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# Comparison of Extractive Nitrogenous Constituents between the Diploid and the Triploid of Oyster *Crassostrea gigas* Whole Body

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In order to investigate the composition of extractive nitrogenous components in the diploid and the triploid oysters, *Crassostrea gigas*, cultured at the south coast of Korea, the whole edible part (whole body) was analyzed into extractive nitrogen, free amino acids, oligopeptides, ATP and its related compounds, quaternary ammonium bases, and guanidino compounds using specimens collected from April to May of 1992. The major free amino acids in the diploid and the triploid were taurine, proline, alanine, glycine, glutamic acid hypotaurine, glutamine, arginine, aspartic acid, and β-alanine. There was no conspicuous difference in the constituents of free amino acids between the diploid and the triploid. A lot of hypotaurine was detected in the diploid and the triploid of oyster and the contents of them were 107 mg and 123 mg/100 g, respectively. The compounds, glycinebetaine, homarine and trigonelline were found in both the diploid and the triploid. Among them, glycinebetaine was the most prominent in all the samples. The amount of protein, glycogen, extractive nitrogen, oligopeptides, ATP and its related compounds, and free amino acids in the triploid was higher than that of the diploid ($p < 0.10$).

Key words: oyster, *Crassostrea gigas*, triploid, extractive nitrogen, free amino acids, oligopeptides, ATP and its related compounds, betaines.

## Introduction

In recent years, as a part of fish and shellfish breeding, the induction of triploid oyster *Crassostrea gigas* has been developed actively. By the way, the triploid which was induced by chromosome operation was reported generally impotence (Beaumont and Fairbrother, 1991). Some strong points of the triploid oyster were higher growth rate, heavier whole body weight, better muscle quality, and lower mortality than the diploid one (Stanley et al., 1981; Tabarini, 1984). Until now, many papers concerning the induction methods (Chation and Allen, 1985; Quillet and Penelay, 1986; Downing and Allen, 1987; Yamamoto and Sugawara, 1988; Yoo et al., 1990), growth, maturation, and reproduction (Akashige and Fushimi, 1992), and gametogenesis (Allen and Downing, 1986; Allen, 1987) of the triploid oyster have been published, but the studies on its extractive components that are closely related to the flavor are scanty. As a part of food chemical and comparative biochemical studies on the extractives in the whole body of the diploid and the triploid oyster of Korea, the experiments were performed to analyze the nitrogenous components in the two kinds of oyster cultured at the southern area of Korea. The objective of this study was to compare the nitrogenous compounds such as the content of extractive nitrogen, free amino acids, oligopeptides, ATP and its related compounds, glycinebetaine, homarine, trigonelline, trimethylamine oxide (TMAO), trimethylamine (TMA), creatine, and creatinine in both diploid and triploid whole body.

## Materials and Methods

### *Samples*

The triploid was induced by heat shocking fertilized eggs of the oyster, *C. gigas*, post fertilization at various time intervals of shock duration

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(caffeine 10 mM+37℃) at a laboratory of the National Fisheries Research and Development Agency, Republic of Korea, in August 1990. They were settled on the ropes in September 1990 and cultured thereafter at the farm. The specimens of the diploid and the triploid oyster cultured at Inpyung-dong, Kyungnam province of Korea were collected from April to May of 1992. For each collection, the whole edible part (whole body) from 7~12 individuals were removed, pooled, and subjected to the analyses. The number of individuals and weight of whole body of the specimens are shown in Table 1.

### *Preparation of Extracts*

For each collection, the whole body was removed from 7~12 specimens and pooled respectively. Each whole body was cut into small pieces and mixed thoroughly by Ace homogenizer (5,000 rpm, 10 min., Nihonseiki Kaisha Ltd.). A part of the mixture (20 g) was extracted with 1% picric acid according to the method of Stein and Moore (1954) to determine extractive nitrogen, free amino acids, oligopeptides, betaines, TMAO, TMA, creatine and creatinine. For the estimation of acid-soluble ATP and its related compounds, the perchloic acid extract was prepared from another part of the mixture (2.5 g) using the method of Nakajima et al. (1961).

### *Analytical Methods*

1) *Proximate Composition*

Moisture, protein, lipid, and ash were estimated by the usual methods (Japanese Society of Food Science and Technology, 1984) and glycogen was determined by the Hanes' method (1929).

2) *Extractive Nitrogen*

Extractive nitrogen was determined by the micro-Kjeldahl method (Japanese Society of Food Science and Technology, 1984).

3) *Free Amino Acids and Oligopeptides*

Amino acids were analyzed with a Hitachi 835 automatic amino acid analyzer (Hitachi, 1987) before and after hydrolysis of the extracts with 6 N HCl at 110℃ for 16 hr in an evacuated sealed tube.

4) *ATP and Its Related Compounds*

The high performance liquid chromatography (HPLC, Hitachi, Japan) employed for the analyses of ATP and its related compounds was the same as described by Kitada et al. (1983). HPLC was carried out using a Hitachi model L-6200 intelligent pump, equipped with a L-4200 UV-VIS ultraviolet detector operated at 254 nm and chromatographic data system Spectra-physis SP-4270 integrator. ATP and its related compounds were separated on a $\mu$ Bondapak $C_{18}$ column ($\phi$ 3.9×300 mm, Waters, USA) at 40℃ with a mobile phase, 2% triethylamine-phosphoric acid (pH 7). The flow rate was 0.8 mℓ/min throughout the elution.

5) *Betaines*

Glycinebetaine, homarine, and trigonelline were analyzed by HPLC (Hitachi, Japan) according to the method of Park et al. (1990).

6) *TMAO and TMA*

TMA was analyzed by the method of Bullard and Collins (1980). TMAO was determined as TMA after reducing with titanous chloride (Bystedt et al., 1959).

7) *Creatine and Creatinine*

Creatine was measured by the colorimetric method described by Niiyama (1961). Creatinine was determined according to the method of Yatzidis (1974) with a slight modification, in which the amounts of sample and buffer solutions were increased to 1 and 9 mℓ, respectively.

8) *Statistics*

The analytical results were calculated by the method of Student's *t*-test (Harris, (1995).

Table 1. Weight and proximate composition*[1] in the whole body of the diploid and the triploid of oyster samples

| | Apr.1992 | | May 1992 | |
|---|---|---|---|---|
| | Diploid | Triploid | Diploid | Triploid |
| n*[2] | 12 | 7 | 11 | 11 |
| Weight*[3] (g) | 3.7~10.5 (7.7 ± 2.2) | 10.2~17.6 (15.2 ± 2.7) | 5.2~10.1 (7.8 ± 1.6) | 5.9~11.8 (8.6 ± 2.0) |
| Moisture*[4] (%) | 83.7 | 76.8 | 82.8 | 79.2 |
| Protein*[4] (%) | 8.8 | 12.1 | 9.0 | 11.3 |
| Lipid (%) | 2.4 | 3.3 | 2.9 | 3.0 |
| Ash (%) | 2.3 | 2.5 | 2.1 | 2.0 |
| Glycogen*[4] (%) | 2.8 | 5.3 | 3.2 | 4.5 |

*[1]Analytical frequency; 3 times.
*[2]Number of individuals; n=7~12.
*[3]Shucked whole body; range (average ± standard deviation).
*[4]Significant level of individual differences between the diploid and the triploid; $p < 0.10$.

## Results and Discussion

### *Proximate Composition*

The proximate composition in the whole body of the oyster is shown in Table 1. When compared

the proximate composition in the whole body between two kinds of samples - the diploid and the triploid, the former was slightly higher in moisture content (average 83.3 versus 78.0 %, $p<0.10$) and somewhat lower in protein (8.9 versus 11.7%, $p<0.10$), and glycogen (3.0 versus 4.9%, $p<0.10$). But the levels of fat and ash were rather constant in all the samples. Akashige and Fushimi (1992) reported almost the same trend in moisture and glycogen content of the diploid and the triploid oysters *C. gigas* cultured in the waters of Hiroshima Japan.

### *Extractive Nitrogen*

The analyzing results of extractive nitrogen in the whole body of the diploid and the triploid oysters showed in Table 2. Extractive nitrogen in the extracts has been used as a quality index of taste-active components for fish and shellfish (Park, 1995). Generally, the amount of extractive nitrogen is higher, the taste of fish and shellfish is better (Suyama and Konosu, 1987). The content of extractive nitrogen in the whole body of the diploid was 467 mg/100 g (the amounts of extractive components are expressed in terms of mg per 100 g of whole body), and in the triploid was 534 mg, higher than that of the diploid ($p<0.01$). Previously, Tsuchiya (1962), Takagi and Simidu (1963a, 1963b), Suyama and Konosu (1987) reported considerably lower levels of extractive nitrogen in whole body of the diploid oyster. However, Murata and Sakaguchi (1986), Sakaguchi and Murata (1988) reported almost the same level of the present data. Probably, the difference of extractive nitrogen content in the oyster would be seasonal and /or regional variation of collected samples (Park et al., 1990).

During the experiment, the amount of extractive nitrogen in the whole body of the triploid oyster which was 534 mg showed the similar to the Japanese abalone in 506mg and top-shell in 507 mg (Suyama and Konosu, 1987). But they showed higher level than the amount of ark-shell in 348~367 mg (Ban, 1997), sea mussel in 382 mg (Lee, 1997), and hard clam in 450 mg (Suyama and Konosu, 1987). On the other hand, they showed lower level than the amount of scallop in 787 mg (Suyama and Konosu, 1987).

### *Free Amino Acids*

Table 2 showed the analyzing result of free amino acids. In the whole body of the diploid, 31 kinds of free amino acids were detected, and the total amount of them was average 2,150 mg/100 g. In the whole body of the triploid, 32 kind of free amino acids were detected, and the total of them was 2,354 mg, a little higher than the diploid ($p<0.10$). The major free amino acids in the diploid were taurine, proline, alanine, glutamic acid, glycine, hypotaurine, glutamine, arginine, aspartic acid, and β-alanine. These 10 kinds of free amino acids occupied 89.7% of the total free amino acids. It has been reported that fresh whole body of the diploid oyster contains large amounts of alanine, glutamic acid, glycine, and proline (Lynch and Wood, 1966; Heavers and Hammen, 1985). This is common to all marine bivalves (Hashimoto, 1965; Konosu and Yamaguchi, 1982).

The principal free amino acids distribution in the triploid were quite similar to those of the diploid. These 10 kinds of free amino acids in the triploid occupied 90.4% of the total free amino acids. Taurine was the most prominent free amino acid in all the diploid and the triploid samples. It occupied average 42.1 and 45.9% of total free amino acids in the diploid and the triploid, respectively. During the experiments, a lot of hypotaurine was detected in the diploid and the triploid samples and the content of it was 107 mg and 123 mg, respectively. Hypotaurine is a precursor of taurine (Kontro and Oja, 1985). It has been found occasionally in some tissues of marine invertebrates (Shibuya and Ouchi, 1957; Amende and Pierce, 1978; Kataoka et al., 1985; Watanabe et al., 1992). Its physiological function shows that it has worked as an antioxidant (Aruoma et al., 1988; Green et al., 1991).

The amount of free amino acids such as valine, histidine, and arginine in the whole body of the diploid and the triploid in April samples were higher level than that of May ones ($p<0.05$) (Table 2). Besides, threonine, alanine, methionine, and leucine in April specimens were higher level than that of May ones ($p<0.10$). On the contrary, β-alanine in April collections was lower level in May samples ($p<0.05$).

The amount of amino acids increased after hydrolysis (oligopeptides) is given in parenthesis of Table 2. Their levels were generally low in the whole body of the diploid and the triploid samples. The amount of oligopeptides was 192 mg in the diploid and 312 mg in the triploid, showing the higher level than that of the diploid ($p<0.01$). Aspartic acid, glycine, β-alanine, and glutamic acid were comparatively rich in the diploid, and glycine, β-alanine, glutamic acid, and aspartic acid were

Table 2. Nitrogenous constituents in the extracts of the whole body in the diploid and the triploid of oyster samples*[1] (mg/100 g)

| | Apr. 1992 | | May 1992 | |
|---|---|---|---|---|
| | Diploid | Tripliod | Diploid | Triploid |
| Extractive nitrogen*[2] | 468 | 570 | 465 | 498 |
| Free amino acids*[2] and oligopeptides*[2] | | | | |
| Phosphoserine | 10 (11) | 9 (18) | 9 (11) | 9 (21) |
| Taurine | 890 | 957 | 1085 | 1017 |
| Hypotaurine | 99 | 128 | 114 | 117 |
| Aspartic acid | 53 (30) | 52 (31) | 54 (31) | 45 (34) |
| Hydroxyproline | − (28) | − (20) | 25 | 31 |
| Threonine | 29 (9) | 33 (15) | 20 (13) | 20 (8) |
| Serine | 40 | 30 (10) | 11 (13) | 21 (14) |
| Glutamic acid | 156 | 172 (15) | 156 (31) | 144 (51) |
| Glutamine | 111 | 144 | 77 | 66 |
| Sarcosine | − | − | − | 5 (1) |
| α-Aminoadipic acid | 2 | 3 | 2 (1) | 1 (1) |
| Proline | 151 | 262 (5) | 197 (12) | 161 (22) |
| Glycine | 132 (15) | 157 (39) | 115 (40) | 243 (55) |
| Alanine | 195 | 191 (12) | 138 (16) | 159 (25) |
| α-Amino-*n*-butyric acid | 8 | 7 | 3 | 4 |
| Valine | 11 (2) | 14 (10) | 6 (8) | 10 (12) |
| Cystine | 5 | − (6) | 4 | 3 (4) |
| Methionine | 15 | 18 | 5 (1) | 11 |
| Cystathionine | 4 (1) | 2 (2) | 2 | 2 |
| Isoleucine | 5 (3) | 8 (9) | 3 (6) | 6 (9) |
| Leucine | 10 (5) | 15 (13) | 5 (10) | 12 (13) |
| Tyrosine | 11 | 12 | 4 (3) | 11 |
| Phenylalanine | 3 (4) | 6 (9) | 5 (3) | 11 (4) |
| β-Alanine | 7 (35) | 11 (63) | 45 (3) | 44 (4) |
| β-Aminoisobutyric acid | − (2) | − (3) | 1 (1) | 2 (1) |
| γ-Amino-n-butyric acid | 5 | 3 | 2 | 1 |
| Ethanolamine | − (6) | − (8) | − (7) | − (11) |
| Ornithine | 18 | 36 | 18 | 17 |
| Lysine | 29 (6) | 25 (16) | 17 (11) | 23 (20) |
| Histidine | 30 | 26 (3) | 14 (5) | 14 (6) |
| Anserine | − | 13 | 11 | 4 |
| Carnosine | 8 | 7 | 3 | 2 |
| Arginine | 75 | 88 | 36 (1) | 57 |
| ATP and its related compounds*[2] | | | | |
| Adenosine 5′-triphosphate | + | + | + | + |
| Adenosine 5′-diphosphate | 2 | + | + | + |
| Adenosine 5′-monophosphate | 21 | 32 | 18 | 15 |
| Inosine 5′-monophosphate | 48 | 50 | 49 | 54 |
| Inosine | 8 | 12 | 7 | 11 |
| Hypoxanthine | 1 | 1 | 1 | 1 |
| Others | | | | |
| Glycinebetaine | 618 | 829 | 749 | 698 |
| Homarine | 173 | 197 | 182 | 187 |
| Trigonelline | 12 | 9 | 7 | 12 |
| Trimethylamine oxide | + | + | + | + |
| Trimethylamine | 35 | 21 | 20 | 10 |
| Creatine | 18 | 15 | 9 | 16 |
| Creatinine | 2 | 13 | 2 | 2 |
| Ammonia | 29 | 31 | 26 | 25 |

*[1]The amounts of oligopeptides are given in parentheses. Marks used: +, trace; −, not detected. Analytical frequency; 3 times.

*[2]Significant level of individual differences between the diploid and triploid; $p < 0.10$.

relatively abundant in the triploid. Amino acids from oligopeptides in the diploid and the triploid occupied 8.9 and 13.9% of the total free amino acids, respectively.

*ATP and Its Related Compounds*

The amount of ATP and its related compounds in the whole body of the diploid and the triploid shown in Table 2. ATP, ADP, AMP, IMP, inosine and hypoxanthine were detected in all the samples. For examining the ATP and its related compounds, this paper deals the amount with the terms of μmol per 1 g of the whole body, because their postmortem changes are quick, but their total amount is almost constant for some time (Park et al., 1990). In the diploid, the amount of total ATP and its related compounds was 2.22 μmol/g, and that in the triploid was 2.67 μmol - higher level than that of the diploid ($p<0.05$). In this study, the total content of ATP and its related compounds in the whole body of the diploid oyster was slightly lower than that in the muscle of the oyster (3.38 μmol/g) reported by Suwetja et al. (1989). This may have resulted from physiological conditions of oysters (Yokoyama et al., 1992), experimental conditions and/or the seasonal variation as reported by Park et al. (1990) in the tissues of the ascidian *Halocynthia roretzi*.

*Betaines*

Glycinebetaine, homarine, and trigonelline were found in all the whole body of the diploid and the triploid samples (Table 2). The amount of total betaines in the diploid and the triploid was 871 mg and 966 mg/100 g, respectively - higher level than that of the diploid ($p<0.01$). Among them, glycinebetaine was the most prominent in all the samples. The content of it was 684 mg in the diploid, and 764 mg in the triploid - almost the same level each other. And, the amount of homarine in the extracts of the diploid and the triploid was 178 mg and 192 mg, respectively; almost the same level each other. The amounts of trigonelline in the diploid and the triploid were 10 mg and 11 mg, respectively. Previously, Abe and Kaneda, 1975) were isolated and identified as glycinebetaine from the basic fraction in whole body of the diploid oyster which was 447 mg. Suwetja et al. (1989) reported almost the same levels of homarine and trigonelline in the whole body of the diploid oyster which were 144 mg and 16 mg, respectively.

*TMAO and TMA*

The TMAO contents in the extracts of the whole body of the diploid and the triploid were extremely low, but the TMA in the diploid and the triploid were 28 mg and 16 mg/100 g, respectively (Table 2). One of the most distinctive characteristics of sea fish is its content of TMAO (Dyer, 1952). Only a little TMAO was detected, and TMA was about 5 mg in the fresh whole body of the diploid oyster by Murata and Sakaguchi (1986). Suyama and Konosu (1987) also reported the content of TMAO in the adductor muscle of oyster which was 2 mg. However, of the Mollusca, the Amphineura, Gastropoda and part of the Pelecypoda (namely, the clams, mussels and oysters), were found not to contain TMAO (Dyer, 1952).

*Creatine and Creatinine*

A small amounts of creatine in the whole body of the diploid (14mg/100g) and the triploid (16mg) were detected in all samples. The content of creatinine was no more than 13mg in all the collections (Table 2).

*Distribution of Nitrogen in the Extracts*

The nitrogen distribution in the diploid and the triploid oyster extracts is presented in Fig. 1, in which nitrogen of extractive components in every group is summed up for each sample. The figure clearly shows that the contribution of free amino acids in the extractive nitrogen is the most important, occuping 60.8~62.0% in the diploid and 58.9~61.6 % in the triploid. They were followed by betaines, oligopeptides, and ATP and its related compounds, which account for 19.8~23.4 % in the diploid and 20.8~21.1% in the triploid, 4.4~6.7% in the diploid and 8.7~9.8% in the triploid, and 2.9~3.1 % in the diploid and the triploid of the extractive nitrogen, respectively. The contribution of TMAO and TMA, creatine and creatinine was very little. The recoveries of extractive nitrogen by those compounds were satisfactory, ranging 92.5~92.6% in the diploid and 95.5~97.7% in the triploid. The high recovery values indicate that the composition of nitrogenous extractives in the whole body was analyzed almost completely.

As mentioned above, the amount of proximate composition (protein and glycogen), extractive nitrogen, oligopeptides, ATP and its related compounds, and free amino acids in the whole body of the triploid was higher than that in the diploid oyster. So the triploid oyster is more palatable than the diploid one.

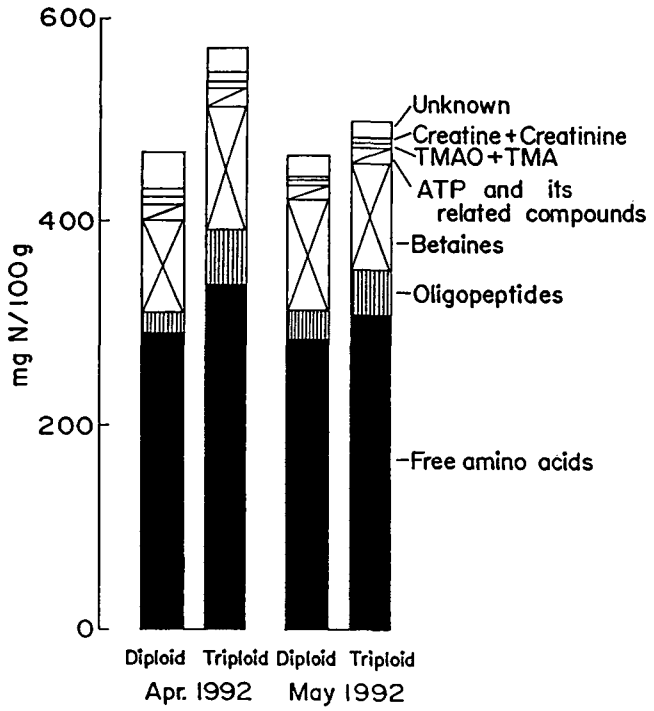


Fig. 1. Nitrogen distribution in the whole body of the diploid and the triploid oyster samples.

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