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Syntheses and Properties of Myofibril Gels of Fish

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The purpose of this study is the preparation of the gels formed by myofibril of fish and evaluation of their chemo-mechanical properties. The muscular protein used was extracted from fish meat. After washing and centrifugal separation, the muscular protein was mixed with 3wt% of NaCl and the mixture was grounded. The gels were prepared by heating the mixtures at 50, 60, 70, 80 and 90 °C. Thermal and mechanical properties of these heated gels were measured using thermogravimetric analysis (TGA) and volumetric phase transition, respectively. In the 0.1N-KOH, 0.1N-KCl or 0.1N-HCl aqueous solution, the sliced specimen with the thickness of ca. 1 mm had a good response under the stimulus of electric field. Volume changes were also observed with the variation of concentration of acetone/water.

Key words: myofibril of fish, heated gels, electric field stimulus, chemo-mechanical

1. INTRODUCTION

Muscles transform the chemical energy, including an external stimulus, into mechanical energy efficiently, resulting in the reversible contraction and relaxation. Myosin, which can change a chemical energy into "a motion" in the body, is the main component of muscle, and is the huge protein with the molecular weight of about 480,000 and a molecule length of 150 nm. All kinds of muscular myosin of animal also change structurally by heating (denaturation), and hydrogel can be formed by heating with a salt. On the contrary, though the synthetic polymers are widely applied to, for example, rubber, plastic, and fiber, they don't respond to the external stimulus reversibly. If the function that responds to an external stimulus is added to these synthetic polymers, it is expected that muscle-like polymers can be embodied.[1)]

The fishery around East Asia has stable marine resources, and many kinds of fish are landed. Although many of these fish are used as food, some of them are abandoned on account of bad taste, smell and size. From these points, we try to utilize these unused fish for development of functional materials.

In this study, fish meat protein was obtained from croakers, and used for the gel preparation. The chemo-mechanical properties of the heated gels obtained were investigated.

2. EXPERIMENT

2.1 Preparation of Heated Gels

A fresh croaker was used as the sample fish. The head and internal organs of a sample fish were removed, and the fish was washed with cold water. This was sliced into two sheets, and then, these were milled using the machine (the size of hole is 5 mm in a diameter) which takes stamp type meat. Next, milled fish meat, a 1 wt% Phosphoric acid sodium (pH=7.5, 0.5M) and 0.001 wt% NaN_3 were added in cold distilled water (10 times volume to fish meat) and then, mixed well. Supernatant of the mixture was removed and centrifugal separation (500 G, 5 min) was carried out. After that, water was removed. This process was performed twice. After mixing this for 60 seconds by the mixer, 3 wt% NaCl was added to fish meat, and then, continued to be mixed for 60 seconds. The tendon was removed by the strainer, and then centrifugal separation was carried out to remove air bubbles. The obtained meat paste was placed in the plastic container (inner size 4.5x5.5 cm, height 5.5 cm) with a lap. The gel was obtained by heating up the fish meat paste for 1 hour from 50 to 90 °C (every 10 °C). After heating, the sample was immediately cooled down with ice water.[2]

2.2 TGA Measurement

Some amount of standard sample (Al_2O_3) and sample minced finely were weighed and replaced into the sample cell, nitrogen flux was 30 ml min^{-1}. The temperature was set to the range from room temperature to 500 °C, and heating rate was 20 °C min^{-1}.

2.3 Tensile Test

The rectangular shape of the heated gel with the size of 40x3x3 mm^3 was cut from heated gel sheet. Measurement was performed 3 times for each sample and the data which showed maximum tensile strength was chosen. Three sides of the sliced sample were measured with a slide caliper, and the averaged magnitude was used for the calculation of the initial cross section area. The test condition was 20 mm of initial length and 5 mm min^{-1} of tensile rate.

2.4 Volume Change with Acetone/Water Solution

The heated gel with the size of 5x5x5 mm^3 was soaked into an acetone/water mixed solvent at room temperature. Relative volume, V/V_0 of heated gel was pursued. Here, V_0 and V denote initial and swollen volumes, respectively. Three sides of the heated gel were measured with a slide caliper. Volume was calculated from the averaged magnitude.

2.5 Volume Change with pH

The rectangular shape of heated gel with the size of 7x4x4 mm^3 was cut from heated gel sheet. The sample was put into 0.1 N-HCl, 0.1 N-KCl, and 0.1 N-KOH solutions and volume change of gel was measured at various immersion times.

2.6 Electric Field Stimulus Response Test

The rectangular shape of the heated gel with the size of 1x5x30 mm^3 was fixed with a stage and immersed into solution (pH=1.1,5.4 and 13.2), and also the sample was placed between two platinum electrodes. The distance between a sample and each electrode was set to be 2.5 cm. Imposed voltage and time were 10 V and 60 s, respectively. Figure 1 shows definition of the bending angle for the electric field stimulus response test.

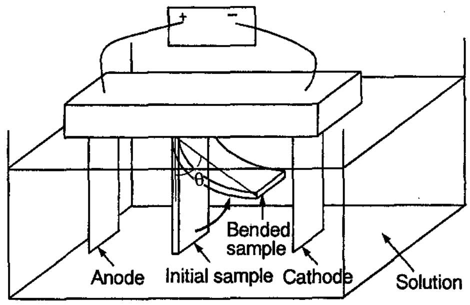

Figure 1. Definition of the bending angle for the electric field stimulus response test. Two platinum electrodes (not shown) were placed both sides of sample.

3. RESULTS AND DISCUSSION

In order to investigate the moisture content in the heated gel, TGA measurement was carried out. Figure 2 shows the TGA curves for the heated gels. The change in the weight of the heated gels almost decreased with increasing preparation temperature. This result indicates that the water content in the heated gel prepared at lower temperatures is higher than that done at higher ones.

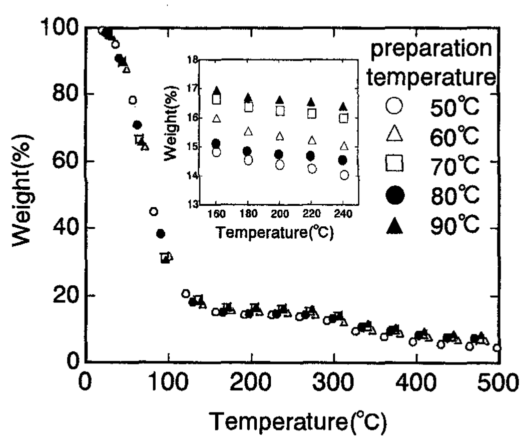

Figure 2. TGA curves of heated gels prepared at various temperatures.

Figure 3 shows the stress-strain curves of the heated gels. Tensile strength, strain at break and Young's modulus are listed in Table 1. Tensile strength and strain at break of the heated gel prepared at 50 °C showed the largest magnitude. Also, these magnitudes decreased with decreasing heating temperature for the other four heated gels. On the contrary, the Young's modulus of the heated gels decreased in the following order of heating temperature: 80>90>50>70>60 °C. These results are related to the cross-linking density of the heated gels. N. Seki et al.[4] reported that glutamic and lysine groups in a protein can be combined via T-glutaminase at around 45 °C. Furthermore, disulfide bond will be formed between cystein groups, and this trend increases with an increasing temperature above 60 °C.[4,5] Therefore, it seems that the heated gels prepared at 50, 80, and 90 °C showed high magnitudes.

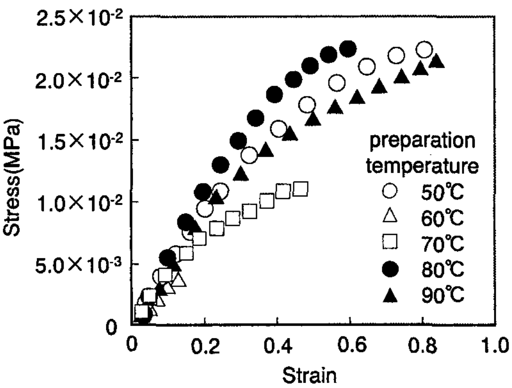

Figure 3. Stress-strain curves of heated gels prepared at various temperatures.

Figure 4 shows relative volume of the heated gels soaked in acetone/water solution. Heated gels showed isotropic contraction. They exhibited constant volume after 1 hour. For all samples, relative volume decreased with an increase in the ratio of an acetone. Moreover, the higher heating temperature, the smaller change in volume. The number of cross-linking point in the heated gel is small when prepared at lower temperatures. Hence, the water molecule cannot be kept in the heated gel and will be diffused into solvent. On the contrary, since the heated gel prepared at higher temperatures has a large number of cross-linking points in the sample, the water molecule will be kept inside.[3]

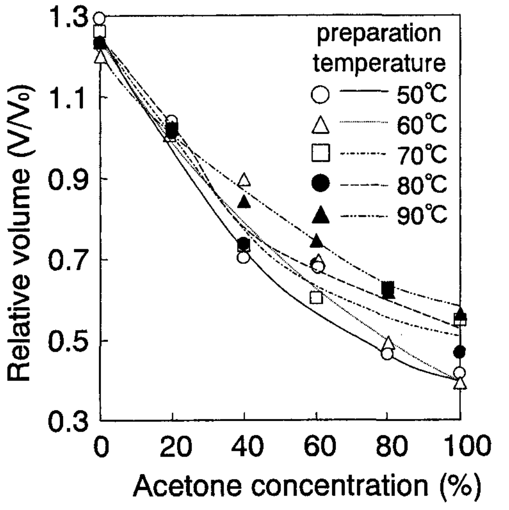

Figure 4. Relative volume of heated gels soaked in acetone/water mixed solution.

Table 1. Mechanical properties of heated gels.

Preparation temperature [℃]	Tensile strength [MPa]	Strain at break	Young's modulus [MPa]
50	2.25×10^{-2}	0.81	5.31×10^{-2}
60	3.91×10^{-3}	0.12	4.15×10^{-2}
70	1.12×10^{-2}	0.46	5.18×10^{-2}
80	2.28×10^{-2}	0.59	8.88×10^{-2}
90	2.14×10^{-2}	0.83	5.40×10^{-2}

Table 2 shows the relative volume of the heated gels in various pH solutions. The relative volume in the pH=1.1 and pH=13.2 solutions showed larger value compared with that in the pH=5.4 one. This result clearly indicates that the volume change is closely related to the electrostatic repulsion of protein.

Table 2. Relative volume (V/V_0) of heated gels in various pH solutions.

Preparation temperature (℃)	0.1N HCl (pH=1.1)	0.1N KCl (pH=5.4)	0.1N KOH (pH=13.2)
50	2.00	1.19	3.48
60	1.81	1.23	3.29
70	1.87	1.12	3.02
80	1.61	1.20	3.20
90	1.93	1.24	3.48

Figure 5 shows bending angle of the heated gels in the electric field stimulus response test. The sample was bent to an anode in acid solution and to opposite in the case of neutral and alkali solution. In acid solution, chlorine ion will be drawn around the anode side, the concentration chlorine ion of around the cathode will decrease. Hence, it seems that ammonium ion (-NH_3^+) at the anode side layer of the sample would be neutralized more in comparison with the other side. This leads to decreasing the volume of the anode side. Thus, it is likely to consider that this volume change between two sides would be why the heated gel was bent to an anode in an acid solution. Similarly, if potassium ions will be drawn around cathode side in alkali solution, the reason why the bending direction is opposite can be explained well. Moreover, in neutral solution, since it is expected that isoelectric point of fish meat seems to be around pH=5, it is considered that the possitively charged protein gel would be bent to the cathode side. The bending angle of the heated gels were following order : acid solution > alkali solution > neutral solution. The smallest bending angle for the neutral solution implies that the isoelectric point of protein is around pH=5.

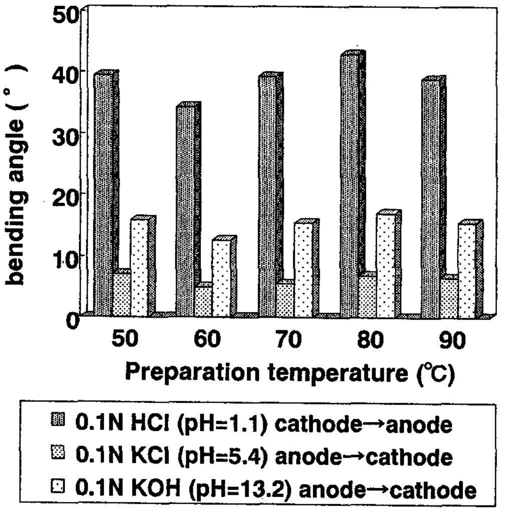

Figure 5. Bending angle of heated gels in electric field stimulus response test.

4. CONCLUSIONS

The heated gels were successfully prepared from unused fish meat. From the relative volume of the heated gels with acetone/water solutions, the cross-linking density is quite dependent on the preparation temperature. Tensile test revealed that the tensile strength, strain at break and Young's modulus of heated gels prepared at 50, 80 and 90 °C exhibited higher magnitude compared with others. This can be explained well with the difference in the cross-linking density. Also, the heated gels showed a good response to the electric field in the acid and alkali solutions. This study suggests one of the most intriguing applications of unused fish, for example, actuators, sensors, biodegradable materials and so on.

5. ACKNOLEGEMENT

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