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Microbiological quality of freshwater prawns during storage

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Abstract: Microbiological quality analysis of freshwater prawns from three sampling sites in Peninsular Malaysia viz: Site 1- Kg. Jumbang, Negri Sembilan; Site 2- Kg. Cangkat Tin, Perak and Site 3- Kg. Cenderiang, Perak for total mesophilic and psychrophilic aerobic counts, proteolytic bacterial counts, histamine producing bacteria, cadaverine producing bacteria and putrescine producing bacteria in the prawns and pond water for the three sites showed that the microbiological quality of freshwater prawns is related to the microflora of pond water in which they were grown. The initial bacterial counts indicated the values were in the range of log 4+ CFU/g for all samples. Total mesophilic and psychrophilic counts of the head regions were higher than that of the body regions for all prawn samples and types of growth media tested. All samples showed an increase in counts with time and temperature of storage up to log 7+ CFU/g for mesophilic counts after 12 hours at ambient, 6 days at 10 ± 2°C and 12 days at iced storage. The samples from Site 2 had relatively higher counts compared to the other two sites which correlated well with the levels determined in the pond water. Similar trends were observed for psychrophilic counts but at lower values for the different types of media studied. Effects of preservatives on quality changes and shelf life of shrimp during iced storage indicated that boric acid, lactic acid and sodium metabisulphite managed to inhibit psychrophilic bacteria and biogenic amines formation in prawns while maintaining the mesophilic counts at lower levels during iced storage.

Keywords: Microbiological quality, freshwater prawns, preservative

INTRODUCTION

Prawns are one of the highest value products in international fisheries trade and as such appears to have good long term potential. Freshwater prawns, *Macrobrachium rosenbergii* are produced throughout Asia, the Pacific Islands, Israel, Central America and parts of the Southern United States. The rapid growth rate of *M. rosenbergii* makes it an ideal species for aquaculture. However, successful marketing of *M. rosenbergii* has been impeded by a shelf life of a few days in refrigerated storage. Not much information is available on the microbiological and biochemical changes during refrigeration storage temperature. The microflora associated with farmed *M. rosenbergii* has been studied (Lalitha and Surendran, 2004). Few studies have been published on the microbiological quality characteristics of farm-reared freshwater prawn stored in ice/stored under refrigeration (Leitào and Rios, 2000) which contrasts the wealth of information available on chemical and sensory characteristics (Ninan *et al.*, 2003; Rodrigues *et al.*, 2000).

In Malaysia, very little is known about the bacteriology of prawns. The little available data, for example, are on *Vibrio spp.* which were from marine fish during occurrence of diseases and severe mortalities and on Pseudomonas sp. isolated from diseased freshwater fish. *Vibrio* and *Pseudomonas* are associated mainly with virulence and pathology. It is an established fact that seafood muscles are different from other muscle protein. However, the factors responsible for the difference and how they affect the microbiological character of seafood products may not be fully appreciated. Some factors have very obvious microbiological implications while others are more subtle. Other studies include microbiological and biochemical changes during ice storage of prawns and on bacteriology during commercial handling wherein most of the reported work has been on cold water species. Ice storage of prawns, is not always done properly, particularly in developing countries where little technological data are available on tropical prawns of commercial importance to Malaysia and nearby countries. Besides, studies on

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microbiological, postmortem quality changes and other quality studies report little information on stability of prawns at the temperature that may be expected during commercial handling.

There are more than 3000 different commercial species of seafood throughout the world. Intuitively, it would seem that this great diversity would give rise to diversity among the bacterial population normally associated with the various fish species. While species difference may be important, evidence seem to indicate that the flora of fish and shellfish is more directly related to environmental factors. Studies indicate that bacteria are a function of the environment where warm water shellfish seem to have more mesophilic gram positive flora (micrococci, bacilli and coryneforms) while cold water shellfish carry predominantly gram negative populations *(Moraxella, Acinetobacter, Pseudomonas, Flavobacterium* and *Vibrio)*. However, spoilage patterns during ice and refrigerated storage are usually quite similar and are caused by proliferation of psychrotrophic bacteria such as *Pseudomonas* and *Alteromonas putrefaciens* (Leitào and Rios, 2000).

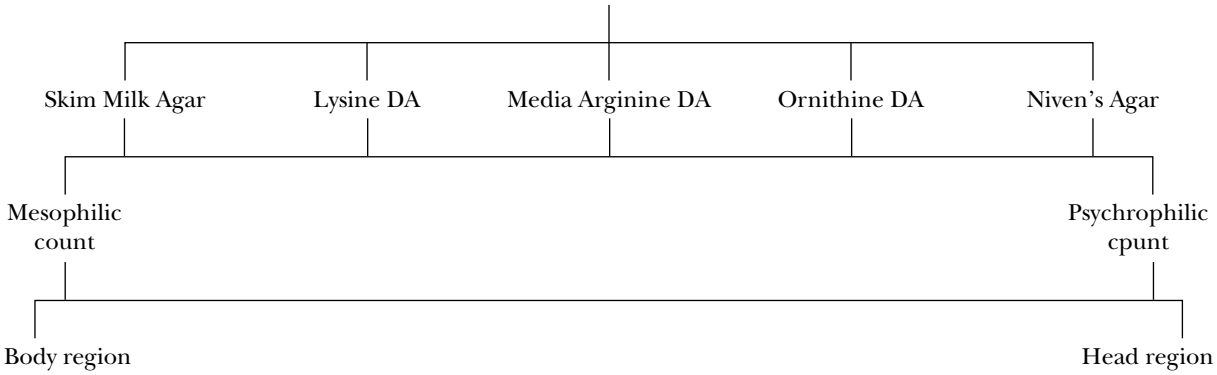
Deterioration of flesh foods during storage has been attributed to both autolytic and bacteriological changes. It was also suggested that there is a shift in the composition of microflora of prawns during storage (Lalitha and Surendran, 2006). Therefore, it is of interest to evaluate the spoilage potential of individual bacterial isolates to determine their contribution to overall spoilage during storage. Cadaverine, putrescine and histamine have also been suggested as chemical indicators. Based on these, the spoilage potential of the organisms could be directly linked to the amount of chemical indicators produced by the organisms during growth in the prawn constituents. Special attention has been paid to histamine because above a certain concentration, it has often been associated with cases of food poisoning. Given the perishable nature of shellfish, a satisfactory method for extending the shelf life of chilled products that ensures quality and a continuity of supply with a minimum of waste has been an ultimate goal. Research with certain preservatives to aid this effort has met with varying degrees of success. The microbiological indicators could therefore be used to relate to different types of preservatives applied during storage. For these reasons and the scant studies in the field of upgrading, control and monitoring the amount of biogenic amines in seafood products, the perfection of quantitative methods and the development of qualitative methods are important for routine controls. The objectives of this study are to evaluate and establish the relationship between pond water quality, total bacterial count and composition of microbial flora involved during storage and to correlate the differences between the sampling sites and the effect of storage temperature on the microbiological changes of freshwater prawns during storage.

MATERIALS AND METHODS

Preparation of the prawns samples and storage conditions

Live cultured freshwater shrimp (*Macrobrachium rosenbergii*) were obtained from three different sites in Malaysia: Site 1- Kg. Jumbang, Negri Sembilan; Site 2 - Kg. Cangkat Tin, Perak and Site 3 - Kg. Cenderiang, Perak (Figure 1). These sites were chosen for this study due to their consistency in breeding and availability of samples throughout the season. Mature four months old adult prawns measuring an average minimum of 13.00 cm and maximum of 18.00 cm in length and weighing between an average of 25.00 g minimum to 71.00 g maximum. Live prawns were brought back to the laboratory with their original pond water in sealed oxygenated bags. Samples from each site were then divided into three groups and immediately placed inside clean styreform boxes. One group of shrimp was kept at ambient temperature (28 ± 2°C) for 20 h. The second group of samples were stored at 10 ± 2°C for 10 days and the remaining prawns were kept in clean crushed ice (4 ± 2°C), for 16 days. Ice was removed and replenished on alternate days throughout the storage period. Ambient storage was chosen to emulate the abuse handling procedure often overlooked by prawn processors. Ten (10) degree Celsius was adapted from the usual commercial refrigerated storage temperature currently practiced by food handlers whilst icing was chosen as the normal handling procedure practiced after samples are harvested. Prawns were sampled at four hourly intervals (0, 4, 8, 12, 16, and 20 hours) for ambient storage, every two days for 10°C storage (0, 2, 4, 6, 8, and 10 days) and every four alternate days for the ice stored samples (0, 4, 8, 12, and 16 days). All samples were subjected to microbiological analyses.

Microbiological analysis



* All mesophilic counts plates were incubated at 30°C for 48 hours.
All Psychrophilic counts plates were incubated at 7°C for 10 days.
DA – decarboxylase agar

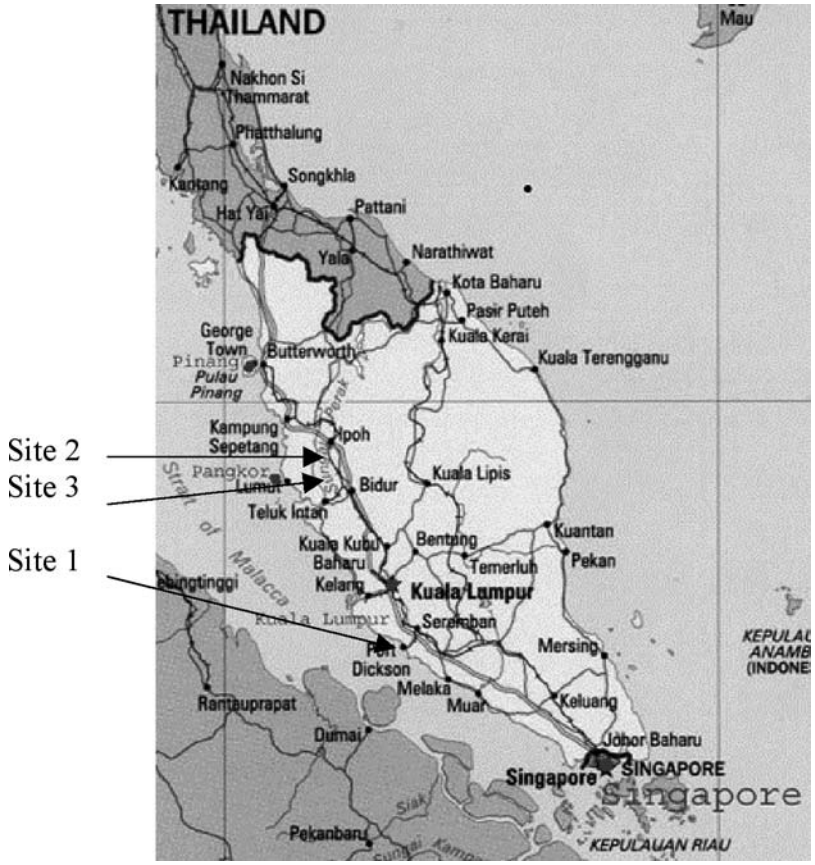


Figure 1: Map of Peninsular Malaysia showing sampling sites for freshwater prawns, *Macrobrachium rosenbergii*

Sample preparation and microbiological media

Microbiological analyses of the samples were done separately for body and head regions of the prawns as follows: two replicate pooled samples of 10 shrimp were blended using stomacher for 60 s. From the pooled samples, 1 g was removed and diluted with 9.0 ml sterile peptone water (0.1% peptone water + 0.9% saline). 0.1 ml aliquots were spread plated onto different selective agar media consisting of plate count agar (PCA) for total aerobic counts using skim milk agar, arginine decarboxylase agar and ornithine decarboxylase agar for putrescine producing bacteria, lysine decarboxylase agar for cadaverine producing bacteria, modified Niven's media for histamine producing bacteria and proteolytic counts were determined by counts on the skim milk agar. Similarly, microbiological analysis of respective pond water from the three sampling sites was also determined using the same media as described above. Pond water agar consisting of 100 ml pooled pond water samples plus bacteriological agar was also tested to determine if there was any growth of bacteria on such medium.

Microbiological quality of preserved prawns

Duplicate samples of 10 prawns were randomly removed from each of the different boxes and blended in sterile 0.1% peptone water + 0.9% saline using stomacher for 60 s. The heads were blended separately from the body regions. Dilutions were done accordingly and 0.1 ml sample homogenates were spread plated onto Plate Count Agar (PCA) for controls and for other chemicals, PCA plates containing individual percentages of the five respective preservatives were used (2% sucrose, 2% sodium chloride, 2% sodium metabisulphite, 2% boric acid, 1% lactic acid). At least six replicate plates were used for each preservative at each incubation temperature. Half of each of the plates were incubated for mesophilic counts whilst the rest were incubated for psychrophilic counts.

RESULTS AND DISCUSSION

Microbiological analysis

(i) Mesophilic counts

The changes in different Mesophilic counts of bacteria (proteolytic, lysine decarboxylase, arginine, ornithine and histidine decarboxylases producing bacteria) shown on Tables 1, 2 and 3 represent an upward pattern throughout the storage periods for all samples and the levels fluctuated and overlapped at some points or time of storage (either ones were higher at one site and not the next) with one type more than the others and vice versa as time proceeded for the different sites and temperature of storage. In general, the overall results for mesophilic counts for all storage times of the three sites increased between two to four log cycles till end of storage period because the prawns are an excellent medium for the growth of bacteria. Fresh prawns normally contain a considerable number of organisms which, when given suitable conditions of elevated temperature, can multiply rapidly to a level that completely spoils the prawns within less than 24 hours at ambient conditions without ice (Zuberi and Qadri, 1991). The relationship between chemical and microbiological changes in freshwater prawns has been established by studies carried out by Ninan *et al.* (2003).

Results of the proteolytic counts showed that they were higher than the other biogenic producing bacterial counts for almost all samples studied (Tables 1, 2 and 3). These values correlated well with the original counts of pond water shown in Table 4 for all the three sites where the recorded levels were about log 4 CFU/ml. (log 4.43, 4.14, and 4.01 respectively). Proteolytic activities in prawns are normally associated with textural changes in a muscle food in relation to changes in proteins (Venugopal, 1990). The results showed that irrespective of the initial microflora, spoilage during storage of *M. rosenbergii* is caused by highly proteolytic bacteria which most probably might be bacteria of the genera *Pseudomonas* and *Aeromonas* spp. According to Cobb *et al.* (1976) the dominant organisms associated with shrimp and prawn spoilage are psychrophilic *Pseudomonas* spp. Even though *Aeromonas* is not a common spoiler of tropical fish and shrimp, Barile *et al.* (1985) reported this organism as constituting a major part of the spoilage flora in fish mackerel. The most likely source is the contaminated water and ice. Above 10°C, *Aeromonas* is a highly proteolytic indole-positive organism which attacks muscle proteins releasing tryptophan which is subsequently converted to indole via bacterial metabolism (Miget, 1991). Typically *Aeromonas hydrophila* is also reported to be present in freshwater environments of tropical countries.

Biogenic amines producing bacteria were indicated by the activity of amino decarboxylation shown by their corresponding amino acids. Histamine producing bacteria were observed on the modified Niven's agar containing histidine, whilst putrescine forming bacteria were shown on arginine and ornithine decarboxylases agar plates and last but not least cadaverine producing bacteria were observed on the lysine decarboxylase agar plates. The results showed that amongst all the three biogenic amines producing bacteria studied,

Table 1: Mesophilic counts of site 1 samples for body and head regions of *M. rosenbergii* during storage at different temperatures as plated on different agar media

Storage temp.	time	Mesophilic Counts (log cfu/g)									
		Skimmed Milk Agar		Lysine DA		Arginine DA		Ornithine DA		Niven's Agar	
		Body	Head	Body	Head	Body	Head	Body	Head	Body	Head
Icing	0d	3.76	3.71	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
	4d	4.67	4.85	6.68	4.59	3.61	3.96	3.39	4.61	4.15	4.85
	8d	5.91	5.85	3.85	4.87	3.92	4.85	4.69	5.43	4.69	4.94
	12d	5.95	6.15	4.92	5.73	4.85	5.28	5.77	5.85	5.92	5.95
	16d	6.99	6.49	5.04	5.49	5.96	6.23	5.04	6.94	5.94	6.00
10°C	0d	3.36	3.71	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
	2d	4.58	4.97	3.64	3.99	4.53	4.97	3.15	3.99	4.41	4.85
	4d	5.69	5.82	4.71	4.96	4.58	5.00	4.49	4.69	4.53	5.57
	6d	5.87	5.90	4.77	5.52	4.94	5.04	4.61	5.52	4.91	5.43
	8d	5.94	6.15	4.63	5.49	5.71	5.85	5.96	6.69	6.00	6.49
	10d	6.95	7.85	6.99	6.53	6.00	6.71	5.96	6.61	5.99	4.28
Ambient	0h	3.36	3.71	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
	4h	4.76	4.43	4.49	4.59	4.25	4.51	3.61	4.91	3.85	4.49
	8h	4.87	4.90	4.53	4.89	4.53	5.58	4.89	5.89	4.71	5.57
	12h	5.49	5.61	5.59	6.53	4.96	5.61	5.23	5.96	4.91	5.32
	16h	5.85	6.85	6.76	6.85	6.00	5.96	5.25	6.04	6.00	6.69
	20h	6.53	6.95	6.85	6.94	5.99	5.36	5.23	6.32	6.15	6.85

Table 2: Mesophilic counts of site 2 samples for body and head regions of *M. rosenbergii* during storage at different temperatures as plated on different agar media

Storage temp.	time	Mesophilic Counts (log cfu/g)									
		Skimmed Milk Agar		Lysine DA		Arginine DA		Ornithine DA		Niven's Agar	
		Body	Head	Body	Head	Body	Head	Body	Head	Body	Head
Icing	0d	4.33	4.57	2.00	2.00	2.00	2.00	2.00	2.00	3.53	4.25
	4d	3.64	3.63	3.23	3.64	3.53	3.56	3.59	3.63	4.28	4.68
	8d	3.35	4.25	4.04	4.08	3.87	4.28	3.63	4.11	4.89	5.04
	12d	4.04	5.15	4.88	5.13	4.79	4.96	4.93	4.93	5.02	5.15
	16d	5.93	6.04	5.80	6.95	5.88	6.14	5.92	6.09	5.11	6.14
10°C	0d	4.33	4.57	2.00	2.00	2.00	2.00	2.00	2.00	3.53	4.25
	2d	3.57	3.91	3.67	3.65	3.58	3.75	3.67	3.91	3.85	4.88
	4d	3.65	4.67	3.77	4.07	3.81	4.99	3.88	4.67	3.86	5.16
	6d	4.78	4.83	4.18	5.11	4.65	5.96	4.75	5.83	4.94	5.20
	8d	5.08	5.20	5.81	6.13	5.87	6.14	5.97	6.20	5.10	6.35
	10d	5.80	6.13	6.82	7.15	6.74	7.15	6.10	6.14	6.88	7.16
Ambient	0h	4.33	4.57	2.00	2.00	2.00	2.00	2.00	2.00	3.53	4.25
	4h	4.97	5.13	4.89	4.96	4.69	4.89	4.61	4.97	4.98	5.16
	8h	5.04	5.20	4.89	4.98	4.85	5.04	4.93	7.15	5.04	5.28
	12h	5.06	6.15	5.93	5.98	5.87	5.32	5.04	6.10	5.15	5.95
	16h	6.20	6.32	6.15	6.24	6.00	6.27	5.96	6.09	6.22	6.98
	20h	6.23	6.69	6.08	6.24	6.08	6.20	6.04	6.23	6.95	7.04

Table 3: Mesophilic counts of site 3 samples for body and head regions of *M. rosenbergii* during storage at different temperatures as plated on different agar media

Storage temp.	time	Mesophilic Counts (log cfu/g)									
		Skimmed Milk Agar		Lysine DA		Arginine DA		Ornithine DA		Niven's Agar	
		Body	Head	Body	Head	Body	Head	Body	Head	Body	Head
Icing	0d	4.04	4.61	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
	4d	4.53	4.87	3.36	3.83	3.61	3.91	3.85	4.63	4.04	4.46
	8d	4.49	4.95	4.53	4.49	4.53	4.04	4.61	5.69	4.85	4.89
	12d	5.95	6.28	4.91	4.97	4.91	5.61	5.15	5.04	5.94	5.87
	16d	6.15	6.25	5.85	6.71	5.85	6.95	5.95	6.23	5.89	6.32
10°C	0d	4.04	4.61	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
	2d	4.69	4.91	3.69	4.61	3.63	3.91	4.63	5.97	3.87	4.64
	4d	4.95	5.23	4.11	4.77	4.04	4.95	4.85	5.23	4.04	4.89
	6d	5.57	5.92	4.71	5.34	4.48	5.87	5.18	5.85	4.53	5.94
	8d	5.91	5.99	5.28	5.95	4.85	6.04	5.61	5.78	5.92	6.04
	10d	5.97	6.49	5.49	6.61	5.99	6.28	6.38	6.92	6.15	6.74
Ambient	0h	4.04	4.61	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
	4h	4.53	4.91	4.85	5.15	4.71	4.96	4.62	4.95	3.94	4.28
	8h	4.95	5.15	4.89	5.25	5.48	5.61	4.91	5.43	4.04	4.89
	12h	5.49	5.85	5.49	5.85	5.59	5.97	5.04	5.53	4.46	5.92
	16h	5.91	6.04	5.69	5.98	5.91	6.04	5.53	6.08	5.69	5.58
	20h	6.00	6.49	6.52	6.75	6.36	6.53	6.85	6.95	6.23	6.87

histamine producing bacteria were higher than the other two. These correlated well with the amounts of the biogenic amine present in prawns as shown earlier in the biochemical changes. However, in general all the three also increased with time and temperature of storage following the same trends of changes with those of the total counts and proteolytic counts of prawns. The results further indicated that the patterns of biogenic amines production were always parallel to the bacterial growth (Figures 2, 3 and 4 with Tables 1, 2 and 3). They also showed that histamine producers were also capable of producing the other two amines in some amounts throughout the storage periods for all samples. This indicates that if bacterial development in the prawns were not blocked, high quantities of histamine may accumulate in the products. Similar trends in results were obtained by studies on semi-preserved anchovies by Rodriguez-Jerez *et al.* (1994).

The formation of high levels of histamine in fishery products was directly correlated with the level of microorganisms present in the product, due to bacterial histidine decarboxylase action on histidine (Niven *et al.*, 1981; Ababouch *et al.*, 1991). The formation of histamine depends on the number of microorganisms rather than on the environment in which they grow (Yoshinaga and Frank, 1982). The significance of putrescine and cadaverine as potentiators of histamine intoxication in fishery products has not been fully established (Taylor and Sumner, 1987). This potentiating effect may be significant at ratios of putrescine plus cadaverine to histamine that are higher than those usually found in spoiled fish. However, putrescine and cadaverine may act in additivity with other undiscovered potentiators. Therefore, the determination of histamine levels alone may not be enough to confirm whether a sample is responsible for histamine poisoning (Wendakoon and Sakaguchi, 1992).

(ii) Psychrophilic counts

Results of the psychrophillic counts are shown on Tables 5, 6 and 7. The results show similar patterns of changes throughout the storage periods as those of the mesophilic counts discussed earlier but at slightly lower counts of about one log cycle. Miget (1991) suggested that differences in the normal microflora for various shrimp studied are due to differences in microflora of surrounding waters and sediments, species differences and post-harvest handling procedures, as well as plating media and incubation temperature. Cobb *et al.* (1976)

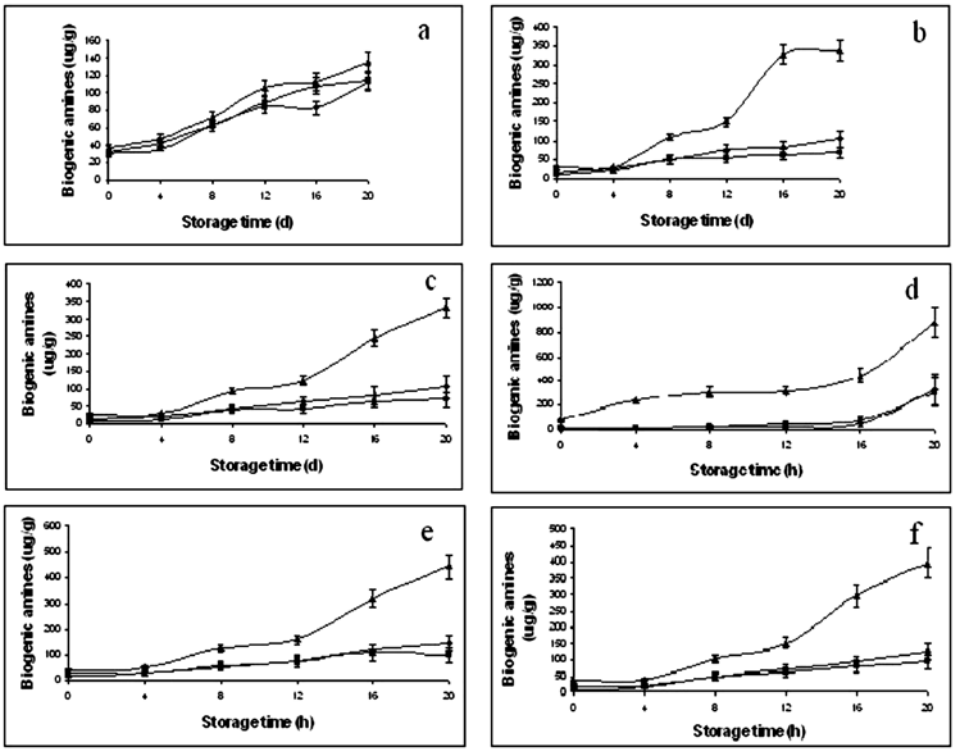


Figure 2: Amount of biogenic amines (putrescine ♦, cadaverine ■ and histamine ▲) produced from the body (a,b,c) and head regions (d,e,f) of *M. rosenbergii at* ambient storage for 20 hours for all three (3) sites. (a,d). Site 1, (b,e). Site 2, (c,f) Site 3.

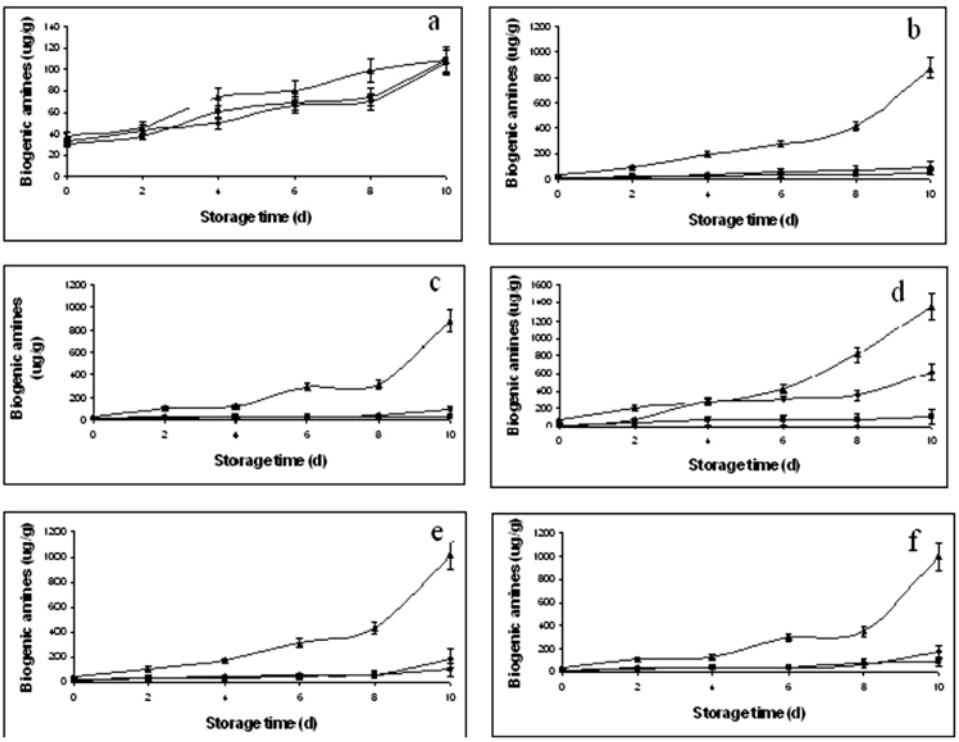


Figure 3: Amount of biogenic amines (putrescine ♦, cadaverine ■ and histamine ▲) produced from the body (a,b,c) and head regions (d,e,f) of *M. rosenbergii at* 10^0C storage for 10 days for all three (3) sites. (a,d). Site 1, (b,e). Site 2, (c,f) Site 3

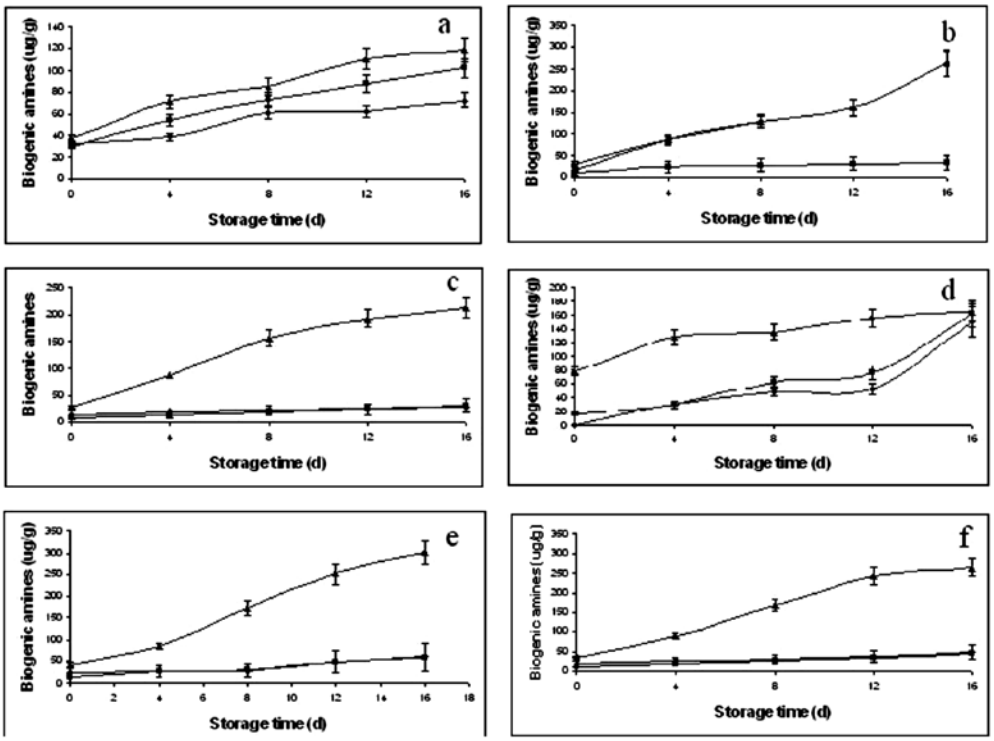


Figure 4: Amount of biogenic amines (putrescine ♦, cadaverine ■ and histamine ▲) produced from the body (a,b,c) and head regions (d,e,f) of *M. rosenbergii at* 10^{0}C storage for 10 days for all three (3) sites. (a,d). Site 1, (b,e). Site 2, (c,f) Site 3.

earlier reported that changes in the nutritional properties on the surface of prawns, or interactive microbial activities are involved in the changes of the microbial flora of prawns during iced storage. The results showed that psychrophilic counts of the different biogenic amines producing bacteria also existed even though the storage temperatures are low (ie. At 10^{o}C and iced storage) indicating that the bacteria could still grow and produce the respective biogenic amines but at lower levels than those of the mesophilic counterparts (Tables 5, 6, and 7). In fact the initial levels at 0 time for almost all samples were less than log 2.0 CFU/g. These also showed that high amounts of biogenic amines particularly histamine was produced as the storage conditions enhanced microbial growth. The general bacteriology of spoilage in most fishery products can be summarised as *Pseudomonas* types having the shortest generation times (and lag periods of growth) at temperatures in the 0-5^{0}C range and with enhanced capability to utilize non-protein nitrogen components of the muscle fluids. This is because growth substrates rapidly outgrow the other bacteria present and become the dominant organisms in a population which increases to over log 6.0 CFU/g. These bacteria initially oxidize amino acids and lactic acids and at later stages produce proteinases and a variety of S-containing compounds mostly derived from cysteine and methionine as well as other compounds variously identified which together produced the objectionable odours perceived as spoilage (Venugopal, 1993).

Microbilogical quality of preserved prawns

Microbiological quality of shrimp revealed that mesophilic aerobic counts shows uptrend profiles throughout the storage period with values reaching log 6 + cfu/g after 20 days of iced storage. The psychrophilic counts however seem to be inhibited in the samples treated with sodium metabisulphite, boric acid and lactic acid respectively (Tables 8 and 9) which followed similar trends as the results obtained for the production of biogenic amines described above. Ninan *et al.* (2003) noticed significant deterioration after 12 days in ice stored cultured *M. rosenbergii.*

Some counts were recorded for the other samples treated with sucrose which was considered the normal sugar solution and sodium chloride which was assumed to be the normal eating salts. Similarly, higher counts were detected for the controls consisting of ice alone wherein results were comparable to those obtained earlier in the storage studies. Results from this study showed that

Table 4: Microbial flora of pond water sampled from the three (3) sites with respect to number, colony types and sizes grown and on different agar media

	Kg. Jumbang, N. Sembilan		*Kg. Changkat Timah, Perak*		*Kg. Cenderiang, Tapah Perak*	
Media	Log cfu/ml	No. of colony types and sizes	Log cfu/ml	No. of colony types and sizes	Log cfu/ml	No. of colony types and sizes
Plate count agar	4.44	76 light yellow < 1mm 155 grey ~2-3mm 47 cream > 5mm	4.17	143 cream ~ 1mm 5 cream > 5mm 1 umbonate yellow	3.66	35 cream ~ 1mm 11 cream > 5mm
Arginine decarboxylase agar	4.25	149 light yellow < 1mm 30 cream ~ 2-3mm 1 orange ~2mm	4.30	101 whitish ~ 1-2 mm 100 grey ~ 1-2 mm	3.92	1 light yellow < 1mm 14 cream ~ 2-3 mm
Ornithin decarboxylase agar	3.17	2 orange ~1mm 13 grey ~2-3mm	3.25	10 grey ~ 2-3mm 8 yellow ~ 1mm	3.08	7 yellow ~ 1mm 5 grey ~ 2mm
Lysine decarboxylase agar	3.54	5 grey ~ 2-3mm 3 white ~ 3mm 21 yellow < 1mm 4 orange ~ 3mm 3 dark orange ~ 2mm	3.90	12 yellow ~ 1mm 67 grey ~ 3-4 mm	3.71	35 yellow < 1mm 16 grey ~ 3-4 mm
Skimmed milk agar	4.43	249 white cream ~ 2mm 14 cream ~ 3-4 mm 7 yellow ~ 2-3 mm 1 umbonate orange ~ 2mm	4.14	135 white cream ~ 2mm 2 cream ~ 3-4 mm	4.01	79 white cream ~ 2mm 24 cream ~ 3-4mm
Niven's agar	-	-	-	-	-	-
Pond H_2O agar	-	-	-	-	-	-

Table 5: Psychrophillic counts of site 1 samples for body and head regions of *M. rosenbergii* during storage at different temperatures as plated on different agar media

Storage temp.	time	Psychrophillic Counts (log cfu/g)									
		Skimmed Milk Agar		Lysine DA		Arginine DA		Ornithine DA		Niven's Agar	
		Body	Head	Body	Head	Body	Head	Body	Head	Body	Head
Icing	0d	3.78	4.08	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
	4d	3.84	4.15	3.49	3.69	3.61	4.15	3.67	4.53	3.53	4.85
	8d	4.61	5.92	4.53	4.88	3.87	4.64	3.91	4.04	4.96	5.04
	12d	5.90	6.00	4.71	5.96	4.61	5.04	4.97	5.85	5.85	6.61
	16d	5.90	6.78	5.96	6.15	5.77	5.95	5.85	5.96	5.60	6.36
10°C	0d	3.78	4.08	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
	2d	4.30	4.32	4.04	4.53	3.59	3.97	4.04	4.49	3.68	3.75
	4d	4.71	5.49	4.32	4.49	4.59	4.04	4.57	4.61	4.61	5.23
	6d	5.11	5.58	4.67	4.67	4.85	5.53	4.68	5.76	4.85	4.49
	8d	5.58	6.15	5.58	5.96	4.97	5.85	5.96	6.96	5.94	5.50
	10d	6.96	6.53	5.97	6.23	5.90	5.97	6.04	6.93	5.60	6.32

Table 6: Psychrophillic counts of site 2 samples for body and head regions of *M. rosenbergii* during storage at different temperatures as plated on different agar media

Storage temp.	time	Psychrophillic Counts (log cfu/g)									
		Skimmed Milk Agar		Lysine DA		Arginine DA		Ornithine DA		Niven's Agar	
		Body	Head	Body	Head	Body	Head	Body	Head	Body	Head
Icing	0d	4.33	4.57	2.00	2.00	2.00	2.00	2.00	2.00	3.53	4.25
	4d	3.53	3.59	3.61	3.72	3.54	3.65	3.78	3.80	3.50	3.66
	8d	3.80	3.87	3.86	3.94	4.00	4.15	3.96	4.08	3.82	3.93
	12d	5.17	5.22	4.94	5.15	4.81	5.18	4.81	5.14	4.97	5.20
	16d	6.18	6.25	6.08	6.18	6.28	6.63	6.20	6.56	6.20	6.32
10°C	0d	4.33	4.57	2.00	2.00	2.00	2.00	2.00	2.00	3.53	4.25
	2d	3.56	3.73	3.59	3.88	3.56	3.76	3.52	3.58	3.73	3.86
	4d	3.75	4.74	3.74	3.94	3.63	3.89	3.67	3.70	3.82	4.10
	6d	4.83	4.97	4.85	5.06	4.59	4.94	4.79	4.81	4.96	5.22
	8d	5.10	5.27	5.75	6.20	4.80	5.10	5.82	5.08	5.83	6.27
	10d	6.87	6.98	6.11	6.19	6.14	6.21	6.11	5.20	6.20	6.32

chemical preservatives were somewhat effective in controlling the psychrophilic counts. These results could be correlated to the absence of histamine production as well as reduction in the amounts of the other two biogenic amines tested. Histamine formation in fish is well known to be associated with growth of bacteria possessing the enzyme histidine decarboxylase. Several histamine producing bacteria are capable of producing hazardous amounts of histamine in a very short period of time when fish are held at elevated temperatures (Taylor and Sumner, 1987).

CONCLUSION

Bacteriological quality of pond water and cultured freshwater prawns, *Macrobrachium rosenbergii,* showed an increase in counts with time and temperature of storage up to log 7 + CFU/g for mesophilic counts after 12 h at ambient conditions, 6 d at 10°C and 12 d at iced storage. All samples were completely spoiled having pinkish, black color, putrid odours, and slimy appearance at the end of storage periods for the respective temperatures. Samples from Site 2 had relatively higher counts as compared

Table 7: Psychrophillic counts of site 3 samples for body and head regions of *M. rosenbergii* during storage at different temperatures as plated on different agar media

Psychrophillic Counts (log cfu/g)											
Storage temp. time		Skimmed Milk Agar		Lysine DA		Arginine DA		Ornithine DA		Niven's Agar	
		Body	Head	Body	Head	Body	Head	Body	Head	Body	Head
Icing	0d	4.53	4.96	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
	4d	4.96	5.15	3.49	3.77	3.71	3.87	2.85	3.61	3.11	3.63
	8d	5.04	5.49	4.04	4.28	4.61	5.49	3.92	4.85	3.49	3.96
	12d	5.11	5.91	5.23	4.61	5.04	5.87	4.97	5.89	4.60	4.23
	16d	6.53	6.00	5.49	5.90	5.53	6.00	5.88	5.99	5.91	5.91
10°C	0d	4.53	4.96	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
	2d	4.61	5.04	3.61	3.69	3.36	3.75	3.61	3.73	3.49	3.92
	4d	4.80	5.91	3.76	3.89	3.78	4.85	4.04	4.49	4.23	4.63
	6d	5.85	5.97	4.78	5.04	4.86	4.96	5.53	5.78	4.49	4.89
	8d	5.97	6.23	4.90	5.28	5.11	5.61	5.68	5.86	4.86	5.96
	10d	6.04	6.59	5.97	6.04	6.15	6.87	6.23	6.57	5.04	6.00

Table 8: Psychrophilic counts of iced stored prawn body samples (log CFU/g) treated with different preservatives

Day of storage	Control	Sucrose	NaCl	$Na_2S_2O_3$	Lactic acid	Boric acid
	Log No. of bacteria (CFU/g)					
Day 0	4.23	4.23	4.23	4.23	4.23	4.23
Day 5	4.23	4.23	4.23	0	0	0
Day 10	5.04	5.01	5.07	0	0	0
Day 15	6.14	5.68	5.46	0	0	0
Day 20	6.41	6.14	6.33	0	0	0
Day 25	6.52	6.25	6.33	0	0	0

• Data are means of 12 readings from duplicate samples, 2 dilutions and triplicate plate

Table 9: Psychrophilic counts of iced stored prawn body samples (log CFU/g) treated with different preservatives

Day of storage	Control	Sucrose	NaCl	$Na_2S_2O_3$	Lactic acid	Boric acid
	Log No. of bacteria (CFU/g)					
Day 0	4.71	4.71	4.71	4.71	4.71	4.71
Day 5	4.99	4.09	4.83	0	0	0
Day 10	5.99	5.38	5.34	0	0	0
Day 15	6.49	5.87	5.61	0	0	0
Day 20	6.89	6.5	6.63	0	0	0
Day 25	6.91	6.53	6.85	0	0	0

• Data are means of 12 readings from duplicate samples, 2 dilutions and triplicate plate

to those of the other two sites which correlated well with the levels determined in their respective pond water results. Similar trends were observed for psychrophilic counts but at lower values for the different types of growth media studied.

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