THE EFFECT OF AGING, THAWING AND FROZEN STORAGE ON THE TENDERNESS OF CHICKEN BROILER MUSCLE

by

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE in the Department of Food Science

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
May, 1974
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Date May 30th, 1974
ABSTRACT

The effects of various aging, thawing and storage methods on the tenderness of frozen broiler Pectoralis major muscle were studied.

Initial experiments were carried out to establish standard methods of freezing, cooking and tenderness evaluation to be used in subsequent experiments. The effects of varying the aging, thawing and storage techniques were then investigated using the established methods.

Whole carcasses were frozen in a liquid nitrogen blast freezer after cooling in ice water for periods of 1 to 10 hours after slaughter, stored for 1 week at -31°C and thawed for varying lengths of time. The P. major muscles were removed and cooked in boiling water between metal plates. Tenderness evaluations were carried out using the Allo-Kramer shear press.

The length of thawing time was shown to greatly influence the degree of toughness of the cooked muscles. When a thawing period of 4 hours in water at 25°C was used, a decrease in toughness took place in carcasses frozen between 1 and 2 hours post-mortem. This was followed by an increase to maximum toughness in birds frozen between 4 and 8 hours post-mortem. Maximum tenderness occurred in birds frozen 10 hours after death.
Thawing birds in air at 4°C for 24 and 48 hours decreased the level of toughness attained after freezing 4 to 8 hours post-mortem. It did not significantly alter the degree of tenderness reached after 10 hours. Similarly, the decrease in toughness in birds frozen between 1 and 2 hours post-mortem, remained significant.

Longer storage (3 months) at -23°C followed by rapid thawing eliminated both the decline in toughness of carcasses frozen between 1 and 2 hours post-mortem and the maximum toughness level attained by carcasses frozen 4 to 8 hours after death.

An attempt was made to explain the decrease in toughness in carcasses frozen between 1 and 2 hours post-mortem in terms of the aging temperature and medium used prior to freezing. No difference in the pattern was observed, however, when other pre-freezing aging techniques were used.

Increases in the sarcomere lengths of muscle frozen at 2 hours post-mortem were observed, corresponding to the increase in tenderness occurring in carcasses frozen at this time. Isometric tension measurements, however, did not correlate well with these observations.

Taste panel members were unable to discern differences in the tenderness of muscle frozen between 1 and 3 hours post-mortem although excellent correlations were
obtained between Allo-Kramer shear press values and sarco-
mere length measurements.

The results of these experiments therefore show that the ultimate tenderness of broiler muscle can be greatly influenced by the interaction of pre-freezing aging time, length of storage and thawing techniques used prior to cooking.
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ACKNOWLEDGEMENTS

The author wishes to express her sincere appreciation to her advisor, Dr. J.F. Richards, Associate Professor, Department of Food Science for his guidance and encouragement during the course of this study.

She is also thankful to the members of her graduate committee: Drs. W.D. Powrie, M.A. Tung and J. Vanderstoep of the Department of Food Science and Professor E. Watson of the Department of Agricultural Engineering for their help, encouragement and interest in the research and for the review of this thesis.

She is also grateful to Miss Lynne Robinson for taste-panel and computer assistance and to her long-suffering taste panel members.

Financial support from the B.C.D.A. Agricultural Sciences Research and Development Fund is gratefully acknowledged.
CHAPTER I. SURVEY OF LITERATURE

Muscle Tenderness

Optimum tenderness, together with optimum flavour, is fundamentally important in the acceptance of all types of meat.

Tenderness is an extremely variable characteristic. It varies from muscle to muscle within an animal and among animals of the same or different species. These variations can be greatly influenced by both ante- and post-mortem events. Reviews of such factors influencing tenderness are given by Briskey (1963) and Marion (1967).

Koonz et al. (1954), deFremery and Pool (1960), and Locker (1960) recognized that the period of time between slaughter and rigor onset, when shortening and loss of extensibility of the muscle occurs, influenced the ultimate tenderness of the final product. The post-rigor resolution of these parameters was also found to be important (Goll, 1968).

It has been recognized that modern processing and utilization practices may influence the tenderness of the final product particularly processing techniques that take place during the period of time between slaughter and the resolution of rigor mortis (Pool et al., 1959; Klose et al., 1971).
Rigor and its Resolution

When an animal is slaughtered, blood circulation ceases thereby terminating the transport of oxygen to the muscle. With the onset of anaerobic conditions in post-mortem muscle, numerous chemical changes occur such as a decrease in ATP concentration, a reduction in pH and the formation of numerous bonds between the actin and the myosin filaments, (Bendall, 1969; deFremery, 1966; Lawrie, 1966).

Such changes lead to alterations in the muscle microstructure and a decrease in the extensibility of the muscle during the first few hours after death. Rigor mortis is the inextensible or rigid state of post-mortem muscle.

Several investigations have shown that muscles shorten during the onset of rigor and, as a consequence, become tougher, (Bate-Smith et al., 1949; Marsh, 1954; Pool, 1963). Newbold and Harris (1972) have extensively reviewed the aspects of pre-rigor shortening.

The extent of shortening is dependent on the storage temperature and the extent of muscle restraint, if any. Smith et al. (1969) showed that shortening of avian muscle stored at 0°C was significantly greater than that which occurred when the muscle was stored at 12-18°C. The amount of shortening was greatest in muscle stored at 20°C.

Jungk and Marion (1970) demonstrated that 'cold
shortening' (shortening induced by allowing glycolysis to proceed rapidly around 0°C) does not occur in turkey breast muscle. However, in 1971, Marion reported 'cold shortening' in turkey thigh muscle. Marsh and Thompson (1958), Locker and Hagyard (1963), and Marsh and Leet (1966) reported that the extent of 'cold shortening' decreased as the period between slaughter and exposure to cold increased.

Muscle shortening has been related to a decrease in sarcomere length (Stromer and Goll, 1968; Buck et al. 1970). Klose et al. (1970) studied the effect of pre-rigor contraction on the tenderness of post-mortem chicken muscle. Electrical stimulation, beating, freeze-thawing and heating all reduced muscle length, in most cases, to between 40 and 50% of the original rest length. Subsequent shear values for the contracted cooked muscle were found to be around half those for the uncontracted controls. The authors suggested that such an extreme contraction caused changes in the sarcomere length which resulted in the myofibrils being more susceptible to shearing stress.

As storage time progresses, at above freezing temperatures the rigid in-rigor muscle reverts to a pliable post-rigor material. This process of post-rigor tenderization in muscle involves a 'resolution' of rigor mortis. Evidence relating to rigor resolution has been reviewed by Goll (1968). The results of Koonz et al. (1954), deFremery and Pool (1960), deFremery (1966), and deFremery
and Streeter (1969), indicate that chicken muscle held at 2°C reaches maximum toughness 3 to 4 hours post-mortem followed by minimum toughness after 12-24 hours. It has also been reported by Gothaard et al. (1966), Stromer and Goll (1967, and Takahashi et al. (1967) that sarcomeres which have undergone extensive post-mortem shortening will, after several days, lengthen again.

Possible explanations for the observed resolution of rigor were given by Goll et al. (1970). The authors postulated that the post-rigor relaxation of sarcomeres occurs through weakened actin-myosin interactions and Z-line degeneration. The gross structural changes occurring in Z-line degradation were explained as a consequence of Ca^{++} release from the sarcoplasmic reticulum after death. Henderson et al. (1970) agreed with these observations and showed that the Z-line of bovine, porcine and rabbit muscle lost its integrity during post-mortem storage. The weakening of the actin-myosin interaction, first observed by Fujimaki et al. (1965) may be effected by a very specific and limited proteolysis of myosin, actin and/or one of the regulatory proteins, (Goll, 1968).

**Thaw Rigor**

During rapid freezing and thawing of pre-rigor muscles, glycolysis occurs at a rapid rate at temperatures around -2°C to -4°C, shortening is severe and the water
holding capacity is reduced substantially (deFremery, 1966; Marsh et al. 1968). This behaviour is known as thaw rigor. Thaw rigor differs from normal rigor in that its time of onset depends only on the rate of thawing. It is always characterized by a more or less powerful contracture, the effect of which is directly related to the ATP content of the muscle. For this reason, muscle frozen pre-rigor contracts to a greater extent on thawing than those which have been frozen with an already depleted ATP level, although limited thaw contracture can occur even at very low levels -- for example, when muscle is frozen after the resolution of normal rigor. When muscle is frozen on the bone, however, the rigid structure prevents a large proportion of the shortening from taking place (Bendall, 1960).

Slow freezing or slow thawing may partially overcome the drastic shortening effect, since the muscle may remain partially frozen for extended periods at temperatures just below its freezing point. Marsh et al. (1968), Bendall, (1960), Jones and Murray (1961) and Behnke et al. (1973) found that at these temperatures, glycolysis occurs at an extremely rapid rate but shortening cannot occur because the muscle is fixed in size and shape by the presence of ice crystals.

Processing and its Effect on Muscle Tenderness

Post-rigor tenderization can occur before freezing or after thawing. The processor can most influence the
tenderness of cooked poultry muscle during the aging period prior to freezing. However, the freezing rate, the length and temperature of frozen storage and the thawing rate may also influence the tenderness of the final product.

Aging

The red and white muscle of poultry require different aging periods to allow them to pass through rigor prior to freezing (van den Berg et al., 1964). This should be taken into account when considering adequate aging times for poultry.

a) Time of Aging

As early as 1948, Lowe demonstrated that chickens (roasters) frozen within 2 hours of killing were less tender than those aged 24 hours before freezing. Stewart et al. (1948) in a study of tenderization of New York-dressed fryers at 35°F found that tenderness developed rapidly after the passing of rigor. Maximum tenderness was found within 24 hours with most of the tenderization taking place within the first 8 hours. Hanson et al. (1942) reported that tenderness in New York-dressed chicken broilers increased rapidly during the first 3 hours of holding at 35°F. Holding beyond this time resulted in continued, gradual, relatively small and diminishing increases in tenderness.

In experiments with frozen broilers Stewart et al. (1945) found no difference in tenderness between groups given pre-freezing chilling periods of 2 hours and 18 hours
respectively, but a 48 hour thawing period prior to cooking may have eliminated any difference that was originally present. Carlin et al. (1949) studied the extent of tenderization in roasters and fowl as a function of hours of holding at refrigerated temperatures and the additional effect of freezing and thawing. For birds aged less than 6 hours, freezing resulted in increased tenderness although it was clearly recognized by Carlin that this may have been due wholly, or in part, to the thawing and additional aging at thawing temperatures (24 hours at 39°F) rather than the freezing itself. Aging, freezing and other processing factors were evaluated by Koonz et al. (1954) for their effect on the tenderness of the principal muscles in the chicken broiler carcass. Ultimate tenderness was reached within 16 to 24 hours of aging at 40°F. Freezing and holding at 20°F for 24 hours essentially arrested any tenderization that would otherwise have occurred during that period. The authors concluded that freezing during the early post-mortem period fixed the state of tenderness existing at that time and complete tenderization was delayed until the tissues were defrosted.

Dawson et al. (1956) reported progressive increases in the tenderization of fryers cooked from the frozen state, as the interval between slaughter and freezing was increased from 40 minutes to 3, 6 and 24 hours respectively.
b) **Temperature of Aging**

Aging temperature in the range from 0°C to average room temperature was shown by Lowe (1948) to have little effect on the rate of post-mortem changes. In fowl cooked 6 hours after slaughter there was no difference in shear force or palatability between those chilled in ice and those held for 6 hours at room temperature.

Pool et al. (1959) found that tenderization of chicken broiler muscle took place within 4 hours at chill temperatures. Very little tenderization took place after 12 hours. No appreciable tenderization occurred at 0°F over a 4 month period, but significant increases in tenderness occurred in frozen carcasses held at 25°C to 27°F for several days. Tenderization arrested by freezing proceeded at a normal rate on thawing. The authors recommended an adequate aging period which potentially could be imposed either in the chilling, frozen storage or thawing periods.

Klose et al. (1961) found that a slow tenderization occurred at 25°C to 27°F when only 70% of the water contained in the muscle was frozen out. All reactions were found by the authors to be arrested at 0°F.

Marion and Goodman (1967) and Welbourne et al. (1968) studied the effects of aging time and chilling treatments on the tenderness of turkey muscle. From results obtained, the authors suggested that if post-mortem
tenderization was not complete prior to freezing, additional increases in tenderness could occur in large turkeys during freezing and thawing.

**Freezing Rate**

Attempts to determine the effect of freezing rate on the tenderness of frozen-thawed muscle have not resulted in complete agreement owing to the fact that other variables such as prefreezing history, depth of freezing and length of storage often influence results.

Dubois et al. (1940) found that beef and poultry muscles frozen in moving air at -40°F were more tender than samples frozen in moving air at -18°F and 23°F. However, Stewart et al. (1945) were unable to detect a significant difference in the palatability of broiler muscle frozen in rapidly moving air at either -68°F or -46°F as compared to broiler muscle frozen in slowly moving air at -21°F. Similarly, Marion and Stadelman (1958) and Miller and May (1965) found no significant difference in the tenderness of poultry muscle frozen at 0°F, -30°F or -90°F.

Barrie et al. (1964), Pickett and Miller (1967) and Streeter and Spencer (1973) compared 'cryogenic' and conventional methods of freezing poultry. No detectable differences in tenderness were found by any of the authors despite the difference in freezing rates.
Thawing Rate

Pool et al. (1959) showed that tenderization arrested by freezing proceeded at about a normal rate upon thawing.

Many methods of thawing have been reported. Klose et al. (1961) showed that wide differences in the rate of thawing of turkeys frozen pre-rigor had no adverse effect on the ultimate tenderness achieved by aging the birds after thawing. A significant difference in tenderness was found, however, between turkeys cooked after thawing and those cooked while still frozen. The authors suggested that holding frozen turkeys for a short period in the intermediate thawing range (20 to 30°F) offered promise of providing tenderness in birds neither chilled long enough nor held long enough in the thawed condition to provide the desired degree of tenderness.

Carlin et al. (1949) recognized that using a thawing temperature of 4°C for 24 hours may have increased the tenderness of chicken broilers frozen 6 hours post-mortem by increasing the aging period upon thawing.

Brodine and Carlin (1968) found no significant difference in the tenderness of turkeys thawed for 4 days at refrigerator temperatures and those thawed for 10 hours in running water. Hoffert et al. (1952) found no difference in the tenderness of broilers thawed in a refrigerator at
5°C, in air at 27°C, in water at 24°C or in an oven at 150°C. Korslund and Essary (1971), however, found that turkeys thawed by immersion in cold water were significantly more tender than those thawed in warm tap water, in a low temperature oven, in a refrigerator or at room temperature. **Temperature of Frozen Storage**

The temperature of frozen storage is a major factor influencing the rate of textural deterioration. As the temperature of storage is lowered, the extent of tenderness loss (when it occurs) is decreased. The textural stability of frozen chicken muscle was found by Miller and May (1965) to be greater at -37°C than at either -28°C or -32°C over a storage period of 6 months. Other workers (Wills et al., 1948 and Klose et al., 1950) have observed that chicken and turkey muscles became less tough as the temperature of frozen storage was lowered to -5°F. **Length of Frozen Storage**

Studies of the effects of varying lengths of frozen storage on the tenderness of muscle, have led to conflicting results. Some investigators have reported that post-rigor muscle of poultry, beef, lamb and fish decrease in tenderness during frozen storage, (Guerrant et al., 1953).

Miller and May (1965) in their study on chicken muscle, found that tenderness generally decreased with
increased time of storage at \(-28^\circ\text{C}\) or \(-32^\circ\text{C}\). Although the difference in tenderness of chicken muscles stored for 1 week and 1 month was not significant, both of these muscles (stored at \(-28^\circ\text{C}\)) were somewhat more tender than those stored at the same temperature for either 3 or 6 months.

Stewart et al. (1945) stored broilers at \(-23^\circ\text{C}\) for periods of time up to 79 days. Using taste panel assessment of texture, he found that the acceptability level decreased as storage time increased.

The large variation in the results obtained from studies of the effect of various aging, freezing and thawing techniques on the tenderness of the final cooked product prompted the research presented in this thesis.

Methods of Measuring Muscle Tenderness

Objective and Subjective Methods

There are a large number of procedures used to measure meat tenderness. Marion (1967) recognized the need to standardize mechanical and sensory methodology so that comparisons of data obtained by different techniques can be made.

Relationships between objective and subjective measurements of meat tenderness have been widely studied, (Deatherage and Garnatz, 1952; Klose et al., 1961; White et al., 1964; Pangborn et al., 1965; Sharrah et al., 1965 a,b; Pool and Klose, 1969; Szczesniak et al., 1970; Larmond and Petrasovits, 1972). Bouton and Harris (1972)
have given a comprehensive comparison of some of the objective methods used to measure meat tenderness.

Most of the objective methods of measuring tenderness use some form of shearing device and record the force required to shear or compress a given amount of sample. Objections to such techniques have been put forward by several authors (Sharrah et al., 1965b; Pool and Klose, 1969; Szczesniak et al., 1970).

Many other methods of measuring meat tenderness have been used such as measurements of the tensile strength of muscle fibres used by Nakamura (1972) and measurement of the electrical impedance of poultry muscle carried out by Zachariah et al. (1971).

Stanley et al. (1972) concluded from their results that there are two major structural contributions of raw muscle to cooked meat tenderness -- a connective tissue factor and a contraction factor -- and that different objective methods are best suited for their evaluation.

Measurement of Sarcomere Length.

Since the report by Locker (1960) that tenderness in beef is influenced by the degree of muscular contraction in the post-mortem muscle, a number of workers have investigated the correlation between sarcomere length, as a measure of the contractile state, and tenderness. The influence of contractile state on texture is demonstrated
in the phenomenon of 'cold shortening' (Locker and Hagyard, 1963; Marsh and Leet, 1966), and in the effect of the configuration of the carcass during rigor mortis on a number of muscles, (Herring et al. 1965b Hostetler et al. 1970).

Many methods have been developed for measuring sarcomere length and these are excellently reviewed in The Meat Research Institute, (England) Bulletin 546 (1972). A method currently in use at the Meat Research Institute (Voyle, 1971) derives initially from observations made in 1874 that a straited muscle acts as a transmission grating when placed in a beam of light. Diffraction patterns are formed on a screen, the separation of the lateral orders being determined by the contractile state of the muscle. A recent development in this technique is the use of a gas laser as a source of coherent, monochromatic light. This has been described by Rome (1967) and Cleworth and Edman (1969).

Isometric Tension Measurement

The first studies in the use of isometric tension measurements were reported by Busch et al. (1967) and Jungk et al. (1967). Before this time, extensibility measurements were used in order to quantitatively follow rigor mortis.

Busch et al. (1972) outlined the advantages of isometric tension measurements over extensibility measurements, the most important of which was the ability to detect
changes which correspond to both onset and resolution of rigor. Busch et al. (1972a) recommended some improvements in the procedure for measuring post-mortem isometric tension.

Jungk and Marion (1970) demonstrated isometric tension development and decline in turkey muscle and established a linear relationship between temperature and tension development in breast muscle.
INTRODUCTION

The effect of freezing on poultry tenderness has been widely studied. Various authors have shown that the tenderness of chicken broiler muscle is dependent on the length of aging time prior to freezing but reported results vary considerably and, in many instances, are difficult to interpret.

With young chickens aged in ice slush, most of the tenderizing effect (determined from the cooked flesh) was found to occur within 5 hours (Hanson et al., 1942; Koonz et al., 1954; Dodge and Stadelman, 1959; Pool et al., 1959.) In a study by Palmer et al. (1965) untrained judges frequently detected undesirable toughness in cooked chicken fryers when a chilling-aging time of less than 4 hours was used prior to freezing.

Stewart et al. (1945) froze birds at 2 and 18 hours postmortem and found no differences in the tenderness of birds subjected to the two treatments. Any differences may have been obviated, however, since a 48 hour thawing time was used prior to cooking and subsequent tenderness evaluation. Carlin et al. (1949) found that for birds
aged less than 6 hours, freezing enhanced the tenderness, although, again, results may have been influenced by a 24 hour aging period upon thawing. Shrimpton (1960) studied 11-week old chickens weighing 3 - 4 lb each and found that chilling-aging times ranging from 0 to 24 hours had no important influence on the tenderness of the cooked product.

deFremery and Pool (1960) reported that freezing and thawing of muscle before the normal onset of rigor induced a very rapid "thaw-rigor" which effected a significant increase in the toughness of the cooked meat. Muscles which had passed through rigor prior to freezing showed no corresponding increase in toughness. The authors concluded that the toughening observed was due to the accelerated onset of rigor upon thawing.

Reasons for the variations and the difficulty of interpretation of published results could be the lack of standardized methods for aging, freezing, thawing, cooking and tenderness evaluation. In view of the diversity found in the published results, it was decided to re-study the effect of various pre-freezing aging times on the ultimate tenderness of chicken broiler muscle. Experiments were also carried out to illustrate the fact that variations in the methods used for aging, thawing and storage time could significantly alter the results obtained.
This chapter therefore describes the establishment of standard methods of freezing, cooking and tenderness evaluation used throughout this thesis.
MATERIALS AND METHODS

Experiment 1 - A Comparison of Subjective and Objective Methods of Tenderness Evaluation.

Muscle Source

The broilers used throughout these studies were obtained from a local processing plant. The birds had been killed by normal packing house procedures and were removed from the processing line before they reached the chiller at approximately 20 minutes postmortem. The carcasses were packed in ice and transported to the University where they were kept in ice water at approximately 0°C until used for freezing. All birds used were commercial broilers and were approximately 8 weeks old at the time of slaughter.

Method of Freezing and Muscle Preparation.

Three groups of 8 carcasses each were separately packed and sealed in polyethylene bags and frozen in an air blast at -31°C. Within each group of 8, two birds were frozen at 1, 3, 6 and 10 hours postmortem, respectively.

After storage for one week at -31°C, the carcasses were thawed for 4 hours in running water at 25°C. The temperature of the water was checked every 30 minutes throughout the thawing procedure.

The P. major muscles were then excised and clamped between two aluminum plates spaced 0.7 cm apart and cooked for 10 minutes in boiling water (deFremery and Pool, 1960).
When cooking was completed, samples were cooled in running tap water for 5 minutes. After removal from the plates, the muscle samples were individually wrapped in Saran wrap until needed.

**Subjective Method of Tenderness Evaluation.**

A five member sensory panel was used. Tenderness was evaluated for each treatment using rank analysis.

Sensory analysis was carried out in a taste panel room seating five people and equipped with individual cubicles. Samples were presented in red light from a 20 W fluorescent tube above each cubicle. Trials were held around 10:30 a.m. and 2:30 p.m. on three days.

For each judge at each panel sitting six small containers were coded with 3-digit random numbers. Three pieces of cooked *P. major* muscle obtained from each of the four treatment groups were coded and placed in the containers. Thus, the six requisite pairs were formed for treatment comparison; i.e., 1:3, 1:6, 1:10, 3:6, 3:10 and 6:10. Samples presented were approximately 2.5 cm² in size and were cut from the same area of each *P. major* muscle. All samples were served at room temperature.

Code numbers were recorded and the pairs were presented to the judges in a random order, together with score sheets. Judges evaluated each sample for tenderness and, for each pair presented, recorded a score of 1 for the
tougher sample and 2 for the more tender sample. Space was provided on the score sheet for written comments.

Thus six panel sessions were conducted for the twenty-four broilers used. The treatment sums of ranks were obtained for all the panel sessions.

Objective Method of Tenderness Evaluation

Measurements of shear were carried out using the Allo-Kramer shear press. Strips of parallel fibres, 1.5 cm wide were cut from samples of the P. major muscle which remained after the panel samples had been removed. A minimum of 8 shear measurements per bird was obtained using a single blade shear cell, 250 lb ring and 9 cm/min cross-head speed. In all cases, attenuation was set at the 10 per cent level.

Experiment 2 - Liquid Nitrogen Blast Freezing

Tests were carried out, using thermocouples, to find the length of time required to freeze whole chicken broiler carcasses to an internal temperature of approximately -60°C in a liquid nitrogen blast freezer.

The blast freezer consisted of an insulated cabinet, divided into four compartments, each compartment separated from the other three by wire mesh. A whole carcass was placed into each of the four sections. A copper constantan thermocouple was inserted into the breast muscle of each
bird at approximately the same position, so that it penetrated to a distance of 0.5 cm from the sternum at the anterior end of the carcass.

A telethermometer was used to record the temperature of the inside of the freezing cabinet. The cabinet was then closed, and liquid nitrogen, under pressure was pumped in via insulated copper tubing. A fan situated in the base of the cabinet caused a rapid flow of nitrogen to circulate throughout the four cabinets.

Temperature readings of the breast muscle of each bird and the inside of the cabinet were taken at regular intervals. The entire system used in this and subsequent studies is shown in Plate I.
Plate I. Apparatus used for liquid nitrogen blast freezing.
RESULTS AND DISCUSSION

Experiment I

Analyses of variance were carried out on the data obtained from the subjective and objective methods of tenderness evaluation. Results of these analyses are shown in Table 1.

TABLE 1. ANALYSES OF VARIANCE FOR SUBJECTIVE AND OBJECTIVE TENDERNESS EVALUATION.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean Squares (Subj.)</th>
<th>Mean Squares (Obj.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>3</td>
<td>1.97***</td>
<td>150.86*</td>
</tr>
<tr>
<td>Error</td>
<td>20</td>
<td>0.24</td>
<td>41.36</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*  p < 0.05
*** p < 0.001

From this table it is seen that in both cases there is a significant treatment effect. This is particularly so in the subjective evaluations.

The treatment means and the results of Duncan's New Multiple Range Tests are shown in Table II. Subjective evaluation means are expressed in terms of panel tenderness scores and have no absolute units; objective evaluation means
are expressed as Allo-Kramer shear press recorder values obtained when the instrument was fitted with a 250 lb ring and attenuation was set at the 10 per cent level. In this case, each unit is equivalent to pounds of force multiplied by a factor of 4.

TABLE II. DUNCAN'S NEW MULTIPLE RANGE TEST ON SIGNIFICANT TREATMENT MEANS FROM TENDERNESS EVALUATIONS – EXPERIMENT 1.

<table>
<thead>
<tr>
<th>Time of Freezing Postmortem, hours</th>
<th>1</th>
<th>3</th>
<th>6</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panel Scores</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Shear Value (lbs x 4)</td>
<td>24.48&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>29.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.62&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Means in the same row with similar superscripts do not differ significantly (p < 0.05).

The panel and shear data both indicate that an increase in toughness occurs in birds frozen between 1 and 6 hours postmortem. In both cases, maximum tenderness is shown to occur in birds frozen 10 hours after death although the difference in the tenderness of birds frozen at 1 and at 10 hours postmortem is not significant.

Both subjective and objective methods of tenderness
evaluation, therefore, show that in this experiment, the length of pre-freezing aging time has an effect on the ultimate tenderness of broiler muscle. These results are in agreement with those of Palmer et al. (1965) and deFremery and Pool (1960). It is probable that the birds used in this experiment, when frozen at 1, 3 and, in some cases, 6 hours postmortem, were either frozen prior to, or during, the normal onset of rigor such that a very rapid "thaw rigor", effecting a significant increase in the toughness of the cooked meat, was induced during rapid thawing. Birds frozen at 10 hours postmortem (and, in certain instances, 6 hours postmortem) had previously passed through rigor and therefore showed no corresponding increase in toughness.

Figures 1 and 2 illustrate these effects. The standard deviations of the shear values and panel scores are shown in these figures and illustrate a relatively large variation in the results obtained for birds frozen at each postmortem time. Variation in results is particularly noticeable for birds frozen after 3 or 6 hours of aging. Several factors may be the cause of this variation. Apart from physiological variation among the birds used, one cause could be the relatively slow method of freezing used in this experiment.
Figure 1. Subjective assessment of toughness of broiler muscle frozen after various postmortem aging periods.
Figure 2. Objective assessment of toughness of broiler muscle frozen after various postmortem aging periods.
Stewart et al. (1945) were unable to detect a significant difference in the palatability of broiler muscle frozen in rapidly moving air at either -55.5° or -43°C as compared to broiler muscle frozen in slow moving air at 29.5°C. Similar results were obtained by Marion and Stadelman (1958), and Miller and May, (1965). However, in all these examples, poultry were frozen after aging periods sufficient to ensure that the muscles had passed through rigor mortis and were therefore no longer subject to the toughening effects of 'thaw rigor'.

In this experiment, birds frozen after 1, 3 and occasionally, 6 hours of aging were approaching the state of rigor at the time of entering the freezer. The rate of glycolysis has been shown to gradually increase in chicken P. major muscle as the temperature is lowered from 0° to -3° or -4°C, and to decrease sharply from -4° to -10°C (Behnke et al. 1973). The rate increase was thought by the authors to be due to a freeze-concentration effect. Air blast freezing at -31°C is a relatively slow method of freezing whole broiler carcasses and may have led to a large variation in the extent of postmortem glycolysis throughout the muscle tissue of birds in the pre-rigor and rigor state. Both the position of the bird in the air blast and the relatively large mass of the carcass may have caused part
of the muscle tissue to remain unfrozen or partially frozen for extended periods at temperatures just below the freezing point, where glycolysis can occur at a rapid rate. Thus, while the surface muscle layer became frozen and thus 'fixed' in its postmortem state, glycolysis and the development of rigor may have continued at a rapid rate in the inner tissues. Such a variation in the postmortem state of the muscle tissue could have affected the tenderness of the cooked product and thus may be a possible reason for the large variation found in the results of the tenderness analyses.

A simple linear correlation analysis was performed on the data from the subjective and objective tenderness tests. The correlation matrix is presented in Table III. It can be seen that there are significant correlations between the two methods of tenderness evaluation particularly when birds frozen after 1 and 10 hours of aging are evaluated.

<table>
<thead>
<tr>
<th>Time of Freezing Postmortem (hours)</th>
<th>Overall Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Subjective</td>
<td>.87***</td>
</tr>
<tr>
<td>Objective</td>
<td></td>
</tr>
</tbody>
</table>

* p = < .1  ** p = < .05  *** p = < .02  **** p = < .01
Relationships between objective and subjective measurements of meat tenderness have been widely studied by many authors. Stanley et al. (1972), did not obtain highly significant correlations between panel evaluations of tenderness and shear values in their studies of porcine muscle. Similarly objections to the use of the Allo-Kramer shear press as an adequate means of determining meat tenderness, have been raised by Sharrah et al. (1965b), Pool and Klose, (1969) and Szczesniak et al. (1970). The basis of the objections involves an exact definition of the qualities that the Allo-Kramer shear press measures. Subjective assessment of tenderness involves cutting, shearing, tearing, grinding and squeezing. Compression, tension and shearing stresses are simulated by this instrument. In addition, certain experimental variables must also be controlled in order to obtain reliable results from the Allo-Kramer shear press.

However, in view of the significant and relatively strong correlations between the subjective and objective methods of tenderness evaluation used in this experiment, and the obvious treatment effect shown by both methods, it was concluded that either method of tenderness assessment could be used in future experiments of this type.
Experiment 2

As previously mentioned, it was thought that the slow rate of freezing of whole broiler carcasses used in Experiment 1 may have partially caused the relatively large variation in the tenderness values obtained for birds frozen prior to or during the onset of rigor.

In an attempt to discount any possible variation in tenderness throughout the *P. major* muscle caused by a slow freezing method, liquid nitrogen blast freezing was tested as a possible method for rapidly freezing the whole carcass, and thus rapidly 'fixing' the postmortem state of the muscle.

Results of this experiment showed that the internal temperature of the *P. major* muscle on the broiler carcass could be lowered to -60°C - a temperature far below that at which any glycolytic changes can occur in the muscle – within 20 minutes. Very little variation was found in the rate of freezing of carcasses placed in each of the four compartments inside the freezer cabinet. This is shown in Figure 3.

It was decided to continue using this method of rapid freezing in the following experiments.
Figure 3. Temperature history of P. major muscle in a liquid nitrogen blast freezer.
CHAPTER III. THE TENDERNESS OF CHICKEN BROILER MUSCLE FROZEN AT VARIOUS POSTMORTEM AGING TIMES - THE EFFECT OF VARYING AGING, THAWING AND FROZEN STORAGE TECHNIQUES.

INTRODUCTION

As previously stated, possible reasons for the variations and the difficulty of interpretation of many of the published results pertaining to the quality of frozen poultry, could be the lack of standardized methods of aging, freezing, thawing, cooking and tenderness evaluation.

Experiment 1 has shown that freezing after various postmortem aging times up to 10 hours after death, has the effect of causing a detectable increase in toughness up to 6 hours postmortem when the previously described methods of freezing, thawing, cooking and tenderness evaluation are used.

A difference in the rate of thawing of the carcass may have a significant effect on the ultimate tenderness of the cooked muscle.

Pool et al. (1959), have shown that tenderization of poultry muscle, arrested by freezing will proceed at a normal rate on thawing. Thus it is conceivable that a long thawing time at low temperatures will have a tenderizing effect on muscle frozen pre-rigor. Postmortem glycolysis proceeds rapidly at temperatures just below the freezing
point (Behnke et al., 1973) and it is therefore possible that holding carcasses in this intermediate thawing range during slow thawing, could greatly increase the ultimate tenderness of the muscle. This fact was recognized by Carlin et al. (1949), who concluded that a thawing temperature of 40°C for 24 hours may have increased the tenderness of chicken muscle frozen 6 hours postmortem. Similar results were reported by Klose et al. (1961).

The method of thawing used in Experiment 1 was rapid, such that the carcass passed through the intermediate thawing range - the temperature range at which postmortem glycolysis is most rapid - in a very short time. The four hour thaw was not sufficient for significant tenderization of the pre-rigor frozen muscle to occur.

The next three experiments therefore describe variations in the thawing method used for poultry frozen after various postmortem aging times, in an attempt to determine an adequate thawing time for pre-rigor frozen muscle.

The temperature of pre-freezing aging has also been suggested to be a possible factor influencing the rate of onset of rigor.

Lowe (1948), found that the temperature of aging in the range from 0°C to average room temperature had little effect on the rate of postmortem changes in fowl.
Conversely, Dodge and Stadelman (1959), showed that both the temperature and the media of aging appeared to affect the pattern of rigor and the level of tenderness at a given time postmortem. Water or ice slush was shown by the authors to be the best medium for aging. Aging in air led to some dehydration and consequent toughening of the muscle tissue. More tenderization of muscle occurred in water at 0° and 12.8°C than in water at 22°C or in air at 3.9° or 12.8°C. Marsh and Leet (1966), also showed that the ultimate tenderness of meat is affected by the temperature during the first few hours postmortem.

Experiment 6 describes a comparative study of two methods of pre-freezing aging to find the effect, if any, of the temperature and media of aging on the ultimate tenderness of broiler muscle.

Studies of the effect of frozen storage on tenderness of poultry muscle have also led to conflicting results. Miller and May (1965), in their study on chicken muscle found that tenderness generally decreased with increasing time of storage. These authors also found that the textural stability of frozen chicken muscle was greater at -35°F than at either -18° or -26°F over a storage period of 6 months. Klose et al. (1950), observed that turkey muscle became less tough as the temperature of frozen storage was lowered to -23°F. Stewart et al. (1945) stored broilers at
-10°F for periods of up to 79 days after which time, the authors found that the texture of the birds had deteriorated.

The effect of temperature and length of frozen storage on the rate and pattern of postmortem glycolysis has not been studied. Muscle frozen pre-rigor may undergo a small amount of glycolysis during long term frozen storage such that the ultimate tenderness of the cooked product is greater than that of the same pre-rigor frozen muscle that has not been stored. Experiment 7 describes a study of the effects of long term storage on the tenderness of broiler muscle frozen pre-rigor.
MATERIALS AND METHODS

Experiment 3 - The Effect of Rapid Thaw on the Tenderness of Chicken Broiler Muscle Frozen at Various Postmortem Aging Times.

Whole carcasses were frozen in a liquid nitrogen blast freezer for 20 minutes (by the method previously described) after aging in ice water for 1, 2, 3, 4, 6, 8 and 10 hours. In each trial, 4 carcasses were frozen for each aging time.

After freezing the carcasses were individually wrapped in polyethylene bags and stored at -31°C. After one week, the carcasses were thawed in running water at 25°C for 4 hours. The P. major muscles were excised and cooked by the method described in Experiment 1. Tenderness was evaluated using the Allo-Kramer shear press. Nine measurements on strips of parallel fibres 1.5 cm wide, were made from each P. major muscle, giving 18 measurements for each carcass used.

This experiment was repeated three times so that, in total, 12 carcasses were used for each aging time studied.

Experiment 4 - The Effect of a 24 Hour Thaw on the Tenderness of Chicken Broiler Muscle Frozen at Various Postmortem Aging Times.

Experiment 3 was repeated. However, in this case, aging times of 1, 2, 3, 4, 6 and 10 hours were used and
carcasses were thawed at 4°C for 24 hours prior to cooking and tenderness evaluation. Four birds were used for each of the six aging times.

Experiment 5 - The Effect of a 48 hour Thaw on the Tenderness of Chicken Broiler Muscle Frozen at Various Postmortem Aging Times.

In this experiment, thawing time at 4°C was increased to 48 hours. Once again, four birds were used for each of the six aging times. (1, 2, 3, 4, 6 and 10 hours).

Experiment 6 - The Effect of Varying the Pre-Freezing Aging Medium and Temperature on the Tenderness of Chicken Broiler Muscle Frozen at Various Postmortem Aging Times.

Five groups of four carcasses each were aged in air at room temperature (23°C) and then frozen in a liquid nitrogen blast freezer at 1, 1 1/2, 2, 2 1/2 and 3 hours postmortem, respectively. Freezing was repeated with a second batch of twenty birds after aging them for 1, 1 1/2, 2, 2 1/2 and 3 hours in ice slush at 0°C. Storage for one week at -31°C was followed by a four hour thaw in running water at 25°C. Muscle excision, cooking and tenderness evaluations were carried out on the two groups of samples.

Experiment 7 - The Effect of Length of Storage on the Tenderness of Chicken Broiler Muscle Frozen at Various Postmortem Aging Times.

Five groups of four birds each were aged in ice
slush at 0°C for 1, 2, 3, 6 and 10 hours postmortem, respectively. After freezing in a liquid nitrogen blast freezer, the carcasses were individually wrapped in polyethylene bags and stored in a freezer at -23°C for 3 months. At the end of this period, the carcasses were thawed in running water at 25°C for 4 hours. Muscle excision, cooking and tenderness evaluations were carried out on each carcass, by methods previously described.
RESULTS AND DISCUSSION

Experiments 3, 4 and 5

Experiments 3, 4 and 5 are similar since they are all concerned with thawing rate. They will therefore be discussed together.

Experiment 3 differed from Experiment 1 only in that the method of freezing used was more rapid and that tenderness was evaluated using the Allo-Kramer shear press only. Table IV lists the means, standard deviations and coefficients of variation of the shear values obtained for muscle frozen at the different aging times in Experiment 3. It may be noted from this table that the mean shear values at each treatment time do not differ appreciably from those found in Experiment 1.

TABLE IV. MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION OF SHEAR VALUES FROM P. MAJOR MUSCLE THAWED FOR FOUR HOURS - EXPERIMENT 3.

<table>
<thead>
<tr>
<th>Time of freezing (hours)</th>
<th>Shear Value (lbs x 4)</th>
<th>sd</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>23.01</td>
<td>7.06</td>
<td>30.70</td>
</tr>
<tr>
<td>2.0</td>
<td>19.36</td>
<td>5.10</td>
<td>26.36</td>
</tr>
<tr>
<td>3.0</td>
<td>24.55</td>
<td>7.63</td>
<td>31.09</td>
</tr>
<tr>
<td>4.0</td>
<td>28.54</td>
<td>6.88</td>
<td>24.09</td>
</tr>
<tr>
<td>6.0</td>
<td>29.12</td>
<td>7.97</td>
<td>27.36</td>
</tr>
<tr>
<td>8.0</td>
<td>28.62</td>
<td>6.41</td>
<td>22.41</td>
</tr>
<tr>
<td>10.0</td>
<td>19.19</td>
<td>5.36</td>
<td>27.91</td>
</tr>
</tbody>
</table>

sd = standard deviation
When a thawing period of 24 hours at 4°C was used in Experiment 4, the mean shear values were lower than those obtained in Experiment 3, for each time of freezing studied. However, in this experiment the coefficients of variation for each observation increased considerably.

The means, standard deviations and coefficients of variation for Experiment 4 are shown in Table V.

TABLE V. MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION OF SHEAR VALUES FROM P. MAJOR MUSCLE THAWED FOR 24 HOURS - EXPERIMENT 4.

<table>
<thead>
<tr>
<th>Time of Freezing (hours)</th>
<th>Shear Value (lbs x 4)</th>
<th>sd</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>21.62</td>
<td>9.14</td>
<td>42.26</td>
</tr>
<tr>
<td>2.0</td>
<td>17.39</td>
<td>7.00</td>
<td>40.27</td>
</tr>
<tr>
<td>3.0</td>
<td>18.85</td>
<td>7.39</td>
<td>39.22</td>
</tr>
<tr>
<td>4.0</td>
<td>20.72</td>
<td>10.16</td>
<td>49.06</td>
</tr>
<tr>
<td>6.0</td>
<td>24.92</td>
<td>11.90</td>
<td>47.75</td>
</tr>
<tr>
<td>10.0</td>
<td>17.85</td>
<td>8.06</td>
<td>45.16</td>
</tr>
</tbody>
</table>

sd = standard deviation

When a 48 hour thawing period was used in Experiment 5, the mean shear values decreased once more. In this experiment, the coefficients of variation of the observation remains higher than those found in Experiment 4. The means, standard
deviations and coefficients of variation for Experiment 5 are shown in Table VI.

TABLE VI. MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION OF SHEAR VALUES FROM P. MAJOR MUSCLE THAWED FOR 48 HOURS - EXPERIMENT 5.

<table>
<thead>
<tr>
<th>Time of Freezing (hours)</th>
<th>Shear Value (lbs x 4)</th>
<th>sd</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>18.15</td>
<td>8.50</td>
<td>46.81</td>
</tr>
<tr>
<td>2.0</td>
<td>15.31</td>
<td>6.43</td>
<td>41.97</td>
</tr>
<tr>
<td>3.0</td>
<td>16.14</td>
<td>4.64</td>
<td>28.72</td>
</tr>
<tr>
<td>4.0</td>
<td>16.62</td>
<td>6.07</td>
<td>36.53</td>
</tr>
<tr>
<td>6.0</td>
<td>18.01</td>
<td>5.53</td>
<td>30.69</td>
</tr>
<tr>
<td>10.0</td>
<td>18.97</td>
<td>6.17</td>
<td>32.50</td>
</tr>
</tbody>
</table>

sd = standard deviation

An analysis of variance and Duncan's New Multiple Range test were performed on the results from each of Experiments 3, 4 and 5. These are presented in Tables VII and VIII.
TABLE VII. ANALYSIS OF VARIANCE OF SHEAR VALUES FROM P. MAJOR MUSCLE
THAWED FOR 4, 24 AND 48 HOURS.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean Squares</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 hour Thaw</td>
<td>24 hour Thaw</td>
</tr>
<tr>
<td>Treatment</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Bird/Treatment</td>
<td>77</td>
<td>18</td>
</tr>
<tr>
<td>Error</td>
<td>1428</td>
<td>408</td>
</tr>
<tr>
<td>Total</td>
<td>1511</td>
<td>431</td>
</tr>
</tbody>
</table>

*** p < 0.001
** p < 0.01
All three experiments show significant variation in shear values for the times of freezing studied. Only Experiment 3 showed a significant variation among the birds used for each treatment time. It is thought that bird to bird variation probably existed in all the experiments carried out. However, the large number of birds used in Experiment 3 resulted in the variation being declared significant.

The results of Duncan's New Multiple Range Test on significant treatment means from Experiment 3, show that two homogenous subsets exist. The mean shear values for birds frozen at 2 and 10 hours postmortem and birds frozen at 4, 6, and 8 hours postmortem do not differ significantly from each other. The same test carried out on results from

<table>
<thead>
<tr>
<th>Time of Freezing (Hours)</th>
<th>Exp 3 4 hour thaw</th>
<th>Exp 4 24 hour thaw</th>
<th>Exp 5 48 hour thaw</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>23.01</td>
<td>21.62&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>18.15&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.0</td>
<td>19.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.0</td>
<td>24.55</td>
<td>18.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.14&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>4.0</td>
<td>28.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.72&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>16.62&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>6.0</td>
<td>29.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.92&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.01&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>8.0</td>
<td>28.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>19.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.97&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Means in the same column with similar superscripts do not differ significantly (p < 0.05)
Experiment 4 shows that three homogenous subsets exist. In this experiment, the mean shear values for birds frozen at 2, 3, 4 and 10 hours postmortem, those frozen at 1 and 4 hours postmortem and those frozen at 1 and 6 hours postmortem do not differ significantly from each other.

Three homogenous subsets also exist in Experiment 5. Birds frozen at 2, 3 and 4 hours postmortem, those frozen at 1, 3, 4, and 6 hours postmortem and those frozen at 1, 6 and 10 hours postmortem do not differ significantly from each other.

The mean shear values together with standard deviations for each freezing time studied are shown in Figures 4, 5 and 6. The least squares cubic fit to the data is shown in each figure. The coefficients of determination, \( R^2 \), are 0.64, 0.79 and 0.67 respectively.

Experiment 3 shows similar results to those found in Experiment 1. However, a larger number of pre-freeze aging times were studied in this experiment and a decrease in toughness was demonstrated in carcasses frozen between 1 and 2 hours postmortem. This is followed by an increase to maximum toughness in birds frozen between 4 and 8 hours postmortem. Increasing tenderness occurs in birds frozen between 8 and 10 hours postmortem. The results of Duncan's New Multiple Range Test on significant treatment means from Experiment 3 imply that carcasses frozen after 2 and 10 hours
Figure 4. The effect of a 4 hour thaw on the tenderness of chicken broiler muscle frozen at various postmortem aging times.
Figure 5. The effect of a 24 hour thaw on the tenderness of chicken broiler muscle frozen at various postmortem aging times.
Figure 6. The effect of a 48 hour thaw on the tenderness of chicken broiler muscle frozen at various postmortem aging times.
of aging do not differ significantly in tenderness when rapid thawing procedures are used. Similarly, carcasses frozen after 4 and 8 hours of aging do not differ significantly in toughness.

The results of Experiment 4 differ slightly from those found in the previous experiment in that the mean shear values are less for every freezing time studied and a decrease in toughness occurs in carcasses frozen between 1 and 4 hours postmortem. Maximum toughness occurs in birds frozen between 6 and 8 hours postmortem.

When a 48 hour thawing time was used, the mean shear values were significantly lower than those found in Experiment 3. Once again, a decrease in toughness occurs in carcasses frozen between 1 and 4 hours postmortem followed by an increase to maximum toughness in birds frozen between 6 and 10 hours postmortem.

The results from these experiments appear to show that the length of thawing time does have a significant effect on the ultimate tenderness of broiler muscle frozen after various postmortem aging times. In an attempt to show this effect more clearly, a combined analysis of variance was carried out on the data obtained in Experiments 3, 4 and 5. The results of this analysis are shown in Table IX. The thawing and freezing treatments and the interaction between the treatments are all shown to be highly significant.
TABLE IX. COMBINED ANALYSIS OF VARIANCE OF SHEAR VALUES FROM P. MAJOR MUSCLE THAWED FOR 4, 24 AND 48 HOURS.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Mean Squares</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thaw</td>
<td>2</td>
<td>7997.30***</td>
<td>Bird/Freeze,Thaw</td>
</tr>
<tr>
<td>Freeze</td>
<td>5</td>
<td>3434.90***</td>
<td>Bird/Freeze,Thaw</td>
</tr>
<tr>
<td>Freeze x Thaw</td>
<td>10</td>
<td>657.47***</td>
<td>Bird/Freeze,Thaw</td>
</tr>
<tr>
<td>Birds/Freeze,Thaw</td>
<td>102</td>
<td>75.26**</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>2040</td>
<td>51.36</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2159</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*** P < 0.001
** P < 0.01
A Duncan's New Multiple Range Test was performed on the significant treatment interaction means. The results of this test are shown in Table X.

**TABLE X. DUNCAN'S NEW MULTIPLE RANGE TEST ON SIGNIFICANT TREATMENT INTERACTION MEANS FROM EXPERIMENTS 3, 4, AND 5.**

<table>
<thead>
<tr>
<th>Times of Freezing (Hours)</th>
<th>Thaw Time (Hours)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>1.0</td>
<td>23.01(^{fg})</td>
<td>21.62(^{efg})</td>
<td>18.15(^{abcd})</td>
</tr>
<tr>
<td>2.0</td>
<td>19.36(^{cde})</td>
<td>17.39(^{abcd})</td>
<td>15.31(^{a})</td>
</tr>
<tr>
<td>3.0</td>
<td>24.55(^g)</td>
<td>18.85(^{bcd})</td>
<td>16.14(^{ab})</td>
</tr>
<tr>
<td>4.0</td>
<td>28.54(^h)</td>
<td>20.72(^{def})</td>
<td>16.62(^{abc})</td>
</tr>
<tr>
<td>6.0</td>
<td>29.12(^h)</td>
<td>24.92(^g)</td>
<td>18.01(^{abcd})</td>
</tr>
<tr>
<td>10.0</td>
<td>19.19(^{cde})</td>
<td>17.85(^{abcd})</td>
<td>18.97(^{bcd})</td>
</tr>
</tbody>
</table>

* Means with similar superscripts do not differ significantly (p < 0.05)

From this table it can be seen that when carcasses are frozen after 1 hour of aging, a thawing time of 48 hours is necessary before any significant lack of toughness is noted. A 24 hour thaw does not appear to affect the toughness in carcasses frozen at this time.

When freezing takes place after 2 hours of aging, a similar decrease in toughness takes place as the thawing time is increased. When carcasses frozen at 3 hours postmortem,
are thawed, a significant decrease in toughness takes place when thawing time is increased to 24 hours. A further decrease in toughness occurs in carcasses thawed for 48 hours.

Large decreases in toughness occur in carcasses frozen 4 and 6 hours postmortem, when the thawing time is increased from 4 to 24 hours. After 48 hours of thawing, carcasses frozen at this time do not differ in toughness with carcasses frozen at 1, 2 or 3 hours postmortem and thawed for the same length of time. No significant differences are observed in the toughness of birds frozen after 10 hours of aging for any of the thawing treatments used.

The effect of varying the thawing time on the tenderness of chicken muscle frozen at various postmortem aging times can be clearly seen in Figure 7.

Data obtained in these experiments support the results of Pool et al. (1959), which show that tenderization of poultry muscle arrested by freezing will continue upon thawing. The rapid thawing method of 4 hours at 25°C used in Experiment 3 does not appear to be sufficient for significant tenderization of the pre-rigor frozen muscle to occur. The rapid freezing and thawing of the pre-rigor muscles have led to some shortening of the muscle on the carcass thereby causing toughening. When the carcasses were held for 48 hours at 4°C, however, the muscle remains at temperatures just below 0°C for a long period of time. At these temperatures,
Figure 7. The effects of a 4, 24 and 48 hour thaw on the tenderness of chicken broiler muscle frozen at various postmortem aging times.
glycolysis occurs at an extremely rapid rate but any shortening which may occur in muscle attached to the carcass, cannot take place because the muscle is fixed in size and shape by the presence of ice crystals. Thus, pre-rigor frozen muscle which is thawed slowly is shown to be less tough than pre-rigor frozen muscle which has been rapidly thawed. Some lack of toughening is also shown to occur after thawing for 24 hours at 4°C but it is debatable whether this length of time at 4°C is sufficient for the muscle to become completely thawed prior to excision and cooking. The fact that the inner muscle tissues may have been partially frozen when cooked could explain the large variation in the shear values for each time of freezing.

Thus, the tenderness of pre-rigor frozen poultry is very dependent on the rate of thawing used prior to cooking and this may explain the variability and the difficulty of interpretation of many of the published results relating postmortem freezing time to tenderness of the cooked product. 

Experiment 6

The decrease in toughness which took place in carcasses frozen between 1 and 2 hours postmortem in Experiment 3 and those frozen between 1 and 4 hours postmortem in Experiments 4 and 5, is an interesting phenomenon which has not been previously reported. It is possible that it could be caused by the temperature and medium used during
the aging period. For this reason, it was decided to study the effect of changing the pre-freezing aging temperature and medium on the tenderness of broiler muscle frozen during the first three hours postmortem.

The analysis of variance was performed on the shear values obtained in this study and is shown in Table XI.

**TABLE XI.** ANALYSIS OF VARIANCE OF SHEAR VALUES FROM *P. MAJOR* MUSCLE AGED IN ICE SLUSH AND IN AIR AT ROOM TEMPERATURE.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean Squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>4</td>
<td>1116.10***</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>1378.10***</td>
</tr>
<tr>
<td>Bird/Treatment/Temperature</td>
<td>30</td>
<td>57.57</td>
</tr>
<tr>
<td>Error</td>
<td>612</td>
<td>78.23</td>
</tr>
<tr>
<td>Total</td>
<td>647</td>
<td></td>
</tr>
</tbody>
</table>

*** p < 0.001

Both the treatment and temperature effect are highly significant. The mean shear values and standard deviations for each treatment-temperature combination are shown in Table XII, together with the results of Duncan's New Multiple Range Test on the treatment mean shear values.
TABLE XII. MEANS, STANDARD DEVIATIONS AND DUNCAN'S NEW MULTIPLE RANGE TEST ON COMBINED MEANS OF SHEAR VALUES FROM EXPERIMENT 6.

<table>
<thead>
<tr>
<th>Time of Freezing (hours)</th>
<th>Shear Value (lbs x 4)</th>
<th>sd</th>
<th>Combined Shear Value (lbs x 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0°C</td>
<td>23°C</td>
<td>0°C</td>
</tr>
<tr>
<td>1.0</td>
<td>23.80</td>
<td>25.00</td>
<td>9.34</td>
</tr>
<tr>
<td>1.5</td>
<td>19.58</td>
<td>23.17</td>
<td>7.30</td>
</tr>
<tr>
<td>2.0</td>
<td>17.40</td>
<td>18.17</td>
<td>6.00</td>
</tr>
<tr>
<td>2.5</td>
<td>16.32</td>
<td>21.06</td>
<td>7.06</td>
</tr>
<tr>
<td>3.0</td>
<td>20.61</td>
<td>26.22</td>
<td>6.93</td>
</tr>
</tbody>
</table>

* Means with similar superscripts do not differ significantly (p < 0.05)

The mean shear values together with standard deviations for each freezing time studied are shown in graphical form in Figures 8 and 9. The coefficients of determination, (R<sup>2</sup>), for the least-squares quadratic fit shown in each of the figures are 0.833 and 0.644 respectively.

The figures show that carcasses aged in air at room temperature are tougher at every freezing time than carcasses aged in ice slush. Also, the standard deviation of the mean at each time of freezing is greater for birds aged in air than for those aged in ice slush. The reason for increased toughness in the carcasses aged in air therefore may be moisture loss from parts of the muscle tissue...
Figure 8. The effect of aging in ice slush on the tenderness of chicken broiler muscle frozen at various postmortem aging times.
Figure 9. The effect of aging in air at 23°C on the tenderness of chicken broiler muscle frozen at various postmortem aging times.
during aging. Such a phenomenon would also account for the large variation of shear values at each freezing time. Dodge and Stadelman (1959), have shown that toughening occurring in muscle tissue aged in air, is due to dehydration.

The decrease in toughness in birds frozen between 1 and 2 hours postmortem found in Experiments 3, 4 and 5 also occurs in this experiment. The temperature and medium of aging are therefore not responsible for this increase in tenderness immediately prior to the onset of rigor. The time at which maximum tenderness is reached, differs slightly with the method of aging used. This may be due to the temperature of the aging environment. Physiological variations among the birds, however, may also be responsible and further experiments using larger numbers of poultry must be carried out to determine the effect of temperature of the aging environment on the tenderness of muscle.

It may be said with reasonable certainty that aging in air at a high temperature leads to a tougher product than one which is aged in ice slush at 0°C, due to dehydration. The temperature and medium of aging do not affect the increase in tenderness observed in birds frozen between 1 and approximately 2 1/2 hours postmortem.
Experiment 7

The temperature and length of frozen storage were shown to have a significant effect on the tenderness of poultry frozen pre-rigor. The analysis of variance was performed on the shear values obtained from poultry subjected to storage at -23°C for 3 months. The results of this analysis are shown in Table XIII.

**TABLE XIII. ANALYSIS OF VARIANCE OF SHEAR VALUES FROM P. MAJOR MUSCLE SUBJECTED TO LONG TERM FROZEN STORAGE.**

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean Squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>4</td>
<td>32.03 ns</td>
</tr>
<tr>
<td>Bird/Treatment</td>
<td>15</td>
<td>121.53 ns</td>
</tr>
<tr>
<td>Error</td>
<td>340</td>
<td>125.87</td>
</tr>
<tr>
<td>Total</td>
<td>359</td>
<td></td>
</tr>
</tbody>
</table>

ns = not significant

This analysis shows that no significant difference exists between the mean shear values of carcasses frozen at any of the five aging times studied, after 3 months of storage at -23°C.

The means, standard deviations and coefficients of variation for the shear values obtained in this experiment are shown in Table XIV.
TABLE XIV. MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION OF SHEAR VALUES FROM *P. MAJOR* MUSCLE STORED AT -23°C FOR 3 MONTHS.

<table>
<thead>
<tr>
<th>Time of Freezing (hours)</th>
<th>Shear Value (lbs x 4)</th>
<th>sd</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>22.79</td>
<td>11.22</td>
<td>49.24</td>
</tr>
<tr>
<td>2.0</td>
<td>23.02</td>
<td>13.09</td>
<td>56.87</td>
</tr>
<tr>
<td>3.0</td>
<td>22.94</td>
<td>9.76</td>
<td>42.53</td>
</tr>
<tr>
<td>6.0</td>
<td>22.51</td>
<td>9.47</td>
<td>42.07</td>
</tr>
<tr>
<td>10.0</td>
<td>21.37</td>
<td>11.33</td>
<td>53.02</td>
</tr>
</tbody>
</table>

sd = standard deviation

The mean shear values together with standard deviations for each time of freezing postmortem studied, are shown in graphical form in Figure 10. The linear fit to the data shown in the figure has a coefficient of determination of 0.87.

In this experiment, four birds were used for each mean shear value shown. Thus, the large standard deviation of each mean shear value, shows that the results of this experiment do not offer conclusive evidence for the tenderization of pre-rigor frozen muscle during long-term frozen storage. However, it is conceivable that such a phenomenon does occur and studies involving frozen storage for longer periods of time may offer further evidence for the occurrence of tenderization at very low temperatures.
Figure 10. The effect of freezing at various postmortem aging times on the tenderness of chicken broiler muscle stored for 3 months.
However, the results of this experiment show that the pattern of tenderness shown in Experiment 3 is obviated after 3 months at $-23^\circ\text{C}$.

Therefore, this group of experiments has shown that the rate of thawing, the aging medium and possibly the temperature of aging and length of frozen storage all affect the tenderness of muscle frozen pre-rigor. A decrease in toughness has also been shown to occur prior to the normal onset of rigor. This phenomenon cannot be explained in terms of the method of pre-freezing aging used. Similarly, a decrease in the rate of thawing does not appear to prevent such an increase in tenderness from occurring although the preceeding increase in toughness is delayed for several hours when a 48 hour thawing method is used.

Further studies carried out in an attempt to explain this phenomenon, are described in Chapter 4.
CHAPTER IV. THE TENDERNESS OF CHICKEN BROILER MUSCLE FROZEN AT VARIOUS POSTMORTEM AGING TIMES - OTHER METHODS OF EVALUATING MEAT TENDERNESS.

INTRODUCTION

There is a need for predicting texture in cooked meat from the characteristics of the uncooked muscle. Many methods of tenderness evaluation have been developed but reported correlations between objective measurements of muscle tenderness and sensory panel values for cooked meat show much variation (Sharrah et al., 1965 a, b).

In Chapter 2, reasonable correlations were obtained between objective measurements of tenderness performed on cooked muscle using the Allo-Kramer shear press and sensory panel evaluations. The reported observations of an increase in tenderness in chicken muscle frozen between 1 and 2 hours postmortem has not been explained in terms of the aging and thawing methods used. Measurements of tension developed during thaw rigor and lengths of sarcomeres at various postmortem times were therefore made in an attempt to explain this phenomenon and to determine whether or not these methods could be used for adequate prediction of tenderness in the cooked meat.

Rigor mortis arrested by freezing, has been shown to proceed at about the normal rate upon thawing. Recent work on turkey and broiler muscle have used the isometric
tension pattern to follow the sequence of physical changes in rigor (Jungk and Marion, 1970; Wood, 1973).

The pattern has been shown to closely parallel the tenderness cycle and illustrates the tendency of muscle to shorten.

Measurements of isometric tension development in muscle frozen before the onset of rigor will therefore determine to some extent, the pre-rigor state of the muscle at the time of freezing. Such measurements could thus be used for predicting the tenderness of the cooked meat. Experiment 8 describes the measurement of isometric tension in muscle frozen prior to the onset of rigor.

Locke (1960) showed that tenderness in beef is influenced by the degree of muscular contraction in the postmortem muscle. Many workers have since investigated the correlation between sarcomere length, as a measure of the contractile state, and tenderness. Herring et al. (1965 a, b) have found that taste panel results are influenced by the degree of contraction as measured by the sarcomere length. Stanley et al. (1972) did not find a significant correlation of these variables although this may have been due to very small differences in the mean sarcomere lengths of the muscle samples used. Experiment 9 describes the use of optical diffraction as a means of determining sarcomere lengths of cooked chicken muscle frozen at various postmortem aging
times. Attempts are made to correlate the sarcomere length measurements with objective and subjective evaluations of tenderness.
MATERIALS AND METHODS

Experiment 8 - The Effect of Freezing at Various Postmortem Aging Times on the Tension Development in Muscle Strips.

Three groups of four birds each were aged in ice slush for 1, 2 and 3 hours postmortem. Muscle for tension measurement was obtained after each aging period by cutting the breast skin and excising muscle strips of parallel fibres, 5 cm long, weighing approximately 1g., from the anterior portion of one P. major muscle on each carcass. Six strips were cut from each bird. The remainder of the carcass, containing one complete P. major muscle to be used for tenderness evaluation using the Allo-Kramer shear press, was frozen with the other three carcasses at the appropriate aging time using the liquid nitrogen blast freezer. At the same time, the twenty-four muscle strips wrapped in Saran wrap, were placed in an air-blast freezer at -31°C.

After freezing, the carcasses were stored at -31°C for one week before thawing at 4°C for 4 hours. The one P. major muscle was removed from each bird, cooked, and evaluated for tenderness using the Allo-Kramer shear press.

Tension development in the muscle strips was measured using an E & M 6-channel physiograph fitted with
isometric transducers. The frozen muscle strips were removed from the freezer after 6 hours and clamped in a plexiglass cylinder. The free upper clamp, holding the frozen muscle strip was attached by fishing line to an isometric transducer. A small amount of phosphate buffer was placed in the base of the plexiglass cylinder and the top was covered in Saran wrap in an attempt to prevent dehydration of the muscle strip during thawing. The physiograph was calibrated so that a 1 cm pen deflection was equivalent to 5 g tension. The muscle strips were allowed to thaw in the cylinders and maximum tension development in grams per square centimetre of muscle tissue was calculated.

Experiment 9 - The Tenderness of Chicken Broiler Muscle Frozen at Various Postmortem Aging Times - Sarcomere Length Measurements as a Prediction of Cooked Meat Tenderness.

Five groups of four birds each were aged in ice-slush for 1, 2, 3, 6 and 10 hours postmortem. Freezing in the liquid nitrogen blast freezer was followed by storage for one week at -31°C. The carcasses were thawed for 4 hours in running water at 25°C after which time the P. major muscles were removed and cooked by the method previously described.

Taste panel evaluations were carried out using the method described in Experiment 1. Using the Allo-Kramer
shear press, shear values were obtained from the P. major muscle which remained after the taste panel samples had been cut. A minimum of 9 shear values per bird were obtained.

The sarcomere lengths of the P. major muscle fibres were determined using the optical diffraction method described by the Meat Research Institute, Bristol, England, (1972). The apparatus consisted of a 1-mW helium - neon laser of a wavelength of $\lambda = 632.8$ nm mounted on an optics bench with a specimen holding device and a screen. The screen consisted of a vertically mounted white card bearing a central millimetre scale.

A small piece of P. major muscle was cut with known orientation of the fibres. Single fibres or small fibre bundles were teased out and mounted between two glass cover slips using a drop of buffered sucrose solution (0.2M sucrose in a phosphate buffer of pH 7.1 and 0.2M). The fibre bundle was then placed vertically in relation to the laser beam to give a horizontal array of diffraction bands on the screen. An example of this is shown in Plate II.

Sarcomere lengths were calculated using the following formula:

$$d = \frac{(632.8 \times 10^{-3})D}{S} \mu m$$

where $d$ is equal to the sarcomere length.
D is the distance in millimetres between the specimen and the screen.

S is the separation between the 0th and the nth order diffraction band.

(Throughout this experiment, D had a constant value of 84 mm.)

Twelve sarcomere lengths were obtained for each carcass. Care was taken to ensure that muscle fibres were taken from the same area of the P. major muscle on each bird.

Experiment 9 was repeated using another 20 broilers. In total, four taste panel sessions were held.
Sarcomere Length = 1.83 μm

Plate II. Diffraction pattern obtained from muscle fibre.
RESULTS AND DISCUSSION

Experiment 8

Analyses of variance were performed on the shear values and tension measurements obtained in this experiment. These are shown in Tables XV.

TABLE XV. ANALYSES OF VARIANCE FOR SHEAR VALUES AND TENSION MEASUREMENTS RECORDED IN EXPERIMENT 8.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean Squares</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Shear Value</td>
</tr>
<tr>
<td>Treatment</td>
<td>2</td>
<td>28.58***</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>2.00</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05
*** p < 0.002

A significant treatment effect is observed for both measurements although this effect is more evident in the shear values than the tension measurements. Duncan's New Multiple Range Tests were performed on both sets of data. The results of these tests are presented with the means and standard deviations in Table XVI.
TABLE XVI. STANDARD DEVIATIONS AND DUNCAN'S NEW MULTIPLE RANGE TEST ON THE SIGNIFICANT TREATMENT MEANS FROM EXPERIMENT 8.

<table>
<thead>
<tr>
<th>Time of Freezing (hours)</th>
<th>Shear Value (lbs x 4)</th>
<th>sd</th>
<th>Tension (g/cm²)</th>
<th>sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>21.23\textsuperscript{a*}</td>
<td>5.94</td>
<td>118.35\textsuperscript{ab}</td>
<td>24.15</td>
</tr>
<tr>
<td>2.0</td>
<td>18.15</td>
<td>5.15</td>
<td>136.95\textsuperscript{a}</td>
<td>34.42</td>
</tr>
<tr>
<td>3.0</td>
<td>23.48\textsuperscript{a}</td>
<td>4.78</td>
<td>88.65\textsuperscript{b}</td>
<td>27.99</td>
</tr>
</tbody>
</table>

* Means on the same column with similar superscripts do not differ significantly (p < 0.05).

One homogeneous subset occurs among the mean shear values while two may be observed among the mean tension measurements. Figure 11 shows the effect of freezing at various postmortem times on the tension developed in muscle strips. Figure 12 shows shear press values from cooked muscle subjected to the same treatment.

A significant simple linear correlation coefficient of -0.7 appears to exist between the two sets of data. However, it is to be noted that muscle strips frozen after 2 hours
of aging develop more tension upon thawing than muscle strips which have been frozen after 1 or 3 hours of aging. This result is the opposite of what would be expected, after observing in previous experiments, the increase in tenderness in carcasses frozen after 2 hours. Wood (1973), found no significant relationship between tension release and shear values and concluded that the tension release observed in individual birds was not indicative of tenderness. Although a relationship between tension development and shear values appears to exist in this experiment, the deviation from the mean tension measurements are large especially in muscle strips frozen after 2 hours of aging. Also, the tension values reported cannot be interpreted as being equivalent to values that would be obtained from muscle left on the carcass during freezing and thawing. Cold shortening and possible moisture loss from the surface layers of the muscle strips during thawing may have led to an increase in the tension values.

Thus, the results of this experiment are not in agreement with the observations made in preceding experiments. Further studies should be carried out using more birds in an attempt to decrease the variation in tension values before the results reported in this experiment can be conclusive.
Figure 11. The effect of freezing at various postmortem aging times on tension development in muscle strips.
Figure 12. The effect of freezing at various postmortem aging times on tenderness in broiler *P. major* muscle.
Experiment 9

Analyses of variance were performed on the three groups of data resulting from this experiment. The results of the analyses are presented in Table XVII.

TABLE XVII. ANALYSES OF VARIANCE FOR PANEL SCORES, SHEAR VALUES AND SARCOMERE LENGTHS RECORDED IN EXPERIMENT 9.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Panel Scores</th>
<th>Shear Value</th>
<th>Sarcomere Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>4</td>
<td>1.34*</td>
<td>16.52**</td>
<td>0.21***</td>
</tr>
<tr>
<td>Error</td>
<td>15</td>
<td>0.18</td>
<td>1.35</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* $p < 0.05$
** $p < 0.001$
*** $p < 0.0001$

A significant treatment effect is observed for all sets of measurements. The significant effect is particularly noticeable for the sarcomere length measurements. Duncan's New Multiple Range Tests were performed at the 10% level on all significant treatment means. Results of these tests are presented in Table XVIII. The 10% level of probability was used in this instance in an attempt to offer better evidence for the existence of significant differences in an experiment.
where only limited numbers of carcasses were used.

**TABLE XVIII.  DUNCAN'S NEW MULTIPLE RANGE TEST ON THE SIGNIFICANT TREATMENT MEANS FROM EXPERIMENT 9.**

<table>
<thead>
<tr>
<th>Time of Freezing (hours)</th>
<th>Panel Scores</th>
<th>Shear Value (lbs x 4)</th>
<th>Sarcomere Length (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>6.20&lt;sup&gt;b*&lt;/sup&gt;</td>
<td>18.50</td>
<td>1.71&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.0</td>
<td>6.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.80</td>
</tr>
<tr>
<td>3.0</td>
<td>5.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.63&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6.0</td>
<td>5.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.46</td>
</tr>
<tr>
<td>10.0</td>
<td>6.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.08</td>
</tr>
</tbody>
</table>

* Means in the same column with similar superscripts do not differ significantly (p < 0.1)

Table XVIII shows that some similarity exists between the three sets of data. The panel scores show that carcasses frozen after 3 and 6 hours of aging are significantly tougher than those frozen at 1, 2 or 10 hours postmortem. The panel was therefore unable to detect any significant differences between the tenderness of birds frozen after 2 hours of aging and those frozen at 1 hour postmortem.

The shear and taste panel values recorded are somewhat less than those obtained in earlier, similar experiments. Although every effort was made to ensure that experimental birds were of approximately the same age and size,
some variation is to be expected. A significant difference is shown to occur between the shear values of birds frozen at 2 and at 10 hours postmortem and those frozen after 1, 3 or 6 hours of aging. Once again, birds frozen at 3 and 6 hours postmortem are tougher than those frozen after 2 and 10 hours postmortem. Carcasses frozen after 1 hour of aging, however, are significantly tougher at the 10% level than those frozen at 2 hours postmortem. Panel members were unable to detect this difference.

Sarcomere lengths reach a minimum in birds frozen after 6 hours of aging. Again, a significant difference exists between the sarcomere lengths of birds frozen at 2 hours postmortem and those frozen after 1 hour of aging. However in this case birds frozen after 1 and after 3 hours of aging have sarcomere lengths which are not significantly different.

Figures 13, 14, and 15, illustrate these observations. The correlation matrix for the three sets of data is presented in Table XIX.

**TABLE XIX.** CORRELATION MATRIX FOR THE THREE PARAMETERS STUDIED IN EXPERIMENT 9.

<table>
<thead>
<tr>
<th></th>
<th>Shear Value</th>
<th>Sarcomere Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panel Score</td>
<td>0.72**</td>
<td>0.60**</td>
</tr>
<tr>
<td>Shear Value</td>
<td>1.00</td>
<td>0.56*</td>
</tr>
</tbody>
</table>

* p < 0.02
** p < 0.01
Figure 13. Subjective assessment of toughness of broiler muscle frozen after various postmortem aging periods.
Figure 14. Objective assessment of toughness of broiler muscle frozen after various postmortem aging periods.
Figure 15. The measurement of sarcomere lengths in broiler muscle frozen after various postmortem aging periods.
A correlation among the three methods of tenderness assessment therefore exists although, from the results of the Duncan's New Multiple Range Tests, the sensitivity of each method of assessing toughness varies. Panel members were unable to discern differences between the toughness of birds frozen at 2 hours postmortem and those frozen after 1 and 10 hours of aging. The Allo-Kramer shear press results show that a significant difference in toughness between birds frozen at 2 and 10 hours and those frozen at other aging times, does exist, however. Similarly, sarcomere lengths are greater in birds frozen after 2 hours of aging than in birds frozen at 1, 3, and 6 hours of aging, indicating that muscle frozen after 2 hours is more tender than that frozen 1, 3 or 6 hours postmortem.

From these results it is not possible to completely explain the phenomenon observed in birds frozen at 2 hours postmortem. The sarcomere length measurements made in this experiment offer some evidence for postmortem changes occurring between 1 and 2 hours after death which could lead to increases in muscle tenderness immediately prior to the onset of rigor. Although taste panel members were unable to detect such a change, objective measurements of tenderness offered evidence that such tenderization takes place.
Further work is obviously necessary to determine what changes could take place in muscle immediately prior to the onset of rigor. This experiment does offer evidence, however, that measurements of sarcomere lengths using the method described could be used as a means of predicting tenderness in cooked muscle.
SUMMARY AND CONCLUSIONS

The results in the previously described experiments show that the ultimate tenderness of broiler muscle can be greatly influenced by the interaction of pre-freezing aging time and thawing time.

When carcasses were frozen at various postmortem times and then rapidly thawed, a characteristic toughness pattern was observed. The pattern consisted of a decline in toughness in carcasses frozen between 1 and 2 hours postmortem followed by an increase to maximum toughness in those frozen between 4 and 8 hours postmortem. Maximum tenderness occurred in muscle frozen 10 hours after death. When the length of thawing time was increased to 48 hours this pattern was eradicated with the exception of the decline in toughness in carcasses frozen after 2 hours of aging.

The aging medium and temperature did not effect the decrease in toughness observed in carcasses frozen at 2 hours postmortem.

After a three month storage period, all carcasses exhibited similar tenderness levels and the pattern of toughness previously observed was no longer evident. Thus, over longer storage periods, some postmortem tenderization appears to take place in the pre-rigor frozen muscle.

Measurements of sarcomere lengths of the muscle fibres of carcasses frozen at various postmortem aging times
correlated well with toughness measurements. Sarcomere lengths were observed to increase in muscle frozen between 1 and 2 hours of aging and decrease in length in carcasses frozen between 4 and 8 hours postmortem. It may therefore be possible to use the measurement of sarcomere lengths as a method for predicting the tenderness in cooked meat.

The sarcomere length measurements offered more evidence for the observed decrease in toughness occurring in muscle frozen between 1 and 2 hours postmortem. This phenomenon has not been previously observed and further work is necessary to determine the changes that take place in muscle immediately prior to the onset of rigor. The results of this thesis do, however, help to explain some of the variability in the published results of work previously carried out in this area.
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