aeruginosa* and *Streptococcus faecalis* were reduced by 65% and the colour and flavour remained similar to the control samples [30].

The reduction of *E. coli* from samples dipped in 10 ppm sodium carbonate ranged between 0.05 and 0.75 log CFU/g (Table 2). The reduction of aerobic plate count from shrimp and prawn was 2.90 (75.59%) and 2.23 (65.6%) log CFU/g, respectively (Table 1). No *Vibrio* sp. was detected from the collected samples (Table 2). It has been reported that *E. coli* O157:H7 population was reduced by 5 and 6 log after a 30-sec treatment with 1 ppm sodium carbonate at 10°C and room temperature, respectively [31]. *Salmonella* sp. on the surface of *P. monodon* was reduced from 5.2 log CFU/cm^2 to nondetectable levels after 15 sec in 15 ppm sodium carbonate [32].

The reduction of *E. coli* from samples dipped in 10 ppm potassium bicarbonate Varied between 0.96 and 2.13 log CFU/g (Table 2). The reduction of aerobic plate count from shrimp and prawn was 2.40 (62.6%) and 1.79 (52.62%) log CFU/g, respectively (Table 1). *Vibrio* spp. was completely eliminated. Slavic *et al.* [13] evaluated the effectiveness of 10 ppm potassium bicarbonate solution on the *M. monoceros* inoculated with *Campylobacter* and found that the reduction was 1.5 log CFU/cm^2. It has also been reported that the aerobic bacterial load was reduced by 2.6 log CFU/cm^2 when *M. dobsoni* were treated by dipping in 2 ppm potassium bicarbonate [21].

Dipping samples in 10 ppm concentration of acetic acid caused a reduction of aerobic plate count in shrimp and prawn by on average of 3.59 (93.35%) and 2.91(83.93%) $\log_{10}$CFU/g, respectively (Table 1), However, this treatment caused almost complete elimination of *E. coli* and *Vibrio* spp. The least significant difference of means was 0.095 for *E. coli* and 0.15 for aerobic plate count. Fernandez *et al.* [33] found that young cephalopod, *Sepia officinalis*, washed with 0.5 ppm acetic acid caused reduction of total bacterial counts by 0.76 log CFU/cm^2 (1989). Anderson *et al.* [34] observed that the total count was reduced by 1.49 log when 3 ppm of acetic acid was sprayed on the beef carcass. Treatment of whole *P. monodon* for 5 min at 21°C with vinegar (7.6 ppm acetic acid) reduced populations of *S. sonnei* more than 7 log CFU/g [35]. In combination with 100 ppm chlorine,

lactic acid or acetic acid was slightly more antagonistic toward *L. monocytogenes* than either acid or chlorine alone. Various combinations of acetic acid, lactic acid and chlorine were observed to reduce populations of *L. monocytogenes* on crabs (*Scylla serrata*) [32].

The comparison of antimicrobial effects of seven treatments revealed treatment of raw shrimp and prawn with calcium hypochlorite is the most effective than the other agents in reduction of total bacterial load as well as elimination of harmful bacteria. Sodium carbonate, potassium bicarbonate, lactic acid and oxalic acid were found to be less effective than acetic acid and trisodium phosphate. However, these chemicals with high concentration as a quality control measures can adversely affect the quality of shellfish due to the presence of its residues in food; moreover they will not be able to eliminate the pathogenic bacteria completely, but will reduce and increase the shelf life and quality to some extent.

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