Improvement in Raw Meat Texture of Cultured Eel by Feeding of Tochu Leaf Powder†

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The muscle of eels fed tochu (Eucommia ulmoides Oliver) leaf powder was 1.8 times harder than the control. The component analysis showed no difference in moisture, lipid, or protein content between the muscles of the control and the tochu-fed eels. Not only the extracted neutral fat but also the compound fat of the raw muscles of the tochu-fed eel and the control had the same TLC patterns and fatty acid composition by gas chromatographic analysis. There was a great difference between the tochu-fed eel and the control concerning the amount of muscle protein stroma fraction which mainly consisted of collagen. The microscopic observation showed that the perimysium and endomysium which were the main components of the stroma fraction of the muscle of the tochu-fed eel were firm and thick compared to those of the control. These findings suggested that the intake of tochu leaf powder hardened the muscle.

The bark of tochu tree (Eucommia ulmoides Oliver) has been known as a herb medicine for its effects such as strengthening the intestinal organs, heart, bone, and muscles and preventing senescence.1) Recently, dried tochu leaf was examined for its medical effects. White laying hens fed tochu leaf powder had a larger average oviposition rate than normal hens.2) Mice fed tochu leaf powder synthesized protein more actively.3) These findings suggested that tochu had some effects on animals. This time, we examined the effects of feeding of tochu leaf powder to strengthen the muscles of cultured eel.

Today, the amount of aquacultural production of fishes amounts to about 300 thousand tons annually, and cultured eels are almost 100% of the total eel production.4) Meanwhile the aquacultural products have been commonly considered to be inferior in quality, especially in texture. On the other hand, the effect of swimming exercise on retardation of weakening of postmortem fish muscle was reported as a method of improving the texture.5) Exercise resulted in the increase of muscle weight, mean muscle fiber diameter, and area of muscle.6) Meanwhile, restricted movement, immobilization, and space flight caused muscle atrophy.7) These reports suggest that exercise might change the muscle tissue, which might cause improvement of meat texture. As for the swimming movement, the musculature of the fish with a flexible body comprises a high proportion of collagen and muscle collagen contributes to the toughness of the sliced raw meat.10) Tochu has been known to make muscle firm.11) Therefore, it was expected to have an effect on the muscle and texture of tochu fed eel meat. In this paper, we examined the effects of the tochu leaf powder on the eel meat and the relationship between the meat texture and eel constituents.

Materials and Methods

Materials. Thousands of eels (Anguilla japonica) were fed a commercial diet for 13 months and eels each weighing less than 200 g were separated into two groups at Hamamatsu, Shizuoka Prefecture. One group was fed a commercial diet containing 2.5% (w/w) dried tochu (Eucommia ulmoides Oliver) leaf powder for the next month. The other group was fed only the commercial diet as a control for the next month. Live specimens which weighed about 200 g were transported from Hamamatsu, Shizuoka Pref. to our laboratory and were filleted and samples were taken from a point 5 cm from the gill at a width of about 10 cm. The dorsal parts of these samples were used for the experiments.

Firmness of eel muscle. Sample blocks (10 x 10 x 5 mm) of the raw muscle were obtained by cutting a strip (10 x 10 mm) parallel to the body axis of the eel and cutting 5 mm in thickness using a sharp thin razor blade. The firmness was measured by a texturometer (Zenken Co., Model GTX-2, General Foods type) with a 50 mm/aluminum plunger pressing the sample blocks from the dorsal to the ventral part. The measurement conditions were as follows: 2.0 mm clearance, 5 or 2 V, and chart speed 750 mm/min.

Measurement of moisture, crude fat, and crude protein contents. Eels muscles were analyzed for moisture, crude fat, and crude protein using standard AOAC methods for meat and meat products.11) Theer compositions of lipid. Neutral fat was extracted with ether from the homogenized and freeze dried eel muscle. The extract was evaporated and separated by silica gel thin-layer chromatography (Merck, HPTLC-plates silica gel 60 F254, hexane–ether–acetic acid (80:20:1). For detection of lipids, thin-layer plates were exposed to I2 vapor. Compound lipid was extracted with chloroform–ether (1:1) from the homogenized and freeze-dried samples. The extract was evaporated and separated by silica gel thin-layer chromatography (Merck, HPTLC-plates silica gel 60 F254), with chloroform–methanol-acetic acid-H2O (25:15:4.2). Thin-layer plates were exposed to I2 vapor for detection of lipids.

Composition of fatty acid. Lipid was extracted with chloroform–methanol (2:1) from the homogenated and freeze-dried eel muscle. The extract was evaporated, saponified with KOH and ethanol, neutralized, and esterified with KOH and ethanol. The fatty acid composition was analyzed by gas chromatography (Shimadzu GC-3BT).

Composition of muscle protein and hydroxyproline content. Eel muscle protein was fractionated by a regular procedure.13) All the operations were done in a cold room at 4°C as quantitatively as possible. The outline is as follows. To 10 g of each muscle was added 100 ml of I = 0.05 phosphate buffer, pH 7.5, (15.6 mM Na2HPO4, 3.5 mM KH2PO4) homogenized with an Ultra-Turrax homogenizer. The homogenate was centrifuged at 5000 x g for 15 min. To the residue was added 100 ml of the same buffer, and the mixture was homogenized and centrifuged again. The supernatants were combined and trichloroacetic acid was added up to 5%. The resulting precipitate was collected by filtration and used as the sarcoplasmic protein fraction. The filtrate was used as the non-protein N containing compound fraction. The above residue was homogenized as above in I = 0.05 buffer

† Studies on the Efficacy of Tochu Leaf on Muscle Protein. Part I.
with 10 vol. of \( I=0.5 \) KCl phosphate buffer, pH 7.5, (0.45 M KCl, 15.6 mM Na\(_2\)HPO\(_4\), 3.5 mM KH\(_2\)PO\(_4\)). The two supernatants were combined and used as the myofibrillar protein fraction. The residue was exhaustively extracted overnight with 0.1 M NaOH under stirring. The mixture was centrifuged and the supernatant was used as the alkali-soluble protein fraction. The final solution was used as the stroma protein fraction. The protein and non-protein N containing fractions provided as above were analyzed for nitrogen by the micro-Kjeldahl method, and the protein composition was calculated using 6.25 as the conversion factor. The hydroxyproline contents were measured by colorimetry by the method of Kivirikko \textit{et al.}\textsuperscript{13} from muscle hydrolyzate by 6 N HCl at 100°C for 24 h.

Table I. Muscle Compositions of Eel Fed the Diet with and without Tochu Leaf Powder

<table>
<thead>
<tr>
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<th>Eel</th>
<th>Water (%)</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
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<tbody>
<tr>
<td>C</td>
<td></td>
<td>63.9 ± 0.6(^*)</td>
<td>17.1 ± 1.7</td>
<td>17.6 ± 0.9</td>
</tr>
<tr>
<td>T</td>
<td></td>
<td>64.2 ± 0.7</td>
<td>16.9 ± 0.8</td>
<td>17.0 ± 0.3</td>
</tr>
</tbody>
</table>

\(^*\) Mean ± SE.

Differences were not significant between eels fed the diet with (T) and without tochu leaf powder (C) in Student's t-test at \( p < 0.05 \). Each group consisted of 5 eels.

Table II. Firmness of Eel Raw Muscle Fed the Diet with and without Tochu Leaf Powder

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>T</th>
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<tbody>
<tr>
<td></td>
<td>0.569 ± 0.045 kg(^*)</td>
<td>1.009 ± 0.062 kg</td>
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</table>

\(^*\) Mean ± SE.

Differences were significant between eels fed the diet with (T) and without tochu leaf powder (C) in Student's t-test at \( p < 0.001 \).

Each group consisted of 4 eels, and the firmness of each muscle was measured twice.

**Microscopic observation.** The samples for microscopic observation were obtained by fixing the raw eel muscles cut in the same way as described in Materials with a pH 7.0 phosphate buffer containing 10% formaldehyde, dehydrating, embedding, and sectioning in the direction of crosscutting the muscle bundle as usual. The samples were stained with alcoholic hematoxyline and eosin.

**Statistical analysis.** Statistical analysis was done on data using Student's t-test.\textsuperscript{14}

**Results and Discussion**

Eels were fed a commercial diet containing 2.5% dried tochu leaf powder or only a commercial diet as a control. The amount of tochu leaf powder added to the diet was 2.5% because in a preliminary experiment the meat texture was the same in the eels fed a diet containing 2.5% and 5.0% tochu powder. The meat of the tochu-fed eels did not show any obvious differences compared to the control in moisture content, lipid content, or crude protein content (Table I). These values were similar to those obtained on eels in the Standard Tables of Food Composition in Japan.\textsuperscript{15} On the other hand, the texturometer analysis of the raw muscle showed that the firmness of the meat of the tochu-fed eel was 1.8 times that of the control (Table II). Tochu seemed to be effective for making the muscle firm.

![Thin-layer Chromatograms of Eel Fats](image)

**Fig. 1.** Thin-layer Chromatograms of Eel Fats.

The fats were extracted with ether (A) or chloroform–methanol (B) from homogenates of eels fed the diet with (T) and without tochu leaf powder (C). TG, triacylglycerol; DG, diacylglycerol; MG, monoacylglycerol; NL, neutral fat; PE, phosphatidylethanolamine; PC, phosphatidylycholine; SM, sphingomyelin. For conditions, see Materials and Methods.

![Gas Chromatograms of the Fatty Acids from the Muscle of Eels Fed the Diet with and without Tochu Leaf Powder](image)

**Fig. 2.** Gas Chromatograms of the Fatty Acids from the Muscle of Eels Fed the Diet with and without Tochu Leaf Powder.

The experimental conditions were as follows. Column, DEG 10% (3 mm × 2 m); injection temp, 144°C; column temp., 132°C; carrier gas, He (25 ml/min); detector, FID.
This difference should be due to the constituents of eel meat other than moisture content, lipid content, or crude protein content.

The difference in the texture between aquacultured and wild horse mackerel was defined to be due to the amount of lipid. Therefore, the lipids and fatty acids of eel muscle were analyzed. The extracted neutral fat of the meat showed the same TLC patterns for the tochu-fed eel and the control and seemed to be composed mostly of triglycerides (Fig. 1A). The extracted compound fat showed the same TLC patterns for the tochu-fed eel and the control and seemed to consist of phosphatidylethanolamine, phosphatidicholine, and sphingomyelin (Fig. 1B). The compositions of fatty acid of the lipids extracted from the meat of the tochu-fed eel and the control were examined by gas chromatography. The chromatography patterns were the same for the tochu-fed eel and the control (Fig. 2). These findings suggest that the firm texture of tochu-fed eel meat originates from the constituents other than fat.

The analysis of the muscle protein compositions showed differences between tochu-fed eel and the control in the myofibrillar fraction and stroma fraction (Table III). Either of the two should influence the firmness of muscle. The amount of the myofibrillar protein of the tochu-fed eel was smaller than that of the control. The smaller quantity of myofibrillar protein cannot explain the firmer muscle in the tochu-fed eel, and the reason for the muscle firmness should be sought in other fractions. The combined amount of the alkali-insoluble fraction ascribable to the denatured sarcoplasmic and myofibrillar protein to the myofibrillar fraction for the tochu-fed eel was still smaller than that of the control. These fractions might not be responsible for

<table>
<thead>
<tr>
<th>Fraction</th>
<th>C</th>
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<tr>
<td>Non-protein N</td>
<td>$10.5 \pm 0.5%$*</td>
<td>$10.8 \pm 0.4%$</td>
</tr>
<tr>
<td>containing</td>
<td></td>
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</tr>
<tr>
<td>Sarcoplasmic</td>
<td>$25.0 \pm 0.7%$</td>
<td>$24.8 \pm 0.9%$</td>
</tr>
<tr>
<td>Myofibrillar**</td>
<td>$52.4 \pm 0.3%$</td>
<td>$43.5 \pm 1.8%$</td>
</tr>
<tr>
<td>Alkali-soluble</td>
<td>$4.5 \pm 0.3%$</td>
<td>$9.0 \pm 1.6%$</td>
</tr>
<tr>
<td>Stroma*</td>
<td>$7.0 \pm 0.5%$</td>
<td>$11.4 \pm 1.3%$</td>
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</table>

Hydroxyproline content* $0.80 \pm 0.03$ $1.04 \pm 0.05$ (mg/wet g)

* Mean ± SE.

** Differences were significant between stroma proteins from eel muscle fed the diet with (T) and without tochu leaf powder (C) and between hydroxyproline contents in Student's $t$-test at $p < 0.05$ (*) and between myofibrillar proteins in Student's $t$-test at $p < 0.01$ (**). Each group consisted of 3 eels for protein composition and 4 eels for hydroxyproline content.

Fig. 3. Microphotographs of Muscle of Eels Fed the Diet with and without Tochu Leaf Powder. C, control; T, Tochu-fed eel. Bars represent 0.8 mm. For conditions, see Materials and Methods.
The muscle firmness of tochu-fed eel.

The quantity of muscle protein of the tochu-fed eel at stroma fraction was 1.6 times higher than that of the control (Table III). The stroma fraction mainly consists of collagen. The collagen content is estimated from the amount of hydroxyproline and Table III showed that the amount of hydroxyproline in the muscle was 1.3 times higher for the tochu-fed eel. Collagen was once considered to be responsible for the toughness of roasted cattle meat.17 Today, it is regarded that the toughness of old cattle meat is due to the quantity and quality of the cross-linking of collagen.17,18 The collagen of young cattle contains less cross-linking and is more soluble than that of old cattle.18,19 These findings suggest that the differences in the quality affect the softness of young cattle roasted meat and the relation between meat texture and collagen. Even in regard to raw fish meat texture, many works refer to collagen. The degradation of raw fish meat by compression starts at the connective tissue.20 The tenderized fish meat stored in a refrigerator showed the degradation of connective tissue while the muscle fiber retained its continuity.21 The tenderization of fish muscle proceeded independently of rigor mortis.22 Sato et al. reported that the V type collagen of fish meat was specifically solubilized by cold storage and they suggested that the tenderization proceeded with the degradation of connective tissue.23 These findings indicate the relation of meat texture and collagen. Hatae et al. reported from the experiment using five kinds of fishes that raw fish meats that contain much collagen were firm compared to meats containing less collagen.24 Sato et al. reported that the muscle collagen contributed to the toughness of the sliced raw meat and that, in the case of six fishes whose muscle collagen contents were low, the texture of raw meat was tender.10 The exercise resulted in the increase of collagen content and made fish meat firm.5 These findings suggest that the increase of collagen content makes meat firm and eel raw meat become firm because of increasing collagen content. The microscopic observation of eel muscles also showed that the perimysium and endomysium surrounding muscle bundles and muscle fibers, respectively, of the tochu-fed eel were firmer and thicker than those of the control and that the size of the muscle bundle was the same (Fig. 3). This supported the hypothesis that the increase of collagen content made the tochu fed eel meat firm.

These findings suggest that tochu leaf powder makes cultured eel muscle firmer and that collagen is responsible for the firmness of the muscle which is in agreement with the effect of the bark of tochu tree to strengthen the muscles. The feeding of tochu leaf powder may offer a new way to improve the quality of cultured fishes. The mechanism by which tochu increases collagen is under investigation.

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References