EFFECT OF AGING AND PROCESSING CONDITIONS ON BEEF QUALITY

by 5509

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B. S., Kansas State University, 1968

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Science and Industry

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1970

Approved by:

Major Professor

ACKNOWLEDGEMENTS

The author wishes to express sincere appreciation to Dr. H. J. Tuma, major professor, for his guidance and sincere interest during the author's masters program. The author also expresses appreciation to Dr. D. H. Kropf and Dr. F. C. Cunningham for their unselfish service on the author's graduate committee.

Acknowledgement is made to National Cylinder Gas Division of Chemetron Corporation, Farmland Industries and Cryovac Corporation for supplying equipment used in the research.

Special thanks is extended to M. C. Hunt for his help in collecting data, to all the graduate students for their fellowship and to R. W. Swanson and G. A. Greathouse for their "academic inspiration" during the graduate program.

I wish to dedicate this work to my wife, Barbara, to my son, Barry and to my parents who have given me the encouragement for the success of my academic career.

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THIS IS AS RECEIVED FROM THE CUSTOMER.

Chapter 1

INTRODUCTION

Recently much interest has been focused around centralized cutting, processing and freezing of red meats. The main advantages cited have been increased efficiency and reduced processing cost per unit of product. Centralized processing and freezing offers increased utilization of labor and equipment, improved sanitation and inspection of processing procedures, extended life of the product, reduced transportation tonnage and vertical integration in the plant from the carcass to the retail cuts.

Through this system retail markets could order meat cuts of type and quality commensurate with consumer demand and at the same time skilled labor and unwanted product inventory would not be necessary. Quality frozen meats are a potential boost not only for supermarkets but to convenience stores which numbered 11,620 at the beginning of 1970, an increase of 21% over the previous year. Freezing would extend the shelf-life of red meat from the present two to five days to two months or more.

There are many unanswered questions concerning the most desirable packaging, freezing, storing, displaying and merchandizing methods for frozen red meats.

Many of these areas have been investigated in the past, but not in the light of newer freezing and packaging systems and other technological developments.

The objectives of this study were:

1. To determine the effect of freezing in liquid nitrogen and frozen storage on the tenderness, palatability and total cooking loss of beef longissimus muscle aged for various periods of time.

2. To determine the effect of cutting room temperature and bloom time on the microbial population of fresh beef aged for various periods of time and to determine the effect of freezing in liquid nitrogen and frozen storage on the microbial population.

3. To determine the effect of bloom time and temperature on the color stability of beef aged for various periods of time and frozen in liquid nitrogen.

Chapter 2

REVIEW OF LITERATURE

Factors Affecting Tenderness

Aging. Beef muscles reportedly become more tender as the aging period is increased, with maximum tenderness being achieved at 10 to 14 days (Lehmann, 1907). Ramsbottom and Strandine (1949) studied the effect of aging for 2, 5, 8, 11 and 14 hours and also 1, 2, 3, 6, 9 and 12 days following slaughter. Objective and subjective measurements of tenderness showed that beef which was chilled in carcass form was more tender than beef which was boned and then chilled to 35°F. This is in agreement with works done by Lowe and Stewart (unpublished data); Koonz, Darrow and Essary, 1954; Paul and Bratzler, 1955; Lowe, 1958; and de Fremery and Pool, 1960. Beef was found to be more tender at two hours following slaughter than at any time thereafter for the next two to six days. By the 12th day following slaughter, beef which was stored at 35°F was considerably more tender than it was at two hours after slaughter.

Gothard et al. (1966) studied the effect of post-mortem muscle contraction on ultimate tenderness of the Semimembranosus (S. M.) and Longissimus dorsi (L. D.) muscle of 12 beef animals of similar weight and grade. Muscle contraction patterns of each animal were plotted through a seven day aging period. State of contraction after seven days appeared to have a greater influence on tenderness than did the state of contraction at the time of maximum rigor mortis. Although contraction did not seem to be the factor most responsible for final tenderness, it did appear to have a significant influence. Considerable lengthening of sarcomeres normally occurred during the aging period. S. M. muscles routinely

contracted less than L. D. muscles during rigor mortis, and were more relaxed after seven days of aging. S. M. muscles were consistently less tender than L. D. muscles at slaughter, but the reverse was usually true after seven days.

Sharp (1963) indicated that muscles contain proteolytic enzymes which operate much more readily at 37°C than at 5°C. Higher temperatures of aging produce a given degree of tenderization in a considerably shorter time than do lower temperatures. Bouton, Howard and Lawrie (1958) found that conditioning for two days at 20°C gave the same degree of tenderizing as 14 days at 0°C, and benefits of aging were more marked with beef of poor quality which was initially tougher although the final degree of tenderness achieved was similar in beef of good and poor quality.

Wilson et al. (1960) employed antibiotics to control bacterial spoilage and were thus able to study temperatures as high as 49°C. Semimembranosus muscles from beef rounds which had been infused with oxytetracycline (30 to 50 ppm) were employed. After appropriate aging periods at 2°C, 38°C, 43°C and 49°C, the meat was cooked and assessed for tenderness by a taste panel. Results indicated that the tenderness score was increased by all conditioning procedures over that of controls. The meat held for two days at 38°C, or for one day at 43°C, was more tender than that kept for 14 days at 2°C. An off-flavor was detected in those samples aged at 49°C and bacterial growth was difficult to control at 38°C. The optimum time and temperature required to achieve the same degree of tenderness as that arising during 14 days at 0°C was 1 day at 43°C. "A greater increase in tenderness was reported in muscles of locomotion than in support muscles."

The effect of aging on various organoleptic characteristics of beef aged for 7 or 14 days in a 3 to 5°C cooler was studied by Lewis, Brown and Heck (1965). Post-mortem aging for 14 days significantly increased the tenderness in the Longissimus dorsi and Quadriceps femoris (Q.F.) muscles and decreased the intensity of aroma in all three muscles and the juiciness scores of the Longissimus dorsi and Quadriceps muscles. The color of the cooked steaks was lighter after post-mortem aging for 14 days.

Cold Shortening. The tenderness of lamb loin is affected greatly by the time-temperature pattern imposed on the dressed carcass during the onset of rigor mortis. Marsh, Woodhams and Leet (1968) found that very significant toughness develops within about 16 hours of slaughter in the Longissimus dorsi muscles of carcasses exposed to low temperatures. This "processing toughness" is shown to be unrelated to the lack of aging. It appears to be due to muscle fiber shortening, earlier demonstrated (Marsh and Leet, 1966) to be responsible for massive toughening in excised muscles. Both cold shortening and thaw shortening are capable of producing toughness, the latter type becoming prominent when meat previously frozen before rigor completion is cooked without a preliminary thaw (Perry, 1950).

The degree of shortening during rigor mortis is temperature dependent. In the muscles of lamb it is minimal at 14 to 19°C. Below this temperature so-called cold stimulus causes shortening (Locker and Hagyard, 1963); above it, shortening is directly proportional to increase of temperature up to 43°C, when there is a very marked increase in shortening accompanied by greater toughness (Marsh, 1962).

Effect of Freezing. The effect of freezing on tenderness of beef muscles representing the primal cuts of beef was studied by Hiner and Hankins (1951). Samples from 52 animals ranging from 10 weeks of age to 12 years were cut into pairs, one being tested fresh and unfrozen after 14 days aging and the pair mate frozen at that time at -18°C. At 24 hours after freezing the samples were thawed at 7.2°C and tenderness objectively evaluated. The foreshank and neck samples were tenderized least by freezing. In no case was the foreshank tenderized significantly; the neck muscle was tenderized to a high degree of significance. The round was highly significantly tenderized in all except veal calves. All other samples from mature animals were tenderized ($P<.01$), whereas those from veal were not tenderized significantly.

Beef aged five days at 34°F and then frozen at 18°F was as tender as similar beef aged for 35 days with no subsequent freezing (Hiner and Hankins, 1947). Beef loins were aged 5 days after slaughter and frozen in still air at 18, 0, -10, -40 and -114°F. Samples were thawed and then cooked to an internal temperature of 158°F. Results indicate that samples were progressively more tender as the freezing temperature was decreased, and associated with this was a decrease in drip during thawing at 45°F.

Smith et al. (1968) compared lamb chops fresh and frozen at -23 or -34°C to determine the effects of freezing on tenderness. Freezing lamb loin chops, in contrast to studies previously cited, resulted in a highly significant increase in loin chop shear force value. Freezing leg roasts resulted in highly significant decreases in flavor, tenderness and overall satisfaction scores. Leg roast shear force values tend to be higher for frozen muscles, although differences were not significant. Freezing lamb rib chops, in contrast, results in a highly significant decrease in

shear force values, indicating an increase in tenderness as a result of freezing.

Smith, Carpenter and King (1969) used beef rib steaks to assess effects of freezing and length of frozen storage on tenderness and found decrease in shear force values to be most pronounced when a longer storage period was involved.

Ramsbottom (1947) reported little observable difference in tenderness between frozen and unfrozen steaks, even with rather long storage periods. Hiner et al. (1951) and Hiner et al. (1947), found that freezing results in a decrease in shear force values, perhaps a result of a longer storage period.

Pearson and Miller (1950) produced contrasting results with steaks obtained from beef carcasses aged for six days and frozen at three different rates. Warner-Bratzler shear values indicated that steaks frozen and stored for 90 days were measurably less tender than those sampled at 0 days.

Rate of Freezing. In a study designed to assess the effects of rate of freezing on the tenderness of beef Longissimus dorsi muscle, Pearson and Miller (1950) froze steaks at a slow rate (approximately 20 hours to reach 20°F); at an intermediate rate (approximately five hours); and at a rapid rate (approximately one hour). The rate of freezing was determined with a thermocouple attached to a potentiometer. Results suggested that rate of freezing did not appear to influence cooking losses, total weight losses, expressible fluid, tenderness or palatability.

Meat frozen at a rate sufficient to freeze the deepest portion of the carcass in 24 hours tends to be less tender after cooking than

corresponding meat chilled for two to three days before freezing. If, however, the rate of blast freezing is increased so that the deepest part of the carcass freezes in only 18 hours, the meat is found to be as tender as corresponding meat frozen after two to three days chilling (Howard and Lawrie, 1957). Since the absence of a chilling period in the former would operate against tenderness, it was suggested that this is more than offset by microstructural changes effected by the faster rate of freezing.

Freezing made postrigor meat more tender when the rate was fast enough to cause intrafibrillar ice formation (Hiner, Madsen and Hankins, 1945; Hiner and Hankins, 1951). However, since even under the most rapid freezing conditions only the outer few mm of a piece of meat are really flash frozen, most of the ice crystals within the meat will be intercellular (Weir, 1960).

Costello and Hendrickson (1964) used a liquid nitrogen freezer maintained at either 0, -65, -130 and -200°F to freeze four inch blocks of beef Biceps femoris (B. F.) muscle. The general sequence of temperature changes within the blocks was the same at all temperatures, but the time required to reach given temperatures was reduced as the compartment temperatures decreased. In addition, two inch beef B. F. steaks were frozen at -10, -60, -110 and -160°F. The internal temperatures of a steak could be reduced to 0°F in one-tenth the time at -160°F as at -10°F. The internal temperature reached -20°F in one-third the time at -160°F as at -60°F. No tenderness data were given.

Whole chicken breasts were frozen at 0, -30 and -90°F and stored at 0, -15 and -30°F (Miller and May, 1965). After one week and one, three and six months storage, the meat was thawed and cooked to 190°F. Results

indicated that rate of freezing did not influence tenderness.

Frozen Storage. The effect of frozen storage for 15 months on beef quality was investigated by Shrewsbury et al. (1945). One half of each carcass was aged 14 days and the other half was aged 21 days. Loin steaks were removed, frozen in still air at -26°F and stored at 0°F for 15 months. At the end of the storage period, steaks were thawed at 40°F for 24 hours before cooking to an internal temperature of 158°F. No significant differences in tenderness resulted from the aging period or frozen storage. Palatability tests showed slight, but definite deterioration in quality of the product after 15 months storage.

Similar results were obtained by Ramsbottom (1947), who estimated maximum storage life of meats from the data on appearance and palatability. Meats stored at 26°F were considered to have reached the end of their storage life in less than 30 days, but most products stored at -10 and -20°F were still rated good after 365 days storage. A storage temperature of 10°F was much more effective than 20°F and 26°F in retaining the original quality of fresh meats. Storage temperatures of 0°F, -10°F and -20°F were progressively more effective than 10°F in preserving quality of fresh meats. Freezer storage at -10°F or lower for seven years did not significantly change the tenderness of beef steaks although some changes in aroma, juiciness and flavor of the lean and a marked deterioration in the appearance of the frozen product and in the flavor of the cooked fatty tissue were noted.

Pearson and Miller (1950) reported that beef steaks stored for 90 days were measurably less tender than steaks tested at day 0, and that storage up to 180 days did not decrease tenderness beyond that measured at 90 days. Weir (1960) reported similar results concerning pork roasts.

Those frozen and stored one year at -12, -18 and -23°C were less tender and had higher shear values than unstored roasts.

More recently, Law et al. (1967) used loins and top-rounds to study the effect of frozen storage on cooked product quality. Steaks were removed eight days post-mortem, quick frozen and stored at -18°C to -23°C for three storage periods; 0, 6 and 9 months. Steaks were sampled at each storage period and cooked to an internal temperature of 70°C in a microwave oven and electric range. Quality was evaluated by chemical tests, sensory evaluations and cooking loss data.

Storage up to six months had little effect on loin steaks with the exception of TBA (thiobarbituric acid) values, but significant changes occurred between 6 and 9 months. Loin steaks had increased cooking losses and decreased juiciness scores, percent moisture and juice content. TBA values increased with each storage period. Top-round steaks at the 9-month period showed a decrease in collagen content and juiciness and flavor scores, and an increase in TBA values. Storage up to 9 months did not influence tenderness in either muscle.

Anatomical Variation. Tenderness variations throughout the Longissimus dorsi muscle are noted. Weir (1953) reported that tenderness of swine L. D. increases from the center to both ends. However, Ramsbottom et al. (1945) indicated that the middle portion of beef L. D. was most tender with the anterior and posterior regions being less tender. Results obtained by Doty and Pierce (1961) support these findings. Blakeslee and Miller (1948) suggested that the rib-end of beef L. D. was least tender, with no significant differences occuring between the middle and posterior portions. In contrast to these, Bray, Vail and Mackintosh

(1942) reported no tenderness differences anywhere in the L. D. muscle of swine.

Results of the study by Smith et al. (1969) suggests that beef L. D. steaks adjacent to the 12th thoracic vertebra are significantly more tender than those nearer the 10th or 11th thoracic vertebrae. Romans, Tuma and Tucker (1965) and Henrickson and Mjoseth (1964) indicated that steaks from the 9th thoracic vertebra were more tender than those from the 11th thoracic vertebra.

Position Effect. Cover, Hostetler and Ritchey (1962) compared six sampling areas and reported that the most tender area in the beef L. D. was the core position nearest the fat cover at the lateral position. Hostetler and Ritchey (1964) also reported that the sample from the lateral position was most tender. Sharrah, Kunze and Pangborn (1965) reported that the area nearest the fat cover and in the center of the L. D. required the least shear force to sever.

Similar results obtained by Smith et al. (1969) suggested that the central position of beef L. D. was most tender and the lateral position least tender. Alsmeyer, Thornton and Hiner (1965) found lowest Warner-Bratzler shear force values from central beef L. D. cores, but the dorsal position was less tender than the lateral.

In contrast, many other workers have reported that the dorsal position was most tender, McBee and Wiles (1967); Alsmeyer (1960); Hedrick et al. (1968); Tuma et al. (1962); and Covington et al. (1970).

Temperature of sample. Hedrick et al. (1968) reported that the mean shear force values of hot and cold sample cores from steaks cooked in deep fat was approximately twice as large for the 2.54-cm cores as for

the 1.27-cm cores. No significant difference was observed between shear force values of 1.27-cm cores that were sheared hot and those sheared cold. However, 2.54-cm cores from broiled steaks that were sheared hot had significantly lower shear force values than similar size cores that were sheared cold.

Cooking

During cooking, two general changes occur: the muscle fibers become tougher and the connective tissue becomes more tender. Thus, for muscles or cuts of meat containing relatively large amounts of connective tissue the toughening of the fibers is less important than softening of the connective tissue, and cooking methods combining a long heating period and a moist atmosphere are selected. For muscles or cuts containing only small amounts of connective tissue, cooking methods involving dry heat for a short time are used to minimize the toughening effect on the muscle fibers (Weir, 1960).

Time and Temperature. Webb et al. (1961) investigated the effect of different internal temperatures and cooking time on the palatability of pork loin roasts. There were four treatments of cooking at 176.6°C; to internal temperatures of 85°C; 73.9°C; 65.6°C; and maintained at 65.6°C for one hour. Results indicated that tenderness scores were reduced as the internal temperature was increased, and slightly increased as time was prolonged. Flavor scores increased as internal temperature was increased. Cooking time, drip loss, evaporation loss and total cooking loss increased as internal temperature and time was increased. Cooking losses were inversely related to juiciness ratings, which suggests that juiciness is a function of cooking loss.

The effect of time and temperature on the shear patterns of small cylinders of choice-grade beef Semitendinosus muscles was studied by Machlik and Draudt (1963). Beef Semitendinosus muscle underwent a marked decrease in shear, approximately one-half completed in 11 minutes at 58°C. Change in shear was time-temperature dependent with high temperature effects predominating. Minimum shear values were obtained when samples were subjected to temperatures in the range 60 to 64°C. The author suggested that in this time-temperature range the collagen shrinkage reaction is completed quickly while the hardening associated with higher temperatures is minimized.

Cover et al. (1962) obtained shear-force values on one-inch steaks from Longissimus dorsi and Biceps femoris muscles cooked to 61 and 80°C by dry heat and to 100°C and held there for 25 minutes in moist heat. In the L. D. the shear force values were significantly higher in each lot at 80°C (well-done) than at 61°C (rare). But moist heat at 100°C resulted in a small and nonsignificant increase above that produced at 80°C. These trends indicated greater toughening at 80 than 61°C, with little further toughening at 100°C. It was suggested that shear-force values in L. D. may be detecting the tightening of the network of the protein structure during denaturation of the meat and may be related to dehydration of the muscle fiber. Trends with meat temperatures of 61 vs. 80°C in both muscles were toward toughening at the higher temperature with shear-force values, but were toward tendering with scores for connective tissue and with collagen content.

In a similar study, the L. D. muscle from U. S. Commercial rib roasts and porterhouse steaks cooked to 70°C was scored significantly more tender

than that cooked to 80°C (Harrison et al., unpublished data). Shear values for rib roasts, but not for porterhouse steaks, were significantly lower when cooked to 70°C than when cooked to 80°C. Moreover, differences in the tenderness scores and shear values for the L. D. muscle from club and T-bone steaks broiled to 70°C were not significantly different from those broiled to 80°C. Juiciness scores and press fluid yields for the rib roasts and loin steaks were significantly higher for meat cooked to 70°C than for that cooked to 80°C.

Cooking from Frozen State. Frozen steaks generally require 10 to 15 minutes more cooking time than comparable unfrozen cuts (Smith et al., 1969). Machlik et al. (1963) stated that changes in shear force or tenderness values produced upon heating are undoubtedly related to time-temperature-dependent protein denaturation processes. If such were the case, it is logical to expect a decrease in tenderness for unthawed steaks because of the additional time required to achieve doneness. However, results obtained by Smith et al. (1969) fail to support such a conclusion since no significant difference resulted when paired steaks were cooked from frozen versus thawed states. The authors postulate that if additional time is required at the beginning of the heating curve it would have little effect on decreases in tenderness related to denaturation.

Color

Chemistry of Fresh Meat Pigments. The chemistry of color in meat is primarily the chemistry of but one pigment, myoglobin. In the live animal myoglobin accounts for only 10% of the total iron, but during slaughter the bleeding process removes most of the iron as hemoglobin. In a well-bled piece of beef skeletal muscle as much as 95% or more of the remaining iron

is accounted for as myoblobin. Because some organs such as heart and liver have greater blood supplies than do skeletal muscles, generally more hemoglobin remains in these organs after bleeding (Giffee et al., 1960).

Other research studies have indicated various hemoglobin percentages in red meats. Fleming, Blumer and Craig (1960) analyzed over 150 beef rib-eyes from two year grass-fed steers and found as high as 18% of heme pigment to be hemoglobin, but the average was only 5%, whereas heart samples averaged 30%. Craig et al. (1966) found hemoglobin to be 11.38 to 13.01% of total pigment present in beef Longissimus dorsi. Rickansrud and Henrickson (1967) indicated that the hemoglobin content of the Longissimus dorsi muscle from seven choice grade steers averaged 20% while the Psoas major muscle averaged 38% of heme pigment. It was suggested that muscle variation accounted for 84.53% of the total variation for total pigment concentration on a wet-tissue basis and 72.74% of the variation for myoglobin concentration on a wet-tissue basis.

Giffee et al. (1960) noted that the oxygen storage role of myoglobin is reflected in the quantities found in various tissues, which quantities are generally functions of (1) the amount of use of the "principally muscular activity tissue", (2) the blood supply, (3) the oxygen availability and (4) the age of the animal. Myoglobin variation is also related to species, breed, sex, age, plane of nutrition and training (Lawrie, 1966).

The myoglobin molecule consists of a haematin nucleus attached to a globin type protein. The haematin portion comprises a ring of four nuclei coordinated with a central iron atom which may exist in both reduced and

oxidized forms. In the ferrous form it can combine with gases such as oxygen and nitric oxide. The ability to combine with oxygen is lost when the globin portion of the molecule is denatured and the tendency for the iron to oxidize to the ferric form is greatly increased. The oxidation of purplish-red myoglobin or of bright red oxymyoglobin to brown metmyoglobin is accelerated by any factor which causes denaturation of the globin (Watts, 1954), by the absence of reducing mechanisms and by low oxygen tension (Lawrie, 1966).

In the presence of oxygen the three pigments, oxymyoglobin, myoglobin and metmyoglobin, are constantly being interconverted (Fox, 1966). The take up of oxygen by myoglobin converts the purple reduced pigment to the bright red oxygenated pigment, oxymyoglobin. This process produces the familiar "bloom" of fresh meats. The red complex, once formed, will undergo no further color change as long as the oxygen remains complexed to the heme. However, the oxygen is continually associated and dissociated from the heme complex, a process which is accelerated by a number of conditions, among them low oxygen pressures. When this occurs the reduced pigment is subject to oxidation by oxygen or other oxidants. As a result, there is a slow and continuous oxidation of the heme pigments to the met form.

Brooks (1938) found that the maximum oxidation of heme pigments occurred at partial oxygen pressures of 4 mm of Hg, but depended upon the pigment, pH and temperature. This effect is important when considering packaging films for fresh meats. As the oxygen permeability of the film decreases, a partial pressure is reached where oxygen utilization by the tissues balances oxygen penetration at a pressure level which favors the oxidation reaction. Landrock and Wallace (1955) determined that a

packaging film must have an oxygen penetration rate of 5 liters of O_2/sq meter/day/atm to prevent such browning.

Bloom Time and Temperature. Round, loin and chuck were used to study the effect of time and temperature on bloom development (Fellers et al., 1963). Results suggested that loin muscle had the highest degree of bloom and chuck the lowest. The bloom color of all cuts held at 70°F reached a peak within 22 minutes of being sliced. Of the three cuts studied, chuck was the most acceptable during vacuum storage (14 days) at 46°F.

Butler, Bratzler and Mallmann (1953) observed that 34°F maintained the desirable red color of meat longer than 40°F. Bratzler (1955) reported that treating fresh meat with oxygen results in brighter color, but that stability is not enhanced. Urbin and Wilson (1958) have described several factors affecting oxygen uptake and penetration of beef steaks including enzymatic utilization, pH, temperature, myoglobin oxygenation and oxygen or solubility in the meat. They concluded that it is advantageous to cut and package meat at 32°F. They further stated that color preservation is benefited by any period in which the meat is cut and left unpackaged in air or in higher oxygen atmosphere at low temperature. The optimum bloom time to be approached at these low temperatures for best color is when all oxygen uptake is due to enzymatic demand, that is, the requirements for saturation and oxygenation of myoglobin have been met.

Freezing Rates. Ramsbottom and Koonz (1939) used freezing temperatures of -12.2, -23.3, -34.4 and -45.6°C and observed a brighter color in beef with each decrease in temperature. Increased freezing rate yielded bright red colors in bovine muscle when comparing slow (20 hours

to reach 20°F), intermediate (5 hours to reach 20°F) and fast (one hour to reach 20°F) freezing rates (Pearson and Miller, 1950). Brissey (1963) reported rapidly frozen meat enhanced bright red color while slowly frozen meat was dark in color. Beef frozen in a liquid nitrogen freezer at 0, -70, -150, -200 and -320°F was lighter in color as the freezing temperature decreased, but no differences in color were observed when these steaks were thawed (Costello, 1964).

Frozen Storage. Color stability of frozen red meat is time and temperature dependent. Brooks (1938) stored frozen meat at -10°C for 16 weeks before oxidation caused discoloration. Ramsbottom and Koonz (1941) stated that the surface color of beef at the end of one year storage was greatly influenced by the amount of "methemoglobin" present. Greater oxidation occured at -12.2°C than at -34.4°C. Ramsbottom (1947) froze beef in a blast freezer at -20°F and stored it at -20, -10, 0, 10, 20 and 26°F for 30, 90, 120, 180, 240 and 365 days. Product stored at 26°F was discolored in less than 30 days, whereas product stored at -20°F was still scored "good" in color and appearance after one year storage. At the higher temperatures the lean meat changed in color from pinkish red to brownish red and the fat lost its bloom and fresh appearance. He also noted that the color stability of ground products was much less than that of steaks.

Beef stored at 6, 2 and -2°C discolored more rapidly at the warmer temperatures (Snyder, 1964). It was also suggested that the reason for discoloration of meat at the colder temperature was due to respiration rather than bacterial action. Townsend and Bratzler (1958) indicated that fluctuating storage temperatures have a detrimental effect on frozen meat color, especially if thawing temperatures were reached. Cyclic defrost

temperatures may produce such results (Brissey, 1963). Winter et al. (1952) stated that constant storage at -18°C or fluctuating temperatures from -17.7 to -10°C had less effect on appearance, flavor, aroma and desiccation of ground pork or beef than storage time, wrapping material or shape of package.

Microbiology of Meat

Microbiology of Fresh Meat.

A freshly slaughtered and dressed meat animal should have very few microorganisms on the surface, and a virtually sterile interior except for lymph nodes which may contain moderate numbers of bacteria. However, as the carcass is subjected to the inevitable handling as it is moved from place to place and cut into smaller units, increasing numbers of microorganisms are introduced on the surface of the meat (Ayres, 1955).

Factors affecting growth of bacteria are temperature, available nutrients, oxygen, moisture, pH and an interrelationship of all factors. Meat provides an ideal environment for microbial growth.

Stringer, Bilskie, and Naumann (1969) recently studied the microbial profiles on fresh beef from time of slaughter to display in the retail store. They found that there was a high bacterial population on the carcasses (180 total), and that more moist areas were most highly contaminated. The population increased slightly after chilling and a larger increase occurred during transportation to the store. Large differences were noted in microbial populations among various lots of cattle. Pseudomonas fragi, P. geniculata and Micrococcus luteus were the main microorganisms present. Shrouds and shroud water contained a large number per square inch. The counts approached the initial level of

contamination on the carcass after slaughter. They also found that scraped steaks had significantly less bacteria than those unscraped.

Ayres (1960) reported that the principal genera of bacteria on meat held at low temperatures are Achromobacter, Micrococci and Pseudomonas. Bacillus, Lactobacillus, Serrotia, Flavobacterium, Chromobacterium and Staphylococcus are sometimes found in surface slimes, according to a review by Peterson and Gunderson (1968). They also state that molds appear on meat primarily as a result of long holding periods and high humidities maintained in the storage rooms.

Contamination of beef ranged from 100 to 100,000 organisms per gram while pork varied from 5,000 to 1,000,000 organisms per gram (Ayres, 1960). According to Jensen (1942), the characteristic flavor of aged beef is due to the growth of microorganisms on the cut surfaces. He concluded that satisfactory organoleptic qualities will not develop without microbial acid.

Temperature. In a review by Evans and Niven (1960), it was stated that most meat bacteria are mesophilic, having an optimum growth temperature of 35-40°C and a minimum growth temperature of near 10-15°C. This has also been reported by Stringer et al. (1969). At any temperature below optimum, growth is slowed down, and below minimum temperature a given microorganism will not grow. Psychrophiles grow quite well at 10°C and below, and some will grow slowly at temperatures as low as -5°C. As a general rule it can be said that fresh meat will spoil at least twice as fast at 10° as at 0°C.

Table 1. GROWTH TEMPERATURE RELATIONSHIPS OF MICROORGANISMS[a]

	Growth Temperatures (°C)		
	Minimum	Optimum	Maximum
Psychrophiles	-5 to +5	20 to 30	35 to 45
Mesophiles	10 to 15	35 to 40	40 to 50
Thermophiles	35 to 40	55 to 60	65 to 75

[a]Evans and Niven (1960)

Rey et al.(1970) indicated that multiplication of microbes was faster at 15°C, although cell yields were highest and survival greatest at 5°C. Since higher numbers of cells and better survival was observed at 5°C than at 15°C, cell production was less inhibited by low temperature than was cell destruction.

Effect of Freezing. A considerable amount of research has been done on the effect of freezing on microbial cells. Birdseye (1929) reported a reduction from 77,000 to 32,000 microorganisms per gram of haddock fillets during quick freezing. Greer, Murray and Smith (1933) found that the average bacterial count of hamburger steak was reduced from 11,600,000 to 2,200,000 organisms per gram by quick freezing. Hucker, Brooks and Emery (1952) noted that freezing and subsequent cold storage caused a reduction in numbers of bacteria found on beans, snap beans and corn. A greater reduction in count during storage was found at -12°C than at -18°C and -23°C. Similar results have been reported by Elliott and Michener (1960). Winter, Burkart and Wrinkle (1951) reported that freezing and storage of liquid egg at -18°C to -29°C resulted in an average destruction of 55% of the standard bacterial count in 12 days, 62% in 30 days, 87% in 60 days and 90%

in 400 days. Rey et al. (1969) also noted that destruction leveled off at about 60 days.

Previous concepts of lethal injury to microbial cells due to penetration of cell membranes by ice crystals or other mechanical causes have yielded to those involving denaturation and flocculation of cellular proteins (Borgstrom, 1955). Luyet (1961) found no evidence for ice formation in cells of Streptococcus Lactis. He suggested that bacteria which survive freezing do not freeze at all, but dehydrate.

Winter and Wilkin (1947) investigated the effect of freezing rate on microbial survival. They found that a greater destruction of bacteria occurred when 30 pound containers of liquid egg were frozen at more rapid rates. Van Esseltine et al. (1948) reported that slow freezing rates would be most lethal to microbial cells but are least desirable in preserving the original fresh qualities of food.

The number of viable microorganisms in a frozen food decreases with lengthening of time of storage in the frozen state. This decrease is gradual and microbes can persist in the frozen state for years. Straka and Combes (1951) found that the microflora of frozen turkey meat steaks was principally composed of micrococci after 8 months storage. A number of studies have demonstrated that considerably more microorganisms are destroyed at $-4^{\circ}C$ than at temperatures $-18^{\circ}C$ or $-24^{\circ}C$. Even temperatures as low as $-193^{\circ}C$ have no additional lethal effect (Luyet and Gehino, 1940).

Effect of Thawing. Thawing is not merely the reverse of freezing, and it also has a lethal effect. One theory states that death occurs during defrosting rather than at freezing. Borgstrom (1961) suggested that rapid warming during thawing is essential to microbial survival. Even

when microorganisms do not die as a result of freezing and thawing, there are other effects on the cell. Sulzbacher (1952) found that freezing and thawing increased the length of the lag growth phase of phychrophilic microorganisms in ground meat, and that their generation time was longer than in unfrozen ground meat.

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Chapter 3

EFFECT OF AGING, FREEZING AND FROZEN STORAGE ON TENDERNESS, PALATABILITY AND TOTAL COOKING LOSS OF BEEF *LONGISSIMUS* MUSCLE

Studies on the relative importance placed upon the various quality factors of beef by the consumer indicate that tenderness is the major quality factor desired (Rhodes, Kiehl and Brady, 1955), however, the consumer probably has more difficulty in determining the degree of tenderness of beef prior to consumption than any other quality factor. Tenderness of beef can be altered by post-mortem aging, however no work has been reported concerning the effect on tenderness of aging for various periods of time and then freezing and storing in the frozen state. There are contrasting reports on the effect of freezing and frozen storage, and only limited work has been done on red meat frozen in liquid nitrogen.

Ramsbottom (1947) and Dawson *et al.* (1959) report that no significant differences in tenderness occur between frozen and unfrozen steaks even with rather long storage periods. In contrast, Hankins and Hiner (1940 and 1941); Hiner, Madsen and Hankins (1945); and Blakeslee and Miller (1948) suggest that beef improves in tenderness when frozen without aging or after only short periods of aging.

Pearson and Miller (1950) reported that steaks sheared at 0 days were significantly more tender than those stored for 90 and 180 days. Ramsbottom (1947), Weir (1960), Dawson *et al.* (1959) and Law *et al.* (1967) reported that frozen storage up to nine months did not influence tenderness. In contrast, Smith *et al.* (1968) and Field, Nelms and Schoonover (1966) found that long periods of frozen storage resulted in significantly

lower shear force values for both lamb and beef.

Independent studies have indicated that freezing and frozen storage results in increased total cooking loss (Smith, Carpenter and King, 1969) and extended post-mortem aging reduces total cooking loss (Doty and Pierce, 1961), however, no recent studies have been conducted to study the combined effect of aging, freezing and frozen storage on the total cooking loss of beef.

The objectives of this study were to determine the effect of various aging times, subsequent freezing in liquid nitrogen and frozen storage on the tenderness, palatability and total cooking loss of beef longissimus dorsi muscle.

EXPERIMENTAL PROCEDURE

Eight choice grade Hereford steers fed a corn-sorghum grain finishing ration for 140 days averaging 486 kg were divided into two equal groups and slaughtered one week apart in the Kansas State University meat laboratory. Following a 24 hour chill in a 0.3°C cooler, ribs and short-loins were removed from the carcass and assigned to one of four aging periods (24, 48, 120, or 240 hours) by a 4X4 Latin square design. At the termination of assigned aging periods, eight 2.54 cm thick steaks were removed from each wholesale cut, avoiding the use of the anterior end of the wholesale rib and the posterior portion of the short-loin. The steaks were assigned to one of three treatments: I, fresh; II, frozen and immediately thawed; and III, frozen and stored for seven weeks.

Treatment I steaks were packaged and placed in a 3.6°C cooler to be evaluated by a taste panel and Warner-Bratzler shear the following day.

Treatment II steaks were packaged, frozen and then placed in the same cooler to thaw and were compared the following day with treatment I steaks to determine the initial effect of freezing upon tenderness and palatability. Steaks assigned to treatment III were packaged, frozen and stored in a chest type deep freeze (-25°C) for seven weeks before being subjected to a taste panel and Warner-Bratzler shear evaluation.

All thawing was done in a 3.6°C cooler for a 20 hour period before cooking.

Packaging and Freezing. Steaks were individually vacuum packaged in L-300 Cryovac bags having an oxygen permeability of about 4,000-5,000 $cc/m^2/24$ hours at one atm. The packaged steaks were heat shrunk for two or three seconds in a water dip tank at 88°C.

The packaged steaks were then frozen in a liquid nitrogen simulator freezer, with each steak placed on edge to allow equal exposure to the nitrogen vapor. The freezing chamber was pre-chilled and programmed to hold -17.8°C for 15 minutes and -40°C for the remainder of the 45 minute cycle. This produced a center steak temperature of -25°C.

Cookery and Shear Evaluation. Steaks were cooked to an internal temperature of 65.5°C in a rotary gas oven pre-heated to 163°C, requiring an average cooking time of 59 minutes. Cooking losses were determined on a weight change basis.

Three 1.27 cm cores (medial, central and lateral; fig. 1) were removed from each steak after they had cooled approximately 20 minutes. These three cores were removed parallel to the muscle fibers. One Warner-Bratzler shear force value was obtained on each core.

Taste Panel. The same steaks used for Warner-Bratzler shear

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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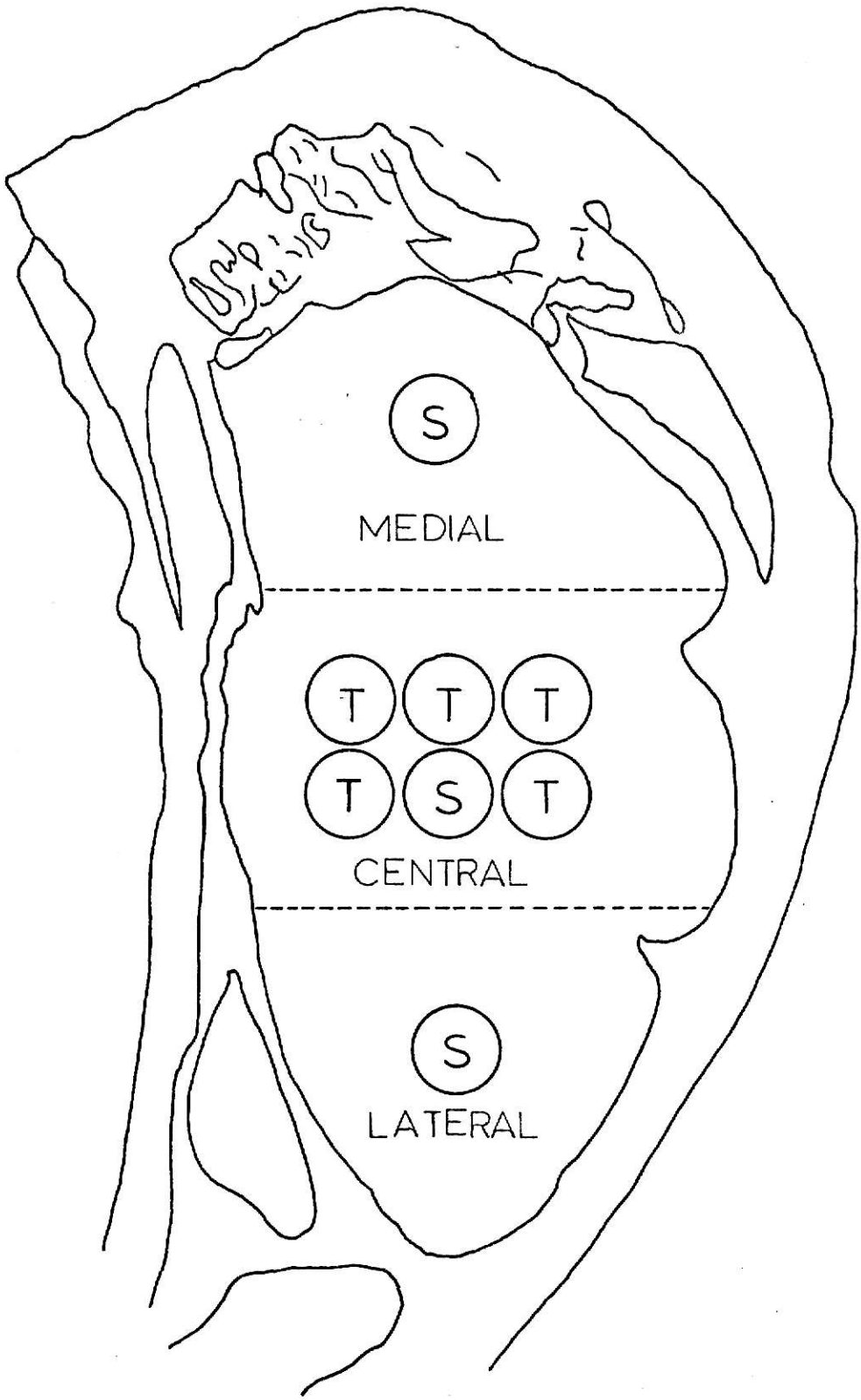

Fig. 1. Core locations of the longissimus muscle used for Warner-Bratzler shear(S) determination and taste panel(T).

evaluation were used for the taste panel. Five cores (fig. 1) were removed from the central portion of each steak for serving to a five-member panel. Panel evaluation scores were recorded on a seven-point hedonic scale with 1 indicating "extremely undesirable" and 7 indicating "extremely desirable" (Appendix Table M).

All steaks in treatment I and II were evaluated by the panel; one-third of those in treatment III were subjected to panel evaluation.

Statistical Analysis. Duncan's New Multiple Range test and analysis of variance (Steel and Torrie, 1960) were employed to determine differences among means.

RESULTS AND DISCUSSION

Shear. There were no significant shear force differences among the longissimus steaks for the three treatments studied (table 2), contrary to the assumption that freezing and frozen storage changes tenderness. This is in agreement with work by Ramsbottom (1947) and Law et al. (1967).

Shear force values (table 2) for the lateral cores were significantly less tender than the medial (dorsal) or central cores, which were similar. This is in agreement with Covington et al. (1970) who reported that no significant differences occurred between medial and central positions, and that the lateral position was least tender.

This study indicated that medial cores obtained from the short-loin steaks had lower shear force values than those from the wholesale rib, (table 3) but the differences were not significant for central or lateral cores. Mean shear force differences between steaks from the short-loin and wholesale rib were 1.09, 0.50 and 0.14 kg for the medial, central and

TABLE 2. MEANS AND STANDARD DEVIATIONS OF WARNER-BRATZLER SHEAR VALUES FOR LOCATION AND TREATMENT 1,2,3

Treatment	No. of samples	Medial		Central		Lateral	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Fresh	32	2.50[a]	0.61	2.60[a]	0.69	2.79[b]	0.56
Frozen	32	2.51[a]	0.82	2.53[a]	0.70	2.91[b]	0.66
Frozen-stored	192	2.60[a]	0.86	2.52[a]	0.73	2.90[b]	0.70

1 Means within the same column did not differ significantly ($P<.01$).
2 Means within the same row with different superscript letters differ significantly ($P<.05$).
3 Mean values are expressed in kilograms.

TABLE 3. MEANS OF WARNER-BRATZLER SHEAR VALUES FOR WHOLESALE CUTS AND AGING TIMES (HRS) FOR THREE SAMPLE LOCATIONS[1]

Item	Frequency	Location means (kg)		
		Medial	Central	Lateral
Overall	32	2.57	2.54	2.88
Right Rib	8	2.96[a]	2.78[a]	3.00[a]
Left rib	8	2.73[a,b]	2.59[a]	2.74[a]
Right loin	8	2.44[b,c]	2.49[a]	2.82[a]
Left loin	8	2.16[c]	2.38[a]	2.78[a]
Time (024)	8	3.13[d]	2.96[d]	3.14[d]
Time (048)	8	2.56[e]	2.54[e]	2.88[e]
Time (120)	8	2.33[e]	2.22[f]	2.72[e]
Time (240)	8	2.47[e]	2.40[e,f]	2.79[e]

[1] Means within same column bearing similar superscript letters are not significantly different ($P<.05$).

lateral positions, respectively. Paul and Bratzler (1955) reported that the anterior portion of the longissimus muscle was more tender than the posterior, while Hankins and Hiner (1940) found that steaks from the short-loin were significantly (6.9%) more tender than those from the wholesale rib. Differences in this study may have been more pronounced if the extreme anterior and posterior portions of the longissimus muscle had been used.

Overall means (table 3) for shear values for the medial, central and lateral locations support the conclusion that no significant differences exist between the medial and central cores, and that the lateral position is least tender ($P<.01$).

Shear values indicated that post-mortem aging had a significant effect on tenderness of the steaks (table 3). Steaks aged for 24 hours were significantly ($P<.05$) less tender than those aged for longer periods of time. No significant differences in shear values were noted between the 48, 120 and 240 hour aging periods for the medial and lateral positions, but a significant difference did occur between the 48 and 120 hour aging times for the central location. Steaks aged for 48 hours did not differ significantly in tenderness, however, from the steaks that had been aged 240 hours.

Steaks aged for 120 hours showed a tendency for greater tenderness than those aged for 240 hours, although the difference was never significant. This is contrary to results obtained by Deatherage (1963), who reported that beef aged for five days had a tenderness score of about 5.4 (10 was most tender) and that aged for 10 days had a score of about 6.0. Ramsbottom and Strandine (1949) reported results similar to this

study; beef steaks aged for six days had shear values 1.0 kg less than those aged for nine days.

Taste panel. No significant differences were noted between fresh and frozen-thawed steaks for all organoleptic qualities evaluated (table 4). A tendency existed for frozen-thawed steaks to be rated slightly higher than fresh steaks, however the differences were not significant. Steaks which had been frozen and stored for seven weeks were significantly ($P<.05$) preferred over the others.

Panel scores indicated no significant differences in tenderness between steaks obtained from the rib or short-loin (table 5). This is in agreement with shear force values from the central and lateral locations but not the medial location. Taste panel samples were taken from the central location so similarity to shear force value results should be expected.

Organoleptic qualities were favorably effected by extended post-mortem aging. Juiciness scores were not significantly affected, although they tended to increase with aging. Flavor and overall acceptability did not differ between the 24 and 120 hour aging periods ($P<.05$), but aging for 240 hours resulted in significantly higher scores for these two characteristics.

Results suggested that tenderness was improved with extended aging. The steaks aged for 240 hours were rated most tender, although the difference between steaks aged 120 and 240 hours was not significant. Those aged for 120 hours had the lowest shear values, but the difference between taste panel rating and shear force values could be a result of overlapping flavor characteristics. Weir (1960) indicated that tenderness is not a

TABLE 4. MEANS AND STANDARD DEVIATIONS OF TASTE PANEL SCORES ACCORDING TO TREATMENT[1]

Treatment	No. of samples	Flavor[2]		Juiciness[2]		Tenderness[2]		Overall accept.[2]	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Fresh	16	5.13[a]	0.39	5.16[a]	0.49	5.44[a]	0.63	5.13[a]	0.40
Frozen	16	5.24[a]	0.60	5.32[a]	0.54	5.46[a]	0.60	5.37[a]	0.46
Frozen-stored	32	5.89[b]	0.53	5.89[b]	0.49	6.06[b]	0.48	5.97[b]	0.49

[1] Means within the same column bearing different superscripts differ significantly ($P<.05$).
[2] Seven indicates most desirable; one least desirable.

TABLE 5. MEANS OF TASTE PANEL SCORES FOR WHOLESALE CUTS AND AGING TIMES[1]

Item	Frequency	Flavor[2]	Juiciness[2]	Tenderness[2]	Overall Accept.[2]
Overall	4	5.53	5.57	5.75	5.61
Right rib	4	5.56^{a}	5.46^{a}	5.84^{a}	5.57^{a}
Left rib	4	5.58^{a}	5.58^{a}	5.75^{a}	5.63^{a}
Right loin	4	5.37^{a}	5.64^{a}	5.62^{a}	5.51^{a}
Left loin	4	5.63^{a}	5.59^{a}	5.80^{a}	5.73^{a}
Time (024)	4	5.35^{b}	5.29^{b}	5.38^{b}	5.47^{b}
Time (048)	4	5.37^{b}	5.50^{b}	5.51^{b,c}	5.34^{b}
Time (120)	4	5.39^{b}	5.62^{b}	5.86^{c,d}	5.54^{b}
Time (240)	4	6.03^{c}	5.86^{b}	6.26^{d}	6.09^{c}

[1] Means within same column bearing similar superscript letters are not significantly different ($P<.05$).

[2] Seven indicates most desirable; one least desirable.

single impression, and Doty and Pierce (1961) have suggested that aroma and flavor increase for about two weeks post-mortem. Favorable flavor and aroma impressions in this study may have raised taste panel tenderness ratings. Also, differences may have not been great enough for the panel to significantly detect.

Total cooking losses. Total cooking losses are defined as the total weight collected as drip and the weight lost through evaporation (Doty and Pierce, 1961). No attempt was made here to separate the two.

Results of this study demonstrated no difference in total cooking losses between fresh (treatment I) and frozen-thawed (treatment II) steaks (table 6). Steaks that had been frozen and stored (treatment III) had significantly higher total cooking losses than those not stored. This is in agreement with Pearson and Miller (1950) and Smith et al. (1969), who reported that total cooking loss becomes greater as the storage time is increased.

Post-mortem aging (table 7) significantly affected total cooking losses. Steaks aged for 24 hours showed less loss during cooking ($P<.05$) with no significant differences between those aged for 48, 120 or 240 hours. Although these results were significant, the difference between the extremes was only 1.68%. This study is contrary to most reports. Bouton, Howard and Lawrie (1958) and Doty and Pierce (1961) reported significant decreases in total cooking losses as a result of extended aging, but their aging was conducted under different conditions than this study.

Differences in this study maybe due to higher evaporative cooking losses from those steaks aged longer periods of time due to more unbound

TABLE 6. MEANS AND STANDARD DEVIATIONS OF TOTAL COOKING LOSSES ACCORDING TO TREATMENT[1]

Treatment	No. of samples	Cooking loss (%)	
		Mean	S. D.
Fresh	32	13.4[a]	0.05
Frozen	32	14.3[a]	0.03
Frozen-stored	192	17.9[b]	0.03

[1] Means within the same column bearing different superscripts differ significantly ($P<.05$).

TABLE 7. MEANS OF TOTAL COOKING LOSSES FOR STEAKS FROM EACH WHOLESALE CUT AND AGING TIME (HRS)

Item	Frequency	Mean (%)[1]
Overall	32	16.94
Right rib	8	16.81[a]
Left rib	8	16.95[a]
Right loin	8	17.13[a]
Left loin	8	16.86[a]
Time (024)	8	15.95[b]
Time (048)	8	16.78[b,c]
Time (120)	8	17.63[c]
Time (240)	8	17.38[c]

[1] Means within same column bearing similar superscript letters are not significantly different ($P<.05$).

juices. Wholesale cuts were aged while loosly wrapped in saran, thus reducing the 10 day weight loss from the conventional 3.65% (Sleeth, Henrickson and Brady, 1957) to less than 1.00%.

Total cooking losses (table 7) did not differ among steaks from the wholesale cuts studied.

SUMMARY

Wholesale ribs and short-loins from eight choice grade steers were removed from the carcass at 24 hours after slaughter and were cut into 2.54 cm thick steaks at 24, 48, 120 or 240 hours post-mortem. Steaks were assigned to one of three treatments: I, fresh; II, frozen at -40°C in a liquid nitrogen freezer and immediately thawed; and III, frozen at -40°C in a liquid nitrogen freezer and stored for seven weeks at -25°C. Tenderness was evaluated by a Warner-Bratzler shear and taste panel, and total cooking losses were determined on a weight change basis. There were no shear force differences due to treatment, but the taste panel favored steaks from treatment III ($P<.05$). Steaks aged for 24 hours had significantly higher shear force values, with no differences occurring between those aged longer ($P<.05$), although steaks aged 120 hours had the lowest shear values. The taste panel rated the steaks aged 240 hours highest in flavor and overall acceptability, with a trend toward improved tenderness with longer aging. Steaks from treatment III had significantly higher total cooking losses. Increased post-mortem aging resulted in larger total cooking losses.

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Chapter 4

EFFECT OF BLOOM TIME AND TEMPERATURE ON THE COLOR AND MICROBIAL POPULATION OF AGED FROZEN BEEF

Color at the retail level is the most important factor for consumer acceptance of red meat. Meat "bloom" is attributed mainly to the oxymyoglobin state of the muscle and is achieved in fresh meat by most retailers.

However, changes to centralized cutting and freezing of red meats are apparent in the retail meat industry due to the need for improved quality control and increased efficiency. Frozen meat generally has had a poor image because quality control procedures have not been practiced, ice crystals form in the package and frozen meat is dark colored. Conditions necessary to produce frozen red meat of acceptable color need to be elaborated.

Microbial standards for meat products are receiving increased attention, yet effects of many variables such as aging, freezing and frozen storage are not clearly established.

The purpose of this study was to determine the effect of aging, bloom time and temperature, freezing with liquid nitrogen, and frozen storage on color stability of beef longissimus muscle and total bacteriological plate counts.

EXPERIMENTAL PROCEDURE

Eight choice grade Hereford steers fed a corn-sorghum grain finishing ration for 140 days averaging 486 kg were divided into equal groups and

slaughtered one week apart. Following a 24 hour chill in a 0.3°C cooler, ribs and short-loins were removed from the carcass and assigned to one of four aging periods (24, 48, 120 or 240 hours) by a 4X4 Latin square. At the termination of assigned aging periods, eight 2.54 cm thick steaks were removed from each wholesale cut, avoiding the use of the anterior end of the wholesale rib and the posterior portion of the short-loin. The steaks were randomly assigned to one of six treatments (table 8).

Packaging and freezing. Steaks were individually vacuum packaged at 10 or 45 minutes after cutting (table 8) in L-300 Cryovac bags having an oxygen permeability of about 4,000-5,000 $cc/m^2/24$ hours at one atm. Packaged steaks were heat shrunk for two to three seconds in a 88°C water dip tank.

Steaks were frozen in a liquid nitrogen simulator freezer, with each steak placed on edge to allow equal exposure to the nitrogen vapor. The freezing chamber was pre-chilled and programmed to hold at -17.8°C for 15 minutes and -40°C for 30 minutes. This produced a center steak temperature of -25°C. Steaks were stored for seven weeks in a chest type deep freeze maintained at -25°C.

Thawing at the end of the storage period was done in a 3.6°C cooler for a 20 hour period.

Total plate count determination. Immediately after being cut, steaks were placed in one of three room temperatures for 10 or 45 minutes before packaging and freezing (table 8). A sterile cotton swab was moistened in a test tube containing 5 ml of phosphate buffer (Difco Laboratories). A one sq cm area was randomly selected for sampling on the cut surface of the longissimus muscle. Appropriate dilutions (0.1 and 0.01) were

TABLE 8. BLOOM TIME AND TEMPERATURE TREATMENT OF STEAKS PRIOR TO PACKAGING

Treatment	Time	Room Temperature (°C)
1	10	-2.0
2	45	-2.0
3	10	7.0
4	45	7.0
5	10	15.5
6	45	15.5

transferred to Petri dishes, poured with Standard Methods Plate Count Agar, and incubated at 25°C for 72 hours. The bacteria were counted on a Quebec Colony Counter.

Hansen (1962) observed that the numbers of bacteria on contaminated meat surfaces showed a very wide variation, while the distribution of the logarithms of the bacterial counts was approximately normal. Therefore, the log of the counts rather than the actual numbers of bacteria were used and reported per sq cm of surface area.

Total plate count was also determined after seven weeks storage and subsequent thawing.

Color determinations. A Bausch and Lomb 600 Spectrophotometer with reflectance attachment was used to determine percent reflectance of surface of frozen steaks from 400-700nm, using a $MgCO_3$ block for 100% reflectance. A scan speed of 250 nm was used and reflectance readings at wavelengths of 474, 525, 568, 572, 600, 610, 620, and 630 nm were marked and read to the nearest 0.1%. Ratios of reflectance readings 474/525 and 572/525 were calculated following suggestions of Snyder (1965). Reflectance scans of frozen steaks were recorded at 0 and 7 days after freezing. Time did not allow scans to be taken before freezing.

RESULTS AND DISCUSSION

Figure 2 presents the log of the means of the numbers of bacteria on steaks aged for 24, 48, 120 or 240 hours, both fresh and also after 7 weeks freezer storage and subsequent thawing. The log mean of bacteria on fresh steaks decreased rather sharply from the 24 to the 48 hour aging period, and the steaks aged 120 hours had a population comparable with those aged

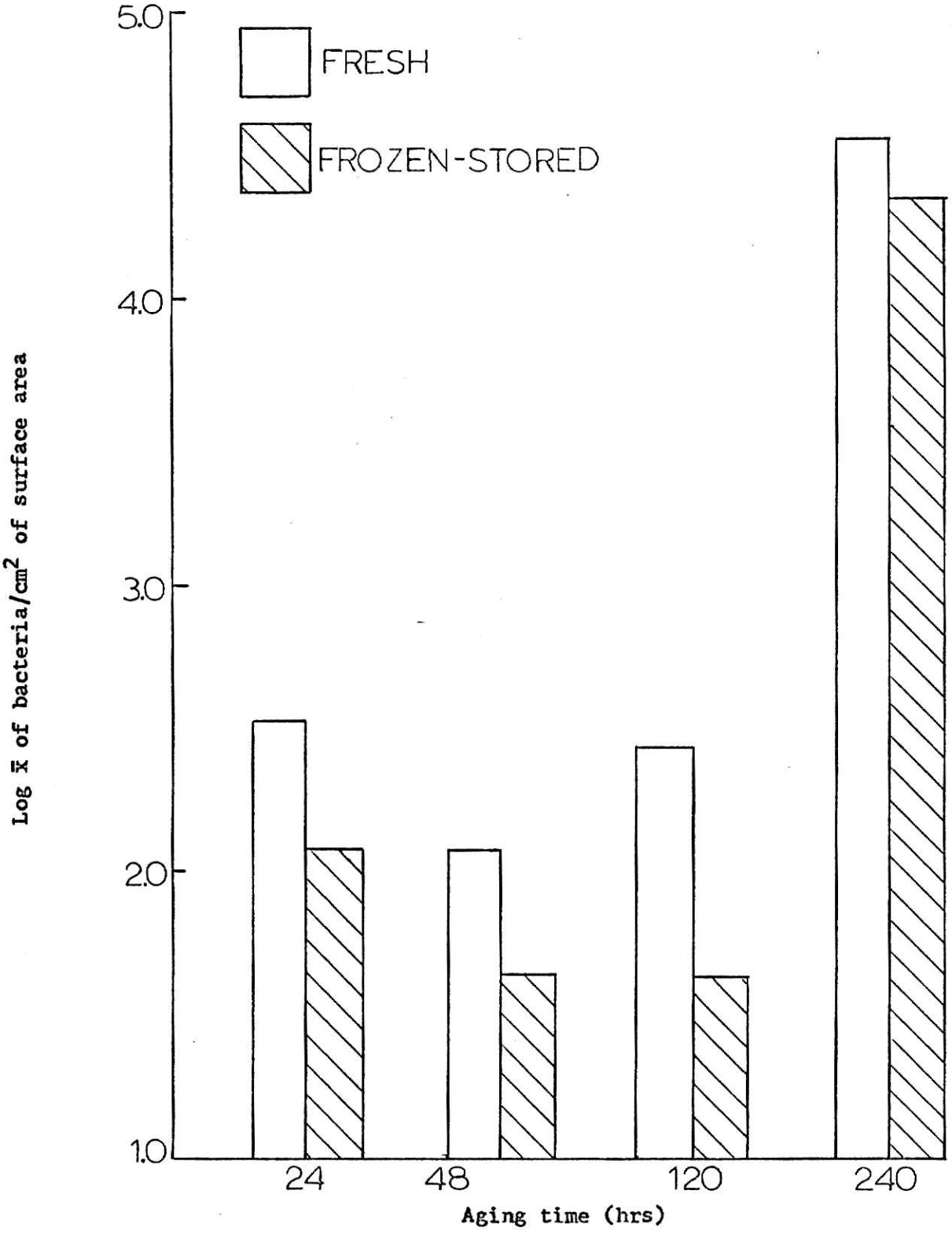

Fig. 2. Comparison of microbial counts before and after freezing and seven weeks storage for 48 steaks in each aging period.

24 hours. Jay (1966) reported that populations on steaks aged 0 days were indentical to those at day six, with day two having a slightly higher value. Rey et al. (1970) indicated that the psychrophile population decreased significantly from day 0 to day 1, and then increased in an almost linear fashion until the end of the seven day aging period. No explanations were offered for decreases in bacterial populations, but a shift from mesophilic to psychrophilic type organisms may be the cause.

Steaks aged 10 days had a much greater bacterial population than samples from other aging periods (figure 2) which is in agreement with Rey et al. (1970) and Jay (1966). This was further evidenced by a stronger but not undesirable aged beef odor.

Freezing and seven weeks storage compared to fresh steak counts (figure 2) decreased microbial populations 65% for steaks aged 24 hours, 62% for those aged 48 hours, 83% for the 120 hour aging period and 37% for those aged 240 hours. Winter, Burkart and Wrinkle (1951) reported that freezing and storage of liquid egg at -18 to -29°C resulted in an average destruction of 55% of the standard bacterial count in 12 days, 62% in 30 days, 87% in 60 days and 90% in 400 days. Rey et al. (1969) also noted that destruction of bacteria on red meat leveled off at about 60 days.

Results shown in figure 2 indicated that the percentage destruction as a result of freezing and storage was lessened as the population on fresh steaks increased.

Bacterial counts of fresh steaks subjected to various bloom times and temperatures (figure 3) did not differ greatly. Counts from steaks in treatment three (10 minutes, 7.0°C) were slightly higher than the remaining treatments. Ingraham (1958) and Straka and Stokes (1960) reported that the

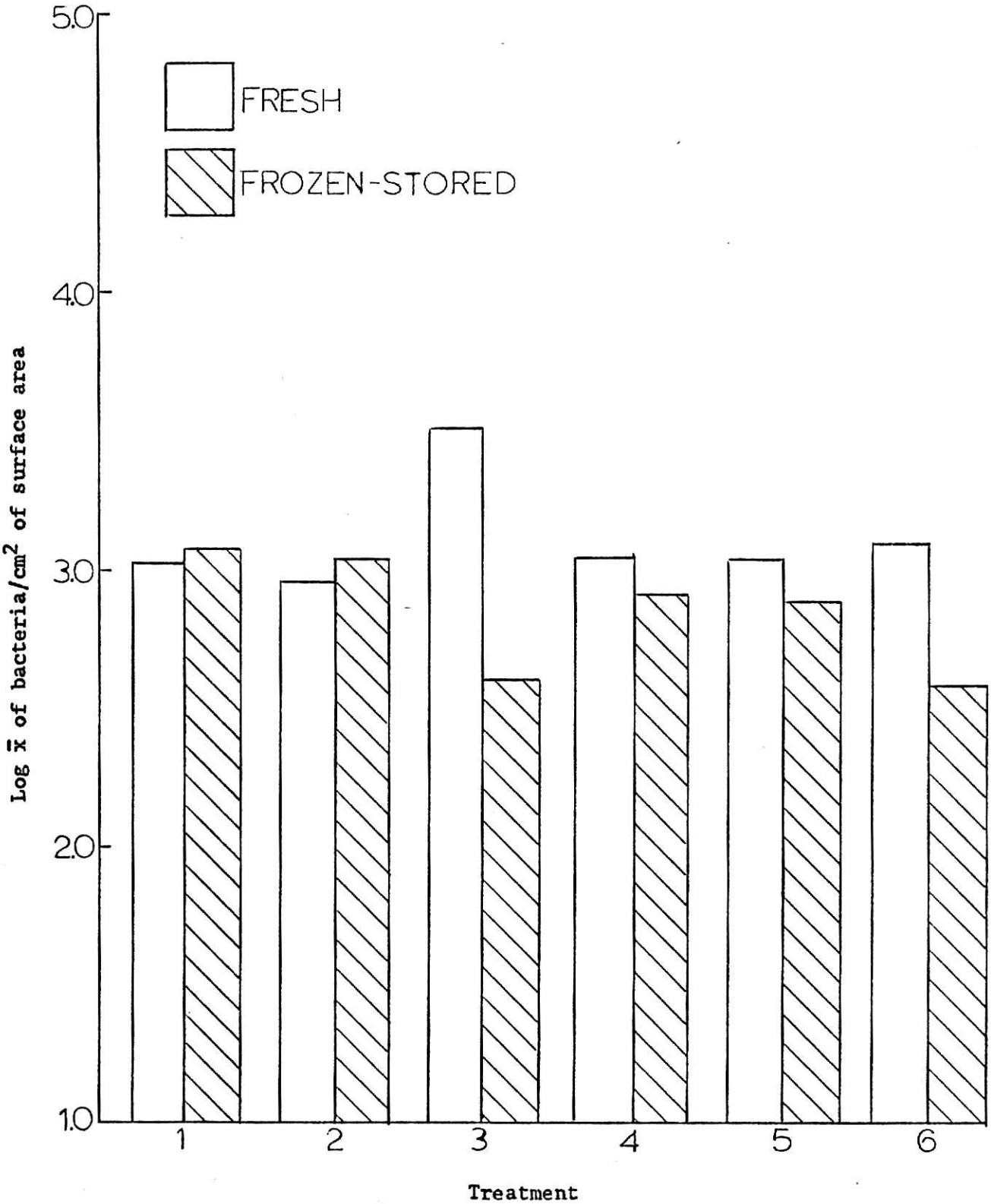

Fig. 3. Comparison of microbial counts before and after freezing and seven weeks storage for 32 steaks in each treatment.

rate of multiplication increased with an increase in temperature, but time in this study was not great enough for bacteria to reproduce logarithmically.

Freezing and frozen storage did not reduce bacterial numbers to a great extent in treatments 1, 2, 4 and 5. In fact there was a slight increase in treatments one (8%) and two (17%), possibly a result of bacterial multiplication during the 20 hour thawing period. Steaks in treatments one and two were both subjected to -2°C bloom temperature before freezing. Freezing and storage resulted in an average destruction of 70, 25, 31 and 70% for treatments 3, 4, 5 and 6, respectively.

Color. Aging had essentially no effect on color as indicated by 474/525 or 572/525 ratios at either day 0 (table 9) or day 7 (table 10).

Calculated overall means for the ratio 474/525 for each treatment were 1.00, 0.99, 0.99, 0.99, 0.99, and 1.01. This lack of noticable difference may be due in part to time required to package and place steaks in the freezer. Fellers et al. (1963) reported that maximum bloom occured at 22 minutes post-cutting, therefore the steaks packaged at 10 minutes were nearly full bloom by the time the color was set as a result of freezing and those packaged after 45 minutes bloom had not yet formed enough metmyoglobin to be noticably different. A lack of any great differences between values for ration 572/525 support this conclusion.

Reflectance data at day 7 (table 10) indicated no major differences from ratio values at day 0. This short storage period before the terminal reflectance data were obtained did not allow autoxidation to form the metmyoglobin pigment. Brooks (1938) stored frozen meat at -10°C for 16 weeks before oxidation caused discoloration. Ramsbottom (1947) indicated that beef stored at temperatures used in this study would retain good color

TABLE 9. MEANS AND STANDARD ERRORS OF REFLECTANCE RATIOS 474/525 AND 572/525 OF FROZEN LONGISSIMUS STEAKS AT DAY 0 ACCORDING TO TREATMENT AND AGING TIME

Treatment	N	Aging time (hrs) 024	048	120	240
1					
474/525	31	1.03[a]±.061[b]	1.01±.034	0.96±.006	0.99±.006
572/525	31	0.85±.040	0.81±.035	0.82±.001	0.85±.027
2					
474/525	31	1.00±.041	1.00±.025	0.97±.013	0.98±.013
572/525	31	0.87±.011	0.84±.033	0.82±.022	0.85±.018
3					
474/525	31	1.04±.069	0.96±.044	0.97±.017	0.97±.025
572/525	31	0.87±.066	0.79±.051	0.85±.037	0.83±.032
4					
474/525	31	1.00±.019	0.99±.020	0.98±.016	0.97±.020
572/525	31	0.85±.033	0.82±.021	0.83±.015	0.78±.097
5					
474/525	31	1.02±.043	0.97±.014	0.97±.011	0.98±.023
572/525	31	0.81±.026	0.82±.028	0.87±.041	0.83±.016
6					
474/525	31	1.03±.043	1.03±.016	0.99±.023	0.99±.005
572/525	31	0.83±.032	0.83±.029	0.88±.064	0.82±.022
Overall					
474/525	186	1.02 ...	0.99 ...	0.97 ...	0.98 ...
572/525	186	0.85 ...	0.82 ...	0.85 ...	0.83 ...

[a] Mean.
[b] Standard error.

TABLE 10. MEANS AND STANDARD ERRORS OF REFLECTANCE RATIOS 474/525 AND 572/525 OF FROZEN LONGISSIMUS STEAKS AT DAY 7 ACCORDING TO TREATMENT AND AGING TIME

Treatment	N	Aging time (hrs) 024	048	120	240
1					
474/525	31	1.02[a]±.034[b]	0.99±.021	0.98±.010	1.09±.145
572/525	31	0.86±.026	0.82±.054	0.85±.010	0.79±.059
2					
474/525	31	1.01±.099	0.97±.022	0.97±.023	0.97±.034
572/525	31	0.91±.101	0.86±.020	0.85±.034	0.82±.101
3					
474/525	31	1.01±.023	0.97±.019	0.99±.016	0.96±.051
572/525	31	0.80±.045	0.83±.021	0.87±.019	0.82±.054
4					
474/525	31	1.01±.010	1.00±.045	0.98±.013	0.98±.042
572/525	31	0.97±.118	0.82±.026	0.86±.019	0.83±.023
5					
474/525	31	0.99±.053	0.99±0.11	0.99±.025	0.96±.020
572/525	31	0.82±.017	0.83±.030	0.87±.014	0.80±.018
6					
474/525	31	1.01±.010	1.01±.028	0.98±.014	0.93±.055
572/525	31	0.81±.032	0.85±.046	0.88±.017	0.78±.064
Overall					
474/525	186	1.01 ...	0.99 ...	0.98 ...	0.98 ...
572/525	186	0.86 ...	0.84 ...	0.86 ...	0.81 ...

[a] Mean.
[b] Standard error.

and appearance for a year without being exposed to light.

Severe "bleaching" noted with -46°C plate freezing (Robertson, 1950) was not observed in this study. Costello (1964) reported rapid freezing with liquid nitrogen produced "lighter" colors in beef steaks. Visual color appraisal of steaks in this study noted a lighter color than fresh beef, but not enough to be objectionable. Ramsbottom and Koonz (1941) attributed the lighter color of meat frozen at lower temperatures to very small ice crystals which reflect more light.

The serratus dorsalis, longissimus costarum, spinalis dorsi and psoas major muscles were noticeably darker in color after freezing than the longissimus dorsi, giving a rib or loin steak a "two-toned" color. Rickansrud and Henrickson (1967) indicated that the hemoglobin content of the longissimus muscle from choice grade steers averaged 20% while the psoas major muscle averaged 38%.

SUMMARY

Wholesale ribs and shortloins from eight beef carcasses were removed 24 hours post-mortem and assigned to 24, 48, 120 or 240 hour aging periods. At the termination of each aging period, eight steaks were removed from each wholesale cut and assigned to one of six bloom time and temperature treatments. Steaks were sampled for total viable plate count immediately before packaging and color reflectance data were obtained after freezing in liquid nitrogen at -40°C and then again 7 days post-freezing.

Results of total plate count determinations indicated that bacterial numbers on steaks aged 48 hours decreased from those aged 24 hours, which had comparable numbers with samples aged 120 hours. Aging for an additional

120 hours greatly increased bacterial numbers. Freezing and seven weeks storage decreased microbial populations 65% for steaks aged 24 hours, 62% for those aged 48 hours, 83% for the 120 hour aging period and 37% for those aged 240 hours.

Bacterial counts on fresh steaks in each bloom time and temperature did not differ greatly. Freezing and frozen storage decreased bacterial numbers in four of the six treatments, however only two were of great significance.

Reflectance data at day 0 and day 7 suggested no differences in color due to time aged, bloom time and temperature or days stored. Visual color appraisal indicated that the color of frozen longissimus muscle were lighter in color than fresh meat, however it was not objectionable.

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APPENDICES

APPENDIX A

STEER CARCASS DATA

Steer no.	Live wt. (kg)	Carcass wt. (kg)	Dressing percent	REA (sq.cm)	Fat thickness (cm)	USDA grade
1	494	313	63.3	73.3	2.39	Choice^{+}
2	499	315	63.1	75.5	1.91	Choice^{+}
3	499	309	62.0	73.8	1.75	Choice^{o}
4	440	272	61.8	69.2	1.91	Choice^{o}
5	485	305	62.8	73.3	1.91	Choice^{o}
6	481	298	62.1	76.5	1.57	Choice^{-}
7	508	315	62.0	69.6	2.39	Choice^{o}
8	481	303	65.1	86.4	1.27	Choice^{o}

APPENDIX B

ASSIGNMENT OF WHOLESALE CUTS TO INDICATED AGING PERIODS BY A 4X4 LATIN SQUARE DESIGN 1,2

Carcass no.	Aging times (hours) 024	048	120	240
1	LR	LL	RR	RL
2	RR	RL	LR	LL
3	LL	LR	RL	RR
4	RL	RR	LL	LR
5	RL	RR	LL	LR
6	LL	LR	RL	RR
7	RR	RL	LR	LL
8	LR	LL	RR	RL

[1] Carcasses one through four are replication one; carcasses five through eight are replication two.

[2] RR=right rib; LR=left rib; RL=right short-loin; LL=left short=loin.

APPENDIX C

ANALYSIS OF VARIANCE OF WARNER-BRATZLER SHEAR VALUES FOR SAMPLE LOCATION ACCORDING TO TREATMENT[1]

		Medial		Central		Lateral	
Source	d.f.	Mean square	F	Mean square	F	Mean square	F
Treatments	2	0.99	0.28	0.43	0.16	0.92	0.37
Error	222	3.54	....	2.77	....	....	....
Total	224	....	....	....	....	....	....

[1] No significant differences were observed ($P<.05$).

APPENDIX D

ANALYSIS OF VARIANCE OF WARNER-BRATZLER SHEAR VALUES FOR REPLICATION, ANIMAL, WHOLESALE CUT AND AGING TIME DIFFERENCES

Source	d.f	Medial		Central		Lateral	
		Mean square	F	Mean square	F	Mean square	F
Replication	1	0.53	0.93	3.19	10.12**	0.73	3.10
Animal within replication	6	1.60	2.81	1.14	3.63*	2.16	9.17**
Wholesale cut within replication	6	2.63	4.63*	1.06	3.35*	0.47	1.99
Time	3	6.75	11.87**	3.90	12.38**	1.34	5.68*
Time x replication	3	0.62	1.10	0.09	0.29	0.30	1.28
Error	12	0.57	....	0.32	.....	0.24	....
Total	31	....	....	....	.....	....	....

* P<.05.
** P<.01.

APPENDIX E

MEANS OF SHEAR VALUES FOR REPLICATIONS AND ANIMALS ACCORDING TO LOCATION

Item	Frequency	Location means (kg)[1]		
		Medial	Central	Lateral
Replication (1)	16	2.63[a]	2.68[a]	2.82[a]
Replication (2)	16	2.52[a]	2.39[b]	2.95[a]
Animal (1 & 5)	8	2.88[c]	2.73[c]	3.17[c]
Animal (2 & 6)	8	2.65[c]	2.53[c]	2.74[d]
Animal (3 & 7)	8	2.37[c]	2.45[c]	2.72[d]
Animal (4 & 8)	8	2.40[c]	2.43[d]	2.91[d]

[1] Means of the same set of observations within the same column bearing different superscripts differ significantly ($P<.05$).

APPENDIX F

ANALYSIS OF VARIANCE OF TASTE PANEL SCORES ACCORDING TO TREATMENT

Source	d.f.	Flavor		Juiciness		Tenderness		Overall accept.	
		Mean square	F	Mean square	F	Mean square	F	Mean square	F
Treatments	2	4.04	12.84**	3.51	11.54**	2.94	8.50**	4.41	18.16**
Error	46	0.31	.....	0.30	.....	0.35	....	0.24	.....
Total	48	....	.....	....	.....	....	....	....	.....

** P<.01.

APPENDIX G

ANALYSIS OF VARIANCE OF TASTE PANEL SCORES FOR ANIMAL, WHOLESALE CUT AND AGING TIME DIFFERENCES

Source	d.f.	Flavor		Juiciness		Tenderness		Overall accept.	
		Mean square	F	Mean square	F	Mean square	F	Mean square	F
Animal	3	0.10	5.12*	0.14	2.29	0.09	1.59	0.07	4.60
Wholesale cut	3	0.05	2.76	0.02	0.36	0.04	0.64	0.03	2.02
Time	3	0.43	22.64**	0.23	3.75	0.63	10.74**	0.44	26.89**
Error	6	0.02	....	0.06	....	0.06	....	0.16	....
Total	15	....	....	....	....	....	....	....	....

* P<.05
** P<.01

APPENDIX H

MEANS OF TASTE PANEL SCORES FOR EACH ANIMAL 1,2

Animal No.	Frequency	Means			
		Flavor	Juiciness	Tenderness	Overall accept.
1	4	5.34[a]	5.82[a]	5.58[a]	5.46[a]
2	4	5.73[a,b]	5.83[a]	5.79[a]	5.79[a]
3	4	5.54[a,b]	5.53[a]	5.71[a]	5.61[a]
4	4	5.53[b]	5.39[a]	5.94[a]	5.58[a]

[1] Means within same column bearing similar superscript letters are not significantly different ($P<.05$).

[2] A value of seven is most desirable; one least desirable.

APPENDIX I

ANALYSIS OF VARIANCE OF TOTAL COOKING LOSSES

ACCORDING TO TREATMENT

Source	d.f.	Mean Square	F
Treatments	2	0.0408	33.89**
Error	222	0.0012	.....
Total	224	.....	.....

** P<.01.

APPENDIX J

ANALYSIS OF VARIANCE OF TOTAL COOKING LOSSES FOR REPLICATION, ANIMAL, WHOLESALE CUT AND AGING TIME DIFFERENCES

Source	d.f.	Mean square	F
Replication	1	0.00000	0.00033
Animal within replication	6	0.00009	0.88680
Wholesale cut within replication	6	0.00006	0.60948
Time	3	0.00045	4.42453*
Time x replication	3	0.00023	2.24242
Error	12	0.00010	.....
Total	31	.....	.....

* P<.05.

APPENDIX K

MEANS OF TOTAL COOKING LOSSES FOR REPLICATIONS AND ANIMALS

Item	Frequency	Mean (%)[1]
Replication (1)	16	16.93
Replication (2)	16	16.94
Animal (1 & 5)	8	17.18
Animal (2 & 6)	8	16.89
Animal (3 & 7)	8	16.99
Animal (4 & 8)	8	16.67

[1] No significant differences were observed ($P<.05$).

APPENDIX L

INSTRUCTIONS TO JUDGES FOR SENSORY EVALUATION OF BEEF LONGISSIMUS DORSI

You may use one cube of meat to score all palatability characteristics for one sample.

Scoring for flavor and juiciness

Record a score for flavor and another for juiciness within a range of 7 to 1 that describes your impression of the sample. See the score card for descriptive terms for specific scores within the range of 7 to 1. Record the score describing your impression of flavor and juiciness at the beginning of the chewing process.

Scoring for tenderness

Count the number of times you chew the ½-in. cube of meat before swallowing. Chew until the cube is masticated completely, then swallow. Record the number of chews required to masticate the cube. Record a score from 7 to 1 that describes your impression of the tenderness of the cube. See the score card for descriptive terms for specific scores within the range of 7 to 1.

Use the number of chews to help you standardize your tenderness scores from day to day. Set up for yourself a range of the number of chews for each score from 7 to 1. For example, if you chew from 15 to 25 times, you might record a score of 7; if you chew 25 to 35 times, a score of 6; 35 to 45, a score of 5; continuing to reduce the score by a given number of increased chews. Each judge sets his own range of chews for a given score.

Over-all acceptability

Record a score that describes your impression of the general desirability of the sample. This is not a total score, i. e., it is not a score obtained by adding the scores for the other factors. Score over-all acceptability within the range of 7 to the same as for each of the other factors listed on the score card.

Comments

Comments about a sample and/or explaining your reason for giving a particular score are helpful.

Take your time to score each sample. Water is provided for rinsing your mouth between samples.

APPENDIX M

SCORE CARD FOR EVALUATING THE PALATABILITY OF BEEF LONGISSIMUS DORSI

Judge_____ Code_____ Date_____

Sample No.	Desirability of Flavor	Juiciness	Tenderness		Over-all Acceptability	Comments
			No. Chews	Score		
1						
2						
3						
4						

Descriptive terms for scoring

Desirability of Flavor	Juiciness	Tenderness	Over-all Acceptability
7 Extremely desirable	7 Extremely juicy	7 Extremely tender	7 Extremely desirable
6 Desirable	6 Juicy	6 Tender	6 Desirable
5 Moderately desirable	5 Moderately juicy	5 Moderately tender	5 Moderately desirable
4 Acceptable	4 Acceptable	4 Acceptable	4 Acceptable
3 Moderately undesirable	3 Moderately dry	3 Moderately tough	3 Moderately undesirable
2 Undesirable	2 Dry	2 Tough	2 Undesirable
1 Extremely undesirable	1 Extremely dry	1 Extremely tough	1 Extremely undesirable

APPENDIX N

COMPOSITION OF PHOSPHATE BUFFER USED IN TOTAL PLATE COUNT DETERMINATIONS [a,b]

Monosodium phosphate
Sodium thiosulfate
Aryl sulfonate complex
NaOH

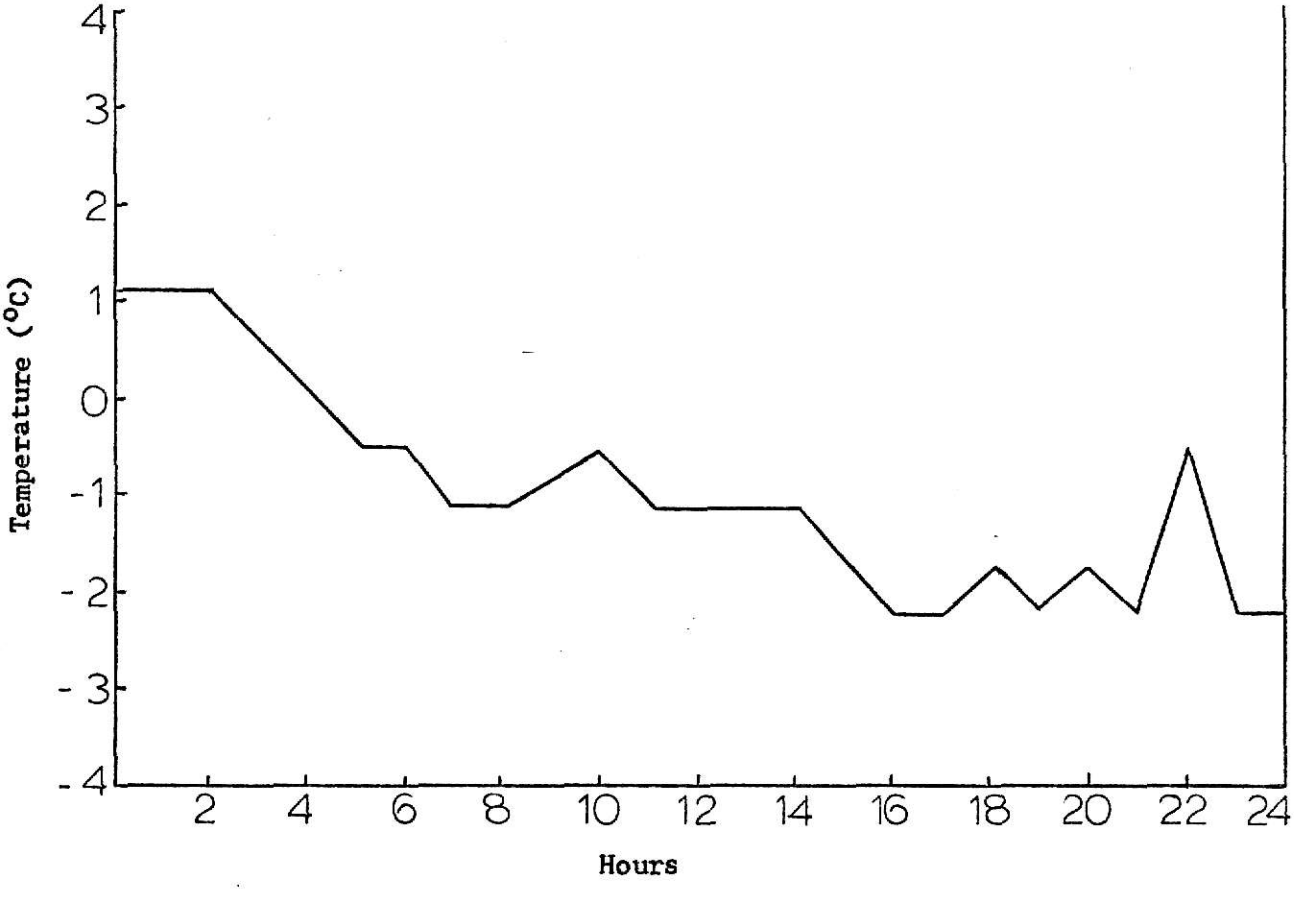
[a] Manufactured by Difco Laboratories
[b] pH 7.2

APPENDIX O

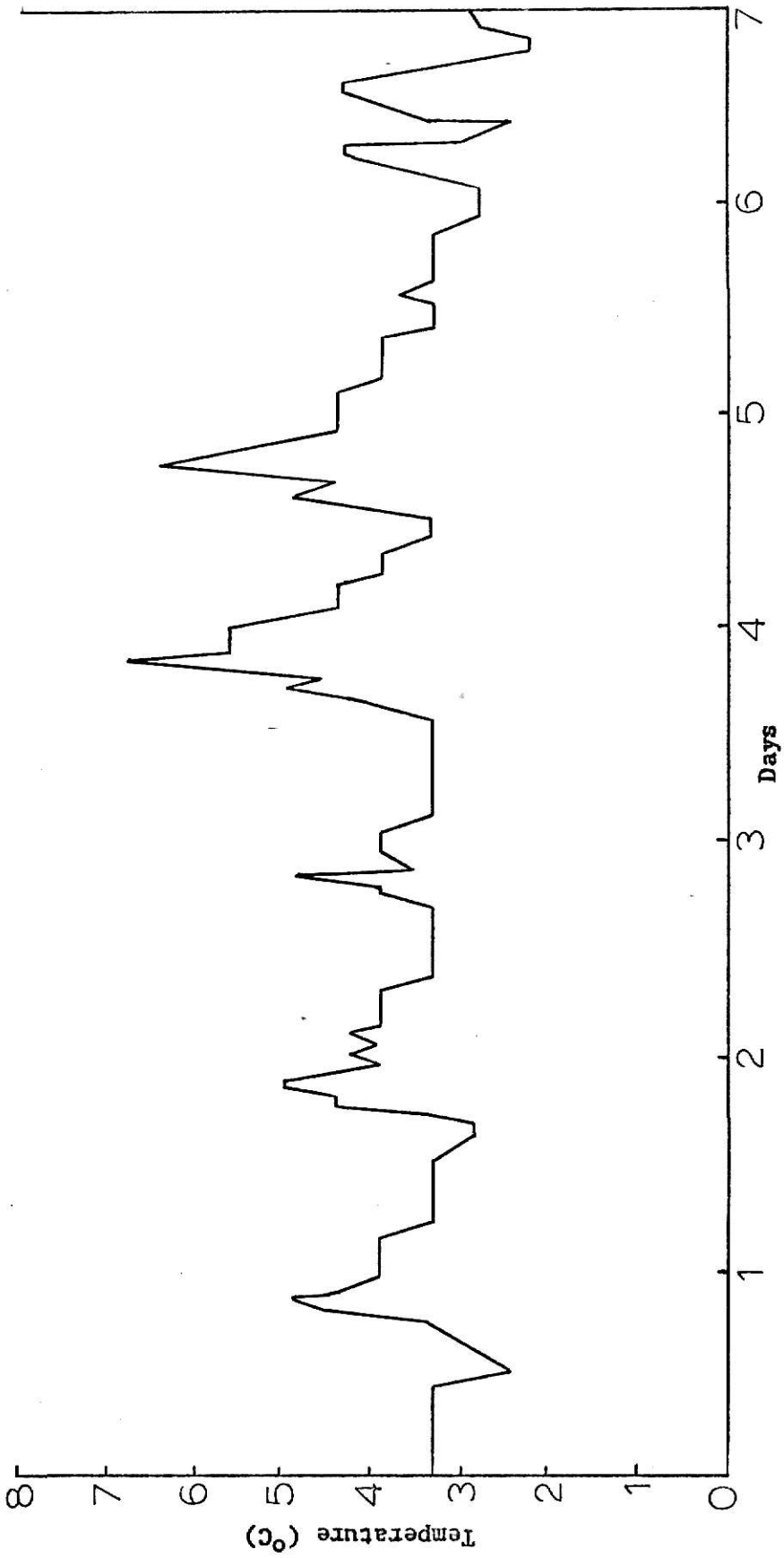
COMPOSITION AND PREPARATION OF TOTAL PLATE COUNT AGAR[1]

Tryptone	5.0 gm.
Yeast extract	2.5 gm.
Glucose	1.0 gm.
Agar	15.0 gm.
Distilled water	1 liter

[1] Boil and then autoclave 15 minutes at 121°C under 15 lbs. pressure.
Final pH 7.0 ($\pm$ 0.1)



APPENDIX FIG. A. Temperature pattern of chill cooler during a 24 hour period.



APPENDIX FIG. B. Temperature pattern of aging cooler during a 7 day period.

EFFECT OF AGING AND PROCESSING CONDITIONS ON BEEF QUALITY

by

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B. S., Kansas State University, 1968

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Science and Industry

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1970

The effect of aging and freezing on tenderness, palatability and total cooking loss of beef longissimus muscle was studied by aging for 24, 48, 120 or 240 hours in saran film and freezing at -40°C in a liquid nitrogen freezer. Steaks were sampled fresh, frozen and immediately thawed and after being frozen in a liquid nitrogen freezer and stored for seven weeks in a -25°C chest type deep freeze. Warner-Bratzler shear data indicated that steaks became more tender with increased aging beyond 24 hours, however differences between the 48, 120 and 240 hour aging periods were not significant ($P<.05$). Freezing and seven weeks frozen storage did not significantly influence shear force values.

Taste panel data indicated a significant preference for steaks frozen and stored and also for steaks that had been aged for 240 hours ($P<.05$). Frozen and stored steaks had significantly higher total cooking losses. Increased post-mortem aging also resulted in significantly larger total cooking losses.

Steaks were also divided into one of six different bloom time (10 or 45 minutes) and temperature (-2.0, 7.0 or 15.5°C) treatments before freezing to determine the effect of environmental conditions on total viable bacteria on each sample and color stability after freezing. Results indicated that steaks aged 48 hours had the least bacteria per cm^2, samples aged for 24 and 120 hours had similar numbers, and aging for 240 hours resulted in greatly increased numbers. No noticable differences in bacteria numbers were observed due to bloom time and temperature treatment. Freezing resulted in 25% to 83% reduction of bacteria.

Reflectance ratio values of 475/525 nm and 572/525 nm indicated no differences in the color of frozen steaks due to bloom time and temperature

treatment or length of aging. The color of frozen steaks was lighter than fresh steaks, but the color was not bleached. Visual appraisal noted that the surrounding muscles in rib and loin steaks were darker in color than the longissimus muscle.