Tenderizing Effect of Low-temperature Long-time Heating on Bovine Muscle

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INTRODUCTION

Two factors have been reported to contribute to the toughness of meat: the contraction stage of the muscle and the connective tissue. So called »actomyosin-toughness» is suggested to be due to configurational changes of actin and myosin (Marsh *et al.*, 1966), which Herring *et al.* (1967) have shown to be highly related with shortened sarcomeres.

Connective tissue of the muscle is mainly collagen, although in certain muscles there are rather large quantities of elastin as well (Ramsbottom and Strandine, 1948). Several studies have indicated that there is no systematic increase in the amount of collagen as age increases (Wilson *et al.* 1954, Goll *et al*, 1963). Hill (1966) suggested that the aging of animal affects the collagen solubility.

There are indications which show some features in the shrinkage of muscle fibers during heating similar to the phenomena occurring in the contraction of living muscle. Hostetler and Landman (1968), when observing the changes in washed isolated muscle fibers in a microscope during heating at a rate of about 1.2 C/min up to 80 C, found that after there had been a decrease in diameter at lower temperatures, a clear and fast decrease in length began at about 50 C and was completed at about 70 C. Sherman (1961) pointed out that the ability of meat to retain fluid by absorption decreases rapidly above 50 C. Ritchey and Hostetler (1964) who cooked longissimus dorsi and biceps femoris muscles in an oven at 177 C, indicated that loss in weight and loss

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in bound water were greatest between 74 and 80 C. Szent-Györgyi (1957) suggested that »heat can be expected to destroy the water structures and heat not only denatures actomyosin but makes it contract and develop not inconsiderable tension».

Kaminer (1962) studied the relationship between contraction and exudation of water in frozen-thawed frog muscle. With maximum shortening, there was a water loss of 35 % of the weight of muscle. By restricting the contraction, it was demonstrated that the amount of water loss was proportional to the degree of shortening, there being no significant loss with isometric contraction. Paul (1965) reported decreases in length of rabbit muscle sarcomeres after cooking.

It is also well known that thet collagenf ibers shorten to about one-third to one-quarter of their initial length when they are heated to about 60 C (Bear, 1952).

With the above information in mind, it seems likely that there could exist a temperature range where the shortening of collagen fibers could be minimal simultaneously with the minimal shrinkage of muscle fibers. The aim of this study was to find out the changes in tenderness and water-holding capacity, coagulation of water-soluble proteins, and the presence of collagenolytic activity when samples from a bovine muscle were heated up to the shrinkage temperature of collagen, namely to 60 C.

MATERIALS AND METHODS

The experimental animals were slaughtered at the Department of Animal Science of Cornell University. Longissimus dorsi, rectus femoris, and semitendinosus muscles from three Hereford steers, age about 20 months, of Prime- or Choice + Grade with warm carcass weight of 286 to 355 kg were used. The carcasses were kept in a cooler at 0 ± 1 C. After 5 to 7 days, the muscles to be studied were separated, packed into Cry-O-Vac bags[1], vacuumized, and kept on the shelf in a cooler at 0 ± 1 C for two weeks. This report will mainly be concerned with the results from semitendinosus muscle.

The muscle to be studied was trimmed aseptically of fat and epimysium. Then it was cut into about 2.5 cm slices weighing 100 ± 30 g. The slices were put into plastic bags, vacuumized, ans sealed. Two experiments were carried out in Vacy gas (ethylenoxide) sterilized polyethylene bags, and one experiment in Cry-O-Vac[2] bags.

A heating program of 0.1 C/min was established to be similar to the rate of temperature rise in a 15.7 kg steamship roast during cooking in a 121 C

[1] Dow Chemical Co, Midland, Mich., U.S.A.
[2] 134 × 425 mm.

oven. The muscle slices in the vacuumized plastic bags were put into a water bath heating at the rate mentioned above. Heating was started at 30 C and ended at 60 C, where it was maintained until 10 hr total cooking time had passed. Samples were taken after 3 or 4 hr, and then each hour, as long as there was enough meat available.

One portion of each muscle was used as a controlled cooking sample following a method similar to that of Marsh, Woodhams and Leet (1966). This sample was first tempered 1 hr at 30 C in a water bath, then heated up to 80 C at a rate of 0.8 C/min, and kept at this temperature for 1 hr.

Tenderness was measured as shear value in lb with Warner-Bratzler apparatus[1]. The diameter of each test core was 2.5 cm, and an attempt was made to keep the axis of the core parallel to the muscle fibers. Three such cores were taken from each portion of meat at each sampling point in the cooking schedule. Each core was sheared first into two halves, and these were sheared again, thus giving nine shear value readings for each sample.

The weight loss during cooking of meat is largely water, especially in low-fat pieces of meat, and therefore the weight loss reflects the water binding capacity of the meat proteins. Meat samples were weighed before and after heating, and the weight loss was computed as percent of the fresh weight.

The water-soluble proteins from the fresh and heated meat samples were extracted at O C (cf. Maier and Fischer, 1966). Fifteen grams of meat was cut into small strips with scissors, and homogenized with 60 ml distilled water for 30 sec in a Waring Blendor. The homogenate was centrifuged for 30 min in a 50-ml centrifuge tube at 2000 $\times$ G in a cold room at 0 $\pm$ 1 C. The supernatant was filtered, freeze-dried, weighed, and stored in polyethylene vials in desiccator in a freezer at —29 C. These samples were subjected to electrophoresis and collagenolytic activity determinations.

Vertical polyacrylamide gel electrophoresis was done in a vertical-gel electrophoresis apparatus[2] according to the method of Thompson *et al.* (1964). Forty milligrams of the freeze-dried water-soluble fraction from the muscle samples was weighed (MacRae and Randall, 1965), and dissolved in 0.5 ml of distilled water for electrophoresis.

Collagenolytic activity in the freeze-dried water-soluble fraction was determined according to a modification of the method by Wünsch and Heidrich (1963), with 4-phenyl-azo-benzyl-oxycarbonyl-L-prolyl-L-leucyl-glycyl-L-prolyl-D-arginine as substrate[1] (Laakkonen, 1969).

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RESULTS AND DISCUSSION

When muscle samples were heated according to the specified heating program, there was a very steep decrease in the shear value readings between the 4th and the 5th hour of heating (temperature rising from 52.0 to 57.0 on the surface, and from 50.5 to 56.0 in the center), from 40.6 lb a the 4th hour to 24.3 lb. at the 5th hour. This is a lower temperature range for tenderizing effect as compared with the range of 56 to 59 C given by Machlik and Draudt (1963), and it seems possible that the shift in the shrinkage temperature of collagen could be due to the effect of collagenolytic activity in the muscle during the low-temperature heating. There was a further decrease in shear value readings after the 9th and the 10th hour of heating, to the values of 17.7 lb. and 15.4 lb., respectively. This may simply be due to further shrinkage of collagen, but since there is a considerable amount of elastin in the semitendinosus muscle (collagen-N 3.26 %, and elastin-N 1.69 %, according to Paul, 1962), and since Solovyov and Karpova (1967) have isolated muscular cathepsis possessing elastase activity, it could be possible that part of the total tenderizing effect has been due to elastase activity.

The shear values in the present study are lower than those given by Paul (1962) for semitendinosus. At the internal temperature of 60 C she found the shear value 2w1.63 lb, and at 77 C it was 22.54 lb. In the presen study the shear value after 6 hr heating (internal temperature 58.5 C) was 19.53 lb, and after 10 hr heating (internal temperature 60 C) it was 15.44 lb.

The increase in weight loss, i.e. decrease in waterholding capacity, was almost linear from the 3rd hr of heating (surface temperature 46.5 C, center 45.0 C) to the 7th hour (60 C), levelling off during the last three hours. The same was true with longissimus dorsi and rectus femoris, but the weight loss in semitendinosus was higher than that of longissimus dorsi, e.g. after 6 hr, 24.2 % and 20.1 %, respectively.

In the 80 C heated control samples, the shear value for semitendinosus was 20.55, and the weight loss was 40.0 %, both considerably higher than the values obtained with the low-temperature long-time heating.

The increase in pH seemed to be slow due to the slow rate of heating.

The water-soluble fraction contained both water-soluble proteins and salts. Heating to 60 C decreases the extractability of sarcoplasmic proteins because of coagulation. Therefore, changes in the amounts of freeze-dried water-soluble fraction reflect the amount of coagulation of the water-soluble proteins. Kronman and Winterbottom (1960) reported 2.51 ± 0.17 % water-soluble proteins in aged muscle. This comes close to the value in the present study, 2.7 %. This value includes other water-soluble substances as well.

The decrease in the amount of the water-soluble fraction was steepest between the 4th and 5th hour of heating. Obviously coagulation of the water-

soluble proteins started more intensively at about 50 C (Sherman 1961, Hostetler and Landman 1968). The electrophoretograms confirmed this result, and showed also that after 6 hr heating, several of the water-soluble proteins were coagulated, but even after 10 hr total heating time there were some of the water-soluble proteins noncoagulated. In the samples from longissimus dorsi, the final value, 10.80 mg freeze-dried water-soluble fraction/g fresh meat, was higher than the value, 6.45 mg/g, for controls heated to 80 C. The electrophoresis did not show any water-soluble proteins in the samples heated to 80 C which indicates that they obviously were all coagulated.

In the aged, fresh semitendinosus muscle, collagenolytic activity equal to. 0.15 *v* collagenase/mg of freeze-dried water-soluble fraction was found. After 6 hr heating at 37 or 45 C the activity was practically unchanged. However, in the drip exuded from meat, at 37 C there was 0.20 *v* collagenase, and at 45 C there was 0.25 *v* collagenase/mg of freeze-dried water-soluble fraction. When the internal temperature of 58,5 C vas reached after 6 hr heating, there was only 0.08 *v* collagenase/mg in the drip, and the water-soluble fraction of the muscle did not show any activity at all.

Houck *et al* (1968) heated normal rat skin in sterile organ culture at 56 C for 16 hr, and found a conversion of the found, inactive collagenolytic function into a free, active collagenase. Similar release of collagenase may occur in muscle during low-temperature long-time heating of meat. The relatively high amount of collagenolytic activity after heating for 6 hr at 37 C and 45 C, and the decrease of activity after heating to 58.5 C may explain Cover's (1941) early finding that heating for 23 hr in a 90 C oven gave shear values of onehalf to one-third of those for meat cooked in 90 C water for 3 hr to the same internal temperature. The better waterholding capacity during low-temperature heating also contributes to the tenderizing effect.

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