ROLE OF MICROORGANISMS IN SHRIMP QUALITY: A RESEARCH SUMMARY

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Role of Microorganisms in Shrimp Quality:

A Research Summary

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I. Introduction

The production and processing of shrimp is an important segment of the United States and Gulf Coast Fisheries. Commercial landings of shrimp in the United States in 1972 were 385 million pounds (heads-on) with a dockside value of $193.2 million. Shrimp accounted for over 27 percent of the total value of U. S. commercial fishery landings. The Gulf States account for 59 percent of the total U. S. shrimp landings and 85 percent of the total value. Texas led all States in both production and value with 97.4 million pounds worth $79.8 million. The United States imported an additional 253.1 million pounds (head-off) of shrimp. It is well established that the organoleptic quality of shrimp often deteriorates in the time between catch and unloading. Additional quality losses frequently occur during further processing. Microbial activity is one of the main causes of quality deterioration of seafoods.

With the establishment in 1968 of a Sea Grant Program at Texas A&M University, a research program was initiated in the Department of Animal Science to determine the role of microorganisms in the quality deterioration of seafoods. An evaluation of the major microbiological problems related to the production and processing of shrimp indicated that a critical need for information existed in four specific areas. These areas can be identified as follows:

1. Role of Microorganisms in the Quality Deterioration of Gulf Coast and Pond-Reared Shrimp.

2. Development of Indicator Tests to Determine the Potential Shelf Life of Shrimp.

3. Role of Vibrio parahaemolyticus in Gulf Coast Seafoods.

4. Quality Deterioration of Microbial Origin in Frozen Breaded Raw Shrimp.

A justification of these research objectives, summary of previous work, and major research accomplishments for the period 1969-1972 are presented in the following sections.

II. Role of Microorganisms in the Quality Deterioration of Gulf Coast Shrimp

ABSTRACT

Bacterial counts of shrimp delivered by fishing vessels to processing plants varied greatly. Aerobic plate counts at 28 C ranged from 870-1,300,000 per gram. Microbial counts at time of catch usually were low and typical spoilage bacteria were not numerous at that time. Either natural seawater or distilled water could be used in media preparation. The use of artificial seawater usually resulted in lower counts. The microbial flora of Gulf shrimp was dominated by coryneforms and species of Pseudomonas, Moraxella, and Micrococcus. Refrigerated storage usually caused an increase in Pseudomonas species. Shrimp inoculated with Pseudomonas species developed a putrid, amine-like odor and became unacceptable sooner than those inoculated with Bacillus or coryneform bacteria.

INTRODUCTION

The number and types of microorganisms on fish at time of landing depend on many factors such as species, season, fishing grounds, methods of catching, handling on board, and time and temperature of storage. Microbial activity is one of the main causes of quality deterioration of shrimp. To determine the significance and role of individual microbial species in this process, a detailed analysis of the microbial flora is useful. Information on this subject is limited. Green (4, 5) reported on the quantitative changes in bacterial populations of shrimp from catching to landing. She determined the influence of various handling procedures such as washing, heading, icing, and storage on the bacterial count. In general, if shrimp was handled under sanitary conditions and iced promptly, low bacterial counts could be maintained for several days. Campbell and Williams (2) and Williams et al. (9) showed that species of Achromobacter, Bacillus, Micrococcus, Flavobacterium, and Pseudomonas predominated in Gulf Coast shrimp. In Pacific shrimp, Acinetobacter-Moraxella species were predominant (6). Information is scarce about the role of individual bacterial species on shrimp quality. Various species have been implicated by inference, i. e., they are part of the predominant microbial flora of shrimp, hence they are responsible for spoilage. This report presents information on the level and type of microbial population on Gulf shrimp and on the effect of individual bacterial species on biochemical and organoleptic changes in shrimp.

MATERIALS AND METHODS

Bacterial counts were determined by plating appropriate dilutions of blended shrimp on Standard Methods agar (SMA, Difco) by the spread-plate method. Plating media were prepared with three different types of water, artificial seawater, natural seawater, and distilled water. The artificial seawaterwas prepared from synthetic sea salts (Aquarium Systems, Inc., Wickliffe, Ohio). The natural seawater was obtained from the Gulf of Mexico at Galveston. Duplicate sets of plates were incubated aerobically at 5 C for 7 days and at 28 C for 2 days. Methods for the determination of microbial types are described by Vanderzant and Nickelson (8). Procedures for protein determination, non-protein nitrogen, total volatile nitrogen and pH are presented by Cobb and Vanderzant (3).

RESULTS AND DISCUSSION

Vanderzant et al. (7) showed that the bacterial counts of fresh Gulf shrimp from commercial vessels varied greatly. They ranged from 870-1,300,000 per gram on SMA plates prepared with distilled water and incubated at 28 C for 2 days (Table 1). In addition to factors such as season, area of catch, and time of trawling, these differences in count most likely reflect differences in the handling of shrimp on the boat, sanitary conditions of hold and ice, and time and temperature of storage. For example, an inspection of the boats revealed that sample 1 was poorly iced and that samples 9 and 11 were from dirty bins in need of repair. These conditions probably contributed to the relatively high bacterial counts of these samples. Additional studies showed that microbial counts at time of catch usually are low and that typical spoilage bacteria are not numerous at that time. When shrimp after catching were handled aseptically and stored on sterile ice, microbial counts at time of catch ranged from 2,000 to 10,000 per g. The microbial flora consisted primarily of coryneforms, Achromobacter, Flavobacterium and Bacillus. Quality losses were not observed until after 21 to 30 days of refrigerated storage.

Aerobic plate counts of fresh shrimp were generally somewhat lower at 5 C than at 28 C and ranged from 400-1,100,000 bacteria per gram on SMA plates prepared with distilled water. When fresh shrimp was stored on ice for 7 days, the bacterial counts increased greatly. Aerobic plate counts on stored shrimp ranged from 34,000 to 70,000,000 per gram on SMA agar prepared with distilled water with plate incubation at 28 C for 2 days. With stored shrimp, a majority of the samples showed similar aerobic plate counts at 28 and 5 C. This could be expected because during refrigerated storage of shrimp only the psychrotrophic bacteria will increase in number. These bacteria can be expected to show up on plating media at both 28 and 5 C. The bacterial counts at both plate incubation temperatures usually were highest on media with distilled water or natural seawater and lowest on those with artifical seawater.

The composition of the microbial flora of fresh and stored shrimp is presented in Tables 2 and 3. Although variations in microbial types were noted between samples, coryneforms and Pseudomonas, Moraxella, and Micrococcus species predominated in fresh and stored shrimp. The microbial flora of fresh shrimp was usually dominated by coryneforms; that of stored shrimp by Pseudomonas species. No consistent differences in type of bacterial flora were observed between the media prepared with seawater or distilled water. The present data do not allow any conclusions about the influence of season on the distribution of the microbial flora in shrimp. The experimental samples came from different boats and the trawling locations and duration of trawling were different. The composition of the microbial flora shown in this study is in some respect different from that of Gulf Coast shrimp reported in 1952 (2,9) and that of Pacific shrimp (6). According to Campbell and Williams, and Williams et al. (2, 9) species of Achromobacter, Micrococcus, Pseudomonas, Flavobacterium, and Bacillus predominated in Gulf Coast shrimp. In Pacific shrimp (6) the initial flora in order of predominance was Acinetobacter, Moraxella, Flavobacterium, Pseudomonas, gram-positive cocci, and Bacillus species. Some of the differences in microflora of shrimp can probably be attributed to differences in shrimp species, marine environment, shrimp handling on board, and time and temperature of storage. The small number of Achromobacter species reported in Gulf shrimp in this study and also in Pacific shrimp (6) most likely reflects a change in taxonomic status. Some of the biochemically inert, gram-negative, short stout rods, formerly classified as Achromobacter species are now frequently reclassified as Acinetobacter or Moraxella species (1,8). In the other studies, Achromobacter or Acinetobacter-Moraxella species predominated. In contrast, coryneforms usually were predominant in this study. Although some of the factors mentioned above may have been responsible for this difference in microflora, it is possible that recovery conditions (composition of plating medium and conditions of plate incubation) are involved. Some coryneforms isolated from shrimp were difficult to maintain on ordinary laboratory media because of their nutritional requirements. This may be another reason for the low numbers reported in other studies.

To determine the effect of individual bacterial species on shrimp quality, white shrimp washed with ethanol and sterile water were inoculated with Pseudomonas, Bacillus and a coryneform bacterium (3). Shrimp inoculated with Bacillus spoiled at the same time as the non-inoculated controls. Addition of coryneform bacteria delayed spoilage. Inoculation of shrimp with Pseudomonas species resulted in higher levels of water-soluble protein, non-protein nitrogen and total volatile nitrogen than in corresponding controls. Inoculated samples spoiled faster and reached higher pH levels sooner than the controls.

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Table 1. Aerobic plate counts (APC) of fresh and stored Gulf shrimp with plate incubation at 28 C for 2 days.

Sample	APC/g of fresh shrimp[a]	APC/g of stored shrimp[b]	Period at sea (days)
1	670,000	13,000,000	2
2	150,000	7,900,000	3
3	870	34,000	1
4	8,100	8,500,000	7
5	51,000	480,000	2
6	65,000	2,500,000	5
7	12,000	4,000,000	5
8	3,800	2,100,000	7
9	1,300,000	15,000,000	6
10	63,000	8,800,000	1
11	130,000	70,000,000	8
12	23,000	2,000,000	2

[a] Fresh shrimp: shrimp as sampled from the boat at time of landing.

[b] Stored shrimp: fresh shrimp stored at 1 C for 7 days.

Data from Vanderzant, Mroz, and Nickelson (7).

Table 2. Distribution of microorganisms from fresh shrimp samples isolated from media with seawater.

Type	Percentage distribution during months of 1969											
	1	2	3	4	5	6	7	8	9	10	11	12
Achromobacter	0	0	0	0	0	0	2.5	0	0	0	0	0
Alcaligenes	5.7	0	2.5	0	0	0	0	0	0	0	0	0
Bacillus	2.9	0	0	0	0	0	0	0	0	0	0	0
Coryneform	22.9	37.5	65.0	45.0	75.7	35.0	25.0	35.0	15.0	42.5	37.5	47.5
Flavobacterium	11.4	5.0	0	2.5	2.7	7.5	7.5	7.5	2.5	45.0	7.5	2.5
Lactobacillus	0	0	0	0	2.7	2.5	7.5	2.5	0	0	0	0
Microbacterium	0	0	0	0	0	0	2.5	0	0	0	0	0
Micrococcus	14.3	10.0	17.5	10.0	18.9	5.0	10.0	2.5	0	2.5	7.5	7.5
Moraxella	8.6	25.0	7.5	12.5	0	12.5	30.0	5.0	2.5	10.0	22.5	32.5
Pseudomonas	31.4	17.5	0	27.5	0	30.0	15.0	47.5	65.0	0	25.0	2.5
Staphylococcus	2.9	5.0	7.5	2.5	0	0	0	0	0	0	0	7.5
Vibrio	0	0	0	0	0	7.5	0	0	15.0	0	0	0

Data from Vanderzant, Mroz, and Nickelson (7).

Table 3. Distribution of microorganisms from stored shrimp samples isolated from media with seawater.

Type	Percentage distribution during months of 1969											
	1	2	3	4	5	6	7	8	9	10	11	12
Achromobacter	0	0	0	0	0	0	0	0	0	0	0	0
Alcaligenes	12.8	2.6	0	2.5	0	0	0	0	0	0	0	0
Bacillus	10.3	2.6	2.5	0	0	0	0	0	0	0	5.0	0
Coryneform	17.9	7.7	52.5	32.5	25.0	15.0	20.0	30.0	7.5	25.0	25.0	12.5
Flavobacterium	0	5.1	0	0	0	2.5	0	0	0	0	0	2.5
Lactobacillus	0	23.0	2.5	0	0	0	20.0	0	0	0	0	0
Microbacterium	0	0	0	0	0	0	0	0	0	0	0	0
Micrococcus	12.8	2.6	12.5	0	2.5	5.0	0	7.5	0	10.0	0	17.5
Mold	0	0	2.5	0	0	0	0	0	0	0	0	0
Moraxella	2.6	41.0	27.5	17.5	37.5	12.5	7.5	22.5	2.5	65.0	10.0	20.0
Pseudomonas	35.9	15.4	0	47.5	35.0	62.5	52.5	37.5	90.0	0	60.0	47.5
Staphylococcus	2.6	0	0	0	0	2.5	0	0	0	0	0	0
Vibrio	5.1	0	0	0	0	0	0	2.5	0	0	0	0

Data from Vanderzant, Mroz, and Nickelson (7).

III. Role of Microorganisms in the Quality Deterioration of Pond-Reared Shrimp

ABSTRACT

Agar plate counts and microbial types are reported for brown shrimp reared in 2-acre natural marshland and in 0.5-acre artificial ponds during June to October 1970. Bacterial counts of pond-reared shrimp ranged from 50,000 to 5,500,000 per g. At final harvest in October bacterial counts ranged from 200,000 to 5,500,000 per g. In marsh ponds, bacterial counts of shrimp were lowest in August when both water temperature and salinity were high. Coryneform bacteria and to a lesser extent Vibrio were the predominant isolates from fresh pond shrimp. Shrimp stored at 3 to 5 C for 7 days were acceptable as judged by appearance and odor. Between 7 and 14 days of refrigerated storage, bacterial counts increased sharply and about 50% of the samples became unacceptable. Refrigerated storage of pond shrimp caused increases in coryneform bacteria and micrococci and decreases in Vibrio, Flavobacterium, Moraxella, and Bacillus species. Pseudomonas species were not significant in fresh or stored pond shrimp. The microbial flora of pond water usually was dominated by coryneform bacteria.

INTRODUCTION

Nearly all shrimp harvested commercially in the United States are caught in nets by trawlers on near-shore fishing grounds. In some other countries, particularly in Asia and the Far East, cultivation of shrimp in ponds is a common practice (3, 5). Although pond cultivation of shrimp in the United States is almost entirely limited to experimental trials, research on shrimp mariculture has become increasingly important in recent years (1, 2, 4, 6, 7, 12). Along the Texas Gulf Coast, natural or artificial ponds filled with brackish water are stocked with either postlarval or young shrimp (juveniles). In ponds, shrimp usually are fed a variety of foods rich in animal proteins. They are harvested after approximately 80 to 120 days. Since microbial activity is one of the main causes of quality deterioration of shrimp, information on the microbial flora of pond-reared shrimp can be useful to assess the potential shelf life of this food. This report presents information on the microbial flora of shrimp from marshland and artificial ponds along the Texas Gulf coast. In addition, data are provided on changes in level and types of bacteria in pond shrimp during refrigerated storage.

MATERIALS AND METHODS

Ponds. The five ponds used in this study are located on the West Galveston Bay shore in Brazoria County, Texas. Two of the ponds (A, B) are natural marshland ponds and were filled and stocked by

natural tidal waters through flood gates. At the beginning of the experiment, the shrimp (Penaeus aztecus) population in pond A (2 acres) was estimated at 20,000 per acre and that of pond B was estimated at 6,000 per acre. Pond A had been treated with rotenone (Chem Fish Kill, Dow Chem. Co.) to remove competitive and predatory fish. Shrimp in these ponds were fed three times a week at a rate of 10% of their body weight. Pelleted feeds were prepared by B&D Mills Inc., Grapevine, Texas. Animal products such as fish meal, poultry meal, blood meal, and meat and bone meal were important ingredients in these feeds.

The others (C, D, E) were 0.5-acre ponds and were located above high tide. In construction of these ponds with the aid of bulldozers, the natural soil was used to make levees. These ponds were filled by pumping brackish water through wire screen from canals in the marshland. They were stocked with 10,000 juveniles (P. aztecus) per pond. Shrimp in these ponds were fed the same feeds at the same times as those in the marsh ponds but at a rate of 3% (pond C), 5% (pond D), and 10% (pond E) of their body weight. All ponds were approximately 3 feet (91 cm) in depth. Shrimp samples were collected by seining. Temperature and salinity of the water in each pond were determined at each feeding period. Salinity was determined by measurement of refractivity.

Microbiological procedure. Samples of shrimp and water were collected in June, July, August, and October 1970. Methods used for the determination of bacterial counts and microbial types are described by Vanderzant and Nickelson (10) and Vanderzant et al. (11).

RESULTS AND DISCUSSION

The changes in bacterial counts of shrimp from marsh ponds from June to October (Fig. 1) probably are related to some extent to changes in characteristics of the water such as temperature, salinity, oxygen level, phytoplankton activity, and pH (8). In August, when the counts were low, the salinity of the water was high (Fig. 2) and the temperature of the water had been 26 to 31 C for at least 2 months (Fig. 3). These factors also may have been responsible for the gradual reduction in coryneform bacteria (Table 1). A marked decrease in water temperature and salinity preceded a rise in counts in October. The percentage of coryneform bacteria, however, remained the same or continued to decline. Similar reasons might be given for the changes in bacterial counts of shrimp in artificial ponds. Oxygen level and pH of the pond waters were not measured in this study. In small ponds (1/20 acre) with excess feed, pH values of as high as 9.0 have been recorded (7). Aeration of these ponds was necessary to maintain a desirable oxygen level.

The bacterial counts of pond shrimp in the present study were considerably higher than those reported previously (11). Changes

in ponds and characteristics of water and shrimp stock could have caused these differences. In addition, minor changes in the agar plate method could have been involved. In the present study, the water used in diluents and plating medium was adjusted to approximate the salinity of the pond water. In the previous study (11), natural seawater, artificial seawater, and distilled water were used.

In the present study, coryneform bacteria were the predominant isolates from fresh pond shrimp (Table 1) and also from pond water. All gram-positive, catalase-positive, nonsporeforming pleomorphic rods were placed in this group. In a separate study, Vanderzant et al. (9) compared these bacteria with type cultures of Corynebacteriaceae. The comparison included 163 morphological, biochemical, and physiological characters. The pond isolates (Fig. 4) exhibited little similarity to type cultures which included animal and plant pathogens. With the aid of numerical analysis, pond isolates could be placed into six major groups based on certain biochemical and physiological tests. About 30% of the pond isolates were able to grow at refrigeration temperature. The role of these organisms in quality deterioration of refrigerated seafood is not known.

Although bacterial counts of pond shrimp in this study were comparable to many commercial boat samples at time of landing, the striking difference in microbial flora between Gulf and pond-reared shrimp was the lack of Pseudomonas in the latter. With similar plating techniques and identification procedures, large numbers of pseudomonads were isolated from Gulf shrimp. The lack of Pseudomonas in pond shrimp also was noted in the spoilage characteristics of pond shrimp when held for 14 to 21 days at 3 to 5 C. A putrid, slimy condition frequently associated with Pseudomonas spoilage in shrimp stored for excessive periods was not observed in pond shrimp. Spoiled pond shrimp usually showed ammoniacal off-odors.

The microbial flora of the water and feed is the main factor controlling the initial microbial flora of marine species. Although purely speculative at present, it is possible that, with increased interest in commercial culture of crustaceans, techniques will be devised to control the microbial population in ponds. This, together with improved sanitary methods of harvesting, handling, and storage, could possibly lead to production of shrimp with a controlled microbial population and, hence, enhanced shelf life.

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Table 1. Distribution of microbial flora of pond-reared shrimp.

Pond	Sample	Percentage distribution									
		Coryne-form	Vibrio	Flavobac-terium	Aero-monas	Achromo-bacter	Moraxella	Alcali-genes	Bacillus	Micrococ-cus	Uniden-tified
A	June	90	2.5	7.5							
	July	72.5	10	12.5	2.5		2.5				
	August	55	22.5	10	7.5			2.5	2.5		
	October	55	20	10			15				
	Stored	91.3								8.7	
B	June	77.5	2.5	7.5			5		5		2.5
	July	85.7	7.1		3.6				3.6		
	August	67.5	20	2.5	5		5				
	October	30	20	45					5		
	Stored	54				15.4				23	7.6
C	June	82.5	7.5	2.5	5		2.5				
	July	80.2	6.6	6.6			6.6				
	August	45	30	2.5	7.5		12.5		2.5		
	October	45	15	5	10		20			5	
	Stored	100									
D	June	82.5	7.5	2.5	5		2.5				
	July	72.5	17.5	5	2.5		2.5				
	August	57.5	20	5	5		2.5		10		
	October	50	20						30		
	Stored	100									
E	June	82.5	7.5	2.5	5		2.5				
	July	87.5	2.5	7.5			2.5				
	August	60	5	2.5			2.5		30		
	October	50.4	8.4	16.8			16.8		8.4		
	Stored	94.5								5.5	

Data from Vanderzant, Judkins, and Nickelson (8).

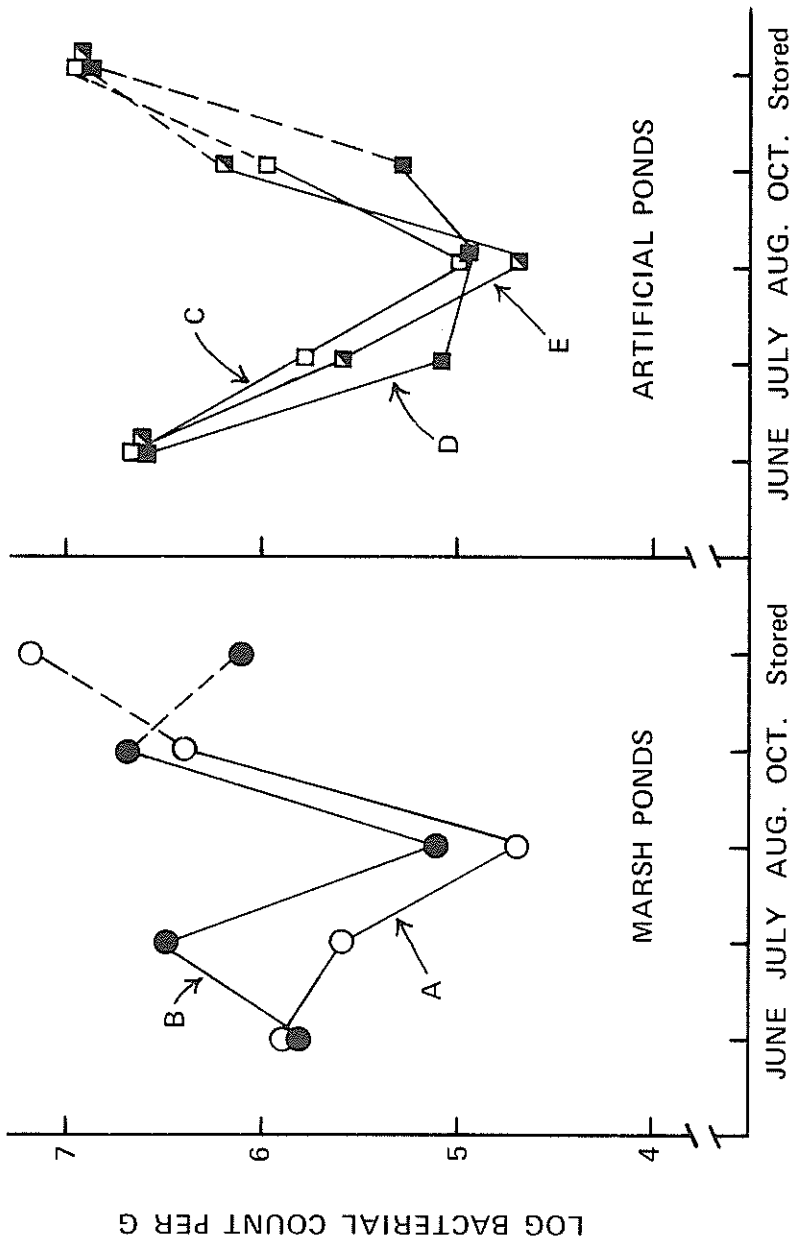

Fig. 1. Agar plate counts of fresh pond shrimp and pond shrimp harvested in October and stored at 3 to 5 C for 14 days (A, B, marshland ponds; C, D, E, artificial ponds).

Data from Vanderzant, Judkins, and Nickelson (8).

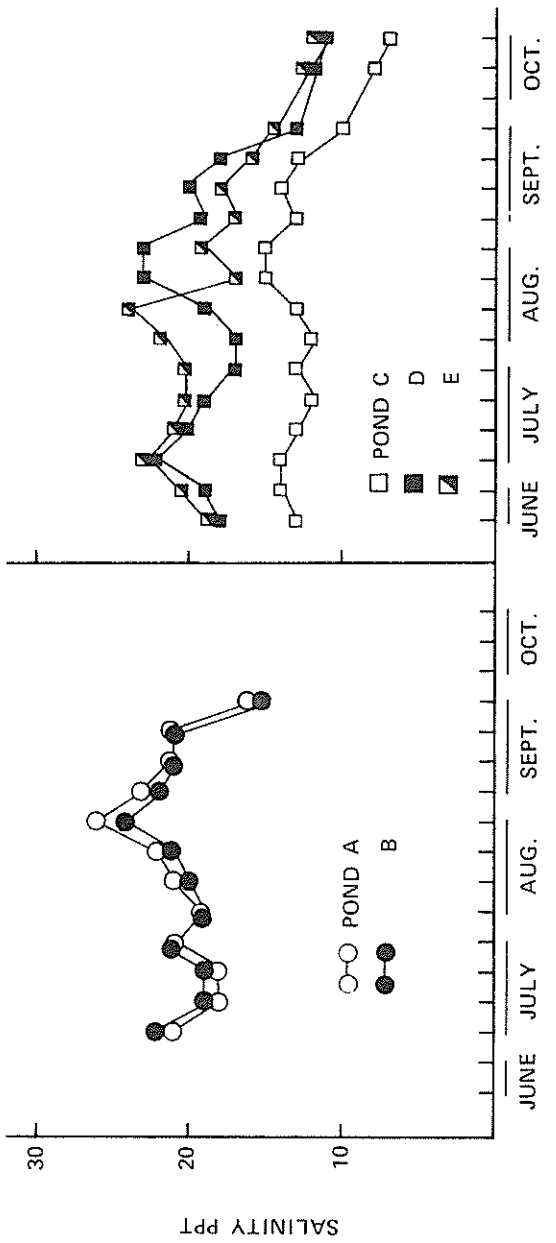

Fig. 2. Salinity of pond water during experimental period.
Data from Vanderzant, Judkins, and Nickelson (8).

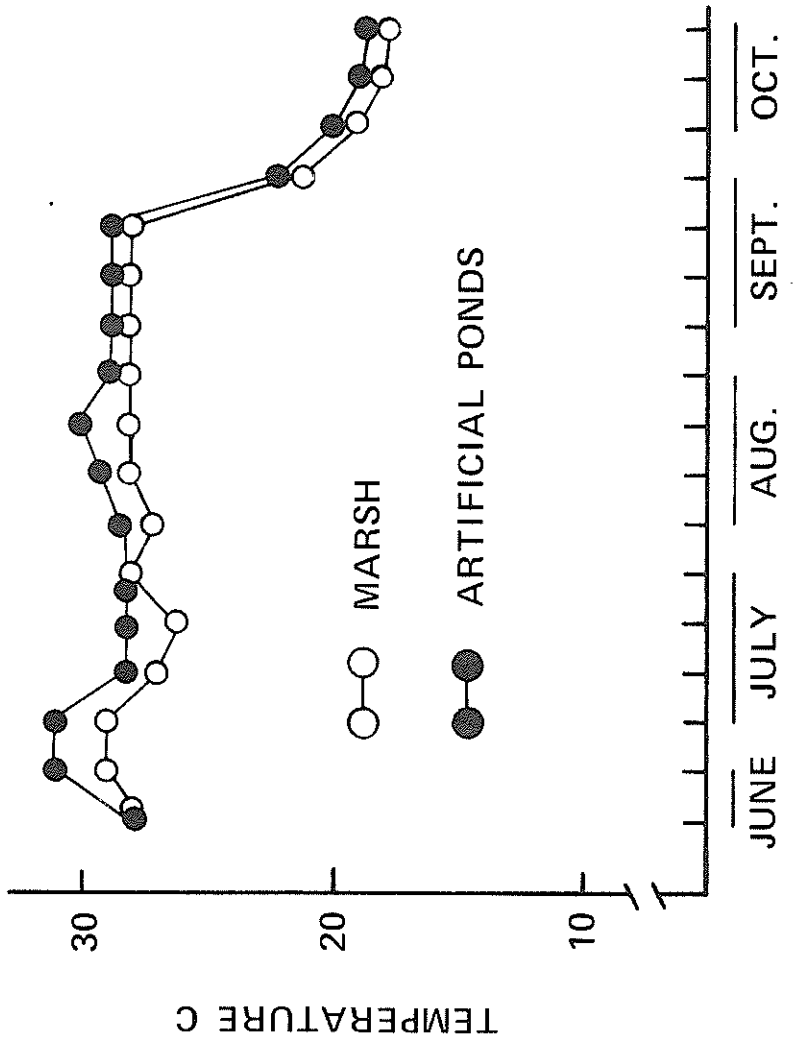

Fig. 3. Temperature of pond water during experimental period.

Data from Vanderzant, Judkins, and Nickelson (8).

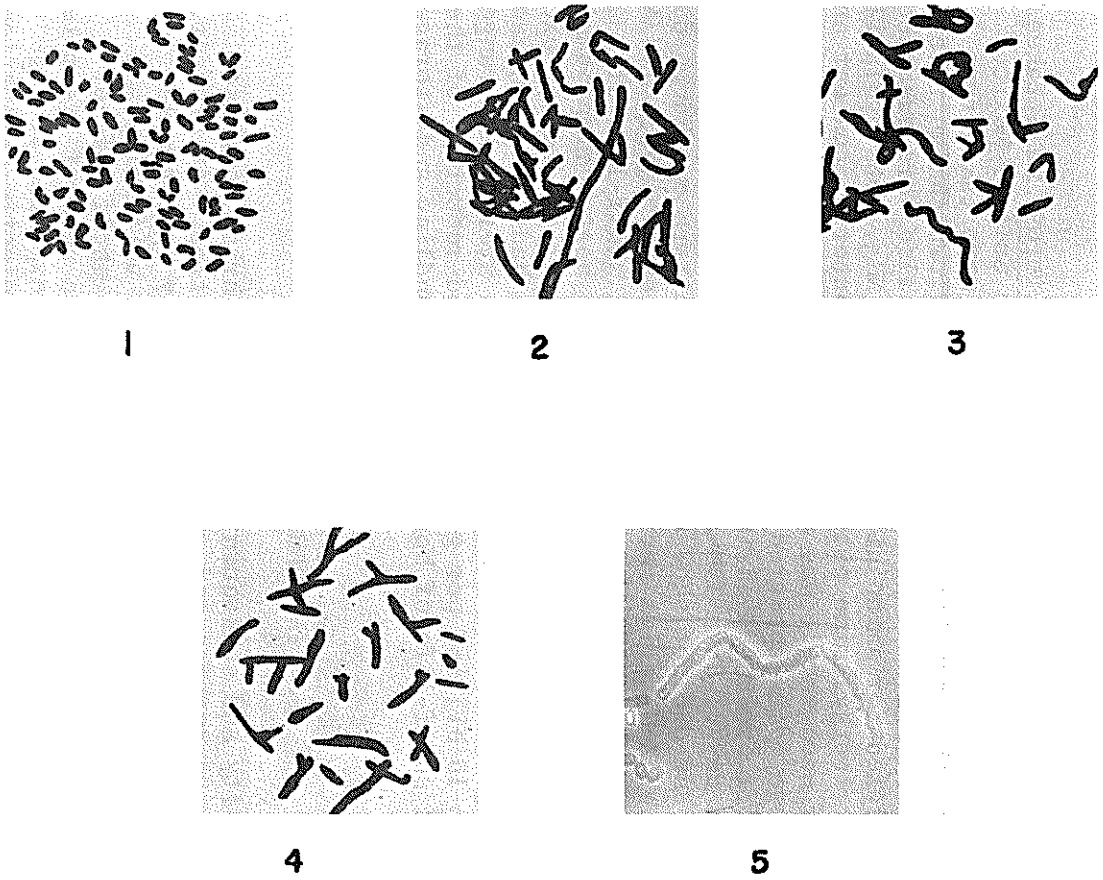

Fig. 4. Morphological characteristics of marine isolates.

Data from paper by Vanderzant, Judkins, Nickelson and Fitzhugh (9).

IV. Development of Indicator Tests to Determine the Potential Shelf Life of Shrimp

ABSTRACT

Freshly harvested white shrimp (Penaeus setiferus) were taken from 13 locations on the northwestern coastline of the Gulf of Mexico. Freshly harvested brown shrimp (Penaeus aztecus) were taken from 3 different water depths near Port Aransas, Tex. Brown shrimp taken from commercial fishing boats at time of landing also were examined. Samples were analyzed for amino nitrogen (AA-N), NH_3, total volatile nitrogen (TVN), trimethylamine nitrogen (TMN), bacterial content and pH. A portion of each sample was placed on sterile ice and allowed to spoil. Spoilage odors appeared in white sea-shrimp after storage for 11-50 days, for brown sea-shrimp in 20-30 days, and in brown boat-shrimp after 0-15 days. Both TVN and AA-N varied considerably from sample to sample and did not show a consistent pattern of change during iced storage. TVN/AA-N ratios increased as samples spoiled. TVN/AA-N ratios greater than 1.3 mg N/mM indicated a short shelf-life of boat shrimp. TMN production was evident in only a few boat-shrimp samples with high TVN levels. Bacterial counts of fresh shrimp did not exceed 10,000 per g. Nine of the 10 boat-shrimp samples had counts in excess of 1,000,000 per g. Counts of samples spoiled on sterile ice ranged from 2,000,000 to 10 billion per g. The estimated reduction of the maximum potential shelf-life of boat-shrimp by handling and storage was 0-15 days.

INTRODUCTION

Loss of quality and subsequent spoilage of shrimp is caused primarily by tissue enzymes and microbial activities. At present, quality of stored iced shrimp is determined mainly by organoleptic tests. These sensory evaluations frequently lack uniformity and contribute little information about the potential shelf life of the product. The shrimp processing industry is in need of a simple, rapid test to determine the sanitary quality and expected storage life of the raw product. When used with an organoleptic evaluation, it would enable processors to determine with some degree of uniformity the suitability of the product for processing. Various tests have been proposed for this purpose. Included are levels of glycogen-sugar content, lactic acid, acid-soluble orthophosphate, trimethyl-amine nitrogen, amino nitrogen, hydration of water-insoluble protein, pH, and bacterial count (1). These tests, as such however, have little application in establishing the potential shelf life of shrimp. For example, high bacterial counts are unacceptable but do not always indicate the extent of loss of quality or spoilage. This is caused by differences in biochemical activities of the individual bacterial species, particularly on the proteins and lipids of shrimp.

During the post-mortem period of storage of shrimp on ice, changes in the nitrogenous components occur. Increases in total volatile nitrogen (TVN) have been reported (2, 7, 8, 9). Trimethylamine (TMA) formation has been noted, especially during the period when spoilage odors appear (2, 8, 9). Both amino nitrogen (AA-N) increases (2) and decreases (7, 12) have been observed during the ice-storage period. This report describes rapid methods for estimating TVN and AA-N and their value in estimating quality characteristics and potential shelf life of shrimp.

MATERIALS AND METHODS

Freshly harvested white shrimp (Penaeus setiferus) were taken from different locations on the northwestern coast of the Gulf of Mexico between Galveston and Port Isabel, Tex. Freshly harvested brown shrimp (Penaeus aztecus) were taken from different locations in the Gulf of Mexico adjacent to Port Aransas, Tex. Brown boat-shrimp were obtained from shrimp boats on arrival at different locations on the northwestern coast of the Gulf of Mexico. Freshly harvested samples were deheaded using sterile rubber gloves. They were placed on sterile ice in an ice chest. Shrimp tails obtained from fishing boats at time of landing were immediately placed on sterile ice. Water was drained from the chambers and ice was added when needed. Samples were withdrawn daily with sterile forceps and examined for appearance and off-odors by a trained three-member panel. Shelf-life was defined as the number of days of refrigerated storage until off-odors developed. At that time refrigerated storage was terminated.

Microbiological and chemical analyses. Aerobic plate counts were determined on Standard Methods Agar as described in a previous report (11). Plates were incubated for 3 days at 25 C.

Both total volatile nitrogen (TVN) and trimethylamine (TMN) analyses employed the microdiffusion procedure of Conway (6) with saturated Na_3PO_4 as releasing agent. Values were multiplied by 1.3 to correct for incomplete distillation (3). Amino nitrogen (AA-N) and NH_3 were determined by the use of a Beckman Model 121C fully Automated Amino Acid Analyzer. Prior to analysis, shrimp extracts were frozen and recentrifuged to remove residual protein. Measurement of pH was made with a combination electrode on five or more shrimp blended with water (2 ml/g shrimp).

RESULTS AND DISCUSSION

Marked differences in AA-N levels and TVN levels existed between samples of freshly harvested white shrimp from different locations (Table 1). TVN levels were below 19 mg N/100g and AA-N levels were either at or above 19 mM/100g. In 50% of the samples the values for TVN/AA-N and NH_3/AA-N were similar indicating that most of the TVN was

NH_3. In the other samples, NH_3 levels exceeded TVN levels. The value of TVN/AA-N of freshly harvested white shrimp was always less than 0.90 mg N/mM. TMN was not detected. Bacterial counts were normal for freshly harvested shrimp ranging from 250 to 10,000 per g.

The white shrimp taken from different locations were placed on sterile ice until spoilage odors appeared. The time at which serious off-odors appeared varied considerably (Table 2) but for most samples ranged from 25 to 37 days when bacterial counts reached levels of 2 million to 3 billion per g with a geometric mean of 50 million per g. No relationship existed between potential shelf-life on sterile ice (Table 2) and initial bacterial count, AA-N, TVN, NH_3/AA-N or TVN/AA-N. Most samples maintained a commercially acceptable appearance during the test period. Little development of melanosis occurred. The main spoilage odor was "musty".

A comparison of the data on the chemical analyses of the freshly harvested (Table 1) and spoiled white shrimp (Table 2) shows that on spoilage (a) AA-N decreased sharply, (b) TVN increased in most samples and (c) values for TVN/AA-N and NH_3/AA-N increased. Decreases in AA-N most likely were the result of the washing action of melted ice. Only two samples had a TVN content greater than 30 mg N/100g shrimp, the limit of acceptability used in some sections of the Australian and Japanese markets (9). In the spoiled samples the TVN/AA-N values exceeded those of NH_3/AA-N suggesting the presence of other amines. TMN, however, was not detected. Portions of certain samples were removed after 14 days of storage and analyzed. The NH_3/AA-N ratios for shrimp from Aransas Pass, Brownsville (ship channel entrance) and Port Isabel were at that time 1.14, 1.33, and 1.13 mg N/mM respectively. These figures correspond to NH_3/AA-N increases of 0.038, 0.034, and 0.040 mg N/mM/day respectively. These increases are in excellent agreement with increases in NH_3/AA-N of 0.032 mg N/mM/day calculated from analyses on stored sterile shrimp juices (4) and suggest that these resulted primarily from tissue enzyme activity. In the final phase of the storage of shrimp from Aransas Pass, Brownsville, and Port Isabel, the average daily increases in NH_3/AA-N ratio were 0.062, 0.219, and 0.130 mg N/mM. These increases probably resulted from tissue enzyme and microbial activities. The pH of spoiled shrimp ranged from 7.7-8.4, values close to 8.0 which some investigators (1) consider indicative of spoilage.

The results of the chemical analyses of freshly harvested brown sea-shrimp were similar to those of freshly harvested white shrimp. Although the number of samples was limited, the shelf-life of brown sea-shrimp on sterile ice was shorter (ranged 20-30 days, average 24 days) than that of white shrimp (ranged 11-50 days, average 33 days).

The results of the analyses on brown boat-shrimp differed considerably from those of freshly harvested shrimp. In general, AA-N levels were low (Avg. 18.43) and TVN levels high (Avg. 26.76) compared

with freshly harvested white or brown shrimp (Avg. AA-N 22.45-23.97; Avg. TVN 13.36-15.73). The values for TVN/AA-N and NH_3/AA-N exceeded those of freshly caught white or brown shrimp. Bacterial counts of just landed brown boat shrimp ranged from 500,000 to 100 million per g with a geometric mean of 12 million per g. A comparison of the values for the chemical and microbial indices of just landed boat shrimp and those of fresh and spoiled sea-shrimp indicates that some quality deterioration had occurred in the boat shrimp. This is also apparent from the limited additional shelf-life of brown boat shrimp on sterile ice which ranged from 0 to 15 days. AA-N levels of the spoiled brown boat shrimp were higher (ranged 11.63-16.58 mM/100g, average 14.2) than those of spoiled brown sea-shrimp (range 2.75-14.61, average 7.8) or spoiled white sea-shrimp (<1-11.86). All but one sample has TVN levels above 30 mg N/100 g. TMN production was evident in the samples with the highest TVN level. These samples were taken from boats where sanitary handling of the shrimp was poor. Values for TVN/AA-N and NH_3/AA-N of spoiled brown boat shrimp were usually high, TVN/AA-N values were larger than those for NH_3/AA-N. Bacterial counts of spoiled samples were greater than 100 million per g. The predominant spoilage odor was putrid. With freshly caught shrimp stored on sterile ice the predominant off-odor was "musty".

The shelf-life of white or brown sea-shrimp on sterile ice was called maximum potential shelf life (MPSL). With the aid of this figure some estimation can be made of the reduction in shelf-life of boat shrimp through commercial handling and storage practices. The average MPSL of brown sea-shrimp was 22 days. The brown boat shrimp except for one sample had been on board an average of 7 days. Differences between the average MPSL of brown sea-shrimp and Estimated Total Shelf Life (ETSL = days on board plus days on sterile ice) of commercial brown boat shrimp were -1 to 15 days (average 7.1 days). Figures for ETSL usually were high for shrimp from boats with excellent sanitary handling practices and storage.

The value for TVN/AA-N of freshly harvested brown or white shrimp was always <0.9, for spoiled sea-shrimp >1.64 and for spoiled boat shrimp >2 mg N/mM. The value for TVN/AA-N may be useful in conjunction with an evaluation of appearance and odor as a screening test to determine shrimp quality. In a limited field trial, commercial boat samples with TVN/AA-N values greater than 1.3 mg N/mM were usually evaluated as poor (based on appearance and odor) by plant quality control personnel. The method of AA-N analysis used in this study is not suitable for the average shrimp processing plant. However, in subsequent studies simple and rapid methods have been devised (3). Analysis of TVN was with a steam distillation procedure with Na_3PO_4 as releasing agent. AA-N was determined with a colorimetric procedure employing a modification of the copper procedure of Spies and Chambers (10).

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Table 1. Chemical analyses and aerobic plate count (APC) of freshly harvested white shrimp taken from different locations on the northwestern coastline of the Gulf of Mexico.

Location of catch	AA-N[a] mM/100g	TVN[b] mg N/100g	NH_3/AA-N mg N/mM	TVN/AA-N mg N/mM	Log APC per g
Galveston (West Bay)	22.30	10.34	0.62	0.46	3.8
(Harbor)	24.33	18.65	0.91	0.77	3.8
Bacliff	21.39	9.53	0.78	0.45	4.0
Matagorda	26.83	17.02	0.70	0.63	3.4
Palacios	26.54	15.39	0.58	0.58	2.8
Port Lavaca	23.88[c]	14.68	0.60	0.61	3.5
	19.60[d]	7.33	0.28	0.37	3.9
Indianola	24.65	9.78	0.48	0.40	2.5
Aransas Pass	26.74	11.92	0.60	0.45	2.5
Brownsville (ship channel near entrance)	31.46	15.69	0.85	0.50	3.0
(Mid-channel)	20.26	16.51	0.90	0.82	2.4
Port Isabel	23.32	11.01	0.63	0.47	2.8

Table 1. (continued)

Location of catch	AA-N[a] mM/100g	TVN[b] mg N/100g	NH_3/AA-N mg N/mM	TVN/AA-N mg N/mM	Log APC per g
Seabrook	25.46	14.98	0.58	0.59	2.8
	18.97	14.27	-	0.75	2.9

[a] α-amino, proline and ammonia nitrogen

[b] Total volatile nitrogen

[c] Harvested in June

[d] Harvested in August

Data from Cobb, Vanderzant, Thompson and Custer (5)

Table 2. Shelf life, chemical analyses and aerobic plate count (APC) of freshly harvested white shrimp kept in sterile ice until spoilage occurred.

Location of catch	Shelf life days	pH	AA-N[a] mM/100g	TVN[b] mg N/100g	NH_3/AA-N mg N/mM	TVN/AA-N mg N/mM	Log APC per g
Galveston (West Bay)	35	-	1.87	15.79	5.78	8.44	8.2
(Harbor)	50	8.4	<1	26.81	>8	>8	10.1
Bacliff	50	7.8	<1	NM[c]	>8	NM	LA
Matagorda	32	-	2.91	19.77	4.90	6.79	6.7
Palacios	25	-	2.95	16.82	3.94	5.70	9.5
Port Lavaca	34[d]	-	3.15	20.17	4.65	6.40	6.3
	11[e]	8.0	11.86	-	1.53	-	6.8
Indianola	35	-	5.00	19.27	3.05	3.85	6.5
Aransas Pass	28	-	11.86	30.17	2.01	2.54	7.5
Brownsville (ship channel near entrance	30	-	8.05	60.70	4.84	7.54	6.5
(Mid-channel)	37	7.7	<1	7.33	>8	>8	8.7
Port Isabel	35	8.1	4.52	20.79	3.91	4.59	8.5

Table 2. (continued)

Location of catch	Shelf life days	pH	AA-N[a] mM/100g	TVN[b] mg N/100g	NH_3/AA-N mg N/mM	TVN/AA-N mg N/mM	Log APC per g
Seabrook	27	7.8	5.66	9.27	1.20	1.64	8.6
	27	8.3	7.62	-	3.18	-	8.0

[a] α-amino, proline and ammonia nitrogen

[b] Total volatile nitrogen

[c] Level too low for accurate measurement

[d] Harvested in June

[e] Harvested in August

Data from Cobb, Vanderzant, Thompson and Custer (5)

V. Role of *Vibrio parahaemolyticus* in Gulf Coast Seafoods

ABSTRACT

A procedure employing a direct plating technique, with or without prior enrichment, was designed for the isolation and enumeration of *V. parahaemolyticus*. The plating medium consisted of 2.0% peptone, 0.2% yeast extract, 1.0% corn starch, 7% NaCl, and 1.5% agar, with the pH adjusted to 8.0. The enrichment broth was Trypticase Soy Broth (TSB) with 7% NaCl. Dilutions of food homogenates were either spread directly on the plates or inoculated into enrichment broth. TSB enrichments were incubated at 42 C for 18 hr. A loopful of the TSB tubes then was streaked onto the direct plating medium. Incubation of plates was at 42 C for 24 to 48 hr. Smooth, white to creamy, circular, amylase-positive colonies were then picked as suspect *V. parahaemolyticus*. Confirmation of gram-negative, fermentative, oxidase-positive, pleomorphic rods sensitive to pteridine 0/129 was made by a fluorescent-antibody technique. With this procedure, a satisfactory quantitative recovery of known *V. parahaemolyticus* from inoculated seafoods was made possible. *V. parahaemolyticus* was not isolated from other salted foods. When *V. parahaemolyticus* was inoculated into whole shrimp and shrimp homogenate, large decreases in viable population occurred during storage for 2 days at 10 to -18 C. However, survivors were present even after 8 days. No significant differences were observed in the population changes of inoculated whole shrimp as compared with shrimp homogenates. Low populations ($5x10^2$ per ml) of *V. parahaemolyticus* were destroyed by heating shrimp homogenates at 60, 80, and 100 C for 1 min. With larger populations ($2x10^5$ per ml), some survivors were present after heating at 60 and 80 C for 15 min. None survived 1 min at 100 C. *V. parahaemolyticus* was very sensitive to pH values below 6.0.

INTRODUCTION

Vibrio parahaemolyticus is a major cause of gastroenteritis in Japan where it is associated with the consumption of raw seafood, particularly during warm summer months. In recent years, this organism has been isolated from shellfish and marine environments in the United States (1, 2, 4, 7, 10). In addition, mortalities among brown shrimp caused by *V. parahaemolyticus* have been observed in laboratory aquaria (9,10). At present, however, there is no firm indication that this organism is a major problem in pond-cultivated shrimp in Texas. Although presumptive evidence existed for the involvement of *V. parahaemolyticus* in food poisoning in the United States, the first confirmed cases occurred in 1971 which involved the consumption of crab (6). It is likely that other cases were not recognized because present isolation and identification procedures are lengthly and laborious. In order to evaluate the

potential role of V. parahaemolyticus in food poisoning, information is needed concerning the distribution of this organism in seafoods from sub-tropical waters such as the Northern Gulf of Mexico and from mariculture operations. In addition, information is needed about the effect on V. parahaemolyticus in foods of various environmental conditions such as pH, refrigeration, freezing or elevated temperatures. This report presents information on (a) the development of a rapid and reliable procedure for the isolation and enumeration of V. parahaemolyticus and (b) the survival of V. parahaemolyticus in shrimp tissue under various environmental conditions.

MATERIALS AND METHODS

The source of the 28 cultures of V. parahaemolyticus, diagnostic procedures, isolation of antigenic substance from V. parahaemolyticus and preparation of labeled antibody are described in detail by Vanderzant and Nickelson (7,8). Survival studies were carried out in whole and homogenized shrimp. Homogenates were prepared by blending equal amounts of peeled deveined shrimp and sterile 3% NaCl. Homogenates were inoculated with appropriate dilutions of a 24-hr. Trypticase Soy Broth (TSB, with 3% NaCl) culture to give approximately 50,000 cells per ml. Whole peeled deveined shrimp were inoculated by injecting 0.1 ml of a TSB culture into three to four sites to give approximately 10^5 cells per shrimp. Inoculated shrimp were stored in sterile petri dishes, and homogenates were stored in sterile screw-capped tubes. No V. parahaemolyticus could be detected in the test samples before inoculation. Counts of V. parahaemolyticus were made by spreading appropriate dilutions on either MT medium (modified Twedt; C. Vanderzant and R. Nickelson, Bacteriol. Proc., p. 20, 1971) or TSA (with 3% NaCl) plates. Plate incubation was at 35 C for 24 hr. The data presented are representative of three independent trials.

RESULTS AND DISCUSSION

Morphological, biochemical, and serological characteristics. All V. parahaemolyticus cells were short, gram-negative rods exhibiting pleomorphism. Curved, straight, coccoid, and swollen forms were observed. There was also a strong tendency towards bipolar staining. All cultures were motile, had a single polar flagellum, and hydrolyzed starch. With few or no exceptions, cultures of V. parahaemolyticus (a) showed a weak catalase reaction; (b) were positive for cytochrome oxidase; (c) utilized glucose fermentatively; (d) were sensitive to pteridine (0/129) and novobiocin; (e) did not grow in TSB without NaCl; (f) grew well in TSB with 3 or 7% NaCl; and (g) produced an alkaline slant, acid butt, and no H_2S or gas in TSI. Growth in 10% NaCl varied. Hemolytic activity of the test strains varied greatly, depending on the type of blood and concentration of NaCl. With the commercial antisera obtained from Japan only 8 of 28 known strains

were typable.

Immunofluorescent reaction. When tested with the conjugated globulin, all V. parahaemolyticus cultures (except 7BW, 8C, A4280, A6540, and A7606) were positive. These five cultures differed in one or more characteristics from the majority of V. parahaemolyticus cultures. Some enteric pathogens such as Providence group, Proteus, and Salmonella showed positive reactions. Of the V. anguillarum cultures, only one (14181S) exhibited a positive reaction. Other species of Vibrio (including V. alginolyticus) exhibited slight or no fluorescence.

Isolation and identification procedure. Thirteen plating media and three enrichment broths were tested for recovery and isolation of V. parahaemolyticus. Plating media were inoculated by spreading 0.1 ml of appropriate dilutions of TSB cultures on the surface of the plates. In addition, seafoods with a natural microbial flora were inoculated with V. parahaemolyticus cultures and plated in a similar manner. All plating and enrichment media were incubated at 35 and 42 C. With few exceptions, a modification of a medium recently proposed for V. parahaemolyticus by Twedt et al. (Bacteriol. Proc., p. 6, 1970) was best suited for recovery and selective isolation of V. parahaemolyticus. This medium (MT) contained 2.0% peptone, 0.2% yeast extract, 1% corn starch, 7% NaCl, and 1.5% agar (pH 8.0). Enrichment broths were compared by subsequent plating on MT medium and on TSA with 3% NaCl. Enrichment was best in TSB with 7% NaCl adjusted to pH 7.3. Cultures of V. parahaemolyticus on MT medium were white to creamy, circular, smooth, and amylase-positive . The isolation procedure selected is as follows. Food (50 g) to be examined for V. parahaemolyticus was blended for 2 min with 450 ml of 7% NaCl. For direct plating, serial dilutions were made in sterile 7% NaCl. Quantities (0.1 ml) of appropriate dilutions were spread over the surface of MT agar plates. Enrichment consisted of placing 10-, 1-, and 0.1-ml quantities of homogenate into TSB with 7% NaCl. After 18 hr at 42 C, the tubes were streaked onto MT agar plates with a wire loop. All MT agar plates were incubated aerobically at 42 C for 24 to 48 hr. White to creamy, circular, smooth, amylase-positive colonies were picked as suspect V. parahaemolyticus. These isolates were then tested for Gram reaction, morphology, glucose utilization, presence of cytochrome oxidase, and sensitivity to pteridine 0/129. Confirmation of these isolates was made by fluorescent-antibody technique.

Recovery efficiency of plating and enrichment media. Seafoods (50 g) were blended with 450 ml of sterile 7% NaCl and inoculated with V. parahaemolyticus. The population level of the inoculum (TSB culture) was determined by plating on MT medium and on TSA with 3% NaCl. The number of cells recovered was determined by direct plating on MT medium. Recovery of V. parahaemolyticus from seafoods inoculated with various isolates was generally acceptable

(Table 1). The average recovery of V. parahaemolyticus from all seafoods was 85%. All enrichment broths which contained seafoods inoculated with V. parahaemolyticus yielded V. parahaemolyticus when subsequently streaked on MT medium. When food homogenates (two samples) were inoculated with very low cell concentrations (calculated 1 to 10 per g), V. parahaemolyticus was not recovered by direct plating on MT medium. However, enrichment in TSB and subsequent plating on MT medium yielded V. parahaemolyticus from these samples.

Incidence of V. parahaemolyticus in other salted food products. Sixteen commercial foods including ham, salt pork, corned beef, stuffed crab, olives, pickles, and some canned seafoods (oysters, clams, sardines, tuna) were checked for the presence of V. parahaemolyticus. No V. parahaemolyticus was isolated from the salted foods.

To determine the potential role of V. parahaemolyticus in foodborne illness in the United States, it is necessary to evaluate its ability to survive under different environmental conditions. It has been reported that V. parahaemolyticus is not psychrotrophic (2,5). Temmyo (5) found no survivors in peptone water or fish extract after 5 days at 4, -2, and -18 C.

The present study indicates that this organism is sensitive to refrigeration and freezing (Fig. 1). Storage of inoculated whole shrimp for 8 days at 10 to -18 C caused reductions in V. parahaemolyticus of 1 to 2 logs. However, even after 8 days at -18 C, about 10^3 cells per shrimp survived when the initial population was 10^5 cells per shrimp. This observation agrees with reports that V. parahaemolyticus can be recovered from frozen shrimp (10) and from frozen marine sediments (11). Digirolamo et al. (3) suggest that bacterial survival in food homogenates should be interpreted cautiously. In their study, survival of Salmonella in oyster homogenates differed from that in whole oysters. In the present study, the only difference in behavior of V. parahaemolyticus in refrigerated whole shrimp and shrimp homogenate was a slight increase of the population in the latter during the first 12 hr of storage.

Low population levels of V. parahaemolyticus in shrimp homogenates were destroyed by heating at 60, 80, and 100 C for 1 min. No survivors were present after heating at 100 C. Temmyo (5) reported that V. parahaemolyticus was destroyed after 5 min at 60 C in peptone water. It is possible that food homogenates afford some protection. V. parahaemolyticus in shrimp homogenates was very sensitive to pH values below 6.0 (Fig. 2). These homogenates were inoculated with 10^2-10^3 cells per ml. Little change in viable population occurred when stored for 2 hr at pH values ranging from 6 to 10.

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Table 1. Recovery of V. parahaemolyticus from various seafoods[a].

Culture	Shrimp		Oysters		Crab Meat		Swordfish Steaks	
0	900[b] / 1800	24,000 / 20,000	3300 / 2400	21,000 / 26,000	1400 / 2400	10,000 / 26,000	400 / 900	8000 / 9400
WL1	2200 / 1600	25,000 / 19,000	2500 / 2800	8,000 / 31,000	1500 / 2800	25,000 / 31,000	1200 / 1000	12,000 / 12,000
5A	3000 / 1900	22,000 / 19,000	500 / 700	9,000 / 7,700	700 / 700	12,000 / 7,700	900 / 1400	9,000 / 9,000
12A	700 / 1000	12,000 / 11,000						
17802	1000 / 1300	7,000 / 14,000						

[a] All enrichments from low and high cell populations were positive for V. parahaemolyticus when streaked on MT medium.

[b] Expressed as number per g recovered/inoculated at low and high cell density.

Data from Vanderzant and Nickelson (7)

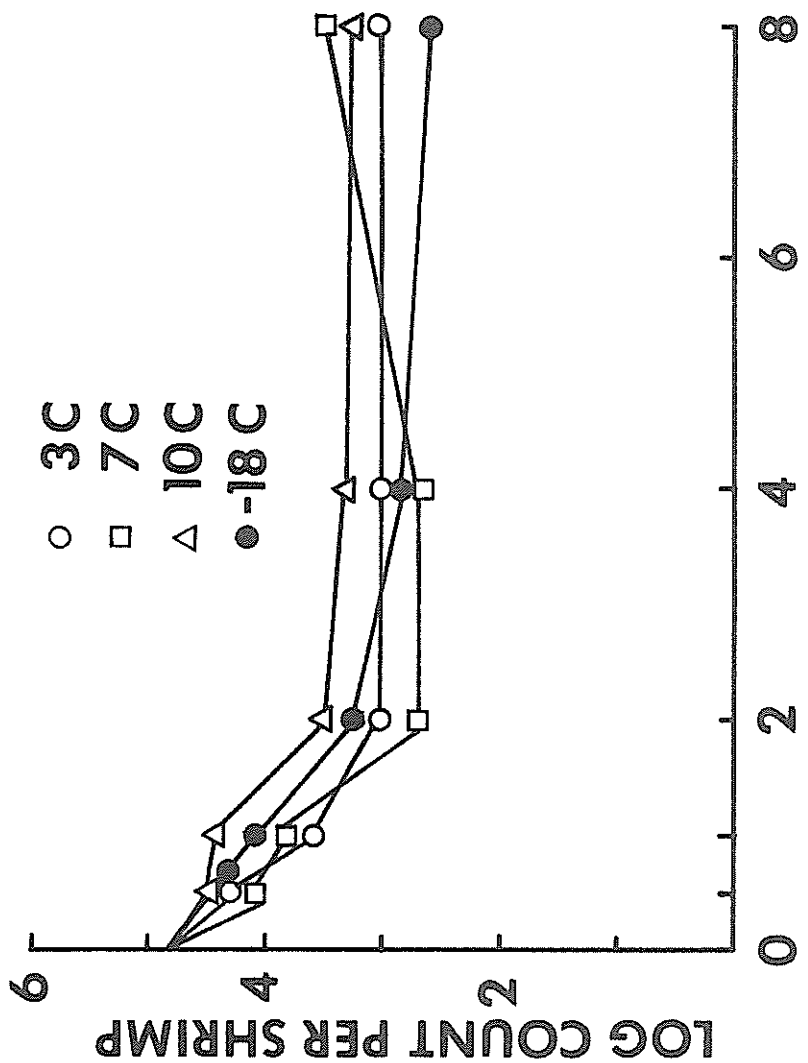

Fig. 1. Survival of *Vibrio Parahaemolyticus* in whole shrimp stored at various temperatures.

Data from Vanderzant and Nickelson (8).

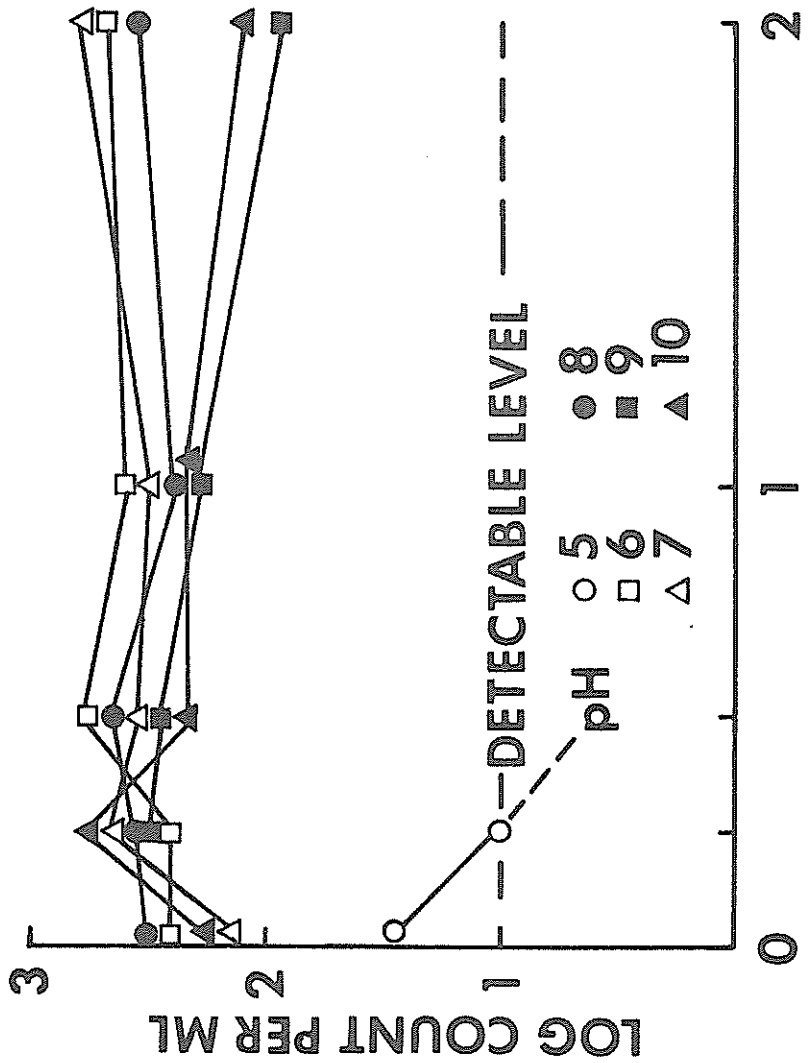

Fig. 2. Survival of *Vibrio Parahaemolyticus* in shrimp homogenates at various pH levels.

Data from Vanderzant and Nickelson (8).

VI. Quality Deterioration of Microbial Origin in Frozen Breaded Raw Shrimp

ABSTRACT

About 30% of the fresh or frozen shrimp received for processing into breaded shrimp had bacterial counts in excess of 10^6 per g. The bacterial counts of 56% of plant processed samples of frozen breaded raw shrimp exceeded 10^6 per g. Counts with plate incubation at 7 or 25 C were significantly higher than at 35 C. Neither Salmonella nor Vibrio parahaemolyticus was isolated from plant processed samples. Frozen storage of breaded shrimp for 3 to 12 months caused minor reductions in aerobic plate count and coliform count and only minor changes in trimethylamine nitrogen (TMN), total volatile nitrogen (TVN), and odor scores. When frozen breaded raw shrimp were exposed to elevated temperatures (0-10 C) extensive increases in count occurred at 0 C after 3-5 days, at 5.5 C after 2-3 days and at 10 C after 1-2 days. Coliform bacteria increased at 5.5 C, Escherichia coli and enterococci at 10 C. In samples held at 0-10 C, marked increases in TMN and TVN and decreases in pH value occurred when bacterial counts had increased sharply and when off-odors became noticeable. Gram-positive forms, Bacillus, Microbacterium, Micrococcus and coryneform bacteria were predominant in retail samples. Many of these samples showed evidence of repeated thawing and refreezing. Over 50% of the retail samples had aerobic plate counts of 10^6 per g or higher.

INTRODUCTION

The production and distribution of breaded shrimp is an important part of the seafood industry. In 1971 production of breaded shrimp was 104,284,000 lb with a value at the processors level of $121 million (16). In the production of frozen breaded raw shrimp, peeled and deveined shrimp are washed, covered with batter and coated with breading material. The breaded shrimp then are packed in cartons and frozen.

In tests made on retail samples of frozen breaded raw shrimp in 1961, Silverman et al. (12) reported that about 28% of individual samples had aerobic plate counts greater than 1,000,000 per g. In a similar study conducted recently, Nickerson and Pollak (9) showed that aerobic plate counts of individual retail samples ranged from 24,000 to 60,000,000 per g. The aerobic plate counts of 65 samples (out of 136) was higher than 1,000,000 per g. In 1967 Surkiewicz et al. (15) reported that breaded shrimp samples collected from plants operating under good conditions of sanitation had an average (geometric) MPN of less than 1,000 coliforms per g, and that 85% had an average (geometric) aerobic plate count of less than

1,000,000 per g. The present report presents detailed information on the microbiological conditions of raw and plant processed products. Included are studies on samples subjected to warm-up treatments to mimic loss of freezer operation.

MATERIALS AND METHODS

Collection of samples. Frozen breaded raw shrimp, except for store samples, were obtained from commercial operations along the Gulf Coast. The frozen samples were packed in dry ice at the processing plant and transported immediately to the laboratory. If additional analyses were required at a later date, sample units were stored at -23 C. Samples of raw and frozen shrimp were packed in dry ice and examined upon arrival in the laboratory. Store samples of frozen breaded raw shrimp were purchased in Fort Worth, Dallas, and Houston and transported to the laboratory packed in dry ice. These samples were from the same commercial sources as the plant samples.

Microbiological examination. Aerobic plate counts of shrimp were determined with the spread plate method by placing 0.1 ml of appropriate dilutions on Standard Method agar (SMA, BBL) plates. Sets of triplicate plates were incubated aerobically at 7 C for 10 days, 25 C for 2 days and 35 C for 2 days. Diagnostic procedures and schemes for identification of the microbial flora were presented previously (19). Previous reports describe the procedures for determining the MPN of coliform bacteria and E. coli (14), enterococci (10), Salmonella (2) and Vibrio parahaemolyticus (21).

Chemical and organoleptic examination. Trimethylamine nitrogen (TMN) and total volatile nitrogen (TVN) were determined as described in a previous paper (1). Organoleptic evaluations were carried out by a trained three-member taste panel. Breaded shrimp were evaluated fried with breading on and also boiled (4 min) with breading removed prior to boiling. Shrimp were fried in Wesson oil for 3 min at 191 C. Data were analyzed using analysis of variance, the mean separation technique of Duncan and simple correlations (13).

RESULTS AND DISCUSSION

Matthys (5) showed that bacterial counts of 276 samples of shrimp (Fig. 1) received for processing into frozen breaded raw shrimp were high. About 30% of the samples had bacterial counts of 1,000,000 per g or higher. Previous studies (18, 20) have shown that bacterial counts of freshly harvested Gulf Coast shrimp ranged from about 100 to 10,000 per g. Low bacterial counts can be maintained if shrimp is handled on the boat under sanitary conditions, is iced promptly and properly and is not held too long. Microbial

activity frequently causes extensive deterioration of quality characteristics particularly when counts reach levels of 1,000,000 per g or greater. The present results indicate a need to improve the bacteriological condition of shrimp that is used for processing.

Although the quality of the raw shrimp undoubtedly is in part responsible for the high counts of many processed breaded products, inspection of plant facilities and processing methods showed that factors related to the processing phase can contribute to additional bacterial contamination. Thorough washing of raw shrimp can reduce the microbial load. If strict sanitary procedures were maintained in the plant and if batter and breading of low bacterial count were applied, one could obtain processed products with bacterial counts lower than those of the initial fresh or frozen shrimp.

Bacterial counts of plant processed samples at 25 or 7 C were significantly higher than those determined with plate incubation at 35 C (Fig. 2, 3, 4,). This has been reported previously (8, 17) with other foods in which psychrotrophic bacteria constitute a significant part of the microbial flora. A high plate incubation temperature probably has a limiting effect on the growth of some psychrotrophic bacteria that are part of the natural flora of the product or enter as contaminants during harvesting, handling, and processing. With plate incubation at 35 C, 17% of the samples had counts in excess of 1,000,000 per g. This figure agrees with that reported by Surkiewicz et al. (15). However, when plates in the present study were incubated at 25 or 7 C, 56% of the samples had counts in excess of 1,000,000 per g. Little relationship existed between coliform or enterococcal counts and aerobic plate counts of breaded shrimp. Nickerson and Pollak (9) made a similar observation.

Frozen storage of breaded raw shrimp for 3 to 12 months at -23 C frequently caused minor reductions in total and coliform count. Freezing can destroy or sublethally injure bacterial cells. The latter often cannot be recovered under regular conditions of medium composition and plate incubation (4,7). This may also account for the somewhat smaller colonies with plate incubation at 35 C, particularly with samples stored frozen for long periods. Poor recovery of sublethally injured cells on selective media with inhibitors may in part explain the decrease in coliform count.

In most samples of breaded shrimp stored for 12 months at -23 C changes in TMN, TVN and odor scores were small. Careful control of the temperature probably kept these changes to a minimum. Repeated changes in temperature, between the frozen state and the melting point, can cause large decreases in bacterial counts of frozen fish (11). Neither Vibrio parahaemolyticus nor Salmonella species were isolated from frozen breaded raw shrimp.

When frozen breaded shrimp were exposed to elevated (0, 5.5, and 10 C) temperatures, extensive increases in bacterial counts were noted at 0 C after 3 to 5 days, at 5.5 C after 2 to 3 days, and at 10 C after 1 to 2 days. Increases in coliform counts occurred at 5.5 C, and in enterococci and E. coli at 10 C. Increases in bacterial populations at 0, 5.5, and 10 C caused decreases in the pH of breaded shrimp. Large increases in TMN and TVN occurred when bacterial counts had increased sharply and flavor scores showed moderate to severe taste and/or odor defects. The lower TMN and TVN values of samples with moderate to severe organoleptic defects were respectively 4.4 mg and 24.2 mg per 100 g shrimp excluding breading material. These values compare favorably with limits of acceptability reported by Montgomery et al. (5 mg TMN, 30 mg TVN) (6) and Iyengar et al. (15 mg TVN) (3). Correlations between TMN, TVN values and odor scores were high.

The initial microbial flora of frozen breaded raw shrimp consisted primarily of Pseudomonas, Achromobacter, Aeromonas, Bacillus, Moraxella, Microbacterium, Micrococcus or coryneform bacteria (Table 1). Holding samples at 0, 5.5, or 10 C caused increases in Microbacterium, Micrococcus, Bacillus or coryneform bacteria. Pseudomonas species, which usually dominate after storage in iced shrimp, did not predominate in refrigerated breaded shrimp. Gram-positive species contributed 50 to 90% of the microbial flora of these samples. The microbial flora of batter and breading which consisted primarily of Bacillus and Microbacterium species, most likely contributed to this condition (Table 2).

Examination of retail samples showed that samples had been held for excessive periods (up to 31 months), and there was ample evidence that repeated thawing and refreezing had occurred. Bacillus, Microbacterium, Micrococcus, and coryneform bacteria were predominant in retail samples. Over 50% of the retail samples had aerobic plate counts of 1,000,000 per g or higher. Coliform counts decreased during frozen storage in wholesale and retail channels.

There is little doubt that handling frozen foods in this way decreases consumer acceptance. The problems associated with the production of high quality shellfish products are not much different from those encountered with other foods. Proper care during harvesting, processing and distribution holds the key to the production of a top quality product.

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Table 1. Percentage distribution of microbial flora of breaded shrimp[a].

Type	Sample 2 C		212	2 K
Pseudomonas	67.5	(47.5)[b]	2.7	2.6
Flavobacterium	7.5	-	-	5.3
Achromobacter	2.5	-	18.9	-
Moraxella	-	2.5	27.1	5.3
Acinetobacter	-	-	2.7	-
Aeromonas	7.5	-	-	10.5
Enterobacteriaceae	-	-	-	2.6
Bacillus	-	(35)	5.4	18.4
Coryneforms	10.0	(5)	5.4	13.2
Microbacterium	5.0	-	32.4	23.7
Micrococcus	-	(10)	5.4	18.4

[a]Plates were incubated at 7 C.

[b]After holding sample 10 days at 0 C.

Data from Matthys (5).

Table 2. Percentage distribution of microbial flora of batter and breading.

Type	Percentage distribution	
	Batter	Breading
Pseudomonas	-	7.5
Moraxella	-	5.0
Bacillus	70.0	50.0
Coryneform	7.5	17.5
Microbacterium	22.5	20.0

Data from Matthys (5).

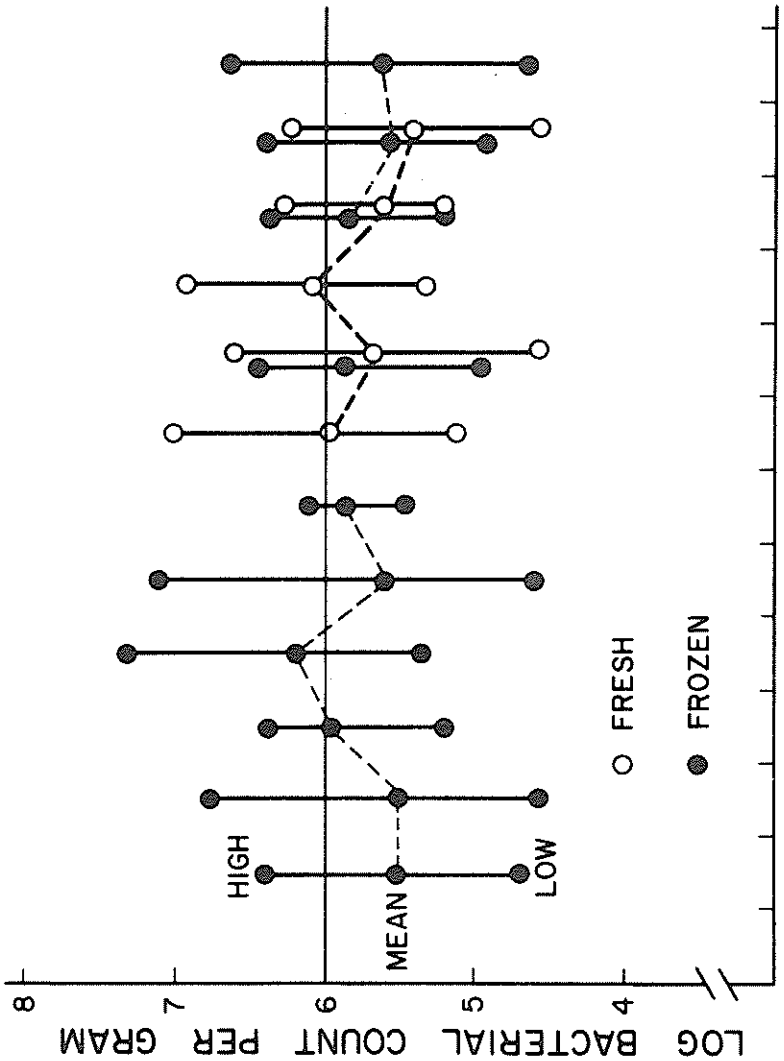

Fig. 1. Aerobic plate count at 35 C of fresh and frozen shrimp used in the manufacture of breaded shrimp.

Data from Matthys (5).

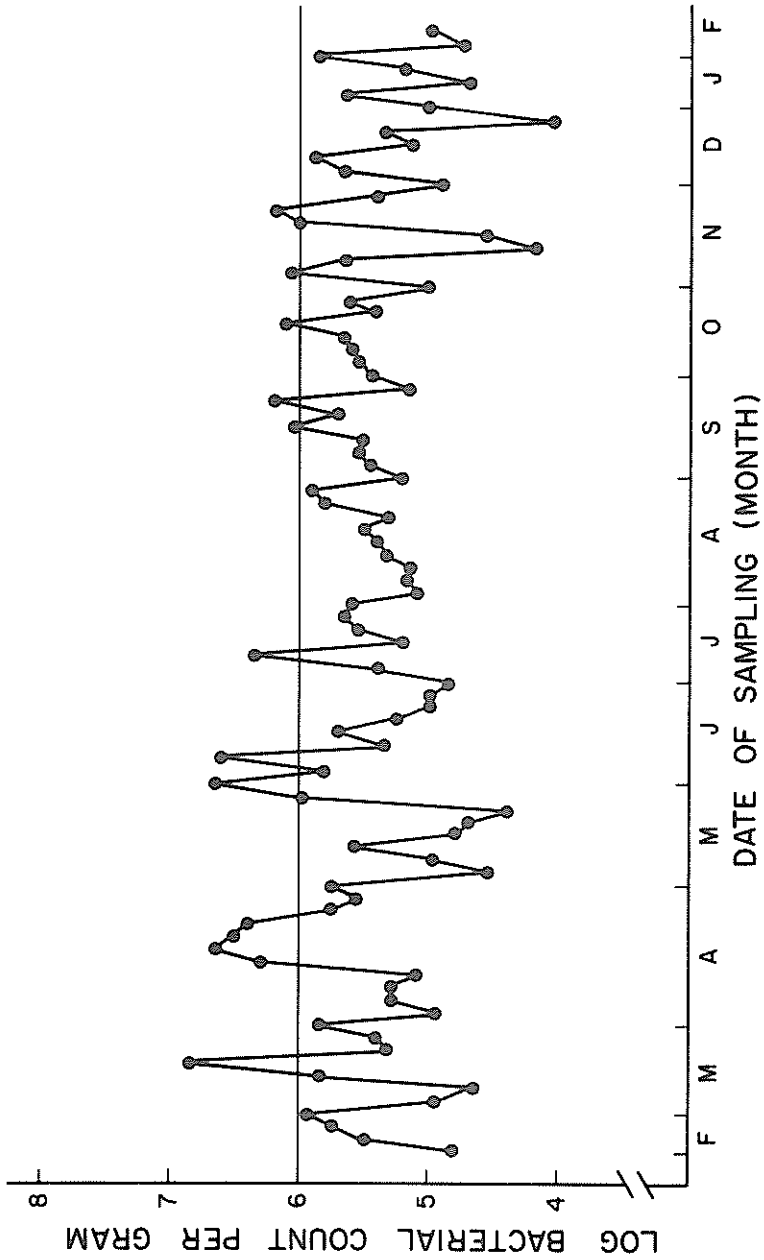

Fig. 2. Aerobic plate count at 35 C of freshly processed frozen breaded raw shrimp.

Data from Matthys (5).

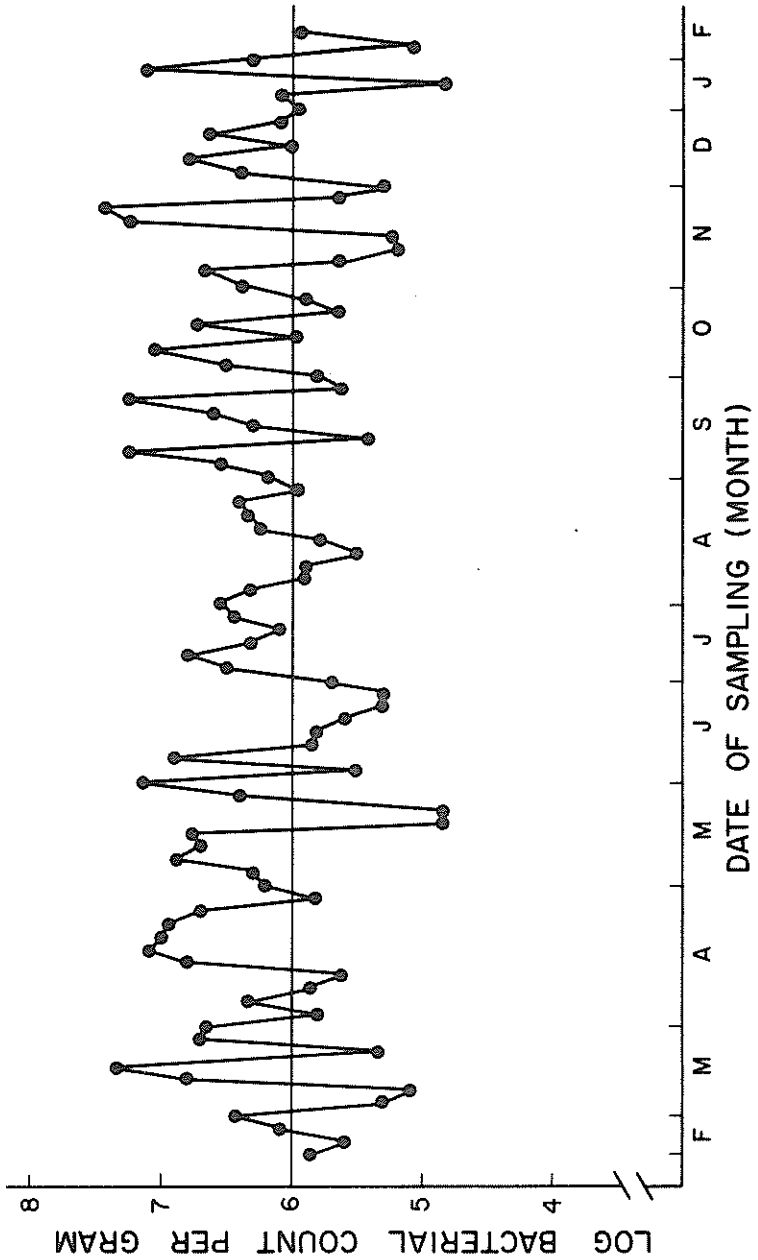

Fig. 3. Aerobic plate count at 25 C of freshly processed frozen breaded raw shrimp.

Data from Matthys (5).

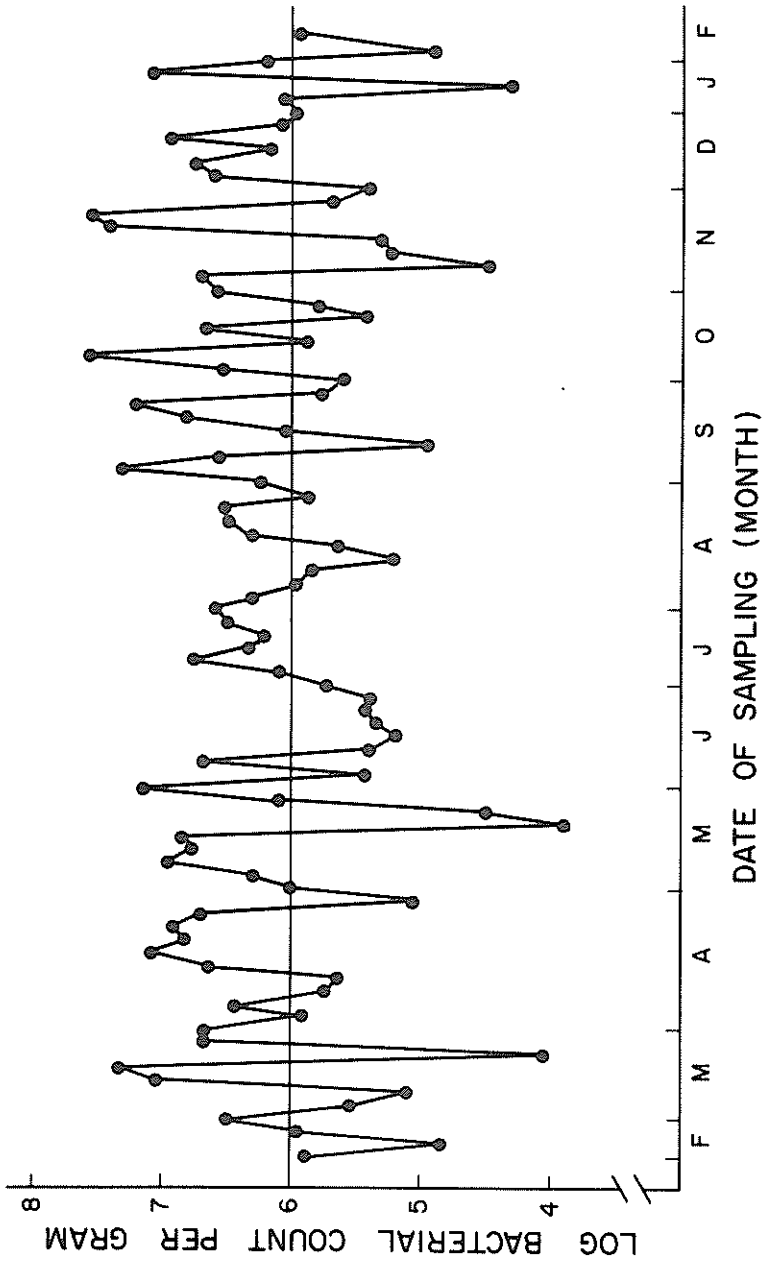

Fig. 4. Aerobic plate count at 7 C of freshly processed frozen breaded raw shrimp.

Data from Matthys (5).