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Compositions of Extractive Nitrogenous Constituents and Their Monthly Variation for Fresh *Capsosiphon fulvescens*

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Abstract

To elucidate the composition of extractive nitrogenous components in the fresh *Capsosiphons fulvescens* cultured off the southern coast of Korea, and to determine the monthly variation of these nitrogenous components, extract samples collected monthly from December to March at Jangheung-gun, Jeonnam Province were analyzed for total nitrogen, free and combined amino acids, ATP and related compounds, betaines, trimethylamine oxide (TMAO) and trimethylamine (TMA). The content of extractive nitrogen was 1,090～1,233 mg/100 g on dry basis. The number of 21～25 ninhydrin-positive substances was detected in the analysis of free amino acids, and their total amount was 3,710～4,788 mg/100 g on dry basis. Among them, free proline, asparagine, glutamic acid, alanine, taurine and glutamine were found to be abundant. The combined amino acids amounted to 1,573～2,121 mg/100 g in total and the total amount of ATP and related compound was 33.8～84.0 mg/100 g (1.06～2.46 μmol/g) on dry basis. Betaine, glycinebetaine, β-alaninebetaine, γ-butyrobetaine, homarine and trigonelline were detected in most of samples. Levels of free and combined amino acids, ATP and related compounds fluctuated from sample to sample, with their contents higher in December and January and lower in March.

Key words: Maesaengi *Capsosiphon fulvescens*, free amino acids, combined amino acids, ATP and related compounds, betaines, TMAO, TMA

INTRODUCTION

Extractive components in the tissues of organisms are divided into nitrogenous and non-nitrogenous compounds (1). The nitrogenous compounds that abound in marine organisms encompass a wide variety of chemical entities, including: free amino acids, combined amino acids, nitrogenous and related compounds, betaines, guanidino compounds, trimethylamine oxide (TMAO), and trimethylamine (TMA) (1-3). Extractive components of seaweeds are mainly low-molecular-weight nitrogenous substances, such as amino acids, oligopeptides, amines, betaines, sulfonic acids, nucleotides and related compounds (4).

The Maesaengi *Capsosiphon fulvescens* is classified as a seaweed and includes the classes Chlorophyta, Chlorophyceae, Ulotrichales, and Ulvaceae. The genetic name is a conjugation of two words; 'kapsa' meaning the tube or box and 'siphon' meaning the tube or pipe (5). *C. fulvescens* is distributed extensively throughout the world. In Korea, it is distributed at various places in southern coastal waters and Jeonnam province and it is used as a food product. It is known to appear in the natural habitat in November and to grow fully in February and decline from March to April (6).

Research into *C. fulvescens's* physico-chemical components looks at its proximate composition (7,8), total amine acids (7,8), free amino acids (7), fatty acids and minerals (7,8), free sugars and organic acids (8). Much of the research into the biologically active substances of C. *fulvescens* is related to its inhibitory effect on melanogenesis (9), lipid metabolism (10), and its hepatoprotective effect (11).

This study was designed to elucidate the composition of taste components in fresh *C. fulvescens* and to determine the monthly variation of these components. Seaweed samples were collected and analyzed to detect the free and combined amino acids, ATP and related compounds, betaines and quaternary ammonium bases as the extractive nitrogenous components closely related to taste.

MATERIALS AND METHODS

Materials

The Maesaengi *C. fulvescens* used for the study was cultured at Jangheung-gun, Jeonnam Province of Korea. After collection once or twice a month from December

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to March, the seaweed was carried to the laboratory in an ice box, and the impurities were removed. Then the seaweed was cut into small pieces and stored in a freezer at -40°C until use.

Preparation of extracts

The *C. fulvescens* was extracted with 1% picric acid according to the method of Stein and Moore (12) as follows: 1% picric acid was added to the sample, homogenized in a homogenizer (Bio-mixer, Nissei, model BM-2, Nihonseiki Co., Ltd., Japan), and centrifuged (20PR type, Hitachi Koki Co., Ltd., Japan), at 10,000 rpm for 10 min. The supernatant was collected and the residue was extracted twice in the same way. The combined extract was passed through Dowex 2×8 (Cl^- form, 200～400 mesh) column to remove picric acid. After washing the column with 0.02 N hydrocholoric acid, the extracts were combined, concentrated under reduced pressure, and then made up to a desired volume. The picric acid extract thus obtained was used for analyzing extractive nitrogen, free and combined amino acids, betaines, trimethylamine oxide (TMAO), and trimethylamine (TMA).

The preparation of perchloric acid extracts for analyzing ATP and related compounds was done according to the method of Nakajima et al. (13), as follows: the sample was centrifuged (10,000 rpm, 10 min), and 5 N potassium hydroxide was added to adjust the pH to 7.0. All of these treatments were done at low temperature in an ice box.

Analytical methods

Proximate composition: Moisture in the fresh *C. fulvescens* was determined according to the air-oven method and protein by the semimicro-Kjeldahl method.

Extractive nitrogen: Extractive nitrogen was measured according to the micro-Kjeldahl method (14).

Free amino acids: Free amino acids were analyzed according to the methods of physiological fluid analysis (15) using an automatic amino acid analyzer (LKB Alpha Plus, Series two, Pharmacia Biochrom Co., Ltd., England). The standard amino acids used were the types of physiological A/N and B made by Pierce Chem. Co., (Illinois, USA).

Combined amino acids: The picric acid extract was hydrolyzed with 6 N hydrochloric acid in an ampule as described above. The increased amino acids after hydrolysis were added in the combined form, except aspartic and glutamic acids, which originated from asparagine and glutamine, respectively.

ATP and related compounds: ATP and related compounds were analyzed using high performance liquid chromatography (HPLC) (16). For HPLC, Waters model 510 HPLC pump, Waters 484 tunable absorbance detector, Waters TCM column oven and Waters 745B data module (USA) were used. Chromatograpic conditions were as follows: Buffer, 2% triethylamine-phosphoric acid (pH 7.0) (17); the flow rate, 1.0 mL/min; detection, at 254 nm; column temperature, 40°C; column, μBondapak C_{18} (3.9×300 mm, USA).

Betaines: Betaines were analyzed using HPLC according to the method of Park et al. (16).

TMAO and TMA: TMA was analyzed according to the Bullard and Collins method (18). TMAO was measured according to the method of Bystedt et al. (19), in which TMAO was reduced into TMA with titanous chloride and quantified increased TMA.

Student's t-test: Student's *t*-test was measured according to the Harris (20).

RESULTS AND DISCUSSION

Proximate composition

The results of the monthly proximate composition of fresh *C. fulvescens* are shown in Table 1. The range of moisture content was 83.7～87.6% (average 85.6%). The ranges of protein, lipid, ash and carbohydrate contents were 4.2～5.5% (average 4.7%), 0.08～0.17% (average 0.14%), 3.4～4.2% (average 3.9%), and 4.5～7.8% (average 5.7%), respectively.

On dry basis, the ranges of protein, lipid, ash and carbohydrate contents were 28.4～35.0% (average 32.6%). 0.6～1.1% (average 0.9%), 25.0～29.4% (average 27.2%) and 35.7～44.4% (average 39.2%), respectively.

Table 1. Proximate composition of the fresh *C. fulvescens* (%)

Sampling date	Moisture	Protein		Lipid		Ash		Carbohydrate[1]	
	a	a[2]	b[3]	a	b	a	b	a	b
Dec.31	87.6	4.2	33.8	0.13	1.0	3.6	29.0	4.5	36.3
Jan.16	85.7	4.9	34.3	0.13	0.9	4.2	29.4	5.1	35.7
Feb.12	84.3	5.5	35.0	0.17	1.1	4.2	26.8	8.8	36.9
Feb.16	83.8	4.6	28.4	0.17	1.0	4.2	25.9	7.2	44.4
Mar. 2	86.4	4.3	31.6	0.08	0.6	3.4	25.0	5.8	42.6
Mean±SD[4]	85.6±1.5	4.7±0.5	32.6±2.7	0.14±0.04	0.9±0.2	3.9±0.4	27.2±1.9	5.7±1.0	39.2±4.0

[1]Carbohydrate by difference. [2]On wet basis. [3]On dry basis. [4]Mean±standard deviation (n=5).

The moisture content of fresh laver *Porphyra yezoensis* (21) in the analysis of proximate composition was 81.9% on average, and the result of Student's *t*-test in the present study, exhibited that there was a high correlation between the moisture content of *C. fulvescens* and that of *P. yezoensis* ($p<0.05$). On dry basis, the contents of protein, lipid, ash, carbohydrate in *P. yezoensis* were 43.6%, 0.74%, 22.3% and 33.4% on average, respectively. These values showed that *C. fulvescens* contained lower amounts of protein than *P. yezoensis* ($p<0.05$), and higher amounts of ash in *C. fulvescens* than *P. yezoensis* ($p<0.01$), which had low correlation regarding to lipid and carbohydrate ($p>0.05$).

The contents of protein, lipid, ash and carbohydrate in *P. dentata* (22) on dry basis were 36.9%, 0.7%, 18.1% and 44.3% on average, respectively. These results showed that there were low correlations between the protein and carbohydrate contents in *C. fulvescens* and those in *P. dentata* ($p>0.05$), and there were high correlations between the lipid ($p<0.01$) and ash ($p<0.05$) contents in *C. fulvescens* and those in *P. dentata.*

The content of protein in *C. fulvescens* was high, whereas carbohydrate levels were low in the middle of January sample and in the beginning of February sample, which indicates high quality for nutritional purposes. Noda (23), who investigated the monthly variation in proximate composition of *P. yezoensis* produced in Japan, pointed out that its quality was best in December, the month of the first harvest, when total protein nitrogens were the highest. He also reported the decrease in total protein nitrogens and the increase in carbohydrate for the samples collected in January and February, which were poor in quality. These monthly variations were similar to those we observed in *C. fulvescens.*

Extractive nitrogen

The extractive nitrogen content of the *C. fulvescens* is shown in Table 2. The amount of extractive nitrogen ranged from 143 to 182 mg/100 g (average 166 mg) on wet basis and from 1,090 to 1,233 mg (average 1,151 mg) on dry basis as shown in Table 3. The content of extractive nitrogen was 1,152 mg on dry basis in the late December sample and increased to 1,233 mg in the middle of January, where it was the highest. Extractive nitrogen levels decreased, however, to 1,123 mg in the middle of February and 1,090 mg in the beginning of March, which was the lowest. The content of extractive nitrogen in *C. fulvescens* showed monthly variation according to the harvesting time. These results indicate that the taste of the seaweed is lated to the season.

Miyazawa et al. (24) reported that the contents of extractive nitrogen were 56.9 mg in a fresh green algae *Ulva pertusa* and 9.4 mg in a fresh *Codium fragile* produced in Japan. Amano and Noda (25) and Noda (26) reported that the contents of extractive nitrogen on dry basis in green algae thalli were 454 mg in *U. pertusa*, 650 mg in *U. fasciata*, 173 mg in *Enteromorpha linza,* and 786 mg in *Monostroma nitidum*. These values are lower than those obtained in *C. fulvescens*.

For *P. yezoensis* (21), it was reported that the range of extractive nitrogen content from January to April was 677～1,175 mg (average 989 mg) on dry basis. The monthly variation of extractive nitrogen content was similar to that of *C. fulvescens*. In *P. dentata* (22), it was reported that the content of extractive nitrogen was 760～872 mg (average 829 mg) on dry basis. The result of Student's t-test showed that there was a low correlation between the content of extractive nitrogen in *C. fulvescens* and *P. yezoensis* ($p>0.05$), while there was a high correlation between the content of extractive nitrogen in *C. fulvescens* and *P. dentata* ($p<0.01$).

Free amino acids

The content of free amino acids collected monthly in fresh *C. fulvescens* is shown in Table 2. Various kinds (about 21～25) of free amino acids were detected, and the total amount was 505～610 mg/100 g (average 576 mg) on wet basis. The content of free amino acids in *C. fulvescens* on dry basis is shown in Table 3. The amount of the total free amino acids was 3,710～4,788 mg/100 g (average 4,020 mg).

Amano (27) reported that the amount of the total free amino acids in *U. pertusa* and *E. linza* were 367 and 345 mg on dry basis, respectively. He also reported that the amount of the total free amino acids in *P. yezoensis* (21) was 1,998～4,580 mg/100 g (average 3,821 mg) on dry basis, and the amount of the total free amino acids in *P. dentata* (22) was 2,404～3,966 mg/100 g (average 3,329 mg). The result of Student's *t*-test showed that there was low correlations between the amount of the total free amino acid of *C. fulvescens* and that of *P. yezoensis*, and between the amount of the total free amino acids of *C. fulvescens* and that of *P. dentata* ($p>0.05$).

The monthly variation of the amount of the total free amino acids in *C. fulvescens* showed that it was the highest (4,788 mg) in late December sample. Thereafter, the amount of the total free amino acids was 4,016 mg in the mid January sample and 3,819 mg in early February sample, which is slightly decreased. The levels of total free amino acids continued to decline to 3,766 mg in the middle of February sample and 3,710 mg in the beginning of March sample, which was the lowest value. The amount of total free amino acid fluctuated according to the harvesting time. In this study, the remarkable de-

Table 2. Nitrogenous constituents in the fresh *C. fulvescens* extracts on wet basis (mg/100 g)

Extractive nitrogen	Dec. 31	Jan. 16	Feb. 1	Feb. 16	Mar. 2
	143	176	182	182	148
Free and combined amino acids					
Taurine	14	23	23	21	19
Phosphoethanol amine	1 (2)[1]	−[2]	−	−	−
Aspartic acid	7	9	17	8	4
Hydroxtproline	− (6)	− (9)	−	−	−
Threonine	3 (9)	3 (14)	3 (7)	1 (13)	3 (19)
Serine	2 (14)	7 (13)	5 (8)	4 (14)	6 (12)
Asparagine	129	168	115	117	120
Glutamic acid	55 (27)	66 (36)	102 (31)	50 (31)	48 (41)
Glutamine	19	7	21	28	18
Sarcosine	−	−	3	2	3
α-Amino adipic acid	8	6	9	8	4
Proline	265	180 (7)	198 (90)	296 (8)	161 (24)
Glycine	7 (19)	10 (18)	9 (11)	5 (16)	9 (19)
Alanine	34 (18)	38 (24)	42 (15)	32 (19)	34 (25)
Citrulline	− (1)	−	− (1)	−	−
α-Amino-*n*-butyric acid	−	−	1	−	−
Valine	2 (11)	5 (15)	5 (15)	4 (12)	5 (17)
Cystine	−	−	− (59)	− (27)	− (20)
Methionine	−	− (11)	5 (12)	− (13)	− (6)
Isoleucine	2 (8)	2 (10)	3 (4)	2 (7)	3 (10)
Leucine	2 (12)	4 (19)	5 (7)	3 (14)	7 (17)
Tyrosine	1 (3)	2 (9)	5 (6)	2 (5)	5 (8)
β-Alanine	2 (1)	1	1	−	2
Phenylalanine	5 (6)	1 (13)	2 (11)	1 (10)	3 (12)
β-Amino iso butyric acid	1	−	− (4)	−	−
γ-Amino-*n*-butyric acid	1 (5)	1	1 (5)	− (2)	3
Ethanolamine	14	6	1 (10)	4 (4)	7
Ornithine	1 (1)	1	1 (2)	1	1 (1)
Lysine	5 (15)	6 (17)	8 (5)	4 (12)	7 (18)
Histidine	− (36)	12 (20)	− (23)	− (27)	15 (15)
Arginine	14 (17)	16 (25)	14 (7)	17 (21)	18 (20)
Subtotal	594 (211)	574 (260)	599 (333)	610 (255)	505 (284)
ATP and related compounds					
Adenosine 5'-triphosphate	−	−	−	−	−
Adenosine 5'-diphosphate	1.5	5.0	1.5	1.0	0.6
Adenosine 5'-monophosphate	6.4	4.2	5.3	2.7	2.0
Inosine 5'-monophosphate	0.7	1.6	0.7	0.9	0.5
Inosine	0.4	0.7	1.3	0.8	0.9
Hypoxanthine	0.7	0.5	0.3	0.2	0.6
Subtotal	9.7	12.0	9.1	5.6	4.6
Betaines					
Glycinebetaine	13.0	32.1	44.2	21.4	−
β-Alaninebetaine	−	0.3	1.7	−	4.9
γ-butyrobetaine	−	0.4	−	−	−
Homarine	14.9	5.3	3.3	17.6	21.6
Trigonelline	3.4	−	−	4.5	5.6
Subtotal	31.3	38.1	49.2	43.5	32.1
Others					
Trimethylamine oxide	49.4	31.6	45.3	45.8	36.7
Trimthylamine	7.7	10.3	6.7	7.2	5.2
Urea	−	10.3	9.5	−	12.4
Ammonia	6.8	11.6	6.7	7.7	9.7

[1]The amounts of combined amino acids are given in parenthesis.
[2]Not detected.

Table 3. Nitrogenous constituents in the fresh *C. fulvescens* extracts on dry basis (mg/100 g)

Extractive nitrogen	Dec. 31	Jan. 16	Feb. 1	Feb. 16	Mar. 2
	1,152	1,233	1,159	1,123	1,090
Free and combined amino acids					
Taurine	113	161	146	130	140
Phosphoethanol amine	8 (16)[1]	–[2]	–	–	–
Aspartic acid	56	63	108	49	29
Hydroxtproline	– (48)	– (63)	–	–	–
Threonine	24 (73)	21 (98)	19 (45)	6 (80)	22 (140)
Serine	16 (113)	49 (91)	32 (51)	25 (86)	44 (88)
Asparagine	1,040	1,175	732	722	882
Glutamic acid	444 (218)	462 (252)	650 (197)	309 (191)	353 (301)
Glutamine	153	49	134	173	132
Sarcosine	–	–	19	12	22
α-Amino adipic acid	65	42	57	49	29
Proline	2,137	1,259 (49)	1,261 (573)	1,827 (49)	1,184 (176)
Glycine	56 (153)	70 (126)	57 (70)	31 (99)	66 (140)
Alanine	274 (145)	266 (168)	268 (96)	198 (117)	250 (184)
Citrulline	– (8)	–	– (6)	–	–
α-Amino-*n*-butyric acid	–	–	6	–	–
Valine	16 (89)	35 (105)	32 (96)	25 (74)	37 (125)
Cystine	–	–	– (376)	– (167)	– (147)
Methionine	–	– (77)	32 (76)	– (80)	– (44)
Isoleucine	16 (65)	14 (70)	19 (25)	12 (43)	22 (74)
Leucine	16 (97)	28 (133)	32 (45)	19 (86)	51 (125)
Tryrosine	8 (24)	14 (63)	32 (38)	12 (31)	37 (59)
β-Alanine	16 (8)	7	6	–	15
Phenylalanine	40 (48)	7 (91)	13 (70)	6 (62)	22 (88)
β-Amino iso butyric acid	8	–	– (25)	–	–
γ-Amino-*n*-butyric acid	8 (32)	7	6 (32)	– (12)	22
Ethanolamine	113	42	6 (64)	25 (25)	51
Ornithine	8 (8)	7	6 (13)	6	7 (7)
Lysine	40 (121)	42 (119)	57 (32)	25 (74)	51 (132)
Histidine	– (290)	84 (140)	– (146)	– (167)	110 (110)
Arginine	113 (137)	112 (175)	89 (45)	105 (130)	132 (147)
Subtotal	4,788 (1,693)	4,016 (1,820)	3,819 (2,121)	3,766 (1,573)	3,710 (2,087)
ATP and related compounds					
Adenosine 5'-triphosphate	–	–	–	–	–
Adenosine 5'-diphosphate	12.1	35.0	9.6	6.2	4.4
Adenosine 5'-monophosphate	51.6	29.4	33.8	16.7	14.7
Inosine 5'-monophosphate	5.6	11.2	4.5	5.6	3.7
Inosine	3.2	4.9	8.3	4.9	6.6
Hypoxanthine,	5.6	3.5	1.9	1.2	4.4
Subtotal	78.1	84.0	58.1	34.6	33.8
Betaines					
Glycinebetaine	104.8	224.5	281.5	132.1	–
β-Alaninebetaine	–	2.1	10.8	–	36.0
γ-butyrobetaine	–	2.8	–	–	158.8
Homarine	120.2	37.1	21.0	108.6	41.2
Trigonelline	27.4	–	–	27.8	
Subtotal	252.4	266.5	313.3	268.5	236.0
Others					
Trimethylamine oxide	398.4	221.0	280.5	282.7	269.9
Trimthylamine	62.1	72.0	42.7	44.4	38.2
Urea	–	72.0	60.5	–	91.2
Ammonia	54.8	81.5	42.7	47.5	71.3

[1] The amounts of combined amino acids are given in parenthesis.
[2] Not detected.

crease of the total free amino acids at the beginning of March may explain the poor quality at the end of *C. fulvescens* farming period.

The seasonal variation of the total amount of the free amino acids in *P. yezoensis* (21) showed that it was the highest in February and March samples, and the lowest in April sample. The seasonal variation of the total amount of the free amino acids in *P. dentata* (22) showed that it was high in January and March sample, and low in February and April sample. Again, the total amount of the free amino acids decreased in March sample, at the end of *C. fulvescens* farming period.

The composition of the free amino acids in extracts significantly varied, even within the same species, according to not only the growth site, growth stage at the time of harvesting and environmental factors, but also the daily conditions (1,28,29). The contents of the free amino acids were the highest in December, the first harvest month, while they were decreased in February and March, according to the lowering of the nutrient salts, such as nitrogen and phosphorus (27).

In a study on free amino acids of *P. yezoensis* collected in November, December, and January in Hokkaido, Japan, Sakai and Kasai (30) reported that the content of free amino acids tended to increase with delayed harvest. Its monthly variation was different from that of *C. fulvescense*. The amount and content of the free amino acids were also remarkably fluctuated with the seasons. The amount of free alanine and glutamic acid, which are the taste components of laver, was the highest from the end of December to the beginning of January.

The important and abundant free amino acids were proline (1,184～2,137 mg/100 g, average 1,534 mg), asparagine (722～1,175 mg/100 g, average 910 mg), glutamic acid (309～650 mg/100 g, average 444 mg), alanine (198～274 mg/100 g, average 251 mg), taurine (113～161 mg/100 g, average 138 mg) and glutamine (49～173 mg/100 g, average 128 mg). Among them, proline, asparagine and glutamic acid exhibited concentration variations according to the harvest time, while alanine, taurine and glutamine showed little fluctuation (Fig. 1).

The patterns of the free amino acids in seaweed showed that alanine, glutamic acid, aspartic acid and their amide form were generally abundant (4), while the composition of amino acids in extracts of the green, brown and red algae significantly differed according to the species.

The ratios of proline (38.3%) and asparagine (22.7%) content to total amount of the free amino acids were high in *C. fulvescens*. In the green algae, the free amino acids glutamic acid, proline, glycine, alanine and taurine were relatively abundant (24,29). The composition of the free amino acids of the important edible seaweeds was generally low in the green algae (32). In the red algae such as *P. yezoensis* (21) and *P. dentata* (22), the contents of proline and asparagine were only 0.2～1.8%.

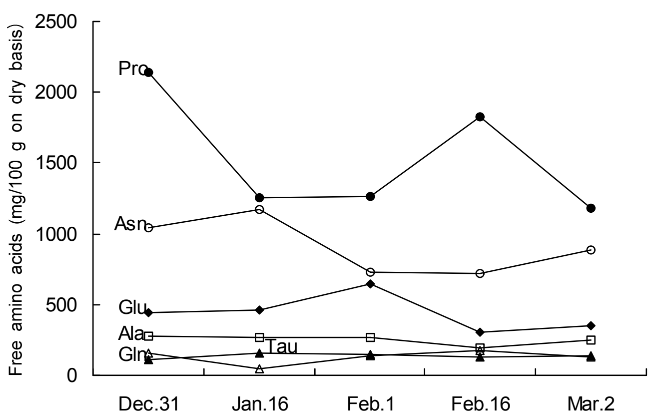

Fig. 1. Monthly variation of free proline, asparagine, glutamic acid, alanine, taurine and glutamine in the fresh *C. fulvescens* extracts.

To examine the effect of content of free amino acids on taste of *C. fulvescens*, the free amino acids detected were divided into umami taste group (aspartic acid, glutamic acid), sweet taste group (threonine, serine, glutamine, proline, glycine, alanine, lysine), and bitter taste group (valine, methionine, isoleucine, leucine, phenylalanine, histidine, arginine) (33) as shown in Table 4. The ratios of umami taste amino acids to the total free amino acids were high; 13.1% in the middle of January sample and 19.8% in the beginning of February sample. The ratios of sweet taste amino acids to the total free amino acids were as high as in the end of December sample and in the middle of February 56.4% and 60.7%, respectively. The average ratios of umami taste amino acids to the total free amino acids and sweet taste amino acids to the total free amino acids in *C. fulvescens* were 12.6% and 51.2%, respectively. The average ratios of umami taste amino acids and sweet taste amino acids to the total free amino acids in *P. yezoensis* (21) were 17.9% and 41.7%, respectively. The result of Student's *t*-test revealed that there was a low correlation between the ratio of the umami taste amino acid group to the total free amino acids in *C. fulvescens* and the ratio of the umami taste amino acid group to the total free amino acids in *P. yezoensis* ($p>0.05$); whereas, in case of the sweet taste amino acids group, a high correlation was shown ($p<0.05$). Because *C. fulvescens* contained more umami and sweet taste amino acids like proline, alanine, glutamine and glycine than *P. yezoensis*, it was thought that *C. fulvescens* had sweeter taste.

Moreover, the average ratios of umami taste amino

Table 4. The amount of total umami, sweet, and bitter free amino acids (FAA) in the fresh *C. fulvescens* extracts on dry basis (mg/100 g)

	Dec. 31	Jan. 16	Feb. 1	Feb. 16	Mar. 2
Total FAA[1)]	4,788 (100%)	4,016 (100%)	3,819 (100%)	3,760 (100%)	3,710 (100%)
Umami[2)]	500 (10.4)	525 (13.1)	758 (19.8)	358 (9.5)	382 (10.3)
Sweet[3)]	2,700 (56.4)	1,756 (43.7)	1,828 (47.9)	2,285 (60.7)	1,749 (47.1)
Bitter[4)]	241 (5.0)	280 (7.0)	217 (5.7)	167 (4.4)	374 (10.1)
Others	1,347 (28.2)	1,455 (36.2)	1,016 (26.6)	956 (25.4)	1,205 (32.5)

[1)]Refer to Table 3.
[2)]Umami: aspartic acid +glutamic acid.
[3)]Sweet: threonine+serine+glutamine+proline+glycine+alanine+lysine.
[4)]Bitter: valine+methionine+isoleucine+leucine+pheylalanine+histidine+arginine.
Amino acids were classified according to Fuke (33) with slight modification.

acids group and sweet taste amino acids group to the total free amino acids in *P. dentata* (22) were reported 12.5% and 44.9%, respectively. The result of Student's *t*-test revealed that there was a low correlation between the ratio of umami and sweet amino acids to the total free amino acid in *C. fulvescense* and the ratio of umami and sweet amino acids to the total free amino acid in *P. dentata* ($p>0.05$).

Combined amino acids

The results of analyzing combined amino acids, i.e., the amino acids originating from oligopeptides, in *C. fulvescens* are shown in the parentheses in Table 2 (on wet basis) and Table 3 (on dry basis). In all the samples, 16～21 kinds of amino acids were increased after hydrolysis of the *C. fulvescens* extracts, while 15 kinds of amino acids were increased after hydrolysis of *U. pertusa*, 8 kinds of amino acids were increased after hydrolysis of *E. linza,* and 4 kinds were increased after hydrolysis of *Cladophora densa* produced in Japan.

As shown in Table 3, the total amounts of the combined amino acids were 1,573～2,121 mg/100 g (average 1,859 mg), which corresponded to an average of 46.2% of the total free amino acids (Fig. 2). The ratio of the total amount of the combined amino acids to the total amount of the free amino acids was 157.5%, 61.9% and 21.1%, respectively (24,29).

The abundant combined amino acids present in the *C. fulvescense* were glutamic acid (191～301 mg/100 g, average 232 mg), histidine (110～290 mg/100 g, average 171 mg), proline (0～573 mg/100 g, average 169 mg), alanine (96～184 mg/100 g, average 142 mg), cystine (0～376 mg/100 g, average 138 mg), and arginine (45～175 mg/100 g, average 127 mg). The abundant combined amino acids present in the *U. pertusa* produced in Japan were glutamic acid, alanine, glycine, aspartic acid and valine, and in *E. linza* were glutamic acid, arginine, proline and alanine, and in *C. densa* were alanine, proline, aspartic acid (24,29). Thus, the total amount and content of the combined amino acids showed a considerable fluctuation according to the species of seaweeds.

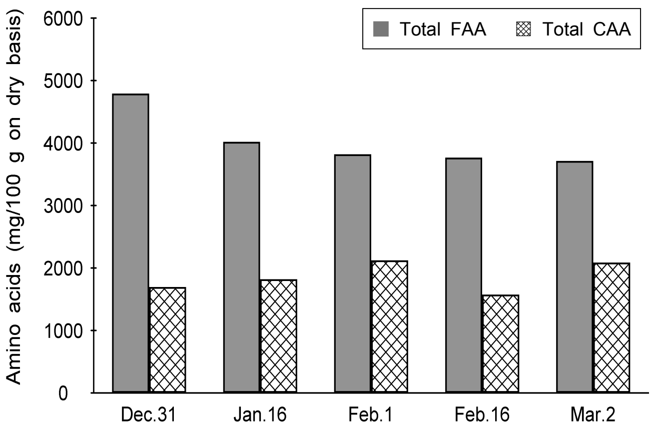

Fig. 2. Total free (FAA) and combinded (CAA) amino acids in the fresh *C. fulvescens* extracts.

The amount of the combined amino acids in *C. fulvescens* at the harvesting time was 1,693 mg in the end of December, and increased to 1,820 mg in the middle of January, was the highest at 2,121 mg in the beginning of February, decreased drastically to 1,573 mg in the middle of February, and recovered to 2,087 mg in the beginning of March. The monthly variation of the total amount of the combined amino acids in *P. yezoensis* (21) showed that the amount of the combined amino acids was high from January to February, and was low from March to April. In case of *P. dentata* (22), the amount of the combined amino acids was high in February and April, and low in January and March.

The ratio of the amount of the combined amino acids to the amount of the free amino acids in *C. fulvescens* was 35.4～56.3% (average 46.9%), which was the lowest in the end of December and the highest in the beginning of March (Fig. 2). The ratio of the amount of the combined amino acids in *P. yezoensis* (21) was 48.9～119.1% (average 76.5%). And in case of *P. dentata* (22), the ratio was 36.0～112.0% (average 65.8%). The result of Student's *t*-test showed a low correlations between the amount of the combined amino in *C. fulvescens* and that in *P. yezoensis* or *P. dentata* (p>0.05). The similar feature of the amount of the combined amino acids in *C. fulvescens*, *P. yezoensis* and *P. dentata* was that the content ratio of them became higher as the harvesting time became late.

ATP and related compounds

The analytical results of ATP and related compounds in the fresh *C. fulvescens* at harvest time are shown in Table 3. ADP, AMP, IMP, inosine and hypoxanthine were detected in all samples. The amount of ATP and related compounds was 33.8～84.0 mg/100 g (1.06～2.46 μmol/g) with the average of 57.7 mg/100 g (1.78 μmol/g). These values were very low when compared with those reported for adenine nucleotides in fish muscle, 4～9 μmol/g (1).

The total amounts of ATP and related compounds in *P. yezoensis* (21) and *P. dentata* (22) were 85.5～119.8 mg/100 g (2.95～4.73 μmol/g) and 73.3～94.4 mg/100 g (2.04～4.43 μmol/g) with the averages of 101 mg/100 g (3.57 μmol/g) and 85.1 mg/100 g (3.1 μmol/g). Thus, the amount of ATP and related compounds in *C. fulvescens* was 50% of the total amount of ATP and related compounds in *P. dentata*. The result of Student's *t*-test showed that there was a high correlation between the total amount of ATP and related compounds in *C. fulvescens* and that in *P. yezoinsis* (21) (p<0.01). However, the result of Student's *t*-test also revealed that there was a low correlation between the total amount of ATP and related compounds in *C. fulvescens* and that in *P. dentata* (22) (p>0.05). It was reported that the total amounts of ATP and related compounds in laver produced in Japan were 0.47 μmol/g in fresh *P. tenera* (34) and 14.0 μmol/g on dry basis in *P. yezoensis* (35).

The concentration of IMP, which is known to play an important role as a flavor component, was 3.7～11.2 mg/100 g (0.11～0.32 μmol/g) with an average of 6.1 mg/100 g (0.18 μmol/g). The ratio of IMP to ATP and related compounds was 6.5～15.1% (average 10.3%), which fluctuated at the harvest time. The contents of IMP in *P. yezoensis* (21) and *P. dentata* (22) were 0.98 and 0.90 μmol/g on average. Thus, the content of IMP in *C. fulvesense* was only 18.4% and 20.0% in comparison with that in *P. yezoensis* (21) and *P. dentat*a (22). The result of Student's t-test showed that there was a high correlation between the content of IMP in *C. fulvescens* and that in *P. yezoensis* (21) (p<0.05) while a low correlation was found between that *fulvescens* and *P. dentata* (22) (p>0.05).

The amount of ATP and related compounds in *C. fulvesense* was 78.1 mg/100 g (2.46 μmol/g) in the end of December sample, 84.0 mg/100 g (2.43 μmol/g) in

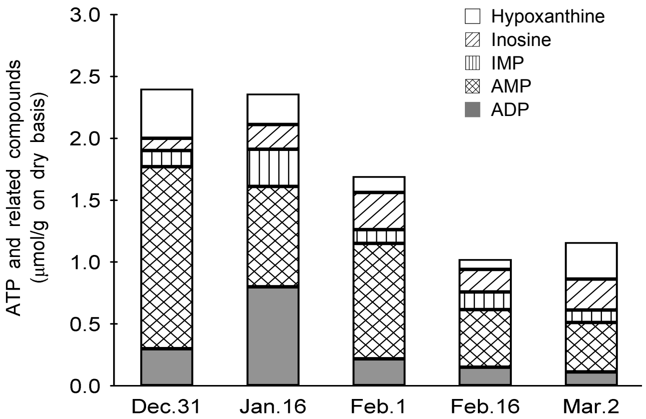

Fig. 3. Monthly variation of ATP and its related compounds in the fresh *C. fulvescens* extracts.

the middle of January sample, which was the highest, and then decreased to 58.1 mg/100 g (1.77 μmol/g) in the beginning of February, thereafter, decreased drastically to 34.6 mg/100 g (1.20 μmol/g) in the middle of February and 33.8 mg/100 g (1.20 μmol/g) in the beginning of March, which showed the seasonal variation clearly (Fig. 3).

Betaines

As shown in Table 2, five kinds of betaines were detected in fresh *C. fulvescens*. Homarine as cyclic betaines was detected in all samples and trigonelline as cyclic betaines was detected in late December and mid February and early March samples. Glycinebetaine, β-alaninebetaine and γ-butyrobetaine as chain betaines were detected in some samples.

The total content of betaines was 31.3～49.3 mg/100 g (average 38.8 mg) in fresh *C. fulvescens* and 236.0～313.3 mg/100 g (average 267.3 mg) on dry basis. The content of glycinebetaine was highest while homarine was the second-highest. The contents were the highest in the middle of February and the lowest in the beginning of March (Fig. 4).

The content of glycinbetaine, which was the main component of betaines in *C. fulvescens* was 104.8～281.5 mg/100 g (average 185.7 mg) on dry basis, which corresponded to 41.5～89.8% of the total betaines (average 66.2%). Homarine content levels were next, at 21.0～15.8 mg/100 g (average 89.1 mg), which corresponded to 6.7～67.3% (average 29.2%) to the total betaines. The content of trigonelline was 27.4～41.2 mg/100 g (average 32.1 mg), which corresponds to 10.4～17.5% (average 12.9%) of the total betaines. The amount of betaines in *P. yezoensis* (21) was 12.0～63.3 mg/100 g (39.8 mg) on dry basis and that in *P. dentata* (22) was 14.4～32.8 mg/100 g (average 20.5 mg). Thus, high correlations are found between the total content of betains in *C. fulves-*

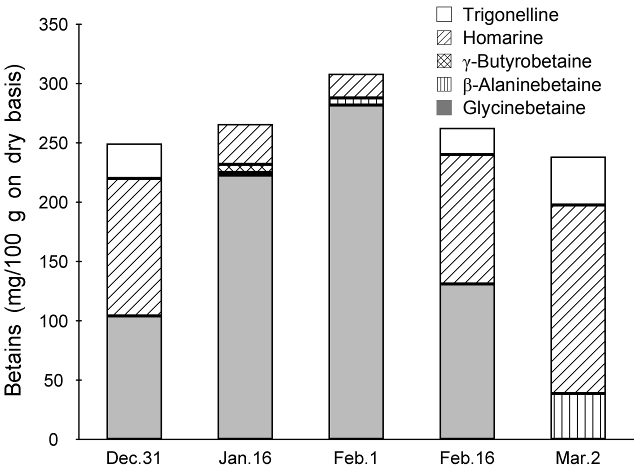

Fig. 4. Monthly variation of betaines in the fresh *C. fulvescens* extracts.

cens and that in *P. yezoensis*, and between that in *C. fulvescens* and that in *P. dentata* ($p<0.001$).

For the first time, 14 mg/100 g of glycinebetaine was isolated from a brown algae *Dictyota dichotoma* (4). And β-alaninebetaine, the substance which reduces the cholesterol in blood, was isolated 0.3 mg/100 g on dry basis in a green algae *M. nitidum* (36). γ-Butyrobetaine was isolated 3.6mg/100 g on dry basis in red algae *P. yezoensis* (37), homarine and trigonelline, as the ultraviolet-absorbing components of red algae, were isolated in *Tichocarpus crinitus* (38).

TMAO and TMA

The results of analyzing TMAO and TMA in *C. fulvescens* are shown in Table 2. TMAO and TMA were detected in all samples. The contents of TMAO and TMA on wet basis were 31.6～49.4 mg/100 g (average 41.8 mg) and 5.2～10.3 mg (average 7.4 mg), respectively. As shown in Table 3, the content of TMAO and TMA on dry basis were 221.0～398.4 mg/100 g (average 290.5 mg) and 38.2～72.0 mg/100 g (average 51.9 mg), respectively.

The content of TMAO in *C. fulvescens* was 15.2 times and 61.8 times higher than that of *P. yezoensis* (21) and *P. dentata* (22), respectively. The content of TMA in *C. fulvescens* was also high, which was 3.0 times and 3.9 times higher than that *P. yezoensis* (21) and *P. dentata* (22), respectively. High correlations were found between the content of TMA in *C. fulvescens* and *P. yezoensis* ($p<0.05$), and between *C. fulvescens* and *P. dentata* ($p<0.001$).

Fujiwara et al. (39) reported that the contents of TMAO and TMA in *P. tenera* were 249～358 and 187～253 mg/100 g on dry basis, respectively. These values were similar to the content of TMAO in *C. fulvescens*, while the content of TMA in *P. tenera* was higher than that of *C. fulvescens*. Monthly variations of TMAO and TMA contents were not considerable.

Nitrogen distribution in the extracts obtained by above analyses is summarized in Table 5, in which the amount of each group of extractive components is shown in terms of the percentage of the extractive nitrogen. The composition of distribution of nitrogen in *C. fulvescens* was similar, regardless of harvesting time. Free and combined amino acids are the major nitrogenous constituents in *C. fulvescens*, occupying 53.0±5.8% and 24.1±1.5%, respectively, and the total of the contents of two components reaches 77.1±6.5%. The nitrogen content of TMAO and TMA is 5.8±1.1%, and that of betaines is 2.6±0.4% and that of ATP and related compounds is 1.1±0.3%. The ratio of recovered nitrogen extracts to total extract components in *C. fulvescens* was 86.6±7.5%.

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Table 5. Nitrogen distribution in the fresh *C. fulvescens* extracts (%)

	Dec. 31	Jan. 16	Feb. 1	Feb. 16	Mar. 2
Free amino acids	62.6	51.8	47.6	49.7	53.2
Combined amino acids	25.3	22.8	25.4	22.1	24.8
ATP and related compounds	1.4	1.3	1.0	0.6	1.0
Betaines	2.0	2.5	3.2	2.7	2.6
TMAO and TMA	7.7	4.7	5.4	5.6	5.8
Unknown	0.6	16.9	17.4	19.3	12.6
Recovery of extractive nitrogen	99.4	83.1	82.6	80.7	87.4

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