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*Received for review March 5, 1971. Accepted January 10, 1972. This work was supported by a grant from the Multinational Programs in Science and Technology of the Organization of American States (OAS).*

# Measurement of Tensile Strength of Muscle Fibers and Its Change during Postmortem Aging of Chicken Breast Muscle

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To study the meat tenderization phenomenon, muscle fibers were prepared from the chicken pectoralis major muscle at a definite time after death and their tensile strength was measured. The tensile strength of muscle fibers increased a little until 6–8 hr postmortem and then decreased greatly. The tensile strength of muscle fibers prepared immediately after death, however, did not decrease during holding at 0°C for 24 hr and incubation with water extract of muscle did not decrease it. The cause of the changes in the tensile strength during postmortem aging is discussed.

Although many workers have been studying the meat tenderization phenomenon, its mechanism is not clarified yet. However, some interesting facts concerning the changes in meat during postmortem aging have been reported. One of these is the breaking of myofibrils into small fragments with mild homogenizing "fragmentation of myofibrils," which was first reported by Takahashi *et al.* (1967). Davey and Gilbert (1969) showed that morphological changes of myofibrils occurred in the region of the z-lines during storage and suggested that meat aging is due to disruption and possible dissolution of z-line material. If such morphological changes of myofibrils occur in stored meat, the tensile strength of muscle fibers can be expected to change during storage.

The tensile strength and the extensibility of muscle fibers were measured by some workers (Wang *et al.*, 1956; Sato *et al.*, 1967). However, no detailed studies have been made of the change in the tensile strength of muscle fibers during postmortem aging.

The major objectives of this study were to establish the measuring conditions of the tensile strength of muscle fibers and to investigate the changes in it during postmortem aging.

## EXPERIMENTAL

**Materials and Procedures.** Twelve- to fourteen-month-old chickens of the White Leghorn (female) strain were used in these experiments. They were raised under similar environmental and nutritional conditions. The bird was slaughtered by cutting the jugular vein and carotid arteries, skinned without scalding, and eviscerated. The muscles were placed in a plastic bag without removing them from the carcass and stored in drained crushed ice.

**Preparation of Muscle Fibers.** Small pieces were cut from the pectoralis major muscle and were transferred into a petri dish containing a large volume of a "muscle preparative solution," such as 50% glycerol, 0.1 *M* KCl, 0.1 *M* KCl with 5 m*M* ATP, and Ringer's solution. (In this report, muscle fibers prepared in each solution were designated as 50% glycerol muscle fibers, 0.1 *M* KCl muscle fibers, and so on.) Then, small fiber bundles of uniform cross section were separated with two tweezers and transferred to another petri dish containing the same muscle preparative solution as the first, and their tensile strength was immediately measured.

The size of fiber bundle cross section was adjusted to that of about 25 single fibers, based on the following preliminary experiment. Muscle fiber bundles with various cross sections were prepared and the number of constituent single fibers was counted in each fiber bundle under a microscope. From this experiment, a definite relationship was noted between the size of the muscle fiber bundle and the number of constituent single fibers.

From the previous experiment using muscle fibers which were prepared in the preparative solution with different pH, it was noted that the tensile strength of muscle fibers was not affected by pH within the range 5.0 to 8.0. So, no buffer solution was used in this experiment for the preparation of muscle fibers.

**Measurement of Tensile Strength.** With scotch tape, 8–10 muscle fiber bundles longer than 2 cm were stuck on a small piece of cardboard (2 × 3 cm) which hung from the lever of

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Table I. Effect of Holding Time at 0°C on the Tensile Strength of Muscle Fibers (g/25 Single Fibers)[a]

| Preparative solution | Preparation time of muscle fibers | Holding time, hr 0 | 1 | 24 |
|---|---|---|---|---|
| Ringer's solution | Immediately after death | 1.0 ± 0.4 | 0.5 ± 0.3 | 0.5 ± 0.3 |
| | 1 hr postmortem | 0.5 ± 0.2 | 0.4 ± 0.2 | 0.4 ± 0.2 |
| 0.1 *M* KCl | Immediately after death | 1.8 ± 0.5 | 0.8 ± 0.5 | 0.6 ± 0.3 |
| | 1 hr postmortem | 0.5 ± 0.2 | 0.4 ± 0.2 | 0.4 ± 0.2 |
| 50% glycerol | Immediately after death | 2.0 ± 0.3 | 2.0 ± 0.3 | 1.9 ± 0.3 |
| | 1 hr postmortem | 2.2 ± 0.4 | 2.1 ± 0.3 | 2.0 ± 0.4 |

[a] Mean ± SD.

Table II. Effect of Carbohydrate Solution for Preparing Muscle Fibers on Their Tensile Strength (g/25 Single Fibers)[a,b]

| None | 30% glucose | 30% galactose | 30% mannitol | 30% sorbitol | 30% sucrose | 50% glycerol |
|---|---|---|---|---|---|---|
| 0.5 ± 0.2 | 1.4 ± 0.3 | 1.4 ± 0.3 | 1.7 ± 0.2 | 2.0 ± 0.2 | 2.0 ± 0.2 | 2.2 ± 0.4 |

[a] Mean ● SD. [b] Tensile strength measurement was carried out after storage of muscle fibers for 3 hr at 0°C.

Table III. Effect of Added ATP on the Tensile Strength of Muscle Fibers (g/25 Single Fibers)[a,b]

| Preparative solution | None | 1 m*M* | 2 m*M* | 4 m*M* | 6 m*M* |
|---|---|---|---|---|---|
| Ringer's solution | 0.5 ± 0.2 | ... | 0.5 ± 0.3 | ... | 0.4 ± 0.3 |
| 0.1 *M* KCl | 0.5 ± 0.2 | 1.0 ± 0.2 | 1.2 ± 0.3 | 2.3 ± 0.2 | 2.5 ± 0.3 |

[a] Mean ± SD. [b] Tensile strength measurement was carried out after storage of muscle fibers for 3 hr at 0°C.

Table IV. Effect of Ca Ions on the Tensile Strength of 50% Glycerol Muscle Fibers (g/25 Single Fibers)[a,b]

| None | 0.1 m*M* | 0.5 m*M* | 1 m*M* | 3 m*M* | 5 m*M* |
|---|---|---|---|---|---|
| 2.2 ± 0.5 | 2.1 ± 0.4 | 1.5 ± 0.3 | 0.7 ± 0.3 | 0.5 ± 0.2 | 0.5 ± 0.2 |

[a] Mean ± SD. [b] Tensile strength measurement was carried out after storage of muscle fibers for 3 hr at 0°C.

a strain gauge (Shinkoh Tsushin Kogyo K.K., Tokyo, Type BU) with a fine wire. One end of each fiber bundle was pulled slowly by tweezers and the force needed to break it was recorded by an automatic recorder (Shinkoh Tsushin Kogyo K.K., Tokyo, Type AS3/A) (Figure 1). The average of 20 readings for each sample was taken as the index of each sample.

Care should be taken not to spend an unnecessarily long time for each measurement. This is especially important for the muscle fibers prepared in other solutions except 50% glycerol. If it takes an exceedingly long time muscle fibers lose moisture during measurement and an abnormally high tensile strength is obtained.

Table V. Tensile Strength of Muscle Fibers with Different Size (g)[a,b,c]

| Experimental no. | Number of single fiber 100 ± 15 | 50 ± 10 | 25 ± 5 |
|---|---|---|---|
| 1 | 5.7 ± 1.7 (0.057) | 2.9 ± 0.6 (0.058) | 1.8 ± 0.4 (0.072) |
| 2 | 7.0 ± 1.1 (0.070) | 3.4 ± 0.9 (0.068) | 2.0 ± 0.4 (0.080) |

[a] Mean ± SD. [b] Muscle fibers were prepared in 50% glycerol immediately after death. [c] Each value in parentheses shows the tensile strength of a single fiber calculated from measured values.

## RESULTS AND DISCUSSION

**Preparation of Muscle Fibers and Measurement of Tensile Strength.** When the sample of muscle fibers was prepared in Ringer's solution, it became opaque and was contracted greatly (>40% of the initial length) during its preparation. The 0.1 *M* KCl muscle fibers also became opaque and were contracted a little (<10% of the initial length). With a microscope, many small creases were observed on the surface of both Ringer's solution and 0.1 *M* KCl muscle fibers, which might be caused by contraction. The tensile strength of both fibers was either indefinite (the sample prepared immediately after death), or very small (the sample prepared at 1 hr postmortem) (Table I).

On the contrary, the sample of muscle fibers prepared in 50% glycerol did not become opaque and was not contracted at all. Its tensile strength was high and the value did not change during holding at 0°C. It had already been noted that muscle fibers prepared in water were contracted and lost their contractility and those in 50% glycerol retained their contractility (Ebashi, 1957). The present result coincides with these facts and the action of glycerol seems to be protec-

Table VI. Tensile Strength of Muscle Fibers Obtained from Stored Muscle (g/25 Single Fibers) (Values are average of Six Birds)[a]

| Preparation time of muscle fibers | Preparative solution | | | |
|---|---|---|---|---|
| | 0.1 *M* KCl | 0.1 *M* KCl + 5 m*M* ATP | 50% glycerol | 50% glycerol + 5 m*M* ATP |
| Immediately after death | 1.8 ± 0.5 | 2.6 ± 0.3 | 2.0 ± 0.3 | 2.1 ± 0.3 |
| 1–3 hr postmortem | 0.5 ± 0.2 | 2.6 ± 0.3 | 2.2 ± 0.4 | 2.6 ± 0.3 |
| 6–8 hr postmortem | 0.4 ± 0.2 | 1.6 ± 0.3 | 2.6 ± 0.4 | 2.8 ± 0.4 |
| 24 hr postmortem | 0.4 ± 0.1 | 1.0 ± 0.3 | 0.4 ± 0.2 | 0.7 ± 0.2 |

[a] Mean ± SD.

tion against some changes in muscle fibers prepared in salt solution.

Similar protective action was noted in the case of the various carbohydrate solutions tested in the experiment reported in Table II. Stromer and Goll (1967) investigated the effect of three kinds of solution, namely, KCl, sucrose, and glycerol solution, on the chemical and morphological properties of myofibrils and showed that 0.25 *M* sucrose solution was the best for preparing muscle fibers although the difference between it and a glycerol solution was relatively small. However, it was noted in this experiment that the tensile strength of sucrose muscle fibers decreased a little during holding at 0°C. For this reason, 50% glycerol was preferred as a preparative solution.

The tensile strength of 0.1 *M* KCl muscle fibers increased to that of 50% glycerol muscle fibers after the addition of ATP to more than 5 m*M*, although it remained opaque and the length of each fiber was not changed with this treatment. The tensile strength of Ringer's solution muscle fibers, however, did not increase at all with the addition of ATP to more than 5 m*M* (Table III). As Ringer's solution contains a small amount of $CaCl_2$, about 2 m*M*, this difference in the tensile strength between two types of muscle fibers may be due to the effect of Ca ions. This is verified in Table IV, which shows that the tensile strength of 50% glycerol muscle fibers decreases in the presence of $CaCl_2$ more than 0.5 m*M*.

When muscles were homogenized in a large volume of 0.1 *M* KCl or Ringer's solution, large amounts of ATP were removed (Nakamura, 1971). Almost all low molecular weight substances in muscles are removed during the preparation of 50% glycerol muscle fibers (Ebashi, 1957). Large amounts of ATP are considered to be absent in the muscle fibers prepared in this experiment. Based on these facts, the above results seem to show clearly that the tensile strength of muscle fibers prepared in salt solution decreases in the absence of ATP and recovers with the addition of ATP when the concentration of the dissolved Ca ions is below 0.1 m*M*. Haga *et al.* (1966) showed that Ca ions are essential to detach actin from the z-lines during the preparation of myofibril from rabbit muscle. This result suggests that the irreversibility of the tensile strength of Ringer's solution muscle fibers might be caused by the destruction of myofibril in the presence of Ca ions.

When measurement was made using a muscle fiber bundle of a different size, the tensile strength values were almost proportional to the number of the fibers in the bundle and the calculated value for a single fiber was almost the same, 0.06–0.08 g, to the three different sizes of bundles shown in Table V. This seems to show that there is no special interaction between each single fiber constituting the bundle, which affects the tensile strength measurement. A single fiber would be ideal for the tensile strength measurement, but its preparation is very difficult and time-consuming. So, in these experiments, tensile strength was measured using a bundle of 25 single fibers, which was easily prepared from muscle pieces.

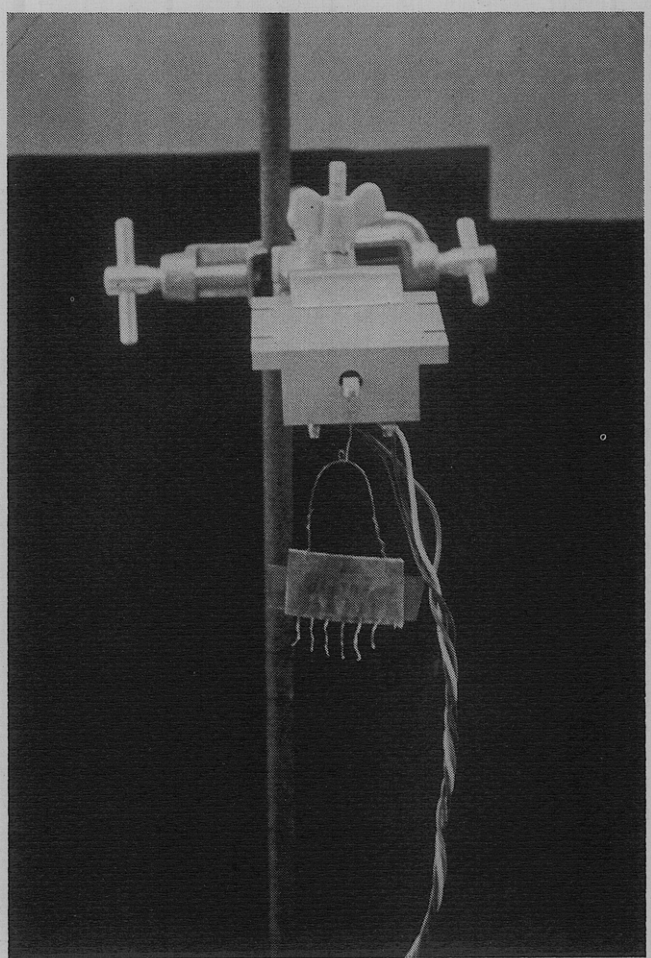

Figure 1. Strain gauge from which a cardboard with muscle fibers hangs

The proportionality of tensile strength to the size of the fiber bundle seems to show the usefulness of the present technique for the tensile strength measurement. Although some different techniques should be used to measure smaller differences in tension, gross differences in tension seem to be measurable by this technique.

**Changes in the Tensile Strength of Muscle Fibers during Postmortem Aging.** The tensile strength of 50% glycerol muscle fibers increased gradually during postmortem aging until 6–8 hr postmortem and decreased greatly thereafter (Table VI). A decrease of tensile strength after 6–8 hr postmortem was also noted in the case of 0.1 *M* KCl +5 m*M*

Table VII. Effect of Incubation with Water Extract of Muscle[b] (0°C, 24 hr) on the Tensile Strength of Muscle Fibers (g/25) Single Fibers)[a]

| Amount of muscle (g) put in 100 ml of water for extraction | Preparative solution | 0.1 *M* KCl | 50% glycerol |
|---|---|---|---|
| 0 | Without ATP | 0.5 ± 0.2 | 2.0 ± 0.3 |
| | With ATP[c] | 2.0 ± 0.2 | 2.1 ± 0.3 |
| 50 | Without ATP | 0.4 ± 0.2 | 1.9 ± 0.2 |
| | With ATP[c] | 2.1 ± 0.3 | 2.0 ± 0.3 |
| 100 | Without ATP | 0.4 ± 0.2 | 2.0 ± 0.2 |
| | With ATP[c] | 2.0 ± 0.3 | 2.0 ± 0.3 |

[a] Mean ● SD. [b] Water extract of muscle was prepared from the pectoralis major muscle immediately after death; a definite amount of muscle was added to 100 ml of water, blended for 1 min in a Waring blender, and then centrifuged. One volume of water extract of muscle was added to 10 vol of 0.1 *M* KCl or 50% glycerol solution in which muscle fibers were suspended. [c] After incubation, muscle fibers were washed with cold distilled water, 5m*M* ATP was added, and they were kept for 30 min at 0°C before the measurement of their tensile strength.

ATP, although the increase in the tensile strength during the first stage of aging was not seen. The tensile strength of 0.1 *M* KCl muscle fibers was always low, except those prepared immediately after death.

Among these changes, a decrease after 6–8 hr postmortem is very interesting in relation to the fragmentation phenomenon. As the tensile strength of both 50% glycerol and 0.1 *M* KCl + ATP muscle fibers prepared immediately after death does not change during holding at 0°C for 24 hr (Table VII, first column), the decrease in the tensile strength after 6–8 hr postmortem is considered to be caused by the action of some water-soluble substances which might be removed during the preparation of muscle fibers. Among these substances, cathepsins are believed to play an important role in such a case. In order to investigate this possibility, 50% glycerol or 0.1 *M* KCl muscle fibers prepared immediately after death were dipped in the water extract of muscle and were kept for 24 hr at 0°C. The result shows that there is no distinct effect of muscle extract on the tensile strength of muscle fibers during holding at 0°C (Table VII).

Many workers have shown that little relationship has been found between the extent of proteolysis and tenderness (Locker 1960; Davey and Gilbert, 1966; Miller *et al.*, 1965; Parrish *et al.*, 1969) and that myofibril itself is not proteolytically cleaved during postmortem storage (Fukazawa and Yasui, 1967). The present results seem to show that proteolysis is not the main cause of the change in the tensile strength during storage.

Another possibility for the cause of the changes in the tensile strength is the loss of ATP from muscle during postmortem aging. The following facts support this thought: the tensile strength of 0.1 *M* KCl muscle fibers is very low and the addition of ATP increases its value; and the ATP content of muscle decreases to almost zero under the aging conditions of this experiment (Nakamura, 1970). This seems to present a good explanation for the change in the tensile strength of muscle fibers described above. However, the tensile strength of 0.1 *M* KCl + ATP muscle fibers prepared at 24 hr postmortem was not so large as those prepared immediately after death, although it was larger than that of 0.1 *M* KCl muscle fibers (Table VI). This suggests that some irreversible changes occur in the intact muscle during postmortem aging, besides the disappearance of ATP.

It was already shown that Ca was released continuously from muscle proteins during postmortem aging (Arnold *et al.*, 1956). As shown in Table III, the tensile strength of Ringer's solution muscle fibers does not increase to that of 50% glycerol muscle fibers with the addition of ATP. It is possible that Ca ions released from muscle proteins during postmortem aging cause the irreversible change in the myofibril. This view is supported by the observation of Davey and Dickson (1970) that the loss of tensile strength during postmortem aging is due to a weakening of the myofibrillar substances at the junction of the I filaments with the z-discs of the sarcomeres.

ACKNOWLEDGMENT

The author thanks Yasushi Sato for his encouragement during this work and Kenji Watanabe for his advice on the measurement of tensile strength.

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*Received for review December 9, 1971. Accepted March 13, 1972.*