Journal of Food and Nutrition Research, 2019, Vol. 7, No. 4, 255-260
Available online at http://pubs.sciepub.com/jfnr/7/4/1
Published by Science and Education Publishing
DOI:10.12691/jfnr-7-4-1

A New Method for Bone Softening and Texture Enhancement of Conger Eel (*Conger myriaster*) Kabayaki

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Received January 06, 2019; Revised February 25, 2019; Accepted March 28, 2019

Abstract The purpose of this study was to develop a processing method to soften intramuscular bones, such as ribs, while maintaining the taste and physical properties of traditional conger eel kabayaki. The steamed product produced by one of the methods for processing kabayaki was evaluated, and it was found that the ribs were hardly softened, and the sensation of foreign bodies in the mouth due to bones was clearly felt. However, the sensory evaluation of the retort products showed that there was no bone-related foreign body sensation, and the ribs of some products were so soft that they could not be detected. In oven-cooked products, the ribs were softened when the oven temperature was set to 140 ℃ and 150 ℃ but not at 130 ℃. Products manufactured in a 150 ℃ oven were found to be overdry on the surface and burnt. The size of the conger eel needed to be less than 253 g to soften the ribs in the 140 ℃ oven (oven-140 product). The moisture content of the retort product was 84.0%, which was relatively higher than that of steamed (65.7%) and oven-140 (66.8%) products. Total free amino acids were the highest when using the retort method to soften bones. The physical properties of conger eel products showed that the oven-140 product is different from the retort product and is similar to the steam product. The sensory evaluation of the products also showed that the sensory properties of the oven-140 product were those of the steamed product but were distinctly different from those of the retort product.

Keywords: *fish bone, softening, texture, conger eel, marinating, kabayaki*

Cite This Article: Jin Gi MIN, Woo Young JUNG, Hong Hee LEE, and Won Kyung LEE, “A New Method for Bone Softening and Texture Enhancement of Conger Eel (*Conger myriaster*) Kabayaki.” *Journal of Food and Nutrition Research*, vol. 7, no. 4 (2019): 255-260. doi: 10.12691/jfnr-7-4-1.

1. Introduction

In Korea, conger eel (*Conger myriaster*) is mainly sold as sashimi or sold and exported as kabayaki processed by marination. In addition to conger eel, freshwater eel such as *Anguilla japonica* is used for kabayaki. Conger eel is caught on the Korean coast, while freshwater eel is mainly cultured. Conger eel kabayaki is generally manufactured in two ways. The first immerses the conger eel fillet in marinade for a certain amount of time and then heats it with steam. The second applies the marinade to the surface of the conger eel fillet, grills it directly, repeating this procedure at least twice. Conger eels are thin cylindrical fish, and their bones are structured with as a backbone, ribs and soft ray. The backbone is easy to remove when preparing conger eel fillet, but small thin ribs are difficult to remove, and thus they sometimes remain in conger eel meat, and complaints are raised from consumers. Although most consumers want the conger eel kabayaki to be manufactured from boneless fillets, removing the ribs completely from the fillets may be an uneconomical activity in the process of manufacturing the kabayaki.

Ribs in conger eel are not softened by traditional manufacturing methods such as steaming and grilling but they do soften under high temperature and high pressure, such as with retort methods [1,2]. Fish bone contains a high amount of calcium and has been developed into a source of calcium mineral supplement [3,4,5]. However, fish bones are limited in their direct application due to their low solubility. The calcium in fish bones is found mainly in the form of hydroxylappatite (HA), which is as difficult to dissolve as calcium-phosphorus [4]. Fish bones can be easily ingested by softening, which is done by adding acetic acid and heating to high temperatures [6].

Retort methods, which are mainly used for fish bone softening, generally decrease the texture, taste, and flavor of fish meat, resulting in lower commercial quality than that of conventional grilled and steamed products.

In this paper, we propose a method to soften the ribs in the eel fillet while maintaining the taste and physical properties of the conger eel kabayaki manufactured by steaming or grilling methods, which are the traditional methods for manufacturing kabayaki.

2. Materials and Methods

2.1. Materials

Conger eels (*conger myriaster*) were purchased at Gijang's fish market in December 2017 and transported to the laboratory within 1 hour. The head, viscera and backbone of the conger eels were immediately removed, washed with distilled water, and then used in the experiment.

2.2. Preparation of the Products

The products were manufactured as three types. The conger eel fillet with ribs was immersed in a 5% acetic acid solution at 5℃ for 24 hours and then steamed at 100℃ for 1 hour to obtain a commercial product, which was designated as the steamed product. The retort product, a traditional bone softening method, was prepared in the same manner as the steamed product, retorted at 115℃ with a controlled pressure of 0.15 MPa for 30 minutes and then cooled. Experimental samples of the third type were prepared by immersing the eel in a 5% acetic acid solution, as in the steamed product, and then heating it a humidity controlled oven (60%) at 130℃, 140℃ and 150℃ for 30 minutes.

2.3. pH and Proximate Components

Ten grams of each conger eel sample was homogenized with 20 mL of distilled water at 12,000 rpm for 1 minute under 5℃. The pH of the homogenate was measured using a pH meter.

Moisture level, crude protein, crude lipid, and ash were determined according to Association of Official Analytic Chemists (AOAC) methods [7].

2.4. Total Volatile Basic Nitrogen (TVB-N)

Two grams of each product was homogenized with 8 mL of 4% trichloroacetic acid (TCA). The mixture was kept at ambient temperature for 30 minutes and then centrifuged at 3,000 rpm for 10 minutes. The supernatant was diluted to 10 mL with 4% TCA and measured for TVB-N according to the method of Hasegawa [8].

2.5. Free Amino Acid

Two hundred ml of 75% ethanol was added to 2 g of each product, homogenized at 10,000 rpm for 5 minutes, and centrifuged at 3,000 rpm for 20 minutes. The precipitate was homogenized again by adding 200 ml of 75% ethanol. Both supernatants were collected and concentrated under vacuum. Five g of perchloric acid (PCA) was added to the mixture, stirred for 30 minutes at room temperature, and then centrifuged at 5,000 rpm for 20 minutes. The supernatant was filtered through a 0.45μm membrane filter and used for analysis. Free amino acid analysis was carried out using an automatic amino acid analyzer (L-8900, Hitachi High Technologies Corp., Tokyo, Japan). The analytical conditions were column size 4 × 150 mm, resin Li + form, lithium citrate buffer (pH 2.85, 3.30, 4.50), flow rate of mobile phase 0.45 ml /min and ninhydrin 0.25 ml / min.

2.6. Texture Analysis

Texture analysis was measured using a texture analyzer (Brookfield, CT3, USA). The shear strength of 20 ribs (see Figure 1) with a diameter of 0.05±0.01 mm was measured using a wire shear plate probe (TA-WSP). The fish meat was measured by a texture profile analysis (TPA) test, which was run on two compression cycles using a 6.35 mm diameter ball probe (TA8) with pretest and posttest speeds of 5 $mm.s^{-1}$ at a 10 kg maximum weight. The shear strength of ribs and the hardness, the cohesiveness and the springiness in the meat were assessed using TexturePro CT software (1.8.31).

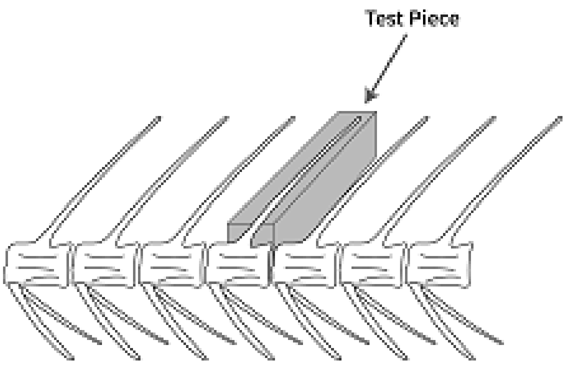

Figure 1. Conger eel ribs used in the experiment.

2.7. Sensory Evaluation

The sensory evaluation of the products was carried out in two ways by 20 panelists of from the staff of Department of Food Science & Engineering, Pukyong National University of Korea. One group investigated the sensory properties of the product against the foreign body sensation, surface dryness, and burnt flavor depending on the oven temperature. Another evaluation compared the sensory characteristics among the oven-140, steamed and retort products. The sensory evaluation of the first group was described as the intensity of each characteristic, and scores ranged from 0 to 5. The foreign body sensation senses the feeling in the mouth felt by the remaining bone in the product, and the surface dryness characteristic indicates the dry state on the surface of the product. The burnt flavor characteristic represents the flavor in the mouth when the product is burned by the heat of the oven. In the second sensory evaluation, the taste and odor of the product were expressed by their intensities, and the texture, color and appearance were indicated by the score of the consumer's preference for the product on a scale of 0 to 9.

2.8. Statistical Analysis

The results from three replications of two trials were subjected to analysis of variance (ANOVA) and Duncan's multiple range test for significant differences at $p < 0.05$ [9].

3. Results and Discussion

3.1. Optimum Softening Condition of Conger Eel Rib

The conger eel fillets were heat-treated in the oven at 130, 140 and 150 ℃ for 30 minutes, and the shear strength of their bones was compared with those of the non-heated (control), steamed and retort products (Figure 2). The shear strength of the bone of the control was 144.9±22.9 g and that of the steamed product was 140.1±17.1 g, which was statistically not different from the control ($p > 0.05$). The shear strength of the bones in the retort product showed the lowest value of 9.9±8.7 g, and some of the bones were so soft that the shear strength could not be measured. The shear strength of the oven-130 product heat-treated in a 130 ℃ oven was 86.0±17.5 g. The shear strength of the oven-140 and oven-150 products were 31.5±14.1 and 28.9±15.8 g, respectively, indicating no significant difference in the shear strength values between the products ($p > 0.05$).

Although fish bones contain high levels of calcium, they are also hard and sharp, so they are rarely eaten as a part of the diet, and most fish bones are disposed of as waste. The calcium in fish bones works as efficiently as calcium carbonate to increase bone mass, and is also known to be nutritionally superior [10,11]. Diets containing whole small fish such as anchovies are nutritionally beneficial with a rich source of calcium. Fish calcium has been shown to be easily absorbed into the body, as was tested in vivo [12]. Fish that have ribs that are difficult to remove,, such as conger eel, should have their bones made physically soft enough to allow the fish meat to be consumed bone-in.

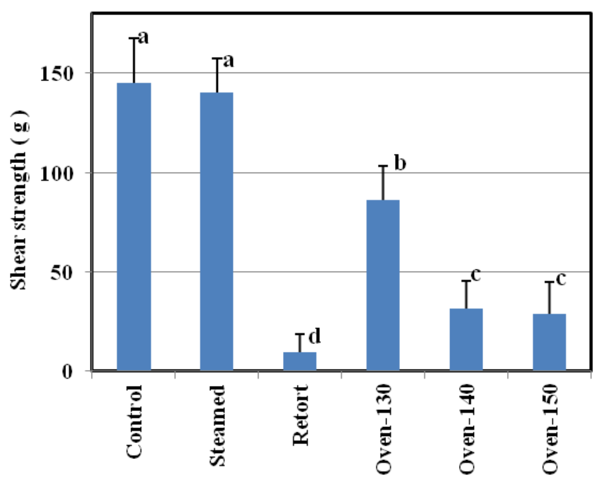

Figure 2. Shear strength of ribs according to the processing methods of conger eel products. Different letters indicate significant differences between processing methods according to Duncan's test ($p < 0.05$)

The bones of the retort product were well softened, and oven-140 and oven-150 products also showed low shear strength values. In the sensory test for foreign body sensation, when the oven-140 and oven-150 products were chewed, there was almost no foreign body sensation in the mouth due to bones (Table 1). However, when the oven-130 product was evaluated, over 90% of the panelists felt a bony foreign body sensation in the mouth. In a foreign body sensation test of the products, 19 of 20 panelists evaluated that the foreign body sensation due to the bones was not felt in the retort product. In the case of steamed products, the intensity of the foreign sensation felt in the mouth was slightly different among the panelists, but almost all the panelists evaluated it as feeling a foreign body caused by the bones. Higher heating temperatures in the oven led to higher surface dryness in the oven product. The oven products were evaluated to have higher surface dryness than the steamed and retort products, although the humidity was adjusted to 60% during the manufacturing process. The oven-150 product was estimated to be more overdry than the oven-130 and -140 products, and the surface dryness score of the oven-150 product was the highest among the products. The burnt intensity of the oven-150 product was very high at 1.5 but was 0.3 and 0.7 for the oven-130 and oven-140 products, respectively, and no burnt flavor was noted in the steam and retort products.

Thus, the most suitable temperature for the oven to soften the ribs while maintaining the merchantability of the conger eel kabayaki was 140 ℃.

Table 1. Sensory characteristics of conger eel products according to processing methods

Products	Foreign body sensation	Surface dryness	Burnt flavor
Steam	4.5 ± 0.6^{a}	1.5 ± 0.6^{a}	0^{a}
Retort	0.3 ± 0.5^{b}	0.5 ± 0.6^{b}	0^{a}
Oven-130	3.3 ± 0.8^{c}	1.2 ± 0.8^{ba}	0.3 ± 0.5^{ab}
Oven-140	1.00 ± 0.9^{b}	1.7 ± 0.5^{b}	0.7 ± 0.5^{b}
Oven-150	1.00 ± 0.9^{b}	3.0 ± 0.6^{c}	1.5 ± 0.8^{c}

Different letters indicate significant differences between processing methods according to Duncan's test ($p < 0.05$).

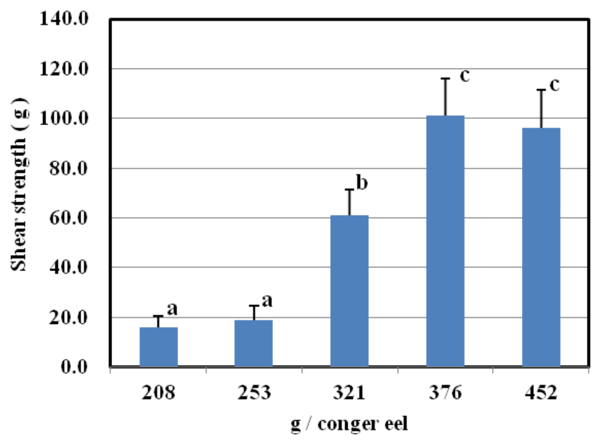

Figure 3. Shear strength of ribs by conger eel size. All conger eel products were processed in a 140 ℃ oven for 30 minutes and the shear strength was measured. Different letters indicate significant differences between processing methods according to Duncan's test ($p < 0.05$)

Eel size is expressed as the number of conger eels that correspond to 1 kilogram when displaying the raw material. The most preferred conger eel size for kabayaki is 4-5 individuals per kilogram or 2-3 individuals per kilogram. Dour to five individuals per kilogram is equivalent to approximately 200-250 grams per conger eel, and 2-3 individuals per kilogram equals 330-500 g per conger eel. Thus, we investigated the degree of softening of the ribs according to conger eel size. The conger eel fillets were processed in the same manner as the "preparation of products" presented in the materials and methods, and then heated in the oven at 140℃ for 30 minutes. The ribs of small conger eels (those less than 253 g each) were soft enough to be eaten with similar shear strength values as those in Figure 1. However, ribs in the muscles of conger eels weighing 321 g or more were shown to have higher shear strength values after heating in a 140℃ oven for 30 minutes (Figure 3). These results indicate that the oven-heating method in the production of kabayaki can be used as a bone softening method to soften the ribs of conger eel that aer equivalent to 4-5 conger eels per kilogram.

3.2. Physico-chemical Characteristics of Conger Eel Products

The proximate composition, pH and volatile basic nitrogen content of conger eel products according to bone softening method are shown in Table 2.

The moisture content of the steam product and the oven-140 product were 65.7 and 66.8%, respectively. The moisture content of the retort product was 84.0%, which is relatively higher than that of steam and oven-140 products. The lower moisture content in the steam and oven-140 products was attributed to the evaporation of water in the fish during the manufacturing process. The high moisture content in the retort product can be explained by the fact that the conger eel fillet was retorted in a sealed retort pouch and therefore retained moisture in the product after the retort.

Crude protein, crude lipid and ash content were also approximately twice as high in steam and oven-140 products than in retort products. The differences in these contents seemed to be due to the high moisture content of the retort product.

We immersed conger eel fillets in 4% acetic acid solution for 24 hours to promote fish bone softening. It is known that fish bones immersed in acetic acid are softened more rapidly when cooked than those immersed in water, it is also known that the higher the acetic acid concentration is, the shorter the cooking time required to soft fish bones must be [6,13,14]. Acetic acid added to soften bones of fish gives a sour taste to the conger eel product, and excessive sour taste in the product has the effect of lowering product palatability. Therefore, pH was measured for each conger eel product according to the manufacturing method. The pH values of the steam, retort and oven-140 products were not significantly different from each other.

Table 2. The proximate composition, pH and TVB-N of conger eel products according to processing methods for bone softening

Items	Bone softening methods		
	Steam	**Retort**	**Oven-140**
Moisture (%)	65.7	84.0	66.8
Crude protein (%)	31.2	14.4	30.5
Crude lipid	2.0	1.2	1.8
Crude ash	1.0	0.4	0.9
pH	4.0	4.3	4.1
TVB-N(mg%)	5.6	8.2	8.6

Total volatile basic nitrogen (TVB-N) includes trimethy; lamine (TMA), dimethylamine (DMA), ammonia and other amines related to the spoilage of aquatic products [15,16]. TMA and DMA are generated from trimethyl amine oxide (TMAO), and TMAO is present in large amounts in fish. The temperature - induced TMA production in the fish's ordinary muscle occurs at temperatures above 80℃, and the increase in TMA concentration increases in proportion to the cooking time. It is known that high concentrations of ammonia, DMA and TMA are produced during the processing of herring at 120℃ for 5 hours [17,18].

The TVB-N of the steam product was 5.6 mg / 100 g, while that of the retort and oven-140 products were 8.2 and 8.6 mg / 100 g, respectively, which were slightly higher than that of the steam product. These results may be related to the processing temperature because the steam product was processed at a relatively low temperature in the production of conger eel products. In our experiment, the TVB-N content of the oven-140 product was approximately 60% higher than that of the steam product, a traditional conger eel kabayaki product, but it was much lower than that of the generally unacceptable products [15,20].

3.3. Free Amino Acids

The free amino acid content of conger eel products according to bone softening method is shown in Table 2. The free amino acids contents of taurine, arginine, and phosphoserine were the highest, regardless of the bone softening method of the conger eel products. However, small amounts of free amino acids such as tyrosine, serine and threonine were detected, and no proline was detected.

Table 3. Free amino acid content of conger eel products on the bone softening methods (mg/100 g, dry weight)

Items	Bone softening methods		
	Steam	Retort	Oven-140
Phosphoserine	44.3	82.5	53.0
Taurine	98.0	107.6	96.4
Aspartic acid	4.7	32.4	9.6
Threonine	4.7	5.2	6.0
Serine	4.8	7.3	7.2
Glutamic acid	9.3	12.8	13.3
Proline	-*	-	-
Glycine	10.5	12.2	12.0
Alanine	8.2	14.9	16.9
Citrulline	16.3	37.6	15.7
α-amino-n-butyric acid	8.2	19.6	9.6
Valine	9.3	15.0	13.2
Methionine	5.8	7.5	6.1
Isoleucine	3.5	6.3	5.8
Leucine	7.0	7.6	10.8
Tyrosine	1.7	3.8	2.4
Phenylalanine	14.0	30.1	18.2
Lysine	14.2	17.3	22.9
Histidine	26.8	45.0	32.1
Arginine	54.8	77.7	67.5
Total	345.8	542.5	418.9

*; not detected.

The conger eel product also contained a significant amount of citrulline, which is known to be useful for increasing exercise capacity and relieving postexercise muscle pain when anaerobic high-intensity exercise is performed. Citrulline is a key intermediate of the urea cycle, a pathway in which mammals convert urea and release ammonia, and it is also produced as a byproduct of the production of nitric oxide synthase from arginine but is not involved in proteins [21,22].

Conger eel has different free amino acid contents due to seasonal effects and the free amino acid content of conger eel caught in summer and winter are approximately 1.5-2 times higher than that of those caught in spring and autumn [23].

Free amino acids except threonine, lysine, leucine and glutamic acid were found to be higher in retort products than in the steam or oven-140 products. Additional, total free amino acids were also highest when using the retort method to soften bones. The total free amino acid content of the oven-140 product was approximately 21% greater than that of the steam product.

3.4. Texture Profile Analysis for Meats of Conger Eel Products

Bone-soft conger eel product is susceptible to changes in the physical properties of the fish meat due to added organic acids and processing at high temperatures and pressures. Therefore, we observed changes in the physical properties of conger eel meat depending on the method of bone softening, and these characteristics are shown in Table 4.

The hardness of conger eel meat was not significantly different between the steam and oven-140 products (1,128 and 1,267 g, respectively), but it was 785 g in retort products, the lowest among three products. Generally, when fish meat is processed at temperatures higher than 100 ℃, the hardness of the fish meat decreases as the heating temperature and the time are increased [24,25,26]. In our experiments, the lower hardness of the retort product processed at 115℃ compared to that of the oven-140 product processed at 140℃ may be related to the higher moisture content in the retort product.

Table 4. Textural characteristics of conger eel meat by bone softening methods

Items	Bone softening methods		
	Steam	Retort	Oven-140
Hardness (g)	1,128±176[a]	785±85[b]	1,267±84[a]
Cohesiveness	0.30±0.12[ab]	0.21±0.08[a]	0.40±0.05[b]
Springiness	3.86±0.21[a]	2.30±0.44[b]	3.67±0.15[a]

Different letters indicate significant differences between processing methods according to Duncan's test ($p < 0.05$).

The cohesiveness of retort and steam products was not significantly different among the products ($p > 0.05$). However, the cohesiveness of the oven-140 product was clearly different from that of the retort products ($p < 0.05$). The springiness also showed similar values between the steam (3.86) and oven-140 (3.67) products ($p > 0.05$), but lower values in the retort product at 2.30 ($p < 0.05$). The physical properties of conger eel products indicate that the oven-140 product is different from the retort product but similar to the steam product.

3.5. Sensory Evaluation

The sensory evaluation of the conger eel products according to the bone softening method is shown in Table 5. Umami taste, one of the typical taste characteristics of sea food products, was slightly higher in the steam and oven-140 products than in retort products in steam and oven-140 products, and there was no significant difference in sour taste among all products ($p > 0.05$). Conger eel sashimi and related products are mainly affected by the content of flavor components such as free amino acids, fatty acids and nucleic acid related compounds, and these components have been reported to have different concentrations depending on the habitat environment and fishing season of the conger eel [27,28]. Free amino acid content was higher in retort products than in steam and oven-140 products, but higher free amino acid content in retort products did not seem to affect umami taste. The oven-140 product still had a slight burnt flavor, and the fishy flavor was somewhat higher in the retort products, but the value was not high.

The texture of conger eel products was similar to that of the TPA test, and the sensory preference of steam and oven-140 products was better than that of retort products.

The color evaluation showed that the oven-140 product had a high score, and the color of these products was somewhat darker than the retort products due to the browning. The browning of such products may be the result of a Maillard reaction, and it was thought that the browning of the product resulted in an increase in the color preference of the product by the panelists.

Table 5. Textural characteristics of conger eel meat by bone softening methods

Items	Bone softening methods		
	Steam	Retort	Oven-140
Taste			
Umami	7.80±0.49[a]	6.91±0.35[b]	7.93±0.60[a]
Sour	2.33±0.57[a]	2.18±0.59[a]	2.10±0.45[a]
Odor			
Burnt	0.77±0.27[a]	0.73±0.36[a]	2.35±0.42[b]
Fishy	2.13±0.27[a]	3.33±0.43[b]	2.08±0.48[a]
Texture			
Chewiness	8.15±0.74[a]	7.08±0781[b]	8.05±0.79[a]
Hardness	7.71±0.66[a]	6.17±0.99[b]	7.33±0.68[a]
Color	6.98±0.76[a]	5.52±0.71[b]	7.27±0.52[a]
Appearance	6.37±0.74[a]	5.83±0.76[a]	6.02±0.72[a]

Different letters indicate significant differences between processing methods according to Duncan's test ($p < 0.05$).

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