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Contribution of cathepsin, calpain and proteasome to the post-mortem proteolysis and tenderisation

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Abstract

Tenderness is one of the most important evaluation indicators of meat quality, and endogenous enzymes in post-mortem muscles play key roles in degradation of myofibrils and tenderness improving. Cathepsin is the first endogenous enzymes family found to be positive in post-mortem ageing, and most studies are focus on cathepsin B, D, H and L. Calpain is the major contributor to post-mortem muscle tenderness, and owns calpastatin to control proteolysis precisely. Proteasome is controversial involving in ageing process, but increasing evidence emerged on the 20S core particle degradation of structural protein even under extreme conditions. In this paper, the characteristics, action mechanism and influencing factors of the three endogenous enzymes were reviewed, and the process of their involvement in meat ageing and tenderness improving was analysed, in order to provide theoretical basis for meat processing.

Keywords

Cathepsin; calpain; proteasome; post-mortem; ageing; tenderness.

1. Introduction

Tenderness is the most intuitive feeling to evaluate the meat quality, and most consumers are willing to choose meat with slightly higher price but guaranteed tenderness [1]. Meat tenderness is mainly determined by the original toughness and enzymatic degradation of intramuscular connective tissue in the complex rigor and post-rigor phase [2]. This process may involve two paths: one is the enzymatic degradation of myofibrils. The other is based on the physicochemical mechanism of post-mortem muscle osmotic pressure rise [3]. The effect of endogenous proteases on meat processing is complicated and of great significance for improving meat processing characteristics. Therefore, the mechanism and characteristics of cathepsin, calpain and proteasome were reviewed in this paper, and the process of their involvement in meat ripening and tenderness improvement were analysed.

2. Cathepsin - the first endogenous enzymes family found to be involved in post-mortem ageing

Cathepsin was discovered and named by Willstaatter in 1929. Since the concept of cathepsin was first proposed, and research progress in cathepsin has been gradually accelerated [4]. The role of cathepsin in hydrolysis is mainly due to the hypoxia of the body after slaughter, the glycolysis in the cytoplasm decreases the pH of cytoplasm, and promotes the activation of acid hydrolase to hydrolyse the lysosomal membrane and the release of cathepsin [5]. Cathepsin has thermal stability and can cut off the internal peptide bonds and degrade proteins into short peptides.

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2.1 Types and properties of cathepsin

Since the discovery of cathepsin C in 1940, more and more cathepsins have been discovered successively, it has been reported from cathepsin A to cathepsin Z. According to the different proteolytic active centres, cathepsin can be divided into three types, namely cysteine proteases (including cathepsin B, H, L, C, M, N, etc.), aspartic proteases (including cathepsin D and E), and serine proteases (including cathepsin A, G and R). These proteases were initially synthesized in the primitive form of enzymes, and only after the n-terminal precursor peptides were hydrolysed off could they become active mature enzymes [6]. At present, at least 11 kinds of cathepsin have been purified and identified from the animal muscles and viscera, namely cathepsin A, B, C, D, E, H, L, L-like and X, S, Z [7-11]. Cathepsin B, D, H and L can degrade myosin, actin, collagen and so on complete protein, therefore the main protease after slaughter [12,13]. Protease A, C, E provide no hydrolytic activity to complete proteins of the muscle, but showed synergy effects with cathepsin B, D, H and L [14].

Most of the studies focused on cathepsin B, D, H and L. Cathepsin B is a kind of mercaptan enzyme, it consists of cathepsin B1 (24-28 kDa) and B2 (47-52 kDa). Cathepsin B combines endopeptidase and exopeptidase activity, and can degrade myosin, desmin and α-actinin [13-15]. Cathepsin D is an aspartic acid protease belonging to endopeptidase and generally exists in the form of an inactive precursor and transfer to be active after 2-5 steps of proteolysis [16]. Studies have shown that cathepsin D can hydrolyse the heavy chain of myosin, tropomyosin and the T and I subunits of troponin [17]. Cathepsin H (28 kDa) displays the activities of mercaptan endonuclease and aminopeptidase, and mainly degrades myosin and troponin [18]. The activation of the cathepsin L depends on its own catalysis, cathepsin D or metalloproteinase [19].

2.2 Factors influencing cathepsin activity

2.2.1 Effects of pH and temperature on cathepsin activity

The optimum pH values of cathepsins are different, and the optimum pH values of cathepsin D and E are lower, namely pH 3.0-4.5 and pH 2-3.5, respectively. Cathepsin A, B, C and H have high activity in the pH 5.0-6.0 [12].

Temperature is an important factor affecting the activity of cathepsin. The cathepsin has good stability, the optimum temperature ranges from 40 ℃ to 55℃, and the low temperature exerts obvious inhibitory effect on enzyme activity. Due to the different sensitivity of organelles to temperature, the activity of cathepsin in different subcellular structures was affected differently by temperature. Research has pointed out that the enzyme activity in myofibril under different freezing storage temperature showed no significant difference, but cathepsin B and B+L activity in the sarcoplasm decreased significantly with temperature [20]. Besides, the cathepsin thermal stability varies with the species, such as cathepsin B of silver carp is not heat-resisting, the optimum temperature is around 30 ℃, only 18% of enzyme activity remains when kept at 35 ℃ for 50 min [21]. Cathepsin L is relatively heat-resistant, and its optimal temperature is 40-50℃. After subjected to 40℃ for 50 min, the enzyme activity remains 46% [22]. But conditions vary from species to species, cathepsin B and L of beef brisket were found to be more heat stable compared to Cathepsin H at sous vide temperatures (50℃ for 24 h, 55 C for 5 h and at 60 C and 70 C for 1 h) [23].

2.2.2 Effects of processing technology and additives on cathepsin activity

In the production of fresh products, ozone is mostly used to reduce the bacteria, especially for fish products, to extend the shelf life of the products in the early treatment. Studies have shown that ozone has strong oxidability and can oxidize part of the active perssad of the cathepsin molecules so as to reduce their enzyme activity [24]. When the ozone concentration reached 1.5 PPM, cathepsin B and L was significantly inhibited [21]. In surimi production and processing, NaCl, pyrophosphate, starch, and exogenous protein will be generally added as additives to improve gel strength. While part of these additives will affect the activity of cathepsin, studies showed that soybean protein and potato starch had great inhibitory effects to cathepsin L of silver carp [25].

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Many processes in meat processing, such as rinsing, electrical stimulation, crushing, ultrasound, extrusion and so on, will have a certain effect on the activity of cathepsins. Bleaching is a key process in the processing of surimi, because it can remove part of endogenous cathepsin, reduce the activities of cathepsin B, H and L, and prevent the degradation of surimi gel [26]. Wu studied the cathepsins activity of four major Chinese carps (black carp, grass carp, silver carps and bighead carp) and found that even though activity of cathepsin B, H and L differed by species, but all of the cathepsins activity decreased after bleaching, and content of cathepsin H was fallen sharply [27]. In Tilapia, the residue rates of cathepsin B, H and L after bleaching were 11.49%, 3.53% and 80.96%, respectively [28]. Meat tenderness can be improved by applying low-pressure electrical stimulation after bloodletting. Chen et al. [29] studied the effects of electrical stimulation on the activity cathepsin B and L of yak. It was found that electrical stimulation doubled the activity of cathepsin in muscle fibres, indicating that electrical stimulation could also be used to enhance the activity of cathepsin to improve the tenderness of meat. Tomas used hydrodynamic pressure processing (HDP) to improved tenderness of beef and suggested that cathepsin was not significantly influenced by HDP, meaning cathepsin would still take part in proteolysis after pressure treatment [30].

2.3 The effect of cathepsin on the processing quality of meat products

2.3.1 Cathepsin brings tender texture in meat

Texture is an important index to evaluate meat quality. After slaughter, the muscle fibre structure is changed by the microbial and enzyme effects, muscle cytoskeleton and extracellular matrix structures are destroyed, myofibres and myoseptums are dissociated from each other during storage resulting in the decreased hardness and elasticity and the quality deterioration [10,31]. Because of the thermal stability and the ability of endopeptidolysis, cathepsin has a great influence on the texture of meat [32,33]. In particular, cathepsin B, D and L contribute greatly to the change of tenderness in the ageing process of meat products after slaughter [34]. Cathepsin B and L have the activity of endopeptidase and have extensive degradation effect on myofibrillar and other proteins. Cathepsin B can hydrolyse myosin, troponin, tropomyosin and actin. Cathepsin L can degrade the T and I subunits of myosin heavy chain, α-actin, actin and troponin [35]. Cathepsin D is also active, which is an important factor affecting the quality of preserved meat [36]. Bahuaud et al. found that cathepsin B and L led to the enlargement of muscle fibre space and accelerated drip loss of Atlantic salmon under micro-freezing conditions [37]. Godiksen et al. studied the changes of myofibril in rainbow trout by electrophoresis, and found that cathepsin D was the main protease, rather than cathepsin B and L, that led to the degradation of the texture [38]. Tian et al. showed the activities of cathepsins L, B and H rose during mortem aging and suggested the potential roles of cathepsins in tenderization process. [39]. Ge et al showed that cathepsin B, L and calpain synergistically isolate and degrade myofibril in a gradual and complementary manner, leading to meat softening of grass carp [40]. The effect of cathepsin on myofibrils degradation and on muscle tissue structure are shown in Table 1.

Table 1. The cathepsin degrading myofibrillar proteins in muscle and the effects on the muscle structure[41]

Myofibril degradation	cathepsin	Impact on organizational structure
α-actin	B、D、L	Z line (disk) weakened
myosin	B、D、L	The myofibril structure is destroyed
Tropomyosin	L	The myofibril structure is destroyed
Troponin T	B、L	The myofibril structure is destroyed
30kDa fragments appear	B、L	The myofibril structure is destroyed
actin	L	The myofibril structure is destroyed

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2.3.2 Cathepsin causes degradation to gel property of surimi

Gel property is one of the important indexes of surimi product quality. Gelation process generally includes protein denaturation and aggregation. During the surimi processing, myosin and actin are dissolved by salting or by grinding, and then hydrate with water, and aggregate to actomyosin gel, finally forming a three-dimensional network of gels after being heated. The content of endogenous protease is closely related with the quality of surimi gel. A large number of research reports suggest the degradation of surimi gel are a result of the degradation of myosin, induced by cathepsins and calpain. Furthermore, cathepsin cannot be completely eliminate in rinsing process, so it plays an important role in degradation of surimi gelation[42], especially the cathepsin B, L, H and L-like [43]. Because cathepsin B, L, H and L-like have endopeptidase activities and can act on a variety of muscle fibrin and other protein substrates [44]. Besides, cathepsin B, L, and H have strong thermal stability, and the optimal temperatures for actomyosin hydrolysing are 45℃, 45℃, and 50℃, respectively, that is close to the soften temperature range of surimi gel [45,46]. In addition, cathepsin B, L and H are not easy to be removed by rinsing, and cathepsin L has strong stability and high activity of endopeptidase, which can degrade the heavy chain (MHC) of myosin and eventually lead to gel softening, which is dominant in gel degradation of surimi [47,48].

Li et al. showed that there was still a part of cathepsin B, L and H left after rinse of silver carp, among which the residue of cathepsin L was up to 25.79%, and that of cathepsin B and H was 11.46% and 6.86% respectively [43]. Carvajal et al. showed that cathepsin L is the main protease in muscle softening of salmon and degradation of Pacific cod surimi [49]. Consequently, researchers also suggested that the gelation strength of surimi could be efficiently reduced adjusted by adding cathepsin L [50].

3. Calpain - a major contributor to post-mortem muscle tenderness

Calpain is a specific calcium-dependent cysteine mercaptopeptidase. To date, 15 different genes encoding caspases have been identified in mammals [51]. After being slaughtered, the muscles of animals will go through four stages, such as rigor, post-rigor, ageing and deterioration. In the rigor stage, sarcoplasmic reticulum is destroyed as pH decreases, and Ca^{2+} content increases to activate calpain in the sarcoplasmic reticulum. Calpain act on the Z-line of muscle fibres, as a result, sarcomeres are disconnected, muscles are softened with improved juiciness and tenderness [52].

3.1 Classification of calpain

The calpain system contains not only proteinase (μ-calpain and m-calpain), but also endogenous regulate proteins (calpastatin and calpain activator) [53]. Calpain exists almost in all eukaryotes and a few bacteria, and is distributed in muscle cells of all vertebrates. It is mainly located near the Z-disks of muscle fibres and on the sarcoplasmic reticulum.

3.2 Structure of calpain

μ-calpain and m-calpain are heterodimers composed of similarly large 80kDa catalytic subunits and identical 28kDa subunits, as shown in Fig. 1. The 80kDa subunit consists of four domains (I, II, III, IV) [53], and the 28kDa subunits comprise two domains (V, VI). The N-terminal domain of domain I has no sequence homology with any known polypeptide, and no regulatory proteolytic activity. Domain II is the catalytic domain of calpain, containing cysteine residues (subdomain IIA) and histidine residues (subdomain IIB), which are situated in relative conservative positions among all cysteine proteases. Domain III is the Ca^{2+} binding domain, which is different from any other known protein source. Domain IV is a calmodulin-like domain, also known as the penta-EF domain, and has 5 EF-calcium binding sites. Domain V is rich in glycine and is the binding site of phospholipid. Domain VI, which is similar with domain IV, also contains EF-hand motifs. Just as planned, the 5^{th} binding site of both domain IV and VI are not offered for Ca^{2+} but for connecting each other, forming a heterodimer of the two calpain subunits [54].

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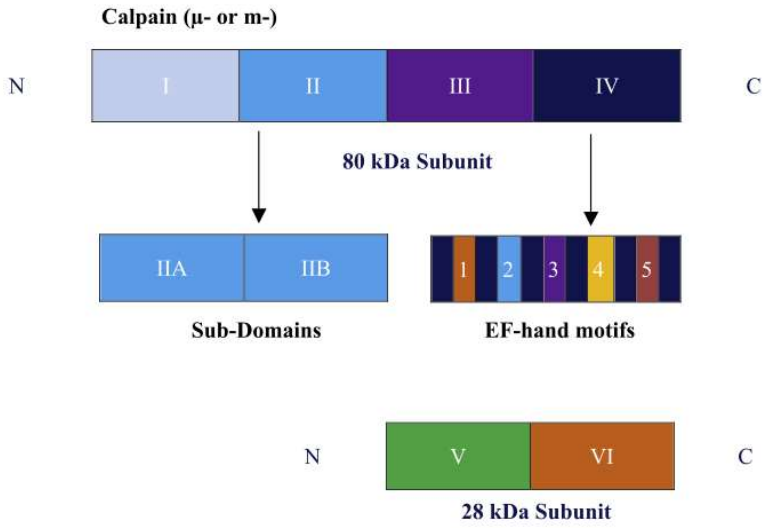

Figure 1. Structure of μ -calpain and m-calpain [54]

3.3 The action mechanism of calpain

When the sarcoplasmic reticulum is destroyed and the concentration of Ca^{2+} in muscle cells accumulate, calpain is awoken by Ca^{2+}, and its two subunits separate. The 28kDa subunit rapidly degrade to 17kDa, calpain starts hydrolysis. The 80 kDa subunit is generally inactive, and only becomes active when it is degraded to 76 kDa (μ -calpain) and to 76 kDa (m-calpain) [54]. The EF-hand motifs of domain IV and VI can bind 3-4 Ca^{2+}, which leads to conformational transformation and dissociation of the small subunits. The core domain IIA and IIB also combine with Ca^{2+}, destroying the salt bridge between glutamic acid (Glu33) and arginine (Arg104) residues in structural domain II, and urging catalytic triplet assembling, (cysteine residues (Cys105) and asparagine residues (Asp262) in domain IIA and histidine residues (His286) in domain IIB). As a result, calpain become more active. Both experiments in vitro and in vivo proved that calpain can hydrolyse skeletal proteins such as connectin, myosin, desmin and troponin T [55]. However, the concentration of Ca^{2+} required to activate m-calpain is much higher than that of μ-calpain [56], so μ -calpain could be activated by Ca^{2+} and degrade myofibroin within 3 days after slaughter, while m-calpain may only play an effect in the last stage of ageing [57,58], therefore it believes that μ -calpain is absolutely dominant in post-mortem ageing [59].

3.4 Calpain's influence on processing characteristics of meat products

Lots of studies discussed the role of calpain system in tenderness of muscles, and calpain was believed to be the main cause of proteolysis in most animals after slaughter, as well as an important driving factor for meat tenderness in the ageing process [60]. Calpain have various effects on muscles in different animals, thus affecting muscle texture differently [61].

Liu studied ageing mechanism of yak after slaughter, results show that the calpain activity was significantly correlated with myofibril fragmentation ($P < 0.01$) [62]. It is supposed to be the result of the joint effect of μ-calpain, m-calpain and calpastatin [63]. Z plates in muscular tissue of livestock and poultry disintegrated thanks to calpain, thus the fragmentation index of myogenic fibres increase, decreasing muscle shear force and increasing chewiness. Flavour compounds are produced simultaneously, thereby improving the edible value and sensory quality of meat. [64].

The fish can go through the ageing process within 2~5h after slaughter, and then autolytic spoilage takes place with the cooperative and complementary action of endogenous enzymes including caipain, cathepsin and proteasome [65]. Of course, people are not willing to watch the products deteriorating, so the activation of calpain during rigor is suppressed to prolong the shelf life. Thankfully, even though the calpain activity is similar, calpastatin activity of fish is 3.9 times higher than that of beef [66].

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4. Proteasome - a controversial endogenous protease

Proteasome is responsible to regulate specific proteins and hydrolyse misfolded proteins, and plays a central role in protein regulation that controls cell cycle progression and apoptosis [67]. The involvement of proteasomes in post-mortem tenderization of meat has been debated for a long time and is discussed in this section.

4.1 Structure and function of proteasome

Proteasome is a 2000 kDa polysubzyme complex widely found in eukaryotic cells. It is called 26S proteasome due to its sedimentation coefficient of 26S. The 26S proteasome consists of a 20S cylindrical catalytic subunit and a 19S capsid regulatory subunit [68]. The 20S proteasome is the centre of proteolytic activity, consisting of two inner β-rings and two outer α-rings. Each of the two inner β-rings contains three proteolytic sites, named for its trypsin-like, peptide-glutamyl peptide hydrolase (PGPH), or chymotrypsin-like activity. One or two 19S regulatory units are at the top of 20S subunit, which aim to recognize ubiquitinated proteins and controls the pathway to proteolytic core. Since the structure of 26S proteasome is very complex and its molecular weight is huge, the structure analysis of 26S proteasome has not been completed currently by using freeze electron microscopy, X-ray and NMR techniques, and only 20S subunit and the crystal structure of partial fragments of the complex have been determined [69].

Ubiquitin-ATP dependent 26S proteasome pathway plays an important role in the proteasome degradation of proteins. Firstly, Gly at 76 site on ubiquitin binds to Cys on ubiquitin activase. Ubiquitin is transferred to Cys of ubiquitin conjugated enzyme by transesterification. And then ubiquitin is transfer to the target protein with assistance by ubiquitin ligase. Polyubiquitin molecules attach to proteins and are recognized by the 26S proteasome and enter the hydrolysis core for degradation. Before degradation, ubiquitin chains are removed and free ubiquitin molecules are recycled [70]. Besides, 19 S and 20S free complexes can be released from the 26 S complex by depleting ATP [71], while 20S can degrade proteins with abnormal structure, misfolding or over oxidation in the absence of ubiquitination target proteins and ATP, forming a non-ubiquitin-ATP dependent 20S proteasome pathway [72]. In addition, activators such as PA28 and PA200 bind to 20S proteasome, which also promote the non-ubiquitin-ATP-dependent 20S proteasome pathway [73].

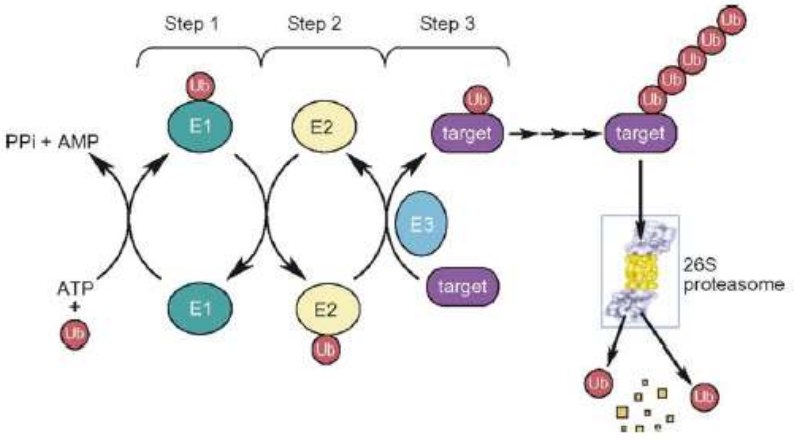

Figure 2. Ubiquitin - ATP-dependent 26S proteasome pathway [68]

4.2 Factors influencing proteasome activity

4.2.1 Effects of pH and temperature on proteasome activity

proteasome was confirmed a wide pH activity range depending on the nature and substrate. However, all reports converge and demonstrate a slightly alkaline optimum pH for the three activities (tryptic-

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like, chymotryptic-like and peptidylglutamyl peptide hydrolase (PGPH) activity) with pH values ranging from 7 to 9 [74-76].

The optimum temperature for peptidase activities relative concentrated at 40-50°C for the chymotryptic-like and the tryptic-like activities, and 45°C for the PGPH activity [77].

Therefore, pH value and temperature optima focused on the pH 7.5–9.0 and 40–50°C for tryptic-like, 7.0–8.0 and 40–50°C for chymotryptic-like, and 7.0–8.0 and 45°C for PGPH activities, respectively [76]. In addition to the above common situation, there are also data reports that differed in pH value and temperature optima.

For the 20S proteasome of ostrich skeletal muscle, the pH optima for tryptic-like was 11.0, temperature optima for chymotryptic-like and PGPH was 60°C and 70°C, respectively [75]. These values are higher than reported values for proteasomes from other species or tissues.it proved that the relatively wide activity range of proteasome.

4.2.2 Effect of processing method on proteasome activity

Refrigeration and freezing are important means to extend the shelf life of meat products after slaughter [78-79]. But the activity of proteasome separated and purified from muscles is extremely sensitive to temperature and storage conditions. Generally, it cannot be stored for a long time at 4℃. At -20℃, glycerol is often needed to ensure the enzyme activity, and it can be stored independently for a long time only below -80℃ (about 4-8 weeks) [74].

Sandra et al. studied the effects of freezing-thawing on Penaeus Vannamei and Crangon Crangon proteasomes and found that the proteasomes of both shrimp species were sensitive to repeated freezing-thawing reactions, with activity loss of about 80-90% [76].

High hydrostatic pressurization is an emerging technology for meat tenderization and acceleration of meat ageing. Yamamoto et al. [80] researched the effects of pressure and post-mortem ageing on in situ proteasome activity in rabbit and bovine skeletal muscles. proteasome was considered to participate in tenderization process together with other endogenous proteinases under pressure. The hydrolytic activity of synthetic peptide of rabbit proteasome remained in muscle after the pressure reached 100mpa. However, when the pressure exceeded 400mpa, the proteasome was significantly inactivated. The proteasome of senescent muscle remained relatively stable during the whole aging process, and the hydrolysis activity of synthetic peptide of bovine muscle proteasome was similar to that of rabbit skeletal muscle proteasome.

4.2.3 Effects of exogenous on proteasome activity

In the processing of livestock and poultry products, dried fish and minced fish, NaCl and other excipients are often needed to improve flavour and inhibit microbial breeding. At this time, the three catalytic activities of proteasome are inhibited by Na+ and other univalent cations, and the degree of inhibition is concentration dependent.

In addition, lipids and phospholipids in the substrate have an activation effect on proteasome activities, while SDS, leupeptin, iodoacetamide, Z-LLL-H (al), TLCK、TPCK and E-64 may show significant inhibited effects [81].

4.3 Effects of proteasome on meat tenderness

4.3.1 Debate on the contribution of proteasome to post-mortem meat tenderness

The hydrolysis of myofibril is the main reason for the change of skeletal muscle microstructure, which is closely related to meat tenderization. The role of proteasome in post-mortem meat myofibrillar proteolysis is controversial, mainly because most researchers consider proteases to be involved in post-mortem meat changes only if they conform to three characteristics: (1) exist in skeletal muscle cells; (2) contact with muscle fibres; (3) hydrolyse proteins that is degraded in the rigor period [1,82]. However, in a series of studies prior to 1995, no convincing results were found for proteasome involvement in post-mortem myofibrillar hydrolysis.

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Proteasome, as a multi-catalytic enzyme complex, has extensive substrate specificity and may be more difficult to enter myofibrillar structure due to its large molecular weight. In 1992, Koohmaraie found that the 20S complex could enter the I zone in vitro and that all myofibrils could easily pass through the 20S proteasome. However, because the 20S proteasome was not proved to be stable existed in vivo like 26S subunit, and the catalytic core of 20S lacked ubiquitin-ATP-dependent regulatory subunits compared with 26S proteasome, so these results were considered insufficient to prove that the proteasome was involved in the hydrolysis of myofibrils in vivo [83]. However, Peters proved in 1994 that the 20S complex can be free-exist in cells and found a large number of 26S proteasomes and their 19S, 20S subcomplexes in the nucleus and cytoplasm of a variety of cells. 19S and 20S were dissociated and in assembly-separation equilibrium with the 26S proteasome [84]. Even so, Taylor et al. used 26S proteasome and calpain purified from sheep skeletal muscle to treat purified myofibril, and found that the calpain could effectively degrade Z line, actin, desmin, troponin-T, troponin-I and α-actinine. Although proteasome can degrade troponin C subunit and sites 2 and 3 of myosin light chain, no morphological effect was shown on myofibrils. Therefore, proteasome was considered noneffective on proteolysis of myofibrils, and may only have regulatory indirect effects in rigor duration [85].Thomas et al. studied the role of 26S proteasome and cathepsin in the tenderizing process of ostrich meat, and found that 74%-82% relatively activity of trypsin-like, peptide-glutamyl peptide hydrolase (PGPH), and chymotrypsin-like activity were remained by 12 days after slaughter, which ensured that proteasome could play a role in the tenderizing process. Proteasome was able to degrade myosin light chain by site of 2 and 3, and nebulin, α-actinine, troponin-I, troponin-C and tropomyosin with the actives SDS or oleic acid, but disappointedly, shear force was not reduced with these changes, meaning proteasome seemed useless towards tenderness [86].

4.3.2 Influence of 20S proteasome on meat tenderness after slaughter

The experimental study on the myofibril decomposition of 20S proteasome changed the situation and is the main basis until now for the recognition of the proteasome's participation in meat tenderness after slaughter. Robert et al. treated the bovine skeletal muscle with 20S proteasome and found that the myofibular structure was rapidly destroyed, and the material loss was mainly from the Z line and the I band. After 24 hours of treatment, myofibrils were ruptured and fragmented, and it was confirmed that nebulin, myosin, actin and tropomyosin were hydrolysed during treatment, and other myofibrils such as actinin were dissolved. It indicated that 20S proteasome can hydrolyse myofibril completely and rapidly in an energy-free manner, suggesting that the 20S proteasome may play a role in muscle ageing independent from 26S [87]. Later, Dutaud used ultrastructure methods to illustrate 20S proteasome's contribution to the tenderization of high pH meat. 20S proteasome caused a series of changes in meat ageing. Firstly, thickness of Z line increased, and a amorphous protein structure was developed and spread to I zone, then the amorphous structure and Z line was degraded totally, and finally crosscutting fragments from myofibril in I zone were detected. This is a protein degradation process that has never been observed in calpain or cathepsin, demonstrating that the 20S proteasome complex has a direct effect on high-pH muscles [88].

An important determination of proteolysis in the 20S free proteasome is based on the structural constraints of the entry aperture of the 20S core particle, which is too narrow to allow the protein to enter the complex. However, Dutaud's research results may be able to explain the problem. If the 20S proteasome can generate amorphous change of myofibril and overcome this obstacle, it can be speculated that the 20S free proteasome stand a chance participating in the proteolysis of myofibrils and contribute to the ageing and tenderization of meat after slaughter [72].

Another study demonstrated the effect of the ubiquitin-proteasome pathway on meat tenderization from another perspective. Ubiquitination inhibitor PYR-41 and proteasome inhibitor MG-132 was injected into loin longus muscle of sheep, respectively. The effects of the injection on myofibril degradation, muscle ultrastructure and sarcomere length were measured. It was found that both proteasome and ubiquitination inhibitor had effects on sarcomere length and reduced damage to

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muscle ultrastructure. Meanwhile, inhibition of the proteasome at 48 h produced different myofibril fragment than the control group. It was suggested that the ubiquitin-proteasome pathway was involved in post-mortem proteolysis and may contribute to meat tenderization [89]. Zeng used proteasome to treat pork myofibrils, and found that the improvement of water holding capacity coincided with the degradation of myofibril, tenderness enhanced [90]. In addition, some studies on proteasome activity can also provide support for its involvement in meat tenderization. Studies of proteasome activity of beef in rigor stage have shown considerable stability, despite a significant decrease in pH, which enables proteasome to participate in the tenderization process of meat in coordination with other proteolytic systems [91,92]. The decrease of proteasome activity during refrigeration has a negative impact on myofibrillar protein degradation, thus affecting its tenderness [93]. The thermal activation of proteasome caused by low temperature cooking can affect the degradation of myofibrils and collagen, leading to a decrease in shear force [94].

5. Conclusion

Protein degradation and tenderness improvement of muscle in the ageing process after slaughter are closely related to calpain, cathepsin and proteasome. Calpain is the main contributor in degradation of myofibril, cathepsin B, D, L also play an important role, especially the leading part in fish texture degradation after slaughter. Rather than arguing about whether proteasomes participate in post-mortem ageing, it is better to regard proteasomes as endogenous proteases capable of stable degradation even under extreme conditions. However, there is no complete mechanism for the relationship and coordination among various enzyme systems at present. Meat products ageing process and the quality change procedure after slaughter are complex, the following aspects are valuable in further studied: (1) Currently, researches point out that autolysis is one of reasons cause the meat texture soften, but cascade reaction mechanism of apoptosis induced myofibril collapse is not clear. Therefore, it is necessary to study the detailed mechanism of meat quality deterioration during post-slaughter, especially during low-temperature storage, to find out the key influencing factors, and further explore the effects of external environment on endogenous enzymes as well as the process of their joint action on the quality change of meat products. (2) Cathepsin B, D, L and calpain are proved to degrade myofibril in a gradual and complementary manner, but studies on the interaction between other endogenous enzymes is still needed to clarify the action mechanism of meat tenderization of multiple endogenous protease systems. (3) Studies on the involvement of proteasomes and the free 20S core particles in degradation of proteins and meat tenderization have been 30 years, but most of the experimental evidence is indirect, clear proof that proteasomes are positive in myofibrillar protein hydrolysis in vivo is lacking. It is because the complex proteasome structure has not been determined completely, not only the combination of substrate, but deubiquitin mechanism is unclear, even assembly and dismantling regulations between the complex need to be explored, so it is difficult to explain the specific protein hydrolysis process, this is also one of the focuses in the future research direction.

Acknowledgments

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