

# EFFECT OF LOW-TEMPERATURE AND LONG-TERM HEATING ON MEAT TENDERNESS

Thomas Carey  
*University of Wisconsin, Madison.*

**Abstract:** Low-temperature heating means heating below normal cooking temperature (45-85°C). The temperature range of meat is between 50-60°C. Low Temperature Long Time (Low Temperature Long Time, LTLT) refers to heating meat at a low temperature of 50°C-60°C for a long time to increase the tenderness of the meat. LTLT Improves meat palatability by improving tenderness and juiciness, as low temperatures do not cause browning or caramelization of the meat surface, allowing a relatively pink color to be retained in the meat, thus optimizing color. Since the tenderness, texture, color and flavor of the meat are greatly improved, this method is becoming more and more popular in the meat processing industry and catering industry. Among them, the texture and color of the meat are the main driving forces behind the popularity of LTLT.

**Keywords:** Food science; LTLT; Review

## 1 EFFECTS OF LTLT ON SENSORY PROPERTIES OF MEAT

As early as 1937, Cover and other scholars found that fully cooked steaks fried at 124°C were more tender than 225°C. Bramblett et al. heated beef to 68°C and 93°C respectively, and found that although the former was more juicy, it was poor, but its tenderness, flavor and appearance are stronger than the latter. Marshall et al. (1960) found that during the low-temperature roasting process, as the temperature in the furnace increases, the internal temperature of the beef increases, the juice loss increases, and the total energy consumption of the oven increases. Schoman (1960) found that roasting beef in a moisture-tight forced convection oven resulted in lower juice loss and lower energy consumption at 121°C than at 149°C. These results indicate that temperature has an effect on the water-binding capacity of meat [1-3].

Dymit proposed "delayed" in 1961 service" (staged heating) method. He heats beef rib meat at 178°C for 1.5 hours and then holds it at 60°C for 3-48 hours. During the first 24 hours, the beef's flavor, tenderness and juiciness improved and remained constant over the next 24 hours. During this period, only 15% of muscle fiber contraction was observed. Gaines (1966) chose a temperature of 218°C for high-temperature heating, and the tenderness decreased, but the beef internal temperature does not reach the melting point of collagen. Funk (1966) chose a higher temperature than Dymit for segmented heating, and the results showed that the beef texture deteriorated. Korschgen et al. (1963) heated the beef in an oven at 149°C to a core temperature of 43°C and then cooled the slices. The slices were grilled on a grill at 204°C for 3 minutes on each side. The results showed that the tenderness was greatly improved. It can be seen from these studies that heating rate can affect various properties of meat related to tenderness.

Laakkonen [4] studied that the end temperature of meat is extremely important in affecting tenderness and cooking loss. If the temperature is lower than the temperature at which collagen shrinks, the tenderness will not be significantly reduced. If the temperature is higher than the shrinkage point of collagen, the cooking loss increases, the contraction of muscle fibers and perimysium increases, and the tenderness decreases. If meat is heated to the point of collagen shrinkage, cooking losses increase and tenderness increases significantly. So during the heating process, the temperature should be kept within the shrinkage point of collagen. Stabursvik and Martens [5] found that connective tissue had a heat absorption peak at 68°C through DSC, and the denaturation temperature was measured at 67°C by separating the connective tissue. The latest research shows that between the heating temperature of 53°C and 63°C, as the heating temperature time increases, the enthalpy change of 68°C ( $\Delta H_{68^\circ\text{C}}$ ) decreases. It shows that the denaturation temperature of connective tissue under LTLT low-temperature and long-term heating is lower than 68°C [2]. However, the denaturation temperature time of connective tissue below 68°C is currently unclear.

L Christensen (2013) [6] conducted low-temperature and long-term heating of old cows and young bulls. The results showed that during all heating treatments, the shear force of old cows was significantly higher compared with that of young bulls. After heating at 63°C for 15 hours, the tenderness of the two was equivalent. Obviously, older cows require higher temperatures and longer heating times to reduce toughness in order to achieve the same tenderness level as younger bulls. An obvious difference between the two is the different heat resistance strength

of connective tissue, which is caused by different degrees of covalent cross-linking of collagen molecules. In young bulls, when the temperature is increased by 2°C from 53°C to 55°C or the heating time is extended for 5 hours at a heating temperature of 53°C, the strength of the connective tissue is significantly reduced. As the heating time is extended and the heating temperature is increased, the solubility of collagen increases. This indicates that the improvement of meat tenderness by LTLT involves the dissolution of collagen. Pulgar (2012)[7] used histological analysis to study the collagen fibers of pork cheeks heated at 60°C and 80°C for a long time. The study showed that most of the tissues were destroyed when heated at 60°C for up to 12h, which was different from heating at 80°C for 12h. Compared with collagen fibers, they are not completely denatured. also, The weakening of connective tissue or changes in protein conformation by proteolytic enzymes during heat treatment may also be one of the reasons for the lower denaturation temperature. Tornberg (2005)[8] reported that the participation of denatured muscle proteins can further tenderize meat. The reason is that denatured proteins can combine muscle fibers and reduce their viscosity, making the meat more fragile and easier to break. Brüggemann [9] used second harmonic microscopy to study heat-induced denaturation of collagen. Research results show that collagen shrinks at 57°C and 59°C, but its triple helix structure does not unfold. Mortensen et al. (2012)[10] found that When the temperature was increased to 60°C, the tenderness of the meat was not that great, and this difference may be due to different heating rates. From this, a small sample sensory analysis was conducted, and it was found that a small sample is more conducive to rapid equilibrium of sample temperature and water bath temperature.

### **1.1 Effect on Color and Flavor**

Studies by Vaudagna et al. concluded that the sensory quality of meat treated with LTLT below 60°C is greatly improved, including tenderness, juiciness, flavor and color. Color is considered an indicator of LTLT. However, myoglobin is stable at 60°C but will denature and precipitate or co-precipitate with other proteins at higher temperatures. The length of time heated at this temperature also affects whether myoglobin is denatured. Therefore, during prolonged heat treatment at temperatures below 60°C, part of the myoglobin is denatured, which may result in a visual improvement in flesh color. At present, little is known about the research on flavor changes under LTLT. Vaudagna[11] et al. studied the off-flavor of beef after LTLT after frozen storage. The study showed that the most significant off-flavor was the taste of blood or metal. .

### **1.2 Effect on Tenderness**

Tenderness is one of the most important palatability indicators, and it can be seen that beef tenderness and quality are higher than nutritional value and cost through consumer preferences and choices [12]. Tenderness is therefore an important factor in ensuring consumers continue to choose the product. Tenderness can be defined as the force required to cut or chew meat and is affected by many factors, the most important of which are muscle fibers and connective tissue. The role of connective tissue content in tenderness is mainly reflected in the process of muscle fiber breakage, because structurally, a complete muscle is surrounded by a layer of connective tissue called the epimysium; the epimysium extends inward, dividing the muscle into Many muscle bundles form the perimysium; the perimysium extends further inward and wraps individual muscle fibers to form the endomysium [13]. Muscle tissue is formed by connecting muscle fibers through connective tissue. Another important factor is the age of the slaughtered animal. As the animal ages, the amount of heat-stable cross-links formed in collagen increases, the nature of the connective tissue becomes more stable, the protein hardness increases, and it is more difficult to decompose [14].

Heating will change the physical properties of muscle tissue. The physiological changes can be thermal denaturation, or chemical or enzymatic denaturation of muscle proteins, leading to the degeneration and dissolution of muscle fibers and connective tissues. So far, LTLT The effects on tenderness remain widely discussed, and there is still some disagreement about the underlying physical and chemical reactions that occur.

## **2 EFFECTS OF LTLT ON MEAT PROTEINS**

### **2.1 Effect on Myofibrillar Protein**

The protein tissue in meat is divided into myofibrils (50-55%), sarcoplasm (30-34%) and connective tissue (10-15%) [8]. Heating thermally denatures these proteins, thereby changing the chemical and physical properties of the muscle. During heating, sarcoplasmic proteins expand and form gels, and myofibrils and connective tissues shrink [8]. The degree of denaturation mainly depends on temperature and time. The first increase in toughness between 40°C and 50°C is attributed to the denaturation and contraction of myosin, the component of the muscle fiber; the second increase in toughness between 65°C and 80°C It is due to the denaturation of collagen in

intramuscular connective tissue. L Christensen[3] found that the tenderness improvement between 50-60°C was due to muscle fibers rather than connective tissue, because an age difference was found between tenderness improvement and connective tissue maturity. By changing the heating time, the age difference can be overcome. Limits to achieve the required tenderness [6]. The transverse contraction of muscle fibers caused by heating is between 45-60°C, and the longitudinal contraction is between 60-90°C. L Christensen et al. [15] conducted an in vitro study to compare the impact of the tensile strength of connective tissue and single muscle fibers on overall tenderness changes under low temperature treatment. Through this experiment, they confirmed that between 50°C -60°C The improvement in tenderness is due to changes in the mechanical strength of the perimysium, while the decrease in tenderness above 60°C that persists to 90°C is due to an increase in the breaking strength of individual muscle fibers.

## **2.2 Effect on Collagen**

Collagen is an abundant structural protein found in animal tissues. It is a hydroxyproline-rich protein composed of repeating Gly-XY A triple helix structure composed of three polypeptide chains surrounding the sequence. When the heating temperature reaches 64°C, the triple helix structure remains relatively stable. However, when the temperature is further raised, the helices begin to denature and unwind. Initially, denaturation causes collagen fibers to shrink, and as heating continues to above 70°C, collagen dissolves and gelatin forms.

The thermal stability of collagen is determined by thermally stable covalent cross-links, and covalent cross-links tend to become stable as the age of the animal increases [16]. LTLT will affect the chemical and physical properties of connective tissue, so beef of different ages must be given different LTLT treatments to achieve similar tenderness. So far, LTLT There is still little research on collagen during heating, and the partial denaturation of specific collagen means that the occurrence of thermal and enzymatic denaturation can be adjusted by combining heating temperature and heating time to achieve the desired tenderness.

## **2.3 Effect on Proteolytic Enzymes**

Proteolytic enzymes refer to enzymes that catabolize proteins. The four main proteolytic enzymes involved in meat tenderization include the proteasome, calpain, cathepsin and caspase systems [17]. Theoretically, if the LTLT temperature is too low, the enzyme is still active, leading to the degeneration of muscle fibers and connective tissue. Laakonen It was found that collagenase remains active during prolonged heat treatment (between 45°C and 60°C), however the activity decreases with increasing temperature. It is further theoretically explained that collagenase can work together with other enzymes. Collagenase catalyzes collagen to unfold the triple helix, which facilitates the attachment and weakening of collagen by other proteolytic enzymes. Collagenase induces the opening of the triple helix structure of collagen by heat. The peptide chain is more easily hydrolyzed by proteases thereby increasing solubility [18]. Calpain is usually excluded from LTLT Except for tenderness investigation, it was completely inactivated after 55°C.

Cathepsins B and L are endonucleases that catalyze the hydrolysis of peptide chains within proteins and are considered to be involved in the weakening of collagen, leading to collagen dissolution [19]. Laakonen et al. found that cathepsin B and L were still active after heating at 55°C for 24 hours, and the activity decreased after further heating to 70°C for 1 hour. Cathepsin B and L, as endopeptidases, can hydrolyze peptide bonds in proteins, so it may play a role in the weakening of collagen. When meat is incubated at 30°C for a long time, as the pH decreases, cathepsin B and L are removed from lysosomes. released [20]. Potential mechanisms of heating-induced connective tissue weakening were analyzed through collagen solubility, calpain B and L activities, and protein denaturation were studied. Studies have found that proteolytic enzymes such as collagenase, calpain B, calpain L, etc. are active during low-temperature and long-term heating, which may also be the reason for the increase in tenderness [21].

L Christensen[6] found that the activities of cathepsin B and L reached the highest when heated at 53°C for 5 hours, and then decreased with increasing temperature and time. After heating at 63°C for 15 h, large amounts of calpain B and L remained active, indicating that these enzymes have the potential to weaken muscle proteins including connective tissue throughout the heat treatment. While other enzyme systems such as collagenase have been inactivated so their effects are negligible, cathepsin B degrades both soluble and insoluble collagen by eliminating intermolecular covalent cross-links. Cathepsin B may increase the solubility of insoluble collagen, thereby lowering the denaturation temperature and promoting the dissolution of collagen.

## **3 CONCLUSION**

Low-temperature and long-term heating can greatly improve meat tenderness for many reasons, including the weakening of muscle fiber tissue caused by the denaturation of myosin, actin, troponin, etc., the loss of thermally unstable covalent cross-links, and The degradation of proteolytic enzymes such as cathepsin B and L leads to the dissolution of collagen and the weakening of connective tissue caused by partial dissolution of collagen. In fact, the degree of shrinkage of connective tissue in LTLT is relatively mild, and the primary condition for shrinkage of connective tissue is the denaturation of procollagen molecules. So the changes in connective tissue in LTLT are due to enzymes breaking down the collagen structure before contraction occurs. The broken down connective tissue then denatures and in turn causes less contraction.

The relationship between cooking temperature and time and tenderness is not linear. However, the shrinkage denaturation of muscle fibers and the partial denaturation of specific collagen that occur at different temperatures and times mean that the occurrence of thermal denaturation and enzymatic denaturation can be adjusted by combining heating temperature and heating time to achieve the desired tenderness.

## COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

## REFERENCES

- [1] Baldwin DE. Sous vide cooking: A review. *International Journal of Gastronomy & Food Science*. 2012, 1(1): 15-30.
- [2] Christensen L, Bertram HC, Aaslyng MD, Christensen M. Protein denaturation and water-protein interactions as affected by low temperature long time treatment of porcine longissimus dorsi. *Meat Science*. 2011, 88(4): 718-22.
- [3] Christensen L, Gunvig A, Tørngren MA, Aaslyng MD, Knøchel S, Christensen M. Sensory characteristics of meat cooked for prolonged times at low temperature. *Meat Science*. 2012, 90(2): 485-9.
- [4] Laakkonen E, Wellington GH, Sherbon JN. LOW -TEMPERATURE, LONG -TIME HEATING OF BOVINE MUSCLE 1. Changes in Tenderness, Water -Binding Capacity, pH and Amount of Water - Soluble Components. *Journal of Food Science*. 1970, 35(2): 175-7.
- [5] Stabursvik E, Martens H. Thermal denaturation of proteins in Post rigor muscle tissue as studied by differential scanning calorimetry. *Journal of the Science of Food & Agriculture*. 1980, 31(10): 1034-42.
- [6] Christensen L, Erbjerg P, Løje H, Risbo J, Berg FWJVD, Christensen M. Relationship between meat toughness and properties of connective tissue from cows and young bulls heat treated at low temperatures for prolonged times. *Meat Science*. 2013, 93(4): 787-95.
- [7] Sánchez DPJ, Gázquez A, Ruizcarrascal J. Physico-chemical, textural and structural characteristics of sous-vide cooked pork cheeks as affected by vacuum, cooking temperature, and cooking time. *Meat Science*. 2012, 90(3): 828.
- [8] Tornberg E. Effects of heat on meat proteins – Implications on structure and quality of meat products. *Meat Science*. 2005, 70(3): 493-508.
- [9] Brüggemann DA, Brewer J, Risbo J, Bagatolli L. Second harmonic generation microscopy: a tool for spatially and temporally resolved studies of heat induced structural changes in meat. *Food Biophysics*. 2010, 5(1): 1-8.
- [10] Frøst MB. Effect of Time and Temperature on Sensory Properties in Low-Temperature Long-Time Cooking of Beef. *Journal of Culinary Science & Technology*. 2012, 10(1): 75-90.
- [11] Vaudagna SR, Sánchez G, Neira MS, Insani EM, Picallo AB, Gallinger MM. Sous vide cooked beef muscles: effects of low temperature–long time (LT–LT) treatments on their quality characteristics and storage stability. *International Journal of Food Science & Technology*. 2010, 37(4): 425-41.
- [12] Kukowski AC, Maddock RJ, Wulf DM, Fausti SW, Taylor GL. Evaluating consumer acceptability and willingness to pay for various beef chuck muscles. *Journal of Animal Science*. 2005, 83(11): págs. 2605-10.
- [13] Chang Haijun. Study on changes in characteristics of intramuscular collagen in beef under different processing conditions and its impact on quality : Nanjing Agricultural University, 2010.
- [14] Lepetit J. A theoretical approach of the relationships between collagen content, collagen cross-links and meat tenderness. *Meat Science*. 2007, 76(1): 147-59.
- [15] Christensen M, Purslow PP, Larsen LM. The effect of cooking temperature on mechanical properties of whole meat, single muscle fibres and perimysial connective tissue. *Meat Science*. 2000, 55(3): 301-7.
- [16] Lepetit J. A theoretical approach of the relationships between collagen content, collagen cross-links and meat tenderness. *Meat Science*. 2007, 76(1): 147-59.
- [17] Kemp CM, Parr T. Advances in apoptotic mediated proteolysis in meat tenderisation. *Meat Science*. 2012, 92(3): 252-9.

- [18] Christensen LL, Ertbjerg PP, Aaslyng MD, Christensen MM. Effect of prolonged heat treatment from 48<sup>o</sup>C to 63<sup>o</sup>C on toughness, cooking loss and color of pork. 2011.
- [19] Agarwal SK. Proteases cathepsins — A view. *Biochemical Education*. 2010, 18(2): 67-72.
- [20] Ertbjerg P, Larsen LM, Moller AJ. Effect of prerigor lactic acid treatment on lysosomal enzyme release in bovine muscle. *Journal of the Science of Food & Agriculture*. 1999, 79(1): 95-100.
- [21] Ertbjerg P, Christiansen LS, Pedersen AB, Christensen L. The effect of temperature and time on activity of calpain and lysosomal enzymes and degradation of desmin in porcine longissimus muscle. 2012.