Changes in Extractive Components and Glycogen in the Edible Meat of Hard Clam *Meretrix lusoria* During Storage at Different Temperatures

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The changes in extractive components and glycogen in hard clam meat during storage at 0, 5, 10, 20 and 30°C were investigated. The initial levels of ATP-related compounds, glycogen, succinic acid and free amino acids in hard clam were $3.34 \pm 0.19 \mu\text{mol/g}$, $1201 \pm 52 \text{ mg/100 g}$, $9.44 \pm 2.55 \mu\text{mol/g}$, and $1226 \pm 106 \text{ mg/100 g}$, respectively. ATP in all samples decreased markedly during storage, while the accumulation of ADP, AMP, inosine, and hypoxanthine varied depending on the 5 storage temperatures. The decrease in glycogen was common for all the samples. In contrast, succinic acid increased with time of storage, and the increase rate was faster for the samples stored at 5 and 10°C. The changes in levels of free amino acids in hard clam during storage at different temperatures were inconsistent. The samples stored at 5 and 10°C showed a relatively high increase in the amounts of free amino acids, particularly in alanine, taurine, and arginine. The enrichment of free amino acids and succinic acid might potentially improve the flavor quality of hard clam stored at 5 and 10°C.

Key words: *Meretrix lusoria*, ATP-related compounds, free amino acids, succinic acid, glycogen, storage temperature

Hard clam *Meretrix lusoria* is one of the important cultured shellfish. Its production in Taiwan was about 18 thousand metric tons in 1995.¹⁾ This bivalve shellfish can survive for several days even it is exposed to the air after harvest.²⁾ Therefore, they are mostly sold in the live state. Many studies have been done on post-mortem biochemical changes in the tissues of shellfish in relation to freshness quality and assessment of freshness indicators. However, little information has been reported on the effects of storage conditions on the quality of live shellfish.³⁾

It is well known that the flavor of fish and shellfish principally originates from the extractive components.^{4,5)} Taurine, glycine, alanine, glutamic acid, arginine, AMP, and glycinebetaine are major constituents in shellfish,^{4,5)} which were considered as taste-active components in abalone,⁶⁾ snow crab,⁷⁾ scallop⁸⁾ and short-necked clam.⁹⁾ Several reports^{10–12)} demonstrated that the extractive components and meat quality of abalones were influenced by handling conditions prior to death, particularly by metabolic stress induced by low environmental oxygen and muscle work. The anaerobic glycolysis catalyzed by pyruvate reductases other than lactate dehydrogenase is the major energy-yielding mechanism in molluscan shellfish during environmental anaerobiosis, and the activity of the enzymes and metabolic end products are species specific.^{13–16)} Watanabe *et al.*¹⁷⁾ found that the glycolytic metabolites and mortality of fasted disk abalones were different from those fed with algae. Summers *et al.*³⁾ indicated that storage temperature affected the rate of D-lactate production and the ability to maintain adenylate energy charge of live clam *Paphies donacina* when stored in air. Recently, Enomoto¹⁸⁾ found

that the adductor muscle of live scallop with anaerobic acclimation was more palatable than that from aerobic acclimation.

As mentioned, hard clam is consumed primarily in the live state and its good flavor quality is generally regarded as a critical factor for consumer acceptance. It is of importance to investigate the changes in extractive components and glycogen of hard clams during storage at different temperatures under exposure of air. Our previous study²⁾ on the changes in freshness quality of live hard clam during storage showed that the initial decomposition of hard clam occurred at 0, 5, 10, 20 and 30°C at about 31, 21, 15, 5 and 1.3 days, respectively. In this study, we report further on the results of changes in the content of ATP-related compounds, glycogen, succinic acid and free amino acids in the edible meat of hard clams during storage at different temperatures.

Materials and Methods

Materials

Specimen of live hard clam *Meretrix lusoria* were collected in July, August and September 1994 from cultural farms in Yunlin prefecture in Southwestern Taiwan. They were transported (10–15°C) to the laboratory within 5–6 h and acclimated in sea water (24–26°C) for 2 h. The body weight and shell length were $13.9 \pm 3.0 \text{ g}$ and $4.0 \pm 0.3 \text{ cm}$, respectively. For storage experiments, the clams were divided into several groups of 1 kg weight (60–90 pieces) each and placed in polyethylene bags without being sealed. The samples were stored in a thermostated room ($\pm 1^\circ\text{C}$ accura-

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cy) set at 0, 5, 10, 20 and 30°C, respectively. A bag of samples was randomly withdrawn at regular intervals, equally divided into two parts, and the samples shucked to take off the edible meat. The meats were held in a stainless sieve (0.1 cm sieve) for 10 min to drain excess body fluid, cut into small pieces, mixed well, and subjected to the following analyses. The mean value obtained from duplicate experiments was expressed for each sample.

Determination of ATP-Related Compounds

A 5 g sample was homogenized in 30 ml of 6% cold perchloric acid (PCA) for 2 min, followed by centrifugation at $4200 \times g$ (4°C) for 20 min. The precipitate was extracted twice in the same way. The combined supernatant was adjusted to pH 6.5 with 1 or 10 N KOH and then placed in an ice bath for 30 min. After filtration, the supernatant was made up to 100 ml (PCA extract). ATP, ADP, AMP, IMP, inosine (HxR), adenosine (AdR), hypoxanthine (Hx) and xanthine (Xan) in the PCA extract were determined by HPLC on a Cosmosil 5C18-MS column (4.6×250 mm).²⁾ The mobile phase consisted of eluent A (50 mM KH_2PO_4 - K_2HPO_4 , pH 6.5) and eluent B (eluent A/methanol=9:1, v/v). Eluent A was applied for 14 min, followed by a linear gradient with an increase of eluent B up to 25% at 18 min, 90% at 22 min, 100% at 25 min, and 100% of eluent B was then kept for another 25 min. The flow rate was 0.7 ml/min. The column temperature was ambient (24–26°C). The detection was monitored at 254 nm.

Measurement of Glycogen

Glycogen was measured by a colorimetric method as described by Carroll *et al.*¹⁹⁾

Determination of Succinic Acid

Succinic acid in the PCA extract was determined by HPLC on an Interaction Ion-300 column (7.8×300 mm) using 0.005 N sulfuric acid as a mobile phase. The flow rate was 0.3 ml/min. The column temperature was set at 60°C and detection was monitored at 210 nm.

Determination of Free Amino Acids

A trichloroacetic acid (TCA) extract was prepared from each sample as described by Konosu *et al.*²⁰⁾ The meat (25 g) was homogenized with 7% cold TCA solution (100 ml) for 2 min using a Polytron PT-3000 homogenizer, followed by centrifugation at $4200 \times g$ (4°C) for 20 min. The precipitate was extracted twice by the same procedure with the exception that 30 ml of 7% TCA was used. The supernatants were filtered, combined, and made up to 200 ml. A portion of 40 ml was transferred into a separation funnel and shaken with an equal volume of diethyl ether. This procedure was repeated 5 times. Finally, the aqueous portion was concentrated under vacuum ($<40^\circ\text{C}$) to dryness and made up to 25 ml. Free amino acids in the TCA extract were determined at 440 nm and 570 nm using a L-8500 High Speed Amino Acid Analyzer equipped with a D-2850 Chromato-integrator (Hitachi, Ltd., Tokyo, Japan). The standard analytical method applied for physiological fluid assay was performed according to the instruction manual provided by the manufacturer.

Results

ATP-Related Compounds

The initial amount of ATP-related compounds in the edible meat of hard clams was $3.34 \pm 0.19 \mu\text{mol/g}$. ATP (0.78 – $1.21 \mu\text{mol/g}$), ADP (0.95 – $1.34 \mu\text{mol/g}$) and AMP (0.68 – $0.75 \mu\text{mol/g}$) were the dominant constituents, and they together accounted for about 90% of the total amount of ATP-related compounds. IMP, HxR (inosine), AdR (adenosine), Hx (hypoxanthine) and Xan (xanthine) were detected in small amounts.

Figure 1 shows the changes in the contents of ATP and its related compounds in hard clam meat during storage at 0°C. ATP decreased gradually with time, while ADP hardly changed until 16 days later. In contrast, AMP increased

Fig. 1. Changes in ATP-related compounds, glycogen and succinic acid in the edible meat of hard clam during storage at 0°C. Arrow indicates the time of onset of initial decomposition.

Fig. 2. Changes in ATP-related compounds, glycogen and succinic acid in the edible meat of hard clam during storage at 5°C. Arrow indicates the time of onset of initial decomposition.

Fig. 3. Changes in ATP-related compounds, glycogen and succinic acid in the edible meat of hard clam during storage at 10°C. Arrow indicates the time of onset of initial decomposition.

Fig. 4. Changes in ATP-related compounds, glycogen and succinic acid in the edible meat of hard clam during storage at 20°C. Arrow indicates the time of onset of initial decomposition.

markedly to the highest level ($1.57 \mu\text{mol/g}$) on the 24th day. Other compounds showed no apparent changes throughout the period of storage. When stored at 5°C (Fig. 2), ATP and ADP decreased rapidly during the first 9 days of storage. AMP increased to a maximum ($1.00 \mu\text{mol/g}$) after storage for 3 days, and decreased thereafter. HxR and Hx accumulated in a considerable amount, but IMP, AdR and Xan changed little during storage. As shown in Fig. 3, the change tendency of ATP-related compounds in the sample stored at 10°C was similar to that of the sample stored at 5°C. During storage at 20°C (Fig. 4), ATP-related compounds remained almost constant before the onset of initial decomposition at the first 3 day of storage. When stored at 30°C (Fig. 5), ATP decreased rapidly after 0.3 day resulting in a sudden increase of ADP. ADP reached $1.37 \mu\text{mol/g}$ on the 0.7th day and decreased thereafter. AMP increased slightly during the first day of

Fig. 5. Changes in ATP-related compounds, glycogen and succinic acid in the edible meat of hard clam during storage at 30°C. Arrow indicates the time of onset of initial decomposition.

storage, but other compounds hardly changed.

Glycogen and Succinic Acid

Figures 1–5 show the changes in the contents of glycogen and succinic acid in hard clam meat during storage at 0–30°C. The initial amounts of glycogen and succinic acid ranged between $1131\text{--}1255 (1201 \pm 52; \text{mean} \pm \text{S.D.}) \text{ mg/100 g}$ and $7.03\text{--}11.77 (9.44 \pm 2.55; \text{mean} \pm \text{S.D.}) \mu\text{mol/g}$, respectively. The decrease in glycogen occurred commonly during storage at different temperatures. As compared to the initial level, the glycogen reduction with a mean value of 28% (ranging between 24–31%) was observed prior to the initial decomposition of hard clam. On the contrary, succinic acid increased gradually with the duration of storage, but its increase seemed to be affected by the storage temperature. Succinic acid increased at a faster rate during storage at 5 and 10°C than at 0, 20, and 30°C.

Free Amino Acids

Table 1 shows the changes in the contents of free amino acids in hard clam meat during storage at 0–30°C. The initial level of free amino acids was $1226 \pm 106 \text{ mg/100 g}$. The most predominant constituent was taurine (Tau), followed by alanine (Ala), glutamic acid (Glu), arginine (Arg) and glycine (Gly). These five amino acids together accounted for 83–89% of the total amount of free amino acids. The other free amino acids were found only in small amounts ($< 35 \text{ mg/100 g}$).

During storage at 0°C, Tau, Glu and Arg decreased considerably. The reduction of these amino acids was directly associated with the decrease of total amino acids. Other free amino acids showed little change or a slight decrease during storage. The change patterns of free amino acids in samples stored at 5 and 10°C were similar. Most of the free amino acids increased prior to the initial decomposition. Particularly, Tau and Ala increased at a higher rate than other amino acids. The total amounts of free amino acids reached the highest level on the 9th day, showing a

Table 1. Changes in free amino acids (mg/100 g) in the edible meat of hard clam during storage at different temperatures

Amino acids	0°C storage (days)								5°C storage (days)							
	0	4	8	12	16	20	24	32	0	1	3	6	9	15	21	
Phosphoserine	7	9	7	8	6	6	6	6	7	6	7	8	10	9	9	
Taurine	596	542	500	490	497	476	431	406	547	529	565	660	648	658	498	
Aspartic acid	34	31	31	29	32	28	24	19	19	21	22	25	33	29	21	
Threonine	15	15	14	15	13	15	13	13	12	10	13	14	20	17	13	
Serine	9	7	7	7	6	7	6	5	8	7	8	8	10	8	5	
Glutamic acid	162	121	110	100	111	97	79	69	132	120	127	136	141	137	103	
Glutamine	9	3	4	3	4	3	2	2	16	15	16	14	25	19	14	
Proline	11	9	11	12	10	10	11	11	5	7	8	9	14	11	11	
Glycine	57	52	50	50	50	49	43	33	32	34	35	44	48	47	34	
Alanine	177	175	180	177	183	178	161	155	129	139	153	193	220	234	187	
Valine	17	17	17	18	17	18	17	17	11	12	15	17	25	22	21	
Cystine	2	1	1	1	1	1	1	1	1	1	2	2	2	1	1	
Methionine	6	4	6	7	6	6	6	7	7	8	8	10	14	10	14	
Isoleucine	9	9	10	11	10	10	10	11	10	13	17	14	24	19	19	
Leucine	13	13	15	16	15	15	15	16	13	14	18	20	32	27	25	
Tyrosine	12	10	13	14	13	13	13	13	11	12	15	16	24	21	21	
Phenylalanine	8	9	10	10	14	10	8	8	10	10	13	14	21	18	18	
β -Alanine	5	4	4	4	4	4	5	5	1	2	2	2	3	3	3	
Ornithine	8	7	8	7	7	7	7	6	8	8	10	10	13	12	23	
Lysine	28	25	25	26	24	25	25	23	23	23	26	26	37	28	24	
Histidine	8	7	7	8	7	8	7	7	5	5	6	7	9	10	7	
Arginine	116	98	94	87	96	93	82	74	80	80	87	98	107	108	58	
Total	1309	1168	1124	1110	1126	1079	972	907	1087	1076	1173	1347	1480	1448	1129	

Amino acids	10°C storage (days)						20°C storage (days)								30°C storage (days)					
	0	1	3	6	9	15	0	0.5	1	1.5	2	3	4	5	0	0.3	0.7	1	1.3	1.7
Phosphoserine	7	8	9	9	10	10	9	10	8	9	9	8	8	10	7	7	7	7	7	8
Taurine	547	622	635	681	677	605	652	661	652	653	655	659	629	651	445	457	442	453	439	430
Aspartic acid	19	23	27	33	37	33	22	28	27	28	29	30	28	30	21	21	19	18	20	19
Threonine	12	12	14	15	19	12	7	9	8	10	9	9	9	10	6	7	7	7	7	7
Serine	8	8	8	8	8	5	5	6	5	6	6	5	5	5	6	6	6	5	5	5
Glutamic acid	132	143	132	140	142	131	150	139	133	134	129	120	112	114	111	118	98	100	96	92
Glutamine	16	16	14	31	30	15	5	4	4	4	5	5	5	5	6	5	3	2	2	3
Proline	5	8	10	11	13	5	7	8	9	8	8	8	8	10	7	7	8	9	9	9
Glycine	32	40	42	47	49	54	60	64	62	71	63	68	69	73	79	88	80	88	86	87
Alanine	129	164	194	248	274	296	206	246	253	272	289	289	299	350	320	338	344	371	390	387
Valine	11	13	16	18	24	18	10	11	11	13	12	12	12	15	12	13	16	14	16	16
Cystine	1	1	2	1	2	1	1	1	1	1	1	1	1	1	trace	trace	trace	1	1	1
Methionine	7	7	7	11	12	18	4	3	4	4	5	4	4	6	3	3	3	3	4	5
Isoleucine	10	11	14	16	21	14	5	6	6	8	7	7	8	10	6	7	7	8	9	9
Leucine	13	15	19	22	29	22	7	8	9	11	11	10	11	15	8	9	10	11	12	13
Tyrosine	11	13	15	18	22	16	6	7	7	9	9	9	9	10	6	6	7	7	7	8
Phenylalanine	10	12	14	16	19	13	4	5	5	8	9	6	7	10	4	5	6	6	6	6
β -Alanine	1	2	2	2	3	3	3	3	3	3	3	3	3	4	6	7	6	7	7	7
Ornithine	8	9	10	11	11	12	6	6	6	6	6	6	6	7	3	4	3	4	4	5
Lysine	23	24	25	26	29	21	12	11	11	12	11	11	12	13	9	10	10	11	12	12
Histidine	5	6	7	9	10	10	4	5	5	6	6	7	7	8	3	3	4	4	4	5
Arginine	80	97	95	110	115	95	89	89	92	92	95	92	90	99	66	70	67	70	70	65
Total	1087	1254	1311	1483	1556	1469	1274	1330	1321	1368	1377	1369	1342	1456	1134	1191	1153	1206	1213	1199

36 and 43% increase of the initial level, respectively, during storage at 5 and 10°C. However, the changes in free amino acids in hard clam during storage at 20 and 30°C were not apparent, except that Ala increased in a relatively high amount. Consequently, the total amounts of free amino acids showed little change throughout the period of storage at 20 and 30°C.

Discussion

The changes in ATP-related compounds, glycogen, succinic acid and free amino acids in the edible meat of hard clams during storage appeared to be affected by storage temperature. The increase of succinic acid was common for all the samples stored at different temperatures. Konosu *et al.*²¹⁾ reported that succinic acid in short-necked clam markedly increased when exposed to the air after col-

lection, and the rate of accumulation was higher in summer than in winter. The accumulation of succinic acid in large amounts during anaerobiosis was also reported for the clams such as *Rangia cuneata*,²² *Mytilus edulis*,²³ *Tellina planata*²⁴ and *Mytilus galloprovincialis*.²⁵ The decrease in glycogen was also the common feature during storage at different temperatures. The glycogen in New Zealand abalone¹¹ and mussel²⁵ showed no significant variations within 24 and 36 h of anoxia, respectively. However, glycogen in mussel was utilized as the substrate of anaerobic metabolism during long-term anoxia.²⁵ Ogunsua *et al.*²⁶ found that glycogen decreased with storage time, and was a reliable quality index for harvested periwinkle *Tympanostomus fuscatus* during storage at 26°C for 6 weeks.

With regard to the degradation pattern of ATP-related compounds of hard clam as influenced by storage temperature, the accumulations of AMP, and HxR and Hx were found in the samples stored at 0°C (Fig. 1), and 5 (Fig. 2) and 10°C (Fig. 3), respectively. The degradation pattern at 5°C storage in this experiment is similar to that in foot muscle of hard clam stored at the same temperature as described by Yokoyama *et al.*²⁷ However, the pattern at 0°C storage is different from that of the muscle of hard clam stored in ice.²⁸ This may be partly due to the variations in tissues and state of specimens used.²³ A series of enzyme systems is responsible for the degradation of ATP-related compounds in the tissues of fish and shellfish. As reported by Yokoyama *et al.*,²⁶ the difference in degradation patterns in ATP-related compounds among the 4 species of shellfish examined was associated with the difference in activities of the degrading enzymes. Iwamoto *et al.*²⁹ indicated that the changes in ATP-related compounds in striated adductor muscle of itayagai scallop during storage at 5°C were different from those in the unshucked specimen. Regardless of the individual compounds, the decrease of the total amount of ATP-related compounds in hard clam meat during storage at different temperatures was not apparent before the onset of initial decomposition. The decrease rate of nucleotides (ATP + ADP + AMP + IMP) was relatively faster at 5 or 10°C storage than at other storage temperatures. The initial level of nucleotides in hard clam meat ranged from 2.8 to 3.1 µmol/g. However, the least amount still remained over 1.8 µmol/g on the 15th and 9th day for 5 and 10°C storage, respectively.

The 5'-nucleotides such as AMP, IMP and GMP are known to have a synergistic effect on umami taste with Glu.³⁰ AMP has been confirmed as a taste-active component in abalone,⁶ snow crab,⁷ scallop⁸ and short-necked clam.⁹ Matsumoto *et al.*³¹ indicated that the increase of umami taste in the muscle of kuruma prawn by "arai" treatment was attributed to the accumulation of large amounts of AMP and IMP. Yamamoto and Kitao³² reported that the contents of AMP, free amino acids, succinic acid and glycogen in the soup of hard clam were increased with elongated cooking time, and the preference of soup was improved. In this connection, it was expected that the contribution of flavor by AMP might not be different among the hard clams stored at different temperatures because they are commonly eaten after cooking.

The change patterns of free amino acids in hard clam during storage at different temperatures were not inconsistent with each other (Table 1). The reason why the changes

in free amino acids in hard clam vary with storage temperature is still not clear. The major free amino acids in hard clams were Tau, Ala, Glu, Arg, and Gly. They have been confirmed as taste-active components in shellfish.⁶⁻⁹ By judging the taste-active components in 5 kinds of seafoods that quantitatively resembled each other, Fuke and Konosu³³ demonstrated that not only combinations of taste-active components but that the relative quantities among them are important in producing the specific flavor of each seafood. They also indicated that Ala was listed as a taste-inactive component in abalone⁶ and short-necked clam⁹ due to its comparatively low content. The initial content of Ala in hard clam was about 3.6 times higher than that of Gly, suggesting that Ala is superior to Gly as a flavor contributor in hard clam. On the other hand, it is worth noting that the free amino acid profile in the hard clam of this study is very similar to that of short-necked clam,⁹ in which succinic acid, Tau, Gly, Glu, Arg, and AMP have been found to be taste-active components. As mentioned above, the increases of succinic acid, Ala, Tau, and Arg, and the total amount of free amino acids were much higher during storage at 5 and 10°C than at other storage temperatures. In this connection, the enrichment of these components might potentially improve the flavor quality of hard clams stored at 5 and 10°C. The increase in Ala is a common feature in mollusks during anaerobic metabolism.^{13,14} However, Kluytmans *et al.*²⁴ reported that *Tellina planata* was the only specimen with a strong accumulation of Tau, lactate, Ala, and Gly among 6 kinds of Mediterranean bivalves examined.

Yokohama *et al.*²⁷ reported that the K' value was the most suitable freshness index for foot muscle of hard clam during storage at 5°C. Our previous study² showed that the use of pH, volatile basic nitrogen, and K' value as freshness indicators for hard clams could not cover all the storage temperatures between 0–30°C. The results of this study showed that storage at 5–10°C was most suitable for hard clams because of the enrichment of free amino acids and succinic acid. The 0°C storage could prolong the shelf life of clams, but resulted in an increase of mortality.^{2,3}

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References

- 1) Taiwan Fisheries Bureau: Fisheries Yearbook Taiwan Area in 1995, Taiwan Fisheries Bureau, Department of Agriculture and Forestry, Provincial Government of Taiwan, Taipei, 1995, p. 145.
- 2) T. K. Chiou, J. F. Lin, and C. Y. Shaiu: Changes of freshness quality of cultured hard clam (*Meretrix lusoria*) during storage. *J. Chin. Nutr. Soc.*, **21**, 95–109 (1996) (in Chinese).
- 3) G. Summers, R. J. Wong, and M. L. Rogers: Effect of storage temperature on stress levels in live clams (*Paphies donacina*, tuatua). *J. Aqua. Food Product Technol.*, **5**, 7–23 (1996).
- 4) S. Konosu and K. Yamaguchi: The flavor components in fish and shellfish, in "Chemistry & Biochemistry of Marine Food Products" (ed. by R. E. Martin, G. L. Flick, C. E. Hebard, and C. R. Ward), The AVI Co., Westport, Connecticut, 1982, pp. 367–404.
- 5) S. Fuke: Taste-active components of seafoods with special reference to umami substances, in "Seafoods: Chemistry, Processing Technology and Quality" (ed. by F. Shahidi and J. R. Botta), Blackie Academic & Professional, Glasgow, UK, 1994, pp. 115–

- 139.
- 6) S. Konosu: Taste of fish and shell fish with special reference to taste producing substances. *Nippon Shokuhin Kogyo Gakkaishi*, **20**, 432-439 (1973) (in Japanese).
- 7) T. Hayashi, K. Yamaguchi, and S. Konosu: Sensory analysis of taste-active components in the extract of snow crab meat. *J. Food Sci.*, **46**, 479-483&493 (1981).
- 8) S. Konosu, K. Watanabe, K. Koriyama, T. Shirai, and K. Yamaguchi: Extractive compounds of scallop and identification of its taste-active components by omission test. *Nippon Shokuhin Kogyo Gakkaishi*, **35**, 252-258 (1988) (in Japanese).
- 9) S. Fuke: Taste-active components of a few species of bivalves, in "Society for Research on Umami Taste '89 Forum" (ed. by Y. Kawamura), Society for Umami Research on Umami Taste, Tokyo, 1989, pp. 85-91 (in Japanese).
- 10) J. Olley and S. J. Thrower: Abalone—an esoteric food, in "Advances in Food Research" (ed. by C. O. Chichester, E. M. Mark, and G. F. Stewart), Academic Press, New York, 1977, pp. 143-186.
- 11) J. Baldwin, R. M. G. Wells, E. Low, and J. M. Ryder: Tauropine and D-lactate as metabolic stress indicators during transport and storage of live paua (New Zealand abalone) (*Haliotis iris*). *J. Food Sci.*, **57**, 280-282 (1992).
- 12) J. M. Ryder, R. M. G. Wells, and J. Baldwin: Tauropine and D-lactate as indicators of recovery of live paua (New Zealand abalone) (*Haliotis iris*) from handling stress, and of postmortem quality. *Food Australia*, **46**, 523-526 (1994).
- 13) A. De Zwaan and T. C. M. Wusman: Anaerobic metabolism in bivalvia (mollusca), characteristics of anaerobic metabolism. *Comp. Biochem. Physiol.*, **54B**, 313-324 (1978).
- 14) S. Sakaguchi: Formation of octopine and its physiological roles in the muscle of molluscs. *Bull. Res. Inst. Food Sci. Kyoto Univ.*, **41**, 1-7 (1978) (in Japanese).
- 15) G. Gäde and M. K. Grieshaber: Pyruvate reductases catalyze the formation of lactate and opines in anaerobic invertebrates. *Comp. Biochem. Physiol.*, **83B**, 252-272 (1986).
- 16) D. R. Livingstone: Origins and evolution of pathways of anaerobic metabolism in the animal kingdom. *Amer. Zool.*, **31**, 522-534 (1991).
- 17) H. Watanabe, H. Yamanaka, and H. Yamakawa: Influences of hypoxia on changes in content of glycolytic metabolites in the muscle of juvenile disk abalone with different algae or starved. *Nippon Suisan Gakkaishi*, **60**, 241-246 (1994).
- 18) T. Enomoto: Umami of scallop. *Seibutsu To Kagaku*, **32**, 765-766 (1994) (in Japanese).
- 19) N. V. Carroll, R. W. Longley, and J. H. Roe: The determination of glycogen in liver and muscle by use of anthrone reagent. *J. Biol. Chem.*, **220**, 583-593 (1956).
- 20) S. Konosu, K. Watanabe, and T. Shimizu: Distribution of nitrogenous constituents in the muscle extracts of eight species of fish. *Bull. Jap. Soc. Sci. Fish.*, **40**, 909-914 (1974).
- 21) S. Konosu, M. Shibota, and Y. Hashimoto: Concentration of organic acids in shellfish with particular reference to succinic acid. *Eiyo To Shokuryo*, **20**, 186-189 (1967) (in Japanese).
- 22) T. M. Strokes and J. Awapara: Alanine and succinate as end-products of glucose degradation in the clam *Rangia cuneata*. *Comp. Biochem. Physiol.*, **25**, 883-892 (1968).
- 23) J. H. Kluytmans, A. M. T. De Bont, J. Janus, and T. C. M. Wijsman: Time dependent changes and tissue specificities in the accumulation of anaerobic fermentation products in the sea mussel *Mytilus edulis* L. *Comp. Biochem. Physiol.*, **58B**, 81-87 (1977).
- 24) J. H. Kluytmans, A. M. T. De Bont, E. C. J. Krutwagen, H. J. L. Ravesteyn, and P. R. Veenhof: Anaerobic capacities and anaerobic energy production of some mediterranean bivalves. *Comp. Biochem. Physiol.*, **75B**, 171-179 (1983).
- 25) G. Isani, O. Cattani, M. Zurzolo, C. Pagnucco, and P. Cortesi: Energy metabolism of the mussel, *Mytilus galloprovincialis*, during long-term anoxia. *Comp. Biochem. Physiol.*, **110B**, 103-113 (1995).
- 26) A. O. Ogunsua, C. C. Ariahu, and M. B. Adebona: Post-harvest changes in periwinkle (*Tympanostomus fuscatus*) at tropical ambient storage conditions. *Lebensm.-Wiss. u.-Technol.*, **23**, 343-348 (1990).
- 27) Y. Yokoyama, M. Sakaguchi, F. Kawai, and M. Kanamori: Chemical indices for assessing freshness of shellfish during storage. *Fisheries Sci.*, **60**, 329-333 (1994).
- 28) I. K. Suwetja, K. Hori, K. Miyazawa, and K. Ito: Changes in content of ATP-related compounds, homarine, and trigonelline in marine invertebrates during ice storage. *Nippon Suisan Gakkaishi*, **55**, 559-566 (1989).
- 29) M. Iwamoto, H. Yamanaka, S. Watabe, and K. Hashimoto: Changes in ATP and related breakdown compounds in the adductor muscle of "itayagai" scallop *Pecten albicans* during storage at various temperatures. *Nippon Suisan Gakkaishi*, **57**, 153-156 (1991).
- 30) S. Yamaguchi: The synergistic effect of monosodium glutamate and disodium 5'-inosinate. *J. Food Sci.*, **32**, 473-478 (1967).
- 31) M. Matsumoto, H. Yamanaka, and K. Hatae: Effect of "arai" treatment on the biochemical changes in the kuruma prawn muscle. *Nippon Suisan Gakkaishi*, **57**, 1383-1387 (1991).
- 32) Y. Yamamoto and N. Kitao: Effects of cooking time on the free amino acid content and the taste of soup of hard clam (ushiojiru). *Chori Kagaku*, **26**, 214-217 (1993) (in Japanese).
- 33) S. Fuke and S. Konosu: Taste-active components in some foods—a review of Japanese research. *Physiology & Behavior*, **49**, 863-868 (1991).