Meat and meat products microstructure and their eating quality

Massami Shimokomaki, 1 Elza I. Ida, 1 Talita Kato, 1 Mayka R. Pedrão, 1 Fabio A. G. Coró and 2 Francisco J. Hernández-Blazquez

1 Universidade Tecnológica Federal do Paraná – UTFPR - Câmpus Londrina, Avenida dos Pioneiros, 3131 – CEP 86036-370 – Londrina, PR, Brazil
2 Depto de Medicina Veterinária e Preventiva, Depto de Ciência e Tecnologia de Alimentos, Universidade Estadual de Londrina, Londrina, PR, Brazil

Microscopy techniques are undoubtedly powerful tools to investigate changes that occur in the transformation of muscle to meat. During the aging period, protease activities in sarcomere components are crucial for meat maturational and subsequently for tenderization. In both cases, microscopy has been used to illustrate the endogenous biochemical events within the muscle to obtain tender meats, thereby facilitating comprehension of the complexity of the aging phenomena. Moreover, these techniques have been applied to understand other abnormal conditions, such as the development of pale, soft and exudative (PSE) meat, which influences meat taste and causes economic problems for meat industries. The conditions in which birds are raised and transported from the farm to the abattoir as well as the technologies applied for slaughter through deboning and during storage have been evaluated in detail. One of the several ideas that emerged from these studies is the excessive liberation of intramuscular calcium ions, which jeopardize broiler breast meat qualities. Furthermore, structural alteration of the charqui meat product in South America has been observed. All these changes can only be visually noted using microscopy techniques.

Keywords: microscopy; rhomboideus m; charqui meat; PSE meat; sarcoplasmic reticulum; tenderness.

1. Meat structures and conversion of muscle into meat

The description of muscle structure and its basic components is of extreme importance for the understanding of the biochemical phenomena that occur during the transformation of muscle into meat dictating its quality, particularly tenderness. Muscle cells are striated, which means that distinct banding patterns or striations are observed when muscle cells are viewed under a microscope. This aspect is due to the intracellular arrangement of their cytoskeleton filaments into cylindrical bundles, the myofibrils. These structures are as long as the muscle fibers (myofibers) and are made up of repeating units known as sarcomeres, which are the contractile cell “machinery” and made up of repeating units, known as sarcomeres. All of the structural elements needed to perform the physical act of contraction at the molecular level are contained in each sarcomere [1]. As illustrated in Figure 1, the sarcomere is divided into four major compartments as follows: Z-disk, I-band, A-band and M-line [2]. A recent proteomic analysis estimates that more than 65 proteins comprise the structure of the sarcomere [3]. However, the actual number is likely far greater than this because the analysis did not take into account multiple protein isoforms [1]. Other muscular cell components include mitochondria, which utilize oxygen and other substrates to produce energy in the form of adenosine triphosphate (ATP), and the endoplasmic reticulum, which is responsible for regulating calcium within the myofiber, thereby exerting a strong influence on the control of muscle contraction. Within muscle cells, this organelle is referred to as the sarcoplasmic reticulum [4]. Rigor mortis is the first step in the conversion of muscle to meat, and it involves biochemical, physical and structural changes [5]. Currently, the conversion of muscle into meat is assumed to occur through the following three steps: pre-rigor, rigor and tenderization [6]. The onset and extent of rigor mortis are biochemically characterized by the content of energy-rich compounds, including ATP, creatine phosphate and glycogen, together with the activities of ATPase, kinase and glycolytic enzymes in the muscle [5]. After animal bleeding, all cells are in anoxia and receive no more nutriments [6]. The products of anaerobic metabolism (glycolysis) cannot be removed and, therefore, accumulate in the tissue resulting in the buildup of lactic acid, which causes a gradual decline in the tissue pH from approximately 7.0 to 5.6. At the same time, the temperature of the carcass falls from approximately 37°C to 2°C over a 12-h period (in cattle) [7,8].

Muscle is highly sensitive to both ATP and Ca2+, which are both involved in the contraction-relaxation process. Consequently, when ATP is depleted and the Ca2+ levels rise postmortem, muscle contraction (actomyosin complex) becomes final meaning there is no more energy (ATP) to undo the connection to allow muscle relaxation [5,10]. In such conditions, each cell can decide to die by initiating the apoptotic process. Apoptosis induces several structural and biochemical changes in dying cells, which are likely found in postmortem muscle [6]. Muscle does not become extensible again, thus resulting in pre-rigor. Thick and thin filaments remain locked together by myosin cross-bridges, and sliding does not occur because the filaments do not regain the ability to slide over one another. Instead, the structure of myofibrils begins to break down, particularly in Z-disk regions [11]. The exact point at which the conversion of muscle to meat is complete is difficult to assess although the establishment of rigor mortis is generally accepted to be at this point. However, while the functional role of skeletal muscle is lost, and rigor has been established, the metabolic
activity of the tissue has not ceased. Various biochemical processes may still occur, and some of these have significant implications for the quality of postmortem muscle as food [4]. Tenderness is an attribute of meat quality considered to be important by the consumer [12,13,14,15]. Meat tenderness is the sum total of the mechanical strength of skeletal muscle tissue and its weakening during postmortem aging of meat. The former depends on the species, breed, age, sex and individual skeletal muscle tissue of the animal.

Meat tenderness originates from the structural and biochemical properties of skeletal muscle fibers, especially myofibrils and intermediate filaments, and from intramuscular connective tissues, including endomysium and perimysium, which are composed of collagen fibrils [16]. The most striking effect of postmortem aging is the improvement of meat tenderness, which is almost exclusively caused by endogenous proteases in the muscle that disrupt myofibrils, but the exact mechanism of postmortem tenderization still remains controversial [15]. Clearly, the majority of the changes that occur in skeletal muscle that lead to the disruption of the muscle cell and meat tenderization are the result of proteolysis [4,14]. There are many proteases in skeletal muscle, but only calpains and certain lysosomal enzymes have been shown to degrade myofibrillar proteins. As shown in Figure 2, current evidence suggests that postmortem meat tenderization is primarily the result of calpain-mediated degradation of key myofibrillar and cytoskeletal proteins [12].

These proteins are involved in the following processes: inter-myofibrill linkages (e.g., desmin and vinculin), intra-myofibrill linkages (e.g., titin, nebulin, and troponin-T), linkage of myofibrils to sarcolemma by costameres (e.g., vinculin and dystrophin), and attachment of muscle cells to the basal lamina (e.g., laminin and fibronectin). Proteolytic
degradation of these proteins causes weakening of myofibrils and, thus, tenderization [14]. There are at least three possible mechanisms through which calcium ions can exert their effect on postmortem tenderization as follows: protein solubilization due to a salting-in action by calcium chloride; non-enzymatic weakening of structural proteins involved in the stability of Z-disk proteins; and activation of calpains [12]. Ca²⁺-dependent proteases are present in two distinct forms with one requiring 50-70 µM Ca²⁺ (µ-calpain) and the second requiring 1-5 mM Ca²⁺ (m-calpain) for activation [19].

Postmortem aging of meat is an important period because it has a significant effect on the meat microstructure and quality traits, especially texture, tenderness and water-holding capacity [21]. The texture of meat is one of the most important quality attributes, which has been studied for many years in different aspects [3,21,22,23].

2. Meat tenderness

Meat texture is dictated by several factors, including the amount of intramuscular fat, connective tissue, and actomyosin complex, as well as its water holding capacity [24]. Collagen and its crosslinking are also important factors to consider [25,26]. Nishimura et al. [27] reported that abundant marbled intramuscular fat is the main factor affecting the meat texture of Japanese Black cattle. Earlier studies have indicated that the marbling degree accounts for 3 to 10.0% of the variation in texture in a relatively small amount of beef intramuscular fat [28]. Pedrão et al. [29] described the influence of lipid content, collagen and collagen crosslinking on the texture of hump muscle meat (Rhomboideus m.). Figures 3A and 3B show differences in the distribution of muscle (M) and fat (F) cells of L. dorsi (LD) and Rhomboideus (RB) muscles analyzed by light microscopy. Chemical composition analyses have shown that there is a much higher incidence (12-15%) of intramuscular fat in RB muscle than in LD muscle. Collagen fiber is also distributed at the perimysium sheaths in both samples.

![Fig. 3 A) Photomicrograph of Bos indicus L. dorsi m. Note the distribution of fat (F) and muscle cells (M) along the perimysium (P). 200X. B) Photomicrograph of Bos indicus Rhomboideus m. Note the higher quantity of fat (F) cells in relation to muscle (M) cells when compared with A. (P). 200X. Source: [29].](image)

Analysis of collagen and its crosslinking with hydroxylysylpyridinium (HP) indicates that there is 22.9% more collagen and 14-fold more HP in RB muscle than in LD muscle. In contrast to expectations, the tenderness of fresh samples evaluated by Warner Bratzler shear force measurements leads to values of 8.05 and 5.81 Kgf for the LD and RB muscles, respectively (p<0.05). These results show that the abundant fat in fresh RB acts as a lubricant for the needle penetration irrespective of the quantity and quality of collagen fibers present.

3. Animal welfare and meat qualities evaluated by microscopy

Meat quality is an extremely complex subject that involves not only muscle anatomy and metabolism but also engineering, psychology and marketing [30]. Meat quality can be affected by the cumulative effects of chronic or continued environmental stressors, including primary production, pre-slaughter handling and post-slaughter handling [31,32]. Thus, the production process with animal welfare monitoring is important to ensure quality final products for the consumer [33]. For instance, the exposure of chickens to higher temperatures results in respiratory alkalosis, which causes a drop in growth performance [34]. Factors, such as the seasons related to high temperature and humidity, contribute to the onset of heat stress throughout pre-slaughter management [35,36,37]. The causes and consequences of broiler breast pale, soft, and exudative (PSE) meat have recently been the subject of experimental studies conducted by several research groups [38,39], and these studies have suggested that PSE meat is the result of the poor welfare of chickens with an emphasis on pre-slaughter stress and its direct relation to meat quality. Our research group in Brazil has found that factors related to pre-slaughter management, such as transport conditions from the farm to the commercial abattoir, holding period where the birds are left at rest while they are submitted to a water mist, ventilation for 60 min
before slaughtering and lighting ambience at the moment of slaughtering, are crucial for the welfare of birds leading to stress. PSE meat has become a model to directly measure the level of animal stress conditions [36,37,40]. Because stress can cause PSE meat, several attempts have been taken to diminish its onset, such as showering the birds before slaughter at the commercial processing plant, which calms the birds down and contributes to the reestablishment of muscle Ca\(^{2+}\) homeostasis [10]. Transportation conditions from the farm to the commercial abattoir can also influence the formation of PSE meat, and showering during the holding period also decreases animal stress [44,45].

The addition of vitamin E to the diet of birds reduces the formation of PSE meat [9] and increases phospholipase A2 activity [42], which may enhance lipid oxidation in PSE meat [43]. Ultrastructural studies have revealed that robust sarcomere shrinking within the muscle fibrils takes place in PSE meat [46]. Guarnieri et al. [40] conducted an experiment with male chickens of the Cobb lineage (42 days old), and they observed the effect of water shower treatment immediately before slaughtering on the biochemical and ultrastructural profiles of broiler breast meat. Light microscopic analysis has indicated that samples from a treated group (TG) present typical fluid-filled spaces at the endomysial and perimysial levels (Fig. 4A). Figure 4B shows that muscle samples from an untreated group (UG) have greater extracellular spaces between muscle cells and collagen fibers at both the perimysium and endomysium sheaths compared with the TG samples. The average cell diameter of PSE samples is approximately 10.0% less (p<0.001) than normal samples. This result in addition to the results of a drip loss experiment has suggested that there is a loss of meat water holding capacity, which may be due to protein denaturation. Thus, water moves from myofibrillar compartments to interfibrillar compartments and then to extracellular compartments, and drip is then finally formed on the meat surface giving the watery appearance of PSE meat samples. The longitudinal muscle section from TG (Fig 5A) is characterized by a normal myofilaments structure and does not show apparently any destructive changes. Regular transverse striation is also visible with evident I-bands, A-bands, H-zones, Z-lines and M-lines. The sarcomeres are of equal length and width, but the faint Z-lines indicate that there is some protease activity within the muscle. On the other hand, UG samples show complete sarcomere disorganization. The typical dark and light banding pattern is not evident, and Z-lines are more pronounced (Fig. 5B). Myosin filaments predominate within sarcomeres, and they even touch the Z-lines. Moreover, actin filaments are seldom observed. Open spaces are also visible, and a super muscle contraction is evident. One of the most affected muscle regions is at the triad where the sarcoplasmic reticulum is present and at the region where Ca\(^{2+}\) is released promoting the super contraction. This contraction draws some of the sarcomere components toward the Z-lines, making them dense.

Fig. 4 Photomicrograph of Pectoralis major m. cross-sections of treated group (A) and untreated group (B) samples. The perimysium is indicated by arrows, and the endomysium is indicated by arrowheads. Note the shrinking of muscle fiber as well as an increase at the endomysium spaces. Magnification 131.25x. Source [40].

Fig. 5 Electronmicrograph of Pectoralis major m. from (A) treated group and (b) untreated group samples. Note the disorganization of A- and I-bands in (B). The M-bands disappeared, and a super contraction was evident. (Z, Z-line; M, M-line). The triad is indicated by an arrow. Magnification 23000x. Source [40].
Wilhelm et al. [47] investigated the protease activities in PSE broiler breast meat by means of tenderness, biochemical properties, and ultrastructural profiles (Figure 6). Figure 6A is an electronmicrograph from the first post-mortem hours of the control samples. The myofilament structure is normal, without any apparent destructive changes. The myofilament structure is normal without any apparent destructive changes. I-bands, A-bands, H-zones, Z-lines and M-lines are evident. At 72 h post-mortem (Fig 6B), this pattern is somewhat altered. The myofibril sarcomere is depolymerized, and some lacunas are evident within the bands, thereby indicating protein fragmentation (Fig. 6B). Conversely, in Figure 6C, PSE samples show complete sarcomere disorganization. The typical dark and light pattern is not evident, and the Z-lines are more pronounced. Myosin filaments predominate within sarcomeres and even touch the Z-lines. Open spaces are visible, and muscle contraction is evident drawing some sarcomere components towards the Z-lines, which increases their density. These results are similar to findings described by Guarnieri et al. [40]. When comparing Figure 6A to 6B, however, the calpain systems initiate their activity in PSE samples much earlier than in the control samples, which can be explained by an increase in the concentration of \( \text{Ca}^{2+} \) in the PSE samples probably even before slaughtering. This hypothesis is supported by consistently lower pH values with respect to control samples [42]. In fact, excess \( \text{Ca}^{2+} \) within the tissue at this stage prematurely enhances \( \mu \)-calpain activity in birds compared with mammals. In mammals, a certain amount of time is necessary to initiate the maturation assembly presumably because the \( \text{Ca}^{2+} \) concentration is not high enough to induce muscle catabolism [48]. It is fair to note that there may be a specific relation between the initiation of the enzyme activity and animal species. It is clear that of all the proteases present in skeletal muscle tissue, \( \mu \)-calpain is the only enzyme capable of acting on myofibril proteins because lysosome enzymes are inactive at the physiological pH level. Furthermore, proteasomes cannot digest myofibril components because they need to be unfolded [49]. Thus, it is the calpain system that acts first to degrade the sarcomere entities. Figure 7 shows transversal sections of 24 h post-mortem control (7A) and PSE (7B) samples. Open spaces are clearly present in the endomysial network (arrow 1), and faint collagen fibers are present in the PSE samples. Open spaces are present both intramuscularly and within the endomysium.

Muscle cell shrinking of approximately 10.0% occurs due to the low pH and relatively high temperature conditions, which denature muscle proteins and impair their functional properties, thus preventing intra- and intermuscular water retention. Simultaneously, higher protease activities might render these proteins further non-functional and synergistically promote an increased loss of water. It is reasonable to believe that in this region, water starts to move from the intracellular compartment to the endomysium (Fig. 7B) and finally to the surface of the meat as previously described. Speculation regarding the biological postmortem myofibril digestion in pig PSE muscle based on the 72 h postmortem micrograph in Figure 8 can be suggested. Arrow 1 points to the first biological condition for calpain to act. The myofibrils appear disarranged and depolymerized throughout the A- and I-bands. The Z-lines are fragmented showing that the enzyme acts upon both the thin and thick filaments, particularly on nebulin and titin, as previously reported [50]. Finally, arrow 3 shows the collapsed sarcomere structure, which makes the meat relatively tender. Excessive \( \text{Ca}^{2+} \) concentration during the onset of broiler PSE meat promotes high protease activity, which may occur before the broiler slaughtering process, affecting the integrity of the muscle fiber structure and, thus, impairing meat protein functionality.
Fig. 6 Longitudinal electronmicrograph of *Pectoralis major* m. from control group 1h30 (A) and 72h (B) post-mortem and PSE group 1h30 (C) and 72h (D) post mortem. Note a disorganization of A and I bands in (C and D). The M bands disappeared and a super contraction of the Z (Z) line is evident. Scale Bar = 700 nm. Source [47].

Fig. 7 Electronmicrograph of a transverse section of 72-h post-mortem broiler *Pectoralis major* m. Control sample (A) and PSE meat sample (B). Arrow 1: endomysium. Arrow 2: intracellular space. Scale Bar = 200nm. Source [47].
4. Intermediate moisture meat products (IMMPs) visualized with microscopy

The work of Biscontini et al. [51] is a good example of microscopic changes that occur to IMMPs due to the dehydration of meat. Charqui and its derivatives are typical tropical IMMPs formulated using hurdle technology, which is a concept described by Leistner [52,53]. Salt, sodium nitrite, dehydration and packaging are hurdles applied in sequence to inhibit deteriorating microorganisms as well as possibly selecting for desirable flora. Biochemical and physicochemical changes occur during charqui processing [54,55]. The intermediate moisture nature of this product has been established [55]. Figure 9A shows typical fluid-filled channels at the endomysial and perimysial regions in control samples as described by Offer et al. [56]. Differences are observed in charqui meat samples (Fig. 9B), and the harsh processing conditions do not completely destroy the myofibers. Clear spaces are observed between the muscle cells and endomysium. Moreover, fewer collagen fibers are observed within the perimysium (Fig. 9B). As a consequence of sample shrinking in charqui meat, the cell number in a specific area diminishes by 20-30%, and the area occupied by these cells decreases by 30-40%.

Fig. 8 Electron micrograph of 72 h postmortem pig Longissimus dorsi. A) Control sample. B) PSE meat showing three phases of PSE development due to proteolysis. Arrow 1 indicates depolymerized sarcomere components, particularly in the Z-line region. Arrow 2 indicates the fragmentation of the Z-line. Arrow 3 indicates the collapsed sarcomere structure. Scale Bar = 500nm. Source [47].

Fig. 9 Photomicrographs of Sternomandibularis muscle. Cross-sections of control (a) and charqui meat (b) samples. In (b), the amount of connective tissue of the perimysium (P) is lower than observed in control samples. Note the shrinkage of muscle fibers as well as an increase of endomysium spaces (arrows). Magnification: 64X. Source [51].
Electron micrographs of *Sternomandibularis* muscle. Cross-sections of control (A) and charqui meat (B) samples. The A- and I-bands are disorganized in (B). The M-band is not well defined. The arrowhead indicates dilated T-tubules. Magnification: 13950X. Source [51].

Electron microscopy shows some intracellular compartments. Figures 10A and 10B show a longitudinal section of muscle fibers. In the charqui meat samples, the typical dark and light banding patterns present in the controls are not evident, and the Z-lines are more pronounced. In addition, the M-band is not clear, and the T-tubule system is dilated.

Both intracellular and extracellular components are affected by the harsh conditions of IMMP processing. The high salt concentration (approximately 15-20%) and drying conditions at temperatures in the range of 30-40°C for 5 days cause structural changes in *Sternomandibularis*. On a dry weight basis, it is estimated that the total protein lost is approximately 35-40%, and solubilized collagen represents 40-45% of this amount. Consequently, the intercellular space shrunk by 20-30%, and the extracellular space increased, which aids drainage of the saline solution. Secondarily, as the temperature during processing reaches 35-40°C, myosin denatures [57], and consequently, water is drained until an equilibrium is reached ($A_w=0.70-0.75$) [55]. Moreover, in the control samples, collagen fibrils are combined with a strong electron dense material (proteoglycans), which is not present in the charqui meat samples. Presumably, proteoglycans are solubilized by the saline solution, which aid dehydration because proteoglycans are hydrophilic. The results of the ultrastructural studies carried out by Biscontini et al. [51] reflect the events that take place during IMMP preparation, notably extraction of A-bands, disaggregation of myofibrils and partial solubilization of extracellular matrix components.

**Conclusion**

Undoubtedly the application of the microscope is a valuable tool in order to visualize the biochemical and physiological events and to explain the actual changes within the striated muscle therefore finding fundamental basis for the understanding of the phenomena of meat and meat product qualities in particular their tenderness.

**References**


