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Development of enhanced value-added processed food of chum salmon *(Oncorhynchus keta)* dorsal muscle using *koji* and its chemical changes during the fermentation

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Abstract

The *koji* was prepared using two kinds of *koji* molds for the production of soy sauce and pure sake. The acid protease, acid carboxypeptidase, α-amylase, and glucoamylase activities on the *koji* were extremely high, and the activities for pure sake were slightly higher than those for soy sauce in any case. The development of the processed food tried to prepare using two kinds of *koji* and the dorsal muscle of chum salmon. During the period of fermentation, the enzyme activities on the *koji* decreased, but the endogenous total protease activities on the muscle increased with the passage of the day. Particularly, the endogenous trypsin-like protease contributed largely to the autolysis on the muscle during the fermentation. The breaking strength on the muscle decreased, but the contents of alanine, glutamic acid, and aspartic acid increased. The total peroxide values and total acid values tests showed that the degradation of the fats and oils contained in the sample was not observed during the storage and the fermentation at all. As a result of the color analysis and the sensory evaluation, it became possible to develop enhanced value-added processed food with sweetness, umami, softness, and thickness as well as clear red color of the carotenoid pigment astaxanthin using the *koji* for pure sake (IV-2) and the dorsal muscle on chum salmon.

Introduction

Chum salmon *(Oncorhynchus keta)* belongs to Salmonidae and is widely distributed in all parts of the world. Chum salmon is one of most popular fish in Japan, and is consumed mainly in sushi ingredient and grilled fish of home cooking, box lunch (bento sold in the convenience store or the station), school lunch, and traditional Japanese multiple course meal, and as one of rice ball filling. Also, chum salmon has been used as a material of the processed foods such as dried salmon, smoked salmon, flake, canned salmon (Fukuda, Yamazawa and Okazaki, 2005), soy sauce (Yoshikawa *et al*., 2006), and kamaboko (fish-paste product) (Saeki *et al*., 1995, Wan *et al*., 1995a, 1995b, Yasunaga *et al.,* 1998). Owing to the progress of coastal hatching project, the fish catches of chum salmon is increasing year by year (Isobe *et al,* 1990). However, the number of chum salmon captured during spawning migration is also increasing accordingly. The qualities of these salmon are generally inferior to that are captured in the sea by the muscular proteolysis (Akino *et al*., 2010). It is also desire to develop the effective utilization method of these salmon.

In recent year, the fish eating draw an international attention to have functions to maintain the health and to prevent lifestyle related diseases such as arteriosclerosis, diabetes, heart disease, hypertension, and metabolic syndrome (Murata *et al.*, 2004). Generally, the consumer has been became health and economical conscious as the potential trend in foods. Due to significantly increasing demand of consumers to high qualities of the cooking foods and the processed foods, it is deeply needed to develop new processed foods.

From ancient times, the *koji* molds have been used in the production of various processed foods

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such as miso, soy sauce, vinegar, pickles, sake, and distilled spirits. The enzymes such as amylases, glucoamylases, proteases, peptidases, and lipases degrade the starches, proteins, and lipids are produced in the *koji* molds, resulting in the improvement of the appearance, taste, aroma, and nutritional values of foods by the enzyme actions. Among the *koji* molds, *Aspertillus oryzae, A. sojae, A. luchuensis var. awamori, A. luchuensis* mut. *kawachii* were certified as the National Fungi by the Brewing Society of Japan in 2006. Harada *et al*. (2008) reported the preparation of fish-miso using the malted rice by *A. sojae* whole bodies of small horse mackerel softened the bone by citrate treatment. They revealed that combination of these treatments is improved the quality of fish-miso. Yoshikawa *et al.* (2006) tried to prepare the fish sauce from gutted chum salmon using the *koji* from barley and halo-tolerant microorganisms such as *Candida versatilis, Tetragenococcus halophilus,* and *Zygosaccharomyces rouxii.* The smelling of fish of the sauce was less than that of *nampla*, and the sauce had a soy sauce like flavor. Thus, it is possible to develop high quality of the processed foods using chum salmon by the *koji* and to also utilize these captured during spawning migration. The objectives of our research were to develop the processed food using chum salmon dorsal muscle with excellent sensory characteristics by the *koji*, and were to investigate the chemical changes on foods during the fermentation.

Materials and Methods

Samples

The *koji* molds for the production of soy sauce and pure sake (IV-2) were obtained from Akita Konno Shoten Co., Ltd., Akita, Japan. Non-glutinous rice (*Haenuki* produced in Yamagata Prefecture, Japan) was purchased from a local wholesale market, Yamagata, Japan. The frozen fillets (male, grade: red, size: L) of chum salmon were obtained from Maruka Asakura Shoten Co., Hokkaido, Japan. All other reagents were of analytical grade.

Preparation of koji

The rice after polishing the non-glutinous rice using a rice-polishing machine (MR-E500, TWINBIRD Co., Niigata, Japan) was washed with running water three times and was soaked in water overnight. After draining with a sieve, the rice was steamed using a steamer for 40 min. The steamed rice was cooled to approximately at 36°C, and then the *koji* mold (0.1% w/w) was sprinkled *(tanekiri).* These were kept warm at 32°C (humidity 90%) in a fermenter *(momiage)*, and were mixed after about 12 h from starting of the *koji* production *(seigiku).* Next, after about 32 h these were spread to lower the temperature and to supply the oxygen *(ichiban-teire)*. Moreover, after about 40 h these were spread similarly *(niban-teire)*. After about 48 h, these were finally taken out from a fermenter *(dekoji)*. The *koji* (the malted rice) was cooled overnight at 15°C in a cool incubator (CN-25C, Mitsubishi Electric Engineering Co., Ltd., Tokyo, Japan), and was used in the following tests.

Preparation of enzyme solution from koji

The enzyme solution from *koji* was prepared by the homogenized extraction method (Commentary Editorial Committee, 1993). That is, the *koji* was added 5 volumes of 10 mM acetate buffer (pH 5.0) containing 85.6 mM NaCl and was homogenized in ice. The homogenates were centrifuged at 30,000 x *g* for 5 min at 4°C, and the supernatants were dialyzed overnight against 10 mM acetate buffer (pH 5.0) at 4°C. The dialysate obtained was diluted to twice with cold distilled water. The solution was used as enzyme solution for the measurement of each enzyme activity.

Measurement of enzyme activity on koji

The enzyme activity on *koji* during the period of fermentation was determined as described by Commentary Editorial Committee (1993).

Acid protease activity

A 0.15 ml of casein solution and 0.1ml of McIlvaine buffer (pH 3.0) were mixed in an Eppendorf tube and were pre-incubated at 40°C for 5 min. The enzyme reaction was started by the addition of 0.05 ml of enzyme solution. After incubation at 40°C for 60 min, the reaction was stopped by addition of 0.3 ml of 0.4 M trichloroacetic acid. After the centrifugation at 800 x *g* for 5 min, the supernatants (0.1 ml) were mixed with 0.5 ml of 0.4 M sodium carbonate and 0.1 ml of phenol reagent in an Eppendorf tube. After incubation at 40°C for 30 min, the absorbance of the mixture was measured at 660 nm. L-Tyrosine was used as the standard. One unit of the enzyme activity was defined as the activity that produces 1 μg of L-Tyrosine at 40°C for 60 min.

Acid carboxypeptidase activity

Acid carboxypeptidase activity was determined by the glutamy L-Tyrosine method (Commentary Editorial Committee, 1993). A 0.1 ml of 0.5 mM Z-Glu-Tyr was pre-incubated in an Eppendorf tube at 30°C for 5 min. The enzyme reaction was started

by the addition of 0.01 ml of enzyme solution. After incubation at 30°C for 20 min, the reaction was stopped by the addition of 0.05 ml of ninhydrin solution. Next, the mixture was boiled for 15 min, and then was cooled in running water. The solution was mixed with 0.5 ml of ethyl alcohol. After 5 min, the absorbance of the solution was measured at 570 nm. L-Tyrosine was used as the standard. One unit of the enzyme activity was defined as the activity that separates 1 μg of L-Tyrosine at 30°C for 60 min.

α-Amylase activity

α-Amylase activity was measured by the soluble starch method (Commentary Editorial Committee, 1993). That is, a 1.0 ml of starch solution in an Eppendorf tube was pre-incubated at 40°C for 5 min, and then the enzyme reaction was started by the addition of 0.05 ml of enzyme solution. A 0.01 ml of the reaction mixture was sequentially mixed with 1.0 ml of iodine solution in the other Eppendorf tube. The absorbance of the mixture was determined at 670 nm. One unit of the enzyme activity was defined as the quantity of 1% soluble starch solution that was decomposed at 40°C for 30 min.

Glucoamylase activity

Glucoamylase activity was determined by the soluble starch method (Commentary Editorial Committee, 1993). A 0.5 ml of starch solution was mixed with 0.1 ml of 0.2 M acetate buffer (pH 5.0) in an Eppendorf tube, and then the mixture was pre-incubated at 40°C for 5 min. The enzyme reaction was performed by addition of 0.05 ml of the enzyme solution at 40°C for 20 min. The reaction was stopped by the addition of 0.05 ml of 1 M sodium hydroxide. After 30 min, the mixture was neutralized with 0.05 ml of 1 M HCl. Next, the amount of glucose produced by the enzyme reaction was measured by the glucose oxidase method. That is, a 0.005 ml of the reaction stop solution in an Eppendorf tube was mixed with 0.35 ml of 0.06 M phosphate buffer (pH 7.1) containing 5.3 mM phenol and 1.08 mM 4-aminoantipyrine, 0.2 ml of 25.3 U/ml glucose oxidase from *Aspergillus niger,* and 0.2 ml of 1.83 U/ml peroxidase from horseradish. After 37°C for 5 min, the absorbance of the solution was measured at 505 nm. D-Glucose was used as the standard. One unit of the enzyme activity was defined as the activity that produces 1 mg of D-glucose from soluble starch at 40°C for 60 min.

Preparation of processed food using the dorsal muscle of chum salmon

The frozen fillets of chum salmon were partially thawed, and then were removed skins, bones, and red muscle. The dorsal muscle was cut (length 30 mm, width 60 mm, thickness 5 mm) and was added the *koji* of half weight against the muscle, 7% (w/w raw materials) table salt, and 20% (w/w muscle and *koji*) mirin (sweet cooking sake). After mixing in a tupperware (a plastic container), these were stored in cool incubators at 4°C and 10°C. These were aseptically mixed once a week during the fermentation for 60 days.

Measurement of endogenous enzyme activity on the dorsal muscle

The endogenous enzyme activities on the muscle during the period of fermentation were measured as total protease activity, cathepsin-like protease activity, papain-like protease activity, and trypsin-like protease activity. The muscle was homogenized with three volumes of 0.1 M sodium phosphate buffer (pH 7.0) in ice. After centrifugation at 30,000 x *g* for 30 min at 4°C, the supernatants were used as the enzyme solution.

Total protease activity

The total protease activity was determined by the azoalbmin method (Rawdkuen *et al*., 2004). A 0.06 ml of 2% azoalbumin in 0.1 M sodium phosphate buffer (pH 7.0) was mixed with 0.06 ml of the enzyme solution and 0.42 ml of the same buffer in an Eppendorf tube. After incubation at 37°C for 30 min, the enzyme reaction was stopped by the addition of 0.06 ml of 10% trichloroacetic acid. The solution was centrifuged at 25,000 x *g* for 5 min, and then the absorbance of the supernatants was measured at 420 nm.

Cathepsin-like protease, papain-like protease, and trypsin-like protease activities

The cathepsin-like protease, papain-like protease, and trypsin-like protease activities were determined by the fluorescence intensity of 7-amino-4-methylcoumarin (AMC) that produced from the substrate in the enzyme reaction (JASCO CO, 2011). Z-Arg-Arg-MCA, Z-Phe-Arg-MCA, and BOC-Phe-Ser-Arg-MCA were used as each substrate, respectively. A 0.1 ml of enzyme solution was mixed with 1.0 ml of 10 mM sodium phosphate buffer (pH 7.0) and 0.8 ml of distilled water in an Eppendorf tube, and then was pre-incubated at 37°C for 5 min. The enzyme reaction was started by the addition of 0.1 ml of the substrate solution dissolved in DMSO. After 37°C for 30 min, the fluorescence intensity of the solution was measured at Ex/Em = 380/440 nm using a spectrofluorometer (FP-777, JASCO Co.,

Tokyo, Japan). AMC was used as the standard. Each enzyme activity was expressed as the concentration of AMC that was calculated by the change of the intensity per min.

Analysis of free amino acid composition

The free amino acid composition on the sample during the period of fermentation was analyzed on a Shimazu LC-VP amino acid analysis system by on-line postcolumn labeling method with *o*-phthalaldehyde. The Ex/Em wavelengths were set at 350/450 nm, respectively. Eluents were filtered through Millipore membranefilters (pore size 0.45 μm). The sample was added 3 volumes of distilled water, homogenized in ice, and then centrifuged at 12,900 x *g* for 15 min at 4°C. The supernatants (0.5 ml) were diluted twice with 10% perchloric acid, and were centrifuged at 12,900 x *g* for 30 min at 4°C. The pH of the supernatants was adjusted at 7.0 with 10 M potassium hydroxide. The solution was filled up to 5.0 ml with distilled water, and then used to the determination of free amino acid concentration.

Color measurement

Color analysis was performed using a colorimeter (NR-11A; Nippon Denshoku Industries Co. Ltd., Tokyo, Japan) with illuminant D65 calibrated to black and white standards. The CIE $L^*a^*b^*$ system was used as the relation to human eye response to color. Color was measured on five different spots and the results were shown as the mean of these measurements. The color difference (ΔE^*ab) was also calculated by the following formula: $\Delta E^*ab = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$

Texture analysis

The texture analysis (determination of breaking force) on the muscle during the period of fermentation was performed using a rheometer (TPU-2, YAMADEN Co., Ltd., Tokyo, Japan) equipped with a cylindrical plunger (5 mm diameter, 2.5 mm/sec depression speed). The muscle sample was removed *koji* on the surface of the muscle with a paper towel, and then was tested.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed by the method of Laemmli (1970).

Sensory evaluation

The sensory qualities of the samples were evaluated on the basis of appearance, color, gloss, thickness, aroma, sweetness, bitterness, saltiness, sourness, umami, astringency, and overall acceptability by a panel of 4 panelists on a 7-point Hedonic scale.

Storage tests

Coliform and Vibrio parahaemolyticus tests

The refrigerated samples during the period of fermentation were used in storage tests. Coliform and *Vibrio parahaemolyticus* tests were performed according to the rule. Desoxycholate agar and TCBS agar were used as the media for bacterial growth, respectively.

Monitoring of lipid oxidation

The total peroxide values (PVs) and the total acid values (AVs) of the muscle samples during the period of fermentation were investigated as described by Seto and Fujimoto (1993) and Fujita and Yamada (2011), respectively.

Statistical analysis

Except for color analysis, each assay was repeated 3 times independently and the mean was reported. Data was analyzed by ANOVA using SPSS 16.0 with the significant level at $p < 0.05$.

Results and Discussion

Measurement of enzyme activity on koji

The *koji* was successfully prepared using two kinds of *koji* molds for the production of soy sauce and pure sake. The enzyme solution was prepared and the enzyme activities were measured. As a result, acid protease activity, acid carboxypeptidase activity, α-amylase activity, and glucoamylase activity were as follows: [29,810.5 (U/g), 41,382.4 (U/g), 1,500.0 (U/g), and 312.5 (U/g)] and [31,326.3 (U/g), 51,088.2 (U/g), 2,000.0 (U/g), and 436.3 (U/g)], respectively. Acid protease is proteolytic enzyme having the optimum pH in acid condition, such as pepsin and chymosin. Acid carboxypeptidase (EC 3.4.16.1) is an exo-type enzyme (exopeptidase) that cleavage the peptide bond of protein from C-terminus and liberates the amino acids. α-Amylase (EC 3.2.1.1) is an end-type enzyme that break α-1,4 bond of the starch and glycogen structure in a random manner. Glucoamylase (EC 3.2.1.3) is an enzyme that hydrolyze α-1,4 bond of the sugar chain to digest the dietary starches and produce one molecule of

Table 1. Changes of the enzyme activities on the *koji* and the muscle of chum salmon during the period of fermentation

Enzyme	Days	0	1	4	7	14	21	30	45	60
Acid protease (U/g *koji*)	A	20,621.1^{b}	16,105.3^{b}	19,452.6^{a}	19,579.0^{b}	16,926.3^{b}	17,431.6^{b}	15,157.9^{a}	14,147.4^{c}	14,021.1^{c}
	B	22,610.5^{c}	23,368.4^{c}	21,347.4^{a}	16,926.3^{a}	16,800.0^{b}	17,684.2^{b}	15,410.5^{b}	12,252.6^{a}	13,263.2^{b}
	C	18,379.0^{a}	13,894.7^{a}	23,115.8^{b}	16,294.7^{a}	15,915.8^{a}	19,073.7^{c}	14,526.3^{a}	12,884.2^{b}	12,884.2^{b}
	D	20,210.5^{b}	14,526.3^{a}	20,715.8^{a}	16,294.7^{a}	16,294.7^{a}	15,915.8^{a}	15,536.8^{b}	11,873.7^{a}	11,747.4^{a}
Acid carboxy-peptidase (U/g *koji*)	A	18,147.1^{a}	16,676.5^{a}	11,088.2^{a}	14,323.5^{b}	19,029.4^{b}	22,264.7^{c}	21,088.2^{a}	25,205.9^{b}	18,441.2^{b}
	B	18,147.1^{a}	17,264.7^{a}	16,382.4^{b}	20,794.1^{c}	17,852.9^{b}	19,323.5^{b}	21,088.2^{a}	16,088.2^{a}	13,735.3^{a}
	C	29,911.8^{b}	26,088.2^{b}	20,205.9^{c}	10,500.0^{a}	17,558.8^{b}	18,147.1^{b}	24,029.4^{b}	16,088.2^{a}	22,264.7^{c}
	D	33,735.3^{b}	24,323.5^{b}	13,441.2^{a}	16,382.4^{b}	12,558.8^{a}	15,205.9^{a}	23,441.2^{b}	17,264.7^{a}	11,970.6^{a}
α-Amylase (U/g *koji*)	A	666.7^{a}	545.5^{a}	400.0^{a}	375.0^{a}	300.0^{a}	352.9^{a}	333.3^{a}	333.3^{b}	300.0^{a}
	B	857.1^{b}	500.0^{a}	428.6^{a}	352.9^{a}	300.0^{a}	352.9^{a}	300.0^{a}	272.7^{a}	272.7^{a}
	C	857.1^{b}	750.0^{b}	750.0^{c}	428.6^{b}	461.5^{b}	500.0^{c}	500.0^{c}	428.6^{c}	428.6^{b}
	D	1,200.0^{c}	857.1^{c}	545.5^{b}	461.5^{b}	428.6^{b}	400.0^{b}	428.6^{b}	428.6^{c}	400.0^{b}
Glucoamylase (U/g *koji*)	A	214.7^{a}	171.5^{a}	162.9^{b}	157.1^{a}	114.0^{a}	134.1^{a}	116.8^{a}	76.6^{a}	62.2^{b}
	B	298.1^{c}	226.2^{b}	137.0^{a}	145.6^{a}	102.5^{a}	128.4^{a}	125.5^{a}	108.2^{b}	33.4^{a}
	C	217.6^{a}	243.5^{c}	272.2^{c}	180.1^{b}	116.8^{a}	183.0^{b}	191.7^{b}	177.3^{c}	73.7^{b}
	D	243.5^{b}	260.7^{c}	185.9^{b}	177.3^{b}	128.4^{b}	200.3^{b}	108.2^{a}	148.5^{b}	145.6^{c}
Total protease (420nm)	A	0.016^{b}	0.119^{c}	0.109^{b}	0.073^{a}	0.120^{a}	0.138^{b}	0.135^{b}	0.152^{a}	0.210^{a}
	B	0.027^{c}	0.098^{b}	0.133^{c}	0.126^{b}	0.156^{b}	0.134^{b}	0.140^{b}	0.172^{a}	0.202^{a}
	C	0.010^{b}	0.084^{a}	0.074^{a}	0.094^{a}	0.096^{a}	0.118^{a}	0.102^{a}	0.186^{a}	0.218^{a}
	D	0.004^{a}	0.105^{b}	0.070^{a}	0.087^{a}	0.116^{a}	0.133^{b}	0.122^{b}	0.228^{b}	0.222^{a}
Cathepsin like-protease (nM AMC)	A	188.9^{c}	127.9^{b}	71.4^{a}	112.8^{c}	52.1^{a}	95.8^{c}	131.4^{c}	122.1^{c}	88.1^{a}
	B	210.5^{c}	129.8^{b}	59.7^{a}	137.1^{c}	65.8^{b}	74.8^{b}	84.4^{a}	93.9^{b}	98.3^{b}
	C	51.8^{a}	64.9^{a}	133.8^{b}	130.4^{c}	72.2^{b}	48.6^{a}	86.2^{a}	86.7^{b}	104.4^{b}
	D	130.8^{b}	50.9^{a}	67.0^{a}	86.1^{a}	65.8^{b}	110.4^{c}	119.7^{b}	38.5^{a}	87.0^{a}
Papain like-protease (nM AMC)	A	233.8^{b}	174.2^{b}	29.1^{b}	55.5^{a}	126.5^{b}	72.9^{c}	115.4^{b}	37.2^{a}	81.6^{b}
	B	307.1^{c}	236.4^{c}	11.5^{a}	63.4^{a}	136.8^{b}	74.0^{c}	50.4^{a}	73.7^{c}	83.2^{b}
	C	192.2^{b}	41.9^{a}	48.4^{c}	55.1^{a}	96.1^{a}	18.9^{a}	50.4^{a}	54.2^{b}	73.4^{b}
	D	163.5^{a}	16.7^{a}	51.9^{c}	93.9^{b}	152.8^{c}	59.5^{b}	64.6^{a}	30.0^{a}	43.8^{a}
Trypsin like-protease (nM AMC)	A	309.0^{b}	322.0^{c}	544.2^{a}	449.4^{a}	572.6^{b}	863.5^{b}	668.8^{b}	690.6^{a}	684.0^{a}
	B	460.4^{c}	367.8^{c}	596.3^{a}	727.6^{c}	487.3^{a}	826.4^{b}	1,114.3^{c}	795.3^{b}	807.9^{b}
	C	129.0^{a}	184.6^{a}	839.6^{c}	502.0^{b}	574.9^{b}	660.3^{a}	490.4^{a}	810.9^{b}	846.5^{b}
	D	141.9^{a}	229.5^{b}	702.0^{b}	437.2^{a}	693.0^{c}	953.9^{c}	1,201.4^{c}	843.3^{b}	993.5^{c}

A: *koji* for soy sauce at 4°C fermentation; B: *koji* for soy sauce at 10°C fermentation; C: *koji* for pure sake at 4°C fermentation; D: *koji* for pure sake at 10°C fermentation. Different lower case letters in same column indicate significant differences by ANOVA ($p < 0.05$).

glucose. These results indicate that the *koji* with highly enzyme activities can effectively digest the starches of the rice and the proteins of the muscles.

Enzyme activity on koji and the muscle during the period of fermentation

Acid protease activity

The acid protease activity on *koji* was measured during the period of fermentation. The activity on *koji* for soy sauce at 10°C fermentation decreased over time. The activities on *koji* for soy sauce at 4°C fermentation and these for pure sake at 4 and 10°C fermentation drastically decreased for 1 day, increased till 4 days, and then moderately decreased (Table 1).

Acid carboxypeptidase activity

The acid carboxypeptidase activity on *koji* was determined during the period of fermentation. The activities on *koji* for soy sauce at 4°C fermentation and for pure sake at 4 and 10°C fermentation rapidly decreased for 4 days, and then moderately increased (Table 1). On the other hand, the activity on *koji* for soy sauce at 10°C did not almost changed during the period of fermentation. It is known that the acid protease and acid carboxypeptidase are satisfactorily produced around 32°C. It was suggested that these enzymes were not almost produced in *koji* under low-temperature environment such as 4 and 10°C.

α-Amylase activity

The α-amylase activity on *koji* was investigated

during the period of fermentation. As a result, the activities gradually decreased under any condition for 7 days, and then did not almost changed during the period of fermentation (Table 1).

Glucoamylase activity

The glucoamylase activity on *koji* was measured during the period of fermentation. Generally, the activities were slowly decreased in spite of condition during the period of fermentation (Table 1). It suggested that the glucoamylase was not produced in *koji* under low-temperature condition, as the glucoamylase was produced around 38°C.

Endogenous total protease activity

The endogenous total protease activity on the muscle was determined during the period of fermentation. As a result, it was not almost recognized the activity before the fermentation (Table 1). However, the activities suddenly increased for 1 day, and then did not almost changed till 30 days under any condition. The activities subsequently increased till 60 days. It suggested that the autolysis on the muscle effectively occurred by the endogenous protease during the period of fermentation.

Endogenous cathepsin-like protease activity

Cathepsin-like protease is a general term of protease having the optimum pH in acid condition. These are classified in endo- and exo-peptidase, and are contained the enzyme having both types of activities such as cathepsin B and H. The endogenous cathepsin-like protease activity on the muscle was investigated during the period of fermentation. The activities drastically decreased for 4 days, and then did not almost changed during the period of fermentation under any condition (Table 1).

Endogenous papain-like protease activity

The papain-like protease is classified in cysteine protease that catalyzes cysteine and histidine residues in protein molecules. The endogenous papain-like protease activity on the muscle was measured during the period of fermentation. Except for the muscle using *koji* for pure sake at 4°C fermentation, the activities rapidly decreased for 4 days, and then did not almost changed during the period of fermentation (Table 1). The activity of that using *koji* for pure sake at 4°C fermentation gradually increased till 4 days, maintained till 7 days, decreased till 21 days, and then increased again till 60 days.

Endogenous trypsin-like protease activity

The trypsin-like protease is classified in serine protease, such as proteinase K, trypsin, chymotrypsin, and elastase. These catalyze the peptide bonds of lysine and arginine residues in C-terminal side. The endogenous trypsin-like protease activity on

Table 2. Changes of free amino acid composition on the samples during the period of fermentation

Amino acid (mg/100g)	Days											
	0				30				60			
	A	B	C	D	A	B	C	D	A	B	C	D
Aspartic acid	18.1^{b}	17.2^{b}	12.0^{a}	13.9^{a}	83.7^{a}	118.0^{b}	81.9^{a}	166.7^{c}	137.6^{a}	247.5^{b}	141.4^{a}	246.4^{b}
Threonine	29.7^{b}	26.0^{b}	18.4^{a}	22.1^{a}	80.6^{b}	91.9^{b}	66.7^{a}	117.5^{c}	112.5^{a}	165.1^{b}	102.9^{a}	150.7^{b}
Serine	35.0^{b}	30.5^{b}	22.8^{a}	26.4^{a}	93.1^{a}	105.0^{b}	80.5^{a}	138.0^{c}	133.3^{a}	190.8^{b}	123.0^{a}	174.5^{b}
Glutamic acid	61.4^{b}	52.2^{b}	47.0^{a}	45.2^{a}	166.5^{b}	206.7^{c}	130.0^{a}	249.1^{c}	243.6^{b}	390.0^{c}	197.6^{a}	329.3^{c}
Proline	19.5^{b}	17.3^{b}	12.0^{a}	12.4^{a}	54.6^{b}	60.6^{b}	40.2^{a}	79.5^{c}	65.6^{a}	94.4^{b}	66.2^{a}	92.1^{b}
Glycine	20.1^{b}	20.6^{b}	8.1^{a}	20.2^{b}	60.4^{b}	70.0^{b}	47.6^{a}	84.5^{c}	79.9^{a}	117.8^{b}	75.5^{a}	107.8^{b}
Alanine	70.7^{b}	69.3^{b}	53.3^{a}	62.2^{a}	154.6^{b}	172.4^{b}	122.3^{a}	215.0^{c}	203.7^{b}	285.3^{c}	187.0^{a}	264.4^{c}
Valine	46.9^{b}	43.4^{b}	33.3^{a}	36.7^{a}	119.6^{b}	133.8^{b}	86.3^{a}	163.5^{c}	155.9^{a}	226.5^{b}	143.9^{a}	208.8^{b}
Cystine	0.6^{b}	0^{a}	0^{a}	0^{a}	1.4^{b}	1.8^{b}	0.7^{a}	1.9^{b}	1.7^{a}	3.2^{b}	1.9^{a}	2.9^{b}
Methionine	39.0^{b}	31.5^{a}	25.4^{a}	28.8^{a}	88.9^{b}	90.1^{b}	59.8^{a}	108.5^{c}	108.7^{a}	140.8^{b}	97.0^{a}	118.6^{a}
Isoleucine	38.7^{b}	31.2^{a}	26.5^{a}	28.4^{a}	95.6^{b}	105.0^{b}	68.1^{a}	129.8^{c}	123.8^{a}	178.4^{b}	115.0^{a}	155.8^{b}
Leucine	86.2^{c}	76.9^{b}	59.3^{a}	68.2^{a}	217.8^{b}	233.1^{b}	156.9^{a}	283.1^{c}	279.2^{b}	374.9^{c}	249.8^{a}	319.2^{b}
Tyrosine	34.3^{b}	33.1^{b}	25.2^{a}	29.5^{a}	110.0^{b}	51.2^{a}	60.5^{a}	113.1^{b}	87.8^{b}	97.8^{b}	112.0^{c}	77.2^{a}
Phenylalanine	50.2^{c}	43.9^{b}	35.3^{a}	39.0^{a}	129.0^{b}	114.4^{b}	84.2^{a}	151.8^{c}	154.7^{b}	192.9^{c}	149.3^{a}	159.3^{b}
Histidine	18.7^{b}	20.5^{b}	13.1^{a}	28.6^{c}	42.9^{b}	46.0^{b}	30.6^{a}	53.0^{c}	55.4^{a}	78.8^{b}	49.9^{a}	68.7^{b}
Lysine	146.5^{c}	122.7^{b}	92.7^{a}	119.1^{b}	283.0^{b}	276.1^{b}	198.6^{a}	320.3^{c}	351.8^{b}	453.3^{c}	315.3^{a}	376.7^{b}
Arginine	64.3^{b}	56.6^{b}	18.3^{a}	19.3^{a}	73.2^{a}	144.9^{b}	87.8^{a}	168.2^{b}	122.4^{b}	160.4^{c}	94.8^{a}	187.9^{c}
Total	779.9^{c}	692.9^{b}	502.7^{a}	600.0^{a}	1854.9^{b}	2021.0^{b}	1402.7^{a}	2543.5^{c}	2417.6^{a}	3397.9^{b}	2222.5^{a}	3040.3^{b}

A: *koji* for soy sauce at 4°C fermentation; B: *koji* for soy sauce at 10°C fermentation; C: *koji* for pure sake at 4°C fermentation; D: *koji* for pure sake at 10°C fermentation. Different lower case letters in same column indicate significant differences by ANOVA ($p < 0.05$).

Table 3. Changes of the color and the color difference on the muscle surface before and after fermentation for 60 days

Sample	Days						
	0			60			
	*L**	*a**	*b**	*L**	*a**	*b**	*ΔE*ab*
A	41.28[a]	13.04[b]	14.80[a]	44.94[b]	2.94[a]	9.19[b]	73.44[c]
B	42.52[a]	14.28[b]	17.33[b]	43.13[b]	3.12[a]	7.19[a]	113.87[d]
C	40.20[a]	11.01[a]	12.18[a]	41.93[a]	10.30[b]	10.63[b]	2.95[a]
D	46.50[b]	10.44[a]	13.88[a]	41.20[a]	4.65[a]	10.18[b]	37.67[b]

A: *koji* for soy sauce at 4°C fermentation; B: *koji* for soy sauce at 10°C fermentation; C: *koji* for pure sake at 4°C fermentation; D: *koji* for pure sake at 10°C fermentation. Different lower case letters in same column indicate significant differences by ANOVA ($p < 0.05$).

the muscle was determined during the period of fermentation. In general, the activities did not changed for 1 day, and then gradually increased during the period of fermentation under any condition (Table 1). These results indicated that the endogenous trypsin-like protease largely contributed to the autolysis on the muscle during the fermentation.

Free amino acid composition

The free amino acid composition of the sample was measured during the period of fermentation. All free amino acid contents increased along with the progression of the fermentation for 60 days. Particularly, the contents of alanine, glycine, serine, and threonine increased about 4-6 times, that of glutamic acid about 7 times, and that of aspartic acid about 18 times in the samples at 10°C fermentation, respectively (Table 2). The contents of arginine, isoleucine, leucine, lysine, valine, methionine, and phenylalanine remarkably increased. It suggested that the sweetness, umami, and sourness of the samples as well as bitterness of these extremely increased by the fermentation for 60 days. Harada *et al*. (2008) tried to prepare fermented fish-miso using whole bodies of small horse mackerel and malted rice by *A. sojae.* They investigated free amino acid contents during the period of fermentation (20, 60, and 100 days) at 30°C. Except for cystine and histidine, the contents of other amino acids drastically increased about 13-99 times after 20 days, and then these gently increased about 19-134 times till 100 days. Cystine had hardly detected for fermentation as well as our results. On the other hand, histidine contents increased with increasing of the period of fermentation under any condition in our investigation.

Color analysis

The change of the color on the muscle surface was investigated using a colorimeter during the period of fermentation. Except for the muscle using *koji* for pure sake at 10°C fermentation, the mean L^* values of the samples slightly increased by the fermentation for 60 days: it showed the increase of the brightness on the muscle (Table 3). The mean b^* values of the muscles using *koji* for pure sake decreased just a little, but those of the muscles using *koji* for soy sauce remarkably decreased. The mean a^* values on the muscles using *koji* for soy sauce at 4 and 10°C fermentation and that using *koji* for pure sake at 10°C fermentation fairly decreased, while that using *koji* for pure sake at 4°C fermentation did not almost changed during the period of fermentation (Table 3). Next, the color difference was calculated on the samples before and after fermentation for 60 days. The ΔE^*ab value on the muscle using *koji* for pure sake at 4°C fermentation were lowest among these samples (Table 3). That is, it means that the color difference on sample is smallest between before and after fermentation. It is known that muscular proteolysis on chum salmon causes the change of color on its muscle: the mean L^* and b^* values on muscle significantly increase (Akino *et al.,* 2010). On the other hand, the redness (high mean a^* value) is one of an important factor to particularly affect the quality of the processed food on the appearance of the muscle of chum salmon. The carotenoid pigment astaxanthin and its degradation product astacin are substances that exhibit clear red color called salmon-red color on its muscle (Hatano *et al*., 1987). It is considered that astaxanthin on its muscle is held to actomyosin by weak hydrophobic bond (Henmi *et al*., 1987). However, the bond is cleaved by enzymes produced in *koji* and contained in the muscle, resulting decoloration of clear red color on muscle. It suggested that the condition using *koji* for pure sake at 4°C fermentation could produce processed food retained rich red color using the muscle of chum salmon.

Texture analysis

The change of the breaking strength on the muscle was investigated during the period of fermentation. It was observed the rise of the breaking strength after 1 day under any condition, and then the lowering of it with passage of the period of fermentation. In particular, the breaking strength for 60 days fell in half

Table 4. Changes of the breaking strength (N) on the muscle during the period of fermentation

	Days										
Sample	0	1	4	7	14	21	30	45	60	75	90
A	1.93^{a}	3.05^{b}	2.54^{a}	2.41^{b}	2.18^{b}	1.46^{b}	1.47^{b}	0.91^{a}	1.03^{b}	1.40^{b}	1.01^{a}
B	2.15^{a}	2.78^{a}	2.80^{a}	1.72^{a}	1.65^{a}	1.39^{b}	1.45^{b}	1.08^{a}	0.95^{a}	1.08^{a}	1.21^{b}
C	2.25^{b}	2.84^{a}	3.04^{b}	2.61^{b}	1.73^{a}	0.91^{a}	1.14^{a}	1.41^{b}	1.13^{b}	1.30^{b}	1.06^{a}
D	2.14^{a}	3.35^{b}	2.61^{a}	2.34^{b}	2.23^{b}	1.84^{c}	0.85^{a}	0.88^{a}	0.88^{a}	1.72^{c}	0.91^{a}

A: *koji* for soy sauce at 4°C fermentation; B: *koji* for soy sauce at 10°C fermentation; C: *koji* for pure sake at 4°C fermentation; D: *koji* for pure sake at 10°C fermentation. Different lower case letters in same column indicate significant differences by ANOVA ($p < 0.05$).

Table 5. Sensory evaluation of the samples during the period of fermentation

Sample		Appearance	Color	Gloss	Thickness	Aroma	Sweetness	Bitterness	Saltiness	Sourness	Umami	Astringency	Overall acceptance
0 day	A	0.75^{a}	1.88^{a}	1.00^{a}	1.00^{c}	0^{a}	1.25^{b}	-1.63^{b}	1.25^{a}	-0.75^{a}	1.63^{a}	-2.25^{a}	0^{b}
	B	0.75^{a}	2.25^{b}	1.50^{b}	1.38^{c}	0^{a}	0.88^{a}	-2.25^{a}	1.25^{a}	0.38^{b}	2.00^{b}	-2.25^{a}	0.75^{c}
	C	0.75^{a}	2.00^{b}	1.50^{b}	0.25^{a}	0^{a}	0.63^{a}	-2.13^{a}	1.75^{b}	-0.63^{a}	2.00^{b}	-2.13^{a}	-0.13^{a}
	D	0.75^{a}	1.63^{a}	1.75^{c}	0.75^{b}	0.13^{a}	1.38^{b}	-2.25^{a}	2.50^{c}	-0.63^{a}	2.13^{b}	-2.38^{a}	0.25^{b}
1 day	A	1.75^{b}	1.88^{b}	1.75^{a}	0.56^{b}	0.25^{b}	1.00^{a}	-1.63^{b}	0.88^{b}	-1.25^{a}	1.38^{a}	-3.00^{a}	0.13^{a}
	B	0.75^{a}	0.13^{a}	1.50^{a}	1.19^{c}	0^{a}	1.13^{a}	-1.88^{b}	1.00^{b}	-0.88^{b}	1.88^{b}	-3.00^{a}	1.50^{c}
	C	1.50^{b}	1.13^{b}	2.50^{b}	0.56^{b}	0.25^{b}	1.50^{b}	-2.13^{a}	0.63^{a}	-0.75^{b}	1.63^{b}	-3.00^{a}	0.88^{b}
	D	2.75^{c}	2.63^{c}	2.75^{b}	0.19^{a}	0.25^{b}	1.25^{a}	-2.13^{a}	1.13^{b}	-0.75^{b}	1.63^{b}	-3.00^{a}	1.13^{b}
4 days	A	1.50^{a}	1.00^{b}	1.50^{a}	1.00^{b}	0.50^{a}	0.50^{a}	-1.50^{c}	2.50^{c}	-0.50^{b}	1.00^{a}	-2.50^{a}	0.50^{b}
	B	2.00^{b}	1.00^{b}	2.00^{b}	2.00^{c}	0.50^{a}	1.00^{b}	-2.00^{b}	2.00^{b}	-0.50^{b}	1.00^{a}	-2.50^{a}	1.00^{c}
	C	1.50^{a}	0.50^{a}	1.50^{a}	0.50^{a}	0.50^{a}	1.00^{b}	-1.50^{c}	1.50^{a}	-1.00^{a}	1.00^{a}	-2.50^{a}	0^{a}
	D	2.00^{b}	0.50^{a}	2.00^{b}	1.00^{b}	0.50^{a}	1.50^{c}	-2.50^{a}	1.50^{a}	-1.00^{a}	2.00^{b}	-2.50^{a}	2.00^{d}
7 days	A	1.25^{c}	0.50^{c}	1.00^{a}	0.75^{b}	0.25^{a}	0.88^{b}	0.25^{d}	2.25^{b}	0.13^{b}	2.00^{b}	-1.25^{c}	0.50^{a}
	B	0.75^{b}	1.13^{d}	1.00^{a}	1.06^{c}	0.38^{b}	0.38^{a}	-1.25^{b}	1.88^{b}	0.50^{c}	1.75^{a}	-1.50^{b}	1.00^{b}
	C	1.00^{c}	0.25^{b}	1.00^{a}	0.69^{b}	0.63^{c}	1.38^{c}	-1.75^{a}	1.00^{a}	-0.13^{a}	2.13^{b}	-1.75^{a}	1.50^{c}
	D	0.25^{a}	0^{a}	1.25^{b}	0.13^{a}	0.38^{b}	1.13^{b}	-0.25^{c}	0.75^{a}	-0.25^{a}	1.63^{a}	-1.25^{c}	1.38^{c}
14 days	A	-0.25^{a}	0.63^{a}	0.50^{a}	1.75^{a}	1.13^{b}	1.00^{a}	-1.88^{a}	2.00^{c}	0^{a}	1.13^{a}	-1.00^{c}	1.00^{a}
	B	1.50^{c}	1.75^{b}	1.75^{c}	2.06^{b}	0.06^{a}	1.63^{b}	-1.13^{c}	1.00^{a}	0^{a}	1.75^{b}	-1.38^{b}	1.75^{b}
	C	0.50^{b}	0.50^{a}	1.13^{b}	2.31^{b}	1.13^{b}	1.13^{a}	-1.50^{b}	1.13^{a}	0.13^{a}	1.63^{b}	-1.63^{a}	1.75^{b}
	D	0.63^{b}	0.38^{a}	1.13^{b}	1.69^{a}	1.50^{c}	1.63^{b}	-1.38^{b}	1.50^{b}	0^{a}	1.75^{b}	-1.38^{b}	1.75^{b}
21 days	A	2.00^{c}	1.75^{c}	2.00^{b}	1.56^{b}	1.75^{a}	1.13^{b}	-0.25^{b}	1.50^{a}	-0.75^{a}	1.88^{a}	-0.88^{b}	1.38^{a}
	B	1.63^{c}	1.38^{c}	2.00^{b}	2.19^{b}	1.63^{a}	1.13^{b}	-0.50^{b}	2.00^{b}	0^{b}	2.50^{b}	-0.63^{c}	1.88^{b}
	C	0.88^{a}	0.25^{a}	1.63^{a}	1.25^{a}	1.63^{a}	0.75^{a}	-0.88^{a}	1.75^{a}	-0.88^{a}	2.13^{a}	-1.00^{b}	1.38^{a}
	D	1.00^{b}	0.63^{b}	2.00^{b}	1.13^{a}	1.63^{a}	1.25^{b}	-1.00^{a}	1.50^{a}	-1.00^{a}	2.00^{a}	-1.38^{a}	1.75^{b}
30 days	A	1.13^{a}	1.25^{a}	1.75^{a}	1.75^{b}	1.38^{a}	1.38^{b}	0^{c}	2.00^{b}	-0.38^{b}	1.63^{b}	-0.88^{b}	1.75^{b}
	B	1.38^{a}	1.75^{b}	1.38^{a}	1.56^{b}	1.63^{b}	1.25^{b}	-0.38^{b}	1.63^{a}	-0.25^{b}	1.25^{a}	0^{c}	1.00^{a}
	C	1.75^{b}	1.50^{b}	2.25^{b}	1.69^{b}	1.63^{b}	0.88^{a}	-0.63^{a}	1.38^{a}	-1.00^{a}	1.38^{a}	-1.75^{a}	1.88^{b}
	D	2.38^{c}	2.50^{c}	2.63^{b}	1.13^{a}	1.63^{b}	2.13^{c}	-0.88^{a}	1.50^{a}	-1.00^{a}	1.75^{b}	-1.50^{a}	1.88^{b}
45 days	A	1.50^{a}	1.83^{a}	2.33^{b}	1.17^{a}	1.83^{b}	1.50^{a}	-1.33^{b}	1.00^{a}	-1.33^{a}	1.50^{a}	-0.67^{b}	1.50^{b}
	B	1.33^{a}	2.00^{b}	2.17^{a}	1.58^{b}	1.67^{a}	1.50^{a}	-1.83^{b}	1.83^{b}	-1.17^{a}	1.33^{a}	-1.67^{a}	1.33^{a}
	C	1.50^{a}	2.00^{b}	2.33^{b}	1.33^{a}	1.83^{b}	1.83^{b}	-2.33^{a}	0.83^{a}	-1.17^{a}	2.17^{b}	-2.00^{a}	2.33^{c}
	D	2.00^{b}	2.33^{b}	2.50^{c}	1.25^{a}	1.83^{b}	1.33^{a}	-2.17^{a}	1.33^{a}	-1.33^{a}	1.67^{a}	-0.67^{b}	1.17^{a}
60 days	A	-0.50^{a}	0^{a}	0^{a}	1.50^{b}	0.50^{c}	1.50^{b}	-0.50^{b}	1.50^{b}	-1.50^{a}	1.00^{a}	-2.00^{b}	0^{a}
	B	-0.50^{a}	0^{a}	0^{a}	1.00^{a}	1.00^{d}	1.00^{a}	-1.50^{a}	1.00^{a}	-1.50^{a}	2.50^{c}	-2.50^{a}	0.50^{a}
	C	1.50^{c}	1.50^{c}	1.50^{b}	2.50^{d}	0^{b}	2.50^{d}	-2.00^{a}	1.00^{a}	-1.50^{a}	2.50^{c}	-2.50^{a}	2.00^{b}
	D	1.00^{b}	1.00^{b}	2.00^{c}	2.00^{c}	-0.50^{a}	2.00^{c}	-0.50^{b}	2.00^{c}	-1.50^{a}	1.50^{b}	-1.00^{c}	0^{a}

A: *koji* for soy sauce at 4°C fermentation; B: *koji* for soy sauce at 10°C fermentation; C: *koji* for pure sake at 4°C fermentation; D: *koji* for pure sake at 10°C fermentation. Different lower case letters in same column indicate significant differences by ANOVA ($p < 0.05$).

of those before fermentation (Table 4). It suggested that the rise of osmotic pressure by addition of salt contributed to the rise of the breaking strength on the muscle after 1 day, and then the myofibrillar proteins were decomposed by the action of the enzymes produced in the *koji* and contained in the muscle, resulting the increase of softness on the muscle. SDS-PAGE was performed using the myofibrillar proteins prepared from the muscle before and after fermentation for 60 days. As a result, the relative band strength corresponding to myosin heavy chain about 200 kDa molecular weight and actin about 43 kDa grew weaker after 60 days in comparison to those before fermentation (data not shown). It suggested the progression of small pieces on these protein species by the action of proteases. Proteolysis causes the muscle on chum salmon softening. Cathepsins B, H, and L are classified in cystein protease distributes in fish muscles (Yamashita and Konagaya, 1990). Among them, cathepsin L has broad substrate specificity, and shows strong proteolytic activity against muscle proteins such as myosin, connectin, nebulin, actinin, troponin, and collagen (Yamashita and Konagaya, 1991). Hereafter, it needs to observe the behavior of the stroma proteins such as collagen and elastin related to the hardness of the meat.

Sensory evaluation

The sensory evaluation tests of the samples during the fermentation were performed with passage of the day. It was observed to undergo a change on each evaluation. In general, the samples using *koji* for pure sake at 4 and 10°C fermentation got a good valuation in appearance after 45 or 60 days (Table 5). It was similarly obtained high evaluation of color and gloss on the samples using *koji* for pure sake at 4 and 10°C fermentation, but was low on the samples using *koji* for soy sauce. It was not observed the increase on the sourness under any condition during the period of fermentation. The sweetness on the samples using *koji* for pure sake remarkably increased with the passage of the day. Moreover, the umami increased in only the sample using *koji* for pure sake at 4°C fermentation. On the other hand, it was felt slightly bitterness and astringency on the sample using *koji* for pure sake at 4°C fermentation, while these tastes became stronger on other samples with the passage of the day (Table 5). The smelling of fish could not be sensed on all sample species at all. Finally, the overall acceptability was observed on the samples. The evaluation on each sample was overall favorable after 45 days, but felt down after 60 days except for the sample using *koji* for pure sake at 4°C fermentation (Table 5). As it can be seen from the results of amino acid analysis, the contents of either free amino acid increased during the fermentation (Table 2). Among them, the contents of alanine, glycine, serine, glutamic acid, and aspartic acid greatly increased. It was revealed that not only the taste of the processed food, particularly sweetness and umami but the softness and thickness of it was mainly brought out by the enzymatic degradation of the muscle protein.

Storage tests

Coliform and Vibrio parahaemolyticus tests

The coliform and *Vibrio parahaemolyticus* tests were performed on the samples during the period of fermentation. As a result, *Escherichia coli* colony's was negative and *Vibrio parahaemolyticus* was not detected for 90 days in all sample species tested (data not shown).

Table 6. Changes of the total PVs and the total AVs on the samples during the period of fermentation

Sample	Days										
	0	1	4	7	14	21	30	45	60	75	90
PVs (meq/kg)											
A	1.61^{c}	0.39^{a}	0.15^{a}	0.26^{a}	0.93^{b}	0.87^{b}	0.76^{b}	0.87^{b}	0.39^{a}	0.58^{b}	0.68^{a}
B	0.97^{b}	0.77^{b}	0.65^{b}	0.57^{b}	0.93^{b}	0.87^{b}	0.27^{a}	1.74^{c}	0.39^{a}	0.74^{b}	0.80^{b}
C	0.71^{a}	0.86^{b}	0.90^{c}	0.77^{b}	0.33^{a}	0.39^{a}	0.23^{a}	0.87^{b}	1.16^{b}	0.25^{a}	0.65^{a}
D	0.71^{a}	1.16^{c}	0.13^{a}	0.64^{b}	1.10^{b}	0.39^{a}	0.27^{a}	0.38^{a}	0.87^{b}	0.45^{b}	0.61^{a}
AVs											
A	3.16^{a}	3.52^{a}	3.72^{b}	3.76^{b}	3.11^{b}	2.42^{b}	3.62^{b}	2.85^{a}	3.22^{a}	4.19^{a}	4.00^{c}
B	3.37^{b}	3.95^{b}	2.51^{a}	2.68^{a}	4.00^{c}	2.31^{b}	4.59^{c}	4.02^{b}	4.83^{b}	4.99^{b}	3.68^{b}
C	3.16^{a}	3.95^{b}	3.58^{b}	3.22^{b}	3.27^{b}	2.76^{b}	3.22^{a}	3.86^{b}	4.35^{b}	3.86^{a}	3.26^{a}
D	3.01^{a}	3.61^{a}	3.72^{b}	3.54^{b}	2.34^{a}	1.69^{a}	3.14^{a}	2.85^{a}	3.78^{a}	4.67^{b}	3.57^{b}

A: *koji* for soy sauce at 4°C fermentation; B: *koji* for soy sauce at 10°C fermentation; C: *koji* for pure sake at 4°C fermentation; D: *koji* for pure sake at 10°C fermentation. Different lower case letters in same column indicate significant differences by ANOVA ($p < 0.05$).

Lipid oxidation

The total PVs on the samples were measured during the period of fermentation. These were lowest from before fermentation, and it was not observed the increase on it with the passage of the day (Table 6). Next, the total AVs on the samples were determined. It was not observed the increase of the values with the passage of the day (Table 6). That is, it was suggested that the degradation of the fats and oils contained in the samples was not observed during the storage and the fermentation at all.

From an overall analysis of the results obtained, it concluded that it became possible to develop superior quality of highly value-added processed food in terms of overall appearance, color, gloss, thickness, and taste using dorsal muscle of chum salmon in the following condition: muscle (length 30 mm, width 60 mm, thickness 5 mm): *koji* prepared from *koji* mold for the production of pure sake (IV-2): mirin (sweet cooking sake) = 10:5:3, 7% (w/w) table salt, and fermentation at 4°C for 45 days. Still now, chum salmon has been mainly used as a material of the processed foods such as dried salmon, kamaboko (fish-paste product), flake, and soy sauce. However, it also need to produce new processed food using chum salmon, as due to an increased demand of consumers to high qualities of the cooking foods and the processed foods. Therefore, the development of an enhanced value-added processed food such as in the present study may be benefit in household and food and its-related industries in the future.

Conclusion

Enzymes are produced in the *koji* molds improve the properties of foods such as appearance, taste, aroma, and nutritional values. By fermentation treatment using *koji* mold for production of pure sake (IV-2), it can produce a novel and an enhanced value-added processed food with good taste and texture (softness) and rich red color on muscle of chum salmon.

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