연사 6

Searching for new ways to make good use of meat

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The Solubilization of Myofibrillar Proteins of Vertebrate Skeletal Muscle in Water

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Background

Meat, skeletal muscle of livestock and poultry, is a very concentrated source of protein that has a high biological value, because its amino acid composition resembles that of human proteins containing all essential amino acid. Although meat contains high quality proteins for human, utilization of meat protein has been limited. One of the factors limiting utilization is the limited solubility of meat proteins.

The myofibrillar proteins are the major protein fraction of meat and represent about 50% of total proteins of muscle tissue. Myofibrillar proteins are generally considered insoluble in solutions of low ionic strength and relatively high concentration of salt are required to solubilize them. If myofibrillar proteins could be solubilize in water or low salt solutions, they could be used in manyways. For example, they could be used as a liquid diet for elderly people.

Objective

The object of our study is the development of the new ways for effective utilization of meat We, therefore, investigated a method to solubilize myofibrillar proteins in water and some physicochemical properties of water-soluble myofibrillar proteins. Furthermore, to establish a method for preserving water-soluble myofibrillar proteins until use, the resolubilization of freeze-dried water-soluble myofibrillar proteins in water was researched.

Materials and Methods

Materials

Chicken breast and leg muscles, pork loin, lamb and beef shoulder loin were obtained from a

commercial source. Fresh rabbit psoas muscle was also used for preparation of glycerinated muscle fiber bundles

Methods

Preparation of water-soluble myofibrillar proteins

The procedure for preparing water-soluble myofibrillar proteins is divided into two processes; a washing procedure followed by water-solubilization procedure.

<washing procedure>

After removing connective and adipose tissues, muscles were cut into cubes as small as possible. The comminuted muscles were homogenized with ten volumes of a cold solution containing 25 mM NaCl and 5 mM L-histidine for 120 sec at 4℃ in a Waring Blender. After filtering through four-layers medical-use gauze, the suspension (1st wash fraction) was centrifuged for 20 min at 18,000 g. The supernatant was discarded, the precipitate was homogenized with five volumes of a cold solution containing 25 mM NaCl and 5 mM L-histidine for 60 sec (2nd wash fraction) followed by centrifugation for 20 min at 18,000 g. The supernatant was again discarded, and the precipitate was re-dissolved using the same procedure following centrifugation for 20 min at 18,000 g. The precipitate obtained from the third wash fraction was homogenized with five volumes of a cold solution containing 2.5 mM NaCl and 5 mM L-histidine for 60 sec (4th wash fraction), and centrifuged for 20 min at 18,000 g. The precipitate was termed as washed myofibrils.

<water-solubilization procedure>

Three to five volumes (dependent on protein concentration) of distilled water was added to the final precipitate obtained by washing step (washed myofibrils), and re-suspended pellet was subjected to ultrasonication (Sonifier Model 250, Branson, CT, U.S.A.). The Sonifier is composed of three elements, power supply, converter and horn. The top of the horn was dipped to the depth of about 1 cm into solution and sonication was carried out at an output power of 50 watts and ··· kHz for varioustime. To prevent the sample temperature raising, ultrasonic energy to be pulsed, i.e. at 0.5 second intervals. After ultrasonication, the suspension was centrifuged for 20 min at 37,000 g and the obtained supernatant fraction was defined as water-soluble myofibrillar proteins.

Results and Discussion

We established a method to solubilize more than 80% of chicken breast muscle myofibrillar proteins in waterfor the utilization of meat as a source of food protein. SDS-polyacrylamide gel electrophoretic patterns of water-soluble myofibrillar proteins demonstrate that all identified myofibrillar proteins except

connectin/titin are soluble in water. To accomplish solubilization, suspensions were maintained at neutral pH, α-actinin was removed, connectin/titin were degradedby ultrasonication. The solubility of myofibrillar proteins was dependent on ultrasonication time. Myofibrillar proteins of chicken leg, pork loin, beef shoulder loin, and lamb are also solubilized in water using this procedure.

To maintain the solubility of water-soluble myofibrillar proteins, heating at a temperature of more than 70C was required. The solubility of heated water-soluble myofibrillar proteins was examined. The solubility of the proteins was found to be sensitive to ionic strength and pH of the solution. At the ionic strength of less than 12 mM and neutral pH, more than 80% of myofibrillar proteins were solubilized. The solubility of freeze-dried protein powder prepared from water-soluble myofibrillar proteins was also examined, and it was found that addition of trehalose and heating were essential for re-solubilization in water. Amino acid composition of water-soluble myofibrillar proteins was found to be almost the same as that of myofibrillar proteins.

Conclusion and Perspective

We have developed the effective procedure to prepare water-soluble myofibrillar proteins. This procedure might makes possible to utilize or develop meat protein food and supplement. But, the procedure to make the product is very complicated and the production cost is very expensive. Therefore, it is impossible that the drink is made by mass production and come onto the market under the present. To find a solution to the problem, we are going to develop a new method to solubilize meat proteins using sub- and supercritical water reaction.

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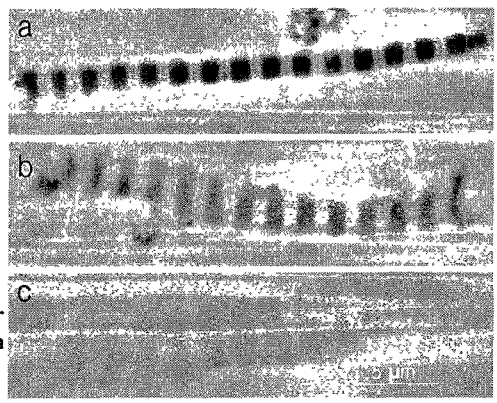

Fig.1. Changes in Myofibrillar Structures during the Solubilization Procedure

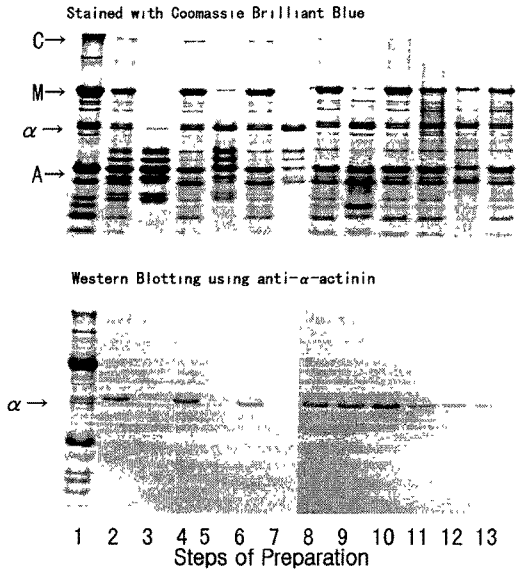

Fig.2. Chicken Breast Muscle Proteins at Various Steps of Preparation of Water Soluble Myofibrillar Proteins

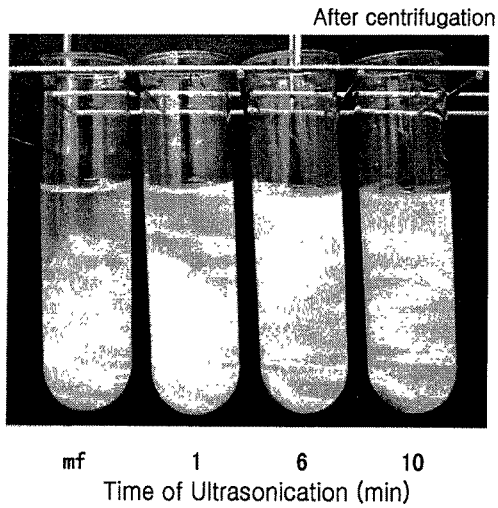

Fig.3. Solubilization of Myofibrillar Proteins by Ultrasonication

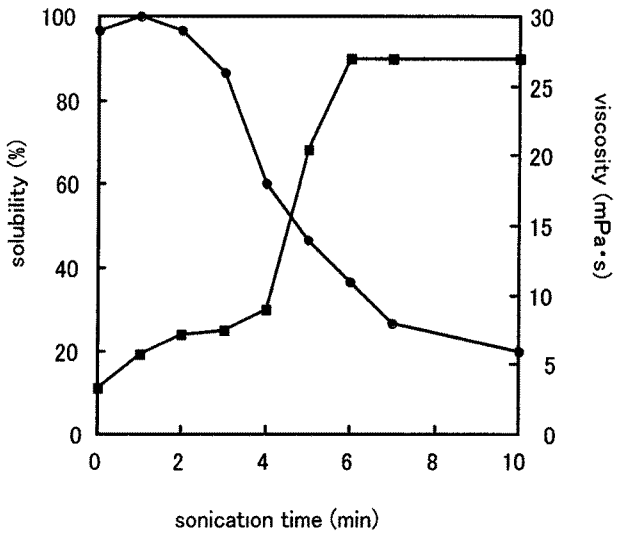

Fig.4. Effect of Ultrasonication on Protein Solubility and Viscosity of Washed Myofibrils

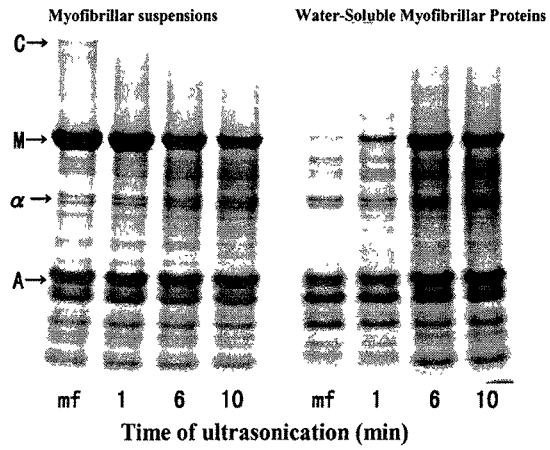

Fig.5. SDS-PAGE of Myofibrillar suspensions after Ultrasonication and Water-Soluble Myofibrillar Proteins Obtained by Centrifugation

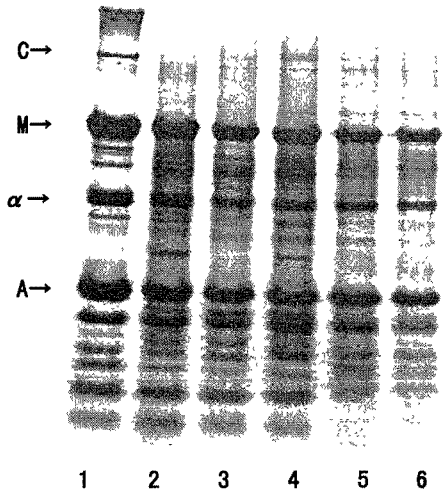

Fig.6. Water-Soluble Myofibrillar Proteins from Various Kinds of Muscle Tissues

Table 1. Effect of heating and incubation on protein solubility

heating temp.(℃)	solubility(%)	
	0 h[a]	24 h[b]
4 (control)	100	36
30	100	36
40	37	34
50	29	24
60	95	90
70	95	95
80	95	95
90	59	95
100	95	95

a, solubility right after heating.
b, solubility passing over night after heating.

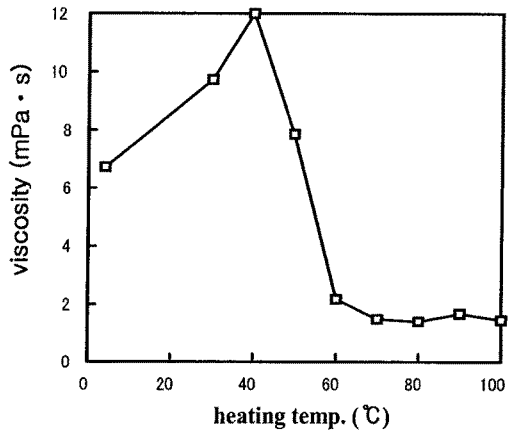

Fig.7. Effect of Heating on Viscosity of Water-Soluble Myofibrillar Proteins

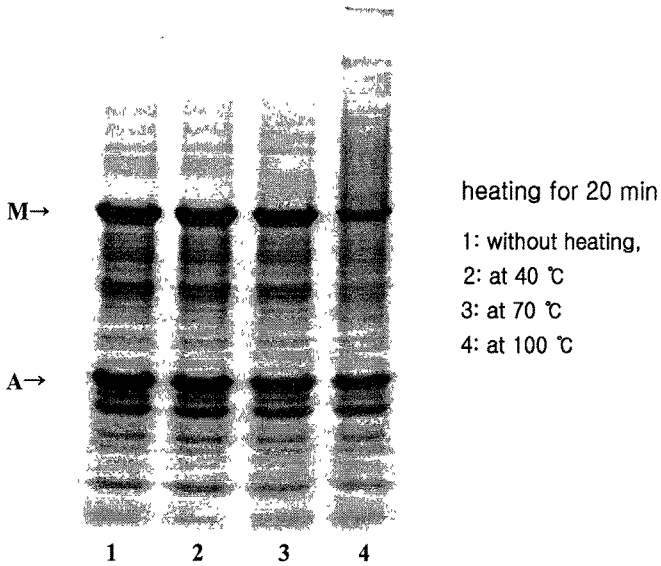

Fig.8. SDS-PAGE of Heated Water-Soluble Myofibrillar Proteins

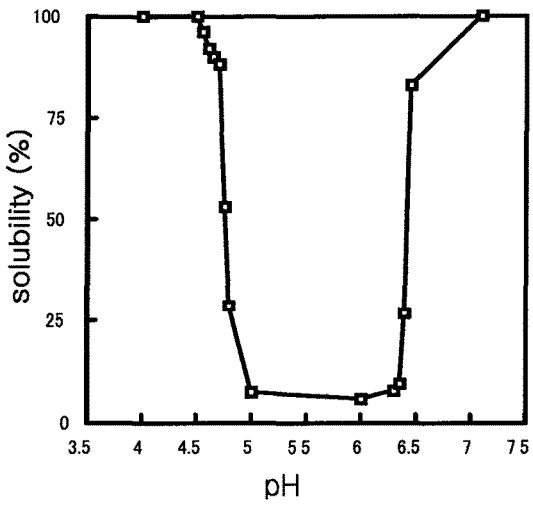

Fig.9. Effect of pH on Solubility of Heated Water-Soluble Myofibrillar Proteins

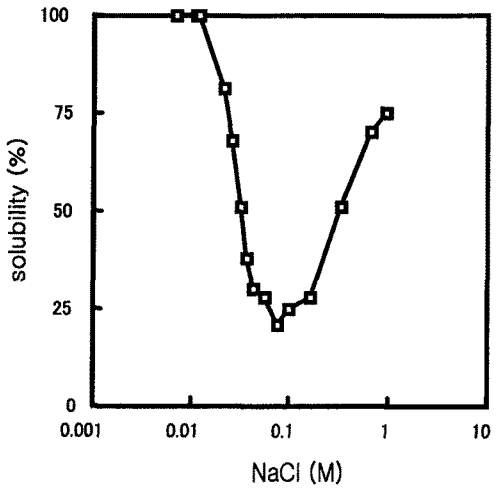

Fig.10. Effect of Ionic Strength on Solubility of Heated Water-Soluble Myofibrillar Proteins

Table 2. Effect of heating and trehalose on freeze-dried protein solubility

Sample	Solubility (%)
WSMF	36
WSMF +heating	59
WSMF +0.5% trehalose	40
WSMF +heating +0.5% trehalose	83

WSMF: water-soluble myofibrillar proteins