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Title INFLUENCE OF AGING TEMPERATURE ON BOVINE SARCOPLASMIC PROTEINS

Abstract approved

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Effects of high temperature aging upon certain characteristics of bovine l. dorsi muscle were studied. Paired wholesale ribs of carcasses were obtained subsequent to slaughter. The left rib of each pair was held at 30° C for 24 hours, then stored at 3° C. Analogous right ribs were immediately stored at 3° C. A sampling schedule of 0, 1, 2, 3, 4, 7, and 10 days was followed.

Muscles held at only the 3° C temperature showed slightly higher pH levels and superior water binding capacity than those subjected to the high temperature aging treatment.

Up to three days storage, extractability of water soluble protein was greatest from muscles held at the elevated temperature. After the third day, however, extractability was greater for muscles held at 3° C. Also during the first three days of aging, tyrosine-tryptophan index ratios indicated protein breakdown to be greatest in muscles subjected to the elevated temperature. Thereafter,
proteolysis appeared to occur more rapidly in the muscles held at 3° C.

Color differences between muscles treated via the two storage temperatures were marked. Spectrophotometric ratios (422/280 m\(\mu\)) of extracts showed that muscles held at the high temperature had higher extractable levels of oxymyoglobin than ribs held at 3° C. This difference remained apparent throughout the aging period.

Results of DEAE-cellulose ion exchange chromatography of the sarcoplasmic proteins showed only minor variations in profiles between the two aging treatments. Alterations did appear with time. Profile alterations did not appear related to anticipated increases in tenderness.
INFLUENCE OF AGING TEMPERATURE ON BOVINE SARCOPLASMIC PROTEINS

by

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INFLUENCE OF AGING TEMPERATURE ON BOVINE SARCOPLASMIC PROTEINS

INTRODUCTION

Meat tenderness is probably the most important factor influencing the acceptability of meat. Attempts to improve or increase tenderness have received considerable attention. While the literature suggests a relationship between tenderness and muscle proteins, the effects of normal or high temperature aging have not been clearly elucidated.

Investigations on the post-mortem changes of meat proteins have been limited to somewhat gross methods due to the lack of more sensitive procedures. The tenderness of meat is generally believed to be determined by the myofibrillar proteins which form the contractile mechanism of muscle (Donnelly, Rongey, and Barsuko, 1966). Sarcoplasmic proteins, however, have been implicated in playing a role in meat tenderness. Bendall and Wismer-Pedersen (1962) postulated that sarcoplasmic proteins can be adsorbed on the myofibrillar proteins during post-mortem changes in pork causing changes in their functional properties in meat. Kronman and Winterbottom (1960) pointed out that the sarcoplasmic proteins contain many enzymes controlling muscle functions so that even though they comprise a small weight percentage of the muscle proteins, the
significance of the reactions they catalyze may render them to be of great importance. Fujimaki and Deatherage (1964) concluded that sarcoplasmic proteins may reflect some aspects of post-mortem changes in meat.

The advent of cellulose ion exchange chromatography has provided a tool with which to fractionate the many proteins in the sarcoplasm. Fujimaki and Deatherage (1964) and Rampton (1965) studied the fractionation of sarcoplasmic proteins although they did not follow the complete aging period but presented data only at the beginning and the final stages of the aging process.

This study is intended to elucidate the changes that occur in sarcoplasmic extracts of beef animals from the pre-rigor state through a normal and high temperature aging process using diethylaminoethyl-cellulose (DEAE-cellulose) ion exchange chromatography.
LITERATURE REVIEW

Shortly after death of an animal, muscles pass into rigor mortis. In this condition, muscles become very rigid and inextensible. Meat from such muscles becomes tough and practically inedible. Following rigor mortis, a gradual softening effect occurs and takes a period of several days to accommodate an improvement in tenderness and acceptability. Aging is the holding of meat to take advantage of these changes. Factors responsible for this tenderizing process are not fully understood even though considerable research has been reported in the literature on the aging of meat. A number of these factors and their resulting effects are reviewed and discussed hereafter.

Animal Factors Affecting the Characteristics of Meat

Considering that meat is obtained from a diversity of animals (i.e., breed, age, sex, etc.) subjected to a variety of environmental conditions, considerable variation in meat tenderness is understandable. Some of these have been held largely responsible for variations in tenderness while others appear to be of less importance.

Age

According to Palmer (1963), age is closely related to
tenderness of the meat. On the basis of taste panel and Warner-Bratzler shear data, Palmer reported that tenderness decreases significantly with increasing age of the animal.

**Breed**

In the same experiments, Palmer (1963) found that tenderness was a heritable characteristic. Huffman et al. (1962) observed that Angus and Angus X Hereford cattle were more tender than other breed groups, and that Brahman cattle were the least tender. In a study involving Angus, Hereford, Jersey, Guernsey, Brahman and Brahman crossbred steers, Cole, Kincaid and Hobbs (1958) showed that the British breeds were significantly more tender than Brahman and Brahman crosses. Dairy breeds were intermediate between the two groups.

**Sex**

It has been generally accepted that sex or sex conditions have little effect on tenderness of young animals. Cahill et al. (1956) found no pronounced tenderness differences between young steers and bulls when half of each group was implanted with diethylstilbestrol and all animals were raised under similar conditions. Palmer (1963) reported data that supported the results of Cahill et al., although he questioned whether mature bulls would produce acceptable meat.
Changes in Meat During Post-Mortem Aging

Physical Changes

Several physical changes are readily observed subsequent to death of an animal. The highly extensible plastic state characteristic of freshly excised muscle is gradually replaced by an inextensible and rigid condition characteristic of rigor. Following maximum rigor, a gradual softening effect occurs over a period of several days.

Bendall (1960) reported that since only a small percentage of the muscle fibers contract at the onset of rigor, contraction and loss of extensibility should not be confused. Conversely, he reported data showing that the decrease in extensibility was inversely related to any shortening that may occur.

Along with the loss of extensibility, Bendall (1960) also reported a change in the "texture" of muscle with the onset of rigor. Muscle is soft and sticky before rigor and later becomes hard and dry as stiffening develops. However, this dry texture may again change to a moist condition. A "weeping" condition may develop following the decrease in muscle rigidity due to sarcolemma breakdown with subsequent fluid loss from muscle fibers. This phenomena apparently has little effect upon fiber extensibility; the extensibility remains low.
The loss of extensibility observed at the onset of rigor can readily be explained by the muscle model of Huxley (1965). This model consisted of actin filaments sliding in and out of globular myosin elements. In resting muscle, actin and myosin are dissociated. When the muscle dies, actin and myosin form the rigid complex, actomyosin, which characterizes the inextensibility of muscle in rigor. A complex series of biochemical reactions are involved in this change, some of which will be discussed later.

The gradual softening of muscle after rigor that proceeds with storage or aging time is not fully understood. Some workers believe that softening may be due to dissociation of the actomyosin complex to the actin and myosin components. Wierbicki et al. (1954) originally suggested that either an extensive dissociation of actomyosin or a limited dissociation of the actomyosin complex coupled with a redistribution of the ionic atmosphere might be responsible for the post rigor changes. They emphasized that whatever the action was, it was closely associated with muscle hydration. In a later report, Wierbicki et al. (1956) stated that tenderization during aging was not due to dissociation of actomyosin or other proteolytic reactions. Alternatively they suggested that the tenderizing effect was due to ion-protein or other protein-protein interactions rather than to proteolysis or dissociation of actomyosin.
Chemical Changes

The most obvious chemical changes occurring during the course of rigor mortis is the gradual acidification of muscle by the production of lactic acid from glycolysis of glycogen. The change commences when oxygen is no longer available for normal muscle metabolism. Stiffening of the muscle is not caused by the production and accumulation of lactic acid, but is due to the loss of ATP (Bendall, 1960). There is a series of complex biochemical reactions leading to the disappearance of ATP. In the absence of ATP the contractile structure of muscle becomes rigid and inextensible as mentioned previously.

Ultimate pH of muscle has considerable influence on the characteristics of muscle. Color, texture, electrical resistance, penetrability by curing salts, and susceptibility to bacterial spoilage are affected by the final pH (Bate-Smith, 1948). The glycogen level in muscle at death regulates the ultimate pH of muscle. In most cases a low pH is desirable since high pH may be detrimental to the color, texture and keeping quality of meat. The glycogen level at death can be influenced by various ante-mortem treatments (Briskey, 1963).

Hamm (1960) reported that the level of hydration of meat has a substantial effect on its subsequent qualities of tenderness and
acceptability. A decrease in pH and ATP disappearance from muscle are two principle factors causing a rapid decline in the level of hydration or water holding capacity of meat.

Normally, the pH of meat in maximum rigor is approximately 5.4. This is very near the isoelectric zone of the actomyosin complex. At the isoelectric point of proteins the net charge is zero. In addition, a maximum number of intermolecular salt linkages are formed and electrostatic repulsion is minimized. Meat having a low pH is characterized by a very low level of hydration. After the development of full rigor, there is a slow rise in muscle pH accompanied by an increase in water holding capacity. Hamm (1960) postulated that one-third of the post-mortem changes in hydration are due to changes in pH. The remainder can be attributed to the loss of ATP. The presence of ATP in muscle has a strong hydrative effect. This effect is apparently due to the chelation of the bivalent alkaline earth ions. These ions are left in a free form with the breakdown of ATP. In the free form they cause strong cross linkages between peptide chains of the muscle proteins and consequently cause a tightening of structure and reduction of water holding capacity (Hamm, 1960).

The slight rise in pH that occurs after full rigor passes is responsible for about 24 to 33 percent of the increase in water holding capacity that is concurrently observed (Hamm, 1960). The
remainder of the increase cannot be fully accounted for although the influence of actomyosin dissociation and proteolysis have been postulated (Hamm, 1960). Dissociation of actomyosin has been discounted by Wierbicki et al. (1956) while the increase of hydration due to proteolysis during aging still remains a possibility.

The role of proteolysis in post-mortem muscle is a controversial topic. Hoagland, McBryde and Powick (1917) found that, as meat became more tender during aging, nonprotein nitrogen accumulated within the tissue. This was believed to be an indication of proteolysis. Zender et al. (1958) reported that the loss of fine structure in meat aged aseptically for long periods of time was due to autolysis. Although Sharp (1963) found evidence of autolysis, he concluded from histological studies that the sarcoplasmic proteins were primarily affected. While Landman (1963) demonstrated catheptic activity in meat extracts using the extract itself as the substrate, Bodwell and Pearson (1964) reported that partially purified bovine muscle cathepsins had no detectable enzymatic action on actin, myosin, or actomyosin preparations from calf L. dorsi muscle. They suggested, however, that proteolytic enzymes could cause transpeptidation which would not be detected by the analytical methods used.

Some workers have given evidence disputing proteolysis in meat during aging. Wierbicki et al. (1954) could not detect an
increase of nonprotein nitrogen (indicative of proteolysis) in beef aged 15 days at 2°C. Using a more sensitive technique, end group analysis, Locker (1960) found no significant increase in N-terminal groups which could be attributed to proteolysis.

Davey and Gilbert (1966) recently showed that proteolysis of bovine l. dorsi muscles amounted to 2.3 percent of the meat proteins degraded into Trichloroacetic acid-soluble (TCA) material. They found this proteolytic activity confined to the sarcoplasmic proteins and that 80 percent of the tenderizing changes associated with aging were completed before proteolytic changes could be detected. Thus they concluded that there was no direct relationship between proteolysis and the increased tenderness due to aging.

**Aging of Beef**

Aging is probably one of the oldest methods of meat tenderization. "Aging" in a classical sense refers to the practice of holding fresh meat in cold storage at temperatures usually between 1 and 4°C for various periods of time to improve meat quality, particularly tenderness (Hoagland, McBryde and Powick, 1917).

Loss of carcass weight during the chilling and holding periods is of major concern to the beef packing industry. Sleeth, Henrickson and Brady (1957) showed that 16 wholesale ribs (U. S. Good) aged 12 days at 1°C averaged 0.4 percent shrinkage daily due
to moisture evaporation. This type of loss, as well as increased storage and trimming losses incurred during aging of beef, prompted early workers to investigate the possibility of reducing the aging time necessary to obtain a suitable degree of tenderness by increasing the storage temperature. Ewell (1940) concluded from detailed tenderizing rate studies that the rate and velocity of the aging process increased as the temperature was increased from 0 to 16° C. Similar degrees of tenderization could therefore be attained in one to three days at 16° C as would normally be found after three or more weeks of aging at 1 to 3° C. Although he reported that the growth of bacteria and mold did not increase as fast as the tenderization processes, growth was sufficient to cause excessive spoilage. He concluded that unless microbial growth could be controlled, high temperature aging was not practical.

One of the early methods employed to control bacterial and mold growth during high temperature aging was the use of ozone (Ewell, 1940). While ozone controlled bacteria and mold growth at normal aging temperatures, it was ineffective at the higher temperatures. He reported aging under a controlled atmosphere of ten percent carbon dioxide doubled the storage life of beef carcasses at 1° C, but was also ineffective at higher temperatures.

Use of ultraviolet irradiation for control of microbial growth on food products was reported by James (1936). He found that meat
aged at 10° C for five days developed little mold or bacterial growth when UV lamps were used. Sleeth, Henrickson and Brady (1957) reported that beef aged two days at 20° C lost 1.67 percent moisture which was comparable to aging three days at 1° C. In later work, they reported that tenderness, flavor, aroma, and juiciness of beef quarters and ribs aged two to three days under germicidal lamps at higher temperatures (20 to 30° C) were comparable to beef aged 12 to 14 days at normal aging temperatures (Sleeth, Kelly, Brady, 1958).

Use of antibiotics for the control of the mold and bacterial growth was investigated in the late 1950's. Wilson et al. (1960) reported that the broad spectrum antibiotic, oxytetracycline, when injected one to three hours ante-mortem into the tail (final muscle tissue concentration of 0.5 to 1.0 ppm) controlled microbial growth for an aging treatment of 43° C for 24 hours. He reported that this aging treatment produced meat that was comparable in tenderness to meat aged two weeks at 2° C. However, taste panel evaluations indicated that the meat aged at 43° C for 24 hours had more of an old, stale and slightly bitter flavor than meat aged at 2° C. The use of antibiotics for such purposes has not been accepted by the Food and Drug Administration in this country so their use for microbial control in high temperature aging of beef is of no commercial significance at this time.
EXPERIMENTAL PROCEDURE

Sample Material and Treatment

Wholesale rib sections (6th through 13th ribs inclusive) were removed from both sides of three beef carcasses about one-half hour subsequent to slaughter at a local meat packing plant. These carcasses were representative of three different quality groups according to the U. S. D. A. grading system for beef animals of the same sex. The first carcass was from a heifer of the good grade, the second from a young cow of commercial grade, and the third carcass from a mature cow of utility grade.

After removal from the carcasses, the rib sections were returned to the laboratory as soon as possible, generally one and one-half to two hours post-mortem. Rib sections from the right side of the carcasses served as controls and were aged at 3° C throughout the study to approximate the normal aging procedure. For the experimental aging treatment, rib sections from the left side of the carcasses were initially held in a tunnel dryer at 35° C for 45 minutes to partially dry the exterior surfaces in order to retard microbial growth. These rib sections were then held at 30° C for 24 hours before being stored at 3° C for the remainder of the aging period.
Sample Preparation

Steaks, one inch in thickness, were removed from rib sections at 1 1/2-2 hours and 1, 2, 3, 4, 7, and 10 days post-mortem. Samples from the L. dorsi muscle were removed for the determination of expressible and total moisture and for total protein analysis.

Fifty gm of tissue were excised from the L. dorsi muscle in small pieces excluding as much intermuscular fat and connective tissue as possible. This tissue was homogenized with 50 ml of distilled water in a water-jacketed Waring blender (semi-micro), cooled with flowing tap water, for one and one-half minutes. With a Beckman Zeromatic pH meter the pH of the homogenate was measured and adjusted to 7.0 with 1 N NaOH followed by homogenization for an additional one-half minute to thoroughly mix the homogenate. After centrifugation of the homogenate at 30,000 x G for 20 minutes, the supernatant was filtered through Whatman No. 12 filter paper to remove the fat layer on top. Four ml of each filtrate was frozen at -30° C, and thus held for the total extractable nitrogen determinations. Another ml was removed for the tyrosine-tryptophan index determinations. The remainder of the sarcoplasmic extract was dialyzed against a 160 fold volume of the chromatographic starting buffer for 22 hours at 4° C, and used for chromatographic analysis.
**DEAE Cellulose Chromatography**

Four DEAE-cellulose ion exchange columns (2 x 50 cm) were prepared for the chromatographic analysis of the sarcoplasmic protein extract. The DEAE-cellulose (Schleicher and Schuell, Selectacel Type 20, 0.83 meq/gm) was screened, and the 100-200 mesh size used. The cellulose was prepared by washing first with 1 N NaOH, then with 1 N HCl and finally washed free of acid with distilled water as described by Rampton (1965).

A suspension of cellulose in 0.1 N NaOH was used to pack the columns (at room temperature) to a height of 40 cm under nitrogen pressure as described by Rampton (1965) with the exception that rotation of the columns was omitted during the packing operation. After packing, the columns were placed in a cold room at 4° C, and washed free of NaOH with the chromatographic starting buffer (0.04 M Tris adjusted to pH 9.0 with concentrated H₃PO₄).

The four chromatographic columns were prepared at the same time so that the columns were subjected to equal conditions. After completion of each chromatographic run, the columns were washed and regenerated for reuse by passing 750 ml of 0.1 M Na₃PO₄-0.2 percent Triton X-100 (a non-ionic detergent) solution through the columns at a flow rate of 80 ml/hour. This wash solution was rinsed from the columns by extensive washing (more than 800 ml).
with starting buffer. Twenty-five ml of 0.1 M Tris-phosphate, pH 9.0, was then passed through the columns followed by 200 ml of starting buffer. These clean-up and re-equilibration procedures were performed after each chromatographic run, and before the initial use of the columns.

The chromatography assembly consisted of a nine chambered Varigrad (variable gradient mixer) connected to a Buchler micro-pump which delivered buffer at the rate of 80 ml/hour to each of two chromatographic columns via polyethylene tubing. Two columns, rather than one, were incorporated into the assembly so the samples from the control and experimental treatment could be fractionated simultaneously. The eluting buffers passed through the columns into flow-through cells of Gilson UV monitors where transmittance of the effluent was measured continuously at 280 mÅ and recorded on either a Beckman Potentiometric or a Texas Instrument "recti-riter" recorder. After passing through the UV monitors, the effluents were collected by two LKB fraction collectors equipped with 10 ml volumetric siphons. Each fraction was marked on the appropriate absorption recording by fraction markers. The chromatographic assembly is shown in Figure 1.

Sarcoplasmic extracts of the 0, 2, 4, 7, and 10 day sampling times were chromatographed. Nine ml of the dialyzed sarcoplasmic extract was placed on top of the column and forced into the cellulose
Figure 1. Chromatographic assembly used in these experiments.
under 5 p.s.i. nitrogen pressure. Eight ml of starting buffer, divided into three portions, was used to completely wash the extract into the column. Ten ml of starting buffer was then placed on top of the cellulose and the elution procedure started.

The elution procedure consisted of 112 ml of starting buffer followed by a gradient mixture beginning at pH 9.0 and 0.04 M Tris-phosphate and ending with a final buffer at pH 3.6 and 0.5 M Tris-phosphate. The elution was completed by placing 300 ml of the final buffer in the last chamber of the Varigrad followed by 500 ml of 0.1 N NaOH. Columns were washed and re-equilibrated at this point.

The gradient was prepared by mixing the following volume percentages of final buffer with the starting buffer in the order of 0, 0.4, 0, 1.6, 0, 3.0, 0, 5, and 100 percent, respectively, for the nine chambers of the Varigrad. Each of the chambers, one through eight, contained 500 ml of solution whereas only 472 ml was placed in the ninth chamber to compensate for its greater specific gravity. The gradient provided is called a concave gradient (Rampton, 1965) which refers to the slope of the increasing salt concentration. All operations were performed at a constant flow rate of 80 ml/hour at a temperature of 4° C.
Moisture

Total moisture was determined by drying, in duplicate, 3-4 gm samples of meat in tared aluminum pans in vacuo at 70° C. The moisture content was calculated by the difference between the wet and dried weight of the meat, and expressed as percentage moisture.

Expressible Moisture Determination

A small sample of the meat (0.2-0.3 gm) was placed mid-position on an 11 cm diameter Whatman No. 1 filter paper previously dried at 85° C for one and one-half hours. The filter paper with the sample was placed between two 3/8 in thick plexiglas plates and pressed in a Carver Laboratory Press under a pressure of four thousand p.s.i. for five minutes. The plates were separated, and the area of the pressed muscle on the filter paper was outlined with a soft pencil. The circumference of the expressed moisture remained distinct so that marking was unnecessary. The areas of the pressed muscle and expressed moisture were then measured with a compensating polar planimeter. The expressible moisture ratio is the ratio of the pressed muscle area to the expressed moisture area (Biskey et al., 1960).
Tyrosine-Tryptophan Index

The tyrosine-tryptophan index is the absorbance of the meat extract measured at 280 μm both before and after TCA precipitation (Zender et al., 1958). Determinations were made in duplicate on the dialyzed and undialyzed sarcoplasmic extracts. One-half ml of the extract was placed in a 25 ml volumetric flask and brought to volume with 0.5 ml of 0.1 N NaOH and distilled water. Absorbance at 280 μm was measured with a Beckman DB spectrophotometer using 1 cm quartz cuvettes. Another 0.5 ml of the extract was placed in a test tube to which 9.5 ml of 15 percent TCA was added. After mixing, the extract was filtered through Whatman No. 42 filter paper and the absorbance of the filtrate was read at 280 μm.

Color-Protein Ratio

A spectrophotometric scan from 750 to 245 μm was made on the diluted dialyzed extract with a Beckman DB recording spectrophotometer. The maximum absorbance of a characteristic peak at 422 μm was used to indicate the amount of oxymyoglobin present. A ratio of absorbance at 422 μm to that at 280 μm was used to express the amount of oxymyoglobin on a protein basis.
Total Extractable Nitrogen

The amount of nitrogen extracted and present in the sarcoplasmic extract before dialysis was determined by the A. O. A. C. micro-Kjeldahl method using the mercuric oxide catalyst and boric acid collection method (Horwitz, 1960).

Vertical Gel Electrophoresis

A vertical gel electrophoresis apparatus (Model E-C 470, E-C Apparatus Corporation) was used to determine the electrophoretic pattern of the sarcoplasmic extract. The buffer used in the procedure was that recommended by the manufacturer. It consisted of 40.0 gm Tris, 4.0 gm Na₂EDTA and 4 liters of distilled water. The pH of the buffer was adjusted to 9.0 with boric acid. An acrylamide gel of 7.5 w/v percent final strength and the Tris buffer of pH 9.0 were used as the aqueous phase. A syringe was used to place 0.05 ml of the dialyzed sarcoplasmic extract on the gel before applying electrical current. A current of 60 ma at 200 volts was then applied for a seven hour period. All of the operating procedures were those recommended by the equipment manufacturer. After the electrophoretic run was completed, the gel was removed from the apparatus and stained with amido black in an aqueous methanol-acetic acid dye solution for one and one-half minutes. The gel was destained in an electrophoretic
destainer (Model E-C 479, E-C Apparatus Corporation) according to manufacturer's operating instructions. The destained gels were then photographed to obtain a permanent record.

**Taste Panel**

Due to size limitations of the l. dorsi muscles and the amount of meat required for analytical purposes, taste panel evaluations were not performed on the meat used in the main part of this study. Thus an experiment was carried out after the completion of the analytical work to obtain information about the effects of high temperature aging upon the organolytic qualities of beef.

Wholesale rib sections of a very mature, utility grade Holstein cow and a mature, commercial grade Hereford cow were removed and treated in a manner similar to samples used for the analytical determinations. At three and seven days post-mortem, roasts (two ribs thick) were removed and roasted at 163° C to an internal temperature of 71° C. The l. dorsi muscle was excised, sliced in $\frac{1}{4}$ in. thick slices, and evaluated subjectively by a trained ten member panel. The panel judged samples on a seven-point descriptive scale for tenderness, juiciness, flavor of lean, and over-all desirability. The scale ranged from 7 (very tender, very juicy, very flavorful, very desirable) to 1 (extremely tough, extremely dry, imperceptible flavor, extremely undesirable) in half-unit divisions. Tenderness of
the cooked meat was measured objectively by Kramer-Allo recording shear press. After the meat had cooled to room temperature, four one-inch cores of the sliced 1. dorsi (¼ in. thick) were placed in the shear box with muscle fibers laying perpendicular to the shear slots. After the initial shear, the meat fragments were returned to the shear box in a random manner and sheared two more times. An average of the three shear force measurements was used as an index of tenderness. Results were expressed as pounds of force required to shear a gram of meat. It should be mentioned at this point that shear force values are inversely related to panel tenderness scores—greater force is required to shear less tender meat than is required for more tender meat.
RESULTS AND DISCUSSION

For the purpose of discussion, the following section has been divided into gross observations and analytical results.

**Gross Observations**

Exposed lean surfaces of the rib sections aged at the high temperature were very dark red which was probably due to the initial drying treatment employed. When the dried surfaces were trimmed away the meat aged by the two methods appeared to be similar in color. After the freshly cut surfaces had been exposed to the air for 20 minutes, the high temperature aged meat showed a brighter cherry red color which was judged by the laboratory staff to be more desirable. An example of this color difference is shown in Figure 2. Although the meat samples in these photographs were not those used for the analytical work, they were obtained from additional carcasses and subjected to the same experimental conditions described previously. The fat layers of the high temperature aged samples appeared more yellow and oily than the normally aged meat.

All homogenates prepared at the first sampling time were just thin enough to be poured from the blender jar. After one day post-mortem, homogenates became less viscous with increasing aging time. Upon adjustment of the pH to 7.0, however, the homogenates
Figure 2. Photographs of paired ribs aged by normal and high temperature methods taken at seven days post-mortem.
of all sampling times increased in viscosity so that their transfer could be accomplished only with the aid of a spatula. All homogenates appeared to have similar viscosities at a pH of 7.0.

Properties of the intramuscular fat appeared to be influenced by the aging treatments. Fat from the high temperature aged meat separated more readily during homogenization and often floated in small clumps on top of the homogenates. After three days post-mortem, homogenates of the high temperature aged meat showed a much brighter pink color than those of the normally aged meat. This color difference was apparent for the remainder of the aging period. After centrifugation, the extracts showed similar color differences.

During dialysis of the extracts, a light flocculant precipitate occasionally appeared to be formed. When this occurred, the dialyzed extract was centrifuged for 20 minutes at 1500 x G before chromatography and electrophoresis. Zender et al. (1958) reported a similar phenomenon. They suggested that the precipitate might be globulin X or other insoluble proteins that had undergone partial proteolysis, although the precipitate was still insoluble at a low salt concentration.
Analytical Results

Effect of pH

The pH changes of the homogenates with aging time are shown in Figure 3. The pH changes of the mature cow and the heifer sample homogenates followed the regular pH patterns that have been reported by many workers (Marsh, 1954; Sayre and Briskey, 1963; Bodwell, Pearson and Spooner, 1965). The pH of the homogenates from the meat subjected to high temperature treatment were slightly lower than those of the control samples at one day post-mortem although the difference decreased with increased aging time. The pH pattern for both the treated and non-treated meat of the cow was much higher than that of the other two animals. After one day post-mortem, the pH of the high temperature treated meat of the cow was consistently lower than that for the normally aged cow meat. This latter relationship was also observed with the other two animals.

The pH-time relationship observed in this study agrees with the results of Marsh (1954) and Cassens and Newbold (1966). The latter workers showed that beef neck muscles (sternomandibularis) held at 37°, 15° and 1° C required 8, 24 and 48 to 72 hours post-mortem, respectively, to reach their ultimate pH. Marsh found that the ultimate pH of the L. dorsi muscle aged at different
Figure 3. pH of L. dorsi muscles of three animals aged by normal and high temperature methods. Legend: High temperature aging——; Normal aging——.
temperatures varied only slightly. He reported that 80 percent of his samples reached an ultimate pH of 5.7 or below while one of every six samples failed to fall below 6.2. He found no significant differences in ultimate pH between grades of beef but did note slight differences between old and young animals. Animals 3-5 years of age had average ultimate pH's of 5.40 ± .06 while 6-8 year old animals averaged 5.55 ± 0.11. No relationship was found between initial and ultimate pH values.

Total and Expressible Moisture

Data for the total moisture determinations are given in Table 1. Results of the analysis of variance (Li, 1964) indicate that differences in moisture levels between the two aging treatments or between the sampling time intervals were not significant at the 5 percent probability level. Since the l. dorsi muscle is well protected from surface drying and only a slight exudate was noticed from any of the meat during the aging period, only minor differences in total moisture contents between treatments and aging time should be expected. The variation in moisture levels between individual animals was probably due to variation in the amount of intramuscular fat.
Table 1. Total moisture contents of L. dorsi muscles of three animals aged at normal and high temperature conditions.

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Heifer</th>
<th>Cow</th>
<th>Mature Cow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N HT</td>
<td>N HT</td>
<td>N HT</td>
</tr>
<tr>
<td>Days Post-Mortem</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>75.48 74.61</td>
<td>74.46 74.64</td>
<td>67.46 68.83</td>
</tr>
<tr>
<td>1</td>
<td>75.27 74.87</td>
<td>73.66 73.39</td>
<td>68.38 67.42</td>
</tr>
<tr>
<td>2</td>
<td>75.39 75.46</td>
<td>74.61 72.69</td>
<td>69.61 68.27</td>
</tr>
<tr>
<td>3</td>
<td>74.97 75.60</td>
<td>74.55 73.92</td>
<td>69.55 67.23</td>
</tr>
<tr>
<td>4</td>
<td>74.59 74.69</td>
<td>74.08 73.82</td>
<td>68.77 69.06</td>
</tr>
<tr>
<td>7</td>
<td>75.38 75.09</td>
<td>74.54 74.00</td>
<td>68.76 67.96</td>
</tr>
<tr>
<td>10</td>
<td>74.36 75.13</td>
<td>74.31 74.30</td>
<td>69.13 66.53</td>
</tr>
</tbody>
</table>

* N = Normal Aging. HT = High Temperature Aging.

Expressible moisture data are presented in Figure 4. These data were tested statistically for differences between the two aging treatments and with aging time. No significant differences (P < 0.05) were found for either case. The graphical presentation of the data, however, indicated that the expressible moisture ratios of the high temperature treated samples were generally slightly higher than those for the normally aged samples. Although these data show considerable variation, there was a trend showing an increase in the expressible moisture ratio from slaughter time to one or two days post-mortem followed by a slight decrease at three or four days post-mortem. After four days post-mortem, a slight increase in the expressible moisture ratios for most samples was noted. The
Figure 4. Expressible moisture ratios of $l.$ dorsi muscles of three animals aged by normal and high temperature methods. Legend: High temperature aging—--; Normal aging—.--.
lower pH values of the high temperature treated samples may indicate a relationship between these two factors. Hamm (1960) reported a sharp decline in the water holding capacity of meat that coincided with the characteristic pH drop in post-mortem muscle.

**Extractable Nitrogen and Tyrosine-Tryptophan Index**

Total crude protein of the samples taken at four days post-mortem were analyzed by the macro-Kjeldahl method (Horwitz, 1960). The results were 21.5, 22.0 and 21.3 percent protein for the normally aged meat of the heifer, cow and mature cow, respectively. Protein contents for the high temperature aged meat were 22.4, 21.8 and 22.2 percent, respectively, for the heifer, cow and mature cow. These data indicated very little difference in protein contents of the various samples.

Determinations for the extractable nitrogen are presented in Table 2. Between the time of sample extraction and completion of the nitrogen determinations, extracts of three sampling periods were inadvertently lost. Since the missing data were from the first four days of the aging period, it is difficult to draw valid conclusions about trends in the extractability of the nitrogenous materials. However, there appeared to be a slight decline in extractability of water extractable materials when measured at the beginning and end of the aging period. In an effort to provide information about this
extractability, the tyrosine-tryptophan index prior to TCA precipitation was used.

Table 2. Percent nitrogen of water soluble extracts of l. dorsi muscles of three animals aged at normal and high temperature conditions.

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Heifer</th>
<th>Cow</th>
<th>Mature Cow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>HT</td>
<td>N</td>
</tr>
<tr>
<td>Days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-Mortem</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.88</td>
<td>0.82</td>
<td>0.82</td>
</tr>
<tr>
<td>1</td>
<td>----</td>
<td>----</td>
<td>0.86</td>
</tr>
<tr>
<td>2</td>
<td>0.76</td>
<td>0.72</td>
<td>----</td>
</tr>
<tr>
<td>3</td>
<td>0.78</td>
<td>0.72</td>
<td>----</td>
</tr>
<tr>
<td>4</td>
<td>0.82</td>
<td>0.73</td>
<td>0.90</td>
</tr>
<tr>
<td>7</td>
<td>0.76</td>
<td>0.69</td>
<td>0.87</td>
</tr>
<tr>
<td>10</td>
<td>0.78</td>
<td>0.66</td>
<td>0.80</td>
</tr>
</tbody>
</table>

*N = Normal Aging.  HT = High Temperature Aging.

Figure 5 shows the tyrosine-tryptophan index before TCA precipitation. The high temperature aged meat had a higher index level for the first three or four days post-mortem after which the normal aged meat showed higher index values. Statistical analysis failed to reveal any significant differences (P < 0.05) in the indices between the two treatments.

Although Zender et al. (1958) described this index as only a relative measure of proteinaceous substances, it can be utilized for following the proteolysis of meat during storage. El-Badawi (1963)
Figure 5. Tryosine-tryptophan index for protein (absorbance at 280 mμ) of undialyzed water extracts of L. dorsi muscles of three animals aged by normal and high temperature methods. Legend: High temperature aging ———; Normal aging ———.
reported that the results of the index were highly correlated to results obtained by the Kjeldahl method. The index after TCA precipitation is used as an indication of nonprotein nitrogenous compounds. Zender et al. (1958) found an initial increase in the index for protein materials at one day post-mortem followed by a gradual decrease for the next 70 days. The index of the nonprotein materials showed a gradual and proportionate increase over the same period.

Results observed for the nonprotein nitrogen indices (Figure 6) followed the same pattern that Zender et al. (1958) showed for the first ten days of their study. The indices for the extracts before TCA precipitation generally followed the data of Zender et al., although they did not show specific data for the time interval between two and ten days.

Sayre and Briskey (1963) found that the solubility of sarcoplasmic proteins was greatest immediately after death. Goll, Henderson and Kline (1964) reported similar findings and proposed that the protein solubilities were altered by post-mortem physiological conditions. Conversely, Zender et al. (1958) observed an increase in sarcoplasmic protein extractibility at one day post-mortem which they attributed to the 1.0 M glycine buffer (pH 8.6) used in their work. The increase in protein solubility observed in the current study was probably due to standardization of the homogenates at pH 7.0 during preparation. Thus the pH was constant for
Figure 6. Tyrosine-tryptophan index for nonprotein nitrogen (absorbance at 280 μm) of undialyzed water extracts of L. dorsi muscles of three animals aged by normal and high temperature methods. Legend: High temperature aging———; Normal aging———.
all extracts rather than being carried out at variable physiological pH's. This difference alone would greatly affect the extractability of the proteins.

**Color-Protein Ratio**

Graphical presentation of the absorbance ratios (422/280 mμ) of the extracts is presented in Figure 7. These ratios were used to depict what was observed visually. There was little difference in the ratios for the two aging treatments for the first two days post-mortem. The heifer and cow extract ratios showed marked differences at the third day post-mortem and the mature cow on the fourth day post-mortem. The differences between the color-protein ratios appeared to remain constant thereafter. Statistical analysis showed significant differences (P < 0.01) between the two aging treatments for the heifer and the cow samples and significant differences (P < 0.01) between the ratios with time for the heifer. Spectrophotometric data were initially collected for the cow samples at three days post-mortem when the color differences were first noted. Consequently, there was not sufficient data for statistical treatment although the difference was observed visually.

Mackinney and Little (1962) reported an absorption maxima of 423 mμ for humpback whale oxymyoglobin with lesser peaks at 542 and 580 mμ. Theorell (1934) showed the absorption maxima to
Figure 7. Absorbance ratios (422/280 m\(\mu\)) of water extracts of L. dorsi muscles of three animals aged by normal and high temperature methods.
be 421 μm for oxymyoglobin. The absorbance ratio (422/280 μm) reported herein was used to express the oxymyoglobin content on a protein basis as measured at 280 μm. This ratio is only a relative index since the absorbance at 280 μm is a crude approximation of the protein concentration. The index, however, indicated what was observed visually. McCarthy and King (1942) showed that the press juice of meat aged by the Tenderay process (48 hours at 16° C) had a greater color density than meat aged at 2° C as measured with an Evelyn type of photoelectric colorimeter using filter No. 540 M. They did not relate the color density to the protein concentration of the press juice.

Color differences observed in the present study can not be readily explained. Differences do not appear to be due to myoglobin concentration since the differences were observed in both the raw meat and extracts. Moreover, the myoglobin concentration should be quite similar in both l. dorsi muscles of the same animal. A possible explanation for the higher color-protein ratio of high temperature aged meat might be that this meat has an ability to oxygenate myoglobin more readily than normally aged meat. Additional research to elucidate the color differences observed is needed.

Column Chromatography

Rampton (1965) reported the usefulness of the chromatographic
scheme used in this study, but interpretation of the protein chromatographic profiles of the various samples must be done with care. Any fluctuations in the experimental chromatographic conditions can easily alter the profile. An attempt was made to keep all conditions standard throughout all of the analyses. The chromatographic profiles are presented with the elution volume shown as ten ml fractions on the abscissa, and the absorbance of the eluent read at 280 m\(\mu\) on the ordinate. No attempt was made to quantitate the results by measuring the profile peak heights or areas under the profiles, although reference will be made to peak heights in a relative sense. The chromatographic profile peaks are referred to as individual peaks with the understanding that a single peak is not necessarily a homogeneous protein fraction, but can consist of several distinct proteins that have similar elution times.

Results of DEAE-cellulose ion exchange chromatographic separation of the sarcoplasmic proteins from the \textit{l. dorsi} muscle of the cow are presented in Figure 8. Differences between the elution profiles of the two treatments at the various sampling times appear to be quite minor. Marked changes, however, can be observed in the chromatograms as storage time increased. Generally, the chromatograms are characterized by a large breakthrough peak followed by three or four small peaks, and then a large peak that was eluted between fractions 64 and 105. This large peak contained
Figure 8. Chromatography of dialyzed sarcoplasmic proteins extracted from cow
l. dorsi muscle at different aging intervals. Chromatograms on left are
for normal aging method and on right for high temperature aging method.
most of the muscle pigments. This pigment peak was followed by 8 to 20 less-defined peaks. These small, often poorly separated, peaks seemed to be eluted at about the same time for the two treatments, although the heights of the peaks were not constant with the different storage times.

The changes that occurred with increasing storage time seemed to take place in and between the breakthrough and the pigment peaks. The most noticeable change in profiles of the sarcoplasmic proteins between zero day and two days post-mortem was the appearance of a double or split peak in place of the single pigment peak. In addition, a very small peak near fraction 35 became more distinct.

The four day post-mortem profile indicates that the breakthrough peak had decreased to about one-half of its initial height. Moreover, the small peak at fraction 35 had increased to form a large peak between fractions 30 and 46 while a small peak appeared near fraction 26. The pigment portion also had been altered so that a smaller peak was eluted before the main pigment peak.

The chromatograms for the seven and ten day extracts both showed an increase in the height of the breakthrough peaks and a decrease in the peaks that appeared between fractions 30 and 46 at the four day sampling time. The seven day profile of the pigment proteins showed only a main peak with one or two shoulders which
appeared to emerge as a distinct peak at 10 days post-mortem. The profiles of the samples held at the elevated storage temperature showed some loss of peaks at fraction 26 and between fractions 30 and 46.

The chromatographic profiles of the heifer are shown in Figure 9. Differences and changes in these patterns were similar to those noted for the cow. The breakthrough and pigment peaks showed marked changes along with minor alterations in the latter part of the elution profile. The peak between fractions 30 and 46 that appeared at four days post-mortem was not evident at 7 days while the breakthrough peak disappeared completely by the ten day post-mortem sampling. The chromatograms showed very small differences between the two temperature treatments.

The chromatograms (Figure 10) for the sarcoplasmic proteins of the L. dorsi muscles of the mature cow showed less variation in the breakthrough and pigment peaks than were noted for the other two animals. The profiles did seem to show, however, more variation with the sampling times than between the two temperature treatments.

As mentioned previously, most of the changes in the chromatograms seem to have occurred in the first 120 fractions of the elution procedure. The changes in the breakthrough and other peaks up to the pigment peak might be due to changes in the extractability
Figure 9. Chromatography of dialyzed sarcoplasmic proteins extracted from heifer 1. dorsi muscle at different aging intervals. Chromatograms on left are for normal aging method and on right for high temperature aging method.
Figure 10. Chromatography of dialyzed sarcoplasmic proteins extracted from mature cow l. dorsi muscle at different aging intervals. Chromatograms on left are for normal aging method and on right for high temperature aging method.
of the particular proteins involved. Such a change as dissociation might cause a shift of one peak to another position. In the case of the heifer chromatograms, the breakthrough peak could have shifted to appear as the peak at fraction 35 as seen on the chromatogram at four days post-mortem. Some differences in the elution times might be due to slight inherent differences between the four chromatographic columns even though they were prepared as equally as possible. The changes in the pigment peak, however, are not clear. There seemed to be a general shifting of this peak in some cases or possibly even the appearance or increase of peaks that may have had similar elution times. Some change of this type occurred in all of the three series of chromatograms. The series of chromatograms for the heifer is a good example. The profile for zero time showed that the major pigment peak at about fraction 84 with a shoulder eluted at fraction 72 for both the normal and high temperature aging treatments. At two days post-mortem the profiles were the same except that the high temperature aging treatment showed another small peak emerging near fraction 96. At the four day sampling time the main pigment peak was eluted near fractions 98-102 with shoulders at fraction 84 which was the position of the original major peak. The final chromatograms at ten days post-mortem showed that the major peak was still at fractions 98-100, but the shoulders mentioned at the four day time had increased to a peak about half of
the height of the main pigment peak. In all of these cases the bulk of the pigmentation always appeared under the largest peak of this group. The reasons for these changes are not clear and merit further investigation.

The literature contains reports on the occurrence of variations in the chromatographic profiles of sarcoplasmic proteins of beef and chicken muscle during post-mortem aging. Fischer (1963) used DEAE-cellulose chromatography to study changes in sarcoplasmic proteins of chicken breast muscle. He found seven distinct protein fractions, one of which increased with the aging time. Fujimaki and Deatherage (1964) used both cellulose-phosphate and DEAE-cellulose chromatography to study the differences in beef sarcoplasmic proteins at one and twelve days post-mortem. Using a stepwise elution scheme, they found that some of the eluted fractions disappeared while others decreased. Rampton (1965) used DEAE-cellulose chromatography with a concave gradient of pH and salt concentration to show that some protein fractions of beef sarcoplasmic proteins disappeared, others diminished while some new components appeared after ten days of aging.

The series of chromatograms obtained in this study not only confirm the observations reported above, but also show clearly that definite changes do occur in the sarcoplasmic proteins as measured by DEAE-cellulose chromatography. Although these changes can be
followed from day to day during the aging period, they do not seem to be significant as a measure of tenderness due to the variation noted between the three animals.

Fujimaki and Deatherage (1964) analyzed certain chromatographic fractions of beef sarcoplasmic proteins fractionated on cellulose-phosphate columns for enzyme activity. They found activities for aldolase, lactic dehydrogenase and myokinase in seven, eight and three fractions respectively of the ten fractions studied. They concluded that the individual chromatographic peaks do not contain a particular homogeneous protein nor is all of any one protein found in a single specific fraction.

In view of Fujimaki and Deatherage's report (1964), it was decided to fractionate aldolase, lactic dehydrogenase (band five) and myokinase by the DEAE-chromatographic procedure to gain information about the elution profiles of these muscle enzymes. These enzymes (grade A purity) which had been extracted from rabbit muscle were purchased from Calbiochem, Los Angeles, California. Approximately 20 mg of aldolase and ten mg of both myokinase and lactic dehydrogenase were chromatographed individually. In addition, a blank chromatogram was obtained by the chromatography of nine ml of starting buffer in place of nine ml of sample extract. The resulting chromatograms are presented in Figure 11.

The chromatogram for the blank showed an absence of UV
Figure 11. Blank and enzyme chromatograms.
absorbing material up to fraction number 250 when a peak was observed followed by a very small peak at fraction number 280. These two peaks appeared after the elution gradient had been completed, and at the time when the pH became strongly alkaline due to the addition of 500 ml of 0.1 N NaOH at the start of the clean-up procedure. The rapid increase in absorbancy at the end of the chromatogram was due to the Triton X-100 detergent used in the final wash solution. The remaining chromatograms indicated that aldolase was eluted as a breakthrough peak while lactic dehydrogenase appeared as three peaks at the beginning of the elution scheme. Myokinase appeared as a single peak near fraction number 200 even though a stabilizer, albumin, had been added to the myokinase by the supplier during preparation of the purified enzyme. The peaks that appeared on the blank chromatogram are also quite evident on chromatograms for the enzymes. Although these enzymes were obtained from rabbit muscle, these results might be indicative for similar enzymes found in beef muscle.

Electrophoresis

The electrophoretograms of the three sample extracts obtained at 0, 4, and 10 days post-mortem are shown in Figure 12. The clarity of the electrophoretograms may have been impaired somewhat by the freezing of the extracts since considerable tailing.
Figure 12. Vertical gel electrophoresis of water extracts of \textit{L. dorsi} muscles of three animals aged by normal and high temperature methods.

\begin{itemize}
  \item \textit{N} = Normal aging
  \item \textit{HT} = High temperature aging
  \item \textit{HT*} = Twice the normal amount of sample used
\end{itemize}
of the individual protein fractions is apparent. The electro-
phoretograms are clear enough, however, to show that the protein
fractions varied only slightly in mobility and intensity between the
two aging treatments and with time. Zender et al. (1958) reported
that the electrophoretic pattern of proteins extracted from the
dorsal muscles of lamb stored aseptically at 25° C was modified
only after 20 days of storage. Although rabbit muscle behaved
similarly, it required 50 to 70 days of storage before modification
of the electrophoretic patterns was observed. They also showed
that when muscles were stored at 38° C the electrophoretic modifica-
tions were apparent nine days post-mortem. Kronman and
Winterbottom (1960) used moving boundary electrophoresis to
study changes in the proteins of beef muscle aged for seven days at
3° C. They reported changes in the relative amounts of the protein
components, but concluded that the changes were very inconsistent.

Taste Panel

Results of the taste panel evaluation are presented in Table 3.
These data indicate that the meat from the mature cow subjected to
the high temperature aging treatment had significantly (P < .01)
higher tenderness and desirability scores at the three days testing
period than did corresponding meat aged under normal conditions.
After seven days of aging, however, differences between these
scores for the treated and non-treated mature cow meat were not significant. Shear force data paralleled taste panel results for tenderness. Flavor and juiciness differences between the two aging treatments were not significant at either the three or seven day testing periods.

Table 3. Mean taste panel scores and Kramer shear values of meat from a cow and a mature cow aged by normal and high temperature conditions.a

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cow</th>
<th>Mature Cow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>HT</td>
</tr>
<tr>
<td>Tenderness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Day</td>
<td>4.65</td>
<td>4.60</td>
</tr>
<tr>
<td>7-Day</td>
<td>5.80</td>
<td>5.15</td>
</tr>
<tr>
<td>Juiciness</td>
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<td></td>
</tr>
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<td>3-Day</td>
<td>5.45</td>
<td>5.55</td>
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<tr>
<td>7-Day</td>
<td>6.15</td>
<td>5.55</td>
</tr>
<tr>
<td>Flavor</td>
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<td></td>
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<tr>
<td>3-Day</td>
<td>5.50</td>
<td>5.45</td>
</tr>
<tr>
<td>7-Day</td>
<td>5.80</td>
<td>5.45</td>
</tr>
<tr>
<td>Desirability</td>
<td>5.25</td>
<td>5.10</td>
</tr>
<tr>
<td>7-Day</td>
<td>5.95</td>
<td>5.30</td>
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<tr>
<td>Kramer Shear</td>
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<tr>
<td>3-Day</td>
<td>17.21</td>
<td>18.32</td>
</tr>
<tr>
<td>7-Day</td>
<td>13.19</td>
<td>19.44</td>
</tr>
</tbody>
</table>

a Means not underlined by the same solid line are different (P < .01)
b N = Normal Aging. HT = High Temperature Aging.
c Expressed as pounds per gm of tissue
Conversely, taste panel results for the cow samples were quite divergent from those described above. At the three day testing period, no significant differences were noted in tenderness, juiciness, flavor and desirability scores for the cow meat aged under normal and high temperature conditions. However, panel scores at the seven day testing time indicated that meat aged under normal conditions was significantly ($P < .01$) more tender, juicy and more desirable than the meat subjected to high temperature aging. Shear force data for the measurement of tenderness paralleled those of the taste panel. It is interesting to note that the shear force data indicated a decrease in tenderness of high temperature aged meat from both animals from the three to seven day post-mortem periods.

In view of conflicting evidence presented by these evaluations and the limited number of samples studied, no conclusions concerning the acceptability of meat aged by the high temperature method can be drawn.
SUMMARY AND CONCLUSIONS

A study of some of the effects of high temperature aging on bovine l. dorsi muscle was completed. Although pH values, expressible moisture ratios, protein extractability levels, color of lean meat and taste panel differences were investigated, major emphasis was devoted to the determination of changes occurring in the sarcoplasmic proteins during the aging treatments. The latter was accomplished by the use of diethylaminoethyl (DEAE) cellulose ion exchange chromatography.

The pH values of muscle homogenates prepared from a heifer, cow and a mature cow followed previously reported patterns. The pH of homogenates of meat aged at a higher temperature was generally lower than that for meat aged at normal temperatures.

While total moisture levels remained constant, expressible moisture ratios of high temperature aged meat appeared generally higher than normally aged meat.

Tyrosine-tryptophan indices of sample extracts before TCA precipitation indicated higher protein extractabilities during the first three or four days followed by lower extractabilities in high temperature samples. Few differences were noted between the indices subsequent to TCA precipitation.

Color-protein ratio data indicated that sample extracts from
high temperature aged meat contained higher concentrations of oxy-
myoglobin per unit of protein than normally aged meat at three to
four days. It was suggested that meat aged at the higher tempera-
ture had greater ability to oxygenate myoglobin.

Taste panel evaluations and Kramer shear data indicated that
rib roasts from the mature cow aged at the higher temperature was
more tender and desirable than paired rib roasts aged at normal
temperature. The reverse was noted in roasts of the younger cow.

Chromatography results of \( \text{I. dorsi} \) muscle extracts made at
prescribed periods showed few differences between profiles of
samples aged by the two methods. Several changes were noted with
increasing time. Most alterations occurred within the first 120
fractions of the elution profile. The chromatographic procedure
appeared to be highly reproducible. Vertical gel electrophoresis
did not appear as sensitive as the chromatographic procedure for
this particular study.

The significance of changes in the sarcoplasmic proteins
observed in this study is not clear at this time. Further studies
designed to clarify the relationship between these changes and those
of myofibrillar proteins in regard to meat tenderness are necessary.
BIBLIOGRAPHY


