CHEMICAL CHARACTERISTICS, BACTERIAL COUNTS, AND POTENTIAL SHELF-LIFE OF SHRIMP FROM VARIOUS LOCATIONS ON THE NORTHWESTERN GULF OF MEXICO[1]

B. F. Cobb III, C. Vanderzant, C. A. Thompson, Jr. and C. S. Custer
Department of Animal Science
Texas Agricultural Experiment Station, College Station 77843

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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 boat-shrimp samples (4 out of 9 samples) with high TVN levels. Bacterial counts of fresh shrimp did not exceed 10^4/g. Nine of the 10 boat-shrimp samples had counts in excess of 10^6/g. Counts of samples spoiled on sterile ice ranged from 2×10^6 to 10^{10}/g. The estimated reduction of the maximum potential shelf-life of boat-shrimp by handling and storage was 0-15 days.

Shrimp are caught in waters varying in many characteristics such as level and type of microbial population, salinity, temperature, organic and inorganic matter, and plankton. Little is known about variations in chemical characteristics, bacterial count, and potential shelf-life of Gulf Coast shrimp harvested from different areas. The microbial flora of shrimp will reflect to some extent the microbiological characteristics of the water. Cobb and Vanderzant *(4)* have shown that different microbial species isolated from shrimp produce different spoilage patterns in refrigerated shrimp. Increased levels of trimethylamine (TMA) in shrimp are often associated with quality deterioration. TMA is generally considered to be the result of bacterial action on trimethylamine oxide (TMAO). Velankar and Govindan *(9)* reported that the TMAO content of shrimp taken from brackish water was lower than in shrimp taken from salt water. Hence, differences in chemical and/or microbiological characteristics of the environment and of the shrimp harvested from these areas could influence the shelf-life during iced storage.

Reduction of the shelf-life of shrimp through tissue enzymes and microbial activity begins during handling on the boat. These activities can be kept to a minimum if shrimp is handled on board under sanitary conditions and is iced promptly and properly. At present, quality of stored iced shrimp is determined mainly by appearance and odor, tests which frequently lack uniformity and provide little information about its potential shelf-life. This study reports on the potential shelf-life and changes in chemical characteristics and bacterial counts of shrimp from different locations in the Northwestern Gulf of Mexico during iced storage. A chemical analysis (TVN/AA-N ratio) is discussed for use in conjunction with appearance and odor evaluation to determine the suitability of shrimp for processing.

Experimental

Shrimp samples

Freshly harvested white shrimp (*Penaeus setiferus*) were taken from different locations (bays and harbors) 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. Freshly harvested shrimp were taken during June to August 1972. Brown boat-shrimp were obtained from shrimp boats on arrival at different locations on the northwestern coast of the Gulf of Mexico during July and August 1972. Freshly harvested samples were placed on a sheet of sterile plastic and were deheaded using sterile rubber gloves. While grasping the carapace with sterile forceps, tails were removed by cutting with sterile scissors. They were placed in sterile containers, rinsed with sea water from which they had just been removed and placed on sterile ice in an ice chest. Sterile ice was prepared by freezing heat-sterilized water in sterile stainless steel pans. Shrimp tails obtained from fishing boats at time of landing were immediately placed on sterile ice. Sanitized (chlorinated) ice chests (40 × 30 × 30 cm, Sears, vacucel insulated) were lined with a sterile plastic bag with holes in the bottom. The ice chests contained a partition which suspended the bag 4 or 5 cm above the bottom. Before inserting the shrimp, a layer (15 cm) of sterile ice was placed in the bag. After insertion of shrimp, it was covered with a 10 cm layer of sterile ice. Water was drained from the chambers and ice was added when needed. Samples

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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/100 g	TVN[b] mg N/100 g	NH_3/AA-N mg N/mM	TVN/AA-N mg N/mM	Log APC/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
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

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 (SMA, BBL) as described in a previous report *(8)*. Plates were incubated for 3 days at 25 C.

Shrimp extracts for chemical analyses were prepared by placing five shrimp in a Waring blendor and blending with 7% trichloroacetic acid solution (2 ml per gram of shrimp) until relatively homogeneous. The mixture was centrifuged to remove insoluble protein. Both total volatile nitrogen (TVN) and trimethylamine (TMN) analyses employed the microdiffusion procedure of Conway *(5)* with saturated Na_3PO_4 as releasing agent to prevent production of extraneous NH_3 during analysis. Values were multiplied by 1.3 to correct for incomplete distillation *(3)*. Amino nitrogen (AA-N) and NH_3 were determined by use of a Beckman Model 121C fully Automated Amino Acid Analyzer. Before 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

Characteristics of freshly harvested white shrimp

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/100 g and AA-N levels were either at or above 19 mM/100 g. 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. This occurred when samples had to be held for several days before amino acid analysis suggesting hydrolysis of compound(s) which yield NH_3. Preliminary experiments indicate that hydrolysis of glutamine is involved. The value of TVN/AA-N of freshly harvested white shrimp was always less than 0.90 mg N/mM. Trimethylamine (TMN) was not detected. Bacterial counts (2.5×10^2 to 10^4/g) were in a range normal for freshly harvested shrimp *(2, 8)*.

The white shrimp taken from different locations were placed on sterile ice until spoilage odors were detectable. The time at which serious off-odors appeared varied considerably (Table 2) but for 11 out of 14 (79%) samples ranged from 25 to 37 days when bacterial counts reached levels of 2×10^6 to 3×10^9/g with a geometric mean of 5×10^7/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. Factors which may have caused differences in shelf-life include type of microbial population, variations in tissue enzyme activity, and differences in chemical characteristics of shrimp of unknown nature. 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 data on chemical analyses of 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

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/100 g	TVN[b] mg N/100 g	NH_3/AA-N mg N/mM	TVN/AA-N mg N/mM	Log APC/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
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

TABLE 3. CHEMICAL ANALYSES AND AEROBIC PLATE COUNT (APC) OF FRESHLY HARVESTED BROWN SEA-SHRIMP TAKEN FROM DIFFERENT DEPTHS IN THE GULF OF MEXICO ADJACENT TO PORT ARANSAS, TEXAS

Location of catch	AA-N[a] mM/100 g	TVN[b] mg N/100 g	NH_3/AA-N mg N/mM	TVN/AA-N mg N/mM	Log APC/g
13 Fathoms[c]	23.27	9.84	0.55	0.42	2.5
18 Fathoms[c]	21.78	19.67	0.87	0.90	2.0
13 Fathoms[d]	21.41	15.18	0.74	0.71	3.2
13 Fathoms[e]	23.62	16.54	0.70	0.70	2.5
18 Fathoms[d]	19.53	16.61	0.69	0.85	3.2
28 Fathoms[d]	25.10	16.51	0.55	0.66	3.4

[a]α-Amino, proline and ammonia nitrogen
[b]Total volatile nitrogen
[c]Harvested June, 1971
[d]Harvested July, 1971
[e]Harvested August, 1971

washing action of melted ice. Only two samples had a TVN content greater than 30 mg N/100 g shrimp, the limit of acceptability used in some sections of the Australian and Japanese markets *(7)*. 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 the shrimp samples from Aransas Pass, Brownsville, and Port Isabel were removed after 14 days of storage on ice 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 *(4)*. The pH of spoiled shrimp ranged from 7.7-8.4, values close to 8.0 which some investigators *(1)* consider indicative of spoilage.

Characteristics of freshly harvested brown shrimp

The results of chemical analyses of freshly harvested brown sea-shrimp (Table 3) were similar to those of freshly harvested white shrimp. AA-N levels were greater than 19 mM/100 g (range 19.53-25.10) and TVN levels were < 20 mg N/100 g (range 9.84-19.67). TVN/AA-N ratios did not exceed 0.90 mg

N/mM. Values for TVN/AA-N ratios were similar to those of NH_3/AA-N. Bacterial counts of freshly harvested samples did not exceed 2.5×10^3/g.

Although the number of samples was limited, the shelf-life of brown sea-shrimp on sterile ice (Table 4) was shorter (range 20-30 days, average 24 days) than that of white shrimp (range 11-50 days, average 33 days). The pattern of changes in AA-N, TVN, NH_3/AA-N, and TVN/AA-N on spoilage of brown shrimp was similar to that when white shrimp spoiled. TMN was not detected in either freshly caught or spoiled brown sea-shrimp. Bacterial counts per gram of spoiled brown sea-shrimp ranged from 2.5×10^6 to $>3 \times 10^9$. The predominant spoilage odor was "musty". The pH of the spoiled samples was relatively high (7.8-8.0).

Characteristics of brown shrimp from commercial boats at time of landing.

The results of analyses on brown boat-shrimp (Table 5) differed considerably from those of freshly harvested shrimp (Tables 1, 3). 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). TVN levels in all but one sample exceeded those of freshly harvested shrimp (20 mg N/100 g). 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 5×10^5 to 10^8/g with a geometric mean of 1.2×10^7/g. A comparison of the values for the chemical and microbial indices of just landed boat shrimp (Table 5) and those of fresh and spoiled sea-shrimp (Tables 1-4) 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 (Sample T) to 15 days (Table 6). AA-N levels of the spoiled brown boat shrimp were higher (range 11.63-16.58 mM/100 g, 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). In samples with high TVN levels, TMN was evident. 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

TABLE 4. SHELF LIFE, CHEMICAL ANALYSES AND AEROBIC PLATE COUNT (APC) OF FRESHLY HARVESTED BROWN SEA-SHRIMP FROM DIFFERENT DEPTHS IN THE GULF OF MEXICO ADJACENT TO PORT ARANSAS, AND KEPT IN STERILE ICE UNTIL SPOILAGE OCCURRED

Location of catch	Shelf life days	pH	AA-N[a] mM/100 g	TVN[b] mg N/100 g	NH_3/AA-N mg N/mM	TVN/AA-N mg N/mM	Log APC/g
13 Fathoms, 18 Fathoms [c, d]	30	—	2.75	10.19	2.07	3.70	6.4
13 Fathoms	21	7.9	12.93	70.2	4.16	5.42	8.8
13 Fathoms	25	—	3.71	—	4.75	—	9.0
18 Fathoms	20	8.0	14.61	53.69	2.84	3.67	>9.5
28 Fathoms	23	7.8	4.99	22.11	3.39	4.43	8.3

[a]α-Amino, proline and ammonia nitrogen
[b]Total volatile nitrogen
[c]For date of catch see Table 3
[d]Samples taken in June pooled because of insufficient amounts of material.

TABLE 5. CHEMICAL ANALYSES AND AEROBIC PLATE COUNT (APC) OF BROWN BOAT-SHRIMP TAKEN FROM COMMERCIAL FISHING BOATS AT LANDING[a]

Sample	pH	AA-N[b] mM/100 g	TVN[c] mg N/100 g	NH_3/AA-N mg N/mM	TVN/AA-N mg N/mM	Log APC/g
R	7.9	20.78	22.62	0.97	1.09	6.3
S	8.1	18.48	24.66	1.11	1.33	7.2
T[d]	—	13.44	45.15	2.36	3.35	>7.5
U	8.2	14.18	24.76	1.35	1.75	6.3
V	7.4	22.21	25.74	0.91	1.15	7.0
W	8.1	18.26	38.43	1.28	2.10	7.9
X	7.3	20.53	19.16	0.91	0.93	7.7
CC	7.5	21.36	22.53	0.67	1.05	5.7
EE	7.1	16.53	20.49	0.86	1.24	7.3
FF	7.2	18.51	24.05	0.99	1.30	8.0

[a]Samples had been on board for a maximum of 6-8 days except for sample CC which had been on board 12 days.
[b]α-Amino, proline and ammonia nitrogen
[c]Total volatile nitrogen
[d]Spoiled at arrival in laboratory

TABLE 6. SHELF-LIFE, CHEMICAL ANALYSES AND AEROBIC PLATE COUNT (APC) OF BROWN BOAT-SHRIMP TAKEN FROM COMMERCIAL FISHING BOATS AT LANDING AND ALLOWED TO SPOIL ON STERILE ICE[a]

Sample	Shelf life days	Estimated total shelf life-days (ETSL)	pH	AA-N[b] mM/100 g	TVN[c] mg N/100 g	TMN mg N/100 g	NH_3/AA-N mg N/mM	TVN/AA-N mg N/mM	Log APC/g
R	11	18	7.9	15.06	97.84	14.98	3.70	6.50	9.3
S	8	15	7.8	11.63	97.89	14.82	4.58	8.41	9.2
U	6	13	8.5	12.41	93.99	16.77	4.64	7.57	9.0
V	5	12	7.5	—	23.44	0	—	—	8.4
W	4	11	7.3	13.39	30.47	0	1.77	2.28	9.0
X	15	22	7.7	15.08	75.82	6.32	3.10	5.03	9.0
CC	11	23	7.5	15.74	32.53	0	1.38	2.06	>8.1
EE	11	18	8.0	16.58	—	—	3.93	—	9.1
FF	3	10	7.7	13.32	31.49	0	1.32	2.36	8.6

[a]Samples had been on board for a maximum of 6-8 days except for sample CC which had been on board 12 days.
[b]α-Amino, proline and ammonia nitrogen
[c]Total volatile nitrogen

samples were greater than 10^8/g. The predominant spoilage odor was putrid. With freshly caught shrimp stored on sterile ice the predominant off-odor was "musty".

Through aseptic handling and storage on sterile ice, brown or white shrimp could be stored for long periods (average values 24 and 33 days) before spoilage odors developed. Even under such conditions the shelf-life is limited because of the activities of the natural microbial flora and tissue enzyme system. 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 caught in July and August was 22 days (Table 4). The brown boat shrimp were also caught in July and August and except for sample CC had been on board an average of 7 days (range 6-8 days). Figures for the estimated total shelf life (ETSL), consisting of the number of days on ice on the boat plus the additional storage on sterile ice before spoilage occurred, are in Table 6. Differences between the average MPSL of brown sea-shrimp and ETSL of commercial brown boat shrimp were −1 (Sample CC) to 15 days (average 7.1 days, Sample T included). Figures for ETSL usually were high for shrimp from boats with excellent sanitary handling practices and storage.

In certain areas of the world high TVN (30 mg/100 g) or TMN (5 mg/100 g) levels are used as an indication of shrimp spoilage *(7)*. In this study 4 of 19 (21%) spoiled sea shrimp samples stored on sterile ice reached this level of TVN and none showed TMN. For spoiled brown boat shrimp the TVN level of 8 out of 9 samples exceeded 30 mg/100 g and 4 samples contained >5 mg TMN/100 g. This discrepancy in TVN and TMN levels of spoiled boat and spoiled sea-shrimp (stored on sterile ice) probably was caused by a difference in microbial activities. *Pseudomonas* species frequently constitute a significant part of the microbial flora of boat shrimp. These species produced large increases in TVN and TMN in shrimp juice *(4)*. On the other hand, microbial counts at time of catch usually are low and typical spoilage bacteria are not numerous at that time. Coryneform bacteria and *Achromobacter* species predominated in sea-shrimp, handled aseptically and stored on sterile ice. This difference in distribution of microbial flora probably is also responsible for the difference in predominant off-odors between spoiled sea-shrimp (musty) and spoiled boat shrimp (putrid).

Some investigators claim that high pH values of shrimp (>7.95) are indicative of spoilage *(1)*. This could not be substantiated in this study.

In general, as spoilage of sea- or boat-shrimp occurred, the value of AA-N decreased and that of TVN increased. TVN and AA-N are produced through tissue enzyme and microbial activities. Losses in TVN and AA-N occur during iced storage when shrimp are continually subjected to washing by water from melting ice. In some samples, increases in TVN after storage were small probably because NH_3 and amines were removed more rapidly than they were produced *(6)*. The value of TVN/AA-N increased, however, because of decreases in AA-N.

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 conjuction with an evaluation of appearance and odor as a screening test to determine shrimp quality. In this relation, the ratio TVN/AA-N is to be preferred over NH_3/AA-N or over TVN and AA-N values alone because *(a)* TVN and AA-N can be measured by simple reliable tests in commercial seafood processing plants *(3)*, *(b)* in spoiled samples TVN consists of NH_3 and various amines, and *(c)* considerable variation existed in AA-N (or TVN) values among freshly harvested samples as well as among spoiled samples. It is recognized that samples of acceptable appearance and odor could have shown certain defects after cooking. Carroll et al. *(2)* reported that some samples of pink and white shrimp rated Grade A with respect to appearance and odor were bitter after cooking.

In a limited field trial with 40 commercial boat samples, samples with TVN/AA-N values >1.3 mg N/mM were usually evaluated as poor (based on appearance and odor) by plant quality control personnel.

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