EFFECT OF COLD STORAGE AND FROZEN STORAGE ON THE PALATABILITY AND HISTOLOGICAL APPEARANCE OF THE LONGISSIMUS DORSI MUSCLE OF BEEF

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This is to certify that the

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EFFECT OF COLD STORAGE AND FROZEN STORAGE

ON THE

PALATABILITY AND HISTOLOGICAL APPEARANCE

OF THE

LONGISSIMUS DORSI MUSCLE OF BEEF

By

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INTRODUCTION

Adequate preservation of meat is necessary to the economy of the commercial packer, the locker operator and to the individual. Meat is a highly perishable product and subject to alteration by enzymes, yeasts, molds, and bacteria which may render it unfit for human consumption. The earliest preservation of meat was by salting and drying, but the growing desire for the superior flavor of fresh meat, and a method for its preservation, led to the development of cold storage - first by the use of ice and later by mechanical refrigeration. Within the past 25 years great progress has been made in the freezing preservation of meat.

For some meats, particularly beef, the practice of a limited period of holding under refrigerated conditions (34 to 36°F.) improves the tenderness and flavor of the meat. A period of aging from one or two days to several weeks has been used in previous studies. In general, it has been customary to age beef prior to freezing. Holding periods, however, are not necessarily indicated for the freezing preservation of beef. Gortner, et al.(1948) suggest that freezing may, and often should be carried out shortly after slaughter. He further states, that during the war Argentine beef destined for England was boned immediately after killing and frozen while still warm. The resulting product was given a high quality rating.

Many recent investigations on beef muscle have indicated that the freezing process itself makes meat more tender and influences other palatability factors. Thus it was deemed worthwhile to study the effect of both cold storage and freezing storage on the palatability and histological appearance of the longissimus dorsi muscle of beef. The specific objectives of this problem were:

- 1. To compare rate of tenderization of beef held at $40-45^{\circ}F$. and at $0^{\circ}F$.
- 2. To note palatability changes, especially in tenderness and flavor, occurring in beef during ripening at 40-45°F. and at 0°F.
- 3. To ascertain the percent cooking losses and cooking time of unfrozen and frozen steaks.
- 4. To determine histological changes during storage of beef and attempt to relate these with palatability changes.
- 5. To determine peroxides and free fatty acids in the raw fat during storage as an indication of development of rancidity of the fat.

DISCUSSION OF LITERATURE Beef Muscle

Structure

Skeletal muscle is made up of striated muscle fibers held together by connective tissue and surrounded by a sheath of heavier connective tissue. The fibers are arranged parallel to each other in bundles called fasciculi. Each fiber is elongate, cylindrical and multinucleated with elliptical shaped nuclei; some fiber ends are tapered; others rounded. The fiber may terminate in a tendon; one end of the fiber may terminate in a tendon and the other end within a muscle; or both ends of the fiber may terminate in the muscle. Each fiber is encased in a thin, colorless, elastic membrane, the sarcolemma. The connective tissue around the fasciculi is called the perimysium, and the sheath of connective tissue around the entire muscle the epimysium(Maximow and Bloom, 1948).

Composition

The principal proteins within the muscle are myosin and myogen. They are present in the colloidal state. The connective tissue consists mainly of collagen which softens and swells in the presence of dilute acid. It is converted into gelatin by boiling or heating with superheated steam, the conversion being assisted by dilute acid(Ewell, 1940). Elastin is also present in connective tissue, but it is resistant to heat and breakdown by hydrolysis(Lowe, 1943).

The proportion of connective tissue present in a muscle is considered by Lowe(1943), and other investigators, as a measure of muscle tenderness. Mitchell, et al.(1928) examined different retail cuts of beef for collagen content. The eye muscle(longissimus dorsi) for calves showed the lowest content of collagen, expressed as total nitrogen. In studying the reliability of four comparable cuts from one animal for obtaining palatability scores, Satorius and Child(1938) concluded that the longissimus dorsi muscles of beef and pork possessed homogeneous physical properties. The longissimus dorsi muscle was chosen for the experimental work in this problem. Sisson and Grossman (1938) have presented a concise, anatomical description of the longissimus dorsi muscle.

Cold Storage Studies

Definition and purpose

Cold storage is generally defined as the holding of fresh beef at $34 - 36^{\circ}F$. for varying periods of time. The primary intention of aging meat after slaughter is to make it more tender(Bate-Smith, 1948). Numerous studies have reported palatability, histological and chemical changes in beef held for various time periods.

Palatability changes

The chief factors of palatability of beef are flavor, aroma, juiciness, texture and tenderness.

<u>Flavor and aroma</u>: Flavor and aroma scores follow similar patterns from analyses of judges' scores. Harrison(1947) and Paul, et al.(1944), found the greatest improvement in flavor and aroma of beef occurred during the first 10 days of storage at $34 - 36^{\circ}$ F. Further storage brought about a "high" or "gamey" flavor in the lean, rancidity in the fat, and a musty odor. Hoagland, et al.(1917) reported that flavor changed in two to four weeks cold storage. Whether or not the change in flavor was in the nature of improvement or deterioration was subject to individual tastes. Lowe(1943) reported that maximum development of desirable flavor in beef roasts was achieved in 20 to 40 days at temperatures a little above freezing.

Juiciness: Juiciness scores from various meat studies showed a gradual increase with aging(Lowe, 1943; Paul, et al., 1944; Anon., 1939b).

<u>Texture</u>: Texture is determined by the size of the bundle fibers and the amount of connective tissue. The larger the fiber bundles the more coarse the texture; conversely, small amounts of connective tissue and indistinct bundles of fibers denote fine texture (Ramsbottom, et al., 1945; Brady, 1937). In a study on cooked longissimus dorsi and other beef muscles aged 10 days, Brady(1937) found that texture was an indication of tenderness and gave a high positive correlation with the tenderness palatability factor.

<u>Tenderness</u> One of the principal desirable effects of aging on the organoleptic properties of beef is usually considered to be a marked increase in tenderness.

A comprehensive review of the principal studies pertaining to storage and aging of beef has been made by Bate-Smith(1948). Particular emphasis is placed on the tenderness of beef at temperatures above freezing. All of the investigators whose work has been reviewed agreed that the process of ripening in cold storage brought about the desired tenderness effect.

Deatherage and Harsham(1947) found that tenderness did not increase linearly with postmortem age of meat. "From tenderness curves

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for each animal it is apparent that not all carcasses increase in tenderness with age throughout the period of observation. The decrease in tenderness during the aging period in some carcasses appears to be quite real as the data are outside the realm of experimental error." Steaks from the loins of some carcasses tested at various periods were found to be more tender than others. The meat of some became less tender at times while meat from others increased progressively in tenderness throughout the aging period. In general, tenderness increased until 17 days; from 17 to 24 days there was no improvement or a slight drop in tenderness. At 24 to 31 days some improvement beyond the 17 day tenderness level was noted. It was concluded that unless beef is to be aged beyond approximately four weeks, it need be aged only two and one-half weeks. Harrison(1947) also found that increase in tenderness during aging was not linear.

Two opposing theories relative to the effect of cold storage and ripening of beef have evolved from the numerous studies presented herein:

- 1) Tenderness is due exclusively to an effect upon muscle fibers(Hammond, 1940; Steiner, 1939; Winkler, 1939).
- 2) Tenderness is based upon the swelling and hydrolysis of collagenous tissue(Ramsbottom, et al., 1945; Lowe, 1943; Ewell, 1940; Anon., 1939b; Moran and Smith, 1929).

Contrary to the findings of most investigators, Mohler(1939) observed little difference in the palatability factors of high grade beef either at 10 or 50 days storage($34^{\circ}F_{\bullet}$).

Histological changes

The histological appearance of muscle has been studied extensively.

However, it has not been reviewed from the particular angle of quality of meat, or the relation of the histological picture to tenderness or toughness of muscle(Howe, 1932).

Paul. et al. (1944) found freshly killed beef fibers were poorly differentiated, and straight to slightly wavy. After one day storage, the fibers were more distinct, with contracture nodes, kinks and crinkles. The nodes were still evident as late as 18 days storage, but in four to nine days the kinks and crinkles had disappeared. Normal rigor produced more dense nodes than those caused by heat. Breaks or disappearance of cross-striae appeared on the second day and increased as storage time progressed. Two types of disintegration occurred: sharp fractures across the fibers and disintegration areas within the fibers. Paul, et al. (1944) found that cooking increased the microscopic evidences of the rigor stages seen in the uncooked meat. Harrison(1947) did not find that cooking intensified the microscopic characteristics of the fibers. The semitendinosus muscle of beef examined showed pronounced macroscopic waves. These waves were present for four days but disappeared by the ninth day of storage. It was postulated that the waves might be evidence of an appreciable amount of elastin in the muscle.

Harrison(1947) found the most outstanding characteristic of the longissimus dorsi muscle to be the prominence of the longitudinal strike in the fibers. Cross-strike of these fibers were evident only under high power. Her conclusion was that it was probable that histo-logical structure of muscle fibers was related to tenderness of beef since there was histological disintegration of the fibers during aging at $34 - 36^{\circ}F$.

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Chemical changes

Chemical changes in fat during aging may be a limiting factor in the storage life of chilled meat.

Oxidation of fat: In a series of studies on the chemical changes in the fat of chilled beef, Lea(1931, 1933, 1935) followed the chemical and palatability changes in beef fat stored in the chilled state for various lengths of time. These experiments involved either: a) storage at 0°C. for 42 days, followed by hanging for four days at 10°C., or b) storage at 0°C. for 60 days. In none of these experiments was atmospheric oxidation of fat sufficient to render it unpalatable, if precautions were taken against exposure to strong sunlight or artificial light.

<u>Free fatty acid</u>: Lea(1931) found that wherever the unpleasant flavor of taint occurred in fat, a correspondingly high value was always found for free acidity(percent oleic acid). Although subsequent Low Temperature Research Station investigations at Cambridge, England have shown that free fatty acid is not itself responsible for taint, it was suggested that free acidity might be taken as an index to the degree of attack by microorganisms capable of splitting fats. Lea(1935) concluded that the life of chilled beef, as determined by absence of unpleasant taste in fat, was limited by the growth of microorganisms. Oxidation (formation of peroxides) played a subsidiary, but still important part. Moran(1929) agreed on these findings.

Scott and Vickery(1939) in the Australian investigations on chilled beef found that a slight hydrolysis of fats occurred during chilling, and that hydrolysis in loin fat was almost invariably greater than in abdominal fat.

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Both Lea(1939) and Moran(1935) stated that beef and mutton fats were relatively resistant to oxidation except when frozen and stored for long periods(longer than 18 months at -10° C.). Rockwood, et al. (1947) reported that free fatty acids and peroxide values had a tendency to increase with increasing storage time and temperature.

pH: The role of pH and its place in the aging of beef during cold storage is a subject not yet clearly defined.

Bate-Smith(1948), Ramsbottom, et.al.(1945), Smorodintsev(1936), and Moran(1935) all stated that the pH value of chilled beef muscle averaged 5.6 to 5.7, and might range from pH 5.3 to 7.0. Winkler(1939) studied therelation between pH and tenderness and reported the maximum toughness of beef occurred about pH 5.0. The pH at which maximum toughness occurred was more variable between different animals in beef than in pork.

Drosdov and Drosdov(1936) found that a rise in pH to 6.2 - 6.3was an early indication of decomposition. Harrison(1947) in her study of aging in beef roasts concluded that there was an acidification of beef muscle postmortem. During storage at $34 - 36^{\circ}F$. the pH of uncooked muscle decreased slightly and then slowly rose.

Bate-Smith(1948) in his comprehensive review of beef muscle activity and special consideration of the problem of ripening, stated, "A common factor in all aspects of the subject is the predominating influence of the acidity of the flesh on its immediate properties and future behavior. This influence is seen in the dependence of the activity of the enzymes responsible for autolysis on pH; this factor in turn, it is presumed, affecting the rate of increase in tenderness during the ripening process."

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Tenderness studies

<u>Shear values</u>: Several investigators have found a high correlation between palatability tenderness and shear force(Mackintosh, et al., 1936; Brady, 1937; Satorius and Child, 1938; Ramsbottom, et al., 1945). The modified Warner-Bratzler shear apparatus(Warner, 1928) has been generally accepted as a reliable measure for the objective determination of tenderness in meat.

<u>Tenderness variation</u>: Many authors have discussed the factors that contribute to tenderness of beef muscle. The sex, age, and grade of the animal, size of the muscle fiber, amount of connective tissue and amount of fat have all been studied in relation to tenderness. The literature on these subjects has been reviewed by Bate-Smith(1948).

Tenderness differences of beef in cold storage between right and left sides of the same animal have not been observed as long as comparable muscles were tested, nor were there observed differences in tenderness from end to end of the longissimus dorsi muscle in the same animal(Deatherage and Harsham, 1947). However, Paul and Bratzler(1949) observed differences between adjacent steaks and between anterior and posterior portions of the same muscle.

Ramsbottom, et al. (1945) in studying the comparative tenderness of representative beef muscles stored at $34.5^{\circ}F_{\cdot}$, found most muscles uniform in tenderness throughout. However, the longissimus dorsi muscle was somewhat less tender from posterior to anterior end of the muscle.

Cooking studies

McLachlan (1937) and Cline, et al. (1930) found that the weight of

a steak determined the rate of cooking; the heavier the steak the less time per pound required for broiling. Total cooking time was determined by weight and thickness of the steak.

Tenderness in cooked meat is the total effect of aging before cooking, heat coagulation of the muscle fiber protein, and changes which take place in connective tissue. Noble, et al.(1934) performing tenderness tests on cooked beef, found by means of the N.Y. Testing Laboratory Penetrometer that the difference between averages of penetrometer values for corresponding right and left sides of wholesale rib cuts were negligible when the cuts were treated in the same manner.

Ramsbottom, et al.(1945) compared tenderness of 25 muscles from each of three heifer carcasses. Tenderness was based on shear readings before and after cooking the 25 muscles. The muscles were ranked according to decreased tenderness in the raw samples. After cooking, shear values showed that 17 of the 25 muscles decreased in tenderness, four did not change significantly, and four became more tender. The longissimus dorsi muscle was ranked as the fifth most tender muscle, and showed a significant decrease in tenderness on cooking as indicated by shear value. Similar findings were noted by Satorius and Child (1938) in beef cooked to an internal temperature above 67°C. This decrease was attributed to the toughening effect of heat in coagulating and hardening the muscle proteins. Coagulation caused the longissimus dorsi muscle to become more tender up to 58°C. In a study made on objective measurements for tenderness, Lowe(1934) found that longissimus dorsi muscle cooked well done was less tender than uncooked muscle when measured by penetrometer and shear stress apparatus. The difference in

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the stage of coagulation of cooked muscle may have caused the discrepancy in the results.

Increased internal temperature of muscle, prolonged storage at $34 - 36^{\circ}F$, and too low an initial cooking temperature of the meat all brought about increased cooking losses as reported by Moran and Smith(1929), Satorius and Child(1938), and Craghead(1938).

Freezing Storage Studies

Definition and purpose

Lean meat(muscle) contains 75 percent water. When muscle begins to freeze at -1° C., the water present is gradually converted to ice as the temperature is reduced. In fresh meat this process is still not quite complete at -20° C. Separation of ice increases the hardness of the frozen carcass. Meat held at -5° C. and below is usually referred to as "frozen", while at -2° C. and above it is classed as "chilled" (Lea, 1939).

Freezing has long been believed to do "something" to meat. But with limited information available on the subject, the effect of freezing was generally regarded as unfavorable. The reputation of frozen meat was not good. It is only in recent years that more extensive research has established the fact that, when properly carried out, freezing may be an asset(Hankins and Hiner, 1944). One major benefit indicated by recent studies on freezing appears to be a tenderizing effect on beef. In general, the lower the freezing temperature the greater the effect, according to these same workers.

Palatability changes

Major palatability factors involved in freezing meat are identical

with those of chilled beef: aroma, flavor, juiciness, texture and tenderness.

Flavor: Hankins and Hiner(1941) stated that flavor of meat was difficult to evaluate in frozen meat quality. However, in the few studies mentioning flavor, the general consensus seemed to be that flavor deteriorated in frozen storage(Lowe, 1945). Off-flavor developed more rapidly when beef aged past the chill period was frozen than when beef was frozen immediately after being chilled 24 - 48 hours (Hickman, 1946). Tressler and Murray(1932) found sirloin steaks aged four days at $33 - 37^{\circ}$ F., then quick frozen and stored at 0° F. for one month or longer, had better flavor than adjacent steaks aged at $33 - 37^{\circ}$ F. for six to seven days and tested immediately without freezing. Contamination and rancidity are probably the most common complaints in the development of off-flavors(Brady, 1945).

<u>Juiciness</u>: Lowe(1945), Anon.(1946a), and Beard and Nelson(1940) found that juiciness as well as flavor decreased in storage. McClurg (1940) concluded that steaks frozen at 0° F. received a higher score for juiciness than those frozen at -10° F.

General palatability: Brady, et al.(1942) determined the effect of freezing rate on the quality of two pairs of U.S. Commercial beef rounds. No significant difference was found between steaks quick frozen(-15°F.) and slow frozen(0°F.) with respect to palatability scores. Beard and Nelson(1940) reported that palatability was little affected by temperature or period of freezing when roasts and steaks were frozen at 0°F. and -10°F. and stored for periods of 30, 60, 90, 120, 240, and 365 days before examination. Child and Paul(1937) found palatability of beef was unaffected by freezing and by use of different - 14 -

thawing temperatures. Artyokh(1940) presents similar results.

<u>Tenderness</u>: Tenderness is generally recognized as one of the most important characteristics of meat. "When meat has a low degree of tenderness, to many people it is quite unsatisfactory, although it may be thoroughly desirable with respect to other characteristics. Therefore, any factor that has an effect on tenderness is of definite concern to the consuming public" (Hankins and Hiner, 1940).

Three approaches have been made to the study of tenderness and freezing of beef: 1) influence of low temperatures; 2) the effect of length of frozen storage; and 3) the effect of aging and subsequent freezing.

In a study on six Hereford steers, Bray, et al.(1942) indicated that little or no change in tenderness of aged beef was due to freezing. This is contrary to other findings in later studies.

Hankins and Hiner(1940, 1941) found that freezing appeared to make meat more tender. Four pairs of beef shortloins were stored 96 hours at $34^{\circ}F$. They were then cut into steaks and frozen at $-6.7^{\circ}C$. $(20^{\circ}F.)$, $-23.3^{\circ}C.(-10^{\circ}F.)$ and $-40^{\circ}C.(-40^{\circ}F.)$. A control was held at $34^{\circ}F.$ The steaks frozen at the two lower temperatures were more tender than those frozen at $20^{\circ}F.$, and showed a definite increase in tenderness over the unfrozen steaks.

The effect of length of frozen storage time and influence of low temperatures upon beef was investigated by Miller, et al.(1947) and Beard and Nelson(1940). The steaks from beef shortloins were frozen at -10° and 0°F. and held in storage at 0, 10 and 15°F. Miller and his associates found that freezing at -8°F. and storing at 0°F. in-

creased tenderness. Beard and Nelson concluded that tenderness increased during the first 120 days of storage and then remained constant.

McClurg(1940) presented the following results on tenderness from his study on steaks and beef roasts held in storage at subfreezing temperatures. The roasts frozen at -10° F. were more tender than the roasts frozen at 0° F. However, these results did not apply to the steaks in the study. The major changes noted in the steaks were evident in other palatability scores.

Several large studies have been made on tenderness of beef as affected by aging with subsequent freezing. Hiner and Hankins(1947, 1944, 1941) aged beef short loins five days at $34^{\circ}F$. and froze them in still air at 418, 0, -18, -40, and $-114^{\circ}F$. The samples were thawed at $45^{\circ}F$. and cooked to $138^{\circ}F$. internal temperature in an electric oven. Cooked samples were tested for tenderness by shear cores. Samples frozen at $+18^{\circ}F$. were more tender than the unfrozen sample; while beef frozen at $-114^{\circ}F$. was the most tender. At temperatures between $-18^{\circ}F$. and $-114^{\circ}F$. tenderness was intermediate, increasing with decreasing freezing temperatures. Similar results have been presented from other studies using different aging and freezing temperatures(Anon., 1946b; Brady, 1945; Tressler and Murray, 1932; Miller, et al., 1947).

Miller, et al.(1947) and Tressler and Murray(1932) noted that lower grades of steaks displayed a more pronounced tendering effect than top grade steaks. Tressler found Grade C sirloin steaks after five weeks of aging plus quick freezing, as tender as Grade A steaks prior to freezing. He concluded that the process of tenderizing quick frozen meat continued during frozen storage. Horne(1937) questioned the reasons for increased tenderness in quick frozen meat. It was his belief that tenderizing of meat was an enzymatic process. This process had been shown to proceed more rapidly at high temperatures than at low temperatures. Tressler and Murray(1932), in agreement with other investigators, acknowledged the fact that tenderizing of beef at temperatures above freezing was brought about by the action of enzymes contained in the tissues. They also pointed out that such activity continued, but at a greatly reduced rate, at temperatures slightly below freezing and more slowly as the freezing temperature was lowered. In addition, Tressler remarked that hammering of meat changed it physically so that it was more easily masticated. Presumably quick freezing acted in a somewhat similar physical manner in conjunction with continued slow enzyme activity to increase tenderness in meat.

Histological changes

Microscopic or histological approach to the effect of freezing on beef has been entirely from the viewpoint of ice crystal formation.

Richardson and Scherubel(1908) examined longitudinal and crosssections from frozen beef knuckle histologically as an aid in determining the deterioration of flesh foods. Ice crystal formation indicated the effect of fast and slow thawing on the tissue. They concluded that the slow thawing produced nearly normal tissue while tissue thawed rapidly at high temperature showed distortion of fibers.

Beard and Nelson(1940) stated that the microscopic structure of frozen meat. was similar under the influence of both 0° and -10°F. freezing temperatures and holding for various storage intervals. Where freezing was delayed in the deep layers, the crystal size of the ice increased.

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A series of studies by Hiner and Hankins(1947, 1946) and Hiner, et al.(1945), with reference to the effect of temperature of freezing on histological characteristics of longissimus dorsi muscle, brought forth the following conclusions:

> Sections from muscle frozen at $18^{\circ}F$. showed large interfibrillar ice crystals which had pushed the fibers into distorted groups. No intrafibrillar ice crystals were visible. As freezing temperatures were lowered, ice crystals became smaller and more numerous. The first intrafibrillar ice was visible as the result of $0^{\circ}F$. freezing. Fiber splitting was observed in longitudinal sections at $-10^{\circ}F$. and became more and more extensive as freezing temperatures were lowered to $-1114^{\circ}F$.

A series of longitudinal and cross-section samples were sectioned at 25 mm. intervals from the exposed surface to the center of the round, a distance of approximately 150 mm. Photomicrographs showed the largest crystals were near the surface and smaller more numerous crystals near the center. This was true for each temperature studied. In general, crystals in rounds frozen at 18° F. were larger than those frozen at 0° F., and so forth for decreasing temperatures. No fiber splitting or intrafibrillar ice crystals were observed in rounds frozen at 18° F. At lower freezing temperatures, fiber splitting was found in longitudinal sections, and became increasingly extensive as temperatures were lowered to the extreme of -1114° F.

Hiner, et al.(1945) observed that, as freezing temperatures were lowered, the fibers became more nearly parallel(less bunched) and were fewer per group.

Hiner and Hankins(1947) believe that the increase in tenderness observed in freezing beef was probably due to increased fiber rupture by intrafiber ice formation, and stretching and rupturing of interstitial connective tissue.

Chemical changes

The development of rancidity in fat tissue has been shown to have considerable influence over the storage life of chilled meat(Lea, 1931). The keeping quality of fat tissue in frozen storage is not much improved over that found in fat tissue maintained in cold storage.

Kiermeier and Heiss(1939) stored beef quarters and half pork carcasses up to 18 months at -8.5, -15, and -21°C. to ascertain the keeping quality of beef and pork fat at low temperatures. The higher storage temperatures were unsatisfactory for periods of even five months, since the fat tissues became rancid; -21°C. was the preferred storage temperature for the product, as the odor at this temperature was better after one year's storage than at the higher temperatures. These men concluded that for long periods of storage, fat tissues should not be subjected to air currents, as the fat becomes rancid more rapidly. Peroxide and aldehyde values increased in the presence of atmospheric oxygen. Storp(1913) found fatty tissues of frozen meat suffered no appreciable change in two months storage.

Brady(1945) remarked that meat stored for long periods in the frozen state became subject to rancidity development. This development was progressive and resulted in a marked diminution of palatability of the product. He attributed this rancidity to 1) the atmospheric oxidation of unsaturated fats and subsequent development of off-flavor and aroma; 2) the action of microorganisms which result in extensive hydrolysis accompanied by unpleasantly flavored metabolic products; 3) the presence of tissue enzymes which led to development of free acidity; 4) picking up of objectionable foreign flavors. Brady concluded that temperature, length of storage, and previous handling all appeared to have a very important effect on the development of rancidity.

Ramsbottom(1947) found that frozen beef, lamb, and pork stored at -10 F., for a period of seven years, was markedly changed in appearance

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and flavor of the fatty tissue. After a storage period of one year, there was no significant change in the free fatty acid content of chops. However, the development of peroxides in the fat was a function of time and temperature. Brady(1945), Shrewsbury, et al.(1945) and McClurg(1940) revealed similar findings. Hankins and Hiner(1941) reported little difference in free fatty acid content between freezerstored meat samples previously chilled at 33°F. and 50°F.

Most investigators of the keeping quality of fat in meat have agreed that beef and lamb fats are more resistant than pork fat to oxidative changes (Moran, 1929; Hankins and Hiner, 1941; Lea, 1939).

<u>pH</u>: In similar studies conducted at a six-year interval, Ramsbottom(1947) and Ramsbottom and Koonz(1941) found no significant change in the pH of beef stored for one year at -10°C. to -30°C. The pH values were given as 5.4 to 6.2, and it was suggested that there was no real relationship between storage condition and pH. McClurg (1940), however, found the difference in pH values between storage periods for steaks were highly significant. Steaks stored at -10°F. were more acid than those stored at zero or 15°F. Both steaks and roasts were more acid after 365 days storage than after 30 days storage.

Beard and Nelson(1940) found the pH value of frozen beef held in storage for different intervals dropped from 6.2 to 5.7 in a 60 to 90 day period; then suddenly rose to 6.6 on the 120th day. McClurg(1940) noted the same rise in alkalinity at 120 to 240 days frozen storage.

Heiss(1936) stated that the pH value influenced freezing alterations in meat and that oxidative changes were accelerated by decreasing pH values. He suggested that freezing of meats should take place as soon as possible after the kill to prevent pH reactions.

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Tenderness studies

<u>Shear value</u>: Samples of beef from several freezing studies have shown a decrease in shear values which represented an increase in tenderness of the meat.

Hankins and Hiner(1940) reported that steaks held at $34^{\circ}F$. had higher shear strength and were less tender than steaks frozen at 20, -10, or $-40^{\circ}F$. Steaks frozen at $20^{\circ}F$. showed a 12 percent decrease in shear value below the steaks held at $34^{\circ}F$. The $-10^{\circ}F$. and $-40^{\circ}F$. samples each showed an 18 percent decrease in shear below the unfrozen steaks. The shear values for each animal reflected the variability within each carcass. A similar study made in 1941 by these same men indicated that length as well as temperature of storage was reflected in a decrease in shear values for frozen short loin steaks.

In 1941 and 1944, Hankins and Hiner concluded that the most rapid tenderizing effect on longissimus dorsi steaks occurred during the first 15 days of storage at 34°F. when the shear value at five days was 25.8, and at 15 days 20.7. Aging for 15 days at 34°F. followed by freezing at -10°F. produced the most tender product.

<u>Tenderness variations</u>: Steiner(1941) pointed out that the age and sex of the animal as well as other factors affect the speed of aging of beef muscle during frozen storage. As in normal aging, toughness increased at first, then decreased to a point under the initial value.

Bray and co-workers(1942) found indications that the change in toughness of aged beef might vary with the animal. Considerable variation was found between animals. The posterior section of the short loin was noted to be more tender than the anterior section. Hankins and Hiner(1940) and Miller, et al. (1947) drew the same conclusions.

Bray, et al.(1942) found considerable variation among the cores taken from the longissimus dorsi muscle; the greatest variation occurred in the lateral core. These investigators also found the right side of the animal to be significantly more tender than the left side. Hankins and Hiner(1940) and others have shown there were no important differences between left and right sides of carcasses in their studies.

Cooking studies

Tenderness in cooked, frozen meat covers the same factors involved in chilled, cooked beef.

McClurg(1940) in a study on quality and palatability of beef after storage in sub-freezing temperatures, found that cooking losses decreased as storage losses increased.

Brady(1945) and Brady, et al.(1942) concluded that steaks cooked from the frozen state had a smaller cooking loss than when thawed first. But palatability scores were not significantly different on either method of thawing.

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EXPERIMENTAL PROCEDURE

Description of meat

The longissimus dorsi muscles from both the right and left sides of two beef animals were obtained immediately after slaughter from the Animal Husbandry Department. Animal I was a four-year old Shorthorn cow and Animal II was a two-year old Shorthorn heifer. Both animals were U.S. Commercial grade.

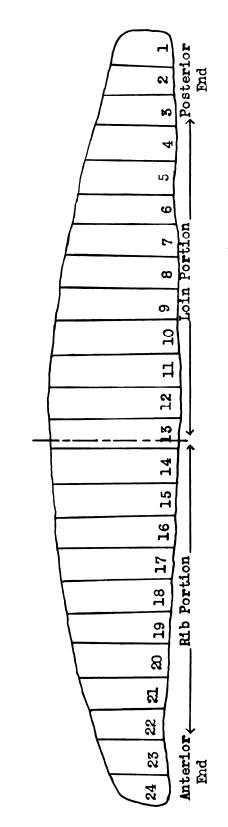
Preparation of samples

Each muscle was divided into 24 one-inch steaks. These were numbered from the posterior or sirloin end to the anterior or rib end of the muscle as shown in Figure 1, page 23. The following table was set up for determining methods of treatment and for analysis of the data.

	Aged, hours	Sam	ples
eatment	at 46°F.	Animal I	Animal II
Unfrozen	0	4 steaks*	4 steaks*
	5	11 11	11 H
	21	u u	11 II
	24 167	81 83	11 EI
Frozen - 1 week	0	4 steaks*	4 steaks*
	5	11 11	11 11
	24	1 t ti	11 H
	167	21 11	t1 11
Frozen - 4 months	0	4 steaks*	4 steaks*
	5	11 11	ti ti
	21	H 11	38 B1
	24 167	11 11	11 11

Table 1.	A	summary	of	aging	and	storage	treatments	on	ste a ks	for
Animal I and Animal II										

I steak of each pair for palatability so l steak of each pair for other analyses





The cold storage periods employed for the 96 steaks were 0, 5, 24, and 167 hours at 46°F. Twelve steaks from each animal were aged at each cold storage period, (Table 1, page 22). At the end of each holding period the 12 steaks received the following treatment:

- 1) Four steaks (2 left and 2 right) were removed from the refrigerator and cooked immediately for palatability and tenderness scores.
- 2) Four steaks (2 left and 2 right) were frozen at -26°C.(-15°F.) and stored for one week at 0°F.
- 3) Four steaks (2 left and 2 right) were frozen at -26°C.(-15°F.) and stored for four months at 0°F.

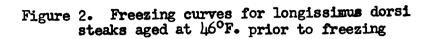
The steaks for freezing were wrapped with a drugstore wrap in cellophane (MSAT 300), sealed with transparent tape, labeled, and frozen. Copper-constantan thermocouples connected to a Brown Electronik Recording Potentiometer recorded the rate of freezing for one pair of steaks from each group. The freezing curves are shown in Figure 2, page 25.

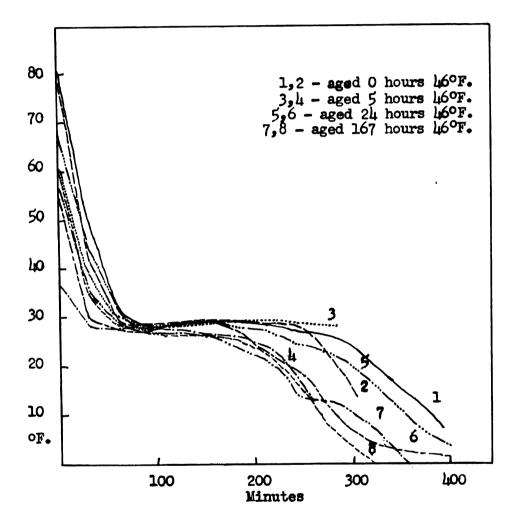
The frozen steaks were over-wrapped with stockinette, tied securely at each end. The one week storage samples were held in a deepfreeze box; the four months storage samples were packed in a corrugated paper carton, sealed, and placed in a commercial locker plant.

At the end of their respective frozen storage periods, the steaks were removed from the stockinette wrapper and placed on the bottom shelf of the laboratory refrigerator to thaw. This required approximately 16 hours at 46° F.

Method of cooking

Each steak was weighed on removal from the refrigerator. The two pair of steaks from each set of four (left and right) were then handled in the following manner: one steak of each pair was tested organoleptically; the second steak of each pair was divided as follows: a portion of the lean was reserved for pH determination, the raw fat set





aside for peroxide and free fatty acid analyses, and the remainder of the lean cooked for shear tests.

Thermometers were inserted into the thickest portion of lean tissue to record internal temperatures of each steak before and during cooking, and maximum temperature reached after cooking. All steaks were deep-fat fried in vegetable shortening at a temperature of 150° C. $(302^{\circ}$ F.) to an internal temperature of 63° C. $(145.4^{\circ}$ F.)(medium rare). On removal from the fryer, each steak was drained for 30 seconds per side on brown paper toweling to remove excess fat, and allowed to come to maximum temperature at which time the cooked weight was recorded.

Data recorded for each steak were: weight before cooking, weight after cooking, cooking time, temperature before cooking, internal cooking temperature, maximum internal temperature after cooking, cooking loss in grams, and percent cooking loss.

Palatability scores

A panel of five experienced judges from the Foods and Nutrition Department scored the cooked steaks for appearance, aroma, flavor of fat and lean, juiciness, tenderness, texture, and general conclusion. Any comments the judges wished to make on the steaks were recorded. A possible high score of seven and a low score of one covered the range for each factor. A sample score sheet is shown on page 67 in the appendix.

Each steak was cut into five one-inch strips the width of the steak with the grain. The judges scored strips from the same position in each steak at each taste period. A piece of fat was included in each strip wherever possible so that the flavor of both the fat and the lean could be tested. All steaks were coded for impartial judging by the panel, and from two to four steaks representing both left and right sides of the animal were scored at one time. Glasses of cold

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water were provided each judge. The O, 24, and 167-hour samples from each treatment were scored at 10:30 A.M., and the 5-hour samples for each treatment at 2:30 P.M.

Shear

Seven cylindrical cores one-half inch in diameter were taken from one left and one right steak in each group at each scoring period. Cores were sheared on a modified Warner-Bratzler shear stress apparatus. The seven readings from each steak were averaged.

Chemical methods

<u>pH</u>: A five gram sample of raw lean tissue was minced with a sharp knife, mixed with 45 ml. of distilled water, and allowed to stand 20 minutes. The liquid portion was decanted and the pH of the liquid determined by a Beckman pH meter. Duplicate readings were made on each steak.

<u>Peroxide number</u>: Determinations of the peroxide number of the raw fat were made by a slight modification of the method described by Watts (1947). The original method was adapted to the problem of raw fat analysis by substituting two grams of minced raw fat plus four grams of anhydrous sodium sulfate for 25 grams of pork sausage meat and 50 grams of anhydrous sodium sulfate. The remainder of the Watts procedure was not altered. Duplicate determinations were made on each sample. Rockwood, et al.(1947) followed essentially the same procedure in determining peroxides in fresh pork loin fat.

Free fatty acid: Free fatty acid content of the raw fat was determined from a portion of the sample used for determining the peroxide number. The method described by Halliday and Noble(1943) was used. Duplicate determinations were made on each sample.

Histological studies

Histological studies were made to determine the changes in the

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muscle fibers during both cold storage and freezing storage of the steaks. Samples from both the raw and cooked meat were preserved in 10 percent formalin and were later embedded in gelatin. The embedded samples were frozen with carbon dioxide, and longitudinal sections cut 10 to 15 microns thick on a Spencer Freezing Microtome. The sections were stained with Harris hematoxylin and mounted in glycerine jelly. This treatment yielded blue muscle fibers while the gelatin frame was a pale blue.

Statistical methods

Correlation coefficients were calculated, and analysis of variance made, according to the methods recommended by Snedecor(1946).

DISCUSSION OF RESULTS

Palatability Changes

Definition of terms

Cold storage in this study refers to the holding of fresh beef at the temperature of 46° F. Frozen storage refers to steaks frozen at -26°C.(-15°F.) and held in storage at 0°F. for periods of one week or four months. "Aging" or "aging period", refers to the 0, 5, 24, and 167-hour time intervals that the steaks were held at 46° F. Treatment applies to frozen steaks in contrast to unfrozen steaks.

Appearance

Little or no difference in scores for appearance of the steaks (Table 2.) resulted from cold storage or frozen storage treatment of the steaks.

Table 2.	Mean scores for appearance	of longissimus dorsi	steaks
	in cold storage and	l frozen storage	

Aged, hours	Treatment		
at 46°F.	Unfrozen	Frozen - 1 week	Frozen - 4 months
O	5•7 5.6	5.5	5•7
24 167	5.0 5.9 5.6	5•7 5•6	5•6 5•5

The judges described the frozen storage steaks for both storage times as dark and dry on the surface.

Aroma

The aroma of the steaks (Table 3, page 30) appeared to increase slightly during aging. The aroma score increased within each treatment,

becoming more desirable with frozen storage. Ramsbottom(1947) found aroma of steaks did not deteriorate unless held in frozen storage at -10° C. or lower for long periods of time.

Table 3.	Mean scores	for aroma of	longissimus	dorsi steaks
	in cold	d storage and	frozen stora	ug e

Aged, hours	Unfrozen	Treatm	ent
at 46°F.		Frozen - 1 week	Frozen - 4 months
0	5.2	5.4	5.4
5	5.2	5.7	5.1
24	5.0	5.5	5.9
167	5.4	5.8	5.9

Flavor

Lean: The flavor score for lean tissue increased slightly at 167 hours aging in cold storage(Table 4.). The set of four steaks aged 0, 5, 24, and 167 hours, and subsequently held in frozen storage for one week at 0° F., followed the same trend of increasing flavor

Table 4. Kean scores for flavor of lean of longissimus dorsi steaks in cold storage and frozen storage

Aged, hours	Unfrozen	Treatm	ent
at 46°F.		Frozen - 1 week	Frozen - 4 months
0	4•7	4.6	5.1
5	4•8	4.7	5.4
24	4•6	4.7	5.3
167	5•2	5.1	5.2

score as the cold storage steaks. The steaks held in frozen storage four months at $0^{\circ}F$. show little change resulting from the four aging periods within the treatment. But the increased length of frozen storage appeared to develop a flavor in the lean tissue slightly more desirable than that found in either the steaks held in frozen storage one week or in cold storage.

One judge consistently commented on a metallic flavor in the lean tissue. "Indistinct" and "lack of flavor" were comments made on the unfrozen and frozen for one week samples. Beard and Nelson (1940) stated that the flavor of lean beef tissue in steaks did not appear to be noticeably affected by low temperature storage(0 and $-10^{\circ}F.$) except at periods beyond 120 days.

<u>Fat</u>: The flavor scores for fat on the longissimus dorsi steaks remained unchanged in cold storage, but increased in desirability in both frozen storage periods; however, the trend of the scores was different within each of the frozen storage periods(Table 5). The score for flavor of fat decreased in value for 167 hours aging and

Table 5. Mean scores for flavor of fat of longissimus dorsi steaksin cold storage and frozen storage

Aged, hours	Unfrozen	Treatm	ent
at 46°F.		Frozen - 1 week	Frozen - 4 months
0	4.5	4.3	5.3
5	4.6	4.9	5.2
24	4.5	4.6	5.3
167	4.5	5.0	4.9

freezing for four months. This lessened desirability of the fat flavor in the latter part of the four-months frozen storage period may be indicative of development of incipient rancidity in the fat.

Texture

Texture scores(Table 6, page 32) showed considerable variation within treatments. In unfrozen storage, the scores dropped consistently from 0 to 5 hours, and from 5 to 24 hours aging, but increased again to the original score after 167 hours aging. The scores for the group

Aged, hours		Treatm	ent
at 46°F.	Unfrozen	Frozen - 1 week	Frozen - 4 months
0	4•4	4•2	3•2
C	3•6	3•8	3•1
24	3•3	4.0	3.4
167	4•5	4.5	4.1

Table 6. Mean scores for texture of longissimus dorsi steaksin cold storage and frozen storage

of steaks frozen one week fluctuated for the first three periods within the group, but increased at the 167-hour period to equal the comparable score(167 hours) in the cold storage group. The scores for four months frozen storage were the lowest of the three storage periods, but again, the last aging period within the group showed improvement over the three earlier periods in the same group. The judges commented on the stringy texture of the steaks throughout the test period.

The texture scores in cold storage and four months frozen storage appeared to follow the same pattern as the tenderness scores. Brady (1937) found that texture of longissimus dorsi beef muscles aged 10 days gave a high positive correlation with tenderness scores.

Tenderness

In Table 7, page 33, the tenderness scores were the most variable of any of the individual palatability factors. The unfrozen steaks were quite tender at zero storage time, but decreased sharply in tenderness during the next 24 hours of cold storage. By the time 167 hours of aging had been completed, the steaks had again increased in tenderness to a score comparable to the tenderness score of the zero storage sample. This tenderness pattern is believed to parallel onset and height of rigor mortis in the meat within the first 24 hours after slaughter of the animals, and the dissolution of rigor after 167 hours aging

Table 7. Mean scores for tenderness of longissimus dorsi steaksin cold storage and frozen storage

Aged, hours	Unfrozen	Treatmo	ent
at 46°F.		Frozen - 1 week	Frozen - 4 months
0	4.0	2•8	2•5
	2.9	2•8	2•3
24	2•2	3•5	3.1
167	4•1	4•5	4.0

at temperatures above freezing(46°F.).

The tenderness of the steaks held in frozen storage for one week at O^oF. presented a different pattern from that found in the cold storage group. The mean scores for the O and 5-hour samples in this group were comparable to the 5-hour samples held unfrozen. The 24 and 167-hour samples in one week frozen storage showed increased tenderness scores over the zero and 5-hour samples within the same treatment. The 167-hour sample scored the most tender and surpassed the tenderness rating of the comparable steak in the cold storage group.

The four months' frozen storage steaks presented a tenderness pattern which paralleled the samples frozen for one week. But all values were consistently lower throughout this storage period. The zero and 5-hour samples in extended frozen storage had tenderness scores comparable with the 24-hour cold storage sample. These were the lowest tenderness scores received in the study. The 24 and 167hour samples frozen four months were more tender than the steaks

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tested at the early aging periods receiving the same treatment. The 167-hour sample was comparable in tenderness value to the 0 and 167-hour unfrozen samples.

The steaks held in frozen storage for four months had apparently reached the same degree of toughness in the 0 and 5-hour samples as the 24-hour sample held in cold storage. This comparable degree of toughness in the two treatments at different aging periods would indicate that the process of aging found in cold storage samples is continued in the frozen meat. Freezing storage periods intermediate between one week and four months would probably clarify the pattern of tenderness development in frozen storage.

The judges commented on the lack of tenderness in the steaks throughout the study, particularly where the lowest tenderness scores are shown.

Extensive work by several investigators(Hankins and Hiner, 1947; 1944; 1941; Willer, et al., 1947; and Tressler and Murray, 1932) has shown that the freezing of meat makes it more tender. This study has shown a definite increase in tenderness in frozen storage at the 24hour cold storage interval, a slight increase in tenderness during freezing at the 167-hour period, but a decided decrease in tenderness at the 0 and 5-hour aging periods. Extended frozen storage treatment did not increase the tenderness of the steaks in this study; one week frozen storage showed tenderness improvement in the two later aging periods.

Gortner(1948) suggested that the tenderizing action of freezing could take over at least a part of the functions of aging. He stated that where meat was to be frozen, the aging period could be shortened by approximately 50 percent, with resultant increased storage life, decreased shrinkage from moisture loss, and reduced trimming losses.

Tenderness is considered to be one of the more important palatability factors in meat, and one greatly influenced by cold storage and frozen storage treatment. Therefore, a statistical analysis of the tenderness data was made to separate variation due to animals, treatment, hours of aging and sides of animals. Table 8, gives the F values which were found to be significant.

Table 8. Sigificant F values from analysis of variance of tenderness scores

Animals	50.13**
Hours	50.13** 36.07**
Animals x treatment	10.13*
Animals x hours	5.47*
Treatment x hours	9.53**
Animals x treatment x hours	5•47* 9•5 3** 7•67*
	Animals x treatment Animals x hours Treatment x hours

* p, less than 0.05, greater than 0.01

The F values show that the differences due to animals and hours of aging were highly significant. Differences between animals are usually attributed to biological and management variations, which could not be controlled in this experiment. The differences due to hours of aging are considered to arise from the onset, height and dissolution of rigor in the muscle after slaughter.

The interaction between treatment x hours was highly significant, indicating that the rate of change in tenderness due to aging was altered by frozen storage. The interactions between animals x treatment, animals x hours, and animals x treatment x hours were significant, showing that there were differences between the two animals used in rate of change of tenderness due to cold storage and frozen storage.

Juiciness

Juiciness scores revealed little effect from treatment, but some effect from aging(Table 9). Several investigators have reported that

Table 9. Mean scores for juiciness of longissimus dorsi steaksin cold storage and frozen storage

Aged, hours		Treatment		
at 46°F.	Unfrozen	Frozen - 1 week	Frozen - 4 months	
0	5.0	4.9	5.1	
5	4.9	5.4	5.1	
214	5.3	5.0	5.1	
167	5•3	5.1	5.2	

juiciness scores increased with aging. The scores in this study were found to increase in the latter periods of cold storage and in one instance in frozen storage for one week. The average juiciness score for each treatment was the same(5.1). If the frozen storage were extended beyond the four months period, it might have shown more effect on this palatability factor. The judges commented that the cooked steaks from both frozen storage periods were "dry".

General conclusion

Palatability scores for general conclusion paralleled the tenderness scores for all three storage periods (Table 10, page 37). The average scores for each treatment were higher than the tenderness scores for these periods.

The general conclusion scores were analyzed to separate the

Table 10.	Mean scores	for general conclusion of longissimus	dorsi steaks
		in cold storage and frozen storage	

Aged, hours at 46°F	The Original States	Treatm	
at 40°r	Unfrozen	Frozen - 1 week	Frozen - 4 months
0	4•3	3•5	3•2
5	3.6	3•4	3-2
24	3.1	3•9	3•5
167	4.6	4.7	4.2

variations due to animals, treatment, hours of aging and sides of animals. Table 11, gives the F values for the factors found to be significant.

Table 11.Significant F values from analysis of variance of
general conclusion scores

Source of variation	F values
Animals	33.88 **
Treatment	10.13*
Hours	38.13**
Animals x treatment	12.50**
Animals x hours	5.38*
Animals x sides	7.63*
Treatment x hours	7.00*
Animals x treatment x hours	7.75*
** p, less than 0.01	
* n. less than 0.05.	greater than 0.01

* p, less than 0.05, greater than 0.01

The differences due to animals and hours of aging were highly significant, probably for the same reasons given under the discussion of tenderness scores: the biological and management variations between animals and the changes in tenderness generally considered to be associated with the onset and dissolution of rigor. The most noticeable differences found within each treatment were for zero aging and for 24-hour aging periods. At zero aging the cold storage sample was superior, at 24-hours aging, it was least desirable. The general conclusion scores reflect the collective influence of the other palatability factors: the scores for appearance, aroma and juiciness were quite uniform throughout the study, the flavor scores for fat and lean increased slightly, but the tenderness and texture scores were variable. The tenderness scores appeared to have the greatest influence on the general conclusion scores as determined by the taste panel, since there was a similar trend for these two factors through each storage period and each aging period.

The interaction between animals x treatment was highly significant; significant interactions were found for animals x hours, animals x sides, treatment x hours, and animals x treatment x hours. These indicate that the differences between the two animals affected the general conclusion scores as well as the tenderness scores.

Shear Keasurements

The relative tenderness of the cooked steaks was evaluated by shear force determinations. Correlation coefficients between shear force readings and the scores on tenderness and texture of the cooked steaks were calculated. These are given in Table. 12.

Table 12. Correlation coefficients between shear force readings and judges' scores on tenderness and texture of cooked steaks

Correlation between of sa	r of pairs Coefficient of mples correlation, r
Shear force and tenderness score	48
	48 -•547*
** p, less	than 0.01 than 0.05, greater than 0.01

A highly significant correlation was found between the judges' scores for tenderness and the shear force values for the steaks, and a significant correlation between texture scores and shear values. The correlation coefficients were negative because the more tender the meat, the higher the judges' scores, but the lower the shear force value. Similar results have been reported by other investigators (Ramsbottom, et al., 1945; Satorius and Child, 1938; Brady, 1937; Mackintosh, et al., 1936).

The significant correlation coefficient obtained between shear value and texture score appears to lend further support to the tenet that texture and tenderness in the longissimus dorsi muscle might be directly related, as reported by Brady(1937).

Cooking Changes

The data on the cooking losses of the steaks are presented in Table 13, page 40. The average values indicated that freezing and storage did not appreciably affect the percentage of total cooking losses. The average percentage of losses for the cold storage steaks was somewhat less than for the steaks which were frozen and held in frozen storage. However, the average total cooking time was also less for the unfrozen than for the frozen steaks. The lower cooking losses for the unfrozen steaks were probably due to the shorter cooking time, which might be attributed to the higher initial temperature of the cold storage steaks when they entered the deep-fat fryer. Their average initial temperature was 21°C., while the average initial temperature of the steaks which had been frozen and thawed was 10°C., lower than

Treatment	Aged, hours at 46°F.	Wgt. of steaks g.	Cooking time min.	Percent o total loss	cooking losses loss per min.
Unfrozen	0	189 .2	3.0	17.96	5.99
	5	192.9	3.9	18.72	4.80
	24	193.6	4.2	23.52	5.60
	167	184.5	4.2	22.77	5.42
Frozen- 1 week	c 0	190.7	4.2	23.96	5.70
	5	189.1	4.0	23.52	5.88
	24	187.1	5.4	27.22	5.04
	167	213.9	6.7	30.07	4.49
Frozen-4 month	ns 0	165.4	3•5	24.33	6.95
	5	198.6	5•4	25.00	4.63
	24	194.7	3•9	25.48	6.53
	167	172.5	4•7	25.05	5.33

Table 13. Summary of averages of combined weights of longissimus dorsi steaks, of cooking time, and cooking losses from Animal I and Animal II

that of the unfrozen steaks immediately before cooking. The coefficient of correlation(\mathbf{r} , 0.713) between total cooking time and percentage of cooking losses was highly significant. This would indicate that the increased losses in the frozen steaks were closely related to the longer cooking time. When the data were calculated on the basis of percent cooking loss per minute cooking time, all three treatments gave similar results which did not appear to be influenced by either storage time or storage temperature.

Chemical Changes

Peroxide values of fat

Table 14, page 42, presents the peroxide values for the raw fat from the samples in cold storage and frozen storage.

It is generally considered that the peroxide values must be greater than 10 milliequivalents per kilogram of fat before obvious rancidity is indicated(Watts, 1947). None of the values found in this study approached this value. This agrees with Storp's results (1913). He found that fatty tissues of frozen meat showed no appreciable change in peroxide value in two month's storage. The values for each animal within each treatment were variable and did not appear to be greatly influenced by the aging time. Animal II showed a more definite trend toward increase in peroxide values than Animal I.

The average values of the combined data for both animals for each storage condition reflected an increase in peroxide values in one week frozen storage over the cold storage samples, but a decrease in values for the fats held in frozen storage for four months. The apparent decrease in peroxide values at four months frozen storage may reflect a decomposition of peroxide at a rate more rapid than its formation. The higher peroxide values for Animal II at the 167-hour

- 41 -

Treatment	Aged, hours at 46°F.	Animal I	Animal II
Unfrozen Kean	0 5 24 167 values	2.95 3.35 4.18 2.54 3.26	2.53 2.92 2.17 3.46 2.77
Frozen - 1 week Mean w	5 24 167	2.55 2.35 1.97 2.82 2.42	3.84 3.77 3.73 5.45 4.20
Frozen - 4 mont	5 24 167	2.66 1.39 1.73 2.03 1.95	3.01 2.02 3.27 5.37 3.42

Table 14. Peroxide values of unfrozen and frozen raw fat from longissimus dorsi muscle of beef 1

Milliequivalents per kilogram of fat

1

Figures are averages for two steaks(right and left sides) from longissimus dorsi muscle from each animal

aging period in both one week and four months frozen storage might indicate development of incipient rancidity. There was also an indication of this in the lowered flavor-of-fat score for this sample. But Lea(1939), Moran(1935), and many other investigators, are agreed that beef fat is relatively resistant to oxidation except when frozen and stored for long periods of time(18 months or longer).

Analysis of variance(Table 15) of peroxide values for differences due to treatment and cold storage, showed that differences due to cold storage were significant.

Table 15. F values from analysis of variance of peroxide values

Source of variation	F values
Treatment	2∙38
Cold storage	2•84*

* p, less than 0.05, greater than 0.01

Free fatty acids in fat

Although free fatty acid development does not necessarily parallel rancidity development, it is a measure of hydrolytic decomposition of the fats. The/fatty acids values for each animal were variable with treatments for the four aging periods. However, there was a progressive increase in free acidity shown from cold storage through both intervals of frozen storage. The values for four months' frozen storage were approximately twice those for cold storage or one week frozen storage. The extremely high value for zero aging and four months' frozen storage might be taken as an index of degree of attack by microorganisms capable of splitting fats(Lea, 1931). But the palatability scores for this same period, did not substantiate this probability, since they indicated a desirable fat flavor. Analysis of variance(Table 16) between free fatty acid values showed that differences due to treatment were highly significant and the differences due to cold storage significant.

Table 16. F values from analysis of variance of free fatty acid values

Source of variation	F values
Treatment Cold storage	50•22 ** 2•96*
** p. less th	an 0.01

** p, less than 0.01 * p, less than 0.05, greater than 0.01

In the discussion on the scores for flavor of fat(page 31), it was brought out that the score for 167 hours aging plus four months frozen storage was somewhat low. The increase of free fatty acids and/or peroxides in fat is usually considered to indicate development of rancidity with associated off-flavor. In comparing the free fatty acid and peroxide data with the scores for flavor of fat, it was apparent that the increased free fatty acid found in Animal I was associated with a low fat score for this sample. However, the increased peroxide value found in Animal II at the same treatment period was not associated with a low score. This was not unexpected, since the peroxide value, although increased, was still below the 10 milliequivalents per kilogram of fat level considered to indicate obvious off-flavor(Watts, 1947). Table 17, page 45, presents the free fatty acid values for this study.

pН

The pH values for both animals(Table 18, page 46) showed a definite increase in acidity with aging in cold storage. The pattern

Treatment	Aged, hours at 46°F.	Animal I	Animal II
Unfrozen Mean	0 5 24 167 values	•30 •27 •23 •31 •28	•28 •30 •27 •40 •31
Frozen - 1 we Mean	ek 0 5 24 167 values	•38 •31 •34 •36 •35	•34 •31 •30 •39 •34
Frozen - 4 mo Mean	onths 0 5 24 167 a values	1•17 •77 •54 •88 •84	•42 •50 •56 •51 •50

Table 17. Free fatty acid values of unfrozen and frozen raw fat from longissimus dorsi muscle of beef¹ Percent Oleic acid

l Figures are averages for two steaks(right and left sides) from longissimus dorsi muscle from each animal

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Treatment	Aged, hours at 46°F.	Animal I	Animal II
Unfrozen	0	6.68	6.54
	5	6.38	6.41
	24	5.81	6.04
	167	5.64	5.77
Frozen - 1 week	0	5.66	5•72
	5	5.66	5•62
	24	5.86	5•52
	167	6.06	5•90
Frozen - 4 months	5 0	5.68	5.83
	5	5.72	5.75
	24	5.69	5.96
	167	5.84	5.81

Table 18.	pH values of unfrozen and frozen raw tissue from
	longissimus dorsi muscle of beef 1

l . Figures are averages for two steaks(right and left sides) from longissimus dorsi muscle from each animal

for each animal was similar in trend. The pH response of the animals during one week frozen storage was variable. At the 167-hour period, three of the four samples from the two frozen storage periods showed an increase in alkalinity. Increased alkalinity after frozen storage of four months has been reported by Beard and Nelson(1940) and McClurg(1940).

Histological Changes

Explanation of descriptive terms

The terms used to describe the microscopic observations made in this study have been used by Harrison(1947) and other investigators in the food research laboratory at Iowa State College.

<u>Rigor node</u>: This term is used to describe a particular kind of contraction of the muscle fiber. Cross-striae are much closer together than in the normal fiber, and on each side of the contracted area is a rarefied area in which cross-striae are much wider than usual or sometimes thrown out of alignment. In the contracted part of the node the fiber bulges so that it is wider than the normal fiber. Paul, et al.(1944), stated that a rigor node indicated a state of active contraction.

<u>Wave</u>: The term wave is self-explanatory. However, waves may be macroscopic or microscopic in size, with V or U bends. They may be deep or shallow, or may be an indentation along the edge like a scallop, or there may be many folds or "S" twists; each fiber shows variations. The waves may be rhythmic in that a pattern is repeated over and over along a fiber, or in that all fibers in certain areas of the sections are involved in the same pattern.

<u>Others</u>: A suddenly rounded curve is referred to as a kink, and multiple kinks as twists. Zig-zag(z-z) contractions describe sharp, angular bends in the fibers which give an accordian pleated effect. The zig-zag contractions, kinks, "S" twists and waves are representative of passive contraction, according to Paul, et al.(1944).

<u>Disintegration</u>: This is a term applied to loss of the histological characteristics of the protoplasm of the fiber, that is, the cross or longitudinal striae. If the sarcolemma remains intact, the disintegrated material within the fiber appears granular. If the sarcolemma is entirely broken, the disintegrated material may have disappeared leaving a blank space in the fibers. With a slight break in the sarcolemma, granular material may exude and be noticeable around the break.

Storage changes

The prominence of the longitudinal striae in the longissimus dorsi muscle fiber was an outstanding characteristic throughout the histological changes. Contrary to the results reported by Harrison (1947), the cross-striae were evident at various times under low power. Harrison found that they were visible only under high power.

<u>Cold storage</u>: The most noticeable microscopic changes which occurred in raw samples in cold storage were as follows;

1. The samples with no storage showed poor differentiation of the fibers and evidence of beginning rigor. The loose z-z effect of passive rigor was noted.

2. After 5 hours storage, a definite pattern of zig-zag effects and "S" twists had become pronounced.

3. At 24 hours cold storage, active rigor nodes were more numerous; z-z and "S" twists were less noticeable. The number of fractures across the fibers had increased over the few scattered ones noted in some samples after 5 hours in cold storage.

4. The samples held 167 hours in cold storage showed extensive evidence of thinning of the cell contents, increased fractures across the fibers and often on each side of rigor nodes, and almost complete disappearance of z-z contractions. The fibers had become straight and fragile. Longitudinal and cross-striations had disappeared in the disintegrated areas. Breaks or fractures across the fibers became increasingly evident from 5 hours through 167 hours aging.

Typical sections for these storage period changes are shown in Figures 3 and 4, pages 51 and 52. Figure 5a, page 53, shows a higher magnification of the rigor node of active contraction, and 5 b, a higher magnification of the "S" twist of passive contraction.

The most prominent microscopic manifestation in the cooked samples was evidence of extreme heat rigor in the 0 and 5-hour storage samples. This type of rigor does not show up in samples aged 24 hours at 46°F. prior to cooking. The other changes found in the cooked samples were the same as those in the uncooked samples (Figures 3, 4, and 5). At times, cooking of the steak samples appeared to intensify the various effects present in the raw samples.

Figure 6, page 54, shows extreme heat rigor produced during deep-fat frying of an unaged steak sample. Figure 6b is a higher magnification of the same effect shown in Figure 6a.

<u>Frozen storage</u>: The four most noticeable histological changes in the raw cold storage samples (Figures 3, 4, and 5) were evident in frozen raw sample. In the frozen samples, the changes frequently

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occurred at an earlier aging period than in the unfrozen samples. It appeared evident that the process of aging continued in frozen storage.

Freezing intensified the microscopic changes found in the raw cold storage samples. It produced an effect similar to cooking of the cold storage samples. Disintegration and fragility of the fibers seemed to appear at earlier aging periods.

Histological sections representing 5, 24, and 167-hours of aging plus frozen storage of the samples are shown in Figures 7 and 8, pages 55 and 56.

The cooked frozen storage samples displayed evidence of heavy granulation outside and within the fibers.

Figure 9a shows noticeable areas of disintegration in the fibers at 5 hours aging plus four months frozen storage. Figure 9b, page 57, is typical of heavy granulation noted in cooking the frozen samples.

Breaks in fibers

The two types of breaks which appeared in the muscle fibers were the sharp fractures and disintegration of the muscle fiber, as found by Paul, et al.(1944). The fractures occurred primarily at the z-z ridges and kinks, and the disintegrated areas were most often evident on each side of the contracture nodes. Disintegration increased considerably as both cold storage and frozen storage time increased. It is noteworthy that the highest tenderness scores in the palatability tests given at the 167-hour aging period for all three storage treatments, were for those steaks in which the greatest number of fractures Figure 3. Changes during cold storage of longissimus dorsi fibers. (longitudinal sections of uncooked samples)

Figure 3a: no storage; fibers poorly differentiated; somewhat wavy; longitudinal striae prominent (125x)

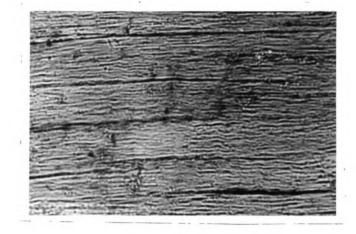
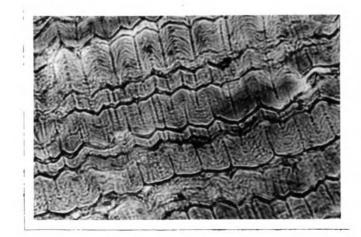


Figure 3b: 5 hours cold storage; definite over-all pattern of zig-zag(z-z) effect; example of passive rigor(125x)



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Figure 4. Changes during cold storage of longissimus dorsi fibers. (longitudinal sections of uncooked samples)

Figure 4a: 24 hours cold storage; fibers show contracture node(lower left center); z-z effect in fiber immediately above node; "S" twists in fibers at top and bottom of picture (125x)



Figure 4b: 167 hours cold storage; fibers show increased fragility; numerous fractures; thinning of cell contents(125x)



Figure 5. Rigor changes in cold storage of longissimus dorsi fibers. (longitudinal sections of uncooked samples)

Figure 5a: 5 hours cold storage; rigor node of active contraction showing contracture bulge and stretched areas on side of node(550x)

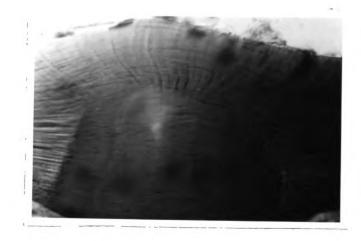


Figure 5b: 24 hours cold storage; "S" twist of passive contraction(550x)



Figure 6. Extreme heat rigor produced in cooked, cold storage sample of longissimus dorsi fibers. (longitudinal sections of cooked sample)

Figure 6a: no storage; showing extreme heat rigor from cooking (125x)

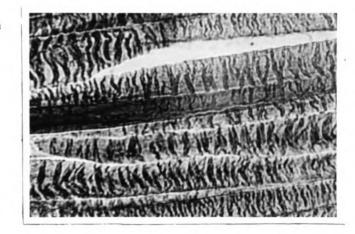


Figure 6b: no storage; higher magnification of extreme heat rigor in Fig. 6a. (550x)

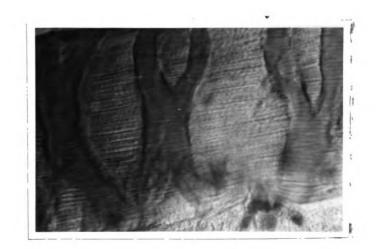
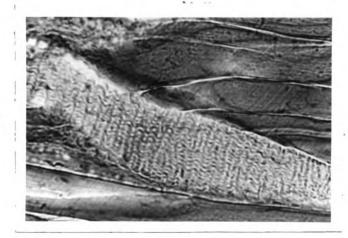


Figure 7. Changes during frozen storage of longissimus dorsi fibers (longitudinal sections of uncooked samples)

Figure 7a: 5 hours cold storage plus 4 months frozen storage; fibers wavy, well differentiated; shows contracture node and crinkles of rigor(125x)



Figure 7b: 24 hours cold storage plus 4 months frozen storage; thinning of fiber areas, longitudinal fracture in lower left-hand corner; tight z-z effect in connective tissue diagonally through center (125x)



167 hours cold storage plus 1 week frozen storage; fibers straight, breaks and disintegrated areas numberous; granulation within and between fibers (125x)

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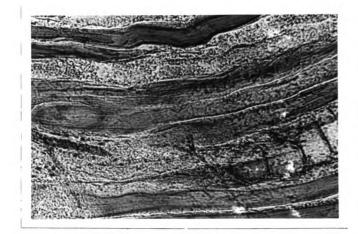


Figure 9. Changes during freezing storage of longissimus dorsi fibers. (longitudinal sections of cooked samples)

Figure 9a: 5 hours cold storage plus 4 months frozen storage; passing z-z effect; fractures and disintegrated areas in fibers (125x)



Figure 9b: 167 hours cold storage plus 1 week frozen storage; evidence of heavy granulation outside and within fibers. Fat cells over fibers in lower righthand corner(125x)



and disintegration within the muscle fibers were found.

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Paul stated that the breaks in the fibers might be the result of either a) autolysis by tissue enzymes, b) physical stress on the stretched areas between the contracture nodes, or c) connective tissue contraction.

SUMMARY AND CONCLUSIONS

The effects of cold storage and frozen storage on the palatability and histological appearance of longissimus dorsi muscle of beef were investigated. Each of four muscles from two animals was divided into 24 one-inch steaks and arranged in randomized right and left pairs. Steaks were aged 0, 5, 24 and 167 hours at 46° F. At the end of each aging period 4 steaks were deep-fat fried at 150°C. to an internal temperature of 63°C.; 8 steaks were frozen at -26°C., and 1) 4 stored for one week at 0°F., and 2) 4 stored for 4 months at 0°F. The cooked steaks were scored subjectively for appearance, aroma, juiciness, flavor of fat and lean, texture, tenderness and general conclusion. The pH of the raw, lean tissue and peroxide values and free fatty acids in the raw fat were determined. Histological sections of raw and cooked storage and frozen storage.

The palatability scores showed that appearance, aroma and juiciness were little affected by cold storage or frozen storage; flavor scores for fat and lean increased with frozen storage; texture, tenderness and general conclusion scores varied within each treatment and decreased in score with extended frozen storage. In comparison with cold storage, this study has shown a definite increase in tenderness at the 24-hour level in frozen storage, a slight increase in tenderness in frozen storage at the 167-hour period, and a decided decrease in tenderness at the 0 and 5-hour aging periods. Correlation between shear value and tenderness score was highly significant, and between shear value and texture score significant. The correlation coefficient between cooking time and percent cooking losses was highly aignificant. Percent cooking loss per minute cooking time was unaffected by cold storage or frozen storage. Peroxide and free fatty acid values increased slightly in frozen storage. The change in free fatty acids was most pronounced after four months frozen storage. The pH values of lean tissue showed increased acidity with cold storage and a slight decrease in acidity in four months frozen storage. The histological changes in muscle fibers for both storage treatments progressed from poor differentiation in fibers through rigor stages to fractures and disintegration. Freezing and cooking both appeared to intensify the changes found in cold storage. The most prominent manifestation in the cooked samples was evidence of extreme heat rigor in samples with little or no cold storage.

On the basis of these results it appears that:

- 1. The palatability factors of appearance, aroma, and juiciness were little affected by either cold storage or frozen storage; flavor of fat and lean, texture, tenderness, and general conclusion factors were more noticeably affected by cold storage.or frozen storage.
- 2. Tenderness scores in cold storage paralleled the onset, height and dissolution of rigor; by comparison with cold storage, tenderness in frozen storage decreased sharply in 0 and 5-hour aging periods, increased markedly at the 24-hour period, and showed slight tenderness increases at the 167-hour period.
- 3. There is an acidification of muscle in cold storage and a slight rise in alkalinity in extended frozen storage.
- 4. Peroxide development within the intervals studied was too small to indicate oxidative rancidity.

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- 5. Free fatty acids showed significant changes in cold storage and frozen storage that might indicate chemical decomposition.
- 6. The changes in tenderness were paralleled by histological appearance of rigor nodes in the least tender samples and by areas of disintegration in the most tender ones.

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AFPENDIX

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Neme		Meat S	Meat Score Card				+		
Factor									
Appearence									
Aroma									
Flavor: fat									1
lean									
Juiciness									
Tenderness			:						
Texture									
General Conclusion	:		: 						
Key: T. excellent 6. very good 5. good 4. medium 3. fair 2. poor 1. very poor			Α	Defects to consider: <u>Appearance</u> : po <u>Aroma</u> : an <u>Flavor</u> : sa <u>Texture</u> : co	isider: 	er: poor color, evidence of freezer burn (extreme drying of surface) any off aroma, foreign, rancid, decayed same as aroma coarsiness, stringiness	freezer tce) rencid,	burn (extı decayed	eme

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Table	

for Animal I and Animal II in cold storage

	Aged,	hours	Aged, hours Animal			1 1	Flavor				General	
Treatment at 46°F.	at 4	6°F	e Se	Appearance	Aroma	Fat	Lean	Juiciness	Tenderness		Texture Conclusion	Shear
Unfrozen	0	•	н	6.0	5.2	4.4	4.6	5•1	4.8	4.9	5.0	7.34
			ㅂ	5.4	5.1	4.5	4.7	4. 8	5.2	3 . 8	5. 6	14.65
2	Mean			5.7	5 . 2	4.5	4.7	5.0	4•0	4•4	4. 3	11-00
	ŝ		н	5.8	5.4	4.8	5.2	4.8	2.8	3.8	3. 8	10.18
		•	日	5.4	5.0	4.4	4.4	4.9	3.0		3.4	16 . ম
F	Mean			5.6	5.2	4.6	4.8	4.9	2°0	3 •6	3 •6	13.25
	24		н	5.9	4. 9	4.7	4.7		1.9		6 ° 2	14.29
			II	5.8	5.0	4.3	4.5	5.3			3 • 3	15.06
	Mean			5 . 9	5.0	4.5	4•6		2•2	3 . 3	3.1	14.68
	167	~	н	5.5 5	4.7		5.2	5.0	3.4		4.1	10.95
			H	5.7	6 •0	4. 8	5.1	5 . 5	4.8		5.1	6.79
4	Меяп			5.6	5.4		5.2	5.3	4.1		4.6	8•87

	Aged, hours	Animal			Flavor	VOF				General	
Treatment	Treatment at 46°F. No.	No.	Appearance	Aroma	Fat	at	Julciness	Tenderness	Texture	Conclusion	Shear
Frozen	0	н			4,5	4. 7					20.43
		Ħ	5.5		4.0	4.4	5.5	2.8			12.63
	Mean		5•5	5.4	4•5	4.6	4.9	2.8	4.2	3•5 5	16.56
	Ŋ	н	5.7		4.9		5.1	1.8	5.8	2.8	23.61
		Ħ	5.7	5•5	4. 8	4.5	5.6	3.7		4. 0	11.52
	Mean		5.7	5.7	4•9	4.7	5.4			3,4	17.57
	24	н	5.7	5. 8	4.8	5.0	4.5	5.1	5-9	5.4	15.88
		H		5.]	4.4	4.4	5.4	3.9	4.0	4. 4	11.89
	Mean		5.7	5.5	4.6	4.7	5.0	3.5	4•0	3 .9	13.89
	167	н	5.7		4.5	4.9	4.8	4.2	4.1	4.5	8.49
		Ħ	5.5	5.7	5.4	5.2	5.4	4.8	4.9	5.0	6.52
	Mean		5.6		5.0	5.1	5.1	4. 5	4.5	4.7	7.51

Summary of mean palatability scores and shear force for

Table 20

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Animal I and Animal II in frozen storage one week - 0°F.

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	Aged, hours Anim	Animel			F1.8	FLavor				General	
Treatment	46 ⁰ F.	No.	Appearance	Aroma	Fat	Lean	Juiciness	Lean Juiciness Tenderness	Texture	Texture Conclusion	n Shear
Frozen -	0	н	5.7	5. S	5.1	5.1	4.9	1.4		2,6	17.31
		ㅂ	5.7	5.4	5 . 5	5.0	5.2	3 •6		3.8	13.67
4 months) Mean	Mean		5.7	5.4	5.3	5.1	5.1	2.5		3•2	15.49
	ŝ	н		4.8	5,5	5.6	5°2		2.5	2.7	16,02
		日		5.4	5.0	5.2	4.9	5.2		5 •6	14.20
	Mean		5.7	5.1	5.2	5.4	5.1	2•3		3.2	15.11
	24	н	5.5		5.5	5.5	4.9	3.1		5 . 5	12.80
		Ħ	5.6		5.2	5.0	5.5	3 •1			9.52
	Mean		5.6	5,9	5 •3	5•5	5.1	3.1	5.4	3.5	11.16
	167	н	5.5	5.8	4.5	5.0	5.3	5.2	3.6	5.7	11.20
·	Veen	H	5. 7. 7.	6.0 8	5.4 4.9	ນ ຄູ	5 	4•7 4-0	4.5 4.1	4°7 4.2	7.18 9.19
					5	2				2	

Summary of mean palatability scores and shear force for

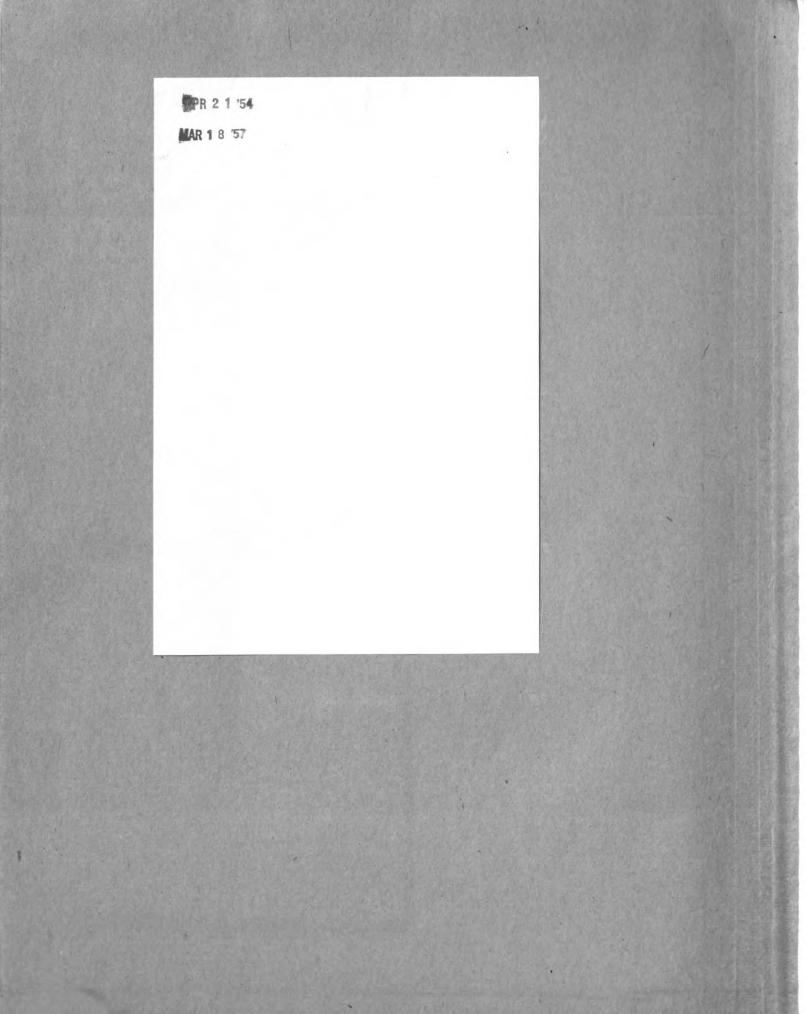
Animal I and Animal II in frozen storage four months - 0°F.

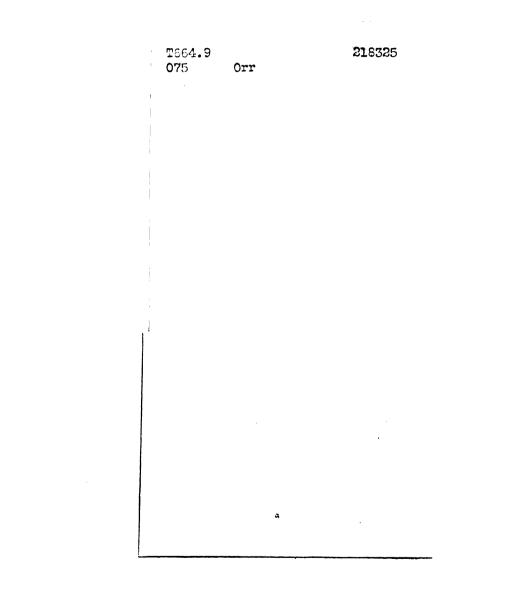
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