Seasonal Variations of Extractive Components in the Muscle of Disk Abalone^{*1}

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Seasonal variations of extractive components were examined in the muscle of disk abalone *Haliotis discus* collected from the coast of Amatsukominato, Chiba Prefecture at intervals of two or three months from July 1990 to July 1991.

The moisture content was highest in December after spawning season, thereafter decreased and became lowest in May. The total amount of ATP and its related compounds was highest in September. AMP was detected in high amounts from July to September. The level of glycogen was high in summer (over 6%), decreased remarkably in the spawning season and low in winter. Succinate was high in amounts from May to September. The total amount of free amino acids was highest in September and decreased to half in other months. The contents of glutamic acid, glycine, and AMP which are taste-active components were highest (470 mg/100 g) in September and lowest (130 mg/100 g) in February. Present study can demonstrate that disk abalones are palatable in summer, and watery and unpalatable in winter, which is generally told in Amatsukominato district.

In fishes and shellfishes there exists the most tasteful season when large amounts of catch is observed. However, little information is available for seasonal variations in the levels of extractive components except for some bivalves such as short-neck clam,¹⁾ freshwater clam,¹⁾ oyster,^{1,2)} and so forth.

In this paper we examined the seasonal variations of extractive components in the muscle of disk abalone in relation to the *umami* taste.

Materials and Methods

Samples

From July 1990 to July 1991, three natural live disk abalones *Haliotis discus* were collected six times on the coast of Amatsukominato, Chiba Prefecture at intervals of two or three months. The averages of shell length and body weight were 123 mm and 247 g, respectively. Five grams of muscle was homogenized with 20 ml of 6% perchloric acid in an ice-cooled homogenizer. The suspension was centrifuged at 3,000 × g for 15 min and the procedure was repeated. The

combined supernatants were made up to 50 ml and neutralized with potassium hydroxide. The extract was used in the following analyses.

Determination of ATP and Its Related Compounds

ATP and its related compounds were determined by high performance liquid chromatography (HPLC) according to the method of Matsumoto and Yamanaka.³⁾ HPLC was performed on an Asahipak GS-320 (Asahi Chemical Industry Co.) column (7.6 × 500 mm) with 200 mM NaH₂PO₄ (pH 2.9) and the absorbance at 260 nm was measured.

Determination of Glycogen

After 0.1 to 0.2 g of muscle was extracted in 2 ml of heated 30% potassium hydroxide, absolute ethanol was added to the solution and glycogen was precipitated. The obtained glycogen was hydrolyzed with 1 M sulfuric acid and glucose formed was determined by the combination of mutarotase and glucose oxidase (Glucose C-Test Wako).

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Determination of D- and L-Lactate

D- and L-lactate were determined by the use of F-kit (Boehringer Mannheim Co.) based on enzymatic analysis.

Determination of Succinate, Malate, and Acetate

The organic acids were determined by HPLC on a Shim-pack SCR-102H column (8.0×150 mm). The elution was performed with 5 mM *p*-toluenesulfonic acid containing 20 mM Bis-Tris and 100 μ M EDTA. The eluate was monitored by a conductivity detector (Shimadzu CDD-6A).

Determination of Free Amino Acids

Free amino acids were determined by an amino acids analyzer (Hitachi 835-50 Type).

Determination of Homarine

Homarine was determined by the same HPLC method and conditions employed in the determination of ATP and its related compounds.

Results and Discussion

The seasonal changes in degree of stoutness of disk abalone obtained by the average of three samples are shown in Table 1. The other results shown below are also the averages of three samples. The degree of stoutness was high in September 1990, February and May 1991. Since sea trumpet, kajime, and wakame on which disk abalone feed, proliferate from February to May, disk abalones are considered to become stout in this season. The growth of gonad was also observed in disk abalone collected in September and December. It should be noted that the low value of stoutness was observed in December. This is due to the spawning season of disk abalones extending from the middle of October to the end

Table 1. Seasonal changes in degree of stoutness and moisture content in the muscle of disk abalone

Period of sampling	Degree of stoutness*	Moisture content (%)
July, 1990	0.115	—
September, 1990	0.130	71.0
December, 1990	0.123	76.5
February, 1991	0.133	75.2
May, 1991	0.136	59.8
July, 1991	0.129	61.5

* Body weight (g) $\times 10^3$ / (shell length (mm))³.

of December. According to the report by Sakai,⁴⁾ the period of stoutness in Yezo abalone *Haliotis discus hannai* was observed from February to July and little growth in winter. The growth of Yezo abalones was also low from August to September when the gonad was matured and the spawning was done. The results of disk abalone (Table 1) were almost the same as those of Yezo abalones.

The results of moisture contents are also shown in Table 1. After spawning season (in December) the moisture content in disk abalones was highest (76.5%) and thereafter decreased to the level of about 60% in May and July. These results substantiate the empirical knowledge that local people perceive disk abalones to be watery in winter, compared with those in summer.

In Fig. 1 are shown the seasonal changes in contents of ATP and its related compounds in the muscle of disk abalone. In September the total amount of ATP and related compounds was

Fig. 1. Seasonal changes in contents of ATP and its related compounds in the muscle of disk abalone.

Fig. 2. Seasonal changes in content of glycogen in the muscle of disk abalone.

highest (5.5 $\mu\text{mol/g}$). ATP and ADP were major components and a fairly large amount of AMP was detected in September and July. Small amounts of IMP and adenosine (AdR) were detected all the year round, indicating that AMP

was metabolized through two pathways. In the muscle of the cultured ascidian³⁾ a marked seasonal change was observed in the sum of nucleotides and related compounds; the value found in September was nearly 4 times as high as that found in January. It has been also reported that smaller amounts of nucleotides

Fig. 3. Seasonal changes in contents of D- and L-lactate in the muscle of disk abalone.

Fig. 4. Seasonal changes in contents of succinate, malate and acetate in the muscle of disk abalone.

Table 2. Seasonal changes in contents of free amino acids in the muscle of disk abalone (mg/100 g)

Amino acids	1990			1991		
	Jul.	Sep.	Dec.	Feb.	May	Jul.
Taurine	1138.6	1281.4	1031.0	1296.6	1173.1	1118.3
Aspartic acid	9.2	53.9	42.1	69.6	15.5	5.5
Hydroxyproline	1.2	3.7	23.9	2.7	5.7	5.7
Threonine	32.7	63.3	20.2	29.0	34.0	34.3
Serine	20.9	138.9	20.6	20.6	23.1	19.4
Glutamic acid	37.6	163.2	77.6	38.2	36.6	32.1
Glutamine	191.1	799.2	214.2	118.9	158.6	107.6
Sarcosine	11.7	3.1	6.2	3.7	1.7	7.4
Proline	32.3	50.4	18.1	20.0	34.4	30.0
Glycine	136.8	278.2	94.5	80.2	82.3	109.1
Alanine	30.5	152.7	57.1	25.6	28.1	27.2
Citrulline	0.2	5.4	1.5	0	0.7	0.3
Valine	12.2	47.2	11.9	9.3	14.0	10.6
Cystine	0.4	2.8	2.2	4.8	0.1	0
Methionine	4.1	6.6	3.7	2.1	4.5	2.7
Isoleucine	6.8	20.7	6.7	4.2	6.0	4.7
Leucine	10.2	235.4	247.4	59.7	8.9	6.6
Tyrosine	18.2	3.6	12.3	4.6	20.1	15.6
Phenylalanine	10.6	16.4	7.7	5.8	12.2	8.3
Tryptophan	9.9	59.3	8.3	7.2	11.1	7.4
Histidine	17.8	10.9	5.8	9.6	11.3	8.9
Ornithine	4.3	8.0	5.9	2.9	4.2	5.0
Ammonia	0.9	8.5	6.9	10.7	3.6	1.4
Lysine	24.2	37.1	8.4	17.6	20.3	29.8
Arginine	198.0	369.7	197.6	126.4	195.0	268.4
Total amounts	1960.4	3819.6	2132.4	1970.0	1905.1	1866.3

were detected in the muscle of mackerel⁶⁾ and ascidian⁷⁾ which were caught in the spawning season.

Seasonal changes in content of glycogen are shown in Fig. 2. Glycogen varied obviously with the seasons. Its level was high in summer (over 6%), decreased remarkably in the spawning season and was lowest (3.2%) in February. These results are similar to those of ascidian muscle;⁸⁾ glycogen contents are high from May to September (ca. 8%) and low from December to March (2–4%), and a high content of glycogen in summer is consistent with the palatable season of ascidian. In the muscle of disk abalone there existed a reverse correlation between moisture content and glycogen level. It is reported that in the spawning season glycogen content decreased markedly and moisture content increased in the muscle of oyster.¹⁾ Glycogen itself is found to be tasteless, but reported to elevate continuity, fullness, complexity, and overall preference, when being added to a synthetic extract from scallop muscle.⁹⁾

Figure 3 shows seasonal changes in contents of D- and L-lactate as the glycolytic end products. D- and L-lactate were detected in high amounts in September and the total amount reached 3.2 $\mu\text{mol/g}$. Sato¹⁰⁾ reported that D-lactate increased noticeably in the muscle of Yezo abalone by removal from sea-water to the air or exhaustion.

The results of seasonal changes in contents of succinate, malate and acetate are shown in Fig. 4. The total amount of these organic acids was highest in September, followed by December. In September and December the ratio of acetate to the total organic acids was very high. Malate level was nearly constant throughout the year. Succinate which is one of taste-active components was high in amounts from May to September and decreased during winter.

In Table 2 are shown the seasonal changes in contents of free amino acids in the muscle of disk abalone. The total amount of free amino acids was highest in September and decreased to half in other months. The major free amino acids were taurine (Tau), arginine (Arg), glutamine (Gln), glycine (Gly), glutamic acid (Glu), alanine (Ala), and leucine (Leu). In September Gln, Arg, Gly, Glu, Ala, and serine were detected in the largest amounts, compared with other months. The seasonal variation of Tau was not observed and Tau was detected at the level of 1,000 to 1,300 mg/100 g throughout the year. As for the

Fig. 5. Seasonal changes in content of homarine in the muscle of disk abalone.

Fig. 6. Seasonal changes in contents of AMP, glutamic acid, and glycine in the muscle of disk abalone.

seasonal variation of free amino acids, there exist the studies on oyster²⁾ and ascidian.^{5,7)} In the spawning season the level of Tau increased in oyster, decreased in ascidian, and the levels of Gly, Ala, and Glu increased in both of them.

In Fig. 5 are shown the seasonal changes in content of homarine in the muscle of disk abalone. Homarine content was highest in September and lowest in December. It is reported that homarine content in the muscle and liver of top shell¹¹⁾ increased remarkably in summer. Homarine has been reported to participate in the regulation of osmotic pressure.¹²⁾

The seasonal changes in contents of AMP, Glu, and Gly which are taste-active components¹³⁾ in the muscle of disk abalone are shown in Fig. 6. The total amount of AMP, Glu, and Gly was highest (470 mg/100 g) in September and lowest (130 mg/100 g) in February. The level of each component was also high in September.

As mentioned above, glycogen level was high (over 6%) between July and September (Fig. 2) and succinate was detected in large amounts from summer to early autumn (Fig. 4). Fur-

thermore, moisture content was very high from December to February (Table 1).

Considering these results inclusively, present study can almost demonstrate that disk abalone is palatable in summer, and watery and unpalatable in winter, which is generally told in Amatsukominato district, Chiba Prefecture. In Amatsukominato district the disk abalone season opens from the middle of May to the middle of September. In this season savoury disk abalone is supplied and Yezo abalone is utilized in winter.

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