IDENTIFYING THE COMPONENTS OF TENDERNESS USING DIFFERENTIAL SCANNING CALORIMETRY

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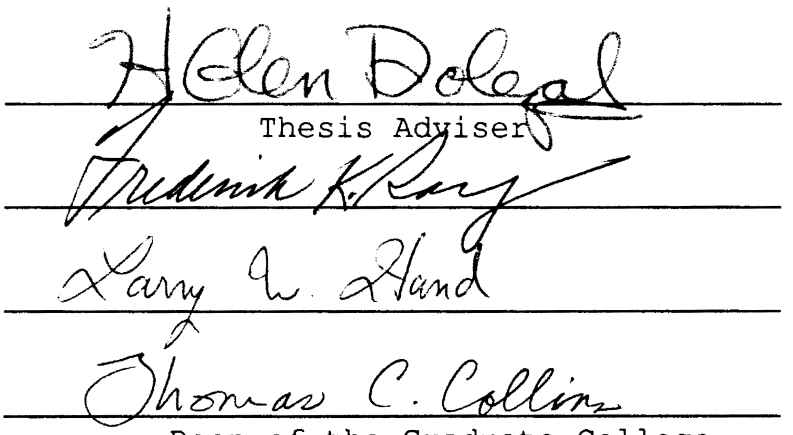

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Several components have been identified as general predictors of beef tenderness. Most of these components include the collagen in the connective tissue, myofibrillar proteins and fat. Berry et al. (1974) reported that muscles rated low in tenderness exhibited a lower percent of soluble collagen content than those with high collagen solubility, while other studies (Kruggel et al. 1970; Smith and Carpenter, 1970; Cross et al, 1973) found lower correlation between total collagen content and tenderness of muscles. In addition, Bouton and Harris (1972b) concluded myofibrils reflected the major differences in tenderness of muscles. Moreover, Cross et al. (1972) and McKeith et al. (1985) reported muscles with higher percent fat tended to be more tender.

Differential scanning calorimetry (DSC) has been used to study the thermal denaturation of proteins in post rigor muscle (Ledward and Lawrie, 1975; Wright et al. 1977; Stabursvik and Martens, 1980; Findlay and Stanley, 1984). Wagner and Anon (1985) determined the thermal denaturation kinetics of myofibrillar proteins in bovine muscle. Also, Wu et al. (1985) investigated thermal transitions of fish

mince and actomyosin from croaker by using DSC. Therefore, the objective of this study was to use differential scanning calorimetry to study thermal denaturation of muscle protein from selected beef muscles differing in tenderness.

CHAPTER II

REVIEW OF LITERATURE

Myofibrillar Protein

Bouton and Harris (1972b) reported that the myofibrillar theory of tenderness can be measured by shear force values, which were believed to reflect myofibrillar rather than connective tissue strength. Furthermore, Marsh and Leet (1966) and Marsh and Carse (1974) explained that shear force values are affected by the changes in actin-myosin interdigitation. Marsh and Carse (1974) also claimed that the changes in shear force values occurring in cold- or rigor-shortened muscle can be explained in terms of actin-myosin or myofibrillar toughness. Shorthouse and Harris (1990) indicated that myofibrillar toughness had a variable and low dependence on animals age and no significance due to muscle differences.

Meat has to be cooked before being eaten and for this reason, the effect of temperature on the structural strength of the meat is important. Davey and Gilbert (1974) found as meat temperature reached 60 °C, the major part of the sarcoplasmic and myofibrillar proteins were denatured. This denaturation is accompanied by an increase in sample rigidity and a very large decrease in the water

holding capacity (Hamm and Deatherage, 1960). However, not only are myofibrillar proteins correlated to tenderness of muscles but also connective tissues played a part in cold-shortened toughness (Bouton and Harris, 1972a).

Connective Tissue and Collagen

Many factors have been shown to be related to beef tenderness. Connective tissue, especially collagen, is one of the most widely studied tenderness-related components. Lehninger (1975) showed that connective tissue is the most abundant group of proteins in the body. King and MacFarlane (1987) reported that collagen is the major constituent of the connective tissue surrounding the whole muscle. Since connective tissue is so plentiful in the body, how it relates to tenderness is very interesting. There are some views of connective tissue (collagen) associated with the tenderness of beef muscles. Cross et al. (1973), Berry et al. (1974), and Light et al. (1985) have reported significant relationships between collagen content of meat and its tenderness. In addition, Bouton et al. (1973) reported that the rate of increase in toughness or tenderness of beef muscle with age was significantly related to their connective tissue strength. Many investigators have shown that meat tenderness is significantly correlated with the degree of connective tissue solubility. Hill (1966) found that the degree of

solubility of the collagen, as well as the total amount, should be considered when biochemical explanations of toughness in meat are considered. Cross et al. (1972, 1973) indicated that the percentage of soluble collagen was significantly related to the contribution of connective tissue to toughness, as assessed by a taste panel. Seideman (1986) reported the amount of collagen was highly related to sensory properties but not instrumental texture properties, whereas, the amount of soluble collagen was less highly related to sensory properties but was strongly related to instrumental texture properties. However, Harris et al. (1992) indicated that total collagen content was a more important characteristic than collagen solubility in explaining variability in tenderness.

Other researchers found that there was a low or non-existent relationship between collagen and the tenderness of beef. Hunsley et al. (1970) reported that although younger animals have a significantly greater amount of collagen solubility than did the older age groups, neither taste panel tenderness measurement nor Warner-Bratzler shear values were related to collagen content of the connective tissue residue. Kruggel et al. (1970) indicated that although the amount of total collagen in epimysial tissue was not correlated with meat tenderness, the type of epimysial acid-soluble collagen (ASC) had some correlation with meat tenderness. Cross et al. (1973) found that the total concentrations of the connective tissue components

(collagen and elastin) were not closely related to ratings for muscle fiber tenderness or amount of connective tissue. McKeith et al. (1985) pointed out that even though the collagen content in the longissimus muscle and psoas major are lower than in the infraspinatus and semimembranosus, total collagen content was not a good predictor of tenderness. Since the role of connective tissue in the tenderness of beef has not been completely revealed, further studies are necessary.

Hill(1966) reported an increase in the number or strength of cross linkages in intramuscular collagen of meat animals as they age. In addition, less collagen becomes soluble during the cooking of meat from older animals compared with meat from younger animals, increasing the sensation of toughness when meat from older animals is consumed. Herring et al. (1967) found that collagen solubility was significantly affected by maturity in both longissimus dorsi and semimembranosus muscles. Smith and Carpenter (1970) found that the percentage soluble collagen was significantly lower in carcasses from more mature cattle. Cross et al. (1972, 1973) and Beltran et al. (1991) showed that as an animal age increases, percentage soluble collagen decreased. Boccard et al. (1979) reported that solubility of collagen decreased markedly between birth and 16 month-old bulls. Beltran et al. (1991) also pointed out that intramuscular collagen from older animals was much more tightly crosslinked.

Sarcomere

Another factor that influences meat tenderness is sarcomere length. Locker (1960) and Locker and Hagyard (1963) reported that meat from relaxed muscle is more tender than meat from contracted muscle. Herring et al. (1965) reported that when muscles were restrained the amount of shortening at the onset of rigor was minimized and these were more tender than contralateral muscles allowed to contract and shorten. Hostetler et al. (1972) indicated that as sarcomere lengths were increased between 2.0 and 2.5 μm, tenderness increased. Stretching muscle sarcomeres beyond 2.5 μm produced little or no additional tenderness in muscles. Harris (1975) reported that as sarcomere length contracted below 1.8-2.0 μm, toughness of meat increased in cooked post-rigor meat until sarcomere length was below 1.2-1.3 μm. Davey and Gilbert(1977) reported that changes in tenderness due to sarcomere length act both along and across the muscle fibers and tensile strength and shear force measurements of meat toughness are quite closely related. Lewis et al. (1977) indicated that there is a significant but low correlation between tenderness and sarcomere length. Also, McKeith et al.(1985) showed that there was a weak relationship between sarcomere length and tenderness. Birkhold et al. (1992) reported that electrical stimulation resulted in significantly longer sarcomeres, which corresponded to an

improvement in tenderness. Harris et al. (1992) showed that top loin steaks were more tender than top sirloin steaks because top sirloin steaks have shorter sarcomere lengths than top loin steaks.

Warner-Bratzler Shear

The Warner-Bratzler shearing (WBS) device has been a widely used instrument for measuring tenderness (Hiner and Hankins, 1950; Tuma et al., 1962; Field et al., 1966; Lewis et al., 1967; Hedrick et al., 1969; Zinn et al., 1970; Bouton et al., 1972a, b, 1978; Smith et al., 1982; Shorthouse and Harris, 1990; Huff and Parrish, 1993). Usually this device has been used to determine the peak force required to shear through a meat sample of fixed cross-sectional area and of known fiber orientation (Bouton et al., 1975). Cross et al, (1973) reported that shear values were significantly correlated with muscle tenderness. Moreover, the difference between initial yield force and peak force values significantly increased with animal age (Bouton et al. 1975).

Age, Sex, and Location

Many researchers reported that meat from older animals is less tender (Hiner and Hankins, 1950; Hunsley et al., 1970; Albaugh et al., 1975, Bouton et al., 1978; and Smith

et al., 1982; Shorthouse and Harris, 1990) Tuma et al. (1962) reported that tenderness of longissimus dorsi steaks, as measured by the Warner-Bratzler shear and panel tenderness, decreased significantly with increase in animal age. For this muscle, the greatest drop in the tenderness was observed between 18- and 42-month old steers. Field et al. (1966) found that bulls 16 to 20 months and 20 to 24 months old were significantly tougher than steers and heifers of comparable age. Hedrick et al. (1969) indicated from WBS values and sensory panel scores for steaks from bulls less than 16 months of age were comparable in tenderness to steaks from steers and heifers of similar chronological age.

Zinn et al. (1970) claimed that muscle location has a significant effect on tenderness of meat. Hiner and Hankins (1950) reported that the psoas major was the most tender in their study and the neck and foreshank were the least tender muscle. Also, semimembranosus muscle was more tender than the longissimus muscle (Zinn et al., 1970). Cross et al. (1973) showed that tenderness differed among muscles from various anatomical locations because of the variation in the traits of factors responsible for tenderness. Shorthouse and Harris (1990), indicated that some muscles such as longissimus dorsi, gluteus medius, and psoas major were more tender than those muscles such as vastus lateralis, rectus femoris and semimembranosus because each of the latter muscles has different connective

tissue strength. Beltran et al. (1991) indicated that intramuscular collagen from older animals was much more tightly crosslinked. Huff and Parrish (1993) reported that animal age and postmortem aging time had more influence on tenderness attributes than did sex of animals.

Seideman (1986) pointed out that although bulls had a higher collagen content than steers, instrumental textural properties were lower for meat from bulls when compared to steers. This was reinforced by sensory tenderness ratings.

FAT

McBee and Wiles (1967), Smith et al.(1970), Campion and Crouse (1975), Jennings et al. (1978), and Tatum et al.(1980) have reported positive relationships between percentage fat and tenderness; however, in most examples, the correlation was low. Previous researchers have emphasized that tenderness was not affected by marbling over a wide range of marbling scores (Walter et al. 1965; Parrish, et al. 1973). Also, Tatum et al. (1980) showed that large differences in marbling affected tenderness but there was not a consistent linear relationship between the two parameters. In addition, Cross et al. (1973) found virtually no relationship between percentage fat and palatability of beef varying in chronological age.

pH

Some researchers reported that an enhancement of tenderness accompanies a lower pH, whereas others indicated that higher pH values are related to more tender muscles. Penny et al. (1963) showed that after freeze-drying treated beef (injected with adrenaline), meat at pH 6.7 was more tender and more juicy than that at pH 5.9. Kauffman et al. (1963) indicated that juiciness and tenderness were significantly enhanced when ham muscles' pH was higher, which was supported by a highly significant negative correlation between pH and shear force. Briskey et al. (1957) and Bouton et al. (1971, 1973) reported that tenderness of meat increased with an increase in pH due primarily to an increase in water holding capacity. On the other hand, Judge et al. (1960) reported that tenderness increased as pH decreased in fresh pork loin.

Stabursvik and Martens (1980) found that myosin was more heat stable, represented by a higher transition temperature, at pH 5.4. The less heat stable myosin component occurs at pH 8.7. They also found that myosin, myosin rod, light meromyosin, heavy meromyosin and subfragment 1 (globular head of HMM) all showed an increase in melting temperature at higher pH values. But subfragment 2 (helical fragment of HMM) showed a decrease in melting temperature at higher pH values. Bouton et al.

(1971) reported that beef muscle tenderness increased as the pH increased from 6 to 7.

Aging

Sayre (1970) identified that meat tenderness increases during postmortem storage. Also, Mitchell et al. (1991) reported both LD and SM steaks increased ($P < .05$) in tenderness with aging for 10 or 21 days. Moreover, Olson et al. (1976) found that PM slightly changed in Warner-Bratzler shear values as aging increased from 2 to 13 days, while LD muscle decreased in shear values during postmortem storage. This result was supported by Koohmaraie et al. (1988) who found psoas major was more tender than biceps femoris and longissimus dorsi muscles at 1-day postmortem, while after 14 days of aging, there were no differences between shear values for the three muscles.

Temperature and Freezing

Olson et al. (1976) reported that storage temperature had little effect on tenderness for the PM muscle. Other studies showed that bovine muscles aged at 16°C improved more in tenderness than those aged at 2°C (Sleeth et al., 1958; Busch et al,. 1967; Parrish et al., 1973). The reason that muscles at a higher aging temperature increased in tenderness may be that a greater fragmentation of

myofibrils occurs near the Z-line which improved tenderness (Parrish et al., 1973). However, Hiner and Hankins (1951) found that the foreshank muscles were not significantly changed in Warner-Bratzler shear values by freezing at -18°C, while the round muscles had high change in tenderness. Moreover, Singh and Wells (1985) reported that patties stored at -12°C or -18°C were less tender than those stored at -35°C over 6-month period, while Cross et al. (1978) found no storage temperature (-12°C, -18°C, -23°C) effects on cooked patty tenderness.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) is gaining acceptance for the thermal study of foods and their components following the development of instrumentation of sufficient sensitivity. DSC is capable of supplying both thermodynamic (heat capacity, enthalpy and entropy) and kinetic data (reaction rate and activation energy) on protein denaturation (Biliaderis, 1983). Wright et al. (1977) and Wu et al. (1985) reported the application of DSC to study the thermal behavior of proteins and biochemical systems. Wagner and Anon (1985) used DSC to monitor the thermal denaturation kinetics of myofibrillar proteins in bovine muscle at pH 5.6. Findlay and Stanley (1984) indicated that DSC can be used to relate the denaturation of muscle proteins to the textural changes caused by

cooking. Parsons and Patterson (1986) showed that DSC appeared to have the potential to yield information about a previous heat treatment applied to meat materials. It also provided a means of studying the process involved in the causes and factors which affect the thermal denaturation of the protein in meat. Ellekjar (1992) suggested that DSC was a potential method for the determination of the extent of treatment to proteins in beef.

The physical state and properties of water in foods has been examined with DSC. Ross (1978) showed that DSC could determine the amount of bound water in several model systems as a function of water activity. Roos (1986) and Wang and Kolbe (1991) indicated that by applying dynamic correction techniques, DSC has great potential as a tool for investigating frozen food thermal properties; in addition, it is an easy and accurate way to obtain initial freezing point, latent heat of fusion and unfrozen water weight fraction.

DSC has been used to study the thermal properties of fish muscle proteins and to measure the extent of their denaturation under various processing conditions. Hastings et al. (1985) showed that fish muscle protein is very unstable and easily irreversibly denatured during handing and processing by DSC analysis. Wu et al. (1985) used DSC to investigate thermal transitions of fish mince (surimi) and actomyosin from croaker. They found out preheating samples containing 3% salt at various temperatures showed

that 40 °C heating caused the first peak to disappear, and preheating at temperatures higher than 50°C caused virtual disappearance of all transition peaks. Beas et al. (1991) studied thermal denaturation of myofibrillar proteins from pre- and post-spawning hake by DSC. Is this study, these authors also pointed out the denaturation enthalpies of all pre-spawning fish muscle extracts were less than those from post-spawning. In addition, between 40°C and 55°C the myosin denaturation rates were greater for post-spawning than for pre-spawning hake.

DSC was also employed in analyzing raw materials with a special interest in any relation of fat melting to emulsion stability (Townsend, 1968). Quinn et al. (1980) used DSC to monitor the changes in structural stability of beef proteins during processing of meat into sausage batter. Clearly, many researchers have found DSC to be a useful instrument in the food science field.

Stabursvik and Martens (1980) reported that the denaturation of a protein is detected as an endothermal peak as a function of increasing sample temperature - a DSC thermal curve. The transition due to denaturation is often referred to in terms of its peak maximum temperature. Thus, DSC can be used to study individual proteins in mixed systems such as meat, provided that the various proteins are denatured in different temperature ranges, yielding distinguishable peaks.

According to the report of Wright et al. (1977), when rabbit muscle was investigated by DSC, post-rigor muscle yielded three endothermic transitions with T_{max} values of 60, 67, and 80°C. Also, Stabursvik and Martens, (1980) showed that there were three major distinguishable peaks in published thermal curves of muscle tissue. Ensor et al. (1991) reported that the DSC thermal curves of beef semimembranosus muscle showed peaks at 58, 67, and 78°C. Compared with the purified proteins, these transitions corresponded to denaturation of myosin, sarcoplasmic proteins, and actin, respectively. Furthermore, the heat change associated with denaturation can be determined by measuring the area under the DSC transition curve (Wright et al, 1977). Stabursvik et al. (1984) pointed out that analyzed myofibrillar tissue using DSC, the thermal curves of myofibrillar tissue from normal pork were characterized by three major peaks with temperature maximums at 58°C and 66°C, associated with myosin denaturation, and 78°C, associated with actin denaturation. Therefore, the temperatures at which these proteins denature are consistent among muscles. Ledward and Lawrie (1975) reported that meat cooked at 80°C has a significantly greater resistance to shear when measured at 20°C than when measured at 70°C. Scanning calorimetry indicated that partial reversion of heat induced collagen in cooled, well cooked meat may explain the increased toughness of the cooled samples. Ensor (1991) found that from DSC thermal

curves, the transition of collagen was lowered from about 67°C to 58°C by the algin/calcium binder. Model systems showed that thermal transition temperatures of myofibrillar and sarcoplasmic proteins were lowered by 7.5°C (55.3 to 47.8°C) and 23.6 °C (68.1 to 44.5 °C), respectively when algin/calcium binder was used.

The DSC thermal curves can be affected by several treatments such as age, salt, or calcium alginate. Beilken and Harris (1987) found that DSC results obtained for samples from young and old animals were similar, but the peak assigned to connective tissue changed from 61°C to 65°C as an animal ages. Sodium chloride, which is the most common additive used in processed meat products, has been shown to affect the thermal properties of muscle proteins. For example, Quinn et al. (1980) found that the effect of the salt is to decrease the heat stability of the muscle proteins so that they denature and coagulate at a lower processing temperature. Barbut and Findlay (1991) pointed out increasing salt resulted in a decrease in myosin transition temperature and enthalpy; on the other hand, actin denaturation temperature tended to increase when salt was added but enthalpy decreased.

From the previously mentioned studies, it seems necessary to look for an improved technology to find out how aging and freezing affect beef muscle tenderness and what are the relationships among different bovine muscles. These factors will be examined in the following chapters.

CHAPTER III

IDENTIFYING THE COMPONENTS OF TENDERNESS USING DIFFERENTIAL SCANNING CALORIMETRY

ABSTRACT

This study was designed to identify the components of tenderness of four different muscles by using differential scanning calorimetry. The psoas major (PM), infraspinatus (IN), longissimus dorsi (LD), and semimembranosus (SM) muscles were taken from the right and left sides of six beef carcasses from cattle varying in biological type. Each muscle was cut into 2.5 cm steaks and one steak from each side was randomly assigned to an aging period of 2, 7, 14 or 21 days. Steaks from the left sides were aged prior to freezing, while steaks of the right side were frozen prior to aging. Warner-Bratzler shear force values were similar ($P > .05$) for both sides. Three cores from each steak were used for differential scanning calorimetry studies. Either two or three peaks were observed in the DSC thermograms. PM and IN muscles had the same beginning temperature (50°C) for the first peak (myosin). PM and IN were also similar between the thermograms displaying either two or three thermal peaks. LD and SM muscles displayed

thermograms having a beginning temperature of 49°C for three peaks and 55°C for two peaks. IN had higher transition temperatures than other muscles in both three and two thermal peaks. Thermograms having three peaks indicated a decrease in the beginning temperature for the first peak beyond 2 days of aging. The third peak had onset and peak temperatures with the lower denaturation temperatures at 21 days of aging. Thermograms having two peaks were not affected by aging except for the onset temperature of the first peak which decreased from 58.4°C to 58.1°C ($P < .05$). This study indicated more tender muscles were more heat stable than less tender muscles. In addition, the actin molecules were more affected by heat than the myosin molecules.

(Key words: Beef, Tenderness, Aging, Temperature, DSC.)

INTRODUCTION

Many studies have been conducted in attempts to identify factors associated with meat tenderness. Goll et al. (1963) found only a small relationship with meat tenderness and total collagen, while Cross et al.(1973) reported a significant relationship between collagen content of meat and tenderness. Other researchers such as

Mitchell et al. (1991) reported that tenderness of longissimus dorsi and semimembranosus steaks were improved with aging. However, Hiner and Hankins (1951) indicated that freezing did not affect the tenderness for the foreshank muscles. The use of differential scanning calorimetry (DSC) may afford a more direct method to study the individual proteins of whole muscle by their thermal behavior (Wright, et al. 1977). In addition, Martens et al. (1982) determined the thermal denaturation kinetics of actin in whole rabbit muscle.

The purpose of this study was to use differential scanning calorimetry to identify the components of tenderness in different beef muscles.

Materials and Methods

Meat Sample Preparation

Four individual beef muscles, psoas major (PM), infraspinatus (IN), longissimus dorsi (LD) and semimembranosus (SM), were obtained from the right and left sides of six beef carcasses from cattle varying in biological type. Muscles were cut into 2.5 cm steaks and assigned to an aging treatment of 2, 7, 14, or 21 days. Steaks from the right side of the carcasses were immediately frozen at -20°C, allowed to thaw for 18 hours, and aged for the 2, 7, 14, or 21 days at 5°C and then frozen at -20°C again. Steaks from the left side were aged

for 2, 7, 14, or 21 days at 5°C and then frozen at -20°C. In total, 48 steaks were examined from each muscle. Each steak was weighed and vacuum packaged.

Twelve steaks were removed from the freezer and three cores were taken from each steak randomly for DSC analysis. A Black and Decker 0.7 cm standing utility drill (The Black and Decker MFG. Co., Towson, MD) equipped with 0.7 cm coring bit was used to excise the cores from each frozen steak. These cores were placed in sterile Whirl-Pak bags (Pittsburgh, PA) and kept frozen. In order to accurately reflect the characteristics of the entire muscle, major fat deposits and connective tissue seams were avoided when removing the cores. Once cores were removed, the remaining steak was trimmed of all excess visible fat and connective tissue and pulverized for further analysis.

Differential Scanning Calorimetry

Cores were kept frozen throughout the study prior to use. Excessive connective tissue or intermuscular fat was avoided by carefully removing slices with a scalpel. Approximately ten mg of lean tissue were then placed in preweighed Perkin-Elmer volatile aluminum sample pans (Perkin-Elmer part No. 0219 -0062, Norwalk, CT) and crimped using a Perkin-Elmer volatile sealer assembly. The tissue was carefully packed in the aluminum sample pans such that the tissue was in direct contact with the entire bottom in order to eliminate possible variation in heat transfer.

Samples were placed in the platinum-iridium sample furnace of a Perkin-Elmer DSC 7 differential scanning calorimeter (Perkin-Elmer part No. N 519-0243, Norwalk, CT). The instrument was calibrated using a sample of bensil (m.p.= 95°C) and indium (m.p.= 156.6°C; ΔH= 6.80 cal/g) at a heating rate of 10°C/min. An empty sealed aluminum volatile sample pan was used as reference. Nitrogen gas (20 cc/min) was used for purging the exhaust away from the DSC sample holder. An ice water bath was used to maintain temperature control. Data collection and analysis were performed using an IBM Personal System/2 Model 55 SX computer equipped with Perkin-Elmer Thermal Analysis Controller (TAC 7/DX). The points at which measurements were taken are indicated along the curve and are associated with the beginning, ending, onset and peak temperatures, respectively. In addition, the transition energy (ΔH) was estimated from area under the curve (Figure 1). After analysis, the sample pans were removed from the DSC and weighed. The sample pans were then placed in a Fisher Scientific Isotemp Oven (Model 655F, Pittsburgh, PA) overnight at 102°C and reweighed for dry weight determinations.

Heat and moisture loss during thermal scanning were evident when the baseline of the thermal curve increased during heating. This loss could be due to over-crimping the sample pan causing a broken seal. These samples were discarded and new samples were run.

Warner-Bratzler Shear Analysis

Steaks were cooked at 190°C using an impingement oven (Model 1022, Food Service Products, Inc., Fort Wayne, IN) for 13 min to an internal temperature of 70°C. Steaks were cooled for two hours at room temperature (20°C). Six to eight 1.25 cm cores were taken with a Black and Decker Standing drill corer parallel to the muscle fibers. Each core was sheared using a Warner-Bratzler shearing device attached to an Instron Universal Testing Machine (Model 4502, Canton, MA). The Instron was fitted with a 1 kN load cell with a crosshead speed of 200 mm/min. Peak force was analyzed as an indication of tenderness.

Solubilization of Intramuscular Collagen

Frozen pulverized meat sample (6 g) was placed in a 50 ml centrifuge tube and 16 ml of a one-fourth Ringer's solution was added (Hill, 1966). Samples were heated in a water bath at 77 °C for 10 min and occasionally stirred. After centrifugation at 3,000 x g, the supernatant was decanted and the residues were washed with 8 ml of a one-fourth Ringer's solution. The supernatants were combined and washed after centrifugation. Supernatants and residues were hydrolyzed in 6 N HCl for 19 hours at 16-19 lbs pressure at 121 °C as described by Goll et al. (1963).

Hydroxyproline analysis

The hydroxyproline content was determined as outlined by Woessner (1961). The collagen content (mg/g) of the residue was multiplied by 7.25 and the supernatant by 7.52 as described by Cross et al. (1973).

Proximate Analysis

A 1.3 cm steak removed from the center portion of right and left sides of each muscle was evaluated for composition. Right side samples were frozen before aging, while left side samples were aged prior to being frozen. Samples were pulverized with liquid nitrogen and saved for analysis. Moisture was determined by drying, and fat by Soxhlet Extraction (AOAC, 1993). Protein was analyzed followed by combustion method for determination of crude protein in meat and meat products (AOAC, 1993).

pH Measurement

Five grams of pulverized meat sample was mixed with 50 ml distilled water using a magnetic stirrer. A probe-type combination electrode (Fisher Standard Ag/AgCl Model, Santa Ana, CA) with a pH meter (Corning 130) was used to measure pH.

Statistical Analysis

Analysis of variance and correlations were performed using the Statistical Analysis System (SAS, 1990). An

alpha level of $P < .05$ was used to determine significance. Multiple regression procedures were used to determine the relationship between tenderness and the objective parameters. The regression equations were built up stepwise starting with the parameter which correlated best and then determining the cumulative proportion of the variation in tenderness explained by the inclusion of other parameters.

Results and Discussion

DSC of the samples from the four different muscles in this study displayed variability in either exhibiting three (Figure 1) or two (Figure 2) thermal transitions. Some steaks from muscles had all three cores display either 2 or 3 transitions or peaks. Other steaks, had only one core display 2 peaks with the other two cores displaying 3 peaks. A possible reason that the first transition peak could not be distinguished as two transitions may be due to the first two peaks merging together because of differences in fiber type or muscle pH (Staburvik and Martens, 1980).

The means of the muscle characteristics for the four selected muscles used in the study showing thermal curves having three or two peaks are presented in Table 1. Shear values for muscles frozen prior to aging were not significantly different ($P > .05$) from the muscles aged prior to freezing. However, results of Warner-Bratzler shear (WBS) evaluations indicated different muscles had different shear values. The semimembranosus muscle had the highest value ($P < .05$), while psoas major had the lowest value ($P < .05$). These results are consistent with the results of McKeith et al. (1985) who reported the psoas major was the most tender muscle and semimembranosus was less tender. McKeith et al. (1985) and Patterson and Parrish (1986) reported that IN and LD were similar in tenderness. In this study, infraspinatus and longissimus

dorsi were not significantly different in WBS values (Table 1).

The pH values varied among muscles (Table 1). Steaks aged before freezing were not significantly different in pH than steaks frozen before aging. IN and PM muscles had the highest pH ($P < .05$) values followed by LD and SM muscles. It is evident that muscles with a higher pH had lower WBS values in this study. Many workers including Kelly et al. (1967), Bouton et al. (1971, 1973) and Penny et al. (1963) found that meat with a high pH was more tender than meat with a low pH. In addition, a greater percentage of samples from steaks having a high pH had 2 transitions (IN and PM), while those muscles (SM and LD) having a lower pH had predominately 3 transitions.

A large variation in composition was observed between muscles (Table 1). IN and PM had a higher ($P < .05$) fat content with SM having the least amount of fat. SM had the highest percent moisture content and PM had the lowest. Initial inspection of the relationship between fat content and WBS values suggested a possible correlation between the two. However, a low correlation was obtained between percent fat and WBS values ($r= -0.32$, $P < .01$). These results agree with Jennings et al. (1978) and Tatum et al. (1980) who indicated that as percent fat content increased, shear force values decreased. However, Parrish et al. (1973) reported that the degree of fat content had no effect on WBS values.

A significant difference in total collagen contents between the muscles was observed (Table 1). PM had the lowest collagen content compared to other muscles. This result agrees with work by Seideman (1986) and McKeith et al. (1985) who concluded that LD and SM had higher total collagen than PM muscle.

The decrease in Warner-Bratzler shear with aging differed between the selected muscles (Table 2). For cores having three peaks aging from 2 to 21 days decreased steaks shear values from 4.55 kN to 2.47 kN and 4.62 kN to 3.49 kN for IN and LD muscles, respectively; while shear values were not significantly changed ($P > .05$) by aging for PM and SM muscles. However, for cores having two peaks, shear values for LD, PM, and SM were not significantly ($p > .05$) affected by aging. The shear values for IN muscle decreased ($P < .05$) with aging from 2 to 7 days, but remained the same after 7 days of aging.

Thermal curves having three peaks had differences in peak characteristics between muscles (Table 3a). In the first peak which has been attributed to myosin (Wright et al. 1977; Stabursvik and Martens, 1980; Findlay and Stanley, 1984), PM and IN had a higher beginning temperature than LD and SM, while PM had a lower end temperature and peak temperatures than LD and SM. All muscles were not significantly different for the onset temperature ($P < .05$) of the first peak. PM had a lower

transition energy (ΔH_1) than LD ($P < .05$), but not significantly different from IN and SM ($P > .05$).

The second peak (Table 3a) which reflects primarily connective tissue and sarcoplasmic proteins (Wright et al. 1977; Stabursvik and Martens, 1980; Findlay and Stanley, 1984) had a beginning temperature that was equal to the ending temperature of the first peak. The IN muscle had a higher ending temperature than other muscles for the second peak, while PM had a lower peak temperature than the LD but was not significantly different ($P > .05$) from IN and SM muscles. The second transition energy (ΔH_2) was not significantly different among muscles.

The third peak (Table 3a), is attributed to actin (Wright et al. 1977; Stabursvik and Martens, 1980; Findlay and Stanley, 1984). IN and PM had a higher ending temperature than LD and SM. Also, IN had the highest onset and peak temperature than other muscles. The third transition energy (ΔH_3) and total transition energy (ΔH_t) were not significantly different among muscles.

If the first peak and the second peak do merge together, then the first peak (Figure 2) reflects both primary myosin and sarcoplasmic protein, while the second peak would reflect actin. The thermal peak characteristic of selected beef muscles having cores showing two peaks are presented in Table 3b. PM and IN had a lower beginning temperature than LD and SM, while PM and IN had a higher beginning temperature when 3 peaks were present (Table 3a).

The peak temperature was not significantly different amongst muscles for the first peak, while IN had a higher ΔH_1 then other muscles (Table 3b).

For the second peak (Table 3b), the IN muscle had a higher onset and peak temperature than other muscles, while ΔH_2 was not significantly different among muscles. Comparing total transition energy, IN, LD and PM had higher values than the SM muscle.

The DSC measurements having three thermal curves as affected by aging period are presented in Table 4a. The beginning temperature of the first peak decreased from 48.7°C to 47.5°C as aging increased from 2 to 7 days, but was not significantly different from 14 or 21 days of aging. Also, the end, onset, and peak temperatures were not significantly different ($P < .05$) between aging periods. The ending, onset, and peak temperatures were not significantly affected by aging for the second peak. The onset and peak temperatures shifted from 75.2°C to 74.8°C and from 79.1°C to 78.8°C as aging increased from 2 to 21 days for the third peak, respectively; while there were no differences for ΔH_1, ΔH_2, ΔH_3, and ΔH_t.

DSC measurements having thermal curves with two peaks are shown in Table 4b. Only the onset temperature decreased as aging increased from 2 to 21 days for the first peak. The other peak characteristics were not significantly affected by aging.

The thermal transitions for the IN muscle cores having three peaks and two peaks as a function of aging period are presented in Table 5a and Table 5b. The onset temperature increased as aging increased for the third peak. Enthalpy was not significantly affected by aging ($P < .05$). According to Findlay and Stanley (1984), the first and third transition decreased as aging increased from 2 to 8 days while the second transition was not affected by aging for beef sternomandibularis muscle. They also found that the energy of each peak and the total energy decreased as aging increased from 2 to 8 days. DSC measurements of the IN muscle showing thermal curves with two peaks indicating that the peak temperature of the second peak (Table 5b) was not significantly changed by aging, while ΔH_1 and ΔH_t decreased as aging increased.

Table 6a and Table 6b showed the DSC measurements of LD muscle showing thermal curves with three and two peaks as affected by aging period. The first and the second transition temperatures were not significantly ($P < .05$) affected by aging, while ΔH_2 decreased as aging increased (Table 6a).

Tables 7a and 7b show the DSC measurements of PM muscle showing thermal curves with three and two peaks as affected by aging period. The beginning temperature of the first peak decreased as aging increased (Table 7a). Moreover, the beginning, onset, and peak temperatures of the second peak decreased as aging increased (Table 7b).

Table 8a and Table 8b show the DSC measurements of SM muscle showing thermal curves with three and two peaks as affected by aging period. In the first peak, beginning temperature decreased as aging increased while peak temperature increased as aging increased (Table 8a). However, there were no significant differences with aging and thermal peak characteristics for cores showing two peaks (Table 8b).

Table 9a and Table 9b show correlation coefficients between the composition characteristics and DSC measurements with three and two peaks, respectively. The pH values and the percent fat were positively correlated to beginning and onset temperatures for the first peak; also, pH values and percent fat were positive correlated with beginning, ending, onset, and peak temperatures for the third peak (Table 9a); whereas the pH values and percent fat were negatively correlated with onset temperatures and peak temperatures for the first peak and negatively correlated with transition energy (Table 9a). On the contrary, Table 9b indicated that pH values and percent fat were negatively correlated with the beginning , onset, and peak temperatures for the first peak and positively correlated with transition energy.

Table 10a through 13b display correlation coefficients between individual muscles and DSC measurements. Total collagen was negatively correlated with the second and the third peaks for the LD but the correlation coefficient was

low. Total collagen showed no significant correlation with the denaturation temperatures on other muscles.

Linear regression coefficients and R^2 values for the regression linking WBS values obtained for characteristics of selected muscle showing thermal curves with three and two peaks are presented in Tables 14 and 15. There was a highly significant but negative linear relationship between pH and WBS values. The relationships were significantly improved by adding a quadratic term. The R^2 improved from 20.5% to 26.9% (Table 14) when moisture, total collagen, pk1 area, and pk2 area were fitted into the equation; while the R^2 improved from 28.4% to 36.4% (Table 15).

The regression equations obtained for individual muscles showing thermal curves with three peaks and two peaks were presented in Tables 16 and 17. The best equation in Table 16 for IN muscle was that when moisture, total collagen, and peak3 onset temperature entered the equations and R^2 went up to 35.6%. The best equation for LD muscle was when pH, moisture, peak1 end, peak1 area, and peak3 peak temperatures entered the equations. The best equation for PM muscle was when pH, peak2 begin, and peak3 end temperature entered the equation, whereas the best equation for SM muscle occurred when fat, total collagen and peak2 peak temperature entered the equation, but R^2 was low (10.5%).

Summary

In this study, shear values for muscles frozen prior to aging were not significantly different ($P > .05$) from the muscles aged prior to freezing, while the more tender muscles were more heat stable than less tender muscles. PM and IN muscles had the same beginning temperature (50°C) for the first peak (primary myosin) and were similar for the thermograms displaying either two or three thermal peaks. On the other hand, the LD and SM muscles displayed thermograms having a beginning temperature of 48°C for three peaks and 55°C for two peaks. Thermograms having three peaks showed a decrease in the beginning temperature for the first peak beyond 2 days of aging, while thermograms having two peaks were not affected by aging except for the onset temperature of the first peak.

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Table 1. Characteristics of steaks in selected muscles showing thermal curves with three and two peaks

Characteristics	Muscle[a] IN	LD	PM	SM	SE[b]
Three peaks					
WBS (kN)	4.00[d]	3.91[d]	3.09[e]	4.78[c]	0.07
pH	5.7[c]	5.5[d]	5.6[c]	5.3[e]	0.004
Fat %	7.1[c]	3.9[e]	6.1[d]	2.9[f]	0.0001
Moisture %	71.5[d]	71.1[d]	70.6[e]	73.0[c]	0.0001
Protein %	20.3[f]	22.9[c]	21.3[e]	22.5[d]	0.0001
Total collagen (mg/g)	12.8[c]	11.8[cd]	5.4[e]	10.9[d]	6.50
n	44	109	56	121	
Two peaks					
WBS(kN)	3.43[e]	4.23[d]	3.19[f]	4.80[c]	0.25
pH	5.7[c]	5.5[d]	5.7[c]	5.3[e]	0.004
Fat %	7.7[c]	3.8[e]	5.0[d]	3.2[e]	0.001
Moisture%	71.0[e]	71.8[d]	71.2[e]	72.5[c]	0.0001
Protein%	20.7[f]	22.7[c]	21.6[e]	22.0[d]	0.0001
Total collagen (mg/g)	11.9[c]	11.5[c]	4.6[d]	10.1[c]	5.90
n	100	35	88	21	

[a]IN=infraspinatus, LD=longissimus dorsi, PM=psoas major, SM=semimembranosus

[b]Standard error

[c,d,e,f]Means in same row with different superscripts differ ($P<0.05$)

Table 2. Warner-Bratzler shear values of selected muscles as affected by aging period

Characteristics	Aging period	Muscle[a] IN	LD	PM	SM	SE[b]
Three peaks	2	4.55[c]	4.62[c]	2.92[c]	4.71[c]	0.07
	7	3.63[d]	3.73[d]	3.25[c]	4.91[c]	
	14	2.90[de]	3.93[de]	3.20[c]	4.75[c]	
	21	2.47[e]	3.49[e]	3.01[c]	4.74[c]	
n		44	109	56	121	
		IN	LD	PM	SM	SE
Two peaks	2	4.22[c]	4.64[c]	3.01[c]	5.00[c]	0.25
	7	3.60[d]	4.04[c]	3.36[c]	4.78[c]	
	14	3.01[d]	4.08[c]	3.28[c]	4.69[c]	
	21	3.29[d]	4.26[c]	3.03[c]	4.31[c]	
n		100	35	88	21	

[a]IN=infraspinatus, LD=longissimus, PM=psoas major, SM=semimembranosus

[b]Standard error

[c,d,e]Means in same column with different superscripts differ ($P<0.05$)

Table 3a. Differential scanning calorimetry measurements of selected beef muscles showing thermal curves having three peaks

DSC measurement	Muscle[a] IN(44[b])	LD(109)	PM(56)	SM(121)	SE[c]
First peak					
Begin(°C)	49.0[d]	47.4[e]	49.2[d]	47.6[e]	2.2
End(°C)	59.2[de]	60.0[d]	58.8[e]	60.0[d]	5.0
Onset(°C)	51.7[d]	50.7[d]	51.5[d]	51.1[d]	6.5
Peak(°C)	55.1[de]	55.4[d]	54.2[e]	55.7[d]	6.5
Second peak					
Begin(°C)	59.2[de]	60.0[d]	58.8[e]	60.0[d]	5.0
End(°C)	74.1[d]	72.9[e]	72.5[e]	72.8[e]	0.9
Onset(°C)	61.2[d]	61.5[d]	60.6[d]	61.6[d]	8.6
Peak(°C)	65.3[de]	66.3[d]	65.0[e]	65.9[de]	5.5
Third peak					
Begin(°C)	74.1[d]	72.9[e]	72.5[e]	72.8[e]	0.9
End(°C)	83.8[d]	82.7[e]	83.9[d]	82.8[e]	2.5
Onset(°C)	76.4[d]	74.9[e]	74.7[e]	74.9[e]	0.8
Peak(°C)	80.1[d]	78.7[e]	78.9[e]	78.8[e]	0.5
ΔH_1[f](j/g)	0.34[de]	0.45[d]	0.29[e]	0.43[de]	0.08
ΔH_2(j/g)	0.89[d]	0.76[d]	0.76[d]	0.86[d]	0.12
ΔH_3(j/g)	0.63[d]	0.67[d]	0.67[d]	0.72[d]	0.08
ΔH_t(j/g)	1.85[d]	1.89[d]	1.73[d]	2.00[d]	0.57

[a]IN=infraspinatus, LD=longissimus, PM=psoas major, SM=semimembranosus

[b]Number of observations

[c]Standard error

[d,e]Means in same row with different superscripts differ ($P<0.05$)

[f]ΔH_1=First peak enthalpy, ΔH_2=second peak enthalpy, ΔH_3=third peak enthalpy, ΔH_t=total enthalpy

Table 3b. Differential scanning calorimetry measurements of selected beef muscles showing thermal curves with two peaks

	Muscle[a]				
DSC measurement	IN(100[b])	LD(35)	PM(88)	SM(21)	SE[c]
First peak					
Begin(°C)	51.4[e]	55.9[d]	51.4[e]	54.7[d]	12.3
End(°C)	74.1[d]	72.9[e]	72.6[e]	72.3[e]	1.8
Onset(°C)	58.3[e]	59.7[d]	58.6[de]	59.6[de]	5.2
Peak(°C)	64.9[d]	65.8[d]	64.8[d]	65.8[d]	3.1
Second peak					
Begin(°C)	74.1[d]	72.9[e]	72.6[e]	72.3[e]	1.8
End(°C)	84.2[d]	83.2[d]	84.4[d]	82.9[d]	5.2
Onset(°C)	76.7[d]	75.3[e]	74.8[e]	74.7[e]	1.0
Peak(°C)	80.5[d]	79.0[e]	79.1[e]	78.8[e]	0.4
ΔH_1[f](j/g)	1.24[d]	0.88[e]	0.91[e]	0.59[e]	0.27
ΔH_2(j/g)	0.68[d]	0.72[d]	0.72[d]	0.64[d]	0.07
ΔH_t(j/g)	1.89[d]	1.60[de]	1.62[de]	1.23[e]	0.57

[a]IN=infraspinatus, LD=longissimus, PM=psoas major, SM=semimembranosus

[b]Number of observations

[c]Standard error

[d,e]Means in same row with different superscripts differ ($P<0.05$)

[f]ΔH_1=First peak enthalpy, ΔH_2=second peak enthalpy, ΔH_t=total enthalpy

Table 4a. Differential scanning calorimetry measurements of selected beef muscles showing thermal curves having three peaks over aging period

	Aging period (day)				
DSC measurement	2(91[a])	7(72)	14(79)	21(88)	SE[b]
First peak					
Begin(°C)	48.7[c]	47.5[d]	47.9[d]	47.8[d]	2.2
End(°C)	59.0[d]	60.2[c]	59.7[cd]	59.8[cd]	5.0
Onset(°C)	50.7[c]	51.3[c]	51.2[c]	51.2[c]	6.5
Peak(°C)	54.6[c]	55.5[c]	55.4[c]	55.5[c]	6.5
Second peak					
Begin(°C)	59.0[d]	60.2[c]	59.7[cd]	59.8[cd]	5.0
End(°C)	73.0[c]	73.1[c]	72.9[c]	72.8[c]	0.9
Onset(°C)	61.2[c]	61.7[c]	61.5[c]	61.5[c]	8.6
Peak(°C)	65.6[c]	66.2[c]	65.9[c]	65.6[c]	5.5
Third peak					
Begin(°C)	73.0[c]	73.1[c]	72.9[c]	72.8[c]	0.9
End(°C)	82.9[c]	83.3[c]	83.2[c]	83.1[c]	2.5
Onset(°C)	75.2[c]	75.3[c]	74.9[cd]	74.8[d]	0.8
Peak(°C)	79.1[c]	79.1[c]	79.0[cd]	78.8[d]	0.5
ΔH_1[e](j/g)	0.32[d]	0.46[c]	0.42[cd]	0.42[cd]	0.08
ΔH_2(j/g)	0.82[c]	0.82[c]	0.83[c]	0.79[c]	0.12
ΔH_3(j/g)	0.64[c]	0.73[c]	0.70[c]	0.68[c]	0.08
ΔH_t(j/g)	1.78[c]	2.00[c]	1.93[c]	1.90[c]	0.57

[a]=Number of observations
[b]=Standard error
[c,d]Means in same row with different superscripts differ ($P<0.05$)
[e]ΔH_1=First peak enthalpy, ΔH_2=second peak enthalpy, ΔH_3=third peak enthalpy, ΔH_t=total enthalpy

Table 4b. Differential scanning calorimetry measurements of selected beef muscles showing thermal curves with two peaks over aging period

	Aging period (day)				
DSC measurement	2 (53[a])	7 (72)	14 (62)	21 (56)	SE[b]
First peak					
Begin (°C)	52.7[c]	52.1[c]	53.7[c]	51.8[c]	12.3
End (°C)	73.1[c]	73.5[c]	73.1[c]	73.2[c]	1.8
Onset (°C)	59.4[c]	58.9[d]	58.5[d]	58.1[e]	5.2
Peak (°C)	65.6[c]	65.0[c]	64.7[c]	64.9[c]	3.1
Second peak					
Begin (°C)	73.1[c]	73.5[c]	73.1[c]	73.2[c]	1.8
End (°C)	84.3[c]	84.0[c]	83.4[c]	84.6[c]	5.2
Onset (°C)	75.4[c]	75.8[c]	75.7[c]	75.6[c]	1.0
Peak (°C)	79.5[c]	79.8[c]	79.4[c]	79.7[c]	0.4
ΔH_1[f] (j/g)	1.05[c]	1.09[c]	0.92[c]	0.99[c]	0.27
ΔH_2 (j/g)	0.77[c]	0.70[c]	0.62[c]	0.72[c]	0.07
ΔH_t (j/g)	1.82[c]	1.75[c]	1.54[c]	1.71[c]	0.57

[a]Number of observations
[b]Standard error
[c,d,e]Means in same row with different superscripts differ ($P<0.05$)
[f]ΔH_1=First peak enthalpy, ΔH_2=second peak enthalpy, ΔH_t=total enthalpy

Table 5a. Differential scanning calorimetry measurements of infraspinatus muscle showing thermal curves having three peaks over aging period

	Aging period (day)				
DSC measurement	2(24[a])	7(7)	14(6)	21(7)	SE[b]
First peak					
Begin(°C)	49.2[c]	48.9[c]	48.9[c]	48.8[c]	4.7
End(°C)	58.7[c]	61.6[c]	58.9[c]	58.7[c]	6.8
Onset(°C)	50.9[c]	53.8[c]	52.5[c]	51.7[c]	11.8
Peak(°C)	54.6[c]	56.5[c]	55.2[c]	55.4[c]	19.0
Second peak					
Begin(°C)	58.7[c]	61.6[c]	58.9[c]	58.7[c]	6.8
End(°C)	73.8[c]	74.3[c]	74.3[c]	74.5[c]	1.3
Onset(°C)	60.8[c]	63.1[c]	62.0[c]	60.0[c]	18.5
Peak(°C)	65.1[c]	67.4[c]	65.4[c]	63.5[c]	45.4
Third peak					
Begin(°C)	73.8[c]	74.3[c]	74.3[c]	74.5[c]	1.3
End(°C)	83.6[c]	83.7[c]	84.2[c]	84.2[c]	0.9
Onset(°C)	76.1[d]	76.2[d]	76.9[c]	77.0[c]	0.2
Peak(°C)	80.0[c]	80.2[c]	80.2[c]	80.4[c]	0.3
ΔH_1[e](j/g)	0.27[c]	0.69[c]	0.33[c]	0.25[c]	0.21
ΔH_2(j/g)	0.86[c]	0.89[c]	1.05[c]	0.84[c]	0.05
ΔH_3(j/g)	0.63[c]	0.69[c]	0.67[c]	0.55[c]	0.03
ΔH_t(j/g)	1.76[c]	2.26[c]	2.01[c]	1.62[c]	0.54

[a]Number of observations
[b]Standard error
[c,d]Means in same row with different superscripts differ ($P<0.05$)
[e]ΔH_1=First peak enthalpy, ΔH_2=second peak enthalpy, ΔH_3=third peak enthalpy, ΔH_t=total enthalpy

Table 5b. Differential scanning calorimetry measurements of infraspinatus muscle showing thermal curves with two peaks over aging period

	Aging period (day)				
DSC measurement	2(12[a])	7(29)	14(30)	21(29)	SE[b]
First peak					
Begin(°C)	50.7[c]	50.0[c]	51.9[c]	52.7[c]	14.2
End(°C)	73.6[c]	74.4[c]	74.1[c]	73.9[c]	1.5
Onset(°C)	59.0[c]	58.3[c]	57.9[c]	58.4[c]	1.7
Peak(°C)	64.4[c]	64.7[c]	64.9[c]	65.1[c]	2.0
Second peak					
Begin(°C)	73.6[c]	74.4[c]	74.1[c]	73.9[c]	1.5
End(°C)	84.0[c]	84.5[c]	84.0[c]	84.3[c]	2.3
Onset(°C)	76.1[e]	76.9[c]	76.9[c]	76.6[d]	0.1
Peak(°C)	80.0[d]	81.0[c]	80.3[d]	80.4[d]	0.2
ΔH_1[f](j/g)	1.52[c]	1.48[c]	1.09[d]	1.04[d]	0.15
ΔH_2(j/g)	0.79[c]	0.77[c]	0.63[c]	0.62[c]	0.07
ΔH_t(j/g)	2.31[c]	2.14[cd]	2.17[cd]	1.66[d]	0.42

[a]Number of observations
[b]Standard error
[c,d,e]Means in same row with different superscripts differ ($P<0.05$)
[f]ΔH_1=First peak enthalpy, ΔH_2=second peak enthalpy, ΔH_t=total enthalpy

Table 6a. Differential scanning calorimetry measurements of longissimus dorsi muscle showing thermal curves having three peaks over aging period

	Aging period (day)				
DSC measurement	2(26[b])	7(24)	14(24)	21(35)	SE[c]
First peak					
Begin(°C)	47.8[c]	46.6[c]	46.7[c]	48.1[c]	4.2
End(°C)	59.2[c]	59.5[c]	59.9[c]	60.7[c]	8.4
Onset(°C)	49.7[c]	50.9[c]	50.1[c]	51.7[c]	13.3
Peak(°C)	54.9[c]	55.4[c]	54.9[c]	56.0[c]	3.3
Second peak					
Begin(°C)	59.2[c]	59.5[c]	59.9[c]	60.7[c]	8.4
End(°C)	72.7[c]	73.2[c]	72.8[c]	72.9[c]	0.6
Onset(°C)	61.5[c]	61.0[c]	61.1[c]	62.1[c]	3.0
Peak(°C)	66.2[c]	66.4[c]	66.3[c]	66.4[c]	1.0
Third peak					
Begin(°C)	72.7[c]	73.2[c]	72.8[c]	72.9[c]	0.6
End(°C)	82.5[c]	83.1[c]	83.2[c]	82.3[c]	1.9
Onset(°C)	75.0[c]	75.4[c]	74.6[c]	74.7[c]	1.9
Peak(°C)	78.8[c]	78.8[c]	78.7[c]	78.6[c]	1.3
ΔH_1[f](j/g)	0.30[d]	0.44[cd]	0.56[c]	0.48[cd]	0.05
ΔH_2(j/g)	0.76[d]	0.83[c]	0.78[cd]	0.70e	0.01
ΔH_3(j/g)	0.68[c]	0.68[c]	0.75[c]	0.61[c]	0.08
ΔH_t(j/g)	1.74[c]	2.00[c]	2.06[c]	1.80[c]	0.17

[a]Number of observations
[b]Standard error of mean
[c,d,e]Means in same row with different superscripts differ ($P<0.05$)
[f]ΔH_1=First peak enthalpy, ΔH_2=second peak enthalpy, ΔH_3=third peak enthalpy, ΔH_t=total enthalpy

Table 6b. Differential scanning calorimetry measurements of longissimus dorsi muscle showing thermal curves with two peaks over aging period

	Aging period (day)				
DSC measurement	2(10[a])	7(12)	14(12)	21(1)	SE[b]
First peak					
Begin(°C)	55.3[c]	55.4[c]	57.6[c]	50.4[c]	41.6
End(°C)	73.2[c]	73.3[c]	72.4[c]	73.2[c]	0.6
Onset(°C)	61.2[c]	58.6[c]	60.2[c]	54.0[c]	15.5
Peak(°C)	73.3[c]	66.1[c]	64.1[c]	67.2[c]	1.4
Second peak					
Begin(°C)	73.2[c]	73.3[c]	72.4[c]	73.2[c]	0.6
End(°C)	84.0[c]	82.9[c]	82.9[c]	82.6[c]	3.2
Onset(°C)	75.6[c]	75.9[c]	74.6[c]	75.2[c]	1.1
Peak(°C)	79.4[c]	79.2[c]	78.5[c]	79.2[c]	0.5
ΔH_1[d](j/g)	1.01[c]	0.78[c]	0.82[c]	1.31[c]	0.95
ΔH_2(j/g)	0.85[c]	0.61[c]	0.72[c]	0.73[c]	0.15
ΔH_t(j/g)	1.86[c]	1.39[c]	1.54[c]	2.10[c]	1.71

[a]Number of observations
[b]Standard error
[c]Means in same row with different superscripts differ ($P<0.05$)
[d]ΔH_1=First peak enthalpy, ΔH_2=second peak enthalpy, ΔH_t=total enthalpy

Table 7a. Differential scanning calorimetry measurements of psoas major muscle showing thermal curves having three peaks over aging period

	Aging period (day)				
DSC measurement	2(15[a])	7(10)	14(18)	21(13)	SE[b]
First peak					
Begin(°C)	50.5[c]	49.1[d]	49.0[d]	48.5[d]	0.7
End(°C)	58.6[c]	59.5[c]	58.3[c]	59.2[c]	1.6
Onset(°C)	51.9[c]	51.4[c]	51.3[c]	51.5[c]	1.1
Peak(°C)	53.8[c]	53.5[c]	54.0[c]	55.0[c]	6.1
Second peak					
Begin(°C)	58.6[c]	59.5[c]	58.3[c]	59.2[c]	1.6
End(°C)	73.1[c]	73.1[c]	71.8[d]	72.4[cd]	0.5
Onset(°C)	60.6[c]	61.7[c]	60.1[c]	60.7[c]	10.4
Peak(°C)	65.3[c]	65.5[c]	64.6[c]	64.8[c]	0.7
Third peak					
Begin(°C)	73.1[c]	73.1[c]	71.8[d]	72.4[cd]	0.5
End(°C)	83.8[c]	85.0[c]	82.6[c]	85.1[c]	13.1
Onset(°C)	74.9[c]	75.0[c]	74.3[c]	74.7[c]	0.7
Peak(°C)	79.2[c]	79.2[c]	78.7[c]	78.7[c]	0.2
ΔH_1[e](j/g)	0.35[c]	0.24[c]	0.25[c]	0.33[c]	0.06
ΔH_2(j/g)	0.89[c]	0.85[c]	0.67[c]	0.72[c]	0.15
ΔH_3(j/g)	0.62[c]	0.70[c]	0.63[c]	0.78[c]	0.10
ΔH_t(j/g)	1.83[c]	1.78[c]	1.55[c]	1.83[c]	0.82

[a]Number of observations
[b]Standard error
[c,d]Means in same row with different superscripts differ ($P<0.05$)
[e]ΔH_1=First peak enthalpy, ΔH_2=second peak enthalpy, ΔH_3=third peak enthalpy, ΔH_t=total enthalpy

Table 7b. Differential scanning calorimetry measurements of psoas major muscle showing thermal curves with two peaks over aging period

	Aging period (day)				
DSC measurement	2(21[a])	7(26)	14(18)	21(23)	SE[b]
First peak					
Begin(°C)	52.5^{cd}	52.2^{cd}	53.4^{c}	50.2^{d}	7.4
End(°C)	73.4^{c}	72.8^{cd}	71.9^{e}	72.3^{de}	0.5
Onset(°C)	59.4^{c}	59.4^{c}	58.1^{c}	57.4^{c}	6.6
Peak(°C)	65.5^{c}	64.7^{c}	64.4^{c}	64.4^{c}	1.5
Second peak					
Begin(°C)	73.4^{c}	72.8^{cd}	71.9^{e}	72.3^{de}	0.5
End(°C)	84.9^{c}	84.3^{c}	82.8^{c}	85.2^{c}	12.1
Onset(°C)	75.4^{c}	74.8^{cd}	74.5^{d}	74.3^{d}	0.6
Peak(°C)	79.6^{c}	79.2^{d}	78.5^{e}	79.1^{d}	0.1
ΔH_1[f](j/g)	0.97^{c}	0.91^{c}	0.74^{c}	0.97^{c}	0.15
ΔH_2[f](j/g)	0.75^{cd}	0.70^{cd}	0.55^{d}	0.85^{c}	0.07
ΔH_t[f](j/g)	1.72^{c}	1.60^{c}	1.29^{c}	1.83^{c}	0.38

[a]Number of observations
[b]Standard error
[c,d,e]Means in same row with different superscripts differ ($P<0.05$)
[f]ΔH_1=First peak enthalpy, ΔH_2=seeond peak enthalpy, ΔH_t=total enthalpy

Table 8a. Differential scanning calorimetry measurements of semimembranosus muscle showing thermal curves having three peaks over aging period

DSC measurement	Aging period (day) 2(26[a])	7(31)	14(31)	21(33)	SE[b]
First peak					
Begin(°C)	48.1[c]	47.4[cd]	48.0[c]	46.9[d]	1.0
End(°C)	59.4[d]	60.6[c]	60.6[c]	59.4[d]	1.8
Onset(°C)	50.8[cd]	51.1[cd]	51.8[c]	50.5[d]	1.9
Peak(°C)	54.7[f]	56.1[d]	56.7[c]	55.3[e]	0.4
Second peak					
Begin(°C)	59.4[d]	60.6[c]	60.6[c]	59.4[d]	1.8
End(°C)	72.4[d]	72.8[cd]	73.2[c]	72.5[d]	0.4
Onset(°C)	61.4[c]	61.9[c]	62.4[c]	61.4[c]	3.0
Peak(°C)	65.7[d]	66.1[cd]	66.5[c]	65.4[d]	0.6
Third peak					
Begin(°C)	72.4[d]	72.8[cd]	73.2[c]	72.5[d]	0.4
End(°C)	82.4[d]	82.8[cd]	83.3[c]	82.9[cd]	0.5
Onset(°C)	74.9[c]	75.0[c]	75.2[c]	74.5[c]	1.1
Peak(°C)	78.6[d]	79.0[c]	79.0[c]	78.6[d]	0.1
ΔH_1[g](j/g)	0.36[c]	0.49[c]	0.42[c]	0.43[c]	0.03
ΔH_2(j/g)	0.80[c]	0.80[c]	0.91[c]	0.91[c]	0.25
ΔH_3(j/g)	0.64[c]	0.79[c]	0.71[c]	0.73[c]	0.14
ΔH_t(j/g)	1.80[c]	2.02[c]	2.04[c]	2.10[c]	1.01

[a]Number of observations
[b]Standard error
[c,d,e,f]Means in same row with different superscripts differ ($P<0.05$)
[g]ΔH_1=first peak enthalpy, ΔH_2=seeond peak enthalpy, ΔH_3=third peak enthalpy, ΔH_t=total enthalpy

Table 8b. Differential scanning calorimetry measurements of semimembranosus muscle showing thermal curves with two peaks over aging period

	Aging period (day)				
DSC measurement	2(10[a])	7(5)	14(2)	21(3)	SE[b]
First peak					
Begin(°C)	53.0[c]	55.5[c]	59.5[c]	56.2[c]	10.1
End(°C)	71.9[c]	72.5[c]	73.8[c]	72.3[c]	7.9
Onset(°C)	58.2[c]	60.5[c]	61.0[c]	62.1[c]	12.6
Peak(°C)	65.5[c]	65.8[c]	67.1[c]	65.6[c]	5.6
Second peak					
Begin(°C)	71.9[c]	72.5[c]	73.8[c]	72.3[c]	7.9
End(°C)	83.5[c]	81.8[c]	83.3[c]	82.6[c]	4.0
Onset(°C)	74.7[c]	74.4[c]	76.1[c]	74.4[c]	1.5
Peak(°C)	78.8[c]	78.4[c]	79.9[c]	78.7[c]	0.7
ΔH_1[d](j/g)	0.69[c]	0.50[c]	0.49[c]	0.48[c]	0.14
ΔH_2(j/g)	0.68[c]	0.54[c]	0.65[c]	0.65[c]	0.03
ΔH_t(j/g)	1.37[c]	1.05[c]	1.14[c]	1.13[c]	0.22

[a]Number of observations
[b]Standard error
[c]Means in same row with different superscripts differ ($P<0.05$)
[d]ΔH_1=First peak enthalpy, ΔH_2=seeond peak enthalpy, ΔH_t=total enthalpy

Table 9a. Correlation coefficients between characteristics of selected muscles and DSC measurements showing thermal curves having three peaks

DSC measurement	pH	Fat	Moisture	Protein	Total collagen	WBS
First peak						
Begin(°C)	0.25**	0.14*	-0.10	-0.16**	-0.13*	-0.12*
End(°C)	-0.14*	-0.15*	0.18**	0.08	0.06	0.03
Onset(°C)	0.13*	0.03	-0.01	-0.06	-0.04	-0.07
Peak(°C)	-0.12*	-0.14*	0.17**	0.06	0.10	0.06
Second peak						
Begin(°C)	-0.14*	-0.15*	0.18**	0.08	0.06	0.03
End(°C)	0.16**	0.21**	-0.06	-0.21**	0.12*	-0.01
Onset(°C)	-0.13	-0.13*	0.15*	0.01	0.08	0.04
Peak(°C)	0.10	-0.09	0.09	0.04	0.14*	0.03
Third peak						
Begin(°C)	0.16**	0.21**	-0.06	-0.21**	0.12*	-0.01
End(°C)	0.16**	0.19**	-0.07	-0.21**	-0.15**	-0.10
Onset(°C)	0.21**	0.20**	-0.01	-0.20**	0.12*	0.01
Peak(°C)	0.26**	0.27**	-0.06	-0.28**	0.05	-0.02
ΔH_1^e(j/g)	-0.16**	-0.10	0.12*	0.10	0.18*	0.01
ΔH_2^e(j/g)	-0.06	0.10	-0.07	-0.05	0.02	0.03
ΔH_3^e(j/g)	-0.30**	0.27**	-0.06	-0.28**	-0.05	0.02
ΔH_t^e(j/g)	-0.13*	-0.01	-0.03	0.03	0.03	0.05
N	320	320	320	320	320	330

*P<0.05
**P<0.01

Table 9b. Correlation coefficients between characteristics of selected muscles and DSC measurements showing thermal curves with two peaks

DSC measurement	pH	Fat	Moisture	Protein	Total collagen	WBS
First peak						
Begin(°C)	-0.23**	-0.19**	0.11	0.13*	0.05	0.12
End(°C)	0.19**	0.28**	-0.10	-0.21**	0.22**	-0.06
Onset(°C)	-0.13	-0.09	0.11	0.02	0.02	0.06
Peak(°C)	-0.10	-0.10	0.06	0.07	0.03	0.14*
Second peak						
Begin(°C)	0.19**	0.28**	-0.10	-0.21**	0.22**	-0.06
End(°C)	0.17**	0.05	0.02	-0.02	-0.09	-0.11
Onset(°C)	0.21**	0.38**	-0.13*	-0.23**	0.34**	-0.10
Peak(°C)	0.24*	0.33**	-0.08	-0.27**	0.30	-0.15*
ΔH_1^e(j/g)	0.22**	0.21**	-0.05	-0.14*	0.09	-0.03
ΔH_2^e(j/g)	0.03	-0.09	0.10	0.19**	-0.06	0.03
ΔH_t^e(j/g)	0.18**	0.13*	-0.01	-0.06	0.05	-0.01
N	241	241	241	241	241	243

*P<0.05
**P<0.01

Table 10a. Correlation coefficients between characteristics of infraspinatus muscle and DSC measurements showing thermal curves having three peaks (N=44)

DSC measurement	pH	Fat	Moisture	Protein	Total collagen	WBS
First peak						
Begin(°C)	-0.20	-0.38*	0.06	-0.29	0.14	0.01
End(°C)	-0.03	-0.21	0.33*	-0.06	0.13	-0.10
Onset(°C)	0.01	-0.33*	0.20	-0.21	0.23	-0.22
Peak(°C)	-0.10	-0.11	0.25	-0.10	0.19	-0.15
Second peak						
Begin(°C)	-0.03	-0.21	0.33*	-0.06	0.13	-0.10
End(°C)	0.13	-0.09	-0.04	-0.22	0.03	-0.22
Onset(°C)	-0.13	-0.30*	0.16	-0.08	0.04	0.01
Peak(°C)	-0.02	-0.17	0.20	-0.09	0.18	-0.03
Third peak						
Begin(°C)	0.13	-0.09	-0.04	-0.22	0.03	-0.22
End(°C)	-0.04	-0.22	0.02	0.03	-0.16	-0.15
Onset(°C)	0.36*	-0.12	-0.07	-0.32*	0.05	-0.25
Peak(°C)	0.31*	-0.12	0.05	-0.15	-0.07	-0.21
ΔH_1^e(j/g)	0.05	-0.05	0.25	0.01	0.19	-0.15
ΔH_2^e(j/g)	0.05	0.13	-0.20	0.14	-0.12	-0.08
ΔH_3^e(j/g)	-0.23	-0.07	-0.14	0.17	-0.27	0.13
ΔH_t^e(j/g)	-0.02	0.03	-0.03	0.13	-0.03	-0.07

*P<0.05
**P<0.01

Table 10b. Correlation coefficients between characteristics of infraspinatus muscle and DSC measurements showing thermal curves with two peaks (N=100)

DSC measurement	pH	Fat	Moisture	Protein	Total collagen	WBS
First peak						
Begin(°C)	-0.02	-0.18	0.22*	-0.03	0.02	-0.03
End(°C)	0.06	0.02	-0.03	-0.15	0.03	-0.11
Onset(°C)	-0.06	-0.08	0.13	-0.05	0.04	0.05
Peak(°C)	-0.01	-0.12	0.09	0.05	-0.02	-0.03
Second peak						
Begin(°C)	0.06	0.02	-0.03	-0.15	0.03	-0.11
End(°C)	0.14	0.01	0.03	0.17	-0.12	0.04
Onset(°C)	0.14	-0.09	0.02	0.12	-0.07	-0.27**
Peak(°C)	0.08	-0.13	0.11	0.02	0.16	-0.06
ΔH_1^e(j/g)	0.14	0.12	-0.12	-0.01	-0.05	0.10
ΔH_2^e(j/g)	0.10	-0.08	0.09	0.38**	-0.12	0.15
ΔH_t^e(j/g)	0.14	0.10	-0.09	0.07	-0.08	0.15

*P<0.05
**P<0.01

Table 11a. Correlation coefficients between characteristics of longissimus dorsi muscle and DSC measurements showing thermal curves having three peaks (N=109)

DSC measurement	pH	Fat	Moisture	Protein	Total collagen	WBS
First peak						
Begin(°C)	-0.01	0.05	-0.01	-0.09	-0.09	0.05
End(°C)	-0.04	-0.09	0.18	-0.02	-0.25**	-0.10
Onset(°C)	0.03	0.08	-0.08	-0.05	-0.22**	-0.06
Peak(°C)	-0.02	-0.01	0.09	-0.04	-0.14	-0.06
Second peak						
Begin(°C)	-0.04	-0.09	0.18	-0.02	-0.25**	-0.10
End(°C)	-0.01	0.23*	-0.13	-0.20*	-0.27*	0.01
Onset(°C)	-0.07	-0.03	0.15	-0.18	-0.20*	-0.06
Peak(°C)	0.04	0.03	0.10	-0.23*	-0.18	0.04
Third peak						
Begin(°C)	-0.01	0.23*	-0.13	-0.20*	-0.27*	0.01
End(°C)	0.01	0.17	-0.06	-0.29**	-0.16	0.01
Onset(°C)	0.02	0.15	-0.07	-0.16	-0.21*	0.06
Peak(°C)	-0.01	0.24*	-0.11	-0.26**	-0.13	0.07
ΔH_1^e(j/g)	0.11	0.07	-0.04	-0.02	-0.22*	-0.10
ΔH_2^e(j/g)	0.11	0.21*	-0.23*	-0.11	-0.06	0.06
ΔH_3^e(j/g)	0.08	-0.08	0.06	-0.11	0.07	0.04
ΔH_t^e(j/g)	0.11	0.11	-0.10	-0.10	-0.06	0.04

*P<0.05
**P<0.01

Table 11b. Correlation coefficients between characteristics of longissimus dorsi muscle and DSC measurements showing thermal curves with two peaks (N=35)

DSC measurement	pH	Fat	Moisture	Protein	Total collagen	WBS
First peak						
Begin(°C)	-0.16	-0.08	0.03	0.23	0.08	-0.13
End(°C)	-0.07	0.08	-0.01	-0.28	-0.20	-0.06
Onset(°C)	-0.18	-0.10	0.13	0.17	0.13	0.01
Peak(°C)	0.21	0.02	0.05	-0.35*	0.05	0.15
Second peak						
Begin(°C)	-0.07	0.08	-0.01	-0.28	-0.20	-0.06
End(°C)	-0.21	-0.23	0.26	0.04	0.06	-0.29
Onset(°C)	-0.10	-0.03	0.10	-0.31	-0.14	-0.23
Peak(°C)	-0.07	-0.02	0.11	-0.34*	-0.19	-0.23
ΔH_1^e(j/g)	0.20	-0.07	0.16	-0.11	-0.09	0.09
ΔH_2^e(j/g)	0.11	-0.17	0.22	-0.10	0.09	0.03
ΔH_t^e(j/g)	0.21	-0.11	0.16	-0.12	-0.06	0.09

*P<0.05
**P<0.01

Table 12a. Correlation coefficients between characteristics of psoas major muscle and DSC measurements showing thermal curves having three peaks (N=56)

DSC measurement	pH	Fat	Moisture	Protein	Total collagen	WBS
First peak						
Begin(°C)	0.30*	-0.09	-0.02	0.43**	-0.22	-0.18
End(°C)	0.01	0.14	-0.07	-0.05	-0.22	-0.28*
Onset(°C)	0.29*	-0.08	0.04	0.21	-0.14	-0.31*
Peak(°C)	0.10	0.03	-0.01	0.03	-0.16	-0.24
Second peak						
Begin(°C)	0.01	0.14	-0.07	-0.05	-0.22	-0.28*
End(°C)	-0.06	0.16	-0.15	-0.08	0.12	-0.13
Onset(°C)	0.03	0.03	-0.01	0.01	-0.08	-0.17
Peak(°C)	-0.01	0.21	-0.23	-0.01	0.07	-0.15
Third peak						
Begin(°C)	-0.06	0.16	-0.15	-0.08	0.12	-0.13
End(°C)	-0.27*	0.04	0.02	-0.16	-0.08	0.01
Onset(°C)	-0.11	0.16	-0.19	-0.04	0.14	0.01
Peak(°C)	-0.14	0.09	-0.09	-0.11	0.08	-0.09
ΔH_1^e(j/g)	-0.17	0.08	0.06	-0.26	-0.14	-0.22
ΔH_2^e(j/g)	-0.10	0.08	-0.01	-0.17	0.11	-0.10
ΔH_3^e(j/g)	-0.20	0.03	0.02	-0.17	-0.15	-0.12
ΔH_t^e(j/g)	-0.17	0.07	0.02	-0.23	-0.06	-0.16

*P<0.05
**P<0.01

Table 12b. Correlation coefficients between characteristics of psoas major muscle and DSC measurements showing thermal curves with two peaks (N=88)

DSC measurement	pH	Fat	Moisture	Protein	Total collagen	WBS
First peak						
Begin(°C)	0.04	0.20	-0.19	-0.01	0.01	-0.09
End(°C)	0.15	0.08	-0.08	0.01	0.06	0.07
Onset(°C)	-0.04	0.05	0.01	-0.06	-0.09	-0.11
Peak(°C)	0.01	0.04	-0.10	0.02	-0.05	0.02
Second peak						
Begin(°C)	0.15	0.08	-0.08	0.01	0.06	0.07
End(°C)	-0.04	-0.12	0.11	0.08	-0.01	0.12
Onset(°C)	0.17	0.10	-0.14	-0.01	0.09	0.02
Peak(°C)	0.05	0.03	-0.05	-0.03	0.11	0.01
ΔH_1^e(j/g)	-0.01	-0.15	0.18	0.03	0.02	0.16
ΔH_2^e(j/g)	-0.04	-0.14	0.13	0.10	0.06	-0.03
ΔH_t^e(j/g)	-0.03	-0.16	0.17	0.07	0.04	0.06

*P<0.05
**P<0.01

Table 13a. Correlation coefficients between characteristics of semimembranosus muscle and DSC measurements showing thermal curves having three peaks

DSC measurement	pH	Fat	Moisture	Protein	Total collagen	WBS
First peak						
Begin(°C)	0.03	0.01	-0.01	-0.05	0.02	-0.01
End(°C)	0.14	-0.08	0.14	-0.01	-0.01	-0.07
Onset(°C)	0.02	-0.07	0.13	0.09	-0.02	0.07
Peak(°C)	0.02	-0.14	0.15	0.03	-0.07	-0.03
Second peak						
Begin(°C)	0.14	-0.08	0.14	-0.01	-0.01	-0.07
End(°C)	0.11	0.04	0.08	-0.01	0.01	-0.03
Onset(°C)	0.03	0.01	0.06	-0.04	0.06	-0.11
Peak(°C)	0.19*	0.01	0.15	-0.11	0.03	-0.14
Third peak						
Begin(°C)	0.11	0.04	0.08	-0.01	0.01	-0.03
End(°C)	0.14	-0.05	0.15	0.10	0.01	0.04
Onset(°C)	0.19*	-0.09	0.22*	0.10	0.02	0.01
Peak(°C)	0.23*	-0.05	0.18	0.08	0.05	0.01
ΔH_1^e(j/g)	0.01	0.04	0.0.5	0.12	0.03	-0.07
ΔH_2^e(j/g)	-0.12	0.13	-0.11	0.03	0.01	-0.03
ΔH_3^e(j/g)	0.03	-0.01	-0.01	0.08	0.01	0.08
ΔH_t^e(j/g)	-0.07	0.06	-0.03	0.10	0.01	-0.04
Observations	111	111	111	111	111	121

*P<0.05
**P<0.01

Table 13b. Correlation coefficients between characteristics of semimembranosus muscle and DSC measurements showing thermal curves with two peaks

DSC measurement	pH	Fat	Moisture	Protein	Total collagen	WBS
First peak						
Begin(°C)	-0.01	0.14	0.23	-0.24	-0.07	-0.08
End(°C)	0.13	-0.23	0.07	0.09	0.45	0.15
Onset(°C)	-0.07	0.18	0.29	0.22	0.12	-0.04
Peak(°C)	0.08	-0.11	0.25	0.16	-0.16	0.15
Second peak						
Begin(°C)	0.13	-0.23	0.07	0.09	0.45	0.15
End(°C)	-0.11	-0.23	0.07	0.09	-0.05	0.15
Onset(°C)	0.13	-0.04	-0.10	0.03	-0.39	0.14
Peak(°C)	0.11	0.11	-0.24	-0.02	-0.22	0.10
ΔH_1^e(j/g)	-0.05	-0.28	-0.11	0.21	-0.30	0.20
ΔH_2^e(j/g)	-0.26	0.19	-0.14	-0.13	-0.14	0.24
ΔH_t^e(j/g)	-0.15	-0.14	-0.15	0.10	-0.17	0.25
Observations	18	18	18	18	18	20

*P<0.05
**P<0.01

Table 14. Linear regression coefficients and R^2 values for the regression equations linking WBS values obtained for characteristics of four muscles showing thermal curves having three peaks

Regression parameters							
Intercept	pH	Moisture	Total collagen	Peak1 area	Peak2 area	R^2 (%)	C(p)
19.85	-2.88	*	*	*	*	20.5	20.9
8.36	-2.38	12.20	*	*	*	24.2	7.52
8.49	-2.31	11.06	0.03	*	*	25.2	5.70
8.83	-2.38	11.31	0.03	-0.35	*	25.9	4.71
7.87	-2.36	12.26	0.03	-0.59	0.29	26.9	2.52

Table 15. Linear regression coefficients and R^2 values for the regression equations linking WBS values obtained for characteristics of four muscles showing thermal curves with two peaks

Regression parameters								
Intercept	pH	protein	Peak1 area	Peak2 peak	Fat	Peak3 end	R^2 (%)	C(p)
20.66	-3.05	*	*	*	*	*	28.4	19.2
17.06	-2.83	11.09	*	*	*	*	32.1	7.93
17.48	-2.96	11.76	0.15	*	*	*	33.3	5.58
15.06	-2.93	11.65	0.18	0.03	*	*	34.5	3.58
14.54	-2.72	9.68	0.19	0.03	-3.40	*	35.4	2.20
16.76	-2.63	9.85	0.26	0.04	-3.58	-0.04	36.4	0.65

Table 16. Linear regression coefficients and R^2 values for the regression equations linking WBS values obtained for characteristics of individual muscle showing thermal curves having three peaks

	Regression parameters							
Muscle type[a]	Intercept	pH	Fat	Moisture	Total collagen	Peak1 end	Peak1 area	Peak2 begin
IN	55.98	*	*	-31.94	-0.05	*	*	*
LD	-54.53	7.55	*	13.97	*	-0.10	-0.36	*
PM	7.48	-0.84	*	*	*	*	*	-0.05
SM	11.50	*	-13.44	*	0.16	*	*	*
	Peak2 peak	Peak3 onset	Peak3 peak	Peak3 end	Total area	R^2 (%)	C(p)	
IN	*	-0.35	*	*	*	35.6	1.47	
LD	*	*	0.17	*	*	34.15	-2.94	
PM	*	*	*	0.04	-0.24	23.92	7.36	
SM	-0.11	*	*	*	*	10.5	9.50	

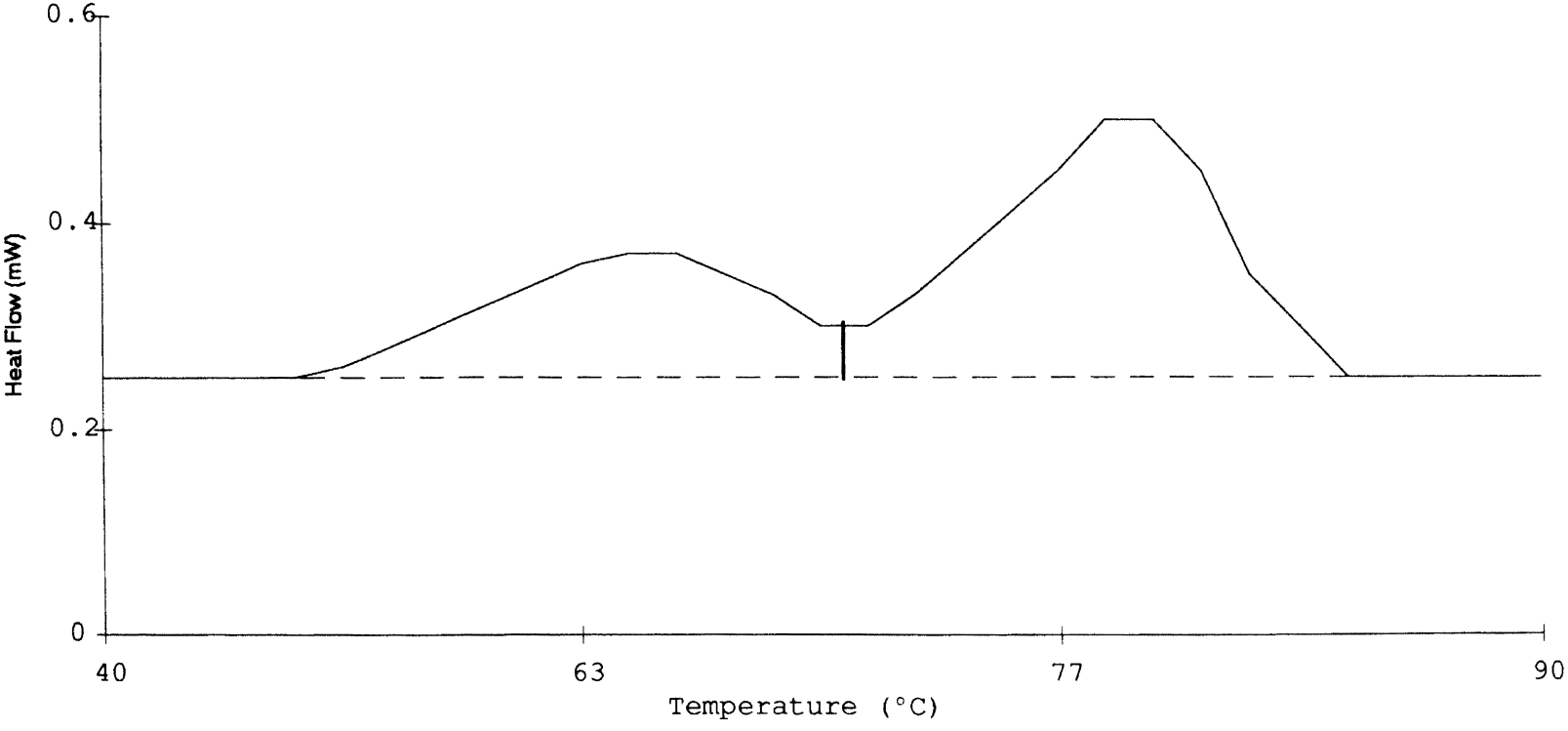
[a]IN=infraspinatus, LD=longissimus dorsi, PM=psoas major, SM=semimembranosus

Table 17. Linear regression coefficients and R^2 values for the regression equations linking WBS values obtained for characteristics individual muscle showing thermal curves with two peaks

	Regression parameters					
Muscle type[a]	Intercept	pH	Fat	Moisture	Total collagen	Peak1 begin
IN	28.35	-1.48	-5.91	*	-0.05	*
LD	-25.58	5.45	*	*	*	*
PM	11.44	*	-18.63	-10.25	*	*
SM	-34.90	-6.57	*	73.64	-0.31	-0.09
	Peak1 area	Peak2 peak	Peak2 onset	Total area	R^2 (%)	C(p)
IN	*	*	-0.20	0.11	29.34	-0.70
LD	*	*	*	*	15.5	-0.68
PM	*	*	*	*	14.4	-1.01
SM	-0.78	0.61	-0.24	*	85.21	13.11

[a]IN=infraspinatus, LD=longissimus dorsi, PM=psoas major, SM=semimembranosus

Figure 2. Differential scanning calorimetry from longissimus muscle demostrating two peaks.



APPENDIXES

APPENDIX A

CRIMPING PROCEDURE

1. Place the meat sample in an aluminum sample pan.
2. Place the sample pan on the base of the standard crimper press.
3. Carefully place a sample pan cover on the sample pan. Be careful not to disturb the sample in the pan.
4. Press the crimper table slightly with one hand to form a shallow recess in the crimper table. Place the sample pan in the recess. Slowly release the table while keeping the sample pan seated in the recess.
5. Gently press the crimper handle down with steadily pressure until the table reaches the crimper base. Hold the handle down for a few second.
6. Release the crimper handle.
7. Press the crimper table to remove the crimpered sample pan. Repeat the crimping procedure if the pan has nor been completely crimped.

APPENDIX B

WARNER-BRATZLER SHEAR VALUES AS AFFECTED BY AGING PERIOD

	Aging period (day)				SE[b]
Shear force (KN)	2	7	14	21	
Three peaks[a]	4.35[c]	4.16[d]	4.00[e]	2.89[e]	0.07
n	91	72	79	88	
Two peaks	4.00[c]	3.67[d]	3.45[de]	3.25[e]	0.25
n	53	72	65	56	

[a]three peaks=showing thermal curves with three peaks in DSC
two peaks=showing thermal curves with two peaks in DSC
[b]Standard error
[c,d,e]Means in same row with different superscripts differ ($P<0.05$)

APPENDIX C

ANALYSIS OF VARIANCE FOR WBS WITH THREE PEAKS IN DSC MEASUREMENTS

Source	df	SS	MS	F
Cattle	5	41.43	8.29	19.76
Aging	3	13.12	4.37	10.43
Side	1	0.21	0.21	0.49
Muscle	3	121.43	40.48	96.52
Aging*Muscle	9	22.60	2.51	5.99

ANALYSIS OF VARIANCE FOR WBS WITH TWO PEAKS IN DSC MEASUREMENTS

Source	df	SS	MS	F
Cattle	5	7.97	1.59	5.79
Aging	3	4.91	1.63	5.94
Side	1	0.68	0.68	2.47
Muscle	3	43.15	14.38	52.23
Aging*Muscle	9	9.28	1.10	4.01

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Candidate for the Degree of

Master of Science

Thesis: IDENTIFYING THE COMPONENTS OF TENDERNESS USING DIFFERENTIAL SCANNING CALORIMETRY

Major Field: Food Science

Biographical:

Personal Data: Born in Taiwan, R, O, C., December 30, 1964, the son of Chin-Shan and Su Wu.

Education: Graduated from Kaohsiung High School, Kaohsiung, Taiwan in May, 1982; received certificate of Food Science from National Pingtung Institute of Agriculture, Taiwan, R. O. C., July, 1987; and a Bachelor of Science degree in Chemistry from Oklahoma City University, August, 1992, respectively. Completed the requirements for the Master of Science degree in Animal Science at Oklahoma State University in December, 1994.

Experience: Quality control at Hi-Pa-Wang frozen food processing plant; salesman for Kennix drinking company; graduate research assistant at Oklahoma State University, 1993-1994.