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REVIEW

Degradation and evaluation of myofibril proteins induced by endogenous protease in aquatic products during storage: a review

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Abstract

Myofibril proteins degradation constitutes an important factor in quality deterioration, procedural activation or inhibition of endogenous protease potential regulates autolytic proteolysis-induced softening of post mortem fish muscle. Based on the brief introduction of myofibril proteins degradation in fish skeletal muscle, a detailed description of the main myofibril degradation properties and the distinct role played by endogenous proteases were proposed, which reflects the limitations and challenges of the current research on myofibril hydrolysis mechanisms based on the varied surrounding conditions. In addition, the latest researches on the evaluation method of myofibril proteins degradation were comprehensively reviewed. The potential use of label-free proteomics combined with bioinformatics was also emphasized and has become an important means to in-depth understand protein degradation mechanism.

Keywords Endogenous protease · Myofibril proteins · Post-mortem · Aquatic products · Spoilage

Introduction

With the dramatic improvement of the resident economic level as well as change of consumption pattern, high quality fish has become an significant animal proteins in daily diet due to the abundant nutrients, high absorption rate and potential preventive effect of cardiovascular diseases, including myocardial infarction, stroke and cardiovascular death (Rahman and Islam, 2020; Vidal et al., 2023). However, the characteristics of various nutrients, such as the high incidence of lipid oxidation and/or protein decomposition caused by microbial contamination and endogenous enzymatic activity, in turn exert negative effects on the storage of fish, one of which reflects in the tissue softening and concomitant quality deterioration during storage (Nie et al., 2022; Umaraw et al., 2020). It has been generally believed that tissue softening is aggravated by inappropriate storage environment, resulting in deterioration of texture properties, taste, loss of nutrients and even obvious decrease in edible value (Prabhakar al., 2020; Wu et al., 2019). There exits close relationship between textural modification and stability of water, resulting in changeable distribution and mobility of water in tissue myofibril, which further negative influences quality attributes.

Vast quantities of studies have shown that tissue softening phenomenon is attributed to complex metabolites reaction including oxidation of macromolecular nutritive components, microbial propagation, activation of endogenous proteolytic proteases (Fan et al., 2021; Yang et al., 2019; Zhuang et al., 2022). Among above mentioned factors, tissue myofibril protein degradation establishes the most significant section to elucidate underlying mechanism of muscle softening. With respect to deterioration indexes of myofibril

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protein, particular importance is given to physicochemical properties, ultrastructure integrity, conformation, intermolecular force as well as functional characteristics (Mehta et al., 2021; Wang et al., 2016). In terms of functional properties, Liu et al. (2023) demonstrated that oxidation enhance surface hydrophobicity of myofibril protein by modifying specific amine acid and decreasing affinity ability to volatile substances and water. From this perspective, the deterioration of water holding capacity (WHC) and flavor trait of aquatic products is closely related to the aggravation of surface hydrophobicity of myofibril proteins during storage. What's more importantly, functional characteristics of myofibril proteins like solubility, emulsifying and gelling properties are highly dependent upon thick filament structure, especially myosin composition (Guo et al., 2019; Mora et al., 2019). In previous studies of fish myofibril proteins, much emphasis is taken on the changes of myosin and actin (Fan et al., 2021; Yu et al., 2018). The most likely explanation is that permanent bonds of two proteins occur causing muscle to not be able to stretch and contract freely and ultimately results in muscle softening during storage (Delbarre-Ladrat, 2006). The most significant change that key myofibril proteins contribute to softening during later storage is the degradation of desmin and titin (Yang et al., 2019). At the same time, the relationship of myofibril and endogenous proteases has aroused widespread attention of research scholars. Ahmed et al. reviewed the impact of endogenous proteases-induced myofibril protein autolysis on fish structural softening during different post-mortem storage stages, especially for the detailed elaboration of main endogenous protease action mechanism. Similar report supported solid evidence that calpains and cathepsins aggravated breakdown of actin and myosin, which had negative effects on structural softening (Cheret et al., 2007). However, the literature lacks systematic summary on the role main key myofibril proteins play in the muscle softening as well as its evaluation indexes. Furthermore, controlled mechanism of endogenous proteases is still unclear due to complex post-mortem physicochemical conditions during different storage time and fish species specific.

In this review, we summarized current study literatures on the changes of myofibril proteins and endogenous proteases, in addition to their relationship contributing to fish muscle tissue softening during storage. Evaluation methods of myofibril protein hydrolysis were also proposed.

Post-mortem changes in fish skeletal muscle myofibrillar proteins

Based on differentiated intracellular physicochemical condition during storage period, postmortem stages are generally composed of four sections: pre-rigor, rigor mortis, post-rigor and spoilage (Roco et al., 2018). During transient pre-rigor stage, the decomposition of lycogen and phosphocreatine for constant ATP supply is of major contribution, which ensures muscle maintain at relaxed state. Once ATP levels decline, accompanied by the sarcomere shortening and myofilament self-assembly, inextensible actomyosin complex generated by interaction between actin and myosin is formed, signaling the onset of rigor mortis (Barido and Lee, 2021). Interestingly, the binding of actin to myosin is considered to be reversible. With the prolongation of storage time, the intermolecular forces existed in myofibril proteins, including ionic bonds, hydrogen bonds, hydrophobic interactions and disulfide bonds, tend to increase or decrease regularly (Wang and Xie, 2019). These chemical bonds changes directly affect the stability of the original myofibril proteins, commonly reflecting in changes of myofilament disassembly, cross-linking aggravation and structural proteins degradation (Ge et al., 2018b; Ko et al., 2016). Increased myofibril proteins degradation brought on by endogenous proteolytic activation may be a primary cause of post-rigor phase, which then causes fish spoilage quickly as evidenced by water loss, tissue softening, unpleasant fishy odor, loss of nutrients and other symptoms (Hussain et al., 2021; Roth et al., 2012; Yu et al., 2021). It is worth noting that the stress response generated by slaughter method and chilling rate might accelerate or retard the onset of myofibril proteins degradation. Therefore, it is important to emphasize how stress reaction affects quality characteristics during storage.

Unlike mammals, endogenous protease-induced myofibril proteins degradation of fish is limited, but reproduction and metabolism of spoilage bacteria have a more prominent effect on the myofibril proteins degradation, especially in the later stage of storage (Shao et al., 2021). This feature undoubtedly causes increased likelihood of unsatisfactory results in actual researches. And the degradation of myofibril proteins is also indirectly impacted by extracellular proteases released by microorganisms (Feng et al., 2021). Furthermore, myofibril proteins degradation is a joint action induced protein hydrolysis coordinated with oxidation. Protein hydrolysis happens as a result of oxidation brought on by proteins and lipids, which conversely affects the oxidation process. Therefore, multiple pathways for myofibril proteins degradation should be clarified, with a focus on the interconnections between various factors.

Endogenous protease-induced myofibril proteins degradation involved in quality deterioration

The degradation of myofibril proteins during fish storage can be sequentially divided into the following three stages: (1) release of peptides and free amino acids, which is called

proteolysis; (2) production of biogenic amines sourced from amino acids; (3) accumulation of organic compounds, such as ammonia, ketoacids, aldehyde acids (Zhuang et al., 2022). The small molecule peptides and free amino acids (FAAs) released in the first step are the products formed along with the gradual degradation of the main structural proteins of myofibrils, causing changes in the structural stability, physicochemical and functional properties of myofibrils. On the other hand, the hydrolyzate will promote the accumulation of subsequent metabolites such as biogenic amines and volatile odor compounds (VOCs) based on the deamination and decarboxylation of amino acid (Zhuang et al., 2021). Given the structural integrity of fish skeletal muscle, apart from actomyosin, brief information on several other significant myofibril structural proteins like nebulin, titin and desmin have been emphasized and analyzed (Table 1).

Myosin

Occupying the main places of thick myofibril filament, myosin is composed of following subunits: S1, S2 (digested from heavy meromyosin (HMM)) and light meromyosin (LMM) from rod domain (Xue et al., 2020). The head domain of S1 region endows myosin with ATPase active and binding site to actin filament. S2 correlated with LMM form a typical double helix structure, which mainly exerts potential beneficial effects on maintaining structural integrity as well as tensile strength of the sarcomere muscle (Liu et al., 2021a, b; Zhang et al., 2022). When subjected to unfavorable factors in the surrounding environment, myosin heavy chain (MHC) and myosin regulatory light chains (MRLCs) are highly susceptible to be dissociated from actomyosin, which sharply exacerbate post mortem disassembly of myofibril filaments (Ge et al., 2018b; Liu et al., 2021a, b). The best explanation of this standpoint might be that myosin regulates ATP hydrolysis, motility production based on the actin migration and muscle contractile ability with the help of MRLCs phosphorylation and Ca^{2+} concentration (Ren et al., 2021; Trybus, 2012). Briefly, factors including pH shift, oxidative stress and endogenous protease activation play a much more prominent role in MHC than those in ELCs section. In connection with the structural characteristics of myosin subunits aforementioned, the S1 portion may serve as the active site and unstable elements responsible for myosin degradation. From this perspective, identification and transfer of the phosphorylation sites and active sites bound to actin may contribute to further elucidate of myosin degradation mechanism. Of course, based on the fact that typical seven residue repeat seen in the C-terminal region of myosin tail is stabilized by hydrophobic residues on the coil surface and highly charged surface (Reed and Park, 2011), it is reasonable to assume that myofibril disorganization is associated with modification of amino acid residues in the rod-shaped region of the myosin subunit, reflected as a significant decline in WHC during fish storage. Similarly, Liu et al. (2022) reported WHC was determined by the relative balance between net negative charge accumulation and protein aggregation: On the one hand, myofibril proteins aggregation attacked by proteins degradation might be prone to cause a significant increase in surface hydrophobicity, accelerating water exudation from triple network structure of myofibril to cytoplasm. On the other hand, with the shift of net charge related to amino acid side-chains modification, isoelectric point of myofibril filament decreases, which reduces cooking loss, drip loss and water migration (Fig. 1). Although the change of the functional properties of myosin is unfavorable for the long-term storage of fish, this feature can be fully utilized to achieve the purpose of improving the quality of various processed aquatic products. For example, mild oxidation, salting, protease inhibitors, and polysaccharides can effectively

Table 1 Basic information on several key myofibrillar structural proteins

Myofibrillar proteins	Molecular weight (kDa)	Content in myofibrillar protein (%)	Role in maintaining myofibrils integrity	References
Myosin	470	50–60	Tightly bind to titin via intermolecular forces to ensure the structural integrity of thick filaments	Liu et al. (2021a, b)
Actin	43–48	15–30	Main components of thin filaments and integral ingredient of Z-discs in skeletal muscle	Lin et al. (2014)
Desmin	212		Linking adjacent myofibrils at z-disk level and maintaining the lateral arrangement of myofibrils	Kim et al. (2013)
Titin	3000–3700	13	Connecting the COOH-terminal portion of myosin filament to the NH_2-terminal end of the z-disk for maintenance longitudinal structure of myofibrillar sarcomere and increase myofibril stiffness	Delbarre-Ladrat (2006) and Huff Lonergan et al. (2010)
Nebulin4	600–900	3–4	Attaching to the Z line and maintaining or regulating actin filaments in a parallel state and be defined as skeletal supporters and stabilizers	Ahmed et al. (2015) and Hanif et al. (2010)

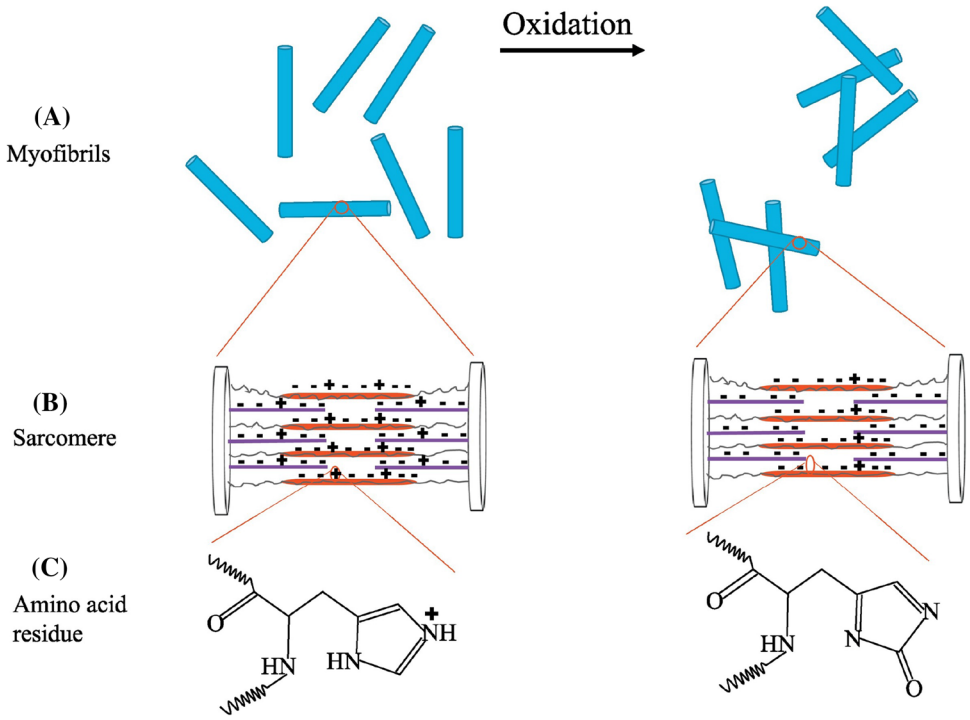

Fig. 1 Model for the effect of myofibril protein oxidation on filament charges, protein aggregation and water-holding (Bao et al., 2018)

alter the site and degree of cross-linking of myosin filaments to improve protein solubility, water-holding capacity, thermal gelation and microrheological properties of the final product (Fang et al., 2021; Fang et al., 2021; Xu et al., 2021). In conclusion, the research on the structural and functional stability of myosin subunits offers a fresh perspective for further exploring the regularity of myofibril degradation during fish storage in the future, and is also a significant means of enhancing quality characteristics of deep-processed aquatic products.

Actin

Actomyosin is a complex formed mainly from actin and myosin by intermolecular forces. As the most significant constituent of thin filament, actin has also been demonstrated to be an integral ingredient of Z-disk in skeletal muscle. Generally, actin can be commonly divided into two different exist forms: α-actinin and α-actinin-2, the former is cross-link skeletal actin filaments (Lin et al., 2014). There is a view that actin amount decreased a small degree even when appearance and texture of fish tissue were severely deteriorated, even throughout post mortem period (Caballero et al., 2009). But it seems a controversial result, more and more lines of evidence suggests that fragmentation of actin shows an upward trend after slaughter and its degradation is more intense during later storage period than that of initial storage (Ge et al., 2018b; Li et al., 2014). Collectively, it is generally agreed that speed of actin proteolysis might vary greatly during different storage period, which is greatly implicated in structural integrity and functional regulation of myofillaments. Longo et al. (2015) demonstrated that detectable actin fragmentation occurred only when stored for a longer period of time. Given the point view, the potential relationship among actin phosphorylation, apoptosis and textural properties during storage was mentioned in another review (Lana and Zolla, 2016). Besides, the dissociation of actin filament is also the main expression form of myofibril proteolysis, which is possibly due to weakened or altered intermolecular interaction with myosin based on the action of endogenous proteases. Li et al. (2012) demonstrated that sarcomere lengthening, brought on by a weakening of the actin-myosin interaction, occurred during the post-rigor period, so calpains were better able to access the I-band and A-band regions and subsequently hydrolyzed associated proteins, causing the disruption of muscle microstructure. Another study pointed out that RLCs phosphorylation may played an important contribution in maintaining the force between myosin and actin via two pathways: (1) stabilizing the intrinsic structure of actomyosin by reducing its kinetic energy; (2) promoting the formation of ionic bonds, hydrogen bonds, and hydrophobic interactions between myosin and actin. This is consistent with the above-mentioned viewpoints about the vulnerability of MRLCs to adverse environmental factors during fish storage.

Desmin

As an important component of intermediate myofibril filaments, desmin breakdown undoubtedly increase the

possibility of enlargement of adjacent myofibril gaps and destruction the original structure of myofibrils (Richardson et al., 2017; Yang et al., 2019). Therefore, it has been emphasized that desmin plays a positive role in maintaining excellent WHC, cooking loss and dripping loss, whose degradation instigates tissue softening and juice loss, ultimately affecting the nutritional value of fish (Qian et al., 2020; Starkey et al., 2015). This tight association is closely related to the fact that desmin contributes to the formation of drip channels, which allows intracellular water to squeeze out into the myofibril space resulting in drip loss (Yang et al., 2021). Indeed, desmin is also demonstrated to be a significant molecular chaperone for myofibril degradation during freeze–thaw cycles, and its concentration is negatively correlated with freeze–thaw cycles number, which may be attributed to the formation of non-uniform ice crystals (Ali et al., 2022). In connection with the fact that different forms of water in muscle, including free water, immobile water, and bound water, exhibit different content changes and migration rates throughout the storage period, the specific mechanism of action of desmin degradation on the decrease in water holding capacity still needs to be further studied. During entire storage of fish fillets, desmin degradation begins early in storage induces a gradual breakage of the myofibrils, which commonly might be explained by followed three different kinds of domains: phosphorylation, ubiquitination and depolymerization by calpain-1 (Cohen, 2020; Ren et al., 2021). For the degradation of myofibrils, calpains are involved in degradation of desmin early postmortem, and desmin degradation is always accompanied by calpain activation. This strong association between desmin and calpain might be determined by the predominance of both being located around the z-disk (Starkey et al., 2015). Similarly, Baron et al. (2004) demonstrated that desmin was mostly cleaved at the head and tail region when it was incubated with calpain, leaving the rod domain largely intact even after a protracted incubation. In addition, desmin is successively broken down into smaller fragments with molecular weights of 50, 47, 45, 43, 39 kDa, respectively (Fig. 2). Furthermore, caspase-3 is also involved in desmin degradation during postmortem based on the mitochondrial apoptosis pathways.

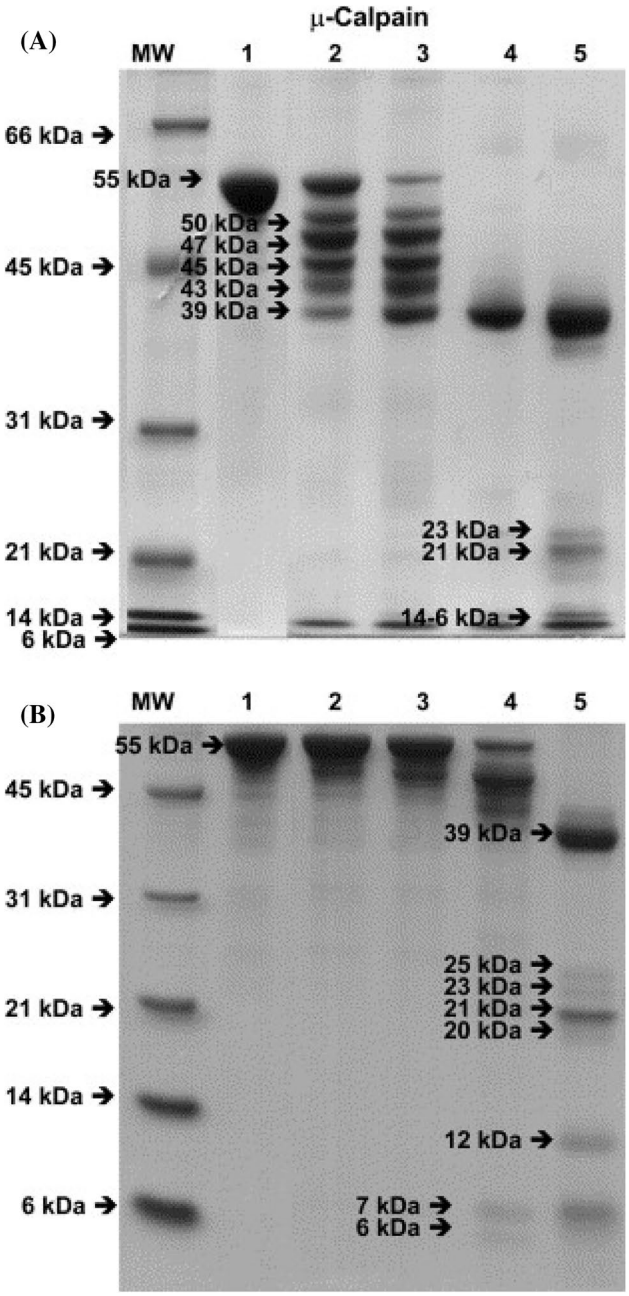

Fig. 2 SDS-PAGE patterns of desmin degradation (Baron et al., 2004)

Troponin

Troponin is localized along entire length of thin filaments and its combination with tropomyosin has a significantly synergistic effect on the contractile interaction between myosin and actin (Lehman et al., 2001). Collectively, as a important calcium-regulatory protein, gradual troponin degradation occurs under the hydrolysis of calpain and are detrimental to myofibrils dissociation (Ohtsuki et al., 2021). Therefore, Ca^{2+} released into the sarcomere has excellent ability to bind regulatory sites on troponin, subsequently inducing conformational changes of troponin and generating muscle contraction (Paul et al., 2009). The phosphorylation of troponin undoubtedly decreases its sensitivity to Ca^{2+}, Martin-Garrido et al. (2018) reported reduced myofibril Ca^{2+} sensitivity and partial calpain-induced proteolysis protection could be provided by troponin-I monophosphorylation at Ser-23/24. Another point of view is that troponin degradation also might be affected by hydrolysis by other endogenous proteases, Jiang et al. (2021) proved that

troponin-T was partially hydrolyzed under the action of caspase-3 in the early stage of storage, and affected by the synergistic effect of cathepsin and calpain with the prolongation of the storage period. Based on this, whether endogenous proteases act sequentially or synergistically in the hydrolysis of troponin needs to be further explored, in order to understand the specific regulation mode and mechanism of its degradation.

Besides, numerous literatures have established strong connection between shear force and troponin, involving the initial microstructure integrity of myofibril and then resulting in quality deterioration (Bao et al., 2018; Sun et al., 2014). Zhang et al. (2020) investigated underlying positive influence of Arg and Lys on troponin fragmentation via maintaining calpain activity, accompanied by physicochemical properties maintenance of fish post-mortem. Of course, oxidation also effectively inhibits the degradation of troponin, which may be due to the different endogenous protease susceptibility and structure changes of troponin-T induced by oxidative modification of myofibrils (Ding et al., 2021). It can be seen that on the basis of recognizing the influencing factors and degradation modes of troponin degradation, it is also of great practical significance to explore effective strategies to slow down troponin degradation.

Nebulin and titin

Titin and nebulin are two significant protein components of the sarcomere matrix in striated muscles. Most existing studies generally agree that nebulin and titin impose much significant role in overall integrity of myofibril protein microstructure by directly interacting with or controlling the interaction between the main proteins located in the thick and thin myofilaments and/or Z-disk, indirectly affecting quality characteristics especially reflected in quality deterioration indicators including shear force and WHC (Huff Lonergan et al. 2010; Li et al., 2012) The difference is that nebulin is more challenging to degrade than titin in the same environmental circumstances, and the latter can be degraded by more than 80% during the entire storage period, which depends largely on the hydrolysis sensitivity of the corresponding endogenous proteases. In a study conducted by Jiang et al. (2021), it was found that both nebulin and titin were hydrolyzed by caspase in the initial several storage days, but with the extension of storage time, titin would continue to be degraded under the action of calpain and cathepsin, while nebulin was hardly affected, Titin and nebulin have relatively low degradation rates in the pH range of 6.0–6.3 (Watanabe and Devine, 1996), and in connection with the fact that the pH of fish tends to increase during storage, it is conceivable that pH is also an important factor in accelerating the degradation rates of both proteins. Similar conclusions were also demonstrated in another study (Huang et al., 2016). Another fact is that titin and nebulin proteolysis is extremely sensitive to calcium (Abidi and Soheilifard, 2020; Huang et al., 2012). However, the mode of hydrolysis of nebulin and titin induced by endogenous proteases has not been thoroughly studied, and whether the hydrolysis is related to the specific active site of its domain and the factors influencing the hydrolysis needs to be further studied.

The process of actomyosin dissociation has been extensively researched in relation to myofibril proteins, whereas studies on the molecular level of other structural proteins, such as titin, desmin, and nebulin, have only focused on the regularity of their destruction. Furthermore, it can be challenging to understand the timing variations between the degradation process and the matching endogenous protease activation, particularly when the condition is specifically affected by outside influencing variables.

Endogenous proteases related to myofibril degradation

The main myofibril proteolysis can be attributed to endogenous protease activity. There are three characterized proteolytic systems to hydrolyze myofibril proteins during post-mortem fish muscle, including calpains, cathepsins and caspases (Jiang et al., 2021; Zeng et al., 2022).

Calpains

Activation of calpains system strongly is contributed to myofibrillar proteins proteolysis, primarily of myofibril structural proteins like desmin, which plays an essential role in accelerating texture deterioration during postmortem (Caballero et al., 2009; Qian et al., 2020). μ-calpain and m-calpain are considered as dominant form of calpains system and continuously degraded influenced by numerous external environments, including storage temperature, ionic strength, pH decline, phosphorylation, Ca^{2+} concentrations and oxidation level (Du et al., 2017; Gerelt et al., 2005). By appropriate associated activators or inhibitors, proteolytic activity of calpain may be orderly regulated, exacerbating quality degradation. Additionally, consistent results show that UK114 increases calpains activity by blocking calpastatin, which is an extremely potent endogenous calpain inhibitor (He et al., 2018a; Liu et al., 2019). It is known that the concentration of Ca^{2+} plays significant effects on the activation mechanism of calpains, substantially resulting in myofibril structural proteins autolysis and muscle microstructure destruction. One study noted that during storage, calpains gradually may translocate from the sarcoplasm to the myofibrils and elevated Ca^{2+} concentration drives the binding of calpain to myofibrils. Once the concentration of Ca^{2+} reaches a particular threshold, this segment of the conjugate

is activated and begins to disintegrate myofibril structural proteins (Lyu and Ertbjerg, 2022). Although the above-mentioned study was conducted on pork, it may, to a certain extent, provide new insights to study the calpain-induced myofibril proteins degradation during storage of fish. It is also worth considering that high Ca^{2+} concentrations may also impact calcium stabilization in tissue cells, which may affect the integrity of organelle membranes such as mitochondria and lysosomes, thus having an indirect effect on the hydrolytic activation of caspases and cathepsins. Calpain activity is proportional to the degree of oxidation within a certain range, involving sulfhydryl, carbonyl and secondary structure changes, whose activity reversely may be inhibited when it exceeds this range (Liu et al., 2021b). The explanatory mechanism for this relationship is that moderate oxidation may enhance electron exchange between Cys and His residues, thence accelerating the calpains autolysis and activation (Zhang et al., 2021). In addition, heat shock proteins (HSP20, HSP27) as well as protein S-nitrosylation has a good protective effect on calpain-induced myofibril degradation (Liu et al., 2016; Lomiwes et al., 2014). All thing mentioned, in addition to the current research generally concerned calpains-induced myofibril degradation patterns, strategies for activating or inhibiting calpains should also be paid more attention to regulate the degradation of myofibrils in a targeted manner to achieve the purpose of better preservation of fish (Delbarre-Ladrat et al., 2004; Yang et al., 2021; He et al., 2018b; Huang et al., 2023).

Lysosomal cathepsins

Lysosomal cathepsins, generally including cathepsin B, cathepsin D and cathepsin L, are demonstrated to trigger myofibril proteolysis and myofilament disassembly (Godiksen et al., 2009; Ladrat et al., 2003). Numerous influencing factors that determine post mortem cathepsins activity were dissected including storage condition such as pH and temperature, exogenously added activators or inhibitors, and metal ions (Wang et al., 2021; Zhang et al., 2020; Zhang et al., 2021). Pre-slaughter stress also appears to increase cathepsin activity, which may be related to the low initial pH of the muscle, which is one of the crucial conditions for cathepsins activity to be activated and release of cathepsins from lysosomes to myofibrils (Bahuaud et al., 2010; Zhang et al., 2019).

It has been known to demonstrate that the activity of cathepsins were activated in the early stage of storage and increased gradually with the extension of storage time, which played a role in the degradation of myofibrillar protein during entire storage period (Jiang et al., 2022). Different kinds of cathepsins might act individually, simultaneously or sequentially to degrade specific myofibril structure proteins, and cathepsins and calpains have obvious synergistic effects on the degradation and disassembly of myofilament (Ge et al., 2018a). This study pointed out that cathepsin B effectively decreased UNC45 and HSP 90 content to manipulate thick myofilament procedural disassembly, boosting the degradation of dissociated MHC induced by calpains, while cathepsin L tended to decline HSP 27 and αb-crystallin concentration to remove the actin from thin filament, to create dissociated actin as substrate supply for calpains. This also reflects the possibility that cathepsins may require the assistance of heat shock proteins to regulate procedure hydrolysis of myofibrils (Ge et al., 2018b). Nonetheless, differences in the hydrolysis pathways of different forms of cathepsins should also be further elaborated, especially in complex fish matrices where they coexist with other endogenous proteases. The construction of an in vitro myofibril protein degradation simulation system based on the co-incubation of endogenous protease and myofibril proteins is an effective research strategy (Zhong et al., 2012). This inevitably puts forward higher requirements on how to effectively obtain highly purified cathepsins to obtain more accurate research results. In addition, based on the fact that cathepsin B, L respectively show high affinity towards Z-Arg-Arg-AMC and Z-Phe-Arg-AMC, whose specific fluorescent substitute could be applied to detect corresponding cathepsins activity (Fidalgo et al., 2020).

Overall, cathepsins-induced myofibril proteolysis has been widely certified, the detailed mechanism of different types of cathepsins leading to the gradual degradation of myofibril proteins needs further research and verification. And the upstream regulators of cathepsins activation need to be further explored based on the proteomics, metabolomics and genomics, including the role played by heat shock proteins, release kinetics from lysosomes and related gene expression.

Caspases

Caspases activation generally regulate programmed apoptotic process by regulating signal transduction and final execution process, generally involving in multiple apoptotic factors: initiator caspases and effector caspases (Longo et al., 2015). With the extensive and in-depth research of caspases, there is unanimous point of view that activated caspase-3 considered as a possible candidate for myofibril proteins degradation interfering with mitochondrial apoptosis pathways (Mohrhauser et al., 2014). When cytochrome c is released from mitochondria to cytosol, apoptosome may be produced by interaction between cytochrome c, Apaf-1 and combine with ATP/dATP, which actuates procaspase-9 and further activates the executor caspase-3 triggering apoptosis cascade reaction (Li et al., 2022). The imbalance in Ca^{2+} homeostasis and reactive oxygen (ROS) accumulation induced by

oxidation maybe the key regulators of caspase-3 activation, which is clearly reflected in the extent of mitochondrial damage and dysfunction. Oxidation may promote the translocation of Hsp27 and cytochrome C from the cytoplasm to the cell membrane and increase caspase-3 activity by mediating their interaction, while having the opposite effect on calpains activity (Ding et al., 2021). Linked to the Ca^{2+}-dependent properties of calpains, there are some potential links between calpain and caspase-3 activity mediated by intermediating protein substances.. There is a point of view: caspase-3 can promote calpastatin degradation or cleave key myofibril proteins during early postmortem period, subsequently inactivated by calpains to merely exert limited influences on myofibril proteins degradation (Smuder et al., 2010). It is well worth noting that lysosomal Fe^{2+} can induce mitochondrial dysfunction, thereby promoting the activity of apoptotic enzymes and promoting the degradation of myofibril proteins, which has also been demonstrated in the report of (Chen et al., 2021). In the view of caspases-induced myofibril disorder, molecular mechanism of how caspases are activated and then take concrete pathways in myofibril disorder are still unclear, especially the procedural activation and inhibition mechanism of apoptotic factors. Although the intrinsic links between apoptase and calpain have been initially established, whether it also has a specific relationship with cathepsins needs further research. In other words, the synergistic effects of caspase-3, the calpains and the cathepsins also undoubtedly need to be further studied.

Taken as a whole, research on endogenous proteases is currently mainly focused on the hydrolysis effects on myofibril proteins, emphasizing downstream events (Fig. 3). Considering the difference of endogenous protease activity in different storage periods, activation pathway of endogenous proteases, the regulation mechanisms of related factors and the interaction mechanism of endogenous proteases are not well established. Moreover, with respect to myofibril proteolysis, the intrinsic molecular structure–function changes involved in exacerbated proteins oxidation, aggregation and degeneration need to be more precisely elucidated in the future studies.

Evaluation method of myofibrils degradation

Traditional qualitative and quantitative evaluation

Myofibril proteins degradation can be qualitatively characterized by traditional physicochemical indicators, the most common of which involves in physicochemical properties, ATPase activities, amino acid residue side chain groups (carbonyl content, S–S and SH group), conformation changes and myofilament microstructure integrity (Table 2). Of course, more functional characteristics,

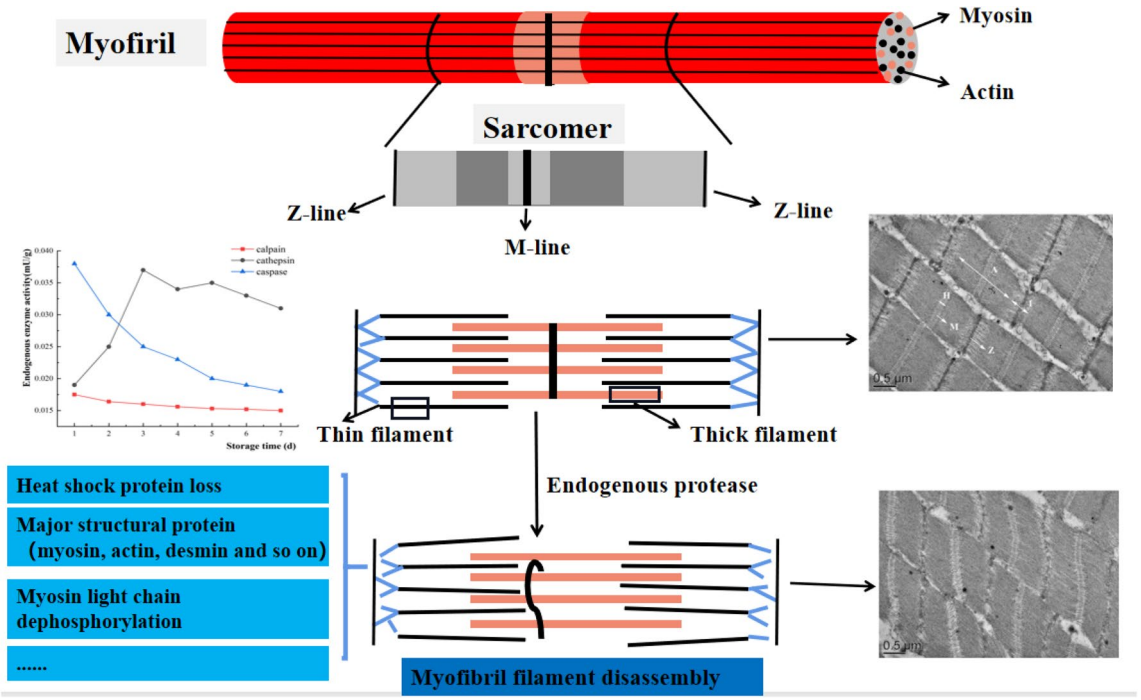

Fig. 3 Schematic diagrams of protein degradation caused by endogenous protease (Jiang et al., 2022; Liu et al., 2021a, b; Ge et al., 2018b)

Table 2 Traditional indexes that have been reported for evaluating myofibril proteins of fillets during storage

Types of fish samples	Storage condition	Evaluation items	Indicators	References
Large yellow croaker	Frozen storage	Physicochemical properties, aggregation and degradation level, conformational change	Turbidity, zeta potential; confocal laser scanning microscopy (CLSM), SDS-PAGE; secondary structure	Tan et al. (2022)
Puffer fish	Papain treatment	Physicochemical properties, functional properties, thermal stability, degradation level, conformational change	Shear force, soluble protein content; rheological properties, thermodynamic parameters; SDS-PAGE; FT-IR	Wang and Xie (2019)
Farmed puffer fish	Chitosan-sodium alginate bioactive coatings combind with MAP	ATPase activities, amino acid residue side chain groups, conformational change, myofibril integrity	Ca^{2+}-ATPase activity; sulfhydryl content, carbonyl groups; secondary structure; TEM images	Li et al. (2021a,b)
Tilapia fillets	Chilled storage	Conformational change, amino acid residue side chain groups, ATPase activities	FT-IR, AFM; disulphide bonds, carbonyl groups, sulfhydryl content; Ca^{2+}-ATPase activity	Zhao et al. (2019)
Grass carp	Chilled storage	Endogenous protease activity, physicochemical properties, actomyosin dissociation,	Cathepsin B, D, L and H activity; SDS-PAGE; myofibril fragmentation index (MFI)	Wang et al. (2016)
Bighead carp	Frozen storage	Microstructure, protein oxidation parameters, and specific modifications of oxidized amino acids	Light microscopy, TEM; carbonyl content, sulfhydryl groups content; intrinsic fluorescence, SDS-PAGE; total number of oxidized amino acids in MHC, actin, enzymes and myosin light chain (MLC)	Liu et al. (2022)

including gelation properties, rheological characteristics and dynamic viscoelastic behaviour, has been applied in evaluating myofibril proteins degradation (Miller et al., 2020). Amino acid residue side chain groups modification is often manifested as the varying aggregation and swelling of myofibril proteins, accompanied by changes in surface hydrophobicity and intermolecular forces of myofibril proteins, which can be attributed to the exposure of hydrophobic amino acid residues buried in the protein (Tan et al., 2022). The gradual accumulation of carbonyl content is based on multiple factors: oxidation, non-enzymatic glycation, oxidative cleavage of the peptide backbone and covalent binding to non-protein carbonyl compounds (Estévez, 2011). The variation trend that S–H groups continuously decrease and S–S groups show opposite direction can be explained by auto oxidation process. In addition, myofibril proteins conformation (secondary structure or/and tertiary structure) can be accurately measured through fourier transform infrared spectroscopy (TF-IR), raman spectroscopy and fluorescence spectrometer. In addition, endogenous protease activity above introduced in this paper has been recognized to understand internal correlations with myofibril proteolysis degree, which is currently mostly detected by the corresponding specific fluorometric assay kit (Mi et al., 2021). Visualization of myofibril proteins microstructure is validly observed by means of scanning electron microscope (SEM), transmission electron microscope (TEM) and atomic force microscopy (AFM) to evaluate information of structural integrity (Li, Mei and Xie, 2021). Information such as myofibril structures (Z-disk, I-bands, and M-lines), sarcomere length and intersarcomere gap can be clearly obtained from the TEM image, which makes it have broad application value in evaluating the integrity of thick and thin myofilaments (Ali et al., 2022; Liu et al., 2022). SDS-PAGE combined with western blot is frequently used to assess myofibril proteins degradation based on relative molecular weight information compared with standard protein to achieve routine quantification (Jiang et al., 2021; Yang et al., 2019). Changes in the noncovalent interactions among myofibril proteins can be assessed by comparing the SDS-PAGE patterns of myofibril proteins with and without EDC crosslinking, which can be well linked to intermolecular forces for a comprehensive analysis of the myofilament stability (Ge et al., 2018). Furthermore, fast non-destructive testing technology has been paid close attention in recent literatures and provides another method to understand myofibril proteins degradation, especially application of spectroscopy technology (Andersen et al., 2017; 2018). According to thermograms and endothermic peaks information, differential scanning calorimetry (DSC) can distinguish between fresh and frozen seabream and determine whether post-mortem storage temperatures were abused (Matos et al., 2011). Two-dimensional electrophoresis (2-DE) has now developed into an effective means

of separating proteolytic peptides based on the differentially abundant spots, identification and quantitative analysis of differential proteins can be further achieved through the highest resolution of matrix assisted laser desorption ionisation time of flight mass spectrometry (MALDI-TOF–MS) (Chen et al. 2013; Feng et al. 2016). Li et al. (2014) identified of proteins and specific peptides via 2DE which could be used as indicators of freshness and quality in fish. It has to be said that electrophoresis is currently an effective tool for separating protein molecules, and it is also the basis for the analysis of myofibril proteins degradation using other techniques, whether it is a relatively simple degradation level analysis or the biological function analysis mentioned below.

However, all the above evaluation methods are still based on the evaluation of the ostensible properties of myofibril proteins, and the evaluation methods at myofibrillar proteins subunit level have not yet well been established due to the the immature reality of mechanism research. In addition, the identification of the binding sites of endogenous proteases to myofibrils also requires powerful tools to be more clearly studied.

Proteomics-based evaluation

With the rapidly advanced development, proteomics has been proposed as a promising strategy to obtain accurate myofibril proteins degradation information based on semi-quantitative data information detected from mass spectrometry (MS) (Gallego et al., 2015; Shui et al., 2021). By high-throughput analysis, label-free proteomics combined with bioinformatics has been validly proven to build molecular connections between differential expression levels of muscle proteins and quality properties deterioration to determine potential protein biomarkers linked to quality traits (Pan et al., 2022). The key elements of proteomics consist of the separation of protein components, the identification of protein components, and analysis and prediction the structure and functional characteristics of identified differential proteins using bioinformatics (Shi et al., 2018; Zhang et al., 2020). After analyzing the comprehensive information obtained from proteomics, the mechanism of quality deterioration can be deeply elaborated by linking to myofibril proteins hydrolysis, differential abundance proteins (DAPs) expression and post-mortem metabolic pathways (Table 3). It can be seen that DAPs generally includes heat shock proteins, metabolic enzymes, structure proteins, oxidative proteins, proteolysis and so on, which have been similarly elaborated in another published review by Huang et al. (2020). Furthermore, proteomics should be combined with other technologies at different research levels (metabolomics, genomics and transcriptomics), which will be used as the main research method to accurately study the mechanism of fish quality deterioration in the future.

Table 3 Examples of proteomic applications to explain myofibril degradation

Types of fish samples	Purpose	Storage condition	DAPs	Bioinformatics	References
Squid	Distinguish two different types of squid	Frozen storage	Dsocitrate dehydrogenase and malic enzyme	Cytoskeleton function, including paramyosin, tropomyosin, and troponin C	Shui et al. (2021)
Sea bass fillets	Identify potential proteomic markers to assess fillet freshness	Epigallocatechin gallate incorporated in agar oligosaccharide treatment	Structural proteins, metabolic enzymes, and proteins involved in protein turnover	Maintaining higher levels of transcription and translation to monitor fillet freshness	Xiang et al. (2021)
Tan mutton	Investigate the effects of oxidized proteins on meat color and water holding capacity	Different low storage temperature	MYL1, MYL3, MYH3, MYH8, MYBPH, NEB, ACTN2, TTN, PKM, MDH1, ALDOC, LDHA, GAPDH, TPI1, HSPA-8	Structural proteins and metabolic enzymes, oxidative modification of metabolic enzymes	Tao et al. (2021)
Bighead carp	Investigate the effects of enzymes in exudates on protein oxidation in thawed fillets	Exudates from different storage condition (fresh, freeze–thaw frozen)	Pyruvate dehydrogenase, β-PDH E1, aldehyde oxidase 1 and dihydrolipoyl dehydrogenase	Biological process, cellular component, and molecular function	Liu et al. (2021a, b)
Litopenaeus vannamei	Investigate proteomic changes correlated with quality deterioration	Partial freezing	Ribosomal proteins, actins, myosin, paramyosin, myosin heavy chains, and tropomyosin	cellular and metabolic process, binding and catalytic activity	Pan et al., (2022)

The advantages of bioinformatics application in proteomics reflected in the analysis of protein–protein interactions and molecular pathways, as well as the systematic study of gene regulation. The most used public bioinformatics databases are gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) analysis, the former of which may provide participated biological processes and effects of DAPs, such as catalytic activity, binding and metabolic processes, while the latter of which mainly investigate signal transduction pathways of myofibril proteins (Gagaoua et al., 2021; Zandonadi et al., 2019). It is undeniable that bioinformatics has promoted tremendous progress of proteomics, which helps explore potential protein markers and provide further insight into the molecular mechanisms and pathways underlying variations in fish spoilage. In addition, it should be noted that the current proteomic research on fish is relatively less than that of livestock meat, and the understanding of the metabolic pathways of post-mortem fish is still relatively limited. However, proper sample preparation can largely guarantee the reliability of proteomics results, so effective specific operation procedures should be further optimized. At present, the specific operation of proteomics is still mainly completed by professional institutions. Researchers usually analyze results provided by the institutions to obtain final reliable experimental results. The high cost problem caused by this is expected to be improved in the future.

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References

Abidi M, Soheilifard R. The effect of calcium binding on the unfolding force of mutated and healthy titin I10 domain: A steered molecular dynamics simulation study. Journal of Molecular Graphics and Modelling. 96: in press (2020).

Ahmed Z, Donkor O, Street WA, Vasiljevic T. Calpains- and cathepsins-induced myofibrillar changes in post-mortem fish: Impact on structural softening and release of bioactive peptides. Food Science and Technology. 45(1): 130-146 (2015)

Ali S, Ammar KM, Rajput N, Naeem M, Zhang W, Li C B, Zhou G. Desmin as molecular chaperone for myofibrillar degradation during freeze-thaw cycles. Food Chemistry. 386, in press (2022)

Andersen PV, Veiseth-Kent E, Wold J P. Analyzing pH-induced changes in a myofibril model system with vibrational and fluorescence spectroscopy. Meat Science. 125:1-9 (2017)

Andersen P V, Wold J P, Veiseth-Kent E. Analyzing mu-Calpain induced proteolysis in a myofibril model system with vibrational and fluorescence spectroscopy. Meat Science. 139: 239-246 (2018)

Bahuaud D, Mørkøre T, Østbye TK, Veiseth-Kent E, Thomassen M S, Ofstad R. Muscle structure responses and lysosomal cathepsins B and L in farmed Atlantic salmon (*Salmo salar L.*) pre- and post-rigor fillets exposed to short and long-term crowding stress. Food Chemistry. 118(3): 602–615 (2010)

Bao Y, Boeren S, Ertbjerg P. Myofibrillar protein oxidation affects filament charges, aggregation and water-holding. Meat Science. 135: 102-108 (2018)

Bao Y, Wang K, Yang H, Regenstein JM, Ertbjerg P, Zhou P. Protein degradation of black carp (*Mylopharyngodon piceus*) muscle during cold storage. Food Chemistry. 308: in press. (2020)

Barido FH, Lee SK. Changes in proteolytic enzyme activities, tenderness-related traits, and quality properties of spent hen meat affected by adenosine 5'-monophosphate during cold storage. Journal of Poultry Science. 100(5): in press (2021)

Baron CP, Jacobsen S, Purslow PP. Cleavage of desmin by cysteine proteases: Calpains and cathepsin B. Meat Science. 68(3): 447–456 (2004d)

Caballero MJ, Betancor M, Escrig JC, Montero D, Espinosa de los Monteros, A, Castro P, Izquierdo M. Post mortem changes produced in the muscle of sea bream (*Sparus aurata*) during ice storage. Aquaculture. 291(3–4): 210–216 (2009e)

Chen C, Guo Z, Ma G, M, J, Zhang Z, Yu Q, Han L. Lysosomal Fe^{2+}contributes to myofibrillar protein degradation through mitochondrial-dysfunction-induced apoptosis. Lwt-Food Science and Technology. 143: in press (2021)

Chen K, Cole RB, Rees BB. Hypoxia-induced changes in the zebrafish (*Danio rerio*) skeletal muscle proteome. Journal of Proteomics. 78: 477-485 (2013)

Cheret R, Delbarreladrat C, Lamballerieanton M, Verrezbagnis V. Calpain and cathepsin activities in post mortem fish and meat muscles. Food Chemistry. 101(4): 1474-1479 (2007)

Delbarre-Ladrat C, Cheret R, Taylor R, Verrez-Bagnis V. Trends in postmortem aging in fish: Understanding of proteolysis and disorganization of the myofibrillar structure. Critical review of food science and nutrition. 46 (5): 409-421 (2006).

Cohen, S. Role of calpains in promoting desmin filaments depolymerization and muscle atrophy. Biochimica Biophysica Acta (BBA)- Molecular Cell Research. 1867(10): in press (2020)

Delbarre-Ladrat C, Verrez-Bagnis V, Noël J, Fleurence J. Proteolytic potential in white muscle of sea bass (*Dicentrarchus labrax L.*) during post mortem storage on ice: time-dependent changes in the activity of the components of the calpain system. Food Chemistry. 84(3): 441–446 (2004).

Ding Z, Wei Q, Zhang C, Zhang H, Huang F. Influence of oxidation on heat shock protein 27 translocation, caspase-3 and calpain activities and myofibrils degradation in postmortem beef muscles. Food Chemistry. 340: in press (2021)

Du M, Li X, Li Z, Li M, Gao L, Zhang D. Phosphorylation inhibits the activity of mu-calpain at different incubation temperatures and Ca^{2+} concentrations in vitro. Food Chemistry. 228: 649-655 (2017)

Estévez M. Protein carbonyls in meat systems: A review. Meat Science. 89(3), 259-279 (2011)

Fan X, Jin Z, Liu Y, Chen Y, Konno K, Zhu B, Dong X. Effects of super-chilling storage on shelf-life and quality indicators of coregonus peled based on proteomics analysis. Food Research International. 143: in press (2021)

Fang M, Luo X, Xiong S, Yin T, Hu Y, Liu R, You J. (2021). In vitro trypsin digestion and identification of possible cross-linking sites induced by transglutaminase (TGase) of silver carp (*Hypophthalmichthys molitrix*) surimi gels with different degrees of cross-linking. Food Chemistry. 364: in press (2021)

Fang M, Xiong S, Yin T, Hu Y, Liu R, Du H, You, J. Proteomic profiling and oxidation site analysis of gaseous ozone oxidized myosin from silver carp (*Hypophthalmichthys molitrix*) with different oxidation degrees. Food Chemistry. 363: in press (2021)

Feng L, Bi W, Chen S, Zhu J, Liu X. Regulatory function of sigma factors RpoS/RpoN in adaptation and spoilage potential of Shewanella baltica. Food Microbiology. 97: in press (2021)

Feng X, Bansal N, Yang H. Fish gelatin combined with chitosan coating inhibits myofibril degradation of golden pomfret (*Trachinotus blochii*) fillet during cold storage. Food Chemistry. 200, 283-292 (2016)

Fidalgo LG, Delgadillo I, and Saraiva JA. Autolytic changes involving proteolytic enzymes on Atlantic salmon (*Salmo salar*) preserved by hyperbaric storage. Lwt-Food Science and Technology. 118: in press (2020)

Gagaoua M, Terlou, EM, Mullen AM, Franco D, Warner RD, Lorenzo JM, Purslow PP, Gerrard D, Hopkins D, Troy D, Picard, B. Molecular signatures of beef tenderness: Underlying mechanisms based on integromics of protein biomarkers from multi-platform proteomics studies. Meat Science. 172: in press (2021)

Gallego M, Mora L, Aristoy MC, Toldra F. Optimisation of a simple and reliable label-free methodology for the relative quantitation of raw pork meat proteins. Food Chemistry. 182: 74-80 (2015)

Ge L, Xu Y, Xia W, Jiang Q. Synergistic action of cathepsin B, L, D and calpain in disassembly and degradation of myofibrillar protein of grass carp. Food Research International. 109: 481-488 (2018a)

Ge L, Xu Y, Xia W, Zhao N, Jiang Q. Contribution of myofibril filament disassembly to textural deterioration of ice-stored grass carp fillet: Significance of endogenous proteolytic activity, loss of heat shock protein and dephosphorylation of myosin light chain. Food Chemistry. 269: 511-518 (2018b)

Gerelt B, Rusman H, Nishiumi, Suzuki A. Changes in calpain and calpastatin activities of osmotically dehydrated bovine muscle during storage after treatment with calcium. Meat Science. 70(1): 55–61 (2005l)

Godiksen H, Morzel M, Hyldig G, Jessen F. Contribution of cathepsins B, L and D to muscle protein profiles correlated with texture in rainbow trout (*Oncorhynchus mykiss*). Food Chemistry. 113(4): 889-896 (2009)

Guo Z, Li Z, Wang J. Zheng B. Gelation properties and thermal gelling mechanism of golden threadfin bream myosin containing $CaCl_2$ induced by high pressure processing. Food Hydrocolloids. 95: 43–52 (2019)

Hanif M, Marttila M, Pelin K, Gronholm M, Wallgren-Pettersson C. Pathogenetic studies of nebulin: a giant myofibrillar protein. Neuromuscular Disorders. 20: 596-680 (2010)

He YF, Huang H, Li LH, Yang XQ, Hao SX, Chen, SJ, Deng JC. The effects of modified atmosphere packaging and enzyme inhibitors on protein oxidation of tilapia muscle during iced storage. Lwt-Food Science and Technology. 87: 186-193 (2018a)

He YF, Huang H, Li LH, Yang XQ. Changes of activated factors and activation of calpain in tilapia muscle during storage. Fisheries Science. 84(5): 889-895 (2018b)

Huang C, Hou C, Ijaz M, Yan T, Li X, Li Y, Zhang D. Proteomics discovery of protein biomarkers linked to meat quality traits in post-mortem muscles: Current trends and future prospects: A review. Food Science and Technology. 105: 416-432 (2020)

Huang JC, Yang J, Huang F, Huang M, Chen KJ, Xu XL, Zhou GH. Effect of fast pH decline during the early postmortem period on calpain activity and cytoskeletal protein degradation of broiler M. pectoralis major. Journal of Poultry Science. 95(10): 2455–2463 (2016)

Huang M, Huang F, Ma H, Xu X, Zhou G. Preliminary study on the effect of caspase-6 and calpain inhibitors on postmortem proteolysis of myofibrillar proteins in chicken breast muscle. Meat Science. 90(3), 536-542 (2012)

Huang XL, Wang H, Tu ZC. A comprehensive review of the control and utilization of aquatic animal products by autolysis-based processes: Mechanism, process, factors, and application. Food Research International. 164: 112325 (2023)

Huff Lonergan E, Zhang W, Lonergan SM. Biochemistry of postmortem muscle-lessons on mechanisms of meat tenderization. Meat Science. 86 (1): 184-195 (2010)

Hussain MA, Sumon TA, Mazumder SK, Ali MM, Jang WJ, Abualreesh MH, Sharifuzzaman SM, Brown CL, Lee H, Lee E, Hasan MT. Essential oils and chitosan as alternatives to chemical preservatives for fish and fisheries products: A review. Food Control. 129: in press (2021)

Jiang QX, Yang F, Jia SN, Yu DW , Gao P, Xu YS, Xia WS. (2021). The role of endogenous proteases in degrading grass carp (*Ctenopharyngodon idella*) myofibrillar structural proteins during ice storage. LWT-Food Science and Technology. 154: in press (2021)

Kim GD, Jung EY, Lim HJ, Yang HS, Joo ST, Jeong JY. Influence of meat exudates on the quality characteristics of fresh and freeze-thawed pork. Meat Science. 95(2): 323-329 (2013)

Ko WC, Shi HZ, Chang CK, Huang YH, Chen YA, Hsieh CW. Effect of adjustable parallel high voltage on biochemical indicators and actomyosin Ca^{2+}-ATPase from tilapia (*Orechromis niloticus*). LWT-Food Science and Technology. 69: 417-423 (2016)

Ladrat C, Verrez-Bagnis V, Noël J, Fleurence J. In vitro proteolysis of myofibrillar and sarcoplasmic proteins of white muscle of sea bass (*Dicentrarchus labrax L.*): effects of cathepsins B, D and L. Food Chemistry. 81(4): 517–525 (2003)

Lana A, Zolla L. Proteolysis in meat tenderization from the point of view of each single protein: A proteomic perspective. Journal of Proteomics. 147: 85-97 (2016)

Lehman W, Rosol M, Tobacman LS, Craig R. Troponin organization on relaxed and activated thin filaments revealed by electron microscopy and three-dimensional reconstruction11Edited by W. Baumeister. Journal of Molecular Biology. 307(3) : 739-744 (2001)

Li PY, Mei J, Xie J. Chitosan-sodium alginate bioactive coatings containing ε-polylysine combined with high CO_2 modified atmosphere packaging inhibit myofibril oxidation and degradation of farmed pufferfish (*Takifugu obscurus*) during cold storage. LWT-Food Science and Technology. 140: in press (2021)

Li S, Xu X, Zhou G. The roles of the actin-myosin interaction and proteolysis in tenderization during the aging of chicken muscle. Journal of Poultry Science. 91(1), 150-160 (2012)

Li T, Li J, Hu W, Chen J, Li H. Protein changes in post mortem large yellow croaker (*Pseudosciaena crocea*) monitored by SDS-PAGE and proteome analysis. Food Control. 41: 49-55 (2014)

Li X, Hu L, Zhu XR, Guo XB, Deng XR, Zhang J. The effect of caspase-3 in mitochondrial apoptosis activation on degradation of structure proteins of Esox lucius during postmortem storage. Food Chemistry. 367 : in press (2022)

Lin XY, Ruiz J, Bajraktari I, Ohman R, Banerjee S, Gribble K, Kaufman JD, Wingfield PT, Grigges RC, Fischbeck KH, Mankodi A. Z-disc-associated, alternatively spliced, PDZ motif-containing protein (ZASP) mutations in the actin-binding domain cause disruption of skeletal muscle actin filaments in myofibrillar myopathy. Journal of Biological Chemistry. 289(19): 13615-13626 (2014)

Liu HT, Zhang H, Liu Q, Chen Q, Kong BH. Filamentous myosin in low-ionic strength meat protein processing media: Assembly mechanism, impact on protein functionality, and inhibition strategies. Trends in Food Science and Technology. 112: 25-35 (2021a)

Liu PP, Zhang ZW, Guo X, Zhu XR, Mao XY, Guo XB, Deng XR, Zhang J. u-Calpain oxidation and proteolytic changes on myofibrillar proteins from Coregonus Peled in vitro. Food Chemistry. 361: in press (2021b)

Liu R, Li Y, Wang M, Zhou G, Zhang W. Effect of protein S-nitrosylation on autolysis and catalytic ability of μ-calpain. Food Chemistry. 213 : 470-477 (2016)

Liu R, Lonergan S, Steadham E, Zhou G, Zhang W, Huff-Lonergan E. Effect of nitric oxide and calpastatin on the inhibition of micro-calpain activity, autolysis and proteolysis of myofibrillar proteins. Food Chemistry. 275: 77-84 (2019)

Liu YY, Zhang LT, Gao S, Bao YL, Tan YQ, Luo YK, Li XM, Hong H. Effect of protein oxidation in meat and exudates on the water holding capacity in bighead carp (*Hypophthalmichthys nobilis*) subjected to frozen storage. Food Chemistry. 370: in press (2022)

Lomiwes D, Hurst SM, Dobbie P, Frost D A, Hurst RD, Young OA, Farouk MM. The protection of bovine skeletal myofibrils from proteolytic damage post mortem by small heat shock proteins. Meat Science. 97(4): 548-557 (2014)

Longo V, Lana A, Bottero MT, Zolla L. Apoptosis in muscle-to-meat aging process: The omic witness. Journal of Proteomics. 125: 29-40 (2015)

Lyu J, Ertbjerg P. Sarcoplasmic and myofibril-bound calpains during storage of pork longissimus muscle: New insights on protein degradation. Food Chemistry. 372: in press (2022)

Martin-Garrido A, Biesiadecki BJ, Salhi HE, Shaifta Y, Dos Remedios CG, Ayaz-Guner S, Kentish JC. Monophosphorylation of cardiac troponin-I at Ser-23/24 is sufficient to regulate cardiac myofibrillar Ca^{2+} sensitivity and calpain-induced proteolysis. Journal of Biological Chemistry. 293(22): 8588-8599 (2018)

Matos E, Silva TS, Tiago T, Aureliano M, Dinis MT, Dias J. Effect of harvesting stress and storage conditions on protein degradation in fillets of farmed gilthead seabream (*Sparus aurata*): A differential scanning calorimetry study. Food Chemistry. 126(1): 270-276 (2011)

Mehta NK, Rout B, Balange AK, Nayak BB. Dynamic viscoelastic behaviour, gelling properties of myofibrillar proteins and histological changes in shrimp (*L. vannamei*) muscles during ice storage. Aquaculture and Fisheries. 8 (2): 180–189 (2023)

Mi J, Zhao XZ, Huang P, Hong JX, Jia R, Deng SG, Yu XX, Wei HM, Yang WG. Effect of hydroxypropyl distarch phosphate on the physicochemical characteristics and structure of shrimp myofibrillar protein. Food Hydrocolloids. 125: in press (2022).

Miller DK, Acevedo NC, Lonergan SM, Sebranek JG, Tarte R. Rheological characteristics of mechanically separated chicken and chicken breast trim myofibril solutions during thermal gelation. Food Chemistry. 307: in press (2020)

Mohrhauser D, Kern S, Underwood K, Weaver A. Caspase-3 does not enhance in vitro bovine myofibril degradation by u-calpain. Meat Science. 96(1): 468 (2014)

Mora L, M G, F T. Degradation of myosin heavy chain and its potential as a source of natural bioactive peptides in dry-cured ham. Food Bioscience. 30: in press (2019)

Nie XB, Zhang RC, Cheng LL, Zhu WB, Li SL, Chen XM. Mechanisms underlying the deterioration of fish quality after harvest and methods of preservation. Food Control. 135: 108805 (2022).

Ohtsuki I, Morimoto S, Kitainda V (2021v). Structural Proteins | Troponin. Vol, III , pp. 695–700, In: Encyclopedia of Biological Chemistry. Jez J(Ed), Oxford: Elsevier.

Pan C, Sun K, Yang X, Wang D, Hu X, Chen S. Insights on Litopenaeus vannamei quality deterioration during partial freezing storage from combining traditional quality studies and label-free based proteomic analysis. Journal of Food Composition Analysis. 112: in press (2022)

Paul DM, Morris EP, Kensler RW, Squire JM. Structure and Orientation of Troponin in the Thin Filament. Journal of Biological Chemistry. 284 (22): 15007-15015 (2009)

Prabhakar PK, Vatsa S, Srivastav PP, Pathak SS. A comprehensive review on freshness of fish and assessment: Analytical methods and recent innovations. Food Research International. 133: in press (2020)

Qian S, Li X, Wang H, Wei X, Mehmood W, Zhang C, Blecker C. Contribution of calpain to protein degradation, variation in myowater properties and the water-holding capacity of pork during postmortem ageing. Food Chemistry. 324: in press (2020)

Rahman MN, Islam A.C onsumer fish consumption preferences and contributing factors empirical evidence from Rangpur city corporation Bangladesh. Heliyon. 6(12): in press (2020)

Reed ZH, Park JW. Thermophysical characterization of Tilapia myosin and its subfragments. Journal of Food Science. 76(7): C1050-C1055 (2011).

Ren C, Hou C, Zhang D, Li X, Xiao X, Bai Y. ATP regulates the phosphorylation and degradation of myofibrillar proteins in ground ovine muscle. Journal of Integrative Agriculture. 20(1): 311-318 (2021).

Richardson E, Bohrer BM, Arkfeld EK, Boler DD, Dilger AC. A comparison of intact and degraded desmin in cooked and uncooked pork longissimus thoracis and their relationship to pork quality. Meat Science. 129: 93-101 (2017).

Roco T, Torres MJ, Briones-Labarca V, Reyes JE, Tabilo-Munizaga G, Stucken K, Lemus-Mondaca R, Perez-Won M. Effect of high hydrostatic pressure treatment on physical parameters, ultrastructure and shelf life of pre- and post-rigor mortis palm ruff (*Seriolella violacea*) under chilled storage. Food Research International. 108: 192-202 (2018)

Roth B, Grimsbø E, Slinde E, Foss A, Stien LH, Nortvedt R. Crowding, pumping and stunning of Atlantic salmon, the subsequent effect on pH and rigor mortis. Aquaculture. 326-329: 178-180 (2012)

Shao L, Chen S, Wang H, Zhang J, Xu X, Wang H. Advances in understanding the predominance, phenotypes, and mechanisms of bacteria related to meat spoilage. Trends in Food Science and Technology. 118 : 822-832 (2021)

Shi J, Zhang L, Lei Y, Shen H, Yu X, Luo YK. Differential proteomic analysis to identify proteins associated with quality traits of frozen mud shrimp (*Solenocera melantho*) using an iTRAQ-based strategy. Food Chemistry. 251: 25-32 (2018)

Shui SS, Yao H, Jiang ZD, Benjakul S, Aubourg SP, Zhang B. The differences of muscle proteins between neon flying squid (*Ommastrephes bartramii*) and jumbo squid (*Dosidicus gigas*) mantles via physicochemical and proteomic analyses. Food Chemistry. 364, 130374 (2021)

Smuder AJ, Kavazis AN, Hudson MB, Nelson W B, Powers SK. Oxidation enhances myofibrillar protein degradation via calpain and caspase-3. Free Radical Biology and Medicine. 49(7): 1152-1160 (2010)

Starkey CP, Geesink GH, Oddy VH, Hopkins DL. Explaining the variation in lamb longissimus shear force across and within ageing periods using protein degradation, sarcomere length and collagen characteristics. Meat Science. 105, 32-37 (2015)

Sun X, Chen KJ, Berg EP, Newman DJ, Schwartz CA, Keller WL, Maddock Carlin KR. Prediction of troponin-T degradation using color image texture features in 10d aged beef longissimus steaks. Meat Science. 96, 837-842 (2014)

Tan, MT, Ding, ZY, Yang DP, Xie J. The quality properties of frozen large yellow croaker fillets during temperature fluctuation cycles: improvement by cellobiose and carboxylated cellulose nanofibers. International Journal of Biological Macromolecules. 194: 499-509 (2022)

Tao Y, Ma L, Li D, Tian Y, Liu J, Liu D. Proteomics analysis to investigate the effect of oxidized protein on meat color and water holding capacity in Tan mutton under low temperature storage. LWT-Food Science and Technology. 146 : in press (2021)

Trybus KM. Smooth Muscle and Myosin Regulation. pp. 268–286, In: Comprehensive Biophysics. Egelman RH (Ed.), Amsterdam: Elsevier (2012)

Umaraw P, Munekata PES, Verma AK, Barba FJ, Singh VP, Kumar P, Lorenzo JM. Edible films or coating with tailored properties for active packaging of meat fish and derived products. Trends in Food Science and Technology. 98: 10-24 (2020)

Vidal NP, Dermiki M, Lordan R. Fish-derived functional foods and cardiovascular health: An overview of current developments and advancements. In I. Zabetakis, A. Tsoupras, R. Lordan & D. Ramji (Eds.), Functional Foods and Their Implications for Health Promotion. 303–316 (2023)

Wang DY, Zhang MH, Deng SY, Xu WM, Liu Y, Geng Z, Sun C, Bian H, Liu F. Postmortem changes in actomyosin dissociation, myofibril fragmentation and endogenous enzyme activities of grass carp (*Ctenopharyngodon idellus*) muscle. Food Chemistry. 197: 340-344 (2016)

Wang L, Li J, Teng S, Zhang W, Purslow PP, Zhang R. Changes in collagen properties and cathepsin activity of beef M. semitendinosus by the application of ultrasound during post-mortem aging. Meat Science. 185: in press (2021)

Wang XY, Xie J. Evaluation of water dynamics and protein changes in bigeye tuna (*Thunnus obesus*) during cold storage. LWT-Food Science and Technology. 108: 289-296 (2019)

Watanabe A, Devine C. Effect of meat ultimate pH on rate of titin and nebulin degradation. Meat Science. 42(4): 407-413 (1996)

Wu L, Pu H, Sun DW. Novel techniques for evaluating freshness quality attributes of fish: A review of recent developments. Trends in Food Science and Technology. 83: 259-273 (2019)

Xiang Y, Sun CF, Zhao YQ, Li LH, Yang XQ, Wu YY, Chen SJ, Wei Y, Li CS, Wang Y. Label-free proteomic analysis reveals freshness-related proteins in sea bass (*Lateolabrax japonicus*) fillets stored on ice. LWT-Food Science and Technology. 155: in press (2021)

Xu YX, Yin YM, Wang R, Zhao HL, Li XP, Yi SM, Li JR, Xie J. Effect of deacetylated konjac glucomannan on heat-induced structural changes and flavor binding ability of fish myosin. Food Chemistry. 365: in press (2021)

Xue Z, Wang M, Chen X, Xu X, Zhou G Conformational and rheological changes of high-pressure processing treated rabbit myosin subfragments during heating. LWT-Food Science and Technology. 122 : in press (2020)

Yang B, Chen T, Li H, Li Y, Yang R. Impact of postmortem degradation of cytoskeletal proteins on intracellular gap, drip channel and water-holding capacity. Meat Science. 176: in press (2021)

Yang F, Jia SN, Liu JX, Gao P, Yu DW, Jiang QX, Xu YS, Yu PP, Xia WS, Zhan XB. The relationship between degradation of myofibrillar structural proteins and texture of superchilled grass carp (*Ctenopharyngodon idella*) fillet. Food Chemistry. 301: in press (2019)

Yu DW, Jing DT, Yang F, Gao, P, Jiang QX, Xu YS, Yu PP, Xia WS. The factors influencing the flavor characteristics of frozen obscure pufferfish (*Takifugu Obscurus*) during storage: Ice crystals, endogenous proteolysis and oxidation. International Journal of Refrigeration. 122: 147-155 (2021)

Yu DW, Regens tein, JM, Zang J, Xia WS, Xu Y, Jiang QX, Yang F. Inhibitory effects of chitosan-based coatings on endogenous enzyme activities, proteolytic degradation and texture softening of grass carp (*Ctenopharyngodon idellus*) fillets stored at 4℃. Food Chemisty. 262:1–6 (2018)

Zandonadi FS, Castañeda Santa Cruz E, Korvala J. New SDC function prediction based on protein-protein interaction using bioinformatics tools. Computational Biology and Chemistry. 83: in press (2019)

Zeng X, Jiao D, Yu X, Chen L, Sun Y, Guo A, Zhu C, Wu JS, Liu JS, Liu HM. Effect of ultra-high pressure on the relationship between endogenous proteases and protein degradation of Yesso scallop (*Mizuhopecten yessoensis*) adductor muscle during iced storage. Food Chemistry. 15: in press (2022)

Zhang B, Mao JL, Yao H, Aubourg SP. Label-free based proteomics analysis of protein changes in frozen whiteleg shrimp (*Litopenaeus vannamei*) pre-soaked with sodium trimetaphosphate. Food Research International. 137: in press (2020)

Zhang LT, Zhang YQ, Jia SL, Li Y, Li Q, Li KF, Hong H, Luo YK. Stunning stress-induced textural softening in silver carp (*Hypophthalmichthys molitrix*) fillets and underlying mechanisms. Food Chemistry. 295: 520-529 (2019)

Zhang M, Li CL, Zhang YM, He LC, Li W, Zhang ML, Pan JJ, Huang JS, Liu YY, Zhang Y, Jin YG, Gao JX, Jin GF, Tang XY. Interactions between unfolding/disassembling behaviors, proteolytic subfragments and reversible aggregation of oxidized skeletal myosin isoforms at different salt contents. Food Research International. 157: in press (2022)

Zhang X, Yang J, Gao H, Zhao Y, Wang J, Wang S. Substituting sodium by various metal ions affects the cathepsins activity and proteolysis in dry-cured pork butts. Meat Science. 166: in press (2020)

Zhang Y, Puolanne E, Ertbjerg P. Mimicking myofibrillar protein denaturation in frozen-thawed meat: Effect of pH at high ionic strength. Food Chemistry. 338: in press (2021)

Zhang ZW, Liu PP, Deng XR, Guo XY, Mao XY, Guo XB, Zhang J. Effects of hydroxyl radical oxidation on myofibrillar protein and its susceptibility to μ-calpain proteolysis. LWT-Food Science and Technology. 137: 110453 (2021)

Zhao X, Zhou Y, Zhao L, Chen L, He Y, Yang H. Vacuum impregnation of fish gelatin combined with grape seed extract inhibits protein oxidation and degradation of chilled tilapia fillets. Food Chemistry. 294: 316-325 (2019)

Zhuang S, Liu X, Li Y, Zhang LT, Hong H, Liu J, Luo YK. Biochemical changes and amino acid deamination & decarboxylation activities of spoilage microbiota in chill-stored grass carp (*Ctenopharyngodon idella*) fillets. Food Chemistry. 336: in press (2021)

Zhuang S, Liu Y, Gao S, Tan Y, Hong H, Luo YK. Mechanisms of fish protein degradation caused by grass carp spoilage bacteria: A bottom-up exploration from the molecular level, muscle microstructure level, to related quality changes. Food Chemistry. 403 : in press (2022)

Zhuang S, Tan Y, Hong H, Li D, Zhang L, Luo YK. Exploration of the roles of spoilage bacteria in degrading grass carp proteins during chilled storage: A combined metagenomic and metabolomic approach. Food Research International. 152: in press (2022)