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Research Article

Seasonal variations in the biochemical composition of sea urchin (*Hemicentrotus pulcherrimus*) roe

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Abstract

Seasonal changes in the biochemical composition of *Hemicentrotus pulcherrimus* roe were analyzed from May 2016 to February 2017 in Korea. Spawning, occurring between April and May, was characterized by relatively low lipid (3.46 g/100 g) and protein levels (13.22 g/100 g). Concurrently, calcium level was increased, while magnesium level was decreased. The roe's polyunsaturated fatty acids (PUFA) content was high, constituting 40% of total fatty acids, with eicosapentaenoic acid (C20:5n-3) and eicosatrienoic acid (C20:3n-3) being the most predominant. During the spawning season, lower levels of ascorbic acid (30.9 mg/kg) and folic acid were observed alongside higher concentrations of riboflavin (7.24 mg/kg) and niacin (232.32 mg/kg). Vitamin E content was at its lowest in May, measuring 0.05 mg/100 g, compared to a range of 1.62–2.22 mg/100 g in other seasons. Overall, the results suggest that *H. pulcherrimus* roe is a valuable source of protein, minerals, vitamins, and glycine, an important flavor component. However, significant variations in its biochemical components were noted during the spawning period compared to other seasons.

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Introduction

Sea urchins, predominantly residing near rocky shores, primarily feed on algae and brown seaweeds (kelps), alongside other invertebrates. Around 950 species of sea urchins are found across various ocean depths and are notably prevalent around Korea, Japan, Russia, and China (Andrew *et al.*, 2002). Korea hosts approximately 30 species of sea urchin, while over 100 species are recorded in Japan. The northwestern Pacific Ocean yields the highest sea urchin catches, with significant fisheries established in Korea, Japan, and Russia. In 2022, Korea produced 3,294 metric tons of sea urchins (Korea Statistical Information Service (KOSIS, 2024). The roe of these sea urchins is commonly consumed raw, served on rice, or preserved, with Japan accounting for about 80-90% of the global consumption of edible sea urchins. Predominantly, Korea and Japan harvest and market species such as *Anthocidaris crassispina*, *Hemicentrotus pulcherrimus*, *Pseudocentrotus depressus*, *Strongylocentrotus intermedius*, *S. nudus*, and *Tripneustes gratilla* (Stefánsson *et al*., 2017). Additionally, for over two centuries, sea urchins have been utilized as model organisms in embryological research (McClay, 2011).Previous studies on edible sea urchins in the Far East have explored aspects like the reproduction and growth of *S. intermedius* and *S. nudus* (Agatsuma, 2020), the ecological parameters for *S. intermedius, S. nudus* and *H. pulcherrimus* (Agatsuma, 2020), and growth and ripeness of *H. pulcherrimus* (Ohgaki *et al.*, 2019). Other research has investigated the impact of size and diet on roe yield and quality in the green sea urchin *S. droebachiensis* (Pearce *et al*., 2004), alongside several studies on the biochemical composition of sea urchin roe (Montero-Torreiro *et al*., 2003; Arafa *et al*., 2012; Ouchene *et al*., 2021) comprehensive research on the nutritional value and functional ingredients of edible sea urchin species still needs to be more extensive.

This study aims to determine the seasonal variations in the biochemical composition of the roe of *H. pulcherrimus*, which is highly prized for its superior flavor, color, and gloss, and is growing in popularity (Stefánsson *et al*., 2017). We anticipate that this research could enhance the popularity of sea urchin roe consumption, potentially benefiting marine environments by encouraging harvesting in areas where excessive sea urchin populations contribute to local ecological damage.

Materials and methods

Sampling

Samples of the sea urchin *H. pulcherrimus* were collected quarterly from Dae-Byun Harbor, Busan, South Korea, from May 2016 to February 2017 (Fig. 1). On the day of collection, shells were removed (Fig. 1A), and the roe (edible portion) was retained (Fig. 1B–Fig. 1E). The average weight and diameter of the sea urchins prior to dissection are presented in Table 1.

Proximate composition

The moisture, crude protein, crude lipid, and ash content of the sea urchin roe were analyzed following the procedures outlined by the Association of Official Analytical Chemists (AOAC, 1995).

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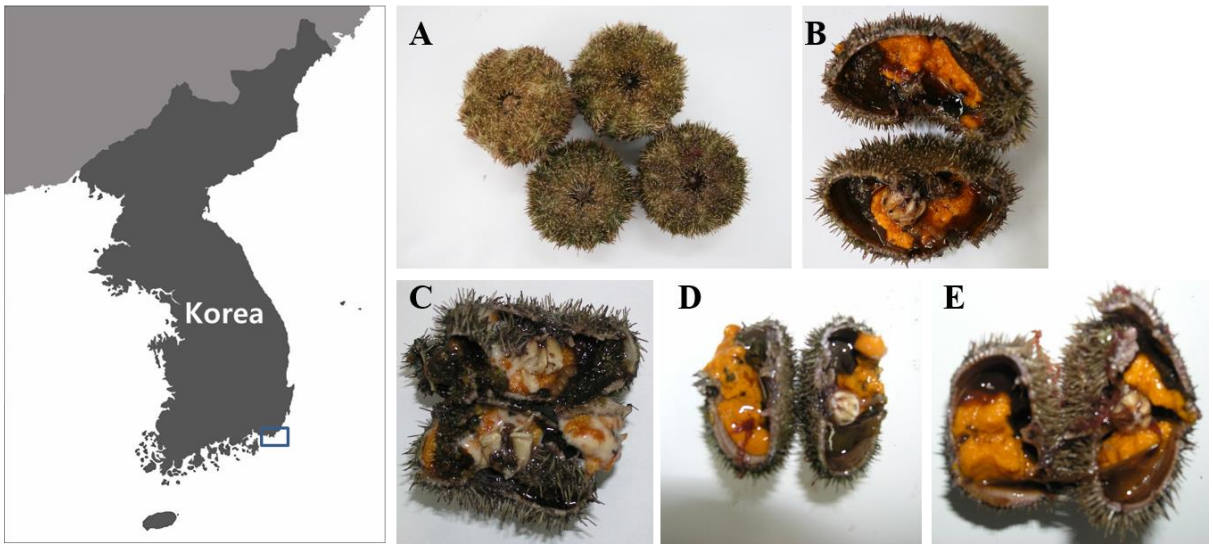

Figure 1: The sampling region and the sea urchin samples collected quarterly from Gijang in Korea. A, Whole; B, February; C, May; D, August; E, November.

Table 1: Capture date and biological data of sea urchin collected from Gijang in Korea.

Species	Sampling Date	No. of sample	Body weight (g)	Body diameter (cm)
Sea urchin (*Hemicentrotus pulcherrimus*)	Feb., 17	25	28.86±5.54	4.14±0.22
	May., 16	25	23.44±7.00	3.84±0.42
	Aug., 16	25	23.19±7.22	3.90±0.44
	Nov., 16	25	26.96±5.97	4.13±0.35

Data expressed as mean±standard deviation.

Minerals and heavy metals

The mineral content (Na, Ca, K, Mg, P, Fe, Cu, and Ze) and heavy metals in the roe were determined using the methods prescribed by the Ministry of Food and Drug Safety (MFDS, 2024). For trace metal analysis, 10 g samples were digested with Suprapur grade nitric acid (HNO_3, Merck, *Darmstadt*, Germany), dried, and subsequently dissolved in distilled water to a final volume of 25 mL. Assays were conducted using an ICP-AES, model Optima 3300 XL (Perkin Elmer, Norwalk, CT, USA).

Fatty acid composition

Lipids were extracted from minced and homogenized sea urchin roe using a chloroform: methanol mixture (2: 1 v/v), based on the Folch *et al*. (1957) method. Fatty acids were then converted into methyl esters using 14% boron trifluoride in methanol under a nitrogen atmosphere. The fatty acid composition was analyzed using a gas chromatograph (Agilent 6890N, Agilent Technologies, USA) equipped with an HP-Innowax capillary column (30.0 m×0.25 mm×0.50 μm, Agilent Technologies). Fatty acids were identified by comparison with known standards, and data were quantified based on the peak area percentage of total fatty acids.

Total amino acids

Approximately 50 mg of sea urchin roe were hydrolyzed in 6N hydrochloric acid (HCl) at 110°C for 24 h. The hydrolyzed solution was then vacuum-dried and

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redissolved in sodium citrate buffer (pH 2.2) to achieve a final volume of exactly 50 mL. This solution was filtered through a 0.20 μm membrane before being analyzed using an amino acid analyzer (Sykam DE/S-433D, *Sykam* GmbH, Germany).

Free amino acids

Free amino acid analysis was performed following the method outlined by Kaneko *et al*. (2012), with minor modifications. To extract free amino acids, 5 g of roe was combined with 15 mL of cold 5% trichloroacetic acid (TCA) and homogenized. The mixture was allowed to stand at room temperature for 30 min before being centrifuged at 8,000× *g* for 15 min. The resulting supernatant was adjusted to a total volume of 25 mL using lithium citrate buffer (pH 2.2). After filtration through a 0.20 μm Puradisc membrane (Whatman PTFE; GE Healthcare, USA), the free amino acid composition was determined using the same amino acid analyzer (Sykam DE/S-433D).

Water-soluble vitamins

Samples weighing 1–10 g were homogenized with 5% metaphosphoric acid to yield 50 mL volumes. These samples were then heated in water at 80°C for 1 h and extracted using a commercial ultrasonic cleaner. Riboflavin required additional heating in water at 80°C for 10–20 min. The extracts were filtered through No. 5A filter paper (*Advantec*, Tokyo, Japan) and a 0.45 μm syringe filter, before being analyzed with a high-performance liquid chromatograph (HPLC) system (Surveyor Plus HPLC, Thermo Scientific, USA). The analysis utilized a Shiseido C18 column (5 μm, 250×4.6 mm) with a water (solvent A)–60% methanol (solvent B) gradient: 0–3 min at 100:0, 3–25 min transitioning from 100:0 to 0:100, and 25–30 min from 0:100 back to 100:0. The mobile phase flow rate was 1 mL/min and the injection volume was 20 μL. UV detection wavelengths were set at 290 nm for pyridoxine and riboflavin, and 270 nm for ascorbic acid, nicotinamide, thiamin, and folic acid (MFDS, 2024).

Vitamins A and E

The content of vitamins A and E in roe tissue was analyzed following the protocol of Lee *et al*. (2011), with sample preparation methods adapted from the MFDA (2024). Three grams of roe tissue were placed into a 250 mL flask, dissolved with 30 mL of ethanol, 1 mL of 10% pyrogallol in ethanol, and 3 mL of 60% potassium hydroxide solution. The mixture was well mixed and saponified for 30 min in a 90°C water bath. After rapid cooling, the solution was transferred to a brown separatory funnel containing 30 mL of water. Then, 50 mL of petroleum ether was added, and the funnel was shaken for 10 min before being allowed to stand for 30 min. The lower aqueous layer was transferred to another funnel and the ether phase was extracted two more times with 30 mL of petroleum ether. The combined ether phases were washed with 50 mL of water repeatedly until the phenolphthalein indicator turned colorless. After removing the water, anhydrous sodium sulfate was added to the ether phase for dehydration, which was then transferred to a brown glass flask and dried at 40°C using a vacuum evaporator. The residue was redissolved in

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5 mL of sample solution and filtered through a 0.2 µm membrane for analysis. Retinol-acetate and α-tocopherol were used as standards for vitamins A and E, respectively. The analysis was performed using a Surveyor plus HPLC system (Thermo Scientific) equipped with a photodiode array detector. A Develosil 5-µm RP-Aqueous C30 column (4.6×250 mm, Nomura Chemical Co., Japan) utilized an isocratic elution of methanol/water at 95:5, with a flow rate of 1.2 mL per min and an injection volume of 10 µL. The photodiode array (PDA) detector wavelength was set at 298 nm.

Statistical Analyses

Data are presented as means±standard deviation. Differences between group means of parametric data were analyzed using one-way ANOVA. Significant differences among means were determined using Duncan's multiple-range test. Means were considered significantly different at a $p<0.05$. All statistical analyses were performed using SAS for Windows (SAS Enterprise Guide ver. 4.3).

Results

Proximate composition

Seasonal variations in the proximate composition of sea urchin roe are presented in Table 2. Significant differences were observed in moisture (67.42–80.92 g/100 g) and crude lipid (3.46–6.60 g/100 g) contents across different seasons. In contrast, variations in crude protein (13.22–15.33 g/100 g) and ash (1.67–3.40 g/100 g) contents were minimal, with no significant seasonal fluctuations. The highest moisture content was 80.92 g/100 g, while the lowest crude lipid and protein contents, 3.46 g/100 g and 13.22 g/100 g, respectively, occurred in May (spawning period), compared to other seasons during the study.

Table 2: Seasonal variation of proximate composition of sea urchin roe collected from Gijang in Korea.

Season	Moisture (g/100g)	Crude lipid (g/100g)	Crude protein (g/100g)	Ash (g/100g)
February	74.78 ± 0.20^{b}	5.10 ± 0.14^{b}	14.17 ± 0.69^{b}	2.51 ± 0.02^{b}
May	80.92 ± 0.25^{a}	3.46 ± 0.24^{c}	13.22 ± 0.06^{c}	2.45 ± 0.07^{b}
August	67.42 ± 0.08^{d}	6.52 ± 0.68^{a}	15.33 ± 0.08^{a}	1.67 ± 0.01^{c}
November	68.46 ± 0.78^{c}	6.60 ± 0.17^{a}	15.22 ± 0.05^{a}	3.40 ± 0.19^{a}

Results are mean±standard deviation, values within the same column are significantly different by Duncan's multiple ranges at $p<0.05$.

Mineral contents

The contents of both macroelements (Na, Ca, K, Mg, and P) and microelements (Fe, Cu, and Zn) in sea urchin roe varied seasonally, as shown in Table 3. Sodium content varied significantly, ranging from 17.34 to 38.75 mg/100 g. Calcium and potassium also showed significant variations, with ranges of 6.60–24.62 mg/100 g and 19.80–35.42 mg/100 g, respectively. There were no notable seasonal differences in the levels of magnesium and phosphorus, or the microelements iron, copper, and zinc. The highest mineral contents were recorded in samples from February of the following

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year, particularly for calcium, which increased substantially during this period. Conversely, the magnesium content in sea urchin roe showed minimal decline post-fertilization, suggesting a potential link between increased calcium levels and fertilization, with only a slight increase observed in magnesium from November measurements.

Table 3: Seasonal variation of mineral contents of sea urchin roe collected from Gijang in Korea.

Season	Na (mg/100g)	Ca (mg/100g)	K (mg/100g)	Mg (mg/100g)	P (mg/100g)	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
February	26.01±0.33^{b}	24.62±0.08^{a}	35.42±0.27^{a}	9.26±0.06^{a}	74.96±0.50^{a}	2.86±0.04^{a}	0.32±0.01^{a}	2.55±0.04^{a}
May	38.75±0.15^{a}	6.60±0.02^{d}	33.83±0.34^{b}	9.29±0.05^{a}	33.80±0.08^{c}	1.38±0.03^{d}	0.11±0.00^{b}	2.36±0.38^{a}
August	20.22±0.07^{c}	8.59±0.02^{c}	19.80±0.07^{d}	8.52±0.01^{b}	34.09±0.04^{bc}	2.47±0.02^{b}	0.02±0.00^{d}	1.48±0.01^{b}
November	17.34±0.14^{d}	16.14±0.07^{b}	22.21±0.18^{c}	8.13±0.01^{c}	34.44±0.12^{b}	1.83±0.05^{c}	0.05±0.00^{c}	1.23±0.01^{b}

Results are mean±standard deviation, values within the same column are significantly different by Duncan's multiple ranges at $p<0.05$.

Fatty acid composition

The fatty acid profile of lipids extracted from sea urchins collected across different seasons is detailed in Table 4. The predominant saturated fatty acids were palmitic acid (16:0) and myristic acid (14:0). Among monounsaturated fatty acids, palmitoleic acid (16:1) and oleic acid (18:1) showed the highest concentrations. Eicosapentaenoic acid (20:5) and eicosatrienoic acid (20:3) were the most concentrated among the polyunsaturated fatty acids. The concentrations of myristic acid and palmitic acid varied significantly, ranging from 13.84% to 16.86% and 19.77% to 24.56%, respectively. Other saturated fatty acids all measured below 3%. For monounsaturated fatty acids, concentrations of palmitoleic acid and oleic acid ranged from 3.91% to 4.78% and 2.61% to 4.86%, respectively. Among polyunsaturated acids, eicosatrienoic acid and eicosapentaenoic acid also showed variations, with ranges from 7.57% to 14.49% and 18.98% to 24.18%, respectively. All other fatty acids were detected in the 1–2% range, indicating negligible seasonal variation. Previous research indicates that palmitic acid (C16:0) and eicosapentaenoic acid (20:5n-3) are the most abundant fatty acids in sea urchins, notably high in omega fatty acids, which are essential components of neuron membranes and retinal tissue, facilitating rapid transmission of electrical stimuli to other cell membranes.

The omega-3 fatty acid content in the sea urchin roe was notably high, exceeding 25%, while omega-6 (n-6) fatty acid accounted for about 10%. The ratio of n-3/n-6 fatty acids ranged from 1.86 to 3.16, indicating a high proportion of n-3. Both n-3 and the n-3/n-6 ratio are significant due to their extensive effects on human physiological functions and are considered crucial nutritional indices in human health.

Total amino acids

In this study, 17 amino acids (excluding tryptophan) were analyzed in sea urchin samples across different seasons. Tryptophan was undetectable because it degraded during hydrolysis with HCl. The total amino acid content varied seasonally:

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it was 888.46 mg/100 g in May, increased to 1,227.31 mg/100 g in August, and reached 1,509.05 mg/100 g in November (doubling the value detected in May, during the spawning period).

Table 4: Seasonal variation of fatty acid composition (area percent) of sea urchin roe collected from Gijang in Korea.

Fatty acids	February (%)	May (%)	August (%)	November (%)
C14:0	16.86	13.84	15.81	15.01
C15:0	0.88	0.99	0.72	0.74
C16:0	21.52	19.77	23.77	24.56
C17:0	0.23	0.22	0.27	0.23
C18:0	2.53	2.61	2.61	3.14
C20:0	0.61	0.51	0.73	0.88
C22:0	1.62	1.22	1.34	1.73
C23:0	0	0	0	0.25
Total SFA	44.25	39.16	45.25	46.54
C14:1	1.39	1.32	1.15	1.06
C15:1	0.13	0.17	0.17	0.16
C16:1	4.77	4.78	4.61	3.91
C17:1	1.69	1.34	2.07	1.56
C18:1	2.61	4.86	4.67	5.31
C20:1	3.57	0.81	0.79	3.72
C22:1	0	0.96	0.89	0.85
C24:1	0.35	0.61	0.39	0.55
Total MUFA	14.51	14.85	14.74	17.12
C18:2	1.03	0.75	1.29	1.21
C18:3	4.96	4.45	6.22	5.05
C20:2	1	0.83	0.43	0.62
C20:3	11.11	14.49	7.81	7.57
C20:4	0.99	0.84	1.28	1.51
C20:5	21.6	24.18	22.03	18.98
C22:2	0	0	0.32	0.37
C22:6	0.55	0.45	0.63	1.03
Total PUFA	41.24	45.99	40.01	36.34
n-3	28.09	29.92	30.16	26.57
n-6	13.14	16.07	9.53	9.4
n-3/n-6	2.14	1.86	3.16	2.83
UFA/SFA	1.26	1.55	1.21	1.15
MUFA/SFA	0.33	0.38	0.33	0.37
PUFA/SFA	0.93	1.17	0.88	0.78
Total	100	100	100	100

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

The content then decreased to 1,199.81 mg/100 g in samples collected in February of the following year (Table 5). The primary amino acids in sea urchins were glutamic acid, glycine, aspartic acid, arginine, and lysine, with glutamic acid

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being the most abundant at approximately 11%. Lysine and leucine, important essential amino acids, comprised 7.3–8.8% of the total amino acids.

Table 5: Seasonal variation of total amino acid content of sea urchin roe collected from Gijang in Korea.

Amino acids	February	May	August	November
Essential amino acid (E) (g/100g)				
Histidine	37.88(3.2)	25.36(2.9)	45.74(3.7)	54.52(3.6)
Isoleucine	63.96(5.3)	43.27(4.9)	71.00(5.8)	84.63(5.6)
Leucine	90.32(7.5)	64.50(7.3)	89.03(7.3)	114.15(7.6)
Lysine	103.22(8.6)	78.46(8.8)	101.87(8.3)	114.99(7.6)
Methionine	35.45(3.0)	22.89(2.6)	41.88(3.4)	54.56(3.6)
Phenylalanine	71.31(5.9)	38.79(4.4)	67.87(5.5)	118.09(7.8)
Threonine	67.60(5.6)	49.83(5.6)	69.55(5.7)	82.83(5.5)
Valine	67.21(5.6)	51.51(5.8)	75.92(6.2)	82.91(5.5)
Total E	536.95(44.8)	374.61(42.2)	562.86(45.9)	706.68(46.8)
Nonessential amino acids (NE) (g/100g)				
Arginine	101.25(8.4)	70.49(7.9)	115.83(9.4)	129.24(8.6)
Aspartic acid	104.42(8.7)	80.06(9.0)	110.38(9.0)	133.18(8.8)
Serine	64.26(5.4)	50.65(5.7)	59.60(4.9)	73.68(4.9)
Glutamic acid	135.49(11.3)	102.73(11.6)	134.26(10.9)	163.43(10.8)
Proline	58.32(4.9)	37.54(4.2)	54.88(4.5)	73.54(4.9)
Glycine	99.68(8.3)	93.26(10.5)	74.91(6.1)	86.38(5.7)
Alanine	73.40(6.1)	57.65(6.5)	53.52(4.4)	69.26(4.6)
Cystein	13.28(1.1)	3.59(0.4)	22.85(1.9)	25.05(1.7)
Tyrosine	12.78(1.1)	17.88(2.0)	38.22(3.1)	48.62(3.2)
Total amino acid	1,199.81(100)	888.46(100)	1,227.31(100)	1,509.05(100)
Total NE	662.88	513.85	664.45	802.38
E/NE ratio	0.81	0.73	0.85	0.88

( ): percentage of each amino acid over the total amino acid.

Although total amino acid levels increased seasonally, the concentrations of individual amino acids were relatively stable throughout the study period. However, glycine content decreased in samples from August and November to 74.91 mg/100 g (6.1%) and 86.38 mg/100 g (5.7%), respectively, compared to 93.26 mg/100 g (10.5%) in May. By February, glycine levels slightly rose to 99.68 mg/100 g (8.3%). Seasonal changes in the content of other amino acids were not significant.

The analysis of nutritionally important essential amino acids in sea urchins showed concentrations in May, August, November, and February of the following year of 374.61 mg/100 g (42.0%), 562.85 mg/100 g (45.8%), 706.66 mg/100 g (46.8%), and 536.95 mg/100 g (44.8%), respectively. These findings underscore that *H. pulcherrimus* roe is a nutritionally dense

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food, containing 42–47% essential amino acids.

Free amino acids

Changes in the content of free amino acids of the sea urchin roe according to season are shown in Table 6.

Table 6: Seasonal changes of free amino acid content of sea urchin roe collected from Gijang in Korea.

Free amino acids (mg/100g)	February	May	August	November
Phosphoserine	5.50(0.27)	18.30(1.37)	2.80(0.09)	4.09(0.12)
Taurine	72.34(3.55)	63.63(4.77)	62.95(1.96)	65.61(1.89)
Phosphoethanolamine	0	0	3.81(0.12)	0
Aspartic Acid	5.26(0.26)	10.57(0.79)	13.90(0.43)	7.24(0.21)
Hydroxyproline	0.02(0)	3.15(0.24)	0	0
Threonine	38.13(1.87)	16.34(1.23)	51.55(1.61)	71.26(2.05)
Serine	41.42(2.03)	22.86(1.72)	49.28(1.53)	73.58(2.12)
Glutamic Acid	91.19(4.47)	64.95(4.87)	131.17(4.08)	97.96(2.82)
Sarosine	0	0	0.24(0.01)	0
α-Aminoadipic	12.03(0.59)	3.13(0.24)	15.29(0.48)	13.04(0.38)
Proline	0.65(0.03)	41.45(3.11)	77.05(2.40)	60.30(1.74)
Glycine	830.05(40.69)	694.71(52.12)	665.71(20.73)	667.43(19.22)
Alanine	191.522(9.39)	156.36(11.73)	187.09(5.83)	232.37(6.69)
Citrulline	2.91(0.14)	6.19(0.46)	38.50(1.20)	9.25(0.27)
α-Aminoisobutyric Acid	3.45(1.17)	9.92(0.74)	8.58(0.27)	2.80(0.08)
Valine	7.75(0.38)	5.10(0.38)	6.32(0.20)	8.37(0.24)
Cystine	0	0.68(0.05)	0	0
Methionine	12.20(0.60)	8.53(0.64)	45.95(1.43)	80.80(2.33)
Cystathionine	12.37(0.61)	3.03(0.23)	56.09(1.75)	36.99(1.06)
Isoleucine	39.10(1.92)	23.09(1.73)	87.29(2.72)	87.80(2.53)
Leucine	23.49(1.15)	13.87(1.04)	111.7(3.48)	122.52(3.53)
Tyrosine	33.88(1.66)	17.56(1.32)	202.81(6.32)	168.11(4.84)
β-Alanine	3.45(0.17)	1.61(0.12)	2.35(0.07)	1.99(0.06)
Phenylalanine	19.96(0.98)	13.40(1.01)	47.54(1.48)	55.13(1.59)
β-Aminoisobutyric Acid	1.16(0.06)	1.66(0.12)	5.78(0.18)	1.52(0.04)
r-Aminobutyric Acid	1.36(0.07)	0.72(0.05)	2.23(0.07)	3.04(0.09)
Ethanolamine	2.44(0.12)	7.86(0.59)	2.07(0.06)	0.93(0.03)
δ-Hydroxylysine	5.6(0.27)	0	0	0.92(0.03)
Ornithine	13.35(0.65)	3.32(0.25)	15.73(0.49)	20.09(0.54)
Lysine	220.23(10.08)	56.40(4.23)	462.55(14.40)	571.04(16.44)
Histidine	42.14(2.07)	10.60(0.80)	100.58(3.13)	169.91(4.89)
3-Methylhistidine	4.09(0.20)	0	7.58(0.24)	8.56(0.25)
Anserine	13.72(0.67)	0	30.78(0.965)	28.89(0.83)
Arginine	289.18(14.18)	53.80(4.04)	715.77(22.29)	801.81(23.08)
Total	2,039.94(100)	1,332.79(100)	3,211.04(100)	3,473.36(100)

( ): percentage of each amino acid over the total free amino acid.

The content was 1,332.79 mg/100 g in May, increasing to 3,473.36 mg/100 g by November—the peak value—before slightly decreasing to 2,039.94 mg/100 g in

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February of the following year. This analysis identified glutamic acid, taurine, glycine, tyrosine, lysine, arginine, and alanine as the predominant free amino acids in the roe. Notably, balanced levels of glutamic acid, glycine, alanine, and taurine are known to enhance sweetness, contribute to nutritional balance, and support metabolic functions. Specifically, the glycine content was highest in February at 830.05 mg/100 g (40.69%), decreasing to 694.71 mg/100 g (52.12%) in May, and slightly lower in August and November, at 665.71 mg/100 g (20.73%) and 667.43 mg/100 g (19.22%), respectively. Glutamic acid levels started at 64.95 mg/100 g (4.87%) in May, rose to 131.17 mg/100 g (4.08%) in August, then decreased to 97.96 mg/100 g (2.82%) in November and 91.19 mg/100 g (4.47%) in February. These findings indicate significant seasonal fluctuations, with the highest concentrations of free amino acids typically occurring from November to February of the following year.

Vitamin contents

The ascorbic acid content in the sea urchin roe varied seasonally, starting at 30.90 mg/kg in May and increasing to 35.21 mg/kg in August, 133.14 mg/kg in November, and reaching a peak of 188.07 mg/kg in February of the following year. Vitamin B1 (thiamin) was detectable only in the cooler months, recording 1.41 mg/kg in November and 4.09 mg/kg in February, while it was undetectable in samples from May and August. The concentration of Vitamin B2 (riboflavin) decreased over time, starting at 7.24 mg/kg in May and dropping to 0.17 mg/kg by the following February. Vitamin B6 (pyridoxine) levels were highest in the warmer months, at 244.51 mg/kg in May and 567.99 mg/kg in August, then decreased to 49.94 mg/kg in November, and were undetectable by February. Vitamin B3 content also showed a declining trend across the seasons, from 232.32 mg/kg in May to 88.21 mg/kg in February. Vitamin B12 (cyanocobalamin) followed a similar pattern, with an initial content of 26.47 mg/kg in May, reducing to undetectable levels by the following February. Folic acid content varied, starting at 5.75 mg/kg in May, decreasing to 4.49 mg/kg in August, increasing significantly to 56.01 mg/kg in November, and peaking at 106.37 mg/kg in February (Table 7).

Table 7: Seasonal changes of vitamin content of sea urchin roe collected from Gijang in Korea.

Vitamins	February	May	August	November
Ascorbic acid (mg/kg)	188.07	30.90	35.21	133.14
Thiamine (mg/kg)	4.09	0	0	1.41
Riboflavin (mg/kg)	0.17	7.24	0.53	0.33
Pyridoxine (mg/kg)	0	244.51	567.99	49.94
Cyanocobalamine (mg/kg)	0	26.47	0.53	0.90
Niacin (mg/kg)	88.21	232.32	181.71	127.89
Folic acid (mg/kg)	106.37	5.74	4.49	56.01
Retinol (mg/100g)	0.63	0.07	0.25	0.35
Tocopherol (mg/100g)	2.22	0.56	1.62	1.97

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This study revealed that the highest levels of vitamins in the B group generally occurred during the spawning period in May and subsequently decreased. However, the contents of ascorbic acid and folic acid notably increased from November, peaking in February. Similar patterns were observed in retinol content, with increments from 0.07 mg/kg in May to 0.63 mg/kg by the following February, and in tocopherol content, which increased from 0.56 mg/100 g in May to 2.22 mg/100 g in February of the following year.

Discussion

The spawning season for *H. pulcherrimus* typically occurs between April and May. Consistent with this, the roe index was lowest during these months (Ohgaki *et al.*, 2019). In our study, sea urchins collected in May also showed the lowest crude lipid content, suggesting an increase in water content during the spawning season as lipid levels decreased. Comparatively, the crude lipid and protein content in the purple sea urchin *Paracentrotus lividus* from the western Mediterranean Sea ranged from 1.87–2.17 g/100 g and 10.6–12.2 g/100 g, respectively (Pais *et al.*, 2011). For *Tripneustes gratilla* in Taiwan, the highest crude lipid content was recorded in March (3.8±0.2%) and the lowest in December (1.8±0.1%) (Chen *et al.*, 2013), indicating that the maturation stage for this species spans from May to August, with roe color reaching optimal acceptability during this period. Typically, moisture and crude lipid contents are inversely proportional; our findings (Shim *et al.*, 2017) showed significant seasonal variations in these components. Furthermore, the crude protein content in this sea urchin was notably lower, and the ash content higher, compared to fish fillet, which typically contains 16–21% crude protein, 0.2–25% crude lipid, 1.2–1.5% ash, and 66–81% moisture.

Minerals are essential for a healthy immune system and normal reproductive processes. Our study found no significant seasonal variations in the macroelements magnesium (Mg) and phosphorus (P), or the microelements iron (Fe), copper (Cu), and zinc (Zn) in sea urchin roe. However, the samples collected in February showed the highest overall mineral content, with a notable increase in calcium (Ca) content during this period.

Previous research indicates that the Ca content in sea urchins surges following fertilization and then stabilizes (Vasilev *et al.*, 2019). Typically, the egg and sperm of sea urchins combine within 30 seconds of fertilization, followed by a rapid production of calcium ions (Ca^{2+}) from the endoplasmic reticulum in the egg protoplasm. Stein *et al.* (2020) discovered that cortical granules at the edge of the protoplasm burst due to the influx of these ions. On the other hand, the Mg content has been shown to decrease slightly post-fertilization (Vacquier, 2011), although our data indicated a slight increase in May compared to November. These changes in Ca and Mg levels are likely closely linked to fertilization events.

It is recommended that individuals consume 0.6-1.0 g of omega fatty acids daily to support normal tissue development, particularly in newborns and teenagers. Deficiencies in omega fatty acids can exacerbate stress and may lead to

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depression, schizophrenia, attention deficit disorder, hyperactivity disorder, amblyopia, and heart disease. The body does not produce n-3 fatty acids; therefore, they must be obtained from dietary sources. This study discovered a significantly high concentration of omega-3 fatty acids in *H. pulcherrimus*, exceeding 25%, while n-6 fatty acids constituted about 10%. Additionally, the ratio of n-3 to n-6 in this sea urchin ranged from 1.86 to 3.16, indicating exceptionally high levels of both n-3 and the n-3/n-6 ratio. The physiological importance of n-3 and n-6 fatty acids underscores their critical role as essential nutrients.

The ideal ratio of essential amino acids to nonessential amino acids in marine products and eggs is suggested to be 0.74 (Iwasaki and Harada, 1985; Mol *et al.*, 2008). The sea urchin species *H. pulcherrimus* analyzed in this study could be a valuable protein source, with the ratio of essential to nonessential amino acids ranging from 0.73 to 0.88.

The composition of free amino acids (FAA) in sea urchin roe varies depending on the species, diet, and environmental conditions. FAA levels are particularly influenced by factors such as seasonality and diet (Sætra *et al.*, 2022). In the sea urchin *Anthocidaris crassispina*, glycine levels were higher in individuals fed an artificial diet compared to those in the wild, while the levels of several amino acids, including valine, methionine, lysine, and arginine, also increased. However, the levels of aspartic acid, glutamic acid, proline, and alanine showed no significant changes (Volpe *et al.*, 2018). During the maturation phase, glycine content increased, whereas valine, alanine, histidine, lysine, and arginine decreased (Chen *et al.*, 2013). Total amino acid and free amino acid content were lowest during the spawning season in May compared to other seasons. Additionally, the levels of valine, methionine, lysine, glutamic acid, alanine, and arginine peaked in November, while glycine content was highest in February during the maturation stage.

Sea urchin roe collected during the spawning season in May displayed lower levels of ascorbic acid (30.9 mg/kg) and folic acid, but higher levels of riboflavin (7.24 mg/kg) and niacin (232.32 mg/kg) compared to samples from other seasons. For context, the ascorbic acid content in the roe of sea urchins from northwestern Spain (Western Mediterranean) averaged 26.57 mg/100 g, while the canned product contained 14.25 mg/100 g (Rodriguez-Bernaldo de Quiros *et al*., 2001).

Vitamin E levels in the roe were also lowest in May (0.05 mg/100 g), contrasting with higher levels found in other seasons (1.62–2.22 mg/100 g). Notably, female fish demonstrated higher vitamin E content in their roe but lower levels in their serum and liver compared to male fish. Palace and Werner (2006) additionally observed that dietary tocopherol supplements promoted multiple spawning events in fish.

Overall, the roe of *H. pulcherrimus* is a valuable source of high-quality protein, minerals, vitamins, and has an acceptable glycine content, which enhances the flavor. However, these biochemical components undergo significant variations during the spawning season.

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Conflicts of Interest

The author declares no conflicts of interest.

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