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CHANGES IN TOTAL COUNTS, BACTERIAL GENERA, TRIMETHYLAMINE AND TOTAL VOLATILE BASES OF LOBSTER (PANULIRUS POLYPHAGUS) MUSCLE DURING ICE STORAGE

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Quantitative and qualitative changes in bacterial flora of twelve lobster *Panulirus polyphagus* tail muscle samples during ice storage for 16 days were studied. The total bacterial population increased from an initial count ranging from $1.0x10^2$ – 3.7×10^6 CFU/g (median 1.0×10^5 CFU/g) to 3.8×10^5 $4.3x10^9$ CFU/g (Median 1.8×10^8 CFU/g) after 16 days storage in ice. In summary, the bacterial flora of fresh lobster in order of predominance was composed of *Moraxella, Micrococcus, Vibrio, Pseudomonas,* Group II and III, *Bacillus, Flavobacterium* and *Alteromonas.* At the end of storage period the flora in order of predominance was composed of *Alteromonas, Moraxella, Pseudomonas* Group II, *Micrococcus, Bacillus* and *Pseudomonas* Group III. Bacterial flora in individual samples was also studied in relation to trimethylamine (TMA) and total volatile bases (TVB) production during storage. In most of the samples where a single or at the most two genera of organisms dominated, significant correlation was obtained between dominant flora and the values of TMA and TVB. While no correlation, between bacterial flora and TMA and TVB values was obtained in other samples particularly where mixed flora or heterogenic bacteria of the same genus dominated during storage.

Key words: Bacterial genera, Trimethyl amine.

INTRODUCTION

Lobster is one of the highly perishable seafoods available around Karachi-Makran coast and its export is an important part of the seafood industry of Pakistan. In 1985, 44 metric tons of lobsters valued at Rs. 4.406 million in foreign exchange were exported mainly in frozen form [1]. Great potential, however, exists for increasing its export in present form as well as in canned and chilled forms.

Quality changes that occur in fresh fishery products during refrigerated and/or ice storage are caused by tissue enzymes and microbial activities. Several reports are available on microbiological and/or biochemical changes in shellfish stored in ice for various lengths of time [2-12]. Fatima and Qadri [13] reported organoleptic, chemical and microbiological changes that occur during storage of lobster (*Panulirus polyphagus*) tail in ice up to 15 days simulating commercial practice. The merits of various changes as objective indices of quality, particularly in relation to taste panel assessment were discussed. Microbiological changes included the changes in bacterial counts during storage. No attempts were made to study the changes in bacterial genera. Zuberi *et al.* [12] studied the bacteriological changes in shrimp, *Penaeus merguiensis* and *Metapenaeus monoceros*, during ice storage. Changes in bacterial populations and generic composition of bacterial flora of two shrimp species were reported. However, the study was confined to bacteriological changes only and no biochemical changes were measured. In general, very little information is available on the changes in bacterial flora during ice storage of tropical lobster.

The purpose of this study was to determine in detail the bacteriological changes in tropical lobster (*P. polyphagus*) tail during ice storage and to see whether any relationship exists between the ultimate bacterial genera that emerge and the subsequent production of trimethylamine (TMA-N) and total volatile bases (TVB).

MATERIALS AND METHODS

Lobsters were obtained live from small fishing boats in Ibrahim Hydri. They were brought by fishermen who operate small boats and make short trips of about 10 hours. Lobsters were transported to the laboratory in clean cotton bags. The lobsters were killed immediately by removing their cephalothorax with a sharp knife to avoid any antimortem degradation due to stress. The abdomen was not freed from the exoskeleton but the gut was removed. The tails that were to be analysed for zero time were removed. Those for storage (shell on tails simulating commercial practice) were packed in perforated polythene bags and stored in ice in the ratio of 1:1 (w/w). The lobster tails were kept in ice in plastic container with holes in the bottom for draining, and these were put into another plastic container and kept in a refrigerator maintained at 4^o.

The lobster tails were constantly kept covered with ice during the storage period, re-iced as required, and removed at 4,8,12 and 16 days of storage for bacteriological and biochemical examination. A total of 12 samples were analysed between Dec. 1985 and Dec. 1986.

Bacteriological analysis. A 10 g portion of lobster muscle was blended with 90 ml of sterile diluent (0.1 % peptone (w/v) (Difco), 0.9 % NaCl (w/v)) for 2 min in a Waring blender. Appropriate dilutions prepared in the same diluent were spread plated in duplicate on tryptone soya agar (TSA, Oxoid). Plates were incubated at 25° for 72 hrs.

To determine microbial types 20-30 colonies were picked up at random from countable plates. Isolated colonies were streaked on TSA slants, identified as described elsewhere [14] and percentages of various genera were determined.

Biochemical analyses. The analyses of trimethylamine nitrogen (TMA-N) and total volatile bases (TVB) were performed on 5 % trichloroacetic acid extract of lobster tails by blending it for 2 min in a sample to solvent ratio of 1:3.

Trimethylamine (TMA-N) was estimated by Dyer's picric acid procedure [15], as modified from Dyers [15] procedure by Hoogland [16].

Total volatile bases (TVB) was determined according to Cobb *et al.* [17].

RESULTS AND DISCUSSION

Table 1 shows the changes in bacterial counts during ice storage of lobster tail. The counts of fresh tail ranged from $1.0x10^2$ to $3.7x10^6$ colony forming units (CFU)/g (Median 1.0×10^5 CFU/g). In general the counts increased with the time of storage. After 4 days the count was $1.1x10^2$ to $2.5x10^8$ CFU/g (Median $1.8x10^5$ CFU/g) showing slight to one log decrease in 6 out of 12 samples. The remaining six samples showed slight to more than one log increase in bacterial numbers. This increase and decrease in bacterial counts may be due to the nature (psychrotrophic and/or mesophilic) of initial flora. After 8 days storage increase in bacterial count was observed only in those samples in which a decrease was obtained after 4 days storage. In samples where an increase was obtained after 4 days storage, amost the same count persisted with very little variation after 8 days storage. After 12 days storage the count ranged from $2.4x10^4$ to $1.5x10^9$ CFU/g (Median $2.1x10^7$ CFU/g) showing a log increase in most of the samples. After 16 days storage further increase in count was noted in most of the samples and the count ranged from $3.8x10^5$ to $4.3x10^9$ CFU/g (Median $1.8x10^8$ CFU/g). At this stage a stale odour developed in lobster tail, the colour became dirty yellow and/or black with slime at the surface.

Table 1. Changes in bacterial count/g of lobster (*Panulirus polyphagus*) during ice storage.

Expt. S. No.	Months 1985-86	Days of storage 0	4	8	12	16
1.	Dec.	$1.8x10^6$	$2.5x10^6$	$1.0x10^8$	$1.1x10^8$	$9.0x10^8$
2.	Jan.	$3.2x10^5$	$1.1x10^7$	$1.2x10^7$	$9.1x10^7$	$3.1x10^8$
3.	Mar.	$3.7x10^6$	$1.1x10^6$	$4.7x10^6$	$3.7x10^8$	$1.5x10^9$
4.	Apr.	$5.1x10^5$	$1.8x10^5$	$4.0x10^6$	$2.9x10^7$	$3.4x10^8$
5.	May	$2.5x10^6$	$2.5x10^8$	$3.2x10^8$	$1.5x10^9$	$4.3x10^9$
6.	Jun	$1.0x10^5$	$5.4x10^6$	$4.4x10^6$	$1.2x10^7$	$1.8x10^8$
7.	Aug.	$1.1x10^4$	$4.5x10^5$	$1.2x10^6$	$2.1x10^7$	$1.8x10^8$
8.	Sep.	$1.7x10^4$	$2.4x10^3$	$2.2x10^4$	$1.3x10^5$	$3.8x10^5$
9.	Oct.	$1.6x10^4$	$2.0x10^3$	$3.6x10^4$	$5.0x10^5$	$5.0x10^6$
10.	Nov.	$5.4x10^4$	$1.8x10^5$	$1.3x10^6$	1.610^6	$4.5x10^6$
11.	Dec.	$5.4x10^2$	$1.1x10^2$	$0.2x10^2$	$2.4x10^4$	$6.6x10^7$
12.	Dec.	$4.1x10^5$	$1.9x10^5$	$1.4x10^6$	$3.1x10^7$	$1.0x10^8$

Table 2 summarizes the changes in the generic composition of bacterial flora of 12 lobster samples during ice storage for 16 days. The microbial flora of fresh lobster in order of predominance was composed of *Moraxella, Micrococcus, Vibrio, Pseudomonas* Group II and III' *Bacillus, Flavobacterium* and *Alteromonas.*

The microbial flora of lobster during storage showed a noticeable increase in population of *Moraxella* sp. The predominant position of this group remained up to 12 days of storage. They also persisted as a second predominant group of organisms on 16 days storage.

Micrococci made up a major proportion of the flora of fresh samples. These organisms gradually decreased as the storage progressed but remained in low proportion throughout the storage.

Pseudomonas Group II and III were often present on fresh samples. *Pseudomonas* Group II increased as the storage progressed and becoming the third dominating organism at the end of storage. However, *Pseudomonas* Group II decreased after 8 days storage.

The genus *Alteronomas* appeared in very low proportion on fresh lobster increased gradually with the storage, appeared in appreciable numbers after 8 days and becoming the most dominating organism after 16 days of storage. At the end of storage period the flora in order of predominance was composed of *Alteromonas, Moraxella, Pseudomonas* Group II, *Micrococcus, Bacillus* and *Pseudomonas* Group III.

During ice storage the genus *Vibrio, Flavobacterium* and *Bacillus* disappeared. These findings, and behaviour of

organisms during storage, are in agreement with those reported for ice stored shrimp [12].

In general, the bacterial populations obtained on fish lobster tail muscle were low if compared with those found in shrimp [12]. This difference may be attributed to the difference in handling of lobster and shrimp. Lobsters were brought alive to the laboratory and were killed before the start of experiment. Shrimp die soon after harvest and only dead shrimp are available for storage. This postmortem commercial handling prior to storage is not involved in the case of lobster. The strong lobster shell may also protect lobster tail muscle from contamination compared to shrimp.

Another aspect of this study was to correlate changes in TMA-N and TVB with changes in bacterial flora during ice storage. There are several reports available which indicate that TMA and TVB production during spoilage of fishery products is due to bacterial action [18-24].

Table 3 shows changes in TMA-N and TVB level of 12 lobster samples during ice storage for 16 days. Marked differences in TMA-N and TVB level existed between different samples at the commencement of storage. The values ranged from nil to 0.55mg/100g and from 9 to 17mg/100g for TMA-N and TVB respectively. TMA was high in samples 3,5,6 and 8 ranged from 0.35 to 0.55 mg/100g. In rest of the samples it ranged from nil to 0.3 mg/100g. It may be seen that in samples with high initial value, TMA-N increased rapidly with the time throughout the storage and ranged from 4 to 5.5 mg/100g at the end of storage period of 16 days. Relatively less increase was obtained in rest of the samples during storage. It ranged from 0.1 to 3.1 mg/100g among different samples. No such variation was observed in the intial TVB values among different samples. It ranged from 8.5 to 17.1 mg/100g irrespective of TMA content.

Changes in percentage distribution of bacterial flora of twelve lobster samples during ice storage are presented in Fig. 1. In general, the initial flora of lobster was found to be closely related, both quantitatively and qualitatively to

Table 2. Summary of changes in percentage of bacterial flora of lobster (*Panulirus polyphagus*) during ice storage.

Days of storage	*Pseudomonas* II	*Pseudomonas* III	*Alteromonas*	*Vibrio*	*Micrococcus*	*Moraxella*	*Bacillus*	*Flavobacterium*
0	11.7	10.8	0.9	19.1	20.5	29.9	6.0	1.1
4	19.6	9.5	7.5	9.5	16.3	32.0	5.9	0.0
8	11.4	11.4	17.6	0.0	13.0	46.6	0.0	0.0
12	28.4	3.7	27.7	0.0	2.7	37.5	0.0	0.0
16	27.5	0.7	36.6	0.0	4.2	28.8	2.2	0.0

Table 3. Changes in TMA, TVB of lobster (*Panulirus polyphagus*) during ice storage.

Expt. S.No.	Months 1985-86	Days of Storage 0		4		8		12		16	
		TMA-N mg/100g	TVB mg/100g	TMA-N mg/100g	TVB mg/100g	TMA-N mg/100g	TVB mg/100g	TMA-N mg/100g	TVB mg/100	TMA-N mg/100g	TVB mg/100g
1	Dec.	nil	17.1	nil	24.5	nil	29.4	0.05	43.12	0.1	55.1
2	Jan.	0.25	14.8	0.4	15.5	0.8	22.5	1.3	43.5	1.66	59.5
3	Mar.	0.5	10.55	1.0	14.75	2.5	19.5	4.0	22.5	4.85	24.75
4	Apr.	0.3	12.5	0.55	14.5	0.95	21.25	2.1	30.3	3.1	37.5
5	May	0.35	10.55	0.9	11.5	1.5	19.5	2.8	32.5	4.0	40.5
6	Jun.	0.55	11.5	0.95	16.5	1.3	19.75	3.05	28.5	4.1	40.5
7	Aug.	0.1	9.0	0.3	14.5	0.8	18.5	1.25	22.5	2.75	25.77
8	Sep.	0.45	11.5	0.75	17.5	0.95	22.5	3.1	33.5	5.5	40.5
9.	Oct.	0.01	9.3	0.01	14.4	0.01	14.5	0.5	17.5	0.55	18.5
10	Nov.	nil	11.0	nil	15.0	0.15	17.0	0.2	18.5	0.3	20.0
11	Dec.	0.1	12.5	0.45	16.0	0.65	18.0	0.75	19.5	0.9	21.5
12	Dec.	0.1	8.5	0.45	11.5	0.8	18.5	0.9	23.0	0.95	31.0

the environment. Wide variations, in the type of flora were however notable in individual samples. The increase and decrease in TMA and TVB appeared to have some bearing on the development of dominant organisms during storage. Referring to sample 1 (Table 3) the TMA level remained almost constant throughout the storage, whereas TVB increased sharply and reached a level of 55.1 mg/100 g after 16 days storage. In this sample the dominating organism was *Pseudomonas* Group II (Fig. 1). Somewhat similar results were observed for sample No. 2. A sharp increase in TVB coincided well with the appearance of *Pseudomonas* Group II after 12 days storage. A value of 59.5 mg/100 g of TVB was obtained after 16 days storage. In this case also, TMA did not increase much and reached to a level of 1.6 mg/100 g after 16 days storage.

It may be noted that in sample No. 9 and 10, both TMA and TVB did not increase much and remained in low acceptable limits throughout the storage. In these experiments dominating flora, throughout the storage, was *Moraxella* and *Micrococusus* (Fig. 1, sample 9 and 10). The spoilage potential of *Alteromonas putrefaciens* is well documented [23,25]. Generally, *A. putrefaciens* was not encountered in fresh lobster and only one of 12 samples was found positive for this organisms. They appeared in appreciable numbers after 8 days of storage and dominated the flora at the end of storage. In samples No. 3, 5 and 6 these organisms appeared as the most dominating organisms after 8-12 days storage and both TMA and TVB increased after 16 days storage in samples 5 and 6. However, sample No. 3 showed comparatively lower level of TVB. Laycock and Regier [19] reported similar observation that there was a linear relationship between the number of *Pseudomonas putrefaciens* and TMA production on haddock fillets during refrigerated storage. It has been reported that 80 % of all the TMA producing organisms represented this species.

It was observed in previous studies that *Alteromonas putrefaciens, Pseudomonas* Group II and III, *Moraxella* and

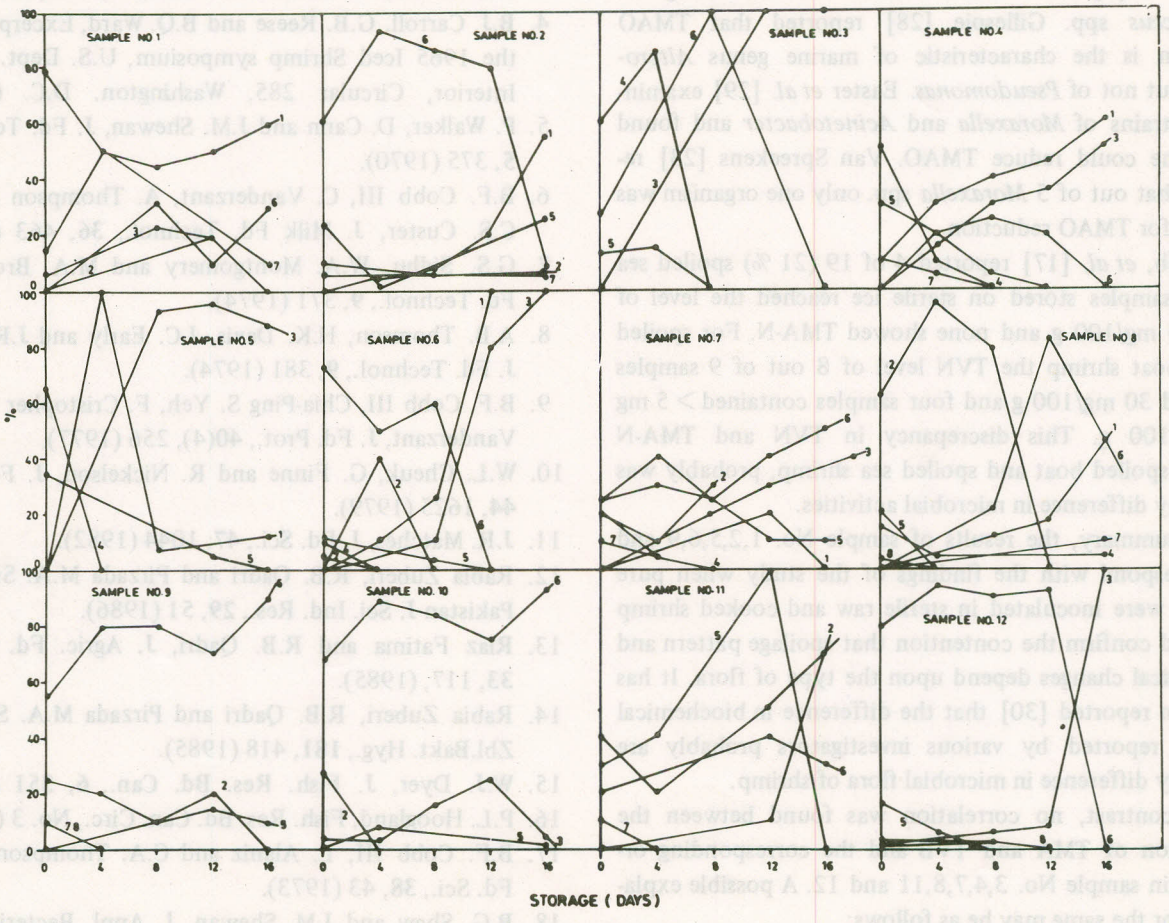

Fig. 1. Changes in percentage distribution of bacterial flora of lobster (*Panulirus polyphagus*) during ice storage.
1 = *Pseudomonas* Group II, 2 = *Pseudomonas* Group III, 3 = *Alteromonas*, 4 = *Vibrio*, 5 = *Micrococcus*, 6 = *Moraxella*, 7 = *Bacillus* and 8 = *Flavobacterium*.

Micrococcus dominate during ice storage of shrimp [12]. In another study where pure cultures were inoculated in sterile raw and cooked shrimp muscles, a sharp increase in TMA and TVB was observed after 14 days of storage at 0° with the inoculation of *Alteromonas* strains, *Pseudomonas* Group II also showed increase in TVB but the TMA value remained almost constant. *Moraxella, Micrococcus* and *Pseudomonas* Group III did produce some changes but not to the extent of *Alteromonas* and *Pseudomonas* Group II [24].

Although these results were obtained during studies of pure culture inoculation in sterile shrimp muscle, the dominating organisms in some samples also behaved in a strikingly similar fashion during ice storage of lobster tail.

Similar to the findings of this study, a variable nature of biochemical potential of different bacterial species has been reported by different workers in many studies. Baird and Wood [6] could not find a single TMAO reducing strain among 104 tested species of *Micrococcus*. Similarly, Lerke *et al.* [7] also found no TMAO reduction among 159 *Micrococcus* spp. Gillespie [28] reported that TMAO reduction is the characteristic of marine genus *Alteromonas* but not of *Pseudomonas*. Easter *et al.* [29] examined 28 strains of *Moraxella* and *Acinetobacter* and found that none could reduce TMAO. Van Spreekens [23] reported that out of 5 *Moraxella* spp. only one organism was positive for TMAO reduction.

Cobb, *et al,* [17] reported 4 of 19 (21 %) spoiled sea shrimp samples stored on sterile ice reached the level of TVN 30 mg/100 g and none showed TMA-N. For spoiled brown boat shrimp the TVN level of 8 out of 9 samples exceeded 30 mg/100 g and four samples contained > 5 mg TMA-N/100 g. This discrepancy in TVN and TMA-N level of spoiled boat and spoiled sea shrimp, probably was caused by difference in microbial activities.

In summary, the results of sample No. 1,2,5,6,9 and 10 correspond with the findings of the study when pure cultures were inoculated in sterile raw and cooked shrimp [24] and confirm the contention that spoilage pattern and biochemical changes depend upon the type of flora. It has also been reported [30] that the difference in biochemical changes reported by various investigators probably are caused by difference in microbial flora of shrimp.

In contrast, no correlation was found between the production of TMA and TVB and the corresponding organisms in sample No. 3,4,7,8,11 and 12. A possible explanation for the same may be as follows:

During storage where mixed flora or flora of different species of same genus develop results in the interaction of numerous factors steming from concurrent or successive activities of various micro-organisms. It is, therefore, very difficult to corelate biochemical changes with bacterial flora. Tissue enzymes and microbial activites of different bacteria makes it a very complex process.

The results of this study demonstrate that if a single or at the most two different genera of organisms dominate during storage, they behave similar to pure cultures inoculated in shrimp [24] showing the characteristic biochemical change. On the other hand where more than two genera share the dominating flora, no characteristic biochemical change is visible possibly due to interaction of numerous factors mentioned earlier.

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