低温貯蔵におけるクルマエビ筋肉の解糖系代謝産物および遊離アミノ酸の消長

誌名	日本水産學會誌
ISSN	00215392
著者	松本, 美鈴 山中, 英明
巻/号	56巻9号
掲載ページ	p. 1515-1520
発行年月	1990年9月

Nippon Suisan Gakkaishi 56(9), 1515–1520 (1990)

Changes in Contents of Glycolytic Metabolites and Free Amino Acids in the Muscle of Kuruma Prawn during Storage*1

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(Received April 25, 1990)

The changes in contents of glycogen, glycolytic metabolites, and free amino acids (FAA) were investigated in the kuruma prawn muscle during storage at 5°C and 0°C.

The concentrations of glycogen and glycolytic intermediates were relatively low. During storage, glycogen and glycolytic intermediates decreased and there were no significant accumulations in these substances. From these results, it was concluded that glycogen was metabolized smoothly in the kuruma prawn muscle.

Six amino acids, Tau, Glu, Pro, Gly, Ala, and Arg, accounted for 97% of the total FAA in the kuruma prawn muscle, especially Gly alone 43%. The total FAA decreased during the early storage and showed maximum levels at the initial stage of decomposition regardless of storage temperatures. These changes in the total FAA were mainly due to the contents of the five major amino acids, except for Tau. Orn was not detected immediately after death and increased gradually during storage. Orn appeared to be useful as a potential index for the freshness of the kuruma prawn muscle.

In the previous paper,[1] we examined the changes in ATP related compounds, polyamines, volatile basic nitrogen (VBN), and lactic acid of the kuruma prawn muscle during storage at low temperatures in relation to its freshness and concluded that the K value, VBN, and lactic acid could be useful indices for freshness and that hypoxanthine and putrescine could be useful indices for decomposition. Our previous experiments revealed the increasing rate of lactic acid depended on the rise of storage temperatures, that is, lactic acid reached the maximum level of around 50 mg/100 g during storage at 5°C, 0°C, and −1°C, after 1, 7, and 9 days, respectively. Since lactic acid is an end product of glycolytic pathway, the changes in lactic acid arouse interest in the clarification of the glycolysis in the kuruma prawn muscle depending on storage temperatures, although the relationship between the degradation of glycogen and the production of lactic acid has remained unclear.

In addition, free amino acids (FAA) are considered as a major part of nonprotein nitrogenous compounds in marine invertebrates and play an important role in taste.[2] Though there have been many reports on the FAA composition in seafoods, the studies on the changes in contents of FAA during storage are comparatively few.

In the present study, we examined the changes in contents of glycogen, glycolytic metabolites, and FAA in the kuruma prawn muscle stored at 5°C and 0°C.

Materials and Methods

Samples

Live cultured kuruma prawn *Penaeus japonicus*, 24–37 g in weight, 15–17 cm in length, were purchased at the Tokyo Central Wholesale Market from April to July in 1989 and were transported to the laboratory in sawdust. After the removal of heads and shells, the muscles were chopped into small pieces. The composite samples were divided into two groups and stored at 5°C and 0°C. At regular intervals, a small amount of muscle was withdrawn from each group and extracted with ice-cold 7% perchloric acid according to the method described previously.[1] The neutralized extract was subjected to the following analyses.

Determination of Glycogen

Glycogen was hyrolyzed with amyloglucosidase, and the glucose formed was determined with hexokinase and glucose-6-phosphate dehydrogenase according to the method of Keppler and Decker.[3]

*1 This study was presented at the annual meeting of the Japan. Soc. Sci. Fish., Miyazaki, October, 1989.

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Determination of Glucose-6-phosphate (G6P) and D-*Glucose*

G6P and D-glucose were determined by the method of Bergmeyer *et al.*[4] At first, G6P was determined with glucose-6-phosphate dehydrogenase on the basis of the increment in optical density of NADPH at 340 nm. D-glucose was converted to G6P with hexokinase and ATP and determined in the same manner mentioned above.

Determination of Glucose-1-phosphate (G1P)

G1P was determined by the method of Bergmeyer and Michal,[5] after conversion to G6P with phosphoglucomutase,

Determination of Fructose-6-phosphate (F6P) and Fructose-1,6-diphosphate (FDP)

F6P and FDP were determined by the method of Michal and Beutler.[6] F6P was converted to G6P with phosphoglucose isomerase, and G6P was determined as stated above. FDP was converted to F6P with fructose-1,6-diphosphatase, and F6P was determined in the same manner.

Determination of α-Glycerol Phosphate (α-GP)

According to the method of Michal and Lang,[7] α-GP was determined with glycerol-3-phosphate dehydrogenase on the basis of the increment in optical density of NADH at 340 nm.

Determination of Lactic Acid

Lactic acid was determined by the method described previously.[1]

Determination of FAA

Thirty five amino acids and dipeptides were analyzed with HPLC equipped with a fluorescence detector (FLC-6A, Shimadzu Co.) on a strong cation exchange Shim-pack ISC-07/S1504 Li (Shimadzu Co.) column according to the method developed by Fujiwara and Ishida.[8]

Results

Glycogen and Glycolytic Metabolites

Fig. 1 shows the changes in contents of glycogen and glycolytic metabolites in the muscle of kuruma prawn during storage at 5°C. Glycogen fell rapidly within the first day, from 63 mg/100 g immediately after death to 22 mg/100 g, and mostly disappeared after 5 days. The concentrations of G1P and G6P immediately after death were 9 mg/100 g and 11 mg/100 g, respectively, and they also disappeared by 5 days of storage. In contrast, lactic acid increased from 5.6 mg/100 g immediately after death to 49 mg/100 g after 1 day of storage and then dropped gradually to 6 mg/100 g after 9 days. The concentrations of glucose, F6P, and FDP immediately after death were 2.5 mg/100 g, 1.2 mg/100 g, and 0.4 mg/100 g, respectively, and they fell to zero after 9 days of storage. α-GP culminated at the 2nd day of storge, dropped to the initial level by 4 days, and then remained at this level until 9 days.

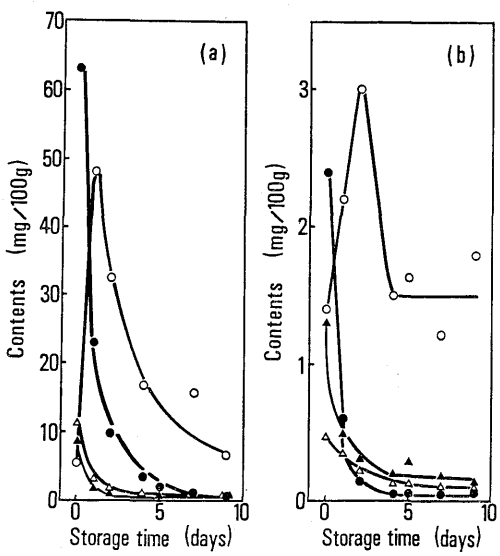

Fig. 1. Changes in contents of glycogen and glycolytic metabolites in the muscle of kuruma prawn during storage at 5°C.
(a) ●—●: Glycogen, ▲—▲: G1P, △—△: G6P, ○—○: Lactic acid.
(b) ●—●: Glucose, ▲—▲: F6P, △—△: FDP, ○—○: α-GP.

In Fig. 2 are shown the changes in contents of glycogen and glycolytic metabolites in the muscle of kuruma prawn during storage at 0°C. The changes in glycogen and glycolytic metabolites were almost the same as those at 5°C. Glycogen and glycolytic metabolites except for α-GP decreased slower than those at 5°C, especially until 4 days and disappeared by 9 days of storage. Lactic acid reached the maximum level of 52 mg/100 g after one week and then decreased.

Free Amino Acids (FAA)

Table 1 shows the changes in contents of FAA in the muscle of kuruma prawn during storage at 5°C. The total amount of FAA immediately after death was 4019 mg/100 g and the major

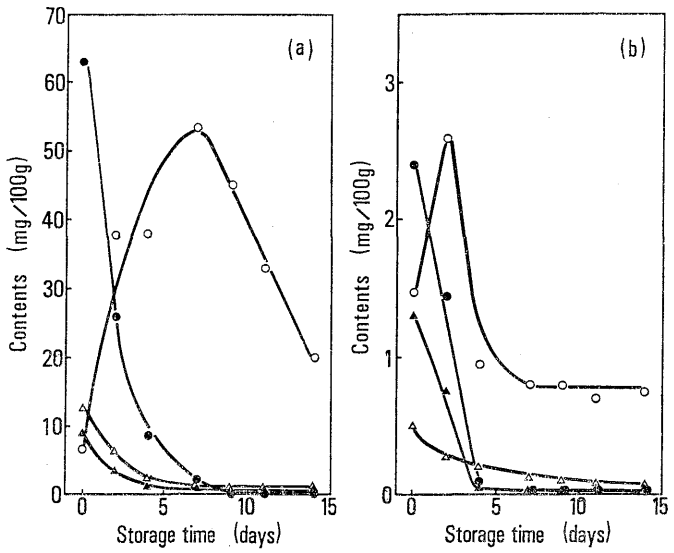

Fig. 2. Changes in contents of glycogen and glycolytic metabolites in the muscle of kuruma prawn during storage at 0°C.
(a) ●—●: Glycogen, ▲—▲: G1P, △—△: G6P, ○—○: Lactic acid.
(b) ●—●: Glucose, ▲—▲: F6P, △—△: FDP, ○—○: α-GP.

FAA were taurine (Tau), glutamic acid (Glu), proline (Pro), glycine (Gly), alanine (Ala), and arginine (Arg). Six amino acids accounted for 97% of the total FAA, especially Gly alone 43%. These FAA decreased during the storage for 2 days, and consequently marked decrease in the total amounts of FAA was observed. After 4 days of storage (the initial stage of decomposition), there was a noticeable increase in the total amount of FAA due to the increase of Pro, Gly, Ala, and Arg. The total amount of FAA decreased again after 9 days of storage and a decrease in all FAA except for ornithine (Orn) was observed. Orn increased from zero immediately after death to 100 mg/100 g after 9 days of storage.

In Table 2 are shown the changes in contents of FAA in the muscle of kuruma prawn during storage at 0°C. The changes in FAA were almost the same as those at 5°C. The total amounts of FAA decreased during early storage, reached the maximum level after 9 days (the initial stage of decomposition), and then dropped after 14 days again. These changes in the total amount of FAA were mainly influenced by the contents of Glu, Pro, Gly, Ala, and Arg. During storage at 0°C, Orn increased and reached the level of 52 mg/100 g after 14 days.

Discussion

Glycogen occurs in large amounts in shellfish such as oyster[9] (1300–5200 mg/100 g), short neck clam[10] (600–1600 mg/100 g), and in fairly high amounts as in fishes, common squid[11] (540 mg/100 g) as well as the Atlantic queen crab[12] (200–400 mg/100 g). On the contrary, the content of glycogen in the kuruma prawn muscle was relatively low (Figs. 1 and 2).

The contents of G6P and G1P were higher than any other glycolytic intermediates in the kuruma prawn muscle (Figs. 1 and 2). G6P in the scallop adductor muscle is found to show the highest level in glycolytic intermediates.[13] On the other hand, Burt[14] stated that FDP was the highest level, followed by G6P in the live cod muscle. Yamanaka[15] also reported that FDP was the highest level and that G6P followed in the skipjack muscle. These evidences suggested the difference of regulation in glycolytic metabolism between marine vertebrate and invertebrate.

As for the changes in glycolytic intermediates during storage, Burt[14] reported G6P and F6P accumulated in the cod muscle during ice-storage. Ehira and Uchiyama[16] also reported the increase of glucose in the plaice muscle during ice-storage. Furthermore, Nakamura *et al.*[17] indicated both glucose and G6P accumulated in the scallop adductor muscle stored at 0°C and 15°C. Naga-

Table 1. Changes in contents of free amino acids in the muscle of kuruma prawn during storage at 5°C (mg/100 g)

Amino acids	Storage time (days)				
	0	1	2	4	9
Phosphoserine	0	0	0	0	0
Taurine	83	65	73	89	43
Phosphoethanolamine	0	0	0	0	0
Aspartic Acid	17	4	4	6	0
Hydroxyproline	0	0	0	0	0
Threonine	3	4	3	2	0
Serine	7	10	12	7	0
Asparagine	17	12	12	52	20
Glutamic Acid	153	110	119	159	40
Sarcosine	0	0	0	0	0
α-Aminoadipic Acid	0	0	0	0	0
Proline	857	583	390	1106	324
Glycine	1736	830	797	1336	1069
Alanine	135	83	77	242	106
Citrulline	0	0	0	0	0
α-Aminobutyric Acid	0	0	0	0	0
Valine	10	7	7	19	0
Cystine	0	0	0	0	0
Methionine	0	trace	4	trace	0
Isoleucine	4	4	4	4	0
Leucine	12	8	8	17	12
Tyrosine	16	17	16	27	0
Phenylalanine	4	5	5	0	0
β-Alanine	0	0	0	0	0
β-Aminoisobutyric Acid	0	0	0	0	0
γ-Aminobutyric Acid	0	0	0	0	0
Histidine	9	10	9	17	9
3-Methylhistidine	0	0	0	0	0
1-Methylhistidine	0	0	0	0	0
Carnosine	0	0	0	0	0
Anserine	0	0	0	0	0
Hydroxylysine	0	0	0	0	0
Ornithine	0	4	11	48	100
Ammonia	0	0	0	7	30
Lysine	10	14	13	21	18
Arginine	946	555	529	1393	267
Total amounts	4019	2325	2093	4552	2038

yama[18] reported both G6P and F6P accumulated in the scallop adductor muscle during ice-storage. However, these accumulations were not observed in the kuruma prawn muscle, so it seemed that the glycolytic intermediates were metabolized smoothly in the kuruma prawn during storage at low temperatures.

In the Atlantic queen crab muscle during ice-storage, the maximum of lactic acid was found when glycogen had disappeared for 2–3 days, though no accumulations of hexose phosphates were observed.[12] On the contrary, in this study, the formation of lactic acid was observed immediately after glycogen was decomposed (Figs. 1 and 2). Furthermore, glycogen and glycolytic metabolites during storage at 5°C decreased more rapidly than those at 0°C, and as a result, lactic acid at 5°C reached the highest level faster than that at 0°C. This evidence also suggested that glycolytic pathway to lactic acid was smooth in the kuruma prawn muscle.

As for FAA in the kuruma prawn muscle, Tau, Glu, Pro, Gly, Ala, and Arg were major FAA, especially Gly accounted for about 2% in the fresh muscle (Tables 1 and 2). Konosu[18] stated that major FAA in the Crustacea muscle were

Table. 2. Changes in contents of free amino acids in the muscle of kuruma prawn during storage at 0°C (mg/100 g)

Amino acids	Storage time (days)				
	0	2	4	9	14
Phosphoserine	0	0	0	0	0
Taurine	83	75	82	79	50
Phosphoethanolamine	0	0	0	0	0
Aspartic Acid	17	4	4	trace	trace
Hydroxyproline	0	0	0	0	0
Threonine	3	trace	4	39	trace
Serine	7	6	16	13	trace
Asparagine	17	15	16	48	trace
Glutamic Acid	153	112	76	196	72
Sarcosine	0	0	0	0	0
α-Aminoadipic Acid	0	0	0	0	0
Proline	857	552	392	968	351
Glycine	1736	483	529	1182	1104
Alanine	135	78	97	261	132
Citrulline	0	0	0	0	0
α-Aminobutyric Acid	0	0	0	0	0
Valine	10	10	11	28	14
Cystine	0	0	0	0	0
Methionine	0	4	9	20	trace
Isoleucine	4	4	4	16	trace
Leucine	12	4	8	35	16
Tyrosine	16	10	11	33	11
Phenylalanine	4	5	5	16	5
β-Alanine	0	0	0	0	0
β-Aminoisobutyric Acid	0	0	0	0	0
γ-Aminobutyric Acid	0	0	0	0	0
Histidine	9	9	5	19	9
3-Methylhistidine	0	0	0	0	0
1-Methylhistidine	0	0	0	0	0
Carnosine	0	0	0	0	0
Anserine	0	0	0	0	0
Hydroxylysine	0	0	0	0	0
Ornithine	0	0	8	20	52
Ammonia	0	0	5	7	7
Lysine	10	8	9	31	18
Arginine	946	830	561	1127	537
Total amounts	4019	2209	1852	4138	2378

Tau, Ala, Gly, Pro, and Arg, especially Gly accounted for more than 1% in the fresh muscle. Fujita *et al.*[19] also reported that Gly was most abundant, followed by Arg and that Pro, Ser, Ala, and Tau showed medium levels in the muscle of eight species of shrimps. These results were in good accordance with our results.

The total amount of FAA decreased during the early storage, rose suddenly after 4 days of storage at 5°C and after 9 days of storage at 0°C when the initial stage of decomposition was observed in the kuruma prawn muscle, and thereafter the total amounts of FAA dropped again as progressed decomposition. These changes were caused mainly by the contents of five major amino acids except for Tau, that is, Glu, Pro, Gly, Ala, and Arg. Ito[20] reported that the amounts of free Arg and Glu decreased, while the changes in other amino acids were not observed significantly in the three species of clam muscle. Sakaguchi *et al.*[21] reported that FAA except for His and Tau increased in the juvenile mackerel muscle during storage. Yamanaka[22] indicated that Arg decreased noticeably in the scallop adductor muscle. Tokunaga *et al.*[23] also reported that the major FAA, Arg, Gly, Ala, Pro, and Tau, decreased in the horsehair

crab muscle during ice-storage and that the decrease of the five amino acids might influence the inferiority of taste and flavor. Miyagawa *et al.*[24] observed that Pro, Gly, and Arg increased until 3 days and then decreased in the snow crab muscle. Cobb *et al.*[25] reported that Arg, Tau, Pro, and Gly were major FAA in the white shrimp muscle and that Tau and Gly levels did not change significantly, whereas Arg and Pro decreased. These results were not in accordance with our results. These discrepancies seemed to be due to the differences of storage condition and species. In the previous experiments,[1] it was observed that putrescine increased rapidly after the initial stage of decomposition. Furthermore, Yamanaka *et al*[20] confirmed that polyamines in the meats of common squid and scallop were produced from amino acids by bacterial decarboxylases. Based on this evidence, the increase in the total amount of FAA observed in our experiments appeared to result from the production of FAA by proteolysis, while the decrease of the total FAA appeared to result from the breakdown of FAA by bacterial decarboxylation and deamination.

Orn is produced from Arg with arginase and is observed to increase during storage in the muscle of white shrimp,[25] horsehair crab,[23] scallop adductor,[22] and snow crab.[24] Furthermore, it is concluded that Orn appears to be useful as a potential index for freshness in scallop adductor[22] and snow crab.[24] In the kuruma prawn muscle, Orn was not detected immediately after death and increased gradually during storage. As decomposition progressed, Orn content reached the levels of 100 mg/100 g and 52 mg/100 mg at 5°C and 0°C, respectively. From these results, Orn seemed to be a useful index for the freshness of the kuruma prawn muscle.

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