MICROBIAL FLORA, CHEMICAL CHARACTERISTICS, AND SHELF LIFE OF FOUR SPECIES OF POND-REARED SHRIMP[1]

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ABSTRACT

Fresh pond-reared shrimp stored on sterile ice developed serious off-odors (musty) and melanosis in 14-17 days. Initial microbial counts were 10^4-10^5/g. Counts were lower after 7 days of refrigerated storage, regained their initial level after 21-22 days, and then continued to increase. The initial microbial flora consisted of *Aeromonas*, *Pseudomonas*, and *Vibrio* species. Coryneform bacteria were predominant after 21 days, and *Acinetobacter* and *Pseudomonas* species after 28 days. Total volatile nitrogen (TVN) levels of freshly harvested pond shrimp were similar to those of fresh Gulf shrimp, amino-nitrogen (AA-N) levels of pond shrimp were about twice as high. During the first 14 days of storage, TVN levels increased and AA-N levels decreased. Values for TVN/AA-N of spoiled pond shrimp were lower than those of spoiled Gulf shrimp and were in the range of fresh white or brown Gulf shrimp. Amino acid analyses showed that during the first 14 days of iced storage increases occurred in lysine-ornithine, histidine, threonine, alanine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine, with sharp decreases in proline and glycine.

Production and processing of shrimp is an important segment of United States and Gulf Coast fisheries. Commercial landings of shrimp in the United States in 1971 were 388 million lb. valued at dockside at $167 million *(10)*. Shrimp accounted for 26% of the total value of U. S. commercial fishery landings. Imports during 1971 amounted to 191 million lb. Nearly all shrimp harvested commercially are caught by fishing vessels on near-shore fishing grounds. In recent years, extensive efforts have been made to rear shrimp in natural and artificial ponds *(8)*. Vanderzant et al. *(12)* reported that the microbial flora of pond-reared brown shrimp (*Penaeus aztecus*) differed from Gulf Coast shrimp and consisted predominantly of coryneform bacteria and *Vibrio* species. Data on chemical characteristics of boat shrimp such as total volatile nitrogen, amino nitrogen, and amino acid distribution are available *(4, 5)*. Similar information on pond-reared shrimp is lacking. This paper reports on the microbial flora, chemical characteristics, and shelf life of four species of pond-reared shrimp stored on ice for 4-5 weeks.

MATERIALS AND METHODS

Shrimp cultivation and sampling

The 0.5-acre artificial ponds used in this study are located on the West Galveston Bay shore in Brazoria County, Tex. Postlarvae (seed stock, mean length 10 mm) of *P. vanami*, *P. occidentalis*, and *P. setiferus* were supplied by the Ralston Purina Company hatchery in Crystal River, Florida; those of *P. aztecus* by the Dow Chemical Co. hatchery in Freeport, Tex. At the time of stocking the estimated number of postlarvae per pond were 50,000 for *P. vanami* and *P. occidentalis*, 20,000 for *P. setiferus*, and 15,000 for *P. aztecus*. Each species was raised in a separate pond. The ponds were stocked in June-July and the shrimp harvested in September 1972. Shrimp in the ponds were fed daily at a rate of 5% of their estimated total body weight. Shrimp were fed 25 and 35% protein rations prepared by Ralston Purina Co. Ponds were approximately 3-5 ft in depth. Detailed information on pond management procedures are presented in another report *(8)*.

Chemical and microbiological procedures

Shrimp were obtained from ponds with a seine and were deheaded aseptically using sterile rubber gloves. The tails were placed in a sterile nylon net bag which was placed in sterile crushed ice in a 40 × 30 × 30 cm insulated ice chest. A perforated plastic partition was placed in each chest, 4-5 cm from the bottom. Water was drained from the ice chests and sterile ice was added when needed. Samples for analysis were withdrawn with sterile forceps. Shrimp were examined by a trained three-member panel 2 to 3 times each week for appearance (melanosis, color, slime) and off-odors.

Total volatile nitrogen (TVN) and amino nitrogen (AA-N) were determined in the trichloroacetic acid extracts as described by Cobb et al. *(3)*. A fully automated Spinco Model 120 C amino acid analyzer was employed for amino acid analysis.

Aerobic plate counts of shrimp were determined with the spreadplate method by placing 0.1 ml of appropriate dilutions on Trypticase Soy agar (TSA, BBL) plates with 3% NaCl. Preparation of samples and dilutions was as described by Surkiewicz *(9)*. Duplicate plates were incubated at 25 C for 2 days. To determine microbial types, approximately 40 colonies were picked at random from countable plates. Diagnostic procedures and schemes for identification of the microbial flora were presented previously *(11)*.

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TABLE 1. DISTRIBUTION OF MICROBIAL FLORA OF POND-REARED SHRIMP DURING REFRIGERATED STORAGE

Storage time (days)	Sample[a]	Percentage distribution									
		Acineto-bacter	*Aero-monas*	*Bacillus*	Coryne-form	*Entero-bacter*	*Flavo-bacterium*	*Micro-coccus*	*Morax-eila*	*Pseudo-monas*	*Vibrio*
0	A		65							30	5
	B		80							5	15
	C		30							60	10
	D		83							13	4
7	A		50							50	
14	A		40				5		15	35	5
21	A		15		65		5		15		
	B		5		95						
	C		30		45			15	10		
	D			10	60				25	5	
28	A	95			5						
	B	97				3					
	C							3		97	
	D	60					15		15	10	

[a]A = *P. vanami*, B = *P. occidentalis*, C = *P. aztecus*, D = *P. setiferus*

RESULTS AND DISCUSSION

Freshly harvested pond shrimp were judged of excellent quality with no detectable defects with respect to appearance or odor. After 7 days some deterioration in appearance (grey flesh, some reddening of pigment, slight melanosis) had occurred in all species except *P. setiferus*. This condition of the 3 species worsened after 14 days. Only a trace of melanosis was detectable on *P. setiferus*. Musty off-odors were detected in *P. vanami*, *P. occidentalis*, and *P. setiferus*. *P. aztecus* had a faint putrid off-odor. After 21 days of ice storage all species exhibited strong musty or musty-amine type off-odors, with dark grey flesh and diffuse greening under the shell. Samples were considered unacceptable in appearance and odor at 17-21 days of storage. Strong putrid off-odors were noticeable after 25 days when significant increases in bacterial count had occurred (Fig. 1).

Bacterial counts of freshly harvested pond shrimp ranged from 3×10^4 to 10^5/g (Fig. 1). With storage on ice, bacterial counts of the samples decreased sharply at 7 days, increased to their initial level after 21 to 22 days and continued to increase with counts at 28 days ranging from 2.5×10^6 to 3×10^7 per g.

The microbial flora of freshly harvested pond shrimp consisted of *Aeromonas*, *Pseudomonas*, and *Vibrio* species (Table 1). *Aeromonas* and *Pseudomonas* species continued to dominate the microbial flora of *P. vanami* after 7 and 14 days of refrigerated storage. A similar distribution of the microbial flora was noted for the other shrimp species at that time. Although minor variations in distribution of microbial types were noted between shrimp species, coryneform bacteria predominated after 21 days and *Acinetobacter* or *Pseudomonas* species after 28 days of refrigerated storage.

The initial bacterial counts of the freshly harvested shrimp in this study were slightly lower than those of *P. aztecus* reported previously *(12)*. *Aeromonas* or *Pseudomonas* species were the predominant isolates of the freshly harvested shrimp, whereas in a previous study *(12)* coryneform bacteria and to a lesser extent *Vibrio* species predominated in *P. aztecus*. Changes in ponds and characteristics of the water and shrimp stock may have caused these differences. In addition, type of feed, feeding rate, and fertilization of the ponds in 1972 differed from those employed in 1970 *(12)*. The reduction in count after 7 days of iced storage probably was caused by the washing effect of the melting ice and the inability of some

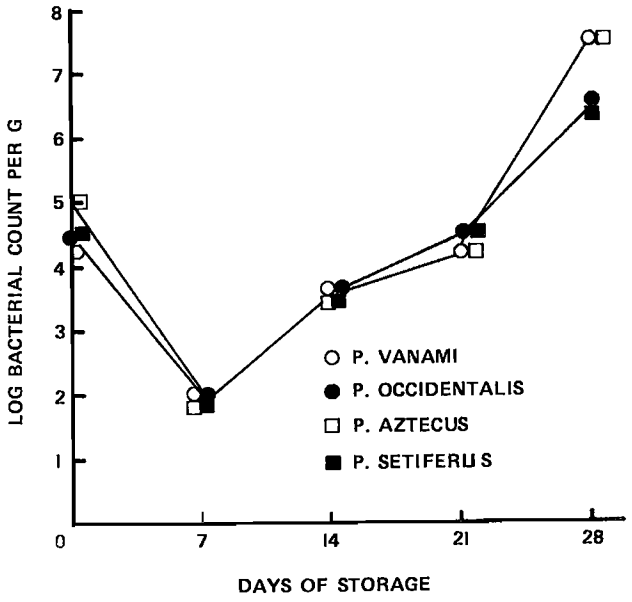

Figure 1. Agar plate counts of pond-reared shrimp stored in ice for 28 days.

microbial species to survive and/or grow at low temperatures. A similar change in microbial population of iced shrimp was reported by Campbell and Williams *(1)* and Carroll et al. *(2)*. Changes in the distribution of microbial flora during refrigerated storage may have been caused by differences in growth rate, symbiotic or antagonistic effects between species, or through reduction of the salinity of shrimp by melting ice. According to Carroll et al. *(2)* some microbial species may be unable to tolerate lowered salinity. Contact of shrimp with melting ice occurs during iced storage on commercial boats and is important in delaying deterioration of quality.

TVN levels of pond shrimp (Table 2) were similar or slightly higher than average values (13.36-15.73 mg N/100 g) for white or brown Gulf shrimp *(5)*. AA-N levels of pond shrimp were approximately twice those of Gulf shrimp (22.45-23.97 mM/100 g). During refrigerated storage, TVN levels increased during the first 14 days, leveled off, and then continued to decrease during the next 7 days. TVN levels of *P. vanami* and *P. occidentalis* increased again after 28-34 days when considerable increases in microbial population occurred. AA-N values for stored samples were lower than the initial values (0 days) particularly for *P. occidentalis*. TVN/AA-N ratios increased sharply during the first 7-14 days of storage, remained about the same for the next 11 days and increased again (*P. vanami* and *P. occidentalis*) after 28 and 34 days of storage. During the first 14 days, TVN/AA-N ratios increased at the rate of 0.017 (*P. vanami*), 0.013 (*P. occidentalis*), 0.017 (*P. aztecus*), and 0.028 (*P. setiferus*) mg N/mM/day. The value for *P. setiferus* is similar to an increase in TVN/

TABLE 2. TOTAL VOLATILE NITROGEN (TVN), AMINO NITROGEN (AA-N) AND TVN/AA-N RATIOS OF FOUR SPECIES OF SHRIMP DURING REFRIGERATED STORAGE

Iced storage (days)	*P. vanami*			*P. occidentalis*			*P. aztecus*			*P. setiferus*		
	TVN[a]	AA-N[b]	TVN/AA-N[c]	TVN	AA-N	TVN/AA-N	TVN	AA-N	TVN/AA-N	TVN	AA-N	TVN/AA-N
0	13.7	50.1	0.27	21.7	55.2	0.39	17.1	46.6	0.37	19.8	47.5	0.42
7	20.2	45.8	0.44	19.7	39.6	0.50	22.1	39.6	0.56	23.8	38.7	0.61
14	25.2	49.3	0.51	25.1	44.0	0.57	26.0	42.3	0.61	37.2	45.8	0.81
17	24.6	43.1	0.57	24.6	37.0	0.66	24.1	40.5	0.60	32.1	37.0	0.87
21	20.6	35.7	0.58	16.9	25.1	0.67	20.7	36.1	0.60	31.0	38.7	0.80
25	21.8	38.7	0.56	20.0	29.1	0.69	24.5	41.4	0.59	28.2	35.2	0.80
28	29.9	39.6	0.76	—	—	—	—	—	—	—	—	—
34	—	—	—	37.6	29.9	1.26	—	—	—	—	—	—

[a]mg N/100 g shrimp
[b]mM/100 g shrimp
[c]mgN/mM

TABLE 3. AMINO ACID ANALYSIS OF EXTRACTS FROM *P. vanami* DURING STORAGE ON STERILE ICE

Amino Acid	mM/100g shrimp						
	0[a]	7	14	17	21	25	28
Lysine-ornithine	0.36	0.58	1.14	1.03	1.09	1.06	1.26
Histidine	0.16	0.19	0.24	0.22	0.17	0.17	0.17
Ammonia	0.75	1.01	2.00	1.86	1.69	1.73	2.77
Arginine	3.74	2.97	3.33	2.97	3.05	2.61	2.14
Taurine	0.47	0.40	0.52	0.46	0.36	0.39	0.33
Aspartic acid	0.01	0.02	0.01	0.02	0.02	0.02	0.04
Threonine	0.16	0.26	0.53	0.46	0.42	0.45	0.42
Serine-glutamine-asparagine	1.36	1.75	1.65	1.44	1.06	1.45	0.66
Glutamic acid	0.36	0.40	0.50	0.43	0.38	0.30	0.42
Proline	8.09	7.20	3.91	5.86	4.00	5.54	5.46
Glycine	26.03	23.20	21.94	13.36	10.18	10.35	16.31
Alanine	4.03	3.60	6.81	5.63	5.36	5.08	4.86
Valine	0.26	0.49	0.87	0.81	0.72	0.83	0.64
Methionine	0.13	0.22	0.31	0.29	0.26	0.29	0.27
Isoleucine	0.21	0.29	0.45	0.43	0.36	0.42	0.36
Leucine	0.33	0.47	0.66	0.62	0.51	0.59	0.50
Tyrosine	0.11	0.17	0.24	0.22	0.22	0.20	0.21
Phenylalanine	0.08	0.18	0.27	0.25	0.03	0.25	0.26
TOTAL	46.64	43.40	45.38	36.36	29.88	31.73	37.08

[a]Days stored on sterile ice

AA-N of 0.032 mg N/mM/day reported for sterile (filtered) shrimp juice *(4)*. This suggests that TVN production during this period was probably caused by tissue enzyme activity. TVN/AA-N ratios for spoiled pond shrimp (after 17-21 days) were within the range of values (<0.9 mg N/mM) reported for freshly harvested Gulf shrimp *(5)* of excellent quality. This difference in TVN/AA-N ratios of spoiled samples probably resulted from high AA-N levels in the pond shrimp. Although all samples were unacceptable after 21 days, only the TVN value of *P. setiferus* exceeded 30 mg N/100g, which in the Australian and Japanese markets is used as indicative of spoilage *(7)*. TVN values of spoiled Gulf shrimp (initially handled aseptically and stored on sterile ice) usually were less than 30 mg N/100g *(5)*. With spoiled boat-shrimp (handled and stored under commercial conditions) TVN values usually exceeded 30 mg N. This difference in TVN level of spoiled boat shrimp and samples handled aseptically and stored on sterile ice may reflect a difference in type of microbial flora and activities.

The amino acid analyses for *P. vanami* are in Table 3. The analyses for the other species were similar. After 14 days increases were noted in the levels of lysine-ornithine, histidine, threonine, alanine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine. The lysine-ornithine component increased sharply probably because of arginase activity *(4)*. Urea was present in the 14-day extracts ranging from 0.317 to 0.700 mM/100g shrimp. Increases in histidine, threonine, leucine, and isoleucine indicate that considerable proteolytic activity occurred. Sharp decreases in proline and glycine occurred during this period. This reduction is not caused by the washing action of the melting ice because taurine which is not metabolized *(4)* did not change. Reduction in the level of glycine could affect the desirable "sweet" flavor of freshly harvested shrimp and result in bitterness *(6)*. This defect has been reported for shrimp stored for long periods in sterile ice *(2)*. The significant changes in chemical characteristics examined in this study occurred during the first 14-17 days. After this period arginase activity and probably urease activity also decreased and hence NH_3 (TVN) production. This may have resulted from the activity of proteolytic enzymes. NH_3 production again was detected when microbial activity had increased sharply (at 28-34 days).

The high AA-N levels in pond shrimp may have resulted from an abundance of protein-rich feed. Deterioration of the appearance of pond shrimp, particularly melanosis, was somewhat faster than that of Gulf shrimp stored under similar condition. In a previous study *(4)* it was noticed that this problem occurred more rapidly in shrimp from waters with high organic matter. The cause and mechanism of the more rapid development of melanosis in shrimp from waters with high organic matter is not known.

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