Effect of Modified Ice Storage on the Quality and Prevention of Darkening Discoloration of Shrimp *Solenccera prominentis*

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To develop a convenient method for preserving shrimp on boat and during processing, several ice storage procedures were investigated. Based on the assessments of microbial growth, volatile base nitrogen (VB-N), extractable nitrogen (AA-N), inosine 5'-monophosphate (IMP) ratio and sensory evaluation, the decomposition of shrimp meat was retarded by storing in an ice-salt mixture (crushed ice and 3% NaCl, w/w), in the ice-salt mixture and 0.1% potassium sorbate, or in the ice-salt mixture, 0.1% potassium sorbate and 0.1% sodium erythorbate. The darkening discoloration was prevented and the freshness, IMP and sensory quality were maintained by the ice-salt mixture, 0.1% potassium sorbate and 0.1% sodium erythorbate during 15 days of storage.

The darkening discoloration of shrimp during storage is considered to be caused by an enzymatic reaction in which tyrosine or tyrosine-like compounds are oxidized in the presence of polyphenol oxidase and oxygen, to produce a dark pigment melanin.¹⁻⁵⁾ So far, much efforts have been made to prevent the development of darkening discoloration.⁶⁻¹⁵⁾ Although controlling pH and immersing in water to exclude oxygen were effective in inhibiting the darkening discoloration,^{2,3,5,9,10)} some physical changes such as loss in natural luster and softening in texture were observed during storage.¹²⁾ Cysteine,⁹⁾ aluminium compounds and ethylenediamine-tetraacetic acid (EDTA)⁵⁾ showed good inhibitory effects on the development of darkening discoloration of shrimp, but it is not commercially used yet due to the cost and limitation of dosage. Other than removing heads, washing and immediate chilling, the use of sulfite agents (potassium bisulfite, etc.) is currently considered to be the most effective method for preventing black spots of fresh shrimp.^{5,14-16)} However, the residual sulfite in shrimp can cause severe allergic reaction in the consumer as well as accelerate the formation of undesirable formaldehyde in shrimp during frozen storage.^{10,11)} Although Ho *et al.*¹⁷⁾ reported that either an ice-salt mixture (a mixture of crushed ice and 3% NaCl) or a mixture of ice-salt and 0.1% potassium sorbate could retard the decomposition of shrimp

during storage, and subsequently extend the shelf-life from 7 days to 15-17 days, the effect of these ice-salt mixtures on the prevention of the development of black spots was still unclear. This study was carried out to investigate the effects of these ice-salt mixtures and combination use of the ice-salt mixture with potassium sorbate and sodium erythorbate on preventing the darkening discoloration of shrimp during storage.

Materials and Methods

Fresh shrimp *Solenccera prominentis* (90-110 shrimps/kg) was purchased from a fishery market in northern Taiwan. These samples were iced for about 14 hr before the study. All shrimps were divided into four groups of 4 kg/each: G-I: stored in crushed ice; G-II: stored in an ice-salt mixture (crushed ice and 3% NaCl); G-III: stored in the ice-salt mixture and 0.1% potassium sorbate; G-IV: stored in the ice-salt mixture, 0.1% potassium sorbate and 0.1% sodium erythorbate. In all groups, the ratio of ice to shrimp was 3.

The ratio of the quantity of chemicals to that of crushed ice (w/w) is expressed as percentage. At a definite time interval, about 500 g samples were taken from each group and subjected to the following assays.

The quality of the shrimp was assessed by measuring the volatile base nitrogen (BV-N),

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extractable nitrogen (AA-N), total viable count, inosine 5'-monophosphate ratio (IMP ratio), sensory evaluation and photographic records. The pool of 500 g shrimp from each group was analyzed in triplicate.

Volatile Base Nitrogen (VB-N)

The VB-N was extracted and determined according to the method of Akiba *et al.*¹⁸⁾ About 5 g shrimp meat was homogenized with 25 ml of 6% trichloroacetic acid (TCA) and the suspension centrifuged at $1000\times g$ for 10 min at 25°C. The precipitates were washed with 20 ml of 6% TCA and the collected supernatants were filtered through a Toyo No. 5B filter paper and made up to 50 ml with 6% TCA. VB-N was determined by Conway's microdiffusion method.

Extractable Nitrogenous Compounds

Shrimp meat (20 g) was homogenized with 50 ml of 7% TCA using a Polytron homogenizer (Kinematica GmbH, Switzerland). The mixture was centrifuged at $1000\times g$ for 10 min. The residue was washed with 30 ml of 7% TCA and centrifuged. After being filtered, the combined supernatant was made up to 100 ml with 7% TCA and subjected to the micro-Kjeldahl analysis. The content of nitrogen, being essentially amino acid-nitrogen (AA-N), was expressed as mg of nitrogen per 100 g shrimp meat.

Inosine Monophosphate Ratio

Nucleotides were extracted by homogenizing 2 g of shrimp meat with 6 ml of 4% chilled perchloric acid (PCA) and centrifuging at $1000\times g$ for 10 min. The precipitates were washed twice with 5 ml of 4% chilled PCA. The combined supernatants were filtered through a Toyo No. 5B filter paper and adjusted to pH 6.5 with 10 N and 1 N KOH. It was filtered again and made up to 20 ml using a neutralized PCA solution (pH 6.5).

The nucleotides were analyzed by high pressure liquid chromatography (HPLC, Instrument: Varian 5020; AX-10, 0.4×30 cm) with three grading solvents¹⁹⁾; 5 mM $\text{NH}_4\text{H}_2\text{PO}_4$ /acetonitrile (13: 87), 5 mM $\text{NH}_4\text{H}_2\text{PO}_4$ and 0.75 M $\text{NH}_4\text{H}_2\text{PO}_4$. The area of each peak was automatically computed. The quantity of the nucleotides were calculated from the peaks by comparing with those of standard materials. The IMP ratio was expressed as a ratio of the quantity of IMP to the sum of IMP, inosine (HxR) and hypoxanthine (Hx).

Microbiological Assessment

To determine the potential bacteriostatic effect of the modified ice storage on microflora of shrimp, 25 g whole shrimp were aseptically homogenized with 225 ml of 0.1% sterile peptone water (Trypticase peptone, BBL). Several dilutions were made with sterile peptone water and duplicate plates were prepared using plate count agar (Difco). Plates were incubated at 37°C for 2 days prior to counting.²⁰⁾

Determination of the Development of Darkening Discoloration

Twenty shrimp from each group were graded individually by 9 panelists as follows: 5: no darkening discoloration; 4: a few black spots on the head, feet and tail; 3: black spots on head, feet and tail; 2: many black spots on head, feet and tail; 1: severe darkening discoloration on whole body. The score of each group was calculated by level of grade $\times$ number of shrimps. The mean of the score from panelists was used to express the degree of discoloration.

Sensory Evaluation

Sensory evaluation was performed by 9 trained panelists. Shrimps from each group were washed and cooked in boiling water for 3 min. Panelists rated them on the basis of overall acceptability using a 9-point hedonic scale. The sensory quality was expressed as: 9: like extremely; 5: acceptable margin; 1: dislike extremely.

Statistical Analysis

Duncan's multiple range test was used for statistical analysis.

Results and Discussion

Changes in the Content of Volatile Base Nitrogen (VB-N), Extractable Nitrogen (AA-N) and VB-N/AA-N Ratio

The VB-N of G-I and G-II decreased during 4 days storage, and increased gradually during the extended storage. The VB-N of G-I exceeded the permitted maximum level for fresh fish and shellfish, 25 mg/100 g, which is administered by the Food and Drug Administration (FDA) of Taiwan, R.O.C., after 11 days storage. That of G-II, G-III and G-IV decreased slowly during 8 days storage and increased during the prolonged storage. The VB-N of G-II, G-III and G-IV were still below the permitted maximum level, 25 mg/100 g, even after 15 days storage.

Table 1. Changes in volatile base nitrogen (VB-N), extractive nitrogen (AA-N), and VB-N/AA-N ratio of the shrimp during various modified ice storage

Treatment* ¹		Storage time (day)					
		0	4	8	11	13	15
VB-N (mg/100 g)	G-I	11.9	10.6	14.8	25.4	33.1	35.4
	G-II	11.9	9.8	12.3	15.7	16.9	17.8
	G-III	11.9	8.5	10.2	14.4	15.2	17.0
	G-IV	11.9	8.0	9.2	12.6	14.1	15.2
AA-N (mg/100 g)	G-I	588	599	330	392	353	274
	G-II	588	560	529	414	448	386
	G-III	588	602	462	459	423	339
	G-IV	588	535	344	375	375	370
VB-N/AA-N ($\times 100^{\circ}$)	G-I	2.02a* ²	1.77a	4.48a	6.48a	9.38a	12.92a
	G-II	2.02a	1.75a	2.33c	3.79b	3.77b	4.61c
	G-III	2.02a	1.41b	2.21c	3.14c	3.59b	5.01b
	G-IV	2.02a	1.50b	2.67b	3.36c	3.76b	4.11c

*¹ G-I: stored in crushed ice (ice: shrimp = 3: 1 w/w); G-II: stored in an ice-salt mixture (crushed ice and 3% NaCl); G-III: stored in the ice-salt mixture and 0.1% K-sorbate; G-IV: stored in the ice-salt mixture, 0.1% K-sorbate, and 0.1% Na-erythroate.

*² Values in the same column with different subscripts differ significantly ($p < 0.01$).

The decrease of VB-N in all samples at the earlier stage of storage might be due to its partial loss with melting ice. The development of VB-N during the extended period of storage was considered to be due to the microbial growth.

The extractable nitrogen (AA-N) of G-I, G-II, G-III and G-IV samples decreased gradually during storage, from an initial level of 588 mg/100 g to 274, 386, 339, and 370 mg/100 g of meat, respectively, after 15 days storage (Table 1). Generally, the development of AA-N of fish muscle is parallel with the deterioration of fish and shellfish because of the degradation of proteins and the growth of bacteria as reported by Chung.¹²⁾ The decrease in AA-N was considered to be lost with the melting ice and the consumption of bacteria.

The ratio value of VB-N/AA-N of G-I sample increased significantly from an initial value of 2.02 to 4.48 and 12.92 ($P < 0.01$), after 8 and 15 days storage, respectively. However, those of G-II, G-III and G-IV increased slowly, and reached to 4.61, 5.01 and 4.11, respectively, after 15 days storage (Table 1). The VB-N/AA-N ratio of control sample (G-I) was significantly higher than that of treated samples after 8 days storage, and much higher than that of treated samples after 15 days storage ($P < 0.01$).

Effect of the Modified Ice Storage on the IMP Ratio of the Shrimp

The IMP content and the accumulation of hypoxanthine were reported to be good indices

Table 2. Effect of the modified ice storage on IMP ratio*¹ of shrimp meat during storage

Treatment* ²		Storage Time (day)			
		0	4	8	13
G-I	content (μ mole/g)				
	Hx* ³	0.000	0.598	2.885	—
	HxR* ⁴	0.468	1.191	0.000	—
	IMP	6.286	1.143	0.000	—
	IMP ratio	93.1%	38.9%	0%	—
G-II	Hx	0.000	0.096	0.252	2.985
	HxR	0.468	0.519	0.882	trace
	IMP	6.286	4.054	2.573	0.000
	IMP ratio	93.1%	86.8%	69.4%	0%
G-III	Hx	0.000	trace	0.537	1.443
	HxR	0.468	0.650	0.930	1.812
	IMP	6.286	4.069	3.057	0.000
	IMP ratio	93.1%	86.2%	67.6%	0%
G-IV	Hx	0.000	0.144	0.241	2.019
	HxR	0.468	0.682	0.552	1.136
	IMP	6.286	5.453	2.323	0.000
	IMP ratio	93.1%	86.9%	74.6%	0%

*¹ IMP ratio = $\frac{\text{IMP}}{\text{IMP} + \text{Hx} + \text{HxR}} \times 100\%$.

*² See Table 1.

*³ Hypoxanthine.

*⁴ Inosine.

for assessing the quality of shrimp.²¹⁾ In the previous study,¹⁷⁾ a significant correlation between IMP ratio and the quality of the shrimp was

observed. The IMP ratio of the fresh sample (0 day) was quite high, 93.1% (Table 2). However, that of G-I sample (control) decreased to 38.9% and 0% after 4 and 8 days storage, respectively. The decrease in IMP ratio of G-II, G-III and G-IV samples showed almost the same patterns: from an initial level of 93.1% decreasing to 86.2–86.9%, 67.6–74.6% and 0% after 4, 8 and 13 days storage, respectively (Table 2). According to these data, no wide difference in the decrease of IMP ratio among the G-II, G-III and G-IV samples was obtained. The potassium sorbate penetrated into the shrimp muscle was determined to be 87–101 ppm, 132–142 ppm and 142–152 ppm, after 4, 8 and 13 days storage in the presence of 0.1% potassium sorbate. These values did not exceed the permitted maximum level of 1000 ppm for fish meat.²²⁾

Changes in Total Viable Count of the Shrimp Meat During Storage

Since the shrimp were not rinsed prior to use in this study, the initial total viable counts was quite high, 2.0×10^8 CFU/g (Table 3). The total

viable counts of G-II, G-III, and G-IV increased slowly and reached 2.5×10^7 , 5.0×10^7 , and 1.6×10^7 CFU/g, respectively, after 14 days storage,

Table 4. Effect of the modified ice storage on melanogenesis of shrimp

Treatment* ¹	Grade* ²	Storage time (day)			
		0	5	10	15
		Number of shrimp			
G-I	5	20	8	0	0
	4	0	12	4	0
	3	0 (100)* ³	0 (88)	8 (56)	4 (40)
	2	0	0	8	12
	1	0	0	0	4
G-II	5	20	20	0	0
	4	0	0	8	8
	3	0 (100)	0 (100)	12 (68)	12 (68)
	2	0	0	0	0
	1	0	0	0	0
G-III	5	20	16	0	0
	4	0	4	8	4
	3	0 (100)	0 (96)	12 (68)	12 (60)
	2	0	0	0	4
	1	0	0	0	0
G-IV	5	20	20	16	12
	4	0	0	4	8
	3	0 (100)	0 (100)	0 (96)	0 (92)
	2	0	0	0	0
	1	0	0	0	0

*¹ See Table 1.

*² 5: no darkening discoloration occurred; 4: few black spots were observed on head, feet and tail; 3: black spots were observed on head, feet and tail; 2: many black spots were observed on head, feet and tail; 1: darkening discoloration occurred on whole body.

*³ Values in the parentheses were obtained from the level of grade X the number of shrimp.

Table 3. Effect of the modified ice storage on microbial growth of shrimp

Treatment*	Storage time (day)			
	0	7	10	14
(CFU/g meat)				
G-I	2.0×10^8	7.9×10^8	4.0×10^8	5.0×10^9
G-II	2.0×10^8	8.0×10^8	1.6×10^7	2.5×10^7
G-III	2.0×10^8	3.1×10^8	6.3×10^8	5.0×10^7
G-IV	2.0×10^8	1.6×10^8	2.5×10^7	1.6×10^7

* See Table 1.

Plate 1. Shrimp before storage.

Plate 2. Melanogenesis of shrimp samples in G-I and II* during storage (* Refer to the footnote in Table 1).

Plate 3. Melanogenesis of shrimp samples in G-III and IV* during storage (Refer to the footnote in Table 1).

whereas, that of control sample increased sharply up to 5.0×10^9 CFU/g after 14 days storage. No significant difference in total viable counts among treated samples was observed after 14 days storage.

Effect of the Modified Ice Storage on Preventing the Development of Darkening Discoloration of Shrimp

Table 4 shows the degree of darkening discoloration of shrimp during storage. A photograph of shrimps before storage is given in plate 1. The darkening discoloration occurred on the feet and tail of few shrimps in G-I (control) and G-III after 5 days storage, however, the discoloration rate in G-III was lower than that of G-I (Table 4). The appearance of the shrimp in G-I changed severely, while that of G-II, G-III, and G-IV samples were still good after 10 days storage. The darkening discoloration occurred on all shrimps of G-II and G-III after 10 days storage (Plates 2 and 3). The degree of darkening discoloration increased slowly in G-II and G-III samples during the prolonged storage (Plates 2 and 3). The development of discoloration in G-III was more severe than that of G-II, while a little or no darkening discoloration was observed in G-IV sample after 15 days of storage (Table 4).

From the view point of color retention, the natural color of the samples in G-IV was retained during 10 days storage, but faded slightly during the extended storage. A little or no darkening discoloration was observed in G-IV sample even after 15 days storage. Although the ice-salt mixture or ice-salt mixture with 0.1% potassium sorbate could retard the decomposition of the shrimp muscle,¹⁷⁾ in this study, (G-II, G-III) were also effective on preventing the decomposition of *Solenccera prominens* shrimp meat (Tables 1 and 5). However, the black spots occurred on all

shrimps of G-II and G-III after 10 days storage. From Table 4, more black spots on shrimps of G-III were observed than on those of G-II after 15 days storage (Plates 2 and 3). The combination use of ice-salt mixture and 0.1% potassium sorbate, 0.1% sodium erythorbate (G-IV) effectively inhibited the development of black discoloration during 15 days storage.

Effect of the Modified Ice Storage on Sensory Quality of Shrimp

Results of sensory evaluations are shown in Table 5. G-II and G-IV samples were still acceptable even after 15 days storage. However, G-I (control) and G-III samples were hardly accepted by most panelists after 8 and 15 days storage, respectively. From these data (Tables 1 and 5), the samples were almost not accepted by panelists, while the ratio value of VB-N/AA-N was over 4.48. In the previous study,¹⁷⁾ this critical value was about 4.0. The difference in this critical VB-N/AA-N value might be due to the difference in species.

It was demonstrated that an ice-salt mixture (crushed ice and 3% NaCl), the mixture plus 0.1% potassium sorbate, the former mixture plus 0.1% sodium erythorbate has an excellent preventing effect on the decomposition and darkening discoloration of shrimp.

In the present and previous studies,¹⁷⁾ potassium sorbate showed an excellent inhibiting effect on the microbial growth in shrimp during storage. When used in combination with the ice-salt mixture, this additive showed again a marked preventing effect on darkening discoloration, but did not show any synergistic effect on keeping the quality of shrimp (Tables 1, 2 and 5).

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Table 5. Effect of the modified ice storage on sensory quality*¹ of shrimp

Treatment* ²	Storage time (day)					
	0	4	8	11	13	15
G-I	8.8a* ³	8.3a	5.3c	3.5c	3.5c	2.3c
G-II	8.8a	8.7a	8.2a	7.2a	6.8b	5.5a
G-III	8.8a	8.3a	7.3b	6.5b	6.5b	4.5b
G-IV	8.8a	8.4a	8.1a	7.4a	7.3a	5.7a

*¹ The sensory quality was evaluated by 9 trained panelists on 9-point hedonic scale (9: like extremely; 5; neither like nor dislike; 1: dislike extremely).

*² See Table 1.

*³ Values in the same column with different subscripts differ significantly ($P < 0.01$).

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