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Extension in the Shelf Life of Shrimp (*Penaeus merguiensis*) Using Cell Free Supernatant of Lactic Culture

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Changes in the sensory quality of raw and cooked shrimp *Penaeus merguiensis*, total bacterial counts, total volatile bases (TVB) and pH were determined in shrimp treated with the lactic culture. The sensory score point averages of treated and untreated samples were found significantly different (p values 0.014 and 0.042 for raw and cooked shrimp, respectively). The average initial bacterial load of fresh shrimp decreased immediately after treatment with the lactic culture. During ice storage, the counts increased with time in the treated samples as compared to the control. Statistical analyses showed significant differences in the counts of treated and untreated samples (p value 0.021) during the storage period. The pH increased with time and reached to 7.43 and 7.85 in treated and untreated samples, respectively. The shelf life of the treated samples was extended, at least, by one week.

Key words: Lactic culture, Shelf life, Shrimp, *Penaeus merguiensis*, LAB.

Introduction

All fishery products are inherently perishable. They get spoiled quickly unless they are properly stored, close to ice temperature (0°C). Even at this temperature their shelf life is relatively short. The reason for such a short shelf life is the enzymes and the presence of a large number of bacteria on freshly harvested fish and shrimp. Freshly caught fish and shrimp commonly have initial bacterial counts of approximately 10^4 to 10^5 per gram (Campbell and Williams 1952; Vanderzant *et al* 1970; Zuberi *et al* 1985, 1987; Shamshad *et al* 1990) and shelf life on a fishing vessel is expected to be 12 to 14 days during ice storage (Cobb *et al* 1976; Zuberi *et al* 1986, 1987; Shamshad *et al* 1990). Reduction in the initial bacterial population on freshly caught fish and shrimp could substantially increase the expected shelf stability.

Besides storage at low temperatures, the use of chemical preservatives such as potassium sorbate and sodium benzoate is a common practice in the fish industry (Einarsson and Lauzon 1995; Yamagata and Low 1995). Due to the increasing awareness of the health hazards of such chemical preservatives, the fish industry is looking for other safe preservation methods. Bio-preservation using lactic acid bacteria (LAB) may serve as an alternative to chemical preservation. Antagonistic actions of lactic acid bacteria (LAB) toward food borne pathogens and spoilage microorganisms have been well documented (Tagg *et al* 1976; Gilliland and Speck 1977; Harris *et al* 1989; Gonzales *et al* 1993; Klaenhammer 1993; Vandenbergh 1993; Einarsson and Lauzon 1995; Jack *et al* 1995; Kato *et al* 1999). Because of their long time association with humans and their food supply, interest has been increasing in examining LAB for the production of antimicrobial substances that could extend the shelf life and improve the margin of safety in food products.

The fish industry of Pakistan mainly thrives on the export of fishery products, especially shrimp. The present study was undertaken with the objective of extending the shelf life of fishery products, in general, and shrimp in particular using LAB and/or their metabolites.

Materials and Methods

Preparation of cell-free supernatant of LAB cultures. A number of LAB strains isolated earlier from different foods and fermented food products were screened for their antibacterial activity against a range of organisms of public health significance and fish/shrimp spoilage flora. Most of these LAB strains were found to be antagonistic to spoilage bacteria of fishery products. The most potent and broad spectrum strain, selected for storage experiments, was identified as *Lactobacillus plantarum* and was isolated from fermented rice. The antagonistic activity of this organism was found to be due to a combination of different metabolites including bacteriocin, as it inhibited a number of organisms of public health significance and fish/shrimp spoilage flora even after negating the effects of other metabolites. This LAB strain was grown overnight at 30°C in 1 litre MRS broth (Oxoid). The pH of the broth culture was determined using a pH meter (Accumet pH meter 915). The supernatant was centrifuged at 10,000 rpm

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for 10 mins (IEC B-20A) at 4°C to get cell-free supernatant of the culture, which was then stored in a chiller at a temperature around 0°C.

Preparation of shrimp samples. Freshly caught shrimp were packed in ice immediately after purchase and were brought to the laboratory within 1-2 h. Deheaded shrimp were divided into two lots. One lot was kept untreated and the other was treated with the cell-free supernatant of the broth culture of *L. plantarum* by immersing the sample in it for 5 minutes. The pH of the broth culture was around 0.4. Shrimp tails were removed from the liquid and distributed into 6-7 clean perforated polythene bags. Approximately 50 g of treated shrimp tails were kept in each bag. Untreated sample was also distributed into clean perforated polythene bags. Both sets were kept in a refrigerator (4°C) covered with ice for the entire period of storage. The shrimp were checked daily for ice during storage and re-iced when required. Bacteriological, biochemical, and organoleptic analyses were done after 0, 1, 2, 3, and 4 weeks of storage. Bacteriological and sensory analyses were done immediately after removing the samples from storage whereas, the samples for biochemical analyses were kept frozen (at -30°C) and were examined later, at a convenient time.

Bacteriological analyses. For bacteriological analysis, 10 g samples were blended in 90 ml of diluent (0.1% peptone + 0.85% NaCl) for 1-2 mins in a Waring blender. Aerobic plate counts and LAB counts were determined with the spread plate method by placing 0.1 ml of appropriate dilutions on Tryptic Soy Agar (TSA, Difco) and MRS agar (Oxiod), respectively. Plates of TSA were incubated at 25°C and that of MRS agar, at 30°C for 24 - 48 h.

Biochemical analyses. TVB were determined according to the method of Cobb *et al* (1976). Shrimp muscles (20 g) were homogenized with 100 ml 7% trichloroacetic acid (TCA) for 1 min and filtered through Whatman No.1 filter paper. The filtrates were used for the determination of TVB.

For the determination of pH, 10 g of the samples were homogenized in 20 ml distilled water and pH was measured using an Accumet pH meter (Fischer Scientific, Model 915).

Sensory analyses. Sensory analyses were done by a panel of eight judges. All prospective panelists were trained in evaluation of fishery products. Panelists were screened, and those who did not like shrimp and/or had food allergies were excluded. Training consisted of ten sessions, until judges knew what constituted quality in raw shrimp. They were also trained in differentiating odor, appearance and texture in ice stored shrimp. Sensory testing was conducted on each sample, the day it was removed from storage. All samples were identified by a three-digit code. Sensory testing was done in a clean, well-ventilated room. Screens were arranged to afford privacy to panel members. The panelists evaluated the samples for odor, texture, and appearance. A point structured scale with a value of 9 for excellent (sweet fresh odor, desirable moisture, good colour and a characteristic firm texture) and 1 being very poor (strong ammoniacal, stale, putrid odour, undesirable discoloration of the muscle and poor texture) was used. A score of 5 was taken as the average score for minimum acceptability. The score of each parameter was calculated in terms of score points awarded by a panel of judges to each sample. Similarly, sensory analyses were also done on cooked shrimp by boiling the samples separately in distilled water for 2 mins. Samples were evaluated for their texture and flavour using the same scale.

Statistical analyses. The significance of the difference between treated and untreated samples was evaluated by paired student's t-test.

Results and Discussion

Figs 1 and 2 present the changes in the sensory quality of raw and cooked shrimp, respectively, during ice storage with and without treatment with LAB culture as a dip solution. The values are the average of six experiments whereas, each point is the mean of the score points awarded by a panel of eight judges for odour, texture and appearance of raw shrimp and texture and flavour of cooked shrimp, respectively. The sensory shelf life of shrimp was determined graphically. According to the sensory panel, the minimum acceptance score point i. e. 5 reached within a week for the untreated samples whereas that for the samples dipped in the culture supernatant of *L. plantarum* did not reach the minimum even after three weeks. The antibacterial substances produced by LAB may have played roles in this extension of shelf life. The score point averages of treated and untreated samples were found significantly different when the paired student's t-test was performed (p values 0.014 and 0.042 for raw and cooked shrimp, respectively).

Total plate counts per gram of shrimp tails during the storage are presented in Fig 3. Fresh shrimp had an average initial bacterial load of 3.9×10^5 colony forming units (CFU) g^{-1} which decreased to 6.8×10^4 immediately after treatment with the lactic culture. This primary inhibition is probably due to acidic pH of the dip solution which is antagonistic to a number of spoilage bacteria and pathogenic organisms (Gilliland and Speck 1972, 1975, 1977). During ice storage the counts increased with time and reached to 4.2×10^8 CFU g^{-1} after four weeks in the treated samples as compared to the untreated ones that had an average count of 6.3×10^8 CFU g^{-1}. Freshly

caught fish and shrimp commonly have initial bacterial counts of approximately 10^4 to 10^5 per gram (Campbell and Williams 1952; Vanderzant *et al* 1970; Zuberi *et al* 1986, 1987; Shamshad *et al* 1990) and shelf life on a fishing vessel is expected to be 12 to 14 days (Cobb *et al* 1976; Zuberi *et al* 1986; Shamshad *et al* 1990). Bacterial counts have been used as an index of sanitary quality. High bacterial counts are unacceptable but do not always indicate extent of quality loss or spoilage. However, fewer than 10^7 $CFUg^{-1}$ are considered acceptable for tropical shrimp (Farooqui *et al* 1978; Shamshad *et al* 1990). The samples treated with lactic culture had bacterial counts within acceptable limits even after 2 weeks whereas in untreated samples the count reached that limit within one week during ice storage. Statistical analyses were also carried out and significant differences were found between treated and untreated samples (p value 0.021).

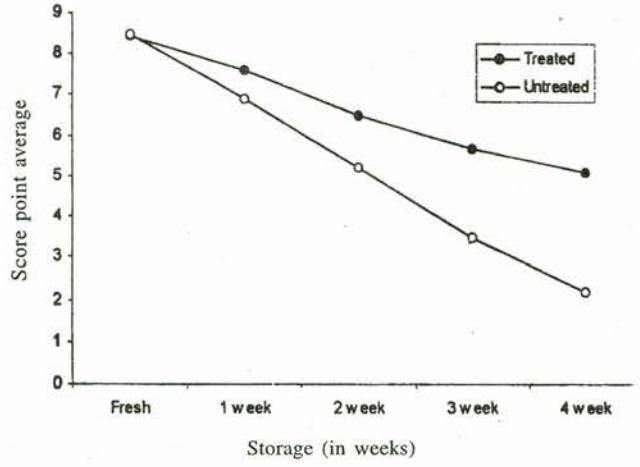

Fig 1. Changes in the sensory quality of raw shrimp (*Penaeus merguiensis*) during ice storage with and without treatment with lactic culture as a dip solution.

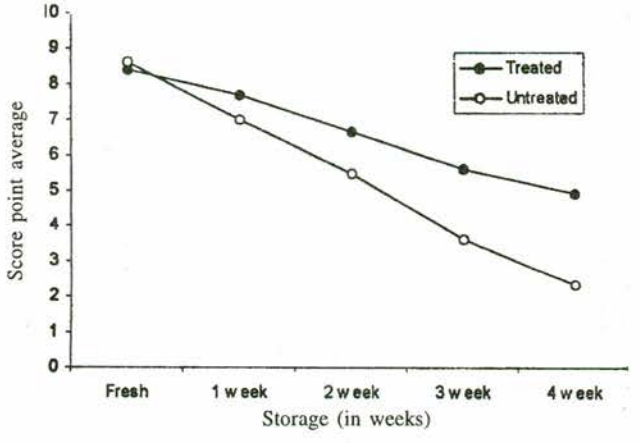

Fig 2. Changes in the sensory quality of cooked shrimp (*Penaeus merguiensis*) during ice storage with and without treatment with lactic culture as a dip solution.

LAB counts were also determined during the study and are presented in Fig 4. LAB counts remained almost the same throughout the storage period. The initial LAB counts were 3.4×10^5 and 6.3×10^3 in treated and untreated samples, respectively. The reason for high initial LAB counts in treated samples might be the left over cells during centrifugation or decanting of the supernatant of the broth culture. After four weeks of ice storage, the LAB counts were 3.8×10^4 and 4.3×10^4 in treated and untreated samples, respectively. This explains the mesophilic nature of lactic acid bacteria for which low temperatures are bacteriostatic. LAB counts were similar in the control and treated samples. Statistical analyses also did not show any significant difference in LAB counts of treated and untreated samples (p value 0.177).

Fig 5 presents the changes in pH of shrimp during ice storage with and without the treatment of lactic culture. Fresh shrimp

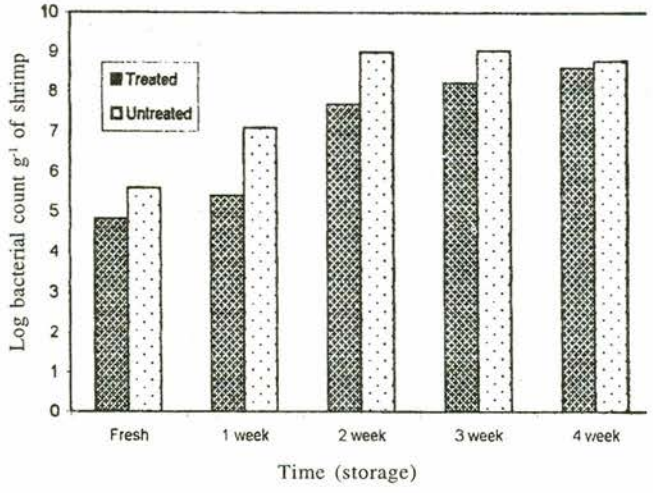

Fig 3. Changes in bacterial counts during ice storage of shrimp (*Penaeus merguiensis*) with and without treatment of lactic culture.

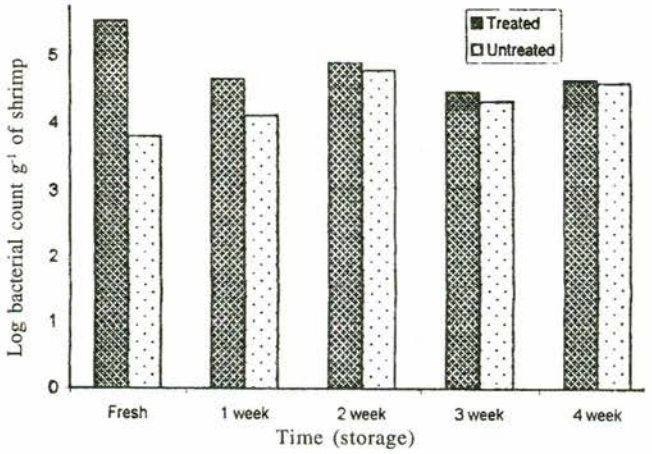

Fig 4. Changes in lactic acid bacterial counts during ice storage of shrimp (*Penaeus merguiensis*) with and without treatment of lactic culture.

had an initial pH of 6.86, which decreased to 6.68 due to treatment with lactic culture. The pH increased with time and reached to 7.43 and 7.85 in treated and untreated samples, respectively. Earlier studies report that shrimp get spoiled when their pH reaches a value of 7.5 or more (Farooqui *et al* 1978; Shamshad *et al* 1990). In this study, treated samples never reached this value even after four weeks. A highly significant p-value (0.001) was obtained by performing the paired student's t-test on the pH values of treated and untreated samples proving the antibacterial activities of LAB cultures against the shrimp spoilage flora which was shown in a previous study.

Changes in TVB are presented in Fig 6. The initial values were 28 mg $100g^{-1}$ and 30 mg $100g^{-1}$, respectively, for treated and untreated samples. The initial values are higher for prime quality shrimp but are acceptable. According to several studies, a value < 69 mg $100g^{-1}$ is considered acceptable (Farooqui *et al* 1978; Shamshad *et al* 1990). During the present study, the samples treated with lactic culture never crossed this limit though the sensory parameters rendered them spoiled. A significant p-value (0.09) was obtained in this parameter when comparing treated and untreated samples.

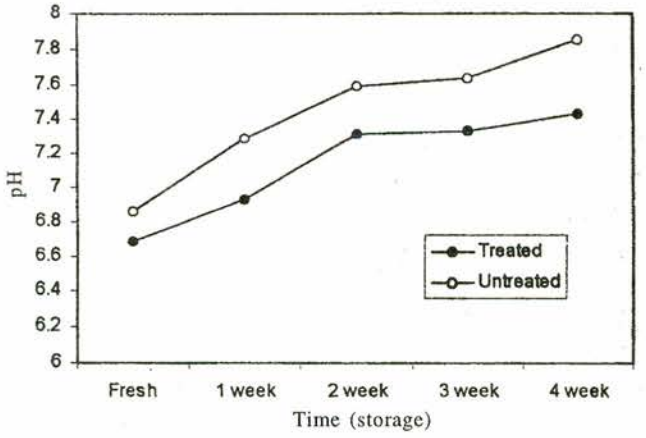

Fig 5. Changes in pH of shrimp (*Penaeus merguiensis*) during ice storage with and without treatment of lactic culture.

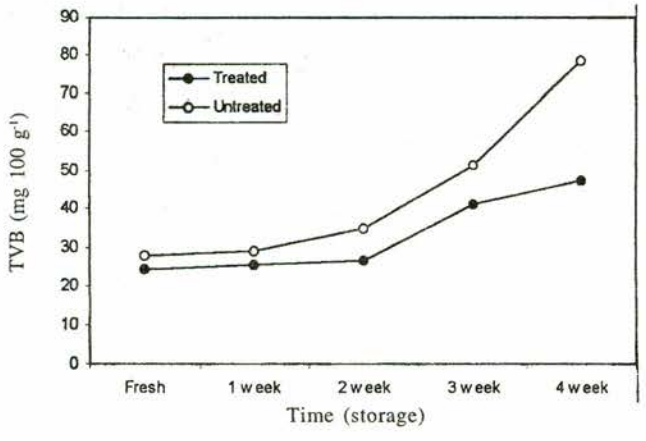

Fig 6. Changes in TVB during ice storage of shrimp (*Penaeus merguiensis*) with and without treatment of lactic culture.

Psychrotrophic microorganisms pose a real threat to preservation at refrigeration temperatures (0°-5°C) resulting in a relatively short shelf life. Lactic cultures are commonly used to improve the shelf life of dairy and meat products. Production of inhibitory substances like lactic acid, propionic acid, diacetyl, hydrogen peroxide and bacteriocins have a profound effect on a variety of Gram-negative spoilage bacteria and foodborne pathogens like *Staphylococcus* and *Salmonella* in dairy and meat products (Gilliland and Speck 1972, 1975, 1977). Fish/shellfish and meat are similar to some extent according to their composition, nutritive properties and types of bacteria present on them. These organisms are mostly Gram-regative, non spore-forming, rod-shaped bacteria belonging to genera *Alteromonas*, *Pseudomonas* and *Moraxella* and cause spoilage of the product at low temperatures (Campbell and Williams 1952; Vanderzant *et al* 1970; Zuberi *et al* 1986, 1987; Shamshad *et al* 1990). In view of the similarities of meat and fishery products, LAB cultures were used to extend the shelf life of fishery products through retarding the deterioration by spoilage bacteria and preventing the growth of undersirable bacteria including pathogens, thereby enhancing the margin of safety for the product.

The present studies indicate that the shrimp treated with cell free supernatant of lactic culture will keep better quality compared to untreated samples even after four weeks of ice storage. This extension in the shelf life may be due to the antibacterial activity of the substances produced as metabolites during the growth of LAB (Price and Lee 1970; Tagg *et al* 1976; Jay 1982; Dahiya and Speck 1986; Klaenhammer 1988; Daeschel 1989; Daba *et al* 1991). These antibacterial substances such as lactic and other organic acids, hydrogen peroxide, diacetyl and antibiotic like substances i.e. bacteriocins may be present in the dip solution used for treating the shrimp samples. There is a need to study the nature and potential of these substances as bacterial growth inhibitors.

Lactic acid bacteria are usually mesophilic in nature and do not grow at refrigeration temperatures. Strains of LAB that are capable of growing at low temperatures could prove more advantageous so far as extension of shelf life of fishery products, in general, and that of shrimp, in particular, is concerned (Moon *et al* 1980). Therefore, studies should be undertaken for isolation and screening of such a strain of LAB. Moreover, genetic manipulation of LAB could also prove beneficial in this regard.

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