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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/m*l* were electrolyzed for up to a maximum of 5 h at 5.5 mA/cm^2, and the contents of total SH (T-SH) and the reactive SH (R-SH), namely exposed SH on the surface of the protein, were determined during electrolysis. 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 evaluated 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 ashi, namely the total assessment of rigidity, elasticity, crispiness, and texture. The ashi depends mainly on the property of network structure of myofibrillar proteins. The formation of the network structure has been reported by many workers[1－7] 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 ashi may be enhanced.

In this report we describe the variation of SH content by electrolysis and kamaboko-gel

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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, *Charcharhinus japonicus*, was used for the experiments. Sar-coplasmic and myofibrillar proteins were prepared according to the methods of Nishioka et al.[8] as shown in Fig. 1. They were dissolved in a Na_2HPO_4-KH_2PO_4 buffer (pH 7.0, I=0.1) and 0.6 M KCl/Na_2HPO_4-KH_2PO_4 buffer(pH 7.0, I=0.1), respectively, to a concentration of 0.5-0.8 mg protein/m*l*.

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.

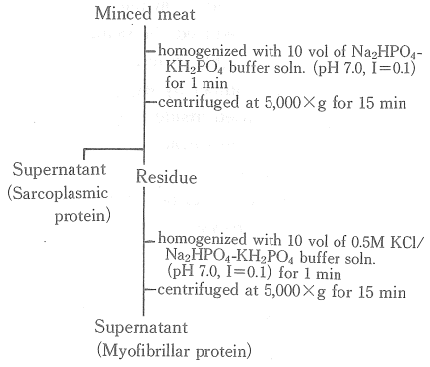

Fig. 1. Preparations of sarcoplasmic and myofibrillar proteins from ground shark muscle[8].

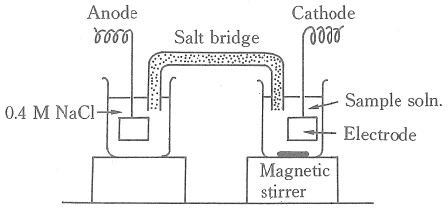

Fig. 2. Apparatus used for the electrolysis of shark muscle proteins.

2.2 Electrolysis Method

Sarcoplasmic and myofibrillar sample proteins were electrolyzed according to the method of Komeyasu and Miura[9] as shown in Fig. 2.

The protein solution in 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 5.5 mA/cm^2. 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/cm^2. During electrolysis, the mince was stirred slowly with a powerful stirrer(Nittoh 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[10] ; A 0.03 m*l* of 10mM 5,5'-dinitrobis(2-nitrobenzoic acid), DTNB in abbreviation, was added to 4.5 m*l* of the diluted sample protein solution, and the mixture was cooled for 1 h at 5 ℃. Amount of the R-SH was determined from the absorbance at 412 nm using a molecular extinction coefficient of 13,600 for the thiophenolate anion.

The content of total SH (T-SH) was determined according to the Buttkus' Method[11]. A protein sample solution in 6 M urea was made by adding 8 M urea, and 0.03 m*l* of 10 mM DTNB was added to 4.5 m*l* of the protein solution and subsequently allowed to stand for 15 min at 40 ℃. The T-SH was determined spectrophotometrically as described above.

The amount of protein was determined by the micro Biuret method[12].

2.4 Preparation of Kamaboko

As the pH of the mince increased to 8.80 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 86%. The dehydrated mince was ground with 3% NaCl for 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 ℃. The paste was heated at 80 ℃ for 30 min.

2.5 Evaluation of Ashi of Kamaboko

The ashi of kamaboko was evaluated by a puncture and a folding tests. In the puncture test, kamaboko samples which had in advance cooled overnight at 5 ℃ were cut into pieces of 3 cm 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-2002J), at a rate of 2 cm/min.

On the other hand, the folding test was performed according to the standards of Noguchi and Matumoto[13] 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 sarcoplasmic protein was slightly higher than that of myofibrillar protein, but the content of R-SH was almost the same level between sarcoplasmic and myofibrillar proteins. The contents of T-SH and R-SH of sarcoplasmic protein were 8.5 and 8 times higher than that of bovine serum albumin (BSA)[14], respectively. The content of T-SH was nearly the same level during electorolysis in either sample protein, being about 8 moles/10^5 g for sarcoplasmic protein, and 6 moles/10^5 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 2 moles/10^5 g for both sarcoplasmic and myofibrillar proteins. These increments were about 4-5 times larger than that of BSA which was approximately 4.5 moles/10^6 g. As a result, shark muscle was considered to be more easily exposed on the surface than BSA by electrolysis. Thus, in an analogy with BSA, the SH group which was buried inside the protein molecules was exposed onto the surface of the molecule, because of the changes in the three dimensional structure of the proteins owing to an increase in pH value by electrolysis.

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[14]. 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 ashi 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 1, 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 tests 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. Consequently, the contribution of SS bond on the elasticity of kamaboko gel was found to be small, which was similar to the results of Niwa et al.[15, 16], Yasui et al.[17], and Samejima et al.[18].

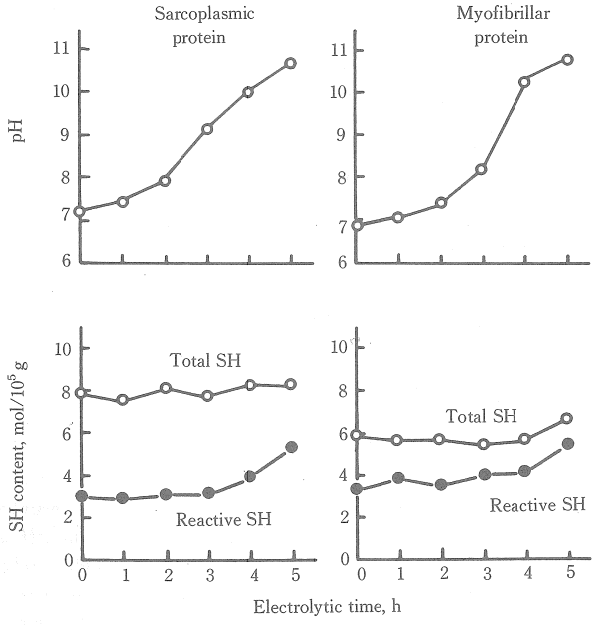

Fig. 3. Variations of SH contents and pH by electrolytic time in shark muscle proteins. Conc. of sample protein, 0.5-0.8 mg/m*l*; electric current density, 5.5 mA/cm^2; electrode, platimum plate.

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

Sample mince	Puncture force*1 in gr	Folding test	Water content in %	pH
Electrolyzed	190±68	AA*2	83.2	7.35
Non-electorolyzed	195±57	AA*2	83.4	7.42

*1 Five test pieces of kamaboko sample were employed for the puncture test.
*2 No crack when folded into a quadrants.

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電気分解によるサメ肉のＳＨ含量とかまぼこ形成能の変化

浜田盛承・石崎松一郎・永井　毅

電気分解(電解)によって魚肉筋肉タンパク質のＳＨ基含量とかまぼこ形成能がどのように変化するかを調べた。メジロザメから筋形質タンパク質と筋原線維タンパク質を調製し，それらの各溶液(0.5-0.8 mg タンパク質/m*l*)に5.5 mA/cm^{2}で最高５時間電解した後，全ＳＨ基(T-SH)含量とタンパク質分子表面のSH基(R-SH)含量の変化を調べた。その結果，電解に伴いR-SHは増加したが，T-SHは変わらなかった。R-SHの増加の原因はSS結合の還元ではなく，電解に伴いタンパク質溶液のpHが上昇したことにあった。一方，サメ筋肉ホモジネートを電解してかまぼこを調製しても，ゲル形成能の向上は認められなかった。