Effect of Ice Storage upon the Free Amino Acid Contents of Tails of White Shrimp (*Penaeus setiferus*)

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The free amino acid and ammonia contents of tails (abdomen) of white shrimp (*Penaeus setiferus*) were measured initially and after short and long term ice storage. Four amino acids, arginine, taurine, proline, and glycine, comprised 93% of the total free amino acids with glycine alone comprising 67%. Glycine content was low in areas of suspected low salinity and high in areas of suspected high salinity. During bag storage (no leaching) significant increases ($P < 0.001$) occurred in the levels of all free amino acids except for taurine, aspartic acid, serine-glutamine-asparagine, and glycine levels which did not change significantly and arginine and proline levels which decreased significantly. The effect of drip on the free amino acid content was not measurable. Free amino acid loss was increased with increasing rate of ice melt. Postmortem free amino acid and ammonia producing enzymic activities could be followed by the relationship $A = 2(Cn_0 - C_0n)/t(n_0 + n)$, where A = enzymic activity in millimoles day^{-1} 100 g^{-1}, t is time in days, C is concentration of the amino acid at t, C_0 is the concentration at $t = 0$, n_0 is concentration of glycine at $t = 0$, and n is concentration of glycine at t. The rate constant k_1, for free amino acid loss due to a particular rate of ice melt could be determined by the relationship $k_1(w)^{1.2}t = \log n_0/n$, where t is time in days, w is weight in grams, n_0 is concentration at $t = 0$, and n is concentration at t.

The shrimp fishery is economically the most important segment of Gulf Coast Fisheries. In 1971 shrimp tail landings in states bordering the Gulf of Mexico were 143 million pounds (USDOC, 1972). Most of the shrimp is stored on board in ice. The majority of boats remain at sea for 4–14 days with some as long as 20 days. It is well established that during handling and storage on board serious losses in quality occur.

The edible portion (tail) of shrimp contains a high level of low molecular weight nonprotein nitrogenous compounds (NPN) (Cobb and Vanderzant, 1971). Amino acids comprise much of the NPN of shrimp and apparently contribute to shrimp flavor (Hashimoto, 1965; Nair and Bose, 1965). During iced storage, the level of some amino acids may be affected by the leaching action of ice, drip (Iyengar *et al.*, 1960), and by the activity of tissue and bacterial enzymes (Cobb and Vanderzant, 1971). Changes in the levels of certain amino acids such as glycine could significantly affect the taste and result in bitterness (Carroll *et al.*, 1968; Hashimoto, 1965).

The purpose of this study was to determine the effect of ice storage on the amino acid contents of the tails of white shrimp (*Penaeus setiferus*). Equations for determining some enzymic activities and predicting amino acid changes in ice-stored shrimp were developed.

MATERIALS AND METHODS

White shrimp (*Penaeus setiferus*) were taken directly from the water at different locations on the northwestern coastline of the Gulf of Mexico. Shrimp taken for long-term storage experiments were handled aseptically and stored on sterile ice as previously described (Cobb *et al.*, 1973b). Shrimp for short-term ice storage experiments were deheaded, dipped in chloramphenicol solution (100 μg ml^{-1}) to retard bacterial growth, and gently blotted dry prior to use. For bag storage, shrimp were placed in Zip-loc plastic bags and placed in ice chests (40 × 30 × 30 cm, Sears Vacucel insulated) in a monolayer with 15 cm of crushed ice beneath the bag and 10 cm above the bag. Shrimp for both long-term and short-term ice storage experiments were stored in the same manner but without bags. For long-term ice storage experiments, ice was replaced as needed. A slow rate of ice melt was maintained by placing ice chests in a 3–5° cold room. For more rapid rates of ice melt, the cold room temperature was maintained at 10°. For long-term storage experiments, shrimp samples were analyzed at 14 days and when musty or putrid off-odors appeared. Bacterial levels were checked as previously described (Cobb and Vanderzant, 1971).

Amino Acid Analyses. Shrimp extracts for analyses were prepared by blending ten or more shrimp tails in a Waring Blendor with 7% trichloroacetic acid solution (2 ml/g of shrimp) until all sizable particles had disappeared. The mixture was centrifuged to remove protein. Amino acid levels were measured by use of a Beckman Model 120C fully automated Amino Acid Analyzer. Analyses were conducted as soon as possible after extraction. All amino acid values were corrected for a moisture content of the shrimp of 80%. Total free amino acid levels and ammonia levels were checked by the procedures of Cobb *et al.* (1973a).

RESULTS AND DISCUSSION

The free amino acid content of tails (abdomen) of freshly caught white shrimp is included in Table I. The shrimp used for these analyses were taken from a small area during January and February and probably represent a homogenous population. Four amino acids, arginine, taurine, proline, and glycine, comprised 93% of the total free amino acids in the shrimp tail. Glycine alone comprised 67% of the free amino acid content.

Without detectable bacterial growth during refrigerated bag storage, significant increases ($P < 0.001$) occurred in the levels of all free amino acids in shrimp tails except for arginine, taurine, aspartic acid, serine-glutamine-asparagine (S-G-A), proline, and glycine. Arginine and proline decreased significantly while taurine, aspartic acid, S-G-A, and glycine did not change significantly. The effect of drip was not measurable.

When ice melted at a slow rate (complete melt in 16 days), except for glutamic acid which did not change, the levels of all free amino acids and ammonia were lower than those in bag-stored shrimp. The levels of ornithine, lysine, and ammonia were 81, 91, and 71% lower, respectively, in the ice-stored shrimp. The remainder of the amino acids were 35 ± 7% lower. When compared with

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Table I. Effects of Short-Term Storage and Rate of Ice Melt on the Free Amino Acid Content (mmol 100 g^{-1}) of White Shrimp Tails

	mmol 100 g^{-1}				
Amino acid	Freshly caught	S.D.	6 day[a] (bag storage)	6 day (ice, slow melt)	6 day (ice, rapid melt)
Orn	0.02[b]	0.004	0.58[c]	0.54[d]	0.33[e]
His	0.05	0.028	0.10	0.06	0.04
NH_3	0.96	0.063	1.42	1.02	0.68
Lys	0.06	0.010	0.16	0.15	0.08
Arg	3.08	0.221	2.39	1.58	1.26
Taurine	1.53	0.108	1.43	0.87	0.51
Asp	0.03	0.007	0.03	0.02	0.01
Thr	0.05	0.010	0.15	0.09	0.06
Ser-Gln-Asn	0.46	0.097	0.56	0.34	0.19
Glu	0.27	0.034	0.38	0.38	0.29
Pro	1.96	0.256	1.57	0.91	0.30
Gly	16.99	1.512	16.43	10.83	5.87
Ala	0.48	0.098	1.23	0.75	0.44
Val	0.07	0.017	0.23	0.13	0.09
Met	0.08	0.267	0.15	0.11	0.08
Ile	0.08	0.009	0.16	0.12	0.09
Leu	0.12	0.018	0.24	0.15	0.12
Tyr	0.05	0.010	0.08	0.05	0.04
Phe	0.02	0.009	0.09	0.05	0.02
Total (less NH_3)	25.34	1.791	25.95	17.11	9.78

[a] Storage time. [b] Mean of six analyses with 15 or 20 shrimp in each. [c] Mean of five experiments with 15 or 20 shrimp in each. [d] Mean of three experiments with 20 shrimp in each. [e] Mean of three experiments with 15 shrimp in each.

Table II. Enzymic Activity in Shrimp Tails Calculated from Changing Amino Acid Levels during Bag and Ice Storage

		Enzymic act., mmol 100 g^{-1} day^{-1}	
Type of act.	Rate of melt	Bag storage	Ice storage
Anabolic			
Arginase	S[a]	0.108[b]	0.107[c]
(from Orn)	R	0.084	0.079
NH_3 producing	S	0.077	0.082
	R	0.087	0.083
Lys producing	S	0.022	0.023
	R	0.015	0.015
Thr producing	S	0.012	0.019
	R	0.010	0.013
Ala producing	S	0.138	0.085
	R	0.120	0.071
Val producing	S	0.026	0.016
	R	0.029	0.016
Ile producing	S	0.016	0.013
	R	0.013	0.014
Leu producing	S	0.021	0.015
	R	0.019	0.019
Catabolic			
Arginase	S	0.124[d]	0.099[e]
(from Arg)	R	0.100	0.062
Pro reducing	S	0.077	0.076
	R	0.100	0.063

[a] Abbreviations: S = slow rate of melt; R = rapid rate of melt. [b] $A = (C - C_0)/t$. [c] $A = 2(Cn_0 - C_0n)/t(n_0 + n)$ (see text). [d] $A = (C_0 - C)/t$. [e] $A = 2(C_0n - Cn_0)/t(n_0 + n)$.

the initial samples, the levels of arginine, taurine, aspartic acid, proline, and glycine decreased while the levels of the other amino acids increased or appeared to remain constant. When the rate of ice melt was approximately doubled, the level of glycine was reduced by 64% from that in the bag-stored shrimp. The level of proline was reduced by 81% while the levels of the other amino acids were reduced at rates comparable to or less than that of glycine.

The concentration (C) of any amino acid at time (t) can be determined by the relationship

$$C = C_0 + At - \text{fraction lost by washing or drip} \quad (1)$$

where t is time in days, C is millimoles 100 g^{-1}, C_0 is millimoles 100 g^{-1} at $t = 0$, and A is enzymic activity in millimoles day^{-1} 100 g^{-1}. For bag-stored shrimp, the fraction lost by drip was not significant. Provided that the amino acid and enzymic activity have the same distribution as glycine (or taurine) the fraction lost by leaching can be approximated by the relationship

$$[C_0 + (At/2)][(n_0 - n)/n_0] \quad (2)$$

where n_0 is the concentration of glycine at $t = 0$ and n is the concentration of glycine at t. Substituting expression 2 into expression 1, we get

$$C = (C_0 + At) - (C_0 + At/2)[(n_0 - n)/n_0] \quad (3)$$

and

$$A = 2(Cn_0 - C_0n)/t(n_0 + n) \quad (4)$$

For calculations involving catabolic (amino acid decrease) rather than anabolic (amino acid increase) activity, the expression becomes

$$C = (C_0 - At) - \left(C_0 - \frac{At}{2}\right)[(n_0 - n)/n_0] \quad (5)$$

and

$$A = 2(C_0n - Cn_0)/t(n_0 + n) \quad (6)$$

If the distributions of glycine and the amino acid and/or enzymic activity differ, expressions 4 and 6 should give values for enzymic activities in ice-stored shrimp which differ from those in bag-stored shrimp. If the distributions are similar, eq 4 and 6 should give activities which agree with those calculated for bag-stored shrimp.

Enzymic activities for the two series of experiments are listed in Table II. Enzymic activities calculated from eq 4 and 6 agree with those for bag-stored shrimp for arginase (from ornithine production), ammonia, lysine, and isoleucine producing activities. Values for arginase activity (from arginine disappearance), alanine, and valine producing activities were lower for the ice-stored samples than for the bag-stored samples. Threonine, leucine producing, and proline reducing activities were the same in one series of bag-stored and ice-stored shrimp experiments but differed in the other series.

The shrimp tail is covered by a hard chitinous shell. Only at the anterior end is flesh exposed to leaching. In the process of removing the cephalothorax, the anterior end could become contaminated with proteolytic or other enzymes such as trypsin (Gates and Travis, 1969) from the digestive tract. Thus the site of major proteolytic activity and the loss of amino acids by leaching would be the same. Proteolytic activity could also be concentrated in the region of the hindgut. If glycine has a uniform distribution in the shrimp tissue, this would explain why eq 4 did not give correct answers with some amino acids.

Table III. Effect of Long-Term Ice Storage on Amino Acid Content of White Shrimp Tails

Amino acid	Freshly caught[b]	S.D.	n/n_0[a] Good, 14 days[c,d]	Spoiled 11 days[e]	Spoiled 25–29 days[f]	Spoiled 30–35 days[g,h]
Orn	0.02		12.80	218.50	35.43	14.417
His	0.13	0.025	0.60	0.81	0.19	0.06
NH_3	1.16	0.350	0.97[i]	3.17	1.18[i]	1.13[i]
Lys	0.10	0.030	1.79	2.12	2.71	0.96
Arg	4.20	0.536	0.40	0.48	0.13	0.06
Taurine	2.41	0.741	0.52	0.46	0.18	0.11
Asp	0.08		0.52	0.15	0.45	0.36
Thr	0.15	0.051	1.13[i]	1.49	0.56	0.35
Ser-Gln-Asn	0.64	0.198	0.40	0.46	0.17	0.06
Glu	0.38	0.102	1.39	1.73	0.75	0.34
Pro	2.00	1.017	0.35	0.33	0.07	0.02
Gly	14.39	3.859	0.46	0.48	0.19	0.08
Ala	1.33	0.399	1.63	0.90	0.50	0.25
Val	0.21	0.058	1.13[i]	1.84	0.61	0.39
Met	0.13	0.031	1.08[i]	1.27	0.64	0.37
Ile	0.10	0.038	1.24	2.94	0.80[i]	0.46
Leu	0.17	0.057	1.13[i]	2.32	0.69	0.38
Tyr	0.08	0.031	1.05[i]	1.13	0.56	0.35
Phe	0.05	0.016	1.44	2.00	1.07[i]	0.63
Total (less NH_3)	27.73	5.558	0.56	0.56	0.216	0.078

[a] n/n_0 = concentration/initial concentration. [b] Ice storage time. [c] Fourteen pooled samples. [d] Four pooled samples. [e] One pooled sample. [f] Four pooled samples. [g] Five pooled samples. [h] Four pooled samples kept for 35–50 days on ice had amino acid levels too low for accurate measurement. [i] Decreased in some experiments and increased in others.

Calculations of arginase activity from arginine disappearance could be affected by either arginine production, by proteolytic enzymes, and/or by arginine utilization by the low level of bacterial activity (Cobb and Vanderzant, 1971).

Long-Term Ice-Storage Experiments. The results of long-term ice-storage experiments are included in Table III. The shrimp used for these experiments were taken during the period of June to August from over 500 km of coast line. The average level of glycine was lower and the levels of arginine and alanine were significantly higher than in the winter-caught shrimp. Shrimp taken from areas of suspected low salinity such as river mouths had lower levels of glycine than those taken from areas with high salinity.

Because the amino acid levels varied in each group of shrimp used for the long-term storage experiments, the fractional changes (n/n_0) rather than actual levels were listed in Table III. On the average, ice melt was slightly slower (18–21 days for complete melt) than for the experiments included in Table I. In the good quality shrimp which had been stored on ice for 14 days, increases in the levels of ornithine, lysine, glutamic acid, alanine, isoleucine, and phenylalanine were evident. The levels of ammonia, threonine, valine, methionine, leucine, and tyrosine increased in some experiments and decreased in others. The levels of arginine, taurine, S-G-A, and glycine were reduced by approximately the same amount (54 ± 6%) while the level of histidine was reduced less than glycine and the level of proline more than glycine.

The "spoiled" shrimp taken at 11 days had a putrid odor while the other "spoiled" shrimp had musty odors.

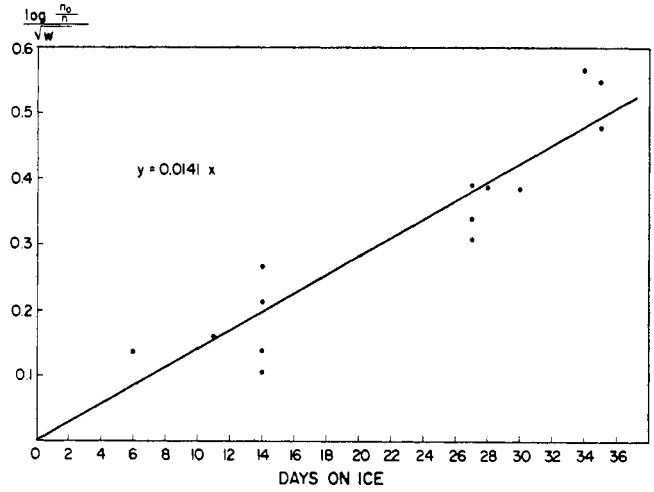

Figure 1. Relationship between shrimp tail weight (w, grams), ice storage time, and glycine content (n_0 = initial concentration, n = concentration at time of sampling).

Considerable proteolytic activity was evident in the sample taken at 11 days. Ammonia levels increased above original levels in some spoiled samples and decreased in others. Ammonia production was indicated in all spoiled samples. Although bacterial levels exceeded 1,000,000 g^{-1}, changes in the amino acid levels in the 25–29 day group and the 30–35 day group appeared to be mainly the result of washing. The anticipated levels of urea, 0.44, 0.66, and 0.46 mmol in the 11, 25–29, and 30–35 day groups, respectively, were not detected suggesting that urea might be the source of much of the ammonia.

Under a relatively constant rate of ice melt, there was a significant relationship ($P < 0.001$) between the log of n_0/n(glycine) divided by the square root of the weight and the time in days (Figure 1). Hence for the size range of shrimp tails used (1.3–7.5 g)

$$k_L t(w)^{1/2} = \log (n_0/n) \quad (7)$$

where k_L is the leaching constant for any particular rate of ice melt, t is ice-storage time in days, w is weight in grams, n_0 is concentration in millimoles 100 $gram^{-1}$ at $t = 0$, and n is concentration in millimoles 100 $gram^{-1}$ at t. Multiplying by -1 and rearranging eq 7 we get

$$\log (n/n_0) = -k_L(w)^{1/2}t \quad (8)$$

and

$$n/n_0 = 10 \exp(-k_L(w)^{1/2}t) \quad (9)$$

Substituting into eq 3

$$C = (C_0 + At) - \left(C_0 + \frac{At}{2}\right)[1 - 10 \exp(-k_L(w)^{1/2}t)] \quad (10)$$

and

$$A = \frac{2\{C + C_0[10 \exp(-k_L(w)^{1/2}t)]\}}{t[1 + 10 \exp(-k_L(w)^{1/2}t)]} \quad (11)$$

The use of eq 10 to monitor arginase activity in shrimp tails (summer caught) during a 12-day ice-storage period is illustrated in Table IV. The slight rate increase during the storage period may have been the result of increase in tissue pH from 7.2 to 7.7. The lower arginase activity than that listed in Table II for winter-caught shrimp may be a seasonal phenomenon as a number of other summer-caught shrimp including those in Table III had arginase activities ranging from 0.20 to 0.65 mmol 100 g^{-1} day^{-1}.

The initial amino acid content of shrimp tails varied with season and with area of catch. The seasonal variation may have been the result of different levels of feeding.

Table IV. Calculated Arginase Activity in Ice-Stored Shrimp

Postmortem age, days	$w^{1/2}$	Ornithine C,[b] mmol 100 g^{-1}	Arginase act., mmol 100 g^{-1} day^{-1}
2	2.130	0.0543	0.027[c]
5	2.287	0.108	0.025
8	2.332	0.178	0.028
9	2.362	0.203	0.030
12	2.467	0.257	0.031

[a] Weight (grams). [b] C_0 = 0.004 mmol 100 g^{-1}. [c] Calculated from the relationship $A = 2\{C + C_0[10 \exp(-k_L(w)^{1/2}t)]\}/t[1 + 10 \exp(-k_L(w)^{1/2}t)]$.

The high level of glycine suggested a physiological function. The lower levels in areas of suspected low salinities suggested that the physiological function might be osmotic pressure regulation.

The rate of loss of amino acids from shrimp tails during ice storage is affected by the rate of ice melt and by the size of the shrimp. Other factors such as position of the shrimp and ice particle size could affect the flow of melt water and, hence, rate of loss of amino acids. The increase in rate of loss with the square root of weight is the opposite direction expected if only diffusion were involved and the entire shrimp tail the site of the leaching action. This is further evidence that amino acid loss is *via* the anterior end of the shrimp tail.

The "natural flora" of shrimp is usually dominated by coryneform bacteria (Vanderzant *et al.*, 1970, 1971) which have little effect upon the free amino acid content of shrimp (Cobb and Vanderzant, 1971). Spoilage odors for all but one sample were not the typical putrid odors evident when shrimp were contaminated with proteolytic bacteria such as *Pseudomonas* species. Shrimp used in this study were checked for bacterial invasion by washing the shrimp tail for 5 min by shaking in water and comparing the bacterial counts with those determined on homogenates. There was no significant difference between the counts determined by the two methods suggesting that most bacteria were located on the surface of the shrimp. These observations probably explain why bacteria appeared to have little effect upon the free amino acid content of most samples.

The equations developed in this study provide methods of estimating some enzymic activities in shrimp tails during ice storage. For the limited size range of shrimp employed, prediction of the half-life of the glycine content and possibly other low molecular weight compounds can be made.

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Decaffeination. A Process to Detoxify Coffee Pulp

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The effect of a decaffeination process on the toxicity and nutritive value of coffee pulp was evaluated in rats. The decaffeination of the material was performed by extraction at 25° and by percolation at 94°. At 20 and 30% of the diet, the pulp subjected to either treatment showed a significantly ($P < 0.01$) lower feed efficiency ratio than the dehydrated coffee pulp. At the 50% level, the percolated pulp caused a significant decrease in the mortality index, in comparison to that observed with the dehydrated coffee pulp and or the decaffeinated pulp at 25°. A significant reduction in mortality was achieved with the latter by applying a complementary alcohol-extraction treatment. A high correlation was found between mortality and tannins, chlorogenic, and total caffeic acids (r = 0.92, 0.94, and 0.97, respectively). Decaffeination of the material could be effected both in the fresh or dehydrated states under equal processing conditions. It is concluded that this process offers an opportunity for the industrial use of coffee pulp.

During coffee processing the coffee pulp (term that includes the cherry peels and the pulp itself), the mucilaginous layer of the seed, and the seed husk are removed. The dry green coffee seed which represents around 20% (w/w) of the whole coffee cherry is the only part of the fruit utilized commercially at present (Bressani *et al.*, 1972).

Coffee pulp represents approximately 42% (w/w) of the whole coffee cherry (Bressani *et al.*, 1972). As an example we can mention that in 1971 approximately 2.7 million

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