Variations of SH Content and Kamaboko-Gel Forming Ability of Shark Muscle Proteins by Electrolysis

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The variations of SH content and kamaboko gel-forming ability of muscle protein by electrolysis were examined using ground shark, Charcharhinus japonicus. Sarcoplasmic and myofibrillar proteins of 0.5-0.8 mg/ml were electrolyzed for up to a maximum of 5 h at 5.5 mA/cm² and the contents of total SH (T-SH) and the reactive SH (R-SH) were measured. The content of R-SH increased with the duration of electrolysis, although those of T-SH did not vary. On the other hand, the pH value of the protein solution at the cathode side, increased with the time duration of electrolysis. Consequently, the increase in content of R-SH was found to be dependent on the exposure of the SH group which was buried inside the molecule by the raised pH, not by the reduction of the SS bond. The kamaboko gel-forming ability of the electrolyzed shark mince was unable to be improved by electrolysis, as evidenced by the results of both puncture and folding tests.

1 Introduction

Kamaboko, one of the traditional and popular seafood products in Japan, is made by heating the minced fish meat kneaded with a small amount of salt. The quality of kamaboko is chiefly evaluated by the strength of ash, namely the total assessment of rigidity, elasticity, crispiness, and texture. The ash depends mainly on the property of network structure of myofibrillar proteins. The formation of the network structure has been reported by many workers to be mainly attributed to the cross linkage between myofibrillar proteins through disulfide bond (SS bond), hydrogen bond, and/or hydrophobic bond. On the basis of the SS bond theory, we deduced following hypothesis: If the free or highly reactive SH groups can be increased in number by exposing the SH groups which are buried inside the myofibrillar protein molecules to the surface, the strength of ash may be enhanced.

In this report we describe the variation of SH content by electrolysis and kamaboko-gel
forming ability of electrolyzed mince of shark.

2 Materials and Methods

2.1 Preparation of Sarcoplasmic and Myofibrillar Proteins, and Minced Muscle

The fresh muscle of ground shark, *Charcharodus japonicus*, was used for the experiments. Sarcoplasmic and myofibrillar proteins were prepared according to the methods of Nishioka et al. as shown in Fig. 1. They were dissolved in a NaHPO₄-KH₂PO₄ buffer (pH 7.0, I=0.1) and 0.6 M KO/N₂HPO₄-KH₂PO₄ buffer (pH 7.0, I=0.1), respectively, to a concentration of 0.5-0.8 mg protein/ml.

To evaluate the effect of electrolysis on the kamaboko-gel forming ability of the electrolyzed muscle, shark muscle specimen was minced in a chopper (pore size 5 mm in diameter), and the mince was washed three times with five volumes of water.

Two sample protein solutions and the mince thus prepared were submitted for the electrolysis as described below.

![Diagram of Apparatus used for the electrolysis of shark muscle proteins](image)

**Fig. 2.** Apparatus used for the electrolysis of shark muscle proteins.

2.2 Electrolysis Method

Sarcoplasmic and myofibrillar sample proteins were electrolyzed according to the method of Kamesaya and Miura as shown in Fig. 2.

The protein solution is a vessel at the cathode side was connected to the anode by a salt bridge (2% agar-0.4 M NaCl), and a direct current was applied between them for up to a maximum of 5 h at 1.4 mA/cm². Platinum plates were employed as electrode.

The minced shark muscle was also electrolyzed in the same manner as above described for a maximum time of 5 h at 1.4 mA/cm². During electrolysis, the mince was stirred slowly with a powerful stirrer (Nittoku Kagaku Co., model 600G).

2.3 Measurement of SH Content

The SH group exposed on the surface of the protein molecule by electrolysis was defined as a reactive SH group (R-SH). Amount of R-SH was determined according to the Ellman's Method; A 0.03 ml of 10mM 5,5'-dinitrobenzoiic acid, DTNB in abbreviation, was added to 4.5 ml of the diluted sample protein solution, and the mixture was cooled for 1 h at 5°C. Amount of the R-SH was determined from the absorbance at 412 nm using a molecular extinction coefficient of 13,600 for the thioarbitrulate anion.

The content of total SH (T-SH) was determined according to the Butkus' method. A protein sample solution in 6 M urea was made by adding 8 M urea, and 0.03 ml of 10 mM DTNB was added to 4.5 ml of the protein solution and subsequently all-well to stand (or 15 min at 40°C). The T-SH was determined spectrophotometrically as described above.

The amount of protein was determined by the micro Buret method.

2.4 Preparation of Kamaboko

As the pH of the mince increased to 8.8 by electrolysis, the mince was adjusted to pH 7 by adding 1 N HCl and was allowed to stand for 2 h. After the mince was dehydrated with a lever-type press, the water content was adjusted to 80%. The hydrated mince was ground with 3% NaCl (30 min), and the paste thus obtained was packed into a polyvinylidene film casing 30 mm in diameter. All the procedures were carried out at 5°C. The paste was heated at 80°C for 30 min.

2.5 Evaluation of Ashi of Kamaboko

The ash of kamaboko was evaluated by a puncture and a folding test. In the puncture test, kamaboko samples which had advance soaked overnight at 5°C were cut into pieces of 3 mm thickness and 3 cm diameter. The breaking force, the force required to penetrate a spherical plunger of 5 mm in diameter into the test piece, was measured with a rheometer (Fudoh Kogyo Co., model NRM-2002), at a rate of 2 cm/min.

On the other hand, the folding test was performed according to the standards of 'Noguchi and Matumoto' by using a slice of 5 mm in thickness. The folding scores were graded as follows: AA, no crack when folded into quadrants; A, no crack when folded in half; B, a crack when folded in half; C, breakage into two pieces when folded in half.

3 Results and Discussion

3.1 Variations of the SH Content and pH during Electrolysis

The SH contents of R-SH and T-SH, and the pH values are shown in Fig.3.

The content of T-SH of sarcomplasmic protein was slightly higher than that of myofibrillar protein but the content of R-SH was about the same level between sarcomplasmic and myofibrillar proteins. The contents of T-SH and R-SH of sarcomplasmic protein were 8.5 and 8 times higher than that of bovine serum albumin (BSA), respectively. The content of T-SH was nearly the same level during electrolysis in either sample protein, being about 8 moles/10⁶ g for sarcomplasmic protein, and 6 moles/10⁶ g for myofibrillar protein. The content of R-SH, on the contrary, increased after 4 h of electrolysis. These findings were almost the same as those of BSA, though the content of R-SH of BSA increased more rapidly with the time of electrolysis.

The increment of R-SH after 5 h of electrolysis was approximately 1.0 x 10⁶ g for both sarcomplasmic and myofibrillar proteins.

The pH value increased with the duration
of electrolysis time in both protein solutions. The rates of pH increase were approximately the same as in either protein, and were higher than that of BSA\textsuperscript{15}. This might be attributed to the lower protein concentration, being 6 to 9 times lower than that of BSA.

3.2 Evaluation of Ashi of Kamaboko made from Electrolyzed Mince

The content of R-SH increased slightly after 5 h of electrolysis as shown in Fig. 3, and therefore small amount of R-SH which was buried inside the protein molecules was exposed by electrolysis. Therefore, the ash of kamaboko made from electrolyzed mince was expected to be improved, assuming that the SS bond forms a strong cross-linkage among myofibrillar proteins and creates the network structure. The kamaboko prepared from the electrolyzed mince was evaluated, and the scores on the evaluation of Ashi are shown in Table I, together with the data on moisture and pH.

As the pH of the mince was increased to 8.80 by electrolysis, the R-SH might be increased in amount as suggested in Fig.3. Both puncture force and scores on the folding test for kamaboko made from electrolyzed mince were almost the same as those from non-electrolyzed mince. That is, the electrolysis of mince did not improve the elasticity of kamaboko.

Table 1. Evaluation of ash of kamaboko gel made from electrolyzed shark muscle mince

<table>
<thead>
<tr>
<th>Sample mince</th>
<th>Puncture force(\times 10^3)</th>
<th>Folding test</th>
<th>Water content in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrolyzed</td>
<td>190 ± 30</td>
<td>83.2</td>
<td>7.35</td>
</tr>
<tr>
<td>Non-electrolyzed</td>
<td>190 ± 37</td>
<td>83.4</td>
<td>7.42</td>
</tr>
</tbody>
</table>

* Five test pieces of kamaboko sample were employed for the puncture test.

Fig. 1. Variations of SH content and pH by electrolytic time in shark muscle proteins.

Electrolysis of Shark Muscle Proteins

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<th>References</th>
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电気分解によるサメ肉のSH含ハ量とかまほこ形成能の変化

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電気分解（電解）によって魚肉筋肉タンパク質のSH基含量とかまほこ形成能がどのように変化するかを調べた。メジョンサメから筋肉タンパク質を粉末研磨し、これらの各粉末（0.5-0.8 ng タンパク質/g）に5 ml/cm\textsuperscript{2}で酸素5時間電解した。全SH基（T-SH基）含量と亜硫酸基および硫酸基の含量を調べた。その結果、電解に伴るR-SH基の増加が観察されなかった。T-SH基の増加の原因はSS結合の遊離ではなく、電解による硫酸基の酸化が示唆された。一方、サメ筋肉ホモジェネートを電解してかまほこを調製しても、アルフレームの向上は認められなかった。