Nutritional Composition Changes in Skate (Raja kenojei) during Different Ripening Periods

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Abstract

In order to suggest fundamental data on the nutritional composition of the Korean traditional food, ripened skate, this study investigated the proximate composition, organic acid, free sugar, fatty acid, total amino acid, and macromolecule levels of skates at different ripening periods. Longer ripening periods resulted in decreased moisture levels and increased protein and ash levels. The total organic acid content increased until the 15th day of ripening, but decreased at 20 days. Phosphoric acid, oxalic acid, and lactic acid were the dominant organic acids. Eight free sugars were detected, ribose, glucose, and sucrose being the dominant forms, which significantly decreased with ripening. Palmitic and linoleic acid were abundant in saturated fatty acids, oleic acid and palmitoleic acid were abundant in monounsaturated fatty acids, and linolenic acid, docosahexaenoic acid, and arachidonic acid were abundant in polyunsaturated fatty acids. The major amino acids were glutamic acid, lysine, aspartic acid, and leucine and the total levels increased with ripening. The most abundant free amino acids were urea, sarcosine, taurine, and β-alanine, which decreased with ripening. Phosphoserine, phosphoethanolamine, α-aminobutyric acid, and cysteine levels increased.

Keywords: Ripening period; Raja kenojei; Organic acid; Free sugar; Fatty acid; Amino acid

Introduction

Skate (Raja kenojei) is a popular Korean cuisine. In the southwestern part of Korea, the preparation of a traditional dish for skate involves allowing the skate to ripen in a ceramic jar without any additives for over 1 week at room temperature, in contrast to products salted and fermented with rice-bran or rice such as Heshiko and Funazushi in Japan [1,2] and to salted product, Jeotgal in Korea [3]. This preparation is preferred because it results in an elastic texture of the dermal fin rays and a unique ammonia-like flavor derived from the large quantity of trimethylamine oxide and/or urea present in the preparation [4-6]. It is not toxic to humans, provided it is kept at the recommended temperature and humidity. According to experienced Korean cooks, the skate hardens during ripening; however, this seems to contradict general knowledge pertaining to raw fish storage, which indicates that softening occurs after only 1 day in chilled storage [7-9]. Both the shark and skate are elasmobranch fish and shark type II collagen has been reported to be an effective treatment against chronic arthritis [10]. As such, there is also a high possibility that ripened skate has the same therapeutic effects as type II collagen. Many studies have been performed on skates with respect to their classification [11], physicochemical changes [12,13], cholesterol and fatty acids [14], muscle, skin, and cartilage collagens [15-19]. Despite the interest in ripened skate, only a few studies have been published on this topic in some Korean local papers. To our knowledge, this is the first study to evaluate the differences in the nutritional composition of skate (Raja kenojei) during ripening periods.

Methods

Sample preparation

Skates (R. kenojei) were purchased from a market (Mokpo, Jeonnam, Korea) and divided into 5 groups (1.5 kg/group), specifically, 1 control and 4 different ripening groups (0, 5, 10, 15, and 20 days). The samples for each ripening group were sealed in 4 different ceramic jars and stored in a cold room at 8°C. The jars were selected randomly at 5 d intervals and the edible parts were separated from the body of the skate. The minced samples were divided into 3 groups (500 g/group) and stored at -80°C until use.

Proximate analysis

For proximate analysis [20] of the skate samples, the samples were dried in an oven, ground, and mixed for analysis in triplicate. The amount of crude protein was determined using the Kjeldahl method (Kjtech auto sampler system 1,035 analyzer; Foss Co., Hillerød, Denmark) and the crude lipid content was determined using the ether-extraction method (Soxtec 2050 auto extraction unit; Foss Co.). The moisture level was determined using an auto moisture system (HR 73; Mettler Toledo Co., Greifensee, Switzerland) and the ash content was determined using a muffle furnace (TMF-3100; EYELEA Co., Tokyo, Japan) at 550°C for 4 h.

Organic acid and free sugar analyses

The standard organic acids and free sugars were prepared with a 1 mg/mL stock solution and quantified by diluting the stock solution. Skate samples (5 g) were homogenized in a tissue grinder (IKA, Germany) with 20 mL of 80% ethanol, and then reflux extracted in a water bath for 3 h by adding 80 mL of 80% ethanol in a 250 mL flask. The suspensions were centrifuged at 4000 ×g for 30 min, and filtered using...
Whatman No. 1 filter papers (Whatman, NJ, USA). The filtered extracts were concentrated to approximately 1 mL by using a rotary evaporator (CCA 1110; Eyela Co. Ltd., Japan) and resuspended with a starting buffer to obtain a volume of 10 mL. The solution (3 mL) was then filtered with a 0.2-μm membrane and analyzed using high performance liquid chromatography (HPLC) (Prominence HPLC, Shimadzu Co. Ltd., Kyoto, Japan) for analyzes of organic acid and free sugar.

Measurement of free sugar content was carried out using HPLC. The HPLC equipment consisted of a LC-20AD HPLC pump, a Sil-20AC auto-sampler, a CTO-20AC oven, and a CBM-20A system controller (Shimadzu Co. Ltd., Kyoto, Japan). The columns used for analysis were an ion exchange Shim-pack ISA-07 analytical column (4.0 mm × 250 mm) and a Shim-pack ISA guard column (4.0 mm × 50 mm). The HPLC was operated at an oven temperature of 65°C with 0.6 mL/min flow of solution A (potassium borate, pH 8.0) and solution B (potassium borate, pH 9.0) for 90 min as the linear gradient elution method. 20 μL of hydrolysate filtered by membrane filter (0.45 μm) was injected. After separation of the saccharides by the column, the arginine/boric acid reagent solution is continuously added to the column eluent to convert the saccharides to fluorescent derivatives for detection [21]. The detector used was the RF-10Axl fluorescence detector set at 320 nm (Ex) and 430 nm (Em). The content of the total free sugars was calculated based on a standard curve while reducing the sugar standard material.

Measurement of the organic acid content was carried out using HPLC. The HPLC equipment consisted of a LC-20AD HPLC pump, a Sil-20AC auto-sampler, a CTO-20AC oven, and a CBM-20A system controller (Shimadzu Co. Ltd., Kyoto, Japan). The columns used for analysis were tandem ion exchange Shim-pack SPR-10H analytical columns (7.8 mm × 250 mm) and a Shim-pack SPR-H guard column (7.8 mm × 50 mm). The HPLC was operated at an oven temperature of 40°C with 0.8 mL/min flow of 4 mM p-toluenesulfonic acid as the post-column method. 10 μL of hydrolysate filtered by membrane filter (0.2 μm) was injected. 16 mM Bis-Tris aqueous solution containing 4 mM p-toluenesulfonic acid and 100 μM EDTA was used for the reactive reagent. The detector used was a conductive detector. The content of the total organic acids was calculated based on a standard curve with an organic acid standard material.

Analysis of fatty acids

Bligh and Dyer extraction was performed using the following method [22]: Briefly, lipids were extracted from 50 g samples by homogenization with 400 mL chloroform and 200 mL methanol. The samples were then filtered and evaporated to remove the solvent. Fatty acid methyl esters (FAMEs) were prepared using boron trifluoride (BF3) according to a method described by the association of official analytical chemists (AOAC) [20]. Quantitative analysis of FAME was carried out on a GC-2010 gas chromatography system (Shimadzu Co. Ltd.) equipped with a split/splitless capillary inlet system and a flame ionization detector (FID) using SP-2560 capillary columns (stationary phase thickness, 0.2 μm; 100 mm (length) × 0.25 mm (i.d.); Supelco, Inc., USA). The sample (0.5 μL) was injected in the split mode using an automatic injection system (AOC-20i; Shimadzu Co. Ltd.). The oven temperature was programmed to increase from 150 to 250°C at 3°C/min with initial and final holds of 5 min. The other operation parameters were as follows: injector temperature=270°C, detector temperature=250°C, helium carrier gas flow=18 cm/s, and split ratio=1:50. The peak areas for the calibration curves and for calculation of the fatty acid composition of oil samples were measured using a GC Solution system (Shimadzu Co. Ltd.).

Total amino acid analysis

Samples (0.5 g) were hydrolyzed with 15 mL of 6 N HCl in vacuum-sealed hydrolysis vials at 110°C for 24 h. The vials were cooled and placed in a rotary evaporator at 45°C to remove HCl from the sample. The residue was then adjusted to pH 2.2 with a 0.2 M sodium citrate loading buffer (pH 2.2), diluted to a final volume of 25 mL with water, and filtered through a 0.45-μm Millipore membrane. The filtrate was analyzed using an amino acid analyzer (Shimadzu Co. Ltd.) equipped with a Shim-pack AMINO-Na column (6.0 × 100 mm). The buffer flow rate and OPA reagent flow rate were set to 0.6 mL/min and 0.3 mL/min, respectively. Fluorescent detection was achieved at 350 nm for excitation and 450 nm for emission.

Free amino acid analysis

Samples (5 g) were homogenized in a tissue grinder with 30 mL of distilled water and 0.2 g of sodium citrate. After 1 h of incubation at room temperature, the samples were centrifuged at 1000 × g for 30 min and the pH of the supernatant was adjusted to 2.2 with 0.2 M lithium citrate loading buffer (pH 2.2). The samples were diluted to a final volume of 25 mL with water and filtered through 0.45 μm Millipore membranes. The filtrate was analyzed with an amino acid analyzer (Shimadzu Co. Ltd.) equipped with a Shim-pack AMINO-Li column (6.0 × 100 mm). The buffer flow rate and OPA reagent flow rate were set to 0.6 mL/min and 0.3 mL/min, respectively. Fluorescent detection was achieved at 350 nm for excitation and 450 nm for emission.

Statistical analysis

All mean values were analyzed via one-way analysis of variance (ANOVA). When differences were found among data, Duncan’s multiple range test was used to compare the mean difference by using the statistical package for social sciences (SPSS) software package version 17 (SPSS Inc., Chicago, IL, USA). Differences were considered significant at p<0.05.

Results and Discussion

Chemical composition

The proximate compositions with different ripening periods are shown in Table 1. Moisture content gradually decreased with ripening and significantly decreased at the 20th ripening day (p<0.05). Crude protein content significantly increased with ripening (p<0.05). These results were obtained due to bacteria or mold that produce small proteins and peptides. Little change could be observed in the crude lipid content; however, the ash content significantly increased with ripening (p<0.05). Carbohydrate content significantly decreased at ripening day 5, but carbohydrates were not detected after ripening day 10. The present study provides evidence that proximate analysis results vary during ripening.

Organic acid composition

Organic acid analyses with different ripening periods are shown in Table 2. The total organic acid level significantly increased on ripening days 10 (515.9 mg/100 g) and 15 (569.1 mg/100 g), but significantly decreased on ripening day 20 (435.1 mg/100 g) compared to that for
the control (p<0.05). The oxalic acid content gradually increased until ripening day 15, but significantly decreased at ripening day 20 (p<0.05). The organic acid content of fish is known to be affected by the fishing method, amount of time since death, conditions after death, and prompt killing methods [23-25]. The lactic acid content of fish is low when they are promptly killed but is high after stressful or hard exercise [24]. Lactic acid was predominant, comprising approximately 90% of the total organic acid content in mackerel, pacific saury, yellow croaker, brown sole, wild and cultured red sea bream, rockfish, and flounder [24]. Lactic acid was not detected.

## Free sugar composition

Free sugar analyses with different ripening periods are shown in Table 3. Among the 8 free sugars detected, the content of sucrose and maltose significantly decreased on ripening day 5 (p<0.05) and these sugars were not detected after ripening day 10. Lactose and mannose content significantly decreased until ripening day 10 (p<0.05), and these sugars were not detected after ripening day 15. Ribose content was significantly decreased with ripening until ripening day 15 (p<0.05), but no significant difference was observed between the content on ripening days 15 and 20. The galactose content significantly decreased by ripening day 5 (p<0.05) but the galactose content on ripening day 10 and 15 was lower than that for the control, significantly higher than that on ripening day 5 (p<0.05), and was not detected after ripening day 20. Xylose was detected in the control but not in the ripening samples. The glucose content was the highest in the control but significantly decreased until ripening day 15 and was not detected at ripening day 20. Glucose and ribose are the dominant free sugars in fish. Glucose is present in high concentrations in the muscle of live fish; glycogen decomposes after death, thereby increasing the glucose content [26]. Moreover, glucose levels in fish have been reported to change with the amount of time since death and conditions after death [27].

### Table 1: Proximate composition of skate during different ripening periods (%).

| Ripening day | 0 | 5 | 10 | 15 | 20 |
|-------------|---|---|----|----|----|
| Moisture    | 81.51 ± 2.40a | 80.45 ± 1.92ab | 79.33 ± 1.53ab | 78.69 ± 2.25ab | 77.25 ± 1.76a |
| Crude protein | 15.36 ± 0.34a | 16.44 ± 0.33ab | 17.51 ± 0.37bc | 17.94 ± 0.57bc | 19.22 ± 0.38c |
| Crude lipid | 0.53 ± 0.01a | 0.52 ± 0.01b | 0.54 ± 0.01b | 0.55 ± 0.01b | 0.55 ± 0.01b |
| Ash | 2.44 ± 0.06a | 2.47 ± 0.08a | 2.62 ± 0.06b | 2.62 ± 0.06b | 2.98 ± 0.08b |
| Carbohydrate | 0.18 ± 0.00a | 0.12 ± 0.00b | - | - | - |

1) Values are means ± SD (n = 3). Values with different superscripts in the same row are significantly different at P<0.05 by Duncan’s multiple range test.
2) Carbohydrate = 100 - (moisture + crude protein + crude lipid + ash).
3) Not detected.

### Table 2: Organic acid contents of skate during different ripening periods (mg/100 g).

| Ripening day | 0 | 5 | 10 | 15 | 20 |
|-------------|---|---|----|----|----|
| Oxalic acid | 142.99 ± 3.26ab | 209.43 ± 5.59c | 250.10 ± 5.17b | 285.90 ± 6.75a | 2.21c |
| Phosphoric acid | 273.96 ± 5.39a | 161.63 ± 3.69c | 156.30 ± 4.37c | 181.89 ± 4.79b | 1.90b |
| Citric acid | 1.81 ± 0.04a | - | - | - | - |
| Tartaric acid | 0.72 ± 0.02a | 3.65 ± 0.08a | 8.50 ± 0.26a | 7.45 ± 0.18b | 0.62a |
| Malic acid | 2.77 ± 0.06a | 1.24 ± 0.03a | 0.84 ± 0.02a | 0.17 ± 0.00b | 0.00a |
| Succinic acid | 4.50 ± 0.13a | 8.50 ± 0.26a | 5.21 ± 0.12a | 6.36 ± 0.14a | 0.25d |
| Lactic acid | 59.53 ± 1.37b | 80.29 ± 2.30a | 74.09 ± 1.58b | 25.74 ± 0.77a | 0.07a |
| Formic acid | 0.26 ± 0.01b | 1.72 ± 0.04b | 3.28 ± 0.08b | 5.94 ± 0.12b | 0.58c |
| Acetic acid | 0.76 ± 0.02b | 7.77 ± 0.09a | 16.19 ± 0.32b | 22.19 ± 0.60b | 0.29b |
| Total | 488.20 ± 12.23b | 476.73 ± 9.29b | 515.86 ± 13.55b | 569.10 ± 11.73a | 6.14d |

1) Values are means ± SD (n = 3). Values with different superscripts in the same row are significantly different at P<0.05 by Duncan’s multiple range test.
2) Not detected.

### Table 3: Free sugar contents of skate during different ripening periods (mg/100 g).

| Ripening | 0 | 5 | 10 | 15 | 20 |
|----------|---|---|----|----|----|
| Sucrose | 4.21 ± 0.12a1 | 0.19 ± 0.00b | - | - | - |
| Maltose | 0.67 ± 0.01a | 0.09 ± 0.00b | - | - | - |
| Lactose | 0.76 ± 0.02a | 0.54 ± 0.01b | 0.18 ± 0.00c | - | - |
| Ribose | 11.25 ± 0.28a | 8.56 ± 0.27b | 4.02 ± 0.10c | 0.16 ± 0.00d | 0.11 ± 0.00d |
| Mannose | 0.37 ± 0.01a | 0.09 ± 0.00b | 0.07 ± 0.00c | - | - |
| Galactose | 0.21 ± 0.00a | 0.05 ± 0.00b | 0.06 ± 0.00b | - | - |
| Xylose | 0.17 ± 0.00a | - | - | - | - |
| Glucose | 6.97 ± 0.15a | 0.83 ± 0.02b | 0.6 ± 0.02c | 0.09 ± 0.00d | 0.11 ± 0.00d |
| Total | 24.61 ± 0.52a | 10.35 ± 0.25b | 4.93 ± 0.16c | 0.31 ± 0.01d | 0.11 ± 0.00d |

1) Values are means ± SD (n = 3). Values with different superscripts in the same row are significantly different at P<0.05 by Duncan’s multiple range test.
2) Not detected.
Values are means ± SD (n = 3). Values with different superscripts in the same row are significantly different at P<0.05 by Duncan’s multiple range test.

### Sugars

| Sugars       | 0                      | 5                      | 10                     | 15                     | 20                     |
|--------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Ribose       | 9.57 ± 0.19            | 9.24 ± 0.21            | 11.04 ± 0.31           | 10.83 ± 0.29           | 11.18 ± 0.12           |
| Glucose      | 8.29 ± 0.07            | 7.52 ± 0.11            | 9.23 ± 0.16            | 9.04 ± 0.13            | 9.51 ± 0.13            |
| Sucrose      | 1.42 ± 0.03            | 1.43 ± 0.04            | 1.28 ± 0.02            | 1.26 ± 0.02            | 1.25 ± 0.02            |

### Fatty acid composition

#### Saturated

| Fatty Acid | 0                        | 5                        | 10                       | 15                        | 20                        |
|------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Palmitic   | 13.53 ± 0.26             | 13.64 ± 0.27             | 13.75 ± 0.28             | 13.86 ± 0.30             | 13.97 ± 0.32             |
| Stearic    | 6.88 ± 0.16              | 6.75 ± 0.17              | 6.62 ± 0.16              | 6.50 ± 0.15              | 6.38 ± 0.14              |
| Lignoceric | 0.41 ± 0.01              | 0.49 ± 0.01              | 0.31 ± 0.01              | 0.33 ± 0.01              | 0.32 ± 0.00              |

### Mono-unsaturated

| Fatty Acid | 0                        | 5                        | 10                       | 15                        | 20                        |
|------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Myristic   | 11.71 ± 0.29             | 11.84 ± 0.30             | 12.03 ± 0.31             | 12.22 ± 0.33             | 12.41 ± 0.35             |
| Palmitoleic| 0.31 ± 0.01              | 0.35 ± 0.01              | 0.40 ± 0.01              | 0.44 ± 0.01              | 0.48 ± 0.02              |
| Oleic      | 8.02 ± 0.04              | 8.25 ± 0.06              | 8.49 ± 0.10              | 8.73 ± 0.12              | 8.97 ± 0.14              |

### Di-unsaturated

| Fatty Acid | 0                        | 5                        | 10                       | 15                        | 20                        |
|------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Myristoleic| 0.02 ± 0.00              | 0.02 ± 0.00              | 0.03 ± 0.00              | 0.03 ± 0.00              | 0.03 ± 0.00              |
| Palmitoleic| 0.01 ± 0.00              | 0.01 ± 0.00              | 0.01 ± 0.00              | 0.01 ± 0.00              | 0.01 ± 0.00              |
| Myristoleic| 0.21 ± 0.00              | 0.27 ± 0.01              | 0.21 ± 0.00              | 0.26 ± 0.01              | 0.26 ± 0.01              |

### Poly-unsaturated

| Fatty Acid | 0                        | 5                        | 10                       | 15                        | 20                        |
|------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Stearic    | 39.69 ± 0.52             | 39.48 ± 1.24             | 39.53 ± 0.91             | 38.77 ± 0.93             | 38.20 ± 0.76             |
| Arachidonic| 57.36 ± 1.44             | 55.92 ± 1.09             | 54.55 ± 1.43             | 53.50 ± 1.10             | 52.71 ± 0.74             |

Values are means ± SD (n = 3). Values with different superscripts in the same row are significantly different at P<0.05 by Duncan’s multiple range test.

### Amino acid composition

The amino acid composition for different ripening periods is summarized in Table 5. Unlike land animals, fish have high protein requirements owing to low utilization of carbohydrates as an energy source. Therefore, the protein content in the body is an important energy source for fish and is also nutritionally important for normal growth [28]. The skate was found to contain high amounts of aspartic acid, glutamic acid, leucine, and lysine. Most amino acid levels increased with ripening except for some fluctuations in glycine, cysteine, and valine levels. The level of glutamic acid was the highest, followed by oleic acid (C18:1) and palmitoleic acid (C16:1) were the dominant forms. No significant differences were observed at ripening day 5 for myristoleic acid (C14:1) and palmitoleic acid but the myristoleic acid level significantly increased and the palmitoleic acid level significantly decreased from ripening day 10 to 20 (p<0.05). Oleic acid and nervonic acid (C24:1) levels significantly increased at ripening day 5 (p<0.05), while the oleic acid level significantly decreased and the nervonic acid level significantly increased from ripening day 10 to 20 (p<0.05). Eicosenoic acid (C20:1) and erucic acid (C22:1) levels significantly decreased at ripening day 5, but significantly increased from ripening day 10 to 20 (p<0.05). Linoleic acid (C18:2) was the dominant form of di-unsaturated fatty acids. All di-unsaturated fatty acids significantly decreased with ripening (p<0.05). In the case of poly-unsaturated fatty acids, docosahexaenoic acid (C22:6) levels were the highest followed by ω3-linolenic acid (C18:3) and arachidonic acid (C20:4) levels. Poly-unsaturated fatty acid levels also significantly decreased with ripening (p<0.05).

### Table 4: Fatty acid composition of skate during different ripening periods (weight, %)

| Fatty Acid     | 0               | 5               | 10              | 15              | 20              |
|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Palmitic       | 13.53 ± 0.26    | 13.64 ± 0.27    | 13.75 ± 0.28    | 13.86 ± 0.30    | 13.97 ± 0.32    |
| Stearic        | 6.88 ± 0.16     | 6.75 ± 0.17     | 6.62 ± 0.16     | 6.50 ± 0.15     | 6.38 ± 0.14     |
| Lignoceric     | 0.41 ± 0.01     | 0.49 ± 0.01     | 0.31 ± 0.01     | 0.33 ± 0.01     | 0.32 ± 0.00     |

**Citation:** Hwang JH, Jung BM, Rha SJ, Kim JM, Kim SJ, et al. (2014) Nutritional Composition Changes in Skate (Raja kenojei) during Different Ripening Periods. J Nutr Food Sci 4: 295. doi: 10.4172/2155-9600.1000295
Table 5: Total amino acid contents of skate during different ripening periods (mg/100 g).

| Amino Acid                  | Ripening Periods | 0       | 5       | 10      | 15      | 20      |
|-----------------------------|------------------|---------|---------|---------|---------|---------|
| Phosphoserine               |                  | 0.76 ± 0.02a | 1.26 ± 0.03b | 0.97 ± 0.02c | 0.41 ± 0.01d | 0.81 ± 0.01c |
| Taurine                     |                  | 65.65 ± 1.46a | 61.00 ± 1.22b | 54.19 ± 1.15c | 41.35 ± 1.30d | 31.28 ± 0.62c |
| Phosphoethanolamine         |                  | 0.83 ± 0.01a | 3.70 ± 0.06b | 4.79 ± 0.06c | 4.92 ± 0.11d | 4.67 ± 0.12c |
| Urea                        |                  | 1041.08 ± 26.11a | 237.46 ± 7.50b | 31.64 ± 0.76c | 22.34 ± 0.51d | 19.63 ± 0.52c |
| Aspartic acid               |                  | 12.85 ± 0.36a | 9.12 ± 0.18b | 7.19 ± 0.21c | 5.09 ± 0.11d | 1.34 ± 0.03c |
| Threonine                   |                  | 24.12 ± 0.50a | 11.31 ± 0.28b | 3.61 ± 0.10c | 0.46 ± 0.01d | 0.39 ± 0.01c |
| Serine                      |                  | 31.99 ± 0.85a | 22.19 ± 0.62b | 11.82 ± 0.26c | 2.78 ± 0.06d | 0.56 ± 0.01c |
| Asparagine                  |                  | 0.60 ± 0.01a | -       | -       | -       | -       |
| Glutamic acid               |                  | 11.35 ± 0.24a | 9.52 ± 0.23b | 3.48 ± 0.11c | 1.48 ± 0.03d | 1.69 ± 0.03c |
| Sarcosine                   |                  | 230.44 ± 7.33a | 195.60 ± 4.53b | 164.14 ± 3.76c | 168.69 ± 3.65d | 154.09 ± 3.86c |
| 7-aminoadipic acid          |                  | 3.28 ± 0.07a | 3.80 ± 0.09b | 3.17 ± 0.10c | 2.64 ± 0.06d | 1.79 ± 0.04c |
| Proline                     |                  | 17.23 ± 0.42a | 9.44 ± 0.19b | 10.75 ± 0.28c | 9.23 ± 0.21d | 7.88 ± 0.15c |
| Glycine                     |                  | 27.89 ± 0.64a | 24.32 ± 0.65b | 24.16 ± 0.50c | 22.78 ± 0.54d | 19.59 ± 0.22c |
| Alanine                     |                  | 23.73 ± 0.47a | 27.49 ± 0.57b | 31.68 ± 0.89c | 31.51 ± 0.83d | 20.22 ± 0.21c |
| Valine                      |                  | 3.20 ± 0.07a | 3.64 ± 0.10b | 3.09 ± 0.08c | 3.44 ± 0.04d | 2.82 ± 0.06c |
| Citrulline                  |                  | 0.30 ± 0.01a | 0.31 ± 0.01b | 0.36 ± 0.01c | 0.42 ± 0.01d | 0.44 ± 0.01c |
| Histidine                   |                  | 5.65 ± 0.12a | 4.78 ± 0.11b | 5.56 ± 0.12c | 5.59 ± 0.13b | 3.50 ± 0.10c |
| Cystine                     |                  | 0.00 ± 0.00a | 0.43 ± 0.01b | 0.34 ± 0.01c | 0.67 ± 0.01d | 0.90 ± 0.03c |
| Methionine                  |                  | 4.29 ± 0.10a | 2.53 ± 0.07b | 2.32 ± 0.05c | 1.02 ± 0.03d | 0.25 ± 0.01c |
| Isoleucine                  |                  | 3.47 ± 0.08a | 2.27 ± 0.05b | 2.33 ± 0.05c | 1.56 ± 0.03d | 0.59 ± 0.02c |
| Leucine                     |                  | 6.41 ± 0.15a | 4.38 ± 0.05b | 4.42 ± 0.09c | 3.67 ± 0.10d | 1.19 ± 0.03c |
| Tyrosine                    |                  | 3.02 ± 0.08a | 2.33 ± 0.05b | 2.87 ± 0.08c | 1.69 ± 0.03d | -       |
| Phenylalanine               |                  | 3.44 ± 0.08a | 3.40 ± 0.06b | 3.29 ± 0.07c | 2.97 ± 0.05d | -       |
| ?-Alanine                   |                  | 32.62 ± 0.62a | 26.71 ± 0.56b | 21.46 ± 0.36c | 22.20 ± 0.47d | 20.96 ± 0.38c |
| ?-Amino-N-butyric acid      |                  | 0.22 ± 0.00a | 0.45 ± 0.04b | 0.44 ± 0.01c | 0.52 ± 0.01d | 0.10 ± 0.00c |
| Histidine                   |                  | 4.53 ± 0.05a | 3.36 ± 0.11b | 3.42 ± 0.10c | 3.15 ± 0.07d | 1.28 ± 0.02c |
| 1-methylhistidine           |                  | 0.21 ± 0.00a | -       | -       | -       | -       |
| Carnosine                   |                  | 0.68 ± 0.02a | 0.72 ± 0.05b | 0.36 ± 0.01c | 0.26 ± 0.01d | 0.25 ± 0.01c |
| Anserine                    |                  | 0.14 ± 0.00a | 0.11 ± 0.06b | 0.18 ± 0.00c | 0.11 ± 0.00d | 0.05 ± 0.00c |
| Ornithine                   |                  | 3.99 ± 0.12a | 4.53 ± 0.09b | 4.93 ± 0.14c | 4.10 ± 0.12d | 2.50 ± 0.05c |
| Lysine                      |                  | 11.96 ± 0.24a | 9.95 ± 0.23b | 9.27 ± 0.21c | 7.31 ± 0.15d | 2.99 ± 0.03c |
| Ethanolamine                |                  | 3.16 ± 0.07a | 2.52 ± 0.08b | 1.65 ± 0.04c | 0.76 ± 0.02d | 4.11 ± 0.08c |
| Arginine                    |                  | 3.23 ± 0.09a | 2.56 ± 0.06b | 2.14 ± 0.07c | 2.32 ± 0.07d | 1.52 ± 0.03c |
| Ammonia                     |                  | 53.28 ± 2.54a | 113.72 ± 22.12a | 1269.45 ± 27.32a | 1307.02 ± 20.22a | 1449.96 ± 33.59a |
| Total                       |                  | 1582.11 ± 34.50a | 688.49 ± 16.15a | 2402.04 ± 9.00a | 375.41 ± 8.42a | 307.50 ± 8.54a |

1) Values are means ± SD (n = 3). Values with different superscripts in the same row are significantly different at P<0.05 by Duncan's multiple range test.
2) Not detected.

Table 6: Free amino acid contents of skate during different ripening periods (mg/100 g).
those of lysine, aspartic acid, and leucine. No significant differences were observed on ripening day 5 for aspartic acid and glutamic acid, but the levels significantly increased from ripening day 10 to 20 (p<0.05). The threonine, serine, proline, alanine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, and arginine levels, as well as the total amino acid content, significantly increased with ripening (p<0.05). The glycine, valine, and cysteine levels also significantly increased with ripening (p<0.05) except on ripening days 5, 15, and 20, respectively. The levels of all the essential amino acids also increased with increase in the ripening period. Taken together, the total amino acid content increased to a level approximately 22% greater than that in the control over 20 ripening days.

**Free amino acid composition**

The contents of individual free amino acids with different ripening periods in skate are shown in Table 6. Free amino acids are important components of bioactive material and impart a characteristic taste to food. In fish, free amino acids are used as a chemical signal to influence behavior, communication, and physiological metabolism [29]. The major free amino acids found throughout the ripening period were urea, sarcosine, taurine, and β-alanine. Although the contents of some individual free amino acids in the skates fluctuated during the ripening period, the levels of most free amino acids, as well as total free amino acids, significantly decreased with ripening. However, the levels of phosphoethanolamine, α-amino butyric acid, and ammonia significantly increased with ripening (p<0.05). Asparagine and 1-methylhistidine were detected in the control, but not in the ripening groups. When compared to the control, the ripening groups showed more than 90% reduction in the urea, aspartic acid, threonine, serine, asparagine, methionine, tyrosine, phenylalanine, and 1-methylhistidine levels over the 20-day ripening period.

In conclusion, we analyzed the nutritional composition changes during different ripening periods. Total ripening increased protein and ash and total organic acid content with the later only were increasing until the 15th day. Moisture content generally reduced during ripening. Moreover, it was shown that ripening over a period of 20 days changed some individual free amino acids in the skates. The threonine, serine, proline, alanine, methionine, tyrosine, phenylalanine, and 1-methylhistidine levels over the 20-day ripening period. The levels significantly increased from ripening day 10 to 20 (p<0.05). The glycine, valine, and cysteine levels also significantly increased with ripening (p<0.05) except on ripening days 5, 15, and 20, respectively. The levels of all the essential amino acids also increased with increase in the ripening period. Taken together, the total amino acid content increased to a level approximately 22% greater than that in the control over 20 ripening days.

**Acknowledgments**

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0077476).

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