Some Observations on the Shrinkage of Beef Muscle Fibers During Heating.

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Heat related changes in muscle systems have been described by a number of investigators (Hamm, 1966; Hamm and Deatherage, 1960) and have been interpreted in terms of changes occurring in the myofibrillar and sarcoplasmic proteins. Of particular interest is the region between 50° and 70° where most changes occur in the properties of proteins, and within which some anomalies have been observed. This region corresponds also to the region of inflection or "flattening" which has been observed in the temperature rise as meat is cooked. (Cover, 1937). While a number of chemical and physical changes in this region are now well known, there still are some effects which require study to understand fully their influence on tenderness, and to substantiate general statements which have been made concerning the cooking of meat for maximum tenderness.

One such effect is the shrinkage of meat fibers. We have been conducting a study of the changes which occur during this shrinkage by observing isolated muscle fibers under the microscope, using ordinary light, phase contrast and polarized light. The changes that have been observed can be interpreted in the light of the altered protein and water relationships already studied by Hamm and others.

Experimental Methods

Isolated meat fibers were obtained by removing samples of suitable size (about 1g.) from post-rigor muscle tissue and blending the sample with cold normal saline (0.9% NaCl) for 30 seconds in a Waring blender. The fiber suspension was filtered and washed with cold saline until no further protein material came through the filter. The fibers were then carefully placed on a microscope slide, suspended in saline and covered with a cover slip. A microscope equipped with a heating stage was used. The temperature of the heating stage was controlled either electrically, or by circulating water at a given temperature through the stage.

When the electrical heating was used, the temperature was gradually raised from 40° to 80°C. To follow the course of the shrinkage of fibers, photomicrographs were taken before the fibers were heated and at intervals throughout the heating period. Usually 5 to 8 fibers were selected from each series of photographs and measurements were made of fiber width and length. Shrinkage was expressed as per cent of initial length or width. In other experiments, the heating stage temperature was held constant for one hour at each selected temperature of 37°, 45°, 53°, 61°, 69°, and 77°. Photomicrographs were taken every two minutes during the time the fiber changes were taking place, and every five minutes thereafter, for the full 60 minutes. By holding the temperature constant in this way, the effect of time of exposure at each temperature could be observed.

In order to study the pH effects, the same general procedures were followed except that the fibers were suspended in buffers at the desired pH values, were washed with the buffer prior to mounting on the slide, and were mounted in the buffer.

The determination of protein in the exudate was carried out as follows- Meat fibers (20-30g.) prepared as described, were diluted to approximately 200 ml. with normal saline. The suspension was filtered by gravity through filter paper and the fibers remaining on the paper were washed with successive 100-ml. portions of saline until no protein could be detected in the filtrate by the Folin-Ciocalteau test. Approximately 1200 ml. of saline was required. Fibers were not stirred, so damage from mechanical shear or pressure could be avoided. After washing was complete, 5.0 g. of the fibers were weighed (as gently as possible) into 50 ml. beakers and 5.0 ml. saline was added. The beakers were incubated in water baths at 25°, 37°, 50°, 55°, and 60° for five minutes after temperature equilibration had been realized. The beakers were removed, cooled to room temperature and allowed to stand until the fibers had settled. Aliquot portions of 1.0 ml. of the clear supernatant solutions were used in the Folin-Ciocalteau test to determine soluble protein content.

For the birefringence studies, a polarizing microscope fitted with a halfwave red interference filter was employed. Color photographs were taken during the course of the heating.

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Results

At temperatures of 25° to 30°, no change in fiber demensions was apparent. As the temperature of the fibers was increased to 37° and higher the first effect noted was a decrease in width. (See Figure 1). This decrease continued until the temperature had reached about 60°, when no further shrinkage occurred. Shortening of fibers appeared to begin when the temperature reached 50°. At this point the fiber length shortened suddenly and continued to shorten rapidly as the temperature between 50° and 65°. Some shortening occurred between 65° and 70° but at a slower rate. At the completion of shrinkage fibers had decreased to about 77 per cent of their initial width and to 70 per cent of their length on the average.

Similar results were more precisely noted when the time of exposure at a given temperature was held constant. For example, at 37° after one hour very little effect could be noted on fiber dimensions. The width had decreased about 5 per cent, while the length had decreased only about 2 per cent.

At 45° the fibers exhibited a decrease in width to about 85 per cent of the initial diameter within 25 minutes. No further effect was noted. At 50°, the fibers decreased in width to 77 per cent of the initial width after 15 minutes. At the higher temperatures, the fibers decreased to the maximal extent within the first five minutes, the time required by the stage to reach the temperature(See Figure 2.).

The shortening of fibers exhibited a different pattern, when studied in this stepwise manner. Not more than 4 per cent shortening occurred at temperatures below 53°, even after one hour. After about 30 minutes' exposure at 61°, about a 16 per cent decrease in length had occurred, but no additional shortening took place in the next 30 minutes. However, at 69° and above, fiber shortening occurred immediately and completely, to a maximum of 30 per cent decrease in length. (See Figure 3-).

The changes in width and length can also be viewed as a volume change, with the assumption that the fiber approaches a cylinder in shape. The

volume decrease appeared to proceed smoothly and slowly until the temperature of 50° was reached, at which point the volume decreased rapidly until the temperature reached 65° or 70°, when the volume change slowed. There also appeared to be a break in the curve at about 55°. The first stage of this volume decrease is due to shrinkage in width or diameter of the fiber; the latter stage is largely due to the shrinkage in length. (See Figure 1).

Changes in fiber diameter and length caused by heat are influenced in a discontinuous manner by pH as shown in Table 1. ...delayed onset of shrinkage occurred in the pH range of 5.25 to 5.75 and at pH 6.75. The most rapid volume change occured at pH 6.0, but the greatest amount of shrinkage took place in the pH range 5.75 to 6.25. Shrinkage was least at pH 5.0. At pH 5.0, which approximates the normal pH of meat, no noticeable shrinkage was apparent until a temperature of 50° had been reached. The shrinkage then followed the usual pattern, its plot lying at a position intermediate between the extremes. The final mean volume of the shrunken fibers was 43 per cent of the original mean volume. At all pH values studied, muscle fibers exhibited the most rapid shrinkage between 45° and 65°.

In all the observations of the volume changes, a background material was noticed, especially as temperatures reached 70°. This material consisted of small particles of an amorphous nature and was visible in the vicinity of the fiber. Studies with exhaustively washed fibers showed this material to be the exudate, or "drip", which was lost when the fiber contracted. Results of the Folin--Ciocalteau method on the filtrates from heat-treated fibers indicated that protein was being released from the fibers. This release began at about 40° and continued to 60°, although the amount was almost maximal at 50°-53°. Heating at temperatures above 60° caused coagulation and decreased the amount which could be recovered as soluble protein in the filtrate. The amounts of protein released from the fibers varied greatly from sample to sample. The significance of this variation is not understood at present.

When viewed under polarized light, muscle fibers appeared brilliantly birefringent. Upon heating, the fibers tended to become more isotropic until at 70°, the entire fiber was isotropic. Fibers heated at 40° for 60 minutes showed no change in birefringence. However, as the temperature reached 50°, the bi--refringence decreased (fibers became more isotropic). The sharpest drop in birefringence occurred between 54° and 56°, and almost no birefringence could be observed at 58°-60°.

Discussion

The changes in muscle fibers observed in these experiments seem to be related most closely to the water-holding properties of muscle proteins as described by Hamm and Deatherage (1960) and Hamm and Iwata (1962). As proteins coagulate, after being denatured by heat, their water-holding capacity is lost. Hamm has stated that the juice lost as drip during this process is that water held immobile by the myofilaments, but is not water of hydration. When the proteins of the myofilaments denature, the immobile water is freed, escapes from the intermyofibrillar space, and carries with it some soluble sarcoplasmic proteins.

This material, in our observations, appeared around the entire fiber, not at the cut ends only. The fiber simultaneously became more narrow, this process being essentially complete at 53°. However, birefringence was only slightly diminished at this temperature. Results of our time-temperature studies on isolated fibers (Figure 2) also showed that the diameter decreased in a manner parallel to the loss of water-holding capacity as demonstrated by Hamm and Iwata. Thus the decrease in diameter may be explained as the early stages of heat denaturation where unfolding of peptide chains has occurred, causing a loss of water-holding capacity but little disturbance in the parallel array of myofilaments.

The changes in length of fibers do not conform to this behavior pattern, but instead resemble more closely the behavior described by the curves Hamm has shown for the decrease or loss of the acidic groups. Fiber shortening does not begin until the temperature reaches 50° or more, when birefringence is noticeably diminishing (54° to 56°). At these temperatures myosin is supposedly coagulated (Locker, 1956) myofibrillar proteins are rearranging and forming stable cross-linkages (Hamm, 1966), and sarcoplasmic proteins are rapidly coagulating (Lee and Grau, 1966). These changes coincide with the observed changes in birefringence and shortening, and suggest that the fiber shortening is associated with the actual coagulation processes of the various proteins in the muscle. The loss of birefringence further indicates that the A-band structure is being disturbed when the fibrillar proteins coagulate and are thrown out of alignment.

The pH studies show that when fibers are in the isoelectric range of the fibrillar proteins, dimensional changes are at a minimum. Hamm has made the same observations for other characteristics.

The shrinkage of collagen in aqueous environment occurs at 60° to 65°. The volume decrease of muscle fibers must then be due to other effects, since most of the change in volume has taken place by the time the temperature reaches this value. However, the shrinkage of collagen may influence the shrinkage of muscle fibers in the final stages of heating.

Of interest may be a final experiment in which isolated washed fibers were heated in a 0.02% papain solution and observed under polarized light. The effects noted were as follows.

Shrinkage in diameter was noticeable at 40°, and continued until the temperature had reached 45° where length also began to decrease. At 48° structural alterations became noticeable, until at 52°, the fibers took on a granular appearance. The shrinkage continued to a greater extent than was exhibited in untreated fibers. At 60° the fibers had become nearly shapeless and were approximately one-tenth the original size. Birefringence began to disappear at 40°, and was rapidly lost through the next 5 to 6 degree increase in temperature. By the time the temperature had reached 48°, the fibers were completely isotropic. When muscle fibers were held at 40° in a papain solution, pronounced deterioration of the fiber wall, along with complete loss of anisotropic character, occurred after thirty minutes' exposure. These effects differ considerably from those observed with heat alone, where no visible changes took place at these temperatures.

S U M M A R Y

Physical changes encountered in cooking and tenderizing meat have been studied using isolated muscle fiber systems, under controlled conditions, and the microscopic changes which the fibers undergo have been observed. The effect of heating washed isolated fibers at successively higher temperatures indicates that fiber shrinkage occurs in two stages. The diameter starts to decrease at a considerably lower temperature than that at which fiber length begins to decrease, and continues until the shortening process begins. The decrease in length is quite marked and much more rapid than the decrease in width, beginning at about 50° and reaching completion at about 70°. This

range corresponds to the region in which the rate of heating changes in dry cooking of meat. During the early stage of fiber shrinkage, additional protein is released into the surrounding solution from the interior of the fiber. Fiber volume decreases in general throughout the heating range of 45° to 80°, but the decrease is most rapid between 50° and 65°.

Changes in fiber diameter and length caused by heat are influenced in a discontinuous manner as pH is increased between 5.0 and 7.0. Both the onset of shrinkage and extent of shrink are influenced by the pH of the fibers.

Birefringence exhibited by muscle fibers disappears when fibers are heated to 50° to 55°. The disappearance of birefringence seems to be a sensitive indicator of changeswithin the muscle fiber, and is apparently reflecting alterations in the proteins of the A-band.

Papain accelerates and intensifies fiber shrinkage. The muscle fiber becomes almost shapeless, with the loss of all detail.

These changes appear to be related to other changes, such as water-binding capacity, denaturation of actomyosin, and ionic charge effects, decribed by Hamm. The decrease in fiber diameter seems to be associated with the effects of protein denaturation and loss of water-holding capacity, while the decrease in length is more closely associated with the actual coagulation of the muscle proteins.

LITERATURE CITED

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Table 1. Effect of pH on Certain Shrinkage Characteristics of Muscle Fibers.

pH	Onset of Shrinkage °C	Final Size % of Original Volume.
5.00	32	57
5.25	38	38
5.50	50	43
5.75	45	26
6.00	30	33
6.25	45	29
6.50	45	51
6.75	61	54

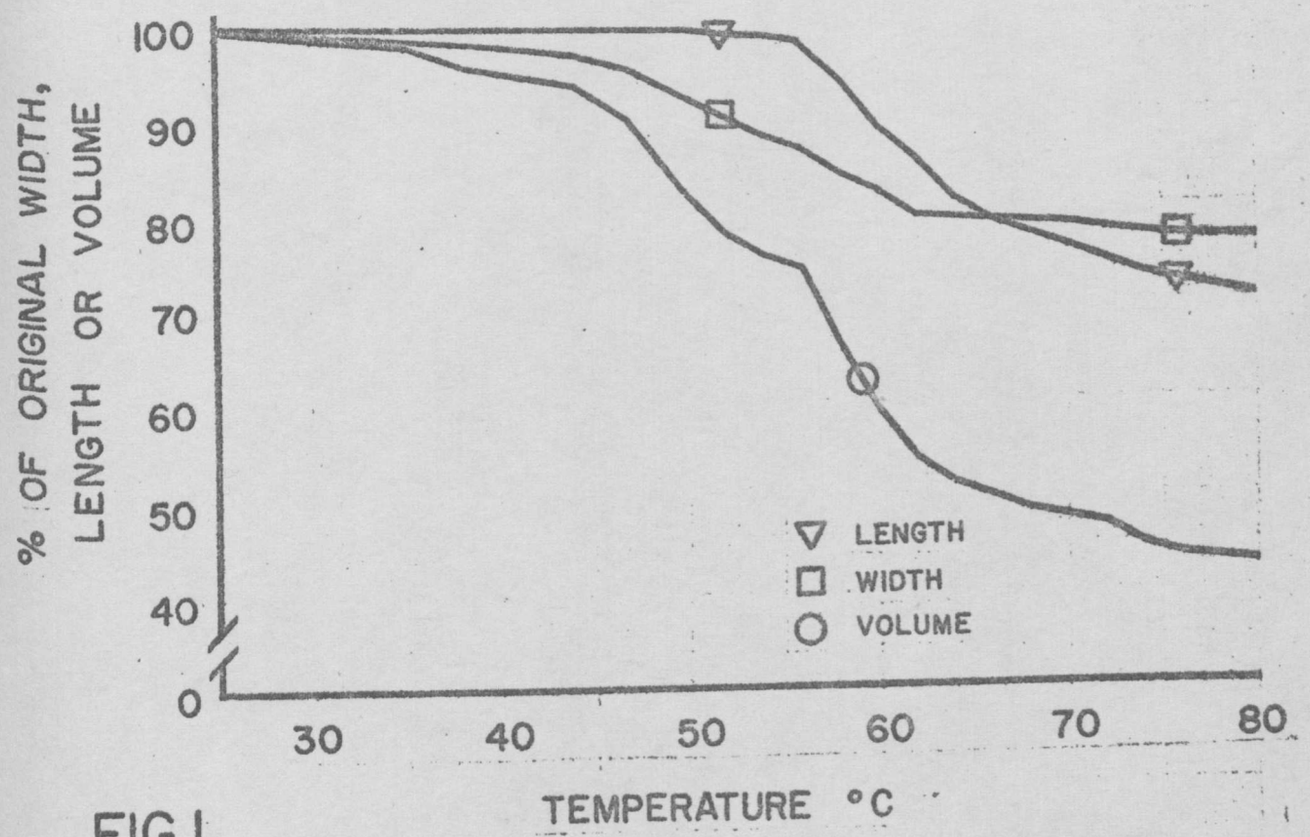

FIG.1

CHANGE IN WIDTH, LENGTH AND VOLUME WITH INCREASING TEMPERATURE

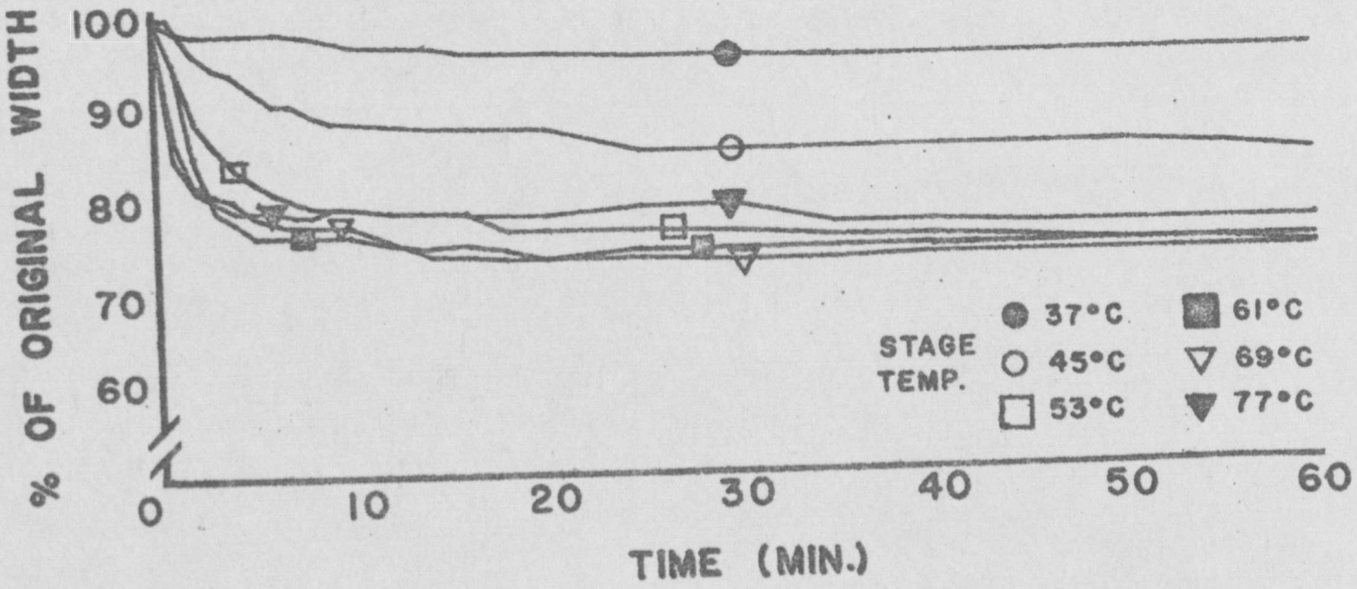

FIG.2 CHANGE IN WIDTH WITH TIME AT DIFFERENT TEMPERATURES

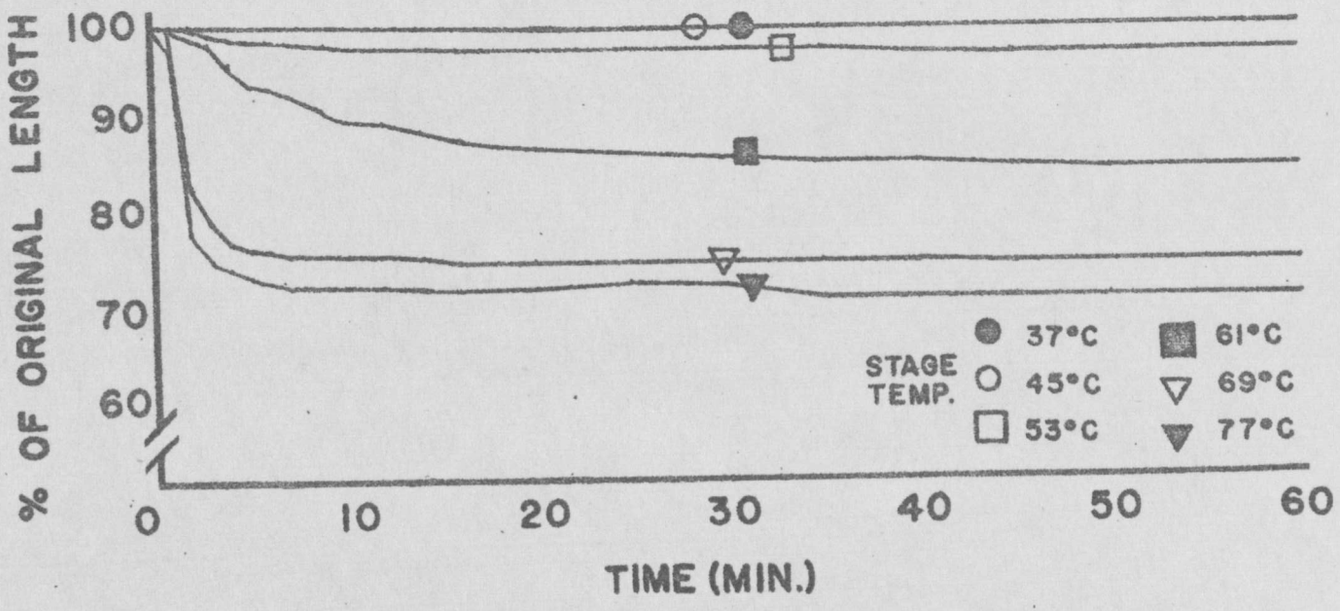

FIG.3 CHANGE IN LENGTH WITH TIME AT DIFFERENT TEMPERATURES