

Article

Correlation Between Sensory Characteristics and Physicochemical Properties of Wild and Farmed Frozen Southern Bluefin Tuna (*Thunnus maccoyii***)**

- 1 Graduate School of Marine Science and Technology, Tokai University, 3-20-1 Orido, Shimizu-ku, Shizuoka 424-8610, Japan
- 2 Department of Fisheries, Faculty of Oceanography, Tokai University, 3-20-1 Orido, Shimizu-ku, Shizuoka 424-8610, Japan
- ³ Department of Applied Life Science, Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Konan-ku, Tokyo 108-8477, Japan
- 4 Artificial Intelligence Laboratory, Fujitsu Limited, 4-1-1 Kamikodanaka, Nakahara-ku, Kawasaki 211-8588, Japan
- 5 Department of Food Science and Technology, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan
- ***** Correspondence: kgoto@tokai.ac.jp

Abstract: In this study, to investigate the quality of wild and farmed frozen southern bluefin tuna, physicochemical analyses and sensory evaluations were conducted. Principal component analysis was then performed using the results obtained to examine the correlation between the bluefin tuna's taste characteristics and physicochemical properties. The sensory evaluation suggested differences in texture and acidity between wild and farmed fish, whereas the principal component analysis indicated differences in fatty acid and amino acid composition. Wild fish contained higher levels of docosahexaenoic acid and monounsaturated fatty acids, while farmed fish had higher levels of saturated fatty acids. Regarding free amino acids and dipeptides, wild fish had higher levels of anserine and alanine, whereas farmed fish showed higher levels of glutamine and histidine, and acidity was observed in farmed fish. Furthermore, based on the results of the principal component analysis, it was inferred that the content of inosinic acid, which is considered an umami component in fish, may have a low impact on palatability. These factors were suggested to influence the differences between wild and farmed tuna.

Keywords: frozen southern bluefin tuna; principal component analysis; fatty acid composition; free amino acids; sensory evaluation; seafood; frozen food; freshness

Key Contribution: This research investigated the relationship between the results of physicochemical analyses and sensory evaluations of wild and farmed frozen southern bluefin tuna using principal component analysis. Various characteristic differences were found between wild and farmed tuna

1. Introduction

In recent years, the widespread adoption of fishing vessels equipped with ultralowtemperature freezers (−60 °C) has led to improved transportation technology, enabling the market distribution of various high-quality fish species for raw consumption. Notably, frozen distribution technology is widely applied to tuna, which ranks second in Japan in terms of seafood consumption [1,2]. Among the tuna species, including bluefin tuna (*Thunnus thynnus*), Atlantic bluefin tuna (*Thunnus atlanticus*), bigeye tuna (*Thunnus obesus*),

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and yellowfin tuna (*Thunnus albacares*), southern bluefin tuna (*Thunnus maccoyii*) is primarily distributed as a frozen product [3]. Southern bluefin tuna, like bluefin and Atlantic bluefin tuna, is highly valued in the market for its fat content and flavor profile, especially for raw consumption as sashimi and sushi.

Wild southern bluefin tuna fisheries off the coasts of Sydney in Australia and Cape Town in South Africa primarily employ the purse seine and longline fishing methods, with the catch frozen on board and then distributed to market. Although the catch volume is not substantial, demand in Japan for this species of tuna is high, and it accounts for approximately 70% of the global catch [4]. Tuna with a high fat content commands premium prices. To meet the demand for *O-toro* (fatty tuna) southern bluefin tuna, aquaculture and related industry operations have been established and are primarily concentrated in the offshore region of Port Lincoln in South Australia. The cultivation process involves capturing juvenile fish with purse seines, transferring them to towing cages for transport to aquaculture sites, and then, rearing them in large circular pens for several months. This short-term intensive feeding results in considerable weight gain and increased fat content before harvesting. The supply of such farmed tuna has rapidly increased in the Japanese market [5].

Wild and farmed southern bluefin tuna are now available in the fresh fish sections of supermarkets and mass merchandisers, increasing consumption opportunities. As a result, the volume of tuna handled has increased, making it one of the most valuable species of marine products distributed domestically and globally. Quality differences between frozen wild and farmed southern bluefin tuna have been reported by Winarni et al. and Bu et al. in terms of their K-values and general composition [6,7]. Other ingredients have also been reported, although to a lesser extent [8]. However, there are no reports on eating quality. Therefore, judgments have been made based on previous experience. In particular, there is a strong consumer preference for wild marine products, which are perceived to be of superior quality. For the stable production and distribution of farmed marine products, including tuna, it is important to add value by further improving their quality. Recent studies on Atlantic bluefin tuna, another premium species, have revealed differences in quality between wild and farmed tuna. Nakamura et al. reported a higher fat content in farmed fish, while inosinic acid content, known to enhance umami flavor, showed no significant difference between wild and farmed fish [9]. However, differences in fatty acid and free amino acid compositions, likely derived from diet, were observed between wild and farmed fish, suggesting potential impacts on the quality of frozen Atlantic bluefin tuna. While such quality differences have been reported in Atlantic bluefin tuna in a few existing studies, research on southern bluefin tuna remains scarce, particularly regarding the correlation between taste and chemical composition.

Although a variety of fish are now farmed, there are reports that cultivated kampachi, a migratory fish like tuna, is inferior to natural fish [10]. Efforts are underway to improve the quality of many farmed fish, and experiments have been conducted to improve meat quality and coloration in yellowtail farming [11]. As mentioned earlier, there are no reports on the eating quality of southern bluefin tuna, but there are many reports of farmed fish being inferior to wild fish.

To address this gap, in this study we aimed to elucidate the taste characteristics of southern bluefin tuna by examining the quality of wild and farmed tuna, focusing on their flavor components. We conducted sensory evaluations and physicochemical analyses on frozen wild and farmed southern bluefin tuna to assess their quality and taste profiles. The samples were subjected to both physicochemical analysis and sensory evaluation to determine their actual quality and taste characteristics. Furthermore, we employed principal component analysis to evaluate correlations between these features based on the results of the physicochemical analysis and sensory evaluation.

2. Materials and Methods

2.1. Materials

The frozen southern bluefin tuna *Akami* (lean meat), *Chu-toro* (medium-fatty tuna), and *O-toro* (fatty tuna) samples used in this experiment were obtained from Toyo Reizo Co., Ltd. (Tokyo, Japan). For farmed samples, each fish was stunned underwater by a diver using an electric shock at the time of landing, and then they were killed on board the boat. After being caught, they were rapidly frozen at −60 °C. Wild-caught samples were also rapidly frozen at −60 °C after being caught. After import, they were processed by Toyo Reizo Co., Ltd., and divided into parts. Samples were kept frozen at −60 °C until analysis. A total of 36 samples were used. Each sample was prepared as a block measuring 7 cm × 15 cm × 2 cm and weighing 200 g. Seven blocks were prepared per sample, representing three individuals from each fishing ground for both the wild and farmed samples, with three parts from each individual. Details of the samples are shown in Table 1. The sizes of the wild individuals (by weight) were matched to those of the farmed tuna.

Table 1. Frozen southern bluefin tuna samples used in this study.

Farming was conducted in the southern hemisphere during the summer months of December and January, when fry (juveniles) were caught, followed by six months of cultivation. YM = Yousyoku Minami maguro (farmed); STM = Sydney Tennen Minami maguro (Sydney, wild); CM = Cape Minami maguro (Cape Town, wild); SIOM = South Indian Ocean Minami maguro (South Indian Ocean, wild).

2.2. Thawing and Sample Preparation

The samples used for sensory evaluation were thawed using the warm saltwater method commonly used in sushi restaurants and fish markets, based on the description in Yoneda et al. [12]. Specifically, the samples were immersed in warm 3% (*w*/*w*) salt water (30 °C) for 5 min, wrapped in paper towels and plastic, and left in a refrigerator at 4 °C for 24 h. Afterwards, they were sliced into sashimi and left in the refrigerator for 30 min before being used for sensory evaluation. Processing was carried out by a licensed chef. The samples were sliced to approximately 1 cm thickness using the hirazukuri technique, where the knife is pulled towards the chef to make flat slices.

For physicochemical analysis, the samples were stored at −20 °C for 12 h, cut into cubes with a knife, and then minced using a food processor to minimize differences between parts. Afterwards, they were stored in sealed containers at −60 °C until used for physicochemical analysis.

2.3. General Component Analysis

Moisture content was determined using the atmospheric pressure thermal drying method [13]. Two grams of minced sample was placed in a low-temperature dryer at 105 °C and heated to a constant weight. The moisture content was then calculated from the initial and final weight after drying.

The crude protein content was determined using the Kjeldahl method and was calculated based on the nitrogen content, which was obtained using a Kjeldahl analyzer (Hanon, Dezhou, China) after heating and decomposing 0.2 g of minced sample in concentrated sulfuric acid. The factor used for converting nitrogen content into protein content was set to 6.25.

The fat content was determined according to a variant of the method described by Hanson and Olley [14] and calculated from the total fat content extracted from 5 g of minced sample using a chloroform/methanol mixture.

The crude ash content was determined using the direct ashing method, where 2 g of the minced sample was ashed by heating it in an electric furnace at 550 °C for 6 h [13], and calculated from the weight before and after ashing.

2.4. Fatty Acid Composition Analysis

Fatty acid analysis was performed according to the method of Hiratsuka et al. [15]. Samples extracted during the fat content analysis described in Section 2.3 were methylesterified, and fatty acids were analyzed by gas chromatography (GC-14, G.L. Science Corporation, Tokyo, Japan) using a capillary column (TC-WAX 30 m × 0.25 mm, G.L. Science Corporation). The initial temperature was 175 $^{\circ}$ C, the heating temperature was 1 $°C/min$, the final temperature was 225 $°C$, the sample injection port temperature was 250 °C, the detector temperature was 270 °C, and the injection mode was split (split ratio 50:1). The results were quantified via comparison with specific standards to obtain composition ratios.

2.5. pH and Salt Content Measurements

The pH was measured according to the method of Takahashi et al. [16]. That is, 27 mL of ion-exchanged water was added to 3 g of sample, and after homogenization, the sample was filtered using filter paper (No. 1, Toyo Filter Paper Co., Ltd., Tokyo, Japan). The pH of the filtrate was measured with a pH meter (LAQAtwin-pH-33, Horiba, Tokyo, Japan).

Salt content was calculated using the Mohr method after measuring the filtrate with a salinometer (PAL-ES2, Atago Corporation, Tokyo, Japan) [17]. Measurements were taken to determine the relationship between salt content and sensory characteristics.

2.6. Adenosine Triphosphate (ATP)-Related Compound Content and K-Value Analysis

The method of Hu et al. was followed [18]. In brief, a 10-fold volume of 5% (v/v) perchloric acid solution was added to 1.5 g of thawed sample and stirred with a glass rod for 15 min. Then, 2.7 mL of 5 M potassium hydroxide solution was added and centrifuged (2200× *g*, 15 min), and 4 mL of 50 mM dipotassium hydrogen phosphate solution was mixed with 2 mL of the obtained supernatant. The samples were then stored at –80 °C until analysis and filtered through a membrane filter (pore size: $0.45 \mu m$) immediately before analysis. The analysis was performed using high-performance liquid chromatography (e2695, Waters Corporation, Milford, MA, USA). An InertSustain AQ-C18 (G.L. Science Corporation) analytical column was used at 40 °C. A 50 mM dipotassium hydrogen phosphate solution was used as the mobile phase at a flow rate of 1.0 mL/min. Detection was performed at 260 nm. The results were quantified for comparison using specific standards. The K-value was calculated from the content of each ATP-related compound obtained [19].

2.7. Analysis of Free Amino Acid, Free Carnosine, Free Anserine, α-Ketoglutaric Acid, and Lactic Acid Contents

Extraction samples were prepared based on the method of Minami et al. [20]. Six milliliters of 5% (*w*/*v*) trichloroacetic acid (TCA) solution was added to 3 g of thawed sample, and they were mixed by grinding with an alumina ball. The sample was then centrifuged (4 \degree C, 8000× *g*, 10 min), and the supernatant was collected. A total of 6 mL of 5% (*w*/*v*) TCA solution was added to the precipitate, the sample was mixed again with an alumina ball and centrifuged under the same conditions as before, and the supernatant was collected. This procedure was repeated three times. The collected supernatant was filtered through 5A filter paper, and the filtrate was diluted to 50 mL with ion-exchanged

water. The sample was then filtered through a membrane filter (pore size: $0.45 \mu m$) and frozen at −30 °C until analysis. The results were quantified for comparison using specific standards.

The free amino acid, free carnosine, and free anserine contents were determined using high-performance liquid chromatography (LC-20AD, Shimadzu Corporation, Kyoto, Japan) using a post-column detection method with o-phthalaldehyde, employing a ISC-30/S 0504 Li trap column (Shimadzu Corporation) and a Shima-pack AMINO-Li analytical column (Shimadzu Corporation) at 37 °C. Excitation and fluorescence wavelengths of 350 nm and 450 nm, respectively, were used for detection. The results were quantified for comparison using specific standards.

The α -ketoglutarate and lactic acid contents were determined using a post-column detection method via high-performance liquid chromatography (LC-20AD, Shimadzu Corporation), modified from the method of Funatsu et al. [21], employing an RSpak KC-G6B guard column (Resonac Holdings Corporation, Tokyo, Japan) and an RSpak KC-811 separation column (Resonac Holdings Corporation) at 60 °C. The mobile phase was 3 mM perchloric acid solution, and ST3-R (Resonac Holdings) was used after the reaction; the flow rate was 1.0 mL/min for both cases. The results were quantified for comparison using specific standards.

2.8. Collagen Content Determination

Collagen was determined using the hydroxyproline quantification method with reference to Woessner [22]. Five grams of minced sample was placed in an autoclave (NCC-16LVB, Azwan Corporation, Osaka, Japan) that reached 120 °C. Total collagen was extracted for 30 min and centrifuged. Then, distilled water and hydrochloric acid were added to the supernatant and hydrolyzed at 130 $^{\circ}$ C for 3 h. After hydrolysis, the hydrochloric acid and distilled water were removed under reduced pressure. The dried sample was dissolved in distilled water, and sodium p-toluenesulfonchloramide and dimethylamidobenzaldehyde were added to allow the sample to develop a color. The color was then measured using a UV–visible spectrophotometer (V-630BIO, Japan Spectroscopic Corporation, Tokyo, Japan) at 557 nm. After measurement, the hydroxyproline content was calculated from the standard calibration curve, and the total collagen content was calculated (unit: %). Hydroxyproline is a unique substance found in collagen, and the collagen content can be determined by using the conversion factor for each organism. As the conversion factor for southern bluefin tuna was unknown, a value of 10 was used in the calculation [23].

2.9. Sensory Evaluation

Sensory evaluation was conducted according to a quantitative descriptive analysis, which is an analytical sensory evaluation method [24]. The evaluation items for the sensory evaluation were the characteristic terms for sashimi on the character wheel created by Sekino et al. [25]. The sensory evaluation was conducted by a panel of 12 trained individuals, each of whom prepared two slices of sashimi for an evaluation of their aroma, texture, and taste. Aroma was evaluated using 7 items, texture was evaluated using 11 items, taste was evaluated using 9 items, and the overall tastiness was evaluated (Table 2). Texture was evaluated based on the feeling while chewing. Tastiness was evaluated subjectively by the panel, who were familiar with eating tuna. Each item was rated on a scale of 1 to 7, where 1 is weak and 7 is strong. Only those items that were sensed were rated, and those that were not sensed were set to 0. Furthermore, in order to suppress the influence of the evaluation environment on the score when performing the sensory evaluation, the following environmental conditions were established: (1) confirmation that there was no foreign odor in the room, (2) the installation of partitions to prevent the evaluators from obtaining visual information, and (3) brightness on the desk set to 2800 lx. The sensory evaluation was conducted with approval from the Ethics Committee of Tokai University (approval number 22157).

Abbr.	Item	Abbr.	Item	Abbr.	Item	Abbr.	Item		
A1	Fat ^{c)}	A29	$C22:1n-9$	A57	Phenylalanineb)	B1	Seashoure		
A2	Protein ^{c)}	A30	$C16:2n-4$	A58	Tryptophan	B2	Fishy		
A ₃	Water content ^{c)}	A31	$C18:2n-6$	A59	Histidine ^{c)}	B ₃	Oily	Smell/aroma	
A4	Ash	A32	C18:3n-3	A60	Lysine ^{c)}	B4	Acidic		
A ₅	Collagen	A33 C18:4n-3		A61 Arginineb)		B ₅	Watery ^{a)}		
A ₆	pH	A34	$C20:2n-6$	A62	Carnosine ^{c)}	B6	Sweet		
A7	Salinity	A35	C20:4n-6	A63	Anserine ^{c)}	B7	Metal		
A8	IMP ^{b)}	A36	C20:3n-3	A64	α -ketoglutaric acid ^{c)}	${\sc B8}$	Fibrous ^{b)}		
A ₉	ATP	A37	C20:4n-3	A65	Lactic acid ^{c)}	B9	Crunchy ^{a)}		
A10	ADP	A38	$C20:5n-3b$	A66	Hardness (load) $[N]^{b}$	B10	Elastic		
A11	Amp.	A39	$C22:5n-6$	A67	Breaking (load) $[N]^{b}$	B11	Softb)		
A12	HxR	A40	C22:5n-3	A68	Fragility (load) [N]	B12	Chewy ^{a)}		
A13	HX	A41	$C22:6n-3c)$	A69	Aggregability	B13	Slippery ^{b)}	Texture	
A14	Total ^{b)}	A42	Aspartic acid	$\rm A70$	Adhesion (load) [N]	B14	Unctuous ^{b)}		
A15	K-valueb)	A43	Theonineb)	A71	Gumming (load) [N]	B15	Fluffy		
A16	C14	A44	Serineb)			B16	Musky		
A17	C15:0	A45	Asparagine			B17	Crumbly ^{a)}		
A18	C16 ^b	A46	Glutamic acid ^{b)}			B18	Soggy		
A19	C17	A47	Glutamine			B19	Metal ^{a)}		
A20	C18:0 ^b	A48	Prolineb)			B20	Rich ^{a)}		
A21	C20:0	A49	Glycine ^{c)}			B21.	Oily ^{b)}		
A22	$C16:1n-7^{b}$	A50	Alanine ^{c)}			B22	Umami ^{a)}		
A23	$C17:1n-8$	A51	Valine ^{b)}			B23	Bitter	Taste	
A24	$C18:1n-9c$	A52	Cysteine			B24	Sour		
A25	$C18:1n-7$	A53	Methionine			B25	Salty		
A26	C20:1n-11	A54	Isoleucineb)			B26	Sweetish ^{b)}		
A27	C20:1n-9b)	A55	Leucine ^{c)}			B27	Astringency		
A28	C 22:1n-11	A56	Tyrosineb)			B28	Tastiness		

Table 2. List of items used in principal component analysis.

Here, the labels a), b), and c) indicate the analysis items with cumulative contributions of 50%, 60%, and 70%, respectively.

2.10. Data Evaluation

2.10.1. Statistical Processing

The results obtained from the physicochemical analysis and sensory evaluation were processed using Excel Statistics 2019 (Microsoft Japan, Tokyo, Japan). Comparisons between data for wild and farmed animals were made using Student's *t*-test (one-sided, *p* < 0.01).

2.10.2. Principal Component Analysis

A principal component analysis was performed using the results of the physicochemical analyses and sensory evaluation. The items used are shown in Table 2. For convenience, the items from the physicochemical analyses are denoted as A1 to A71, and the items from the sensory evaluation as B1 to B28. The statistical processing software R (ver. 4.0.3) was used for the principal component analysis. The analysis items were

filtered due to the large number of items. Because we wanted to investigate the relationship between the results of the sensory evaluation and those of the physicochemical analyses, the standard deviations of all items were calculated, and items with small standard deviations were deleted to improve the cumulative contribution ratio. In this study, a principal component analysis with cumulative contribution percentages of 50%, 60%, and 70% was performed.

3. Results and Discussion

3.1. Physicochemical Analyses

Table 3 shows the general composition (fat content, crude protein content, moisture content, and crude ash content) of each sample. *O-toro* had the highest fat content and *Akami* had the lowest fat content for both wild and farmed samples. The fat content of *Akami* and *O-toro* tended to be lower in the wild samples than in the farmed samples and was significantly lower in *Akami* ($p < 0.05$). On the other hand, the protein content was highest in *Akami* and lowest in *O-toro* in the wild samples, but highest in *Chu-toro* in the farmed samples. Livestock farming is considered to have low crude protein content, which is found in muscle, due to the animals' low physical activity. Moisture content was high for both wild and farmed *Akami,* followed by *Chu-toro* and *O-toro*, with significant differences between wild and farmed *Akami* and *O-toro*. There was no difference in ash content between wild and farmed samples, with *Akami* exhibiting the lowest ash content, followed by *Chu-toro* and *O-toro*. In Pacific bluefin, it has been reported that wild tuna has a higher moisture content and lower fat content than farmed tuna [26]. In this study, similar results were obtained for *Akami* and *O-toro*, but the opposite was true for *Chu-toro*. The reason for this is that the samples used in this study were relatively small, and some samples had more *Akami* in the *Chu-toro*. Since more moisture is contained in *Akami* and the samples used in this study were considered to have a high percentage of *Akami*, the moisture content was high in the *Chu-toro.*

Table 3. General nutritional composition (%).

The data are represented as the mean \pm standard deviation. $*$ indicates a 95% significant difference; ** indicates a 99% significant difference. The asterisk is attached to the higher number.

Table 4 shows the organic acid content of each sample. α-ketoglutaric acid is an intermediate byproduct of the TCA cycle. The α -ketoglutaric acid concentrations were lowest in both wild and farmed *Akami*, followed by *Chu-toro* and *O-toro*. Wild samples for Akami and Chu-toro contained significantly more α -ketoglutaric acid than farmed samples, and wild samples for O-toro contained significantly more α -ketoglutaric acid than farmed samples. Lactic acid was higher in farmed samples than in wild samples for all parts, with significant differences between *Chu-toro* and *O-toro* (*Chu-toro p* < 0.05, *Otoro* $p < 0.01$). For both wild and farmed fish, lactic acid content tended to be lowest in *Akami*, followed by *Chu-toro* and O-toro.

Table 4. Organic acid (µmol/g) content.

The data are represented as the mean \pm standard deviation. $*$ indicates a 95% significant difference; ** indicates a 99% significant difference. The asterisk is attached to the higher number.

It has been reported that lactic acid is produced due to stress when fish are reared at high densities [27]. Therefore, it was thought that the higher levels of lactic acid in farmed fish than in wild tuna in the present study were due to stress during rearing.

The pH, salt content, and collagen content are shown in Table 5. The pH was lower in farmed samples than in wild samples across all parts of the fish (*Akami*, *Chu-toro*, *Otoro*). This could be because lactic acid is more abundant in farmed samples. There were no differences in salt content between wild and farmed samples. The collagen content was higher in farmed fish than in wild fish in all parts of the fish, and the content was highest in the farmed *O-toro*. Japanese eel, which is considered to have a strong texture, is considered to be high in collagen, but the low collagen content in the results of this study suggests that its effect on sensory characteristics is small [28]. Studies on tuna are considered to involve experimental error because tuna has a lot of connective tissues (fiber layers), which tend to mix with the sample during measurement.

Table 5. pH, salt (%) and collagen (%) contents.

The data are represented as the mean \pm standard deviation. $*$ indicates a 95% significant difference; The asterisk is attached to the higher number.

The fatty acid composition of each sample is shown in Table 6. The proportions of palmitic acid (C16:0), oleic acid (C18:1 n-9), and docosahexaenoic acid (C22:6 n-3) were high in all parts of both wild and farmed fish. A fatty acid analysis of the ventral normal muscle of Pacific bluefin tuna conducted by Popovic et al. [26] revealed that oleic acid was most abundant in wild samples, followed by docosahexaenoic acid and palmitic acid. In this study, it was found that southern bluefin tuna also has a high content of oleic and palmitic acids. Palmitic acid was significantly more abundant in farmed *Akami* samples. Docosahexaenoic acid levels in *Akami* were significantly higher in the wild samples than in the farmed samples, and docosahexaenoic acid levels in *Chu-toro* and *O-toro* were significantly higher in the farmed samples than in the wild samples. Palmitoleic acid was significantly higher in farmed samples than in the wild samples in all parts of the fish. Wild samples contained more monounsaturated fatty acids, while farmed samples contained more saturated fatty acids. These results suggest that there are differences in fatty acid composition between wild and farmed fish.

Table 6. Fatty acid composition (%).

The data are represented as the mean ± standard deviation. * indicates a 95% significant difference; ** indicates a 99% significant difference. The asterisk is attached to the higher number.

The free amino acid composition and free anserine and free carnosine contents in each sample are shown in Table 7. The histidine, alanine, and lysine contents were high in all samples. The anserine and carnosine contents were also high, with anserine being more abundant in wild samples and carnosine being more abundant in farmed samples. In particular, the content of anserine was high in both wild and farmed samples. It has been reported that the free amino acid composition of fish varies depending on diet [29]. A diverse intake of fish, crustaceans, cephalopods, and sponges has been reported in Atlantic bluefin tuna [30]. Farmed Atlantic bluefin tuna are fed a diet of sardines, herring, and mackerel [26,31]. The farmed southern bluefin tuna used in this study are fed primarily sardines and mackerel. Although there are few findings and no reports on southern bluefin tuna, there were differences in free amino acids between wild and farmed southern bluefin tuna, suggesting that the differences in feed may have resulted

in differences in free amino acids. In particular, there were large differences in the essential amino acids glutamine acid, glycine, and alanine between wild and farmed fish. In addition to the essential amino acids, there were large differences in taurine and zalcosine between wild and farmed fish.

		Akami	Chutoro		Otoro		
	Wild	Farmed	Wild	Farmed	Wild	Farmed	
Aspartic acid	1.3 ± 0.4	1.3 ± 0.7 *	0.6 ± 0.3	0.9 ± 0.4 *	0.7 ± 0.3	1.2 ± 0.4 *	
Threonine	4.1 ± 1.1	5.0 ± 1.1 *	2.2 ± 0.8	3.2 ± 1.7 *	1.8 ± 0.7	2.7 ± 0.1 *	
Serine	5.4 ± 1.8 *	3.1 ± 1.4	2.5 ± 1.1	2.8 ± 1.3 *	2.1 ± 1.0 *	1.7 ± 0.7	
Asparagine	0.0 ± 0.0	0.0 ± 0.0 *	0.0 ± 0.0	0.0 ± 0.0 *	0.0 ± 0.0	0.0 ± 0.0 *	
Glutamine acid	2.7 ± 2.0	8.3 ± 3.9 *	1.4 ± 0.5 *	6.0 ± 2.4 *	2.1 ± 1.1	5.9 ± 1.1 **	
Glutamine	$0.1 \pm 0.1*$	5.2 ± 5.9 *	$0.1 \pm 0.1*$	$4.0 \pm 3.6*$	0.3 ± 0.3	4.1 ± 1.3 *	
Proline	1.4 ± 0.9	2.4 ± 0.4 *	2.7 ± 1.3 *	1.5 ± 0.5	3.0 ± 1.0 *	2.2 ± 1.1	
Glycine	11.4 ± 2.7 *	7.5 ± 2.1	$8.0 \pm 3.0*$	6.8 ± 1.5	6.3 ± 2.7 *	4.8 ± 1.4	
Alanine	52.2 ± 17.6 *	32.7 ± 10.0	10.3 ± 3.3 *	10.1 ± 3.3	9.8 ± 2.5 *	7.8 ± 1.1	
Valine	6.4 ± 1.6	$7.0 \pm 0.7*$	2.8 ± 1.1	4.5 ± 1.4 *	3.1 ± 0.7	3.5 ± 0.3 *	
Cysteine	0.2 ± 0.1	0.0 ± 0.0	$0.1 - 0.2$	0.0 ± 0.0	$0.1 \pm 0.1*$	0.1 ± 0.1 *	
Methionine	3.3 ± 1.1 *	3.0 ± 1.3	1.8 ± 0.6	2.2 ± 0.4 *	1.4 ± 0.5 *	1.9 ± 0.5 *	
Isoleucine	4.3 ± 1.2 *	3.8 ± 0.7	$1.9 \pm 0.8*$	2.9 ± 1.1 *	1.9 ± 0.6	2.2 ± 0.3 *	
Leucine	11.0 ± 2.9 *	8.1 ± 0.9	3.6 ± 1.1	4.7 ± 1.5 *	3.7 ± 1.1	3.8 ± 0.3 *	
Tyrosine	6.7 ± 1.4 *	5.5 ± 0.9	2.1 ± 0.8 *	$3.8 \pm 0.9*$	1.7 ± 0.6	2.7 ± 0.4 *	
Phenylalanine	3.4 ± 0.9	3.0 ± 0.6	1.8 ± 0.5	2.8 ± 0.8 *	1.5 ± 0.5	1.8 ± 0.5	
Tryptophan	2.0 ± 0.3	0.6 ± 0.2	$0.3 - 0.4$	0.5 ± 0.2 **	0.3 ± 0.2	0.2 ± 0.0	
Histideine		798.7 ± 111.9 1247.5 ± 41.9	577.3 ± 133.8	1080.2 ± 20.3	454.4 ± 124.0	775.5 ± 152.2 *	
Lysine	16.3 ± 10.1	42.7 ± 17.9 *	9.5 ± 3.7	19.8 ± 2.3	11.3 ± 8.4	24.1 ± 3.5 **	
Arginine	1.0 ± 0.7	$8.1 \pm 4.6*$	$0.1 - 0.2$	1.3 ± 0.4	0.2 ± 0.3	2.2 ± 0.3 *	
Essential amino acid Total				932.0 ± 172.9 1394.9 \pm 270.4 629.0 \pm 125.3 1158.1 \pm 234.6	505.7 ± 98.5	1180.6 ± 181.5	
O-Phosphoserine	0.3 ± 0.2	$2.4 \pm 0.4*$	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	
Taurine	10.6 ± 1.6	11.2 ± 0.3 *	49.4 ± 52.8 *	13.9 ± 2.1	$60.6 \pm 44.6*$	21.0 ± 2.7	
Ω							
Phosphoethanolamine	0.2 ± 0.1	0.1 ± 0.0	0.6 ± 0.3	0.2 ± 0.1	1.1 ± 0.7 **	0.3 ± 0.1 *	
Hydroxyproline	0.6 ± 0.2	0.9 ± 0.1 *	0.4 ± 0.2	$0.6 \pm 0.1*$	0.4 ± 0.1	0.6 ± 0.1 *	
Zalcosine	27.1 ± 19.1 **	5.7 ± 5.9	10.4 ± 5.3	2.2 ± 1.6	10.2 ± 6.6 **	2.8 ± 1.7	
2 Aminoadipic Acid	0.0 ± 0.0	$0.0 \pm 0.0*$	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	
Citrulline	0.3 ± 0.1 *	0.2 ± 0.1	0.1 ± 0.1 *	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 *	
2-Aminobutyric acid	1.1 ± 0.2 *	0.8 ± 0.3	0.7 ± 0.7 *	0.4 ± 0.2	0.4 ± 0.1	0.4 ± 0.2	
Cystathionine	0.3 ± 0.2	0.5 ± 0.0 **	1.0 ± 2.3 *	0.4 ± 0.3	0.2 ± 0.1	0.2 ± 0.1	
B-Alanine	0.9 ± 0.3	0.2 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.5 ± 0.2 **	0.2 ± 0.1	
3-Aminoisobutyric acid	0.0 ± 0.0	$0.0 \pm 0.0*$	0.0 ± 0.0	0.0 ± 0.0 *	0.0 ± 0.0	0.0 ± 0.0 *	
4-Aminobutyric acid	0.1 ± 0.1 *	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	
3-Methylhistidine	5.9 ± 1.2	7.9 ± 0.7 *	4.0 ± 0.9	7.9 ± 0.8 **	3.4 ± 0.9	6.0 ± 0.7 **	
1-Methylhistidine	2.6 ± 1.2 **	1.4 ± 0.3	$1.0 \pm 0.8*$	0.4 ± 0.1	$1.2 \pm 0.9*$	0.4 ± 0.2	
Hydroxylysine	0.2 ± 0.1	0.2 ± 0.0	$0.6 \pm 0.4*$	0.3 ± 0.1 *	0.6 ± 0.5	1.1 ± 0.1 **	
Ornithine	0.5 ± 0.1	2.3 ± 1.7 *	$0.3 \pm 0.1*$	0.9 ± 0.3	0.5 ± 0.1	0.6 ± 0.2	
Ammonia ethanolamine	1.5 ± 0.4	1.9 ± 0.3 *	1.1 ± 0.9	1.2 ± 0.1 *	0.7 ± 0.1	$1.0 \pm 0.5*$	
Carnosine	2.9 ± 3.0	12.8 ± 10.1 *	2.5 ± 2.0	8.1 ± 5.1 *	1.5 ± 1.5	8.1 ± 4.7 *	
Anserine					1524.6 ± 148.0 519.1 ± 135.6 929.1 ± 219.2 ** 518.6 ± 137.3 697.3 ± 272.5 **	356.5 ± 55.0	

Table 7. Free amino acids, free anserine, and free carnosine (µmol/100 g).

The data are represented as the mean \pm standard deviation. $*$ indicates a 95% significant difference; ** indicates a 99% significant difference. The asterisk is attached to the higher number.

The results for ATP-related compounds are shown in Table 8. There was no noticeable trend between K-values in wild and farmed samples. The total amount of ATPrelated compounds was also higher in farmed samples (7.5 ± 0.7 of *O-toro* to 13.3 ± 0.4 of Akami μ mol/g) than in wild samples $(6.5 \pm 1.1 \text{ of } O$ -toro to $11.4 \pm 0.9 \text{ of } A$ *kami* μ mol/g), and there were significant differences between *Chu-toro* and *O-toro*. The content of inosinic acid, considered the umami component of fish, was higher in farmed samples $(6.0 \pm 0.7 \text{ of}$

O-toro to 10.6 ± 0.3 of *Akami* μ mol/g) than in wild samples (5.1 ± 1.0) of *O-toro* to 9.3 ± 0.1 of *Akami* µmol/g) for all parts of the fish, with a significant difference in *O-toro*.

	Akami		Chutoro		Otoro	
	Wild	Farmed	Wild	Farmed	Wild	Farmed
IMP	9.3 ± 0.7	10.6(0.3)	6.5 ± 1.0	9.5 ± 0.7	5.1 ± 1.0	6.0 ± 0.7 **
ATP	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
ADP	$0.2 + 0.1$	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.1 *
AMP	$0.3 + 0.3$	0.6 ± 0.3		0.3 ± 0.3 0.5 ± 0.2 **	0.2 ± 0.1	0.3 ± 0.1 *
HxR		0.8 ± 0.2 * 1.1 ± 0.4 *		0.8 ± 0.7 1.1 ± 0.1 **	0.8 ± 0.1	1.0 ± 0.1 *
HX		0.7 ± 0.3 0.7 ± 0.6 0.4 ± 0.0		0.1 ± 0.0	0.3 ± 0.1 *	0.1 ± 0.0
total		11.4 ± 0.9 13.3 ± 0.4	8.1 ± 1.3	11.4 ± 0.9	6.5 ± 1.1	$7.5 \pm 0.7*$
K-Value					12.9 ± 2.9 13.3 ± 2.1 * 14.7 ± 2.6 11.0 ± 0.0 16.7 ± 2.5 *	14.0 ± 0.8

Table 8. ATP-related compounds (µmol/g) and K-values (%).

The data are represented as the mean \pm standard deviation. $*$ indicates a 95% significant difference; ** indicates a 99% significant difference. The asterisk is attached to the higher number.

Table 9 shows the results of the physical property measurements. The wild samples showed higher values for hardness, an indicator of chewiness, than the farmed sample, and there was a significant difference among the farmed *Chu-toro* and the wild *Chu-toro*. Breaking (load) was also higher for the wild sample than for the farmed sample, and significant differences were observed for all parts of the fish. Wild fish did not show higher values than farmed fish in any of the other categories. There were significant differences in all parameters except for gumming in Akami. The physical property measurements showed that wild fish exceeded farmed fish in all items. It was suggested that farmed fish had a weaker texture than wild fish.

The data are represented as the mean \pm standard deviation. $*$ indicates a 95% significant difference; ** indicates a 99% significant difference. The asterisk is attached to the higher number.

3.2. Sensory Evaluation

The results of the aroma sensory evaluation are shown in Table 10. All parts of both wild and farmed fish were watery. The watery characteristic scored higher in *Akami*, with little difference between the farmed (1.7 ± 1.0) and wild (1.7 ± 0.6) samples. *Chu-toro* and *O-toro* scored higher for the sweet characteristic. This characteristic was not detected in *Akami*, which is thought to be related to the fat content of the southern bluefin tuna. Next, we will outline the texture results, which are shown in Table 11. For *Akami* and *O-toro*, wild samples scored higher in terms of items like "slippery" and "fibrous" compared to farmed samples, suggesting they possess chewiness. For *Chu-toro* and *O-toro*, the scores for "unctuous " and "soft" were higher, with this tendency being stronger in wild samples than in farmed samples. In farmed *Chu-toro*, "mushy" (0.7 ± 1.0) and "crumbly" (2.0 ± 1.4) textures were noted. As these textures were not detected in wild samples, they are considered characteristics specific to farmed tuna. Table 12 shows the taste results. Both

farmed and wild samples had a strong metallic taste and strong umami flavor in *Akami*, with the metallic taste being more pronounced in farmed fish and the umami being more pronounced in wild-caught fish. The same trend was observed for *Chu-toro* and *O-toro*, with high scores given to oily, umami, and sweet characteristics. *O-toro*, in particular, received high scores for oily and sweet. Across all parts of the fish, the umami flavor was stronger in the wild samples $(2.1 \pm 0.8 \text{ to } 3.2 \pm 0.5)$ than in the farmed samples $(1.1 \pm 0.8 \text{ to } 3.2 \pm 0.5)$ 2.4 ± 0.3), with *Akami* scoring highest, followed by *Chu-toro* and *O-toro*. The lipid content was highest in farmed *O-toro*, followed by wild *O-toro*, wild *Chu-toro*, and farmed *Chutoro*. This was the same result as for the oily characteristic. The sweetish characteristic also showed a similar trend, but there was no difference in *O-toro*. The absence of data for *Akami*, which has a low lipid content, suggests that high lipid content affects these taste characteristics.

Table 10. Results for sensory evaluation of odor.

The data are represented as the mean ± standard deviation. - shows no valid data. * indicates a 95% significant difference. The asterisk is attached to the higher number.

Table 11. Results for sensory evaluation of texture.

The data are represented as the mean ± standard deviation. - shows no valid data. * indicates a 95% significant difference; ** indicates a 99% significant difference. The asterisk is attached to the higher number.

Table 12. Results for sensory evaluation of taste.

The data are represented as the mean ± standard deviation. - shows no valid data. * indicates a 95% significant difference. The asterisk is attached to the higher number.

The sour flavor was uniquely associated with farmed fish, particularly in the Akami portion. Sour was perceived particularly strongly in *Akami*. There were significant differences in the odor, texture, and taste items perceived in each part of both wild and farmed fish. The results for tastiness are shown in Table 13. This item is important because it evaluates the overall tastiness of the fish. The results for all flavor items were higher for wild (4.6 ± 0.4 for *Akami* and 5.2 ± 0.3 for *O-toro*) than farmed tuna (3.8 ± 0.1 for *Akami* and 4.3 ± 0.1 for *O-toro*). Farmed *Akami* (3.8 ± 0.1) yielded the lowest results, while wild *Chutoro* (5.2 ± 0.3) yielded the highest. There was a significant difference between farmed and wild samples for *Chu-toro*.

Table 13. Sensory evaluation taste results.

The data are represented as the mean ± standard deviation. * indicates a 95% significant difference; The asterisk is attached to the higher number.

3.3. Principal Component Analysis

A principal component analysis was conducted based on the results of the physical and chemical analyses and sensory evaluation. Figure 1 shows the results of the principal component analysis for all samples with a cumulative contribution rate of 50.2%. To increase the cumulative contribution rate to 50.0%, we removed items with a standard deviation of less than 0.07, resulting in a total of 87 items. The principal component analysis chart in Figure 1 shows that the fish were divided into two groups, wild and farmed, and among them, four groups emerged: wild, farmed, only *Akami*, and a mixture of *Chu-toro* and *O-toro*. Higher organization was observed in the wild group than in the farm-raised group. Tastiness (B28), which is a comprehensive sensory evaluation of taste, was highest in the wild *O-toro* group. Looking at the items near palatability, we found characteristics related to physical properties, such as hardness (A66) and gumminess (A71), related to texture. In addition, in the sensory evaluation, the texture was evaluated as soft (B11) and fluffy (B14) for *O-toro* and as slippery (B13) for *Akami*. On the other hand, farmed fish was found to be crumbly (B17) and mushy (B16), indicating that there are differences in texture between wild and farmed meat.

Free fatty acids such as palmitoleic acid (A22), gondoic acid (A27), cetoleic acid (A28), and erucic acid (A29), which are known to be important for taste, were also observed. These results suggest that texture and free fatty acid content contribute to the tastiness of wild southern bluefin tuna. Inosinic acid (A8), which is considered to contribute to the taste of fish, was located between wild and farmed *Akami* and showed no correlation with taste. It was thought that in southern bluefin tuna, inosinic acid content may not contribute significantly to taste. The wild group was characterized by high pH (A6), while the farmed group was characterized by high lactic acid content (A65). In general, as lactic acid accumulates in the body, pH becomes lower. Our observation of lactic acid content moving in the opposite direction to pH, as shown in Figure 1, suggests a correlation relationship between lactic acid and pH in this study as well. The relationship between

lactic acid and acidity was also inferred from the fact that the scores for the sour (B24) characteristic in the sensory evaluation were high in the vicinity of lactic acid.

Figure 1. Principal component analysis chart with cumulative contribution rate of 50.2%. Sample names show abbreviation, sample number, and part of fish (A: *Akami*; C: *Chu-toro*; and O: *O-toro*, respectively). Red vectors correspond to Table 2. Thick line: Wild, farm-raised; Dashed line: *Akami* group; Dotted line: *Chu-toro* and *O-toro* groups.

Furthermore, to investigate the items contributing to group differentiation, items with a standard deviation of less than 0.98 were removed, resulting in a cumulative contribution rate of 60.9% (40 items remained). These results are shown in Figure 2. Although the top–bottom and left–right orientations were reversed, the groupings remained unchanged. A characteristic of farmed tuna was that EPA (A38) extended into the *O-toro* group. Additionally, high levels of free amino acids, such as glutamic acid (A46), glutamine (A47), histidine (A59), lysine (A60), arginine (A61), and carnosine (A62), were observed.

Conversely, in wild *Akami*, free amino acids and dipeptides such as alanine (A50), leucine (A55), and anserine (A63) were found to extend into the farmed group. Free fatty acids such as oleic acid (A24) and gondoic acid (A27) were also identified in the wild *Chutoro* and *O-toro* groups. The sensory evaluation results observed in the wild toro group included characteristics such as soft (B11), melty unctuous (B14), oily taste (B21), and sweet (B26).

Figure 2. Principal component analysis chart with cumulative contribution rate of 60.9%. Sample name shows abbreviation, sample number, and part of fish (A: *Akami*; C: *Chu-toro*; and O: *O-toro*, respectively). Red vectors correspond to Table 2. Thick line: Wild, farm-raised; Dashed line: *Akami* group; Dotted line: *Chu-toro* and *O-toro* groups.

To achieve a cumulative contribution rate of 70.0%, items with standard deviations below 3.0 were removed, resulting in a 70.1% cumulative contribution rate (14 items). The results are shown in Figure 3. When the sensory evaluation results were eliminated, leaving only physicochemical analysis results, the groupings remained largely unchanged from those in Figure 1.

Figure 3. Principal component analysis chart with cumulative contribution rate of 70.1%. Sample name shows abbreviation, sample number, and part of fish (A: *Akami*; C: *Chu-toro*; and O: *O-toro*, respectively). Red vector corresponds to Table 2. Thick line: Wild, farm-raised; Dashed line: *Akami* group; Dotted line: *Chu-toro* and *O-toro* groups.

Wild *Akami* was characterized by higher levels of anserine (A63) and alanine (A50), both associated with physical tuna activity. Wild tuna migrate extensively in the southern hemisphere, while farmed tuna are confined to 50–60 m pens. The limited water flow and reduced need to chase prey in farmed environments may result in less developed musculature. As exercise-related components, alanine and anserine levels are expected to increase with muscle mass, explaining their higher concentrations in wild tuna. Previous studies have reported higher imidazole peptide content in fish with greater physical activity [32], consistent with the higher free anserine levels observed in wild tuna in this study. This suggests that there is a significant difference in physical activity between wild and farmed samples.

Farmed samples exhibited lower pH values and higher lactic acid content than wild samples. Anserine is known to have a pH-buffering capacity [33], and lactic acid is biosynthesized through glycolysis during exercise. While wild southern bluefin tuna likely produce substantial lactic acid due to extensive movement, the lactic acid may be rapidly degraded by the pH-buffering capacity of anserine. In contrast, farmed tuna, with lower anserine levels, may accumulate lactic acid, resulting in a lower pH and higher acidity.

4. Conclusions

Differences between wild and farmed southern bluefin tuna were confirmed by both sensory evaluation and physical and chemical analyses.

Regarding fatty acid composition, wild tuna had higher levels of docosahexaenoic acid and monounsaturated fatty acids, while farmed tuna had more saturated fatty acids. In terms of free amino acid and dipeptide composition, anserine and alanine were more prevalent in wild tuna, while glutamine and histidine were more abundant in farmed tuna. The results of the physical and chemical analyses indicated differences between wild and farmed fish with respect to quality, suggesting that these differences originated from the feed.

Regarding texture, wild tuna were characterized as having a chewy texture, while farmed tuna were evaluated as having no chewy texture. A sour taste was only detected in farmed tuna, suggesting that it might be a characteristic specific to farmed tuna. This was thought to be due to differences in habitat. However, there are no detailed reports on the differences in physical activity between wild and farmed tuna, and further research is anticipated in this area.

In this study, principal component analysis was performed to determine whether there was a correlation between the sensory evaluation and physical and chemical analysis results. From the principal component analysis diagram, it was concluded that fatty acid composition, the physical properties, the presence or absence of acidity, and differences in texture were important factors contributing to the taste of tuna in terms of compounds and sensory aspects. Furthermore, based on the principal component analysis results, it was inferred that inosinic acid, which is considered an umami component in fish, may have a low impact on the taste of southern bluefin tuna. These results suggest that it is possible to improve the quality of farmed tuna by improving the aquaculture environment, including feed. In the future, it will be necessary to further examine the effects of feed and habitat on wild and farmed fish in more detail.

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