Effect of *Planococcus citreus* on Selected Quality Indices of *Penaeus* Shrimp

R. J. ALVAREZ and J. A. KOBURGER

Food Science and Human Nutrition Department, University of Florida, Gainesville, Florida 32611

(Received for publication September 24, 1980)

ABSTRACT

Selected biochemical and microbial changes in *Penaeus* shrimp inoculated with *Planococcus citreus* were examined to determine the potential of this organism to contribute to spoilage of shrimp. Biochemical and microbial studies were conducted following storage of *Penaeus* shrimp at 5°C for 0, 4, 8, 12 and 16 days. Three samples, a control (raw shrimp), an irradiated (600 Krad) control and an irradiated (600 Krad) sample inoculated with *P. citreus*, were analyzed for changes in aerobic plate count, pH, total volatile nitrogen/amino acid nitrogen (TVN/AA-N) ratio, trimethyl amine-nitrogen (TMN) and total extractable protein (TEP). *P. citreus* counts increased in the inoculated shrimp from $3.0 \times 10^9$ bacteria/gram at 0 day to $1.5 \times 10^8$ bacteria/gram at the 16th day. By the 16th day of storage, the pH of the inoculated shrimp was significantly higher than the pH of the other samples. *P. citreus* inoculated onto irradiated shrimp was able to produce a TVN/AA-N ratio of 1.3 by the 10th day of storage, about the same time as that developed by the natural flora on raw shrimp. The increase in TMN content of the control (raw shrimp) and the inoculated sample were not significantly different. *P. citreus* was also able to bring about a significant decrease in the percent TEP of shrimp during storage. These changes indicate the capabilities of *P. citreus* in lowering the overall quality of *Penaeus* shrimp.

In 1978, fishermen in the United States landed a record 477 million pounds of shrimp (7). Of this, a significant but unknown quantity were discarded because of spoilage following harvest. Quality deterioration and subsequent spoilage of shrimp are caused primarily by activities of tissue enzymes and microorganisms (35). Some researchers (10,43) believe that bacterial action plays a more important role than enzymatic autolysis in spoilage of certain fish species.

Various types of bacteria have been reported on freshly caught shrimp. Initially, the microbial flora is a mixture of organisms from both marine and terrestrial environments. Campbell and Williams (12) and Williams et al. (42) isolated species of *Achromobacter, Bacillus, Micrococcus, Flavobacterium* and *Pseudomonas* from Gulf Coast shrimp. Vanderzant et al. (41) reported that the flora of shrimp from the Gulf of Mexico consisted of coryneforms, *Achromobacter, Flavobacterium* and *Bac-

illius*. In Pacific shrimp, *Acinetobacter-Moraxella* species predominated (26). Lee and Pfeifer (31) reported that the flora of Pacific shrimp (*Pandalus jordani*) consisted of *Moraxella, Pseudomonas, Acinetobacter, Arthrobacter* and *Flavobacterium-Cytophaga* species. Cann et al. (13) found that coryneform bacteria were predominant in the bacterial flora of scampi (*Nephrops norvegicus*) with strains of the *Achromobacter-Acinetobacter* group and *Pseudomonas, Cytophaga* and *Micrococcus* also present. Koberger et al. (30) reported that the *Flavobacterium-Cytophaga* group represented most of the organisms on fresh rock shrimp (*Stenopus hispidus)*.

In recent studies (2,3,4), *Planococcus citreus* has been found to be an important component of the natural flora of *Penaeus* shrimp. Alvarez and Koberger (5) described *P. citreus* as a motile gram-positive coccus capable of growth over a pH range of 7-10, at temperatures of 5-35°C and in broth containing 0.5-12% sodium chloride (NaCl), and capable of hydrolyzing gelatin, cottonseed, soy and, more importantly in regard to this work, shrimp protein. The organism was also shown to increase in numbers during iced storage of shrimp (3,4,30). The purpose of this study was to more directly assess the contribution of *P. citreus* to spoilage of *Penaeus* shrimp. A selected group of biochemical tests were chosen to measure quality changes in *Penaeus* shrimp, i.e., pH, total volatile nitrogen/amino acid nitrogen (TVN/AA-N) ratio, trimethyl amine-nitrogen (TMN) and total extractable protein (TEP).

MATERIALS AND METHODS

Samples

Approximately 5.5 Kg of fresh shrimp were obtained from Mayport Beach, Florida for each study conducted. The shrimp were transported on ice to the laboratory at Gainesville, Florida. After washing, the shrimp were divided into lots and placed in sterile Whirl-Pak® bags to a bag. Biochemical and microbial studies were conducted on samples after 0, 4, 8, 12 and 16 days of storage at 5°C. Three samples, a control (raw shrimp), an irradiated (600 Krad) control and an irradiated (600 Krad) sample which was inoculated with a culture of *P. citreus* were analyzed after each storage interval. All analyses were done in triplicate.

Irradiation

A number of preliminary studies were conducted to determine the
A.A. ALVAREZ AND J. KOBURGER

minimum dose which would bring about the maximum destruction of microorganisms on the shrimp. It was finally concluded that approximately 600 Krads were adequate. A Mark III 'Prort' Lodi irradiator designed by Brookhaven National Laboratories and built by Processing Equipment Corporation, Lodi, NJ was used. Whirl-Pak Bags® with the shrimp were placed in the middle of three stainless steel containers. After sealing, the containers were lowered into the water chamber and subjected to gamma rays (cobalt source) for 4 h.

Culture
A culture of P. citreus ‘A-17’ previously isolated from rock shrimp (30) was selected for this study. Cells for inoculation of the irradiated shrimp were grown on Plate Count Agar slants (29) containing an added 0.5% NaCl. Incubation was at 20 C for 5 days. Serial dilutions of the cells were prepared from growth obtained from slants to obtain a concentration of approximately 3 x 10^6 organisms per gram on the inoculated shrimp. Dilutions were prepared in phosphate buffer into which the shrimp were dipped for 1 min before plating and storage.

Microbiological analyses
Total aerobic plate counts were made after 0, 4, 8, 12 and 16 days of storage at 5 C. Plate Count Agar (Difco) with an added 0.5% NaCl was used with incubation at 20 C for 5 days. The methods outlined in the Compendium of Methods for the Microbiological Examination of Foods (6) were followed. The unoinoculated irradiated control shrimp plates only developed white to cream colored colonies; therefore, with the shrimp irradiated and inoculated with P. citreus, only those colonies that showed orange-yellow pigmentation were counted as P. citreus.

Biochemical analyses
Selected biochemical tests were done to assess the potential of this organism to contribute to the spoilage of Penaeus shrimp. Total volatile nitrogen/ amino acid-nitrogen (TVN/AA-N) ratio, trimethyl amine-nitrogen (TMN), pH and percent total extractable protein (TEP) were used. Biochemical tests were done after 0, 4, 8, 12 and 16 days of storage at 5 C.

Shrimp extracts. Shrimp extracts were prepared by blending (for 2 min at low speed) in a Waring Blender at least five shrimp at a ratio of 1 g of shrimp to 2 ml of 7% trichloroacetic acid (TCA). The mixture was then filtered, using Whatman #4 filter paper, and the filtrate was stored at 5 C until used. For amino acid analyses, the supernatant fluid was centrifuged after storage overnight at 5 C to remove additional protein.

Microdiffusion analyses of volatile nitrogen. The modified Conway (19) microdiffusion dish (34) was used for analysis of volatile nitrogen compounds. The procedure outlined by Cobb et al. (16) was followed using NaHCO_3 and KOH as the releasing agents to prevent production of extraneous NH_3 during analyses. For TMN analysis, 0.5 ml of 40% formaldehyde was added to the sample before the releasing agents. Values were multiplied by 1.3 to correct for incomplete distillation (16, 18). Results are expressed as milligrams of N/100 g of shrimp.

Amino acid nitrogen
Amino acid nitrogen was analyzed for by the copper procedure of Spies and Chambers (35) as modified by Cobb et al. (16). Solutions were prepared following the specifications set by Pope and Stevens (37). Results are expressed as millimoles of N/100 g of shrimp.

pH
pH was measured electrometrically, using a Corning Model 130 pH meter. A homogenate containing 2 parts of distilled water to 1 part of shrimp tissue was used.

Total extractable protein
The percent extractable nitrogen attributable to protein was calculated by subtracting the percent non-protein nitrogen (29) from the percent total extractable nitrogen (21, 36). The percent extractable protein was calculated by multiplying percent total extractable protein nitrogen by 6.061 (28).

Statistical analyses
Data from the last day of storage were analyzed using the Statistical Analyses System (SAS) program package for the two-way analyses of variance calculations (9).

RESULTS
Changes in total aerobic plate counts of the control (raw shrimp), irradiated control, and irradiated shrimp inoculated with P. citreus during 16 days of storage at 5 C are shown in Fig. 1. P. citreus counts increased in the inoculated shrimp from 3 x 10^3 bacteria/gram at 0 day to 1.5 x 10^6 bacteria/gram at the 16th day. Bacterial counts in the raw shrimp increased from 1 x 10^6 bacteria/gram at 0 day to 1.8 x 10^6 bacteria/gram at the 16th day. In contrast, the irradiated control sample contained

Figure 1. Aerobic plate counts of Penaeus shrimp stored at 5 C for 16 days. Those attributes followed by the same letter do not differ significantly at the a = 0.05 level.

2.1 x 10^2 bacteria/gram at 0 day (survivors of the irradiation treatment) and 1.2 x 10^5 bacteria/gram at the 16th day.

It has been suggested that an increase in the pH of shrimp indicates loss of quality and onset of spoilage (8). Figure 2 shows the change in pH observed in the three samples. The pH of the P. citreus-inoculated shrimp increased from 7.58 at 0 day to 8.25 by the 16th day. The pH values of the irradiated control and the raw control samples were 7.9 and 8.1, respectively, at the 16th day of storage. Analysis of the data shows that the pH of the inoculated sample at the end of the storage period was significantly different (a = 0.05 level) from the other two samples.

Cobb and Vanderzant (17) observed that the
TVN/AA-N ratio and the logarithm of the bacterial count increased at approximately the same rate after the initial lag phase of bacterial growth. A TVN/AA-N ratio greater than 1.3 indicated that the shrimp would have a relatively short period of acceptability (18). Figure 3 shows that the TVA/AA-N ratio of the P. citreus-inoculated shrimp was above 1.3 by the 10th day of storage. The irradiated control shrimp had a TVN/AA-N ratio of 1.05 at the 16th day. In addition, Figure 3 demonstrates that P. citreus-inoculated shrimp had a similar TVN/AA-N ratio as the control sample after 16 days of storage at 5°C (no statistical difference, α = 0.05). If the TVN/AA-N ratio is an accurate indicator of the freshness of shrimp, P. citreus can markedly affect the quality of Penaeus shrimp.

![Figure 2](http://example.com/figure2.png)

**Figure 2.** Changes in pH of Penaeus shrimp stored at 5°C for 16 days. Those attributes followed by the same letter do not differ significantly at the α = 0.05 level.

Traditional studies investigating shrimp quality have included determination of the trimethyl amine-nitrogen (TMN) content as an indicator of shrimp spoilage. Figure 4 shows the increase of TMN content in shrimp stored at 5°C for 16 days. By the 16th day of storage there was no statistical difference between the TMN content of the control (raw shrimp) and P. citreus-inoculated samples (.275 and .257 mg of N/100 g of shrimp, respectively). These results show that the capability of P. citreus to produce TMN in shrimp tissue is similar to the natural flora present on shrimp.

![Figure 3](http://example.com/figure3.png)

**Figure 3.** Changes in total volatile nitrogen/amino acid nitrogen ratios in Penaeus shrimp stored at 5°C for 16 days. Those attributes followed by the same letter do not differ significantly at the α = 0.05 level.

**DISCUSSION**

Quality changes that occur in shrimp during storage are generally considered to result from the combined action of tissue enzymes and microbial contamination (15,23,24,32,33,39). Cobb and Vanderzant (15) studied selected biochemical changes that took place in shrimp washed with ethanol and sterile water and inoculated with isolates of *Pseudomonas, Bacillus* and coryneform bacteria. They reported that samples inoculated with *Pseudomonas* species became unacceptable 2-3 days sooner than their controls. Shrimp inoculated with *Bacillus* spoiled at the same time as the controls; whereas, addition of coryneform bacteria delayed spoilage. Previous studies (2,3,4,5) have shown the presence of *P. citreus* in iced *Penaeus* shrimp, and one study (30) has shown the ability of this organism to increase in numbers during the ice-storage of rock shrimp (*Sycionia brevirostris*). In our study, *P. citreus* increased from $3.0 \times 10^3$ org/g at 0 day to $1.5 \times 10^8$ org/g by the 16th day at 5°C (Fig. 1). Following irradiation, the control contained 200 bacteria/gram. This was not unexpected in that the National Research Council's Committee on Microbiology of Foods (I) has discussed the radiation-resistance of a variety of organisms such as...
Acinetobacter calcoaceticus, Micrococcus radiodurans, Micrococcus radiophilus, Moraxella osloensis and Streptococcus faecium. In addition, Thornley (48) added some members of the Achromobacter-Alcaligenes group and some Bacillus species as being radiation-resistant. Some of these bacteria are part of the normal flora of Penaeus shrimp (2).

Subjective odor evaluation of the three samples showed a difference in the onset of off-odors in the stored shrimp. The control sample was the first to produce off-odors, followed by the P. citreus-inoculated shrimp. The irradiated control still had an acceptable odor at the 16th day. These observations show that P. citreus is capable of producing undesirable volatile compounds that would contribute to the overall unacceptability of the shrimp by the consumer.

Changes in tissue pH have been regarded as a simple quality test for shrimp. Previous work (8,10,24,25,27) has shown that an increase in pH occurs during the ice-storage of raw shrimp. Bailey et al. (8) suggested a value of 7.70 or below as indicative of prime quality shrimp, those shrimp having pH values from 7.70 to 7.95 as poor quality but acceptable and those shrimp having a pH of 7.95 or above as spoiled. By the 10th day of storage, the P. citreus-inoculated shrimp had a pH of 7.95; whereas, it took almost 12 days for the control shrimp to reach a similar pH (Fig. 2). At the end of the storage period, the pH of the P. citreus-inoculated shrimp was significantly higher than the pH of the other treatments.

In 1973, Cobb et al. (16) reported a high correlation between the total volatile nitrogen/amino acid-nitrogen ratio (TVN/AA-N) and quality of shrimp. Later work (17) suggested that the TVN/AA-N ratio and the logarithm of bacterial count increase at approximately the same rate after the initial lag phase of bacterial growth, and a ratio of 1.3 indicated a limited shelf life of the shrimp. P. citreus was capable of increasing the TVN/AA-N ratio at a similar rate as the control sample (natural flora in shrimp) (Fig. 3). These data show that P. citreus is capable of shortening the shelf life of the shrimp.

Determination of trimethyl amine-nitrogen (TMN) content is one of the traditional tests used to indicate the onset of spoilage in shrimp (10,22). Recent research (14) suggests that TMN is one of the major volatile components associated with the odor of spoiled marine fish, and increases in TMN level are characteristic of spoiling seafood. P. citreus inoculated onto shrimp was capable of increasing the TMN content during the 16 days of storage at a similar rate as the natural flora on the control shrimp. No statistical difference was observed between the TMN content of the control sample and the inoculated shrimp (Fig. 4). In addition, the TMN of the irradiated control sample also increased but at a slower rate, showing that the irradiation survivors are also
capable of producing TMN nitrogen.

During the first 12 days of storage, the total extractable protein (TEP) of the shrimp inoculated with *P. citreus* decreased markedly. The control sample also showed a similar decrease in TEP during this period. However, a marked decrease in TEP was observed with the control shrimp following the 4th day of storage. This is probably due to the change in bacterial flora observed in shrimp after the first few days of ice-storage (5, 4), with *Pseudomonas* spp. increasing in numbers until they account for a large percentage of the bacterial flora present in shrimp. By the 16th day of storage, no difference in TEP was observed between the raw control and the inoculated samples (Fig. 5). These results indicate that *P. citreus* is a highly proteolytic organism that can contribute to the overall deterioration of shrimp quality.

*P. citreus*, a gram-positive coccus of marine origin, appears to be capable of contributing to reduction of shrimp quality during storage. It is often found as part of the normal flora of *Penaeus* shrimp (although not always recognized) and when inoculated onto shrimp, grows rapidly. The potential of *P. citreus* as a "spoiler" of raw shrimp is shown by the rapid increase in the TVN/AA-N ratio, pH and TMN content as well as the reduction in TEP of inoculated samples.

REFERENCES

1. Advisory Board on Military Personnel Supplies, National Research Council Committee on Microbiology of Food. ABMPS Report No. 77, p. 2.
39. Sundsvold, D. C., B. Uppstad, G. W. Ferguson, T. McIachlan, and D. Feely. 1969. The trimethylamine oxide content of...
storage requirements and with ordinary peripheral equipment. This aspect of the matrix product model should make this management control tool available to even the smallest foodservice operations. Finally, applications of this kind of structure can be made to other food processing and service operations, including dairy processing plants, as well as meat and vegetable processing and distribution systems.

REFERENCES


APPENDIX--MATRIX MULTIPLICATION (1)

If A and B are symbols for matrices, several special conditions must be met before it is possible to form the product AB. Suppose that A has n rows and m columns and that B has r rows and s columns. Typically A is said to be an n by m matrix, with B an r by s matrix. The product AB can only be formed if m = r -- the number of columns in the first matrix factor must equal the number of rows in the second matrix factor. If this condition is met, the product matrix will be an n by s matrix with entries determined by combining the rows of A with the columns of B in a special way. For convenience, let C = AB. The entries of the product matrix can be represented by $c_{ij}$ -- the entry in the ith row, jth column. The formula for computing this entry is

$$c_{ij} = \sum a_{ij}b_{ij}$$

Observe that this sum only requires entries from the ith row of A and the jth column from B. Since m = r, we know there are exactly enough entries in the ith row to match with the entries in the jth column of B.

Suppose

$$A = \begin{bmatrix} 2 & 3 & -1 & 2 \\ -2 & 5 & 4 & 7 \end{bmatrix} \quad (2 \times 4)$$

$$B = \begin{bmatrix} 3 & 0 & 0 \\ 4 & 1 & 5 \\ -2 & 6 & 3 \\ 4 & 1 & 5 \end{bmatrix} \quad (4 \times 3)$$

then C = AB so:

$$C = \begin{bmatrix} 28 & -1 & 22 \\ 34 & 36 & 72 \end{bmatrix} \quad (2 \times 3)$$

for example:

$$c_{12} = \sum a_{ij}b_{ij} = 2\times0+3\times1+(-1)\times6+2\times1=-1.$$

**Alvarez and Koburger, con't. from p. 363**