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EFFECT OF ELEVATED TEMPERATURE OF STORAGE ON THE BACTERIOLOGICAL QUALITY OF TROPICAL SHRIMP (*PENAEUS MERGUIENSIS*)

Rabia Zuberi, Seema Ismat Shamshad and R. B. Qadri

PCSIR Laboratories, Off University Road, Karachi-39

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Changes occurring in bacterial populations and in generic composition of bacterial flora of shrimp (*Penaeus merguiensis*) during storage in ice (0°), 10° and 22-30° (room temperature) were studied.

The total bacterial population increased from an initial count ranging from 5.5×10^5 to 4.7×10^7 CFU/g to 10^9 CFU/g after 16 days of storage in ice (0°), $> 10^9$ CFU/g after 12 days at 10° and to $> 10^{10}$ CFU/g after 24 hr. at room temperature (22 -30°).

At the end of the storage period the dominant organisms in shrimp during ice storage (0°) were *Alteromonas, Moraxella, Pseudomonas* Groups II and III and *Micrococcus.* Genus *Micrococcus* dominated at 10° storage together with the flora found at 0° storage. At room temperature (22 -30°) the mesophilic flora composed of *Vibrio, Pseudomonas* Group III, *Bacillus* and *Micrococcus* was dominant.

Key words: Storage; Tropical shrimp; Bacteriological quality.

INTRODUCTION

It is now well recognised that marine catch after hervest must be properly stored in ice as early as possible to prevent rapid bacterial growth which is one of the major causes for the deterioration of quality during subsequent storage. In commercial situations, however, this is not practicable.

Trawling in Pakistan tropical waters normally takes about 10 hr. A shrimp trawl contains shrimp, small fish and other marine animals. Before icing, shrimp are separated from the trash fish and are usually deheaded. Thus, icing which is of vital importance is undertaken usually after several hours of the catch. During this period the shrimp may be held at ambient temperatures ranging from 15°-40°. Furthermore, during handling ashore and in the processing factories, improper ice storage may also be a source of bacterial multiplication. In any event, where shrimp are allowed to remain warm ambient temperatures or poorly iced, degradative changes begin to take place rapidly. The rate of these changes, as may be expected, is temperature dependent.

The effect of high temperature storage on shrimp quality has been investigated by many workers [1,2,3]. Almost all these studies are based on cold water shrimp species. In a previous study [5] these authors reported the changes in bacteriological quality of shrimp (*P.merguiensis*) stored at ice temperature. The purpose of the present investigation was to determine the effects of holding at higher temperatures on the bacteriological quality of shrimp and reports both qualitative and quantitative changes at temperatures which may be encountered during commercial practice in Pakistan. For comparison shrimp were also stored in ice.

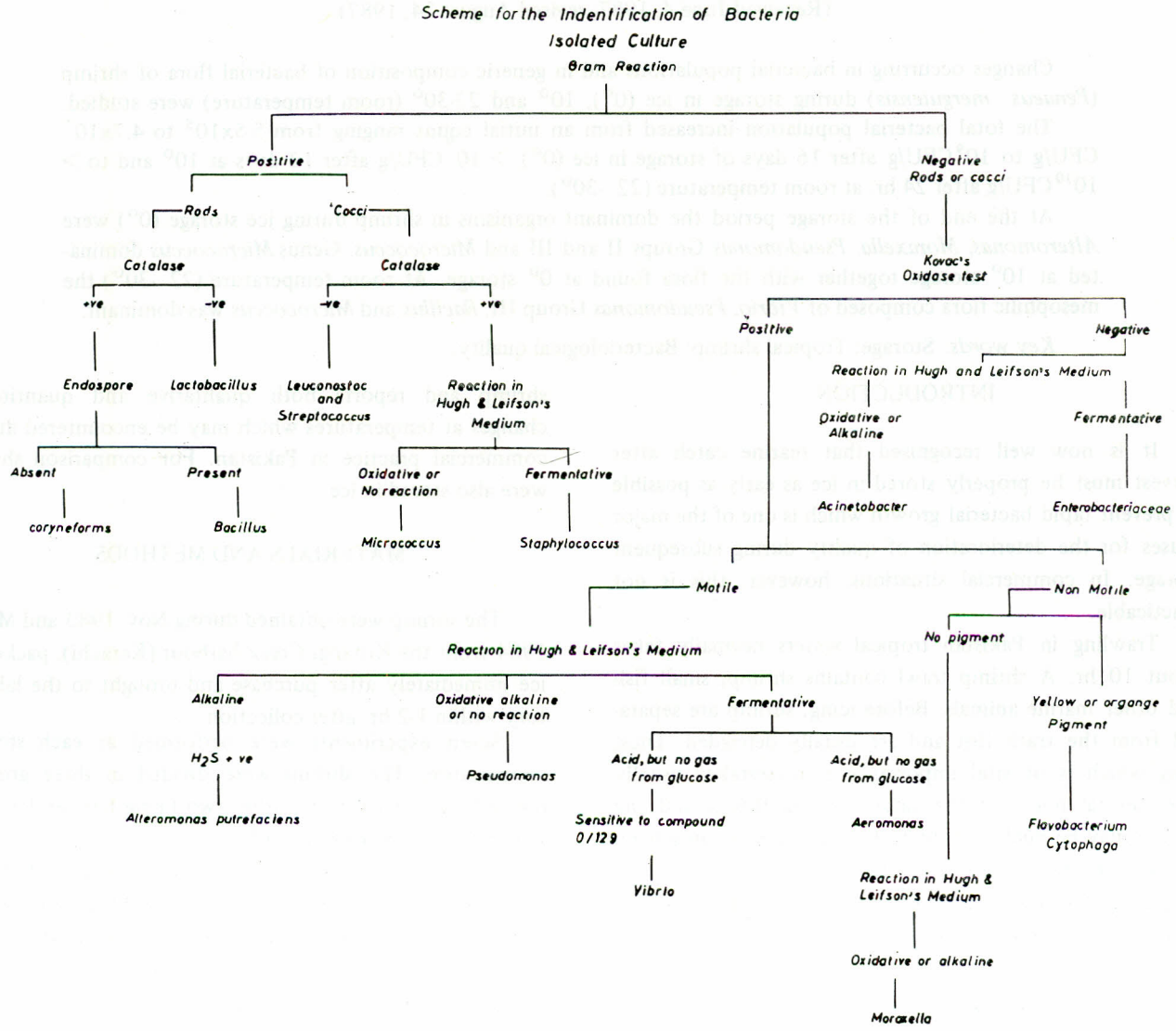
MATERIALS AND METHODS

The shrimp were obtained during Nov. 1983 and March 1984 from the Korangi Creek harbour (Karachi), packed in ice immediately after purchase and brought to the laboratory within 1-2 hr. after collection.

Seven experiments were performed at each storage temperature. The shrimp were divided in three groups: one held in ice (0°), the other two being held at 10° and at room temperature (22 -30°).

For ice storage the deheaded shrimp were distributed in perforated polythene bags containing shrimp tails weighing 75-100 g. One set of five bags was covered with sterile crushed ice and stored in the refrigerator for 16 days. The shrimp were constantly checked for ice during storage and reiced when required. The other set of five bags was stored at 10° in a cold incubator. For room temperature storage, the shrimp samples were kept in covered sterile petridishes. The samples stored in ice (0°) and at 10° were examined bacteriologically after 2,4,8,12 and 16 days of storage. Those stored at room temperature were analysed after 6 and 24 hr. Fifty g. of shrimp sample from each storage were removed and blended with a 450 ml diluent (0.1 % peptone + 0.85 % NaCl) for 1-2 min. in a waring blender. Aerobic plate counts were determined with the spread

plate method by placing 0.1 ml of appropriate dilutions on nutrient agar (Merck) + 1 % NaCl. For each experiment, duplicate series of plates were incubated at 25° for 48 to 72 hr. About 20-30 colonies were picked up at random from appropriate plates for each sample.Isolated colonies were streaked on nutrient agar slant with 1 % NaCl, identified to genus level as described previously [4] and according to the scheme given below.



RESULTS AND DISCUSSION

Quantitative aspects. Table 1 shows the changes in bacterial count during ice storage. The initial counts ranging from $5.5x10^5$ to $4.7x10^7$ CFU/g increased with the time of storage and ranged between $2.1x10^8$ to $7.8x10^9$CFU/g at the end of storage period. In general the changes were similar to those previously reported [5].

Table 2 shows the changes in bacterial counts during storage at 10°. As expected, rapid increase in counts was observed after 4 days of storage as compared to 0° (Table 1), confirming the contention that the higher is the temperature, the more rapid is the growth and shorter shelf-life. After 12 days' storage all the samples were completely spoiled. The bacterial count reached $> 10^9$CFU/g in most of the samples. The experiments demonstrate that shrimp may be stored for 4 days at 10° without any serious loss in quality and the same level of quality may maintained in the shrimp stored for 8-10 days in ice.

Table 3, shows the changes in the bacterial counts at room temperature (22 -30°). After 6 hr. of storage the counts ranged from $4.3x10^6$ to $9.3x10^7$CFU/g showing one log increase in most of the samples. The increase was almost the same as noticed in ice after 4-5 days. After

Table 1. Changes in the bacterial count of shrimp Jaira (*Penaeus merguiensis*) stored at 0°

Experiment No.	TPC/g	Days of storage				
	0	2	4	8	12	16
1	6.3×10^5	*X	4.2×10^5	1.4×10^6	1.7×10^7	7.8×10^9
2	1.2×10^6	8.0×10^6	1.5×10^7	3.9×10^7	9.4×10^8	9.9×10^8
3	9.9×10^6	1.7×10^7	3.4×10^7	5.0×10^8	3.1×10^{10}	7.3×10^9
4	9.8×10^6	2.1×10^7	1.4×10^7	1.3×10^7	7.8×10^8	4.5×10^9
5	4.7×10^7	7.1×10^7	9.6×10^7	3.2×10^8	5.0×10^9	3.2×10^9
6	5.5×10^5	6.2×10^5	7.8×10^6	1.9×10^7	2.6×10^8	2.1×10^8
7	1.4×10^6	8.5×10^5	7.2×10^5	3.5×10^7	6.3×10^8	1.1×10^9

*Not done

Table 2. Changes in the bacterial count of shrimp Jaira (*Penaeus merguiensis*) stored at 10°.

Experiment No.	TPC/g	Days of storage				
	0	2	4	8	12	16
1	6.3×10^5	*X	9.6×10^5	1.0×10^7	4.3×10^8	2.5×10^{10}
2	1.2×10^6	2.1×10^7	1.3×10^8	1.3×10^9	1.5×10^9	3.9×10^9
3	9.9×10^6	9.3×10^7	9.7×10^7	7.2×10^8	2.6×10^9	4.0×10^8
4	9.8×10^6	2.2×10^6	4.6×10^7	8.3×10^8	1.1×10^9	2.7×10^9
5	4.7×10^7	3.8×10^7	5.8×10^9	3.4×10^9	5.5×10^9	5.2×10^9
6	5.5×10^5	6.5×10^5	6.5×10^7	5.0×10^8	3.0×10^8	2.5×10^8
7	1.4×10^6	3.2×10^7	4.6×10^8	1.7×10^9	3.7×10^9	8.6×10^8

*Not done

Table 3. Changes in the bacterial count of shrimp Jaira (*Penaeus merguiensis*) stored at room temperature (22 -30°).

Experiment No.	TPC/g Time of storage		
	0 hr.	6 hr.	24 hr.
1	2.3×10^6	1.5×10^7	3.8×10^{10}
2	6.3×10^5	4.3×10^6	1.4×10^9
3	1.2×10^6	7.6×10^6	1.2×10^9
4	9.9×10^6	7.2×10^7	2.3×10^{10}
5	9.8×10^6	5.3×10^7	6.4×10^{10}
6	4.7×10^7	9.3×10^7	1.6×10^9
7	5.5×10^5	3.3×10^7	5.9×10^{10}

24 hr, very rapid increase in counts was noticed. Most of the samples had a bacterial load of $> 10^{10}$ CFU/g (Table 3). All the samples were completely spoiled having pinkish black colour, putrid odours and slimy appearance. Similar changes were observed after 16-20 days in ice.

Qualitative aspect. Table 4 summarizes the changes in the generic composition of 7 samples of the shrimp during ice storage at 0,2,4,8,12 and 16 days of storage. The microbial flora of fresh shrimp in order of predominance was composed of *Moraxella, Pseudomonas* Group III, *Vibrio, Micrococcus, Flavobacterium, Bacillus,* and *Pseudomonas* Group II. The percentage composition of the flora of fresh shrimp used in these experiments was found to be somewhat different from the flora reported [5] and may be attributed to the difference in sampling time. However, the behaviour of the organisms was similar as already reported [5].

The genus *Vibrio* and *Flavobacterium* disappeared and *Alteromonas* and *Pseudomonas* Group II gradually increased with storage (Table 4). *Moraxella* and *Pseudomonas* Group III dominated both in fresh shrimp and throughout the storage. These organism being psychrotrophic in nature found favourable conditions during ice storage and multiplied at a more rapid rate than the other organisms. This is in agreement with the findings of Cobb and Vanderzant [6] that the spoilage pattern of shrimp depends upon the initial microflora, because usually the same groups of organisms are present during spoilage as are found on freshly caught shrimp. The percentage distribution of organisms, however, alters markedly during storage and the shrimp spoils as a result of increased microbial activity. It may be seen (Table 4) that in these experiments also, the behaviour of *Micrococcus* and *Bacillus* was similar as reported earlier [5].

Table 5 summarizes the changes in the generic composition of the bacterial flora during 10° storage. From the initial flora similar to the samples stored at 0°, somewhat different flora emerged during storage as a result of elevated storage temperature. The genus *Vibrio* increased up to 4 days and then persisted throughout the storage in low proportions in contrast to ice storage, while both *Vibrio* and *Flavobacterium* disappeared during early storage. *Alteromonas putrefaciens* behaved differently if compared with ice storage. This organism appeared after 8 days of storage and persisted throughout the storage in low proportion. *Pseudomonas* Group III persisted in a higher proportion up to 12 days of storage. *Moraxella* was present throughout the storage in a low proportion if compared with the ice stored samples (Table 4). In contrast to the 0° storage, at 10° the genus *Micrococcus* gradually increased as the storage progressed and dominated the flora at the end of storage (Table 5). At the end of storage period the flora in order of predominance was composed of *Micrococcus, Moraxella, Pseudomonas* Group III, *Vibrio, Bacillus, Flavobacterium* and *Alteromonas.* The *Moraxella* group was replaced by the genus *Micrococcus* at 10° storage.

Table 6 summarizes the changes in the generic composition of bacterial flora during storage at room temperature. No significant qualitative changes in the flora were

Table 4. Summary of changes in percentage distribution of bacterial flora of shrimp Jaira (*Penaeus merguiensis*) during storage at 0°.

Days of storage	*Pseudomonas* Group II	*Pseudomonas* Group III	*Vibrio*	*Moraxella*	*Alteromonas*	*Flavobacterium*	*Micrococcus*	*Bacillus*
0	2.9	27.15	23.2	32.0	0.0	4.45	6.2	3.8
2	0.0	33.6	8.6	26.3	1.6	13.47	16.0	0.28
4	o.0	56.6	0.0	24.0	0.0	9.5	9.0	0.83
8	5.3	48.0	0.0	28.5	4.1	0.0	13.0	1.6
12	16.8	32.2	0.0	24.1	18.1	0.0	8.6	0.0
16	18.2	28.0	0.0	14.2	28.4	0.0	11.2	0.0

Table 5. Summary of changes in percentage distribution of bacterial flora of shrimp Jaira (*Penaeus merguiensis*) during storage at 10°.

Days of storage	*Pseudomonas* Group II	*Pseudomonas* Group III	*Vibrio*	*Moraxella*	*Alteromonas*	*Flavobacterium*	*Micrococcus*	*Bacillus*
0	2.9	27.1	23.2	32.0	0.0	4.45	6.2	3.8
2	3.0	21.8	36.1	10.5	2.0	0.0	20.3	6.1
4	6.0	28.8	32.3	11.1	0.0	1.3	16.8	3.5
8	8.8	27.1	14.1	5.5	7.5	0.0	25.6	10.8
12	0.0	21.0	13.0	20.8	3.3	2.1	31.6	8.0
16	0.0	10.6	10.0	12.6	3.0	4.0	49.4	5.8

Table 6. Summary of changes in the percentage distribution of bacterial flora during storage of shrimp Jaira (*Penaeus merguiensis*) at room temperature (22-30°).

Storage time	*Pseudo-monas* Group II	*Pseudo-monas* Group III	*Vibrio*	*Moraxe-lla*	*Micrococcus*	*Flavobac-terium*	*Bacillus*
Fresh	2.9	27.15	23.2	32.0	6.2	4.45	3.8
6 hr.	0.0	25.0	36.6	20.6	13.2	2.0	2.6
24 hr.	0.0	24.4	36.4	0.0	7.4	0.0	21.8

observed after 6 hr. storage at room temperature (22 - 30°). Organisms initially present were observed with slight variations in percentage. However, *Vibrio* increased if initially present. Generally the percentage of *Moraxella* decreased and *Micrococcus* increased while *Pseudomonas* Group III persisted almost in the same proportion. After 24 hr storage mesophilic *Vibrio, Pseudomonas* Group III, *Bacillus* and *Micrococcus* dominated the flora and seem to play an important role in the spoilage of shrimp at room temperature (Table 6).

Shrimp are an excellent medium for the growth of bacteria. Fresh shrimp 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 shrimp within 24 hr. One log increase in the bacterial count after 6 hour at room temperature in most of the samples is of great significance since during commercial practice in Pakistan, the shrimp remain at room temperature for hours without ice at the harbour before auction which results in loss in quality. Therefore, a major amount of the catch reaches the consumers and processing plants in an inferior condition. Rapid bacterial multiplication has been reported at elevated temperatures by other workers. Fieger, Bailey and Novak [1] reported that the bacterial count of shrimp exposed to air temperature (25°-29°) for 6 hr was twice as compared to freshly caught shrimp. However, this initial change was found to exercise a profound effect on bacterial counts compared to controls. Cobb *et al.* [2] reported that the handling of shrimp for 3 hr at 30°-44° caused serious quality deter-

ioration and in general the level of deterioration was comparable with the shrimp held for 6 hr at 20° and 24 hr at 10°.

The results of this study demonstrate that in the storage of shrimp at 0, 10 and 22-30°, the changes in initial flora of fresh samples depend upon the temperature of storage, their appearance, disappearance and changes in percent composition are however, temperature dependent. Similar findings have been reported by Matches [3]. It may be concluded that organisms which dominate and may be considered responsible for the spoilage of shrimp during storage in ice (0°) are *Alteromonas, Moraxella, Pseudomonas* Groups II and III and *Micrococcus.* At 10° storage more mesophilic organisms such as *Micrococcus* persist along with the organisms already mentioned during ice storage and they spoil the shrimp earlier than the ice stored samples. The same may be said for the samples left at room temperature (22-30°) where mesophilic flora composed of *Vibrio, Pseudomonas* Group III, *Bacillus* and *Micrococcus* appears to be responsible for spoilage.

An examination of the data presented enable a number of observations to be made.

The multiplication of organisms occurs at all three storage temperature and the rate of the growth of organisms is temperature dependent. In this study, shrimp were considered spoiled after 12 days in ice (0°), 4-8 days at 10° and between 6 and 24 hr at room temperature. Rapid deterioration and short shelf life of shrimp at 10° clearly indicate that this is not an efffective temperature for the storage of shrimp. The results, therefore demonstrate the need of maintaining as low a temperature as possible during the commercial handling of shrimp. These requirements are in contrast to those found in commercial handling of our shrimp which are handled generally without ice at ambient temperatures.

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