Food Science of Animal Products

Review Article

Research progress on the mechanism of fish quality deterioration during frozen storage and novel control technologies

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Abstract: Frozen storage is a prevalent technique for maintaining the freshness of fish. However, fish quality is susceptible to decline throughout the freezing period, substantially impacting its flavor and nutritive content. Hence, this article examines the mechanisms behind the quality degradation and the control strategies employed to preserve fish during frozen storage. Firstly, the formation of ice crystals and the reasons for the decline of fish meat quality were clarified. Secondly, the factors that may lead to quality deterioration during frozen storage were analyzed, including protein denaturation, fat oxidation and enzymatic reaction. Furthermore, the novel freezing control technologies for the deterioration of fish frozen storage quality were reviewed, including the technology of adjusting the nucleation, growth behavior, and recrystallization of ice crystals, and the technology of improving the freezing rate. Finally, the development direction of the research on the deterioration of frozen fish quality in the future was prospected, and novel technologies and methods to improve the quality of frozen fish were pointed out, which is expected to lay a foundation for higher quality frozen fish meat in the future.

Keywords: fish; frozen storage; ice crystals; freeze-thaw; novel technologies

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1 Introduction

Fish is one of the main sources of protein intake in people's daily lives. China as a major fishing country, it leads the world in fish production and consumption. In 2023, the output value of the national leisure fishery was 93.147 billion yuan (monitoring data excluding Hong Kong, Macao, Taiwan and Tibet, the same below), an increase of 10.99% year-on-year. Since 2023, the output of aquatic products has steadily increased, and the fishery economy has generally maintained a good development trend, making positive contributions to the stable supply of "vegetable basket" products and the development of the agricultural and rural economy. However, due to the perishable nature of fish, effective storage and preservation technologies are usually needed to meet the growing market demand for high-quality aquatic products[1]. Freezing remains the most commonly used and effective method to maintain the freshness of fish, accounting for 35% of fish directly consumed by humans[2]. However, fish quality deterioration still occurs during the frozen storage process, such as fat oxidation, protein degradation, water loss, and decreased muscle quality[3–4]; it even causes the generation of odor substances such as fishy smell, oxidized fish oil smell, and foul odor caused by spoilage[5]. These problems seriously affect the taste, nutritional value, and food safety of fish, causing huge economic losses. Therefore, it is of great significance to study the quality deterioration mechanism and control technology of fish during frozen storage.

With the improvement of people's living standards and changes in dietary concepts, the market demand for frozen fish continues to grow. Frozen fish has the advantages of being easy to transport and not restricted by seasons, which facilitates international trade. However, quality deterioration is inevitable during frozen storage and transportation. Frozen fish requires special equipment and processes for processing, which is relatively expensive. At the same time, appropriate temperature and humidity need to be controlled during storage and transportation to prevent microbial growth and deterioration. Freezing technology is an important part of frozen fish. Through freezing technology, the shelf life of fish products can be extended and the color, freshness and nutritional value of the fish can be maintained.

In recent years, with the continuous progress of science and technology, a lot of research has been conducted on the mechanism and control technology of quality deterioration of frozen fish[1,3–4]. On the one hand, by analyzing the biochemical and microstructural changes of fish during frozen storage, the main mechanism of quality deterioration has been revealed. On the other hand, the use of advanced refrigeration, packaging, antioxidant and preservation technologies has effectively delayed the quality deterioration of fish during frozen storage, improving the quality and food safety of fish products. In general, the research on the mechanism and control technology of quality deterioration during frozen storage of fish has made certain progress, but there are still some challenges and problems that need to be further solved. Future research can focus on exploring more refined quality deterioration mechanisms and developing more efficient frozen storage quality control technologies to provide more scientific basis and technical support for improving the quality and market competitiveness of frozen fish products. This paper discusses the research progress of the mechanism of fish quality deterioration during frozen storage, and

comprehensively reviews the control technology of quality deterioration of frozen fish based on the application of novel freeze-thaw technologies reported at home and abroad in the processing of different fish products, in order to provide certain theoretical guidance for the quality control research of frozen fish.

2 Ice crystal formation in fish meat

2.1 Fish meat structure and water distribution

Fish meat is a highly organized tissue, including muscle tissue, connective tissue, adipose tissue, and bone tissue (Figure 1A). The muscle structure of fish is mainly composed of two categories: striated muscle and smooth muscle. The obvious feature of striated muscle is that it has clear light and dark stripes on its surface, which extend evenly and symmetrically along both sides of the fish spine. The order of decomposition of fish muscle components is muscle tissue, muscle bundles, muscle fibers/myocytes, myofibrils and tiny myofilaments, with sarcoplasm in the middle of the myofilaments (Figure 1B). Myocytes and myofibrils are arranged tightly and regularly (Figure 1C). Myofibrils are slender tubular structures with their long axes parallel to the long axes of muscle fibers. The sarcomere structure in myofibrils can clearly distinguish dark bands (A bands), light bands (I bands), M lines and Z lines (Figures 1D and E)[6]. The delicateness and tenderness of fish meat is determined by the diameter and density of muscle fibers. At the same time, high-density muscle fibers also help maintain the integrity of muscle structure and reduce the damage of muscle cells. Water molecules in the muscle can form hydrogen bonds with surrounding water molecules and bind to protein molecules to form a hydration layer (Figure 1F). Currently, the moisture distribution of fish products is mainly measured by nuclear magnetic resonance (NMR), especially low-field NMR (LF-NMR). In the study of Zhang et al.[7], LF-NMR analysis was used to detect the effect of cellulose treatment on the water distribution of surimi and the water distribution after cooking.

2.2 Formation of ice crystals

One of the reasons for the growth of ice crystals is that the surrounding water or water vapor converges to the surface of the ice crystals and condenses and adheres to them. The physical dynamics of ice crystal formation mainly include crystallization and recrystallization, which are controlled by the molecular surface attachment dynamics and diffusion-limited growth (Figure 2). Due to the existence of pressure difference, water vapor will migrate from the high vapor pressure area to the low vapor pressure area, and continue to condense and adhere to the surface of the ice crystals, causing large ice crystals to grow continuously, while small ice crystals decrease and disappear[8]. Ice crystal growth is also affected by recrystallization. During the frozen storage, the formation of ice crystals is not the final state, and their morphology and distribution are in dynamic change. These ice crystals recrystallize with tiny ice crystals as the core during the frozen storage process. From a thermodynamic perspective, this phenomenon can be attributed to the inherent tendency of the system to pursue the lowest energy state. In particular, when the frozen storage temperature fluctuates, the ice crystals inside the aquatic product recrystallize, which in turn promotes the growth of the ice crystal volume[9]. Under long-term frozen storage, even if the temperature is kept constant, the occurrence of recrystallization is still difficult to avoid. This phenomenon can be attributed to the relatively high surface free energy of water molecules around smaller ice crystals in the ice crystal structure, which makes these water molecules unstable and easily detached from the ice crystal surface. These detached water molecules then redeposit on the surface of larger ice crystals, causing the small ice crystals to melt and the large ice crystals to grow in size[10].

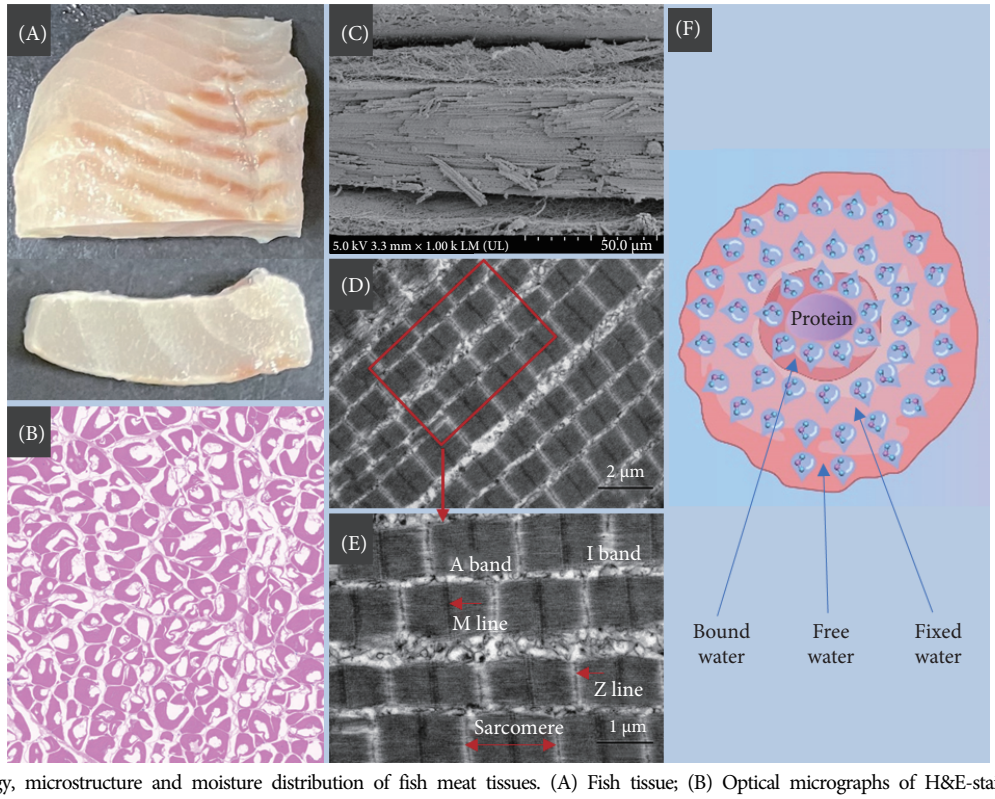

Figure 1 Morphology, microstructure and moisture distribution of fish meat tissues. (A) Fish tissue; (B) Optical micrographs of H&E-stained sections of fish; (C–E) Scanning electron microscope photographs of fish[6]; (F) Moisture distribution in fish.

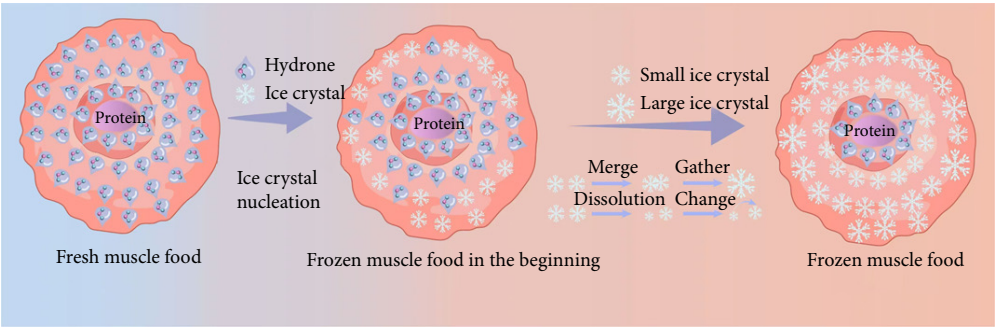

Figure 2 Ice crystal formation mechanism.

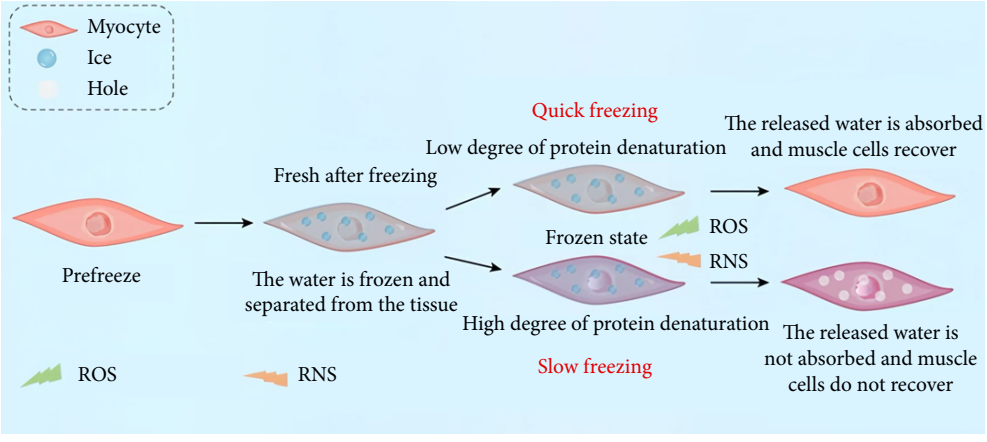

Figure 3 Effect of ice crystals on muscle cells of frozen stored fish. ROS, reactive oxygen species; RNS, reactive nitrogen species.

2.3 Factors that affect ice crystal formation

In the process of in-depth research on fish freezing technology, it was found that the mechanism of ice crystal formation is influenced by a number of factors, which are related to the physical properties of fish such as size, shape, moisture content, and the conditions during the freezing process. During the crystallization process, ice crystals undergo nucleation, growth, and new nucleation. The formation of ice crystals is affected by nucleation temperature, freezing point, freezing rate, and temperature fluctuations. At different freezing rates, the time to pass through the maximum ice crystal formation zone is different. When cooling rapidly, the degree of supercooling is high and the nucleation rate of ice crystals is fast. The time to pass through the maximum ice crystal formation zone and the crystal nucleus formation stage is shortened, the size of the ice crystals formed is smaller and the distribution is more uniform, and the damage to the cell structure is also less[11]. In the process of slow temperature reduction, the low degree of supercooling makes the ice crystal nucleation rate slower, the time to pass through the maximum ice crystal formation zone is longer, the crystal nucleus growth time is sufficient, and the generated crystals are larger. The formation of such large ice crystals aggravates the damage to cell and tissue structure[12]. Xia et al.[13] found that the first two samples with faster freezing rates passed through the maximum ice crystal formation zone within 15 and 30 min, respectively, while the third sample with slower freezing rate took 1.5 h to pass through the maximum ice crystal formation zone. This shows that the freezing rate has an impact on the ice crystal growth rate and the final crystal size, and the faster the freezing rate, the lower the thawing juice loss rate of the sample. As the freezing temperature decreases, the growth dynamics of ice crystals change significantly and the freezing rate accelerates. Under lower temperature conditions, the time it takes for ice crystals to pass through the maximum ice crystal formation zone is effectively shortened, resulting in smaller and more uniform ice crystals. In addition, temperature fluctuations will intensify the recrystallization of ice crystals after nucleation, thereby changing the morphological characteristics of ice crystals. These phenomena might lead to the formation and redistribution of giant ice crystals[14].

2.4 Damage of ice crystals to fish muscles

Inside the muscle with intact cellular structure, a transition between the water and ice phases occurs during freezing and thawing process. When the ambient temperature drops rapidly (fast freezing), the water inside and outside the muscle cells undergoes an *in situ* phase change, accompanied by the formation of a large number of ice crystals. At the same time, the stress type in the muscle core and surface changes, resulting in mechanical damage in the form of microcracks (ice crystal effect)[15]. During the slow cooling process, crystals first appear outside the cells, followed by a rapid increase in the solute concentration in the unfrozen water around the crystals, thereby generating an osmotic gradient across the intracellular and extracellular boundaries; the water inherent in the intracellular environment migrates to the extracellular environment, ultimately leading to cell dehydration[16]. Then, a portion of the supercooled water molecules gradually precipitate onto the developing crystal form driven by the decreased free energy. After the muscle tissue is frozen, the water in the muscle forms ice crystals and separates from the muscle. The degree of protein denaturation is relatively low under the condition of quick freezing, and the muscle cells can recover. While under the condition of slow freezing, the degree of protein denaturation is relatively high. The excessive growth of ice crystals causes irreparable mechanical damage to the muscle cells, directly damaging the highly organized structure of muscle fibers, as shown in Figure 3.

Therefore, during the thawing process, incomplete muscle cells encounter difficulties in recovering migrated water molecules, resulting in increased juice loss. The destruction of cell structure triggers the release of endogenous enzymes (such as cathepsins) and pro-oxidants (such as hemoglobin iron), thereby accelerating the oxidation process[17]. On the other hand, water migration caused by ice crystal growth leads to changes in the microenvironment of muscle proteins (such as increased solute concentration and pH) and changes in protein conformation. These changes are manifested as reduced protein solubility and thermal stability, increased surface hydrophobicity, and disruption of intermolecular forces[18]. Myofibrillar proteins play an important role in the structural development of gel-type muscle foods. However, during the freeze-thaw process, myofibrillar proteins are inevitably denatured and oxidized due to the formation of ice crystals, which may lead to excessive aggregation of myosin molecules with gel-like properties and affect the gel network formed during subsequent heat treatment[19]. Problems such as the loss of juice, softening, microbial reproduction, discoloration caused by the oxidation of dark fish myoglobin, as well as protein denaturation caused by the formation of ice crystals during freezing storage, increased drip loss, and lipid oxidation have greatly affected the commercial value of fish.

3 Effects of freezing on fish meat quality

Although frozen storage can slow down the unwanted metabolic processes in fish, the formation of ice crystals can cause cell rupture and muscle fiber damage, changing the physical properties of the muscle (such as texture and water-holding capacity), chemical processes (such as protein aggregation, denaturation, oxidation, discoloration, fat decomposition, and lipid oxidation), and sensory properties (such as flavor and sensory characteristics). Wang et al.[20] showed that under −18 °C conditions, with the continuous extension of storage time, the carp brightness value (L^*) showed a gradual upward trend, while the yellowness value (b^*) first increased and then stabilized; the redness value (a^*) showed a trend of first decreasing and then slightly increasing, which was related to the reduction of pigment-related substances caused by the loss of water in aquatic products. Further studies have shown that during multiple freeze-thaw cycles, the L^* of fish meat shows a continuous downward trend, while a^* and b^* show a continuous upward trend. In addition, it is worth noting that the minerals contained in fish meat, such as copper and iron, will also cause changes in the color of the meat during the oxidation process[21]. During the freezing, refrigeration and thawing processes, the globulin part of the myoglobin molecule will denature, making myoglobin more susceptible to self-oxidation. Myoglobin, especially the oxygen type, is responsible for the bright red color of fish muscles, especially tuna and dark meat fish, and astaxanthin (AXT) directly determines the color of salmon muscles. Microbial spoilage and chemical changes (e.g., oxidation of lipids/proteins) can lead to auto-oxidation of myoglobin or fading of AXT, ultimately resulting in undesirable color and lower acceptability.

The water-holding capacity of muscle has a direct impact on the juiciness, tenderness, color, aroma, and other sensory qualities of meat, making it a key texture indicator in meat quality evaluation. Juice loss rate and cooking loss rate are two important indicators for measuring the water-holding capacity of fish protein. Sun et al.[22] have found that the juice loss rate increases with the extension of frozen storage time. The three-dimensional structure of myofibrillar protein will change, resulting in a decrease in its water-holding capacity[23]. Zhao et al.[24] have found that the chemical structure of fish protein changes during the freezing process, causing a decrease in the water-holding capacity of muscle tissue. In addition, during the freezing process, the formation of ice crystals causes certain mechanical damage to the cells, resulting in muscle fiber damage, which also causes a decrease in water-holding capacity.

The tenderness of fish meat can be reflected by analyzing the shear force in the texture quality[25]. Slow freezing causes a large amount of water loss, resulting in loose muscle structure, reduced shear force, and thus reduced muscle tenderness[26]. The reduction in shear force of frozen meat is mainly due to the catalysis of enzymes during the hydrolysis of muscle protein. This biochemical process causes muscle fiber rupture, which in turn leads to muscle aging, and ice crystals can also damage the muscle tissue structure. Based on this, it can be considered that under frozen storage conditions, the dual effects of muscle protein degradation and ice crystal formation constitute a factor in the deterioration of fish meat quality[27].

In addition, as the storage time increases, fish meat will experience protein aggregation, denaturation, fat decomposition, and lipid oxidation, and microorganisms will gradually grow and multiply, causing the fish meat to spoil, reducing its nutritional value and even producing harmful substances. The hardness, elasticity, cohesiveness, and chewiness of fish meat all show a continuous downward trend[20].

Flavor loss during freezing and thawing is inevitable. The types and contents of flavor substances in fish meat vary with the freezing time and temperature[28]. Mechanical damage to muscle tissue caused by freezing-induced ice crystals leads to a large loss of flavor substances after thawing[29]. At the same time, flavoring amino acids, especially the umami amino acid glutamate, are also lost, resulting in a decrease in umami taste[30]. The flavor deterioration of frozen fish products is most obvious in seafood, which may be related to its high enzyme content. During frozen storage, proteins, amino acids, and other nitrogen-containing compounds in fish meat decompose to produce a large number of compounds including ammonia, amines, sulfides, polyols, aldehydes, ketones, and organic acids, and under the influence of oxidation, further induce unpleasant flavors and odors[31]. Long-term frozen storage is not conducive to maintaining the original flavor of fish meat. Therefore, it is recommended to adopt more scientific storage methods and technologies such as low-temperature rapid freezing and short-term refrigeration to slow the decline in flavor quality of fish meat[32].

In addition, certain enzymes remain active at low temperatures, which may lead to chemical reactions in fish, such as oxidation, which can affect the freshness and taste of fish meat. During frozen storage and freeze-thaw cycles, nutrients such as fat, protein, and vitamins in the fish may be lost. Fats, in particular, are prone to oxidation reactions during the freezing process, resulting in unpleasant odors. In addition, the loss of nutrients can also occur due to cell damage during freezing. Although the low temperature environment can inhibit the growth of microorganisms, it can still lead to the survival and reproduction of microorganisms in certain situations, such as freezing fast enough or incomplete cold chains. The growth of microorganisms in fish meat will accelerate the spoilage process and affect the eating quality of fish meat.

4 Mechanism of quality degradation of frozen fish

4.1 Damage caused by oxidation

Muscle oxidation is an important cause of food quality degradation. This process mainly involves the oxidation of fat and protein. These chemical changes not only affect the sensory properties of food, such as color and taste, but also have a negative impact on its nutritional value. The hydrolysis of fat and its subsequent oxidation can cause fish meat to emit an unpleasant odor, seriously affecting consumer acceptance[33]. During the frozen storage and transportation, the formation, expansion, melting of ice crystals and repeated freezing and thawing can destroy the cell structure of fish meat, leading to the oxidation of fat in the meat, causing the generation of odor and the loss of nutrients, shortening the shelf life of fish products[34]. In addition, the oxidative balance system in frozen fish meat is destroyed, catalyzing the oxidation and hydrolysis of lipids. The fatty acids in fish muscle tissue, under the catalysis of lipoxygenase, cause fish products to produce a strong fishy odor during storage and processing. At the same time, studies have also revealed that oxidation catalyzed by hemoglobin may be the key cause of food odor[35]. The oxidative decomposition process of fish meat is affected by various factors, such as the degree of muscle tissue damage, differences in fatty acid composition, changes in ambient temperature and water activity, the presence of metal ions, the action of endogenous enzymes, oxygen concentration, and microbial activity. The main oxidation pathways are shown in Figure 4.

Among them, the oxidation process of fat includes enzymatic oxidation, free radical chain reaction, and photosensitive oxidation. The latter two are also called auto-oxidation and are the main forms of lipid oxidation in fish meat. In this chemical reaction, the interaction between fatty acids and oxygen molecules generates poorly stable peroxides and hydroperoxides, which promote the generation of new free radicals and trigger a chain reaction. The auto-oxidation of unsaturated fatty acids in frozen muscle can be divided into three stages: initiation, growth, and termination[36]. Its free radical chain reaction is shown in Figure 5A. The initiation stage includes the activation of triplet oxygen ($^3O_2$), the catalysis of transition metals (Fenton reaction) and the rearrangement of double bonds. The growth stage involves the interaction between lipids and is terminated by the interaction between peroxides, alkyl radicals and/or peroxide radicals. During the oxidation process, energy (light, heat, etc.), trace heavy metals, and free radical peroxides induce the occurrence of active free radicals. The action of energy causes fatty acids to decompose into free radicals and hydrogen ions. As the free radicals combine with other fatty acids for oxidation, hydroperoxides and more free radicals are formed,

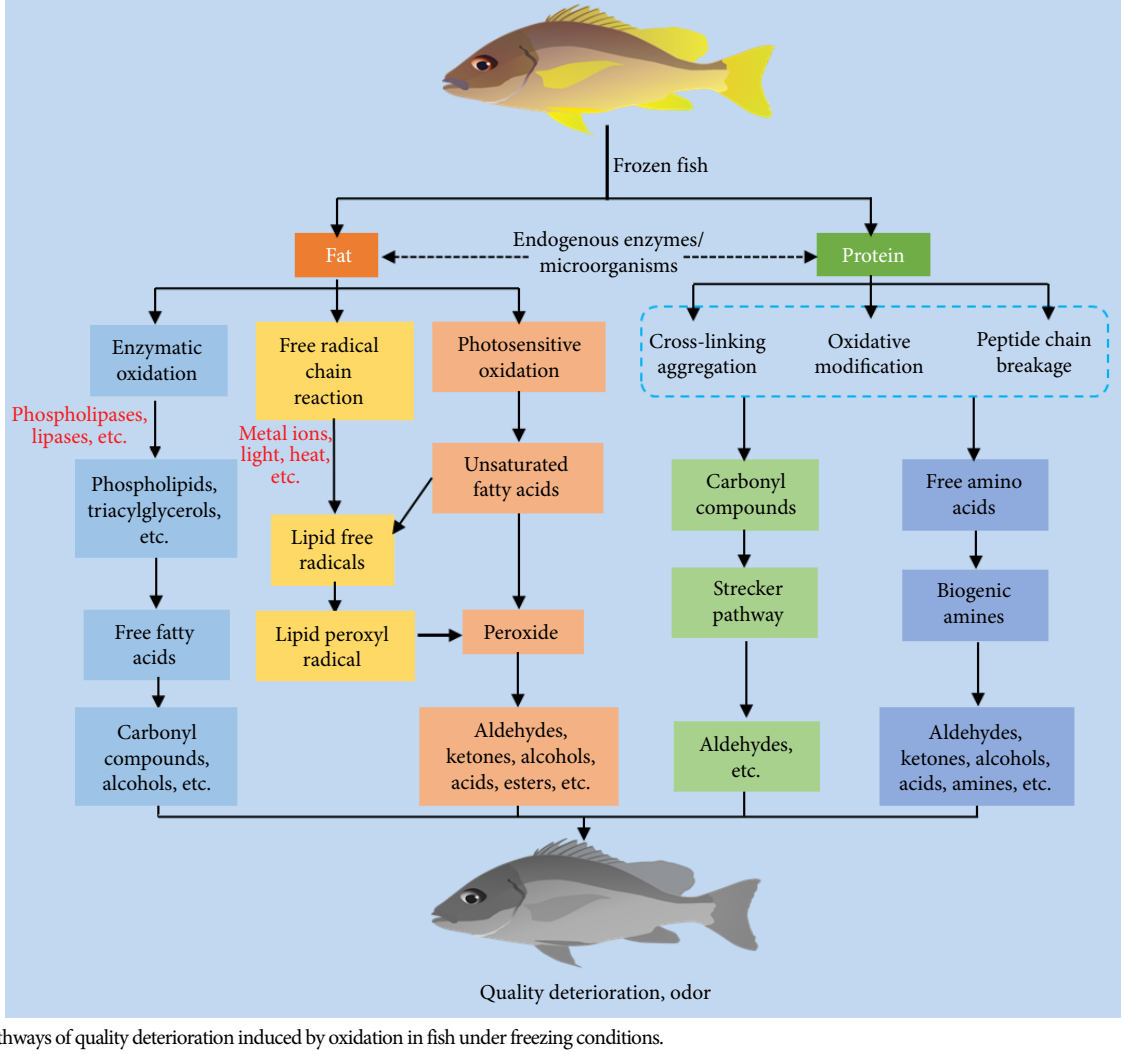

Figure 4 Pathways of quality deterioration induced by oxidation in fish under freezing conditions.

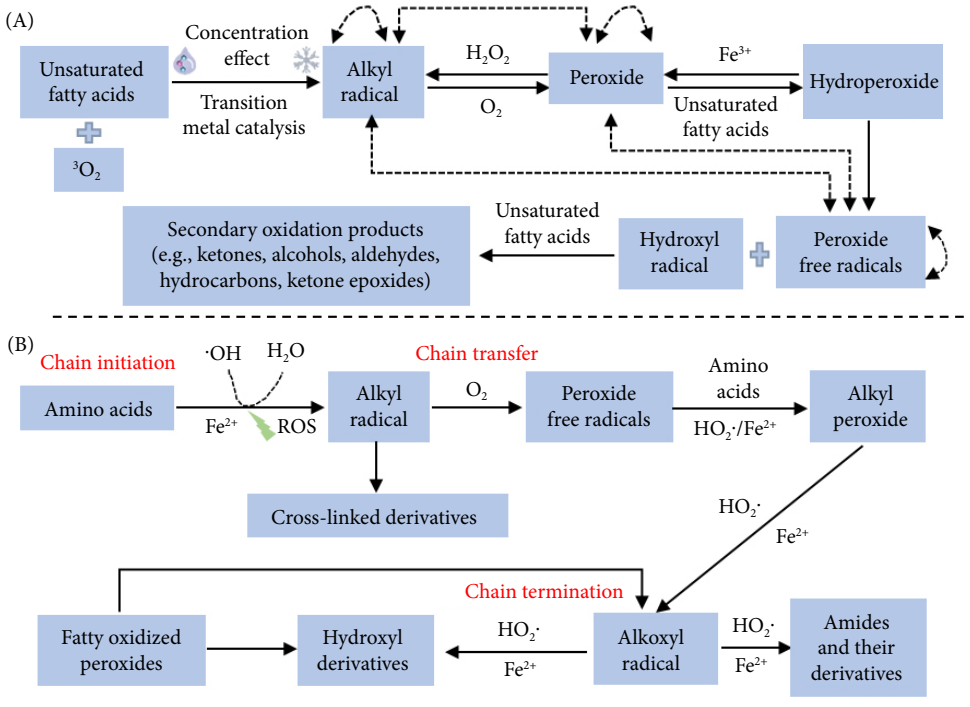

Figure 5 Free radical chain reaction in response to (A) lipid oxidation and (B) protein oxidation in fish. The dotted line indicates the end point of the lipid oxidation free radical chain reaction.

which re-enter the oxidation chain. On the other hand, under the action of energy, hydroperoxides generate oxidized forms of oxo groups and free radicals, and produce more hydroperoxides and new free radicals together with other fatty acids. At the same time, the compounds produced during the decomposition of hydroperoxides are converted into epoxides through *α*-cleavage and internal molecular rearrangement, and the fatty acid free radicals are disproportionated and interact with hydroxyl free radicals and unsaturated fatty acids to generate secondary lipid oxidation products such as ketones, alcohols, aldehydes, hydrocarbons, epoxy ketones, and epoxy hydrogens, thereby triggering the rancidity process of oils and fats. The oxidation reaction is terminated by binding between free radicals or reacting with a deactivator to produce a stable compound. In summary, the oxidation reaction of lipids will have a adverse effect on the appearance, flavor, and nutritional value of meat products, while reducing the shelf life of fish products, and is one of the key factors leading to the decline of food quality.

Protein oxidation occurs regardless of whether lipid oxidation is initiated or not[29]. In the field of biomolecular oxidation research, protein oxidation is a complex and multi-stage chain reaction that can be directly triggered by ROS or RNS, or indirectly initiated by sugar or lipid oxidation derivatives. This process is similar to lipid oxidation and mainly undergoes three consecutive stages: chain initiation, chain transfer, and chain termination, as shown in Figure 5B. Specifically, the protein oxidation chain reaction is initiated by ROS-induced dehydrogenation of protein molecules to form alkyl free radicals. Subsequently, in the presence of oxygen, these alkyl free radicals are converted into peroxide free radicals, which then react with Fe^{2+} or superoxide free radicals, or through the dehydrogenation of another protein molecule to form alkyl peroxides. This substance then participates in further reactions, reacting with peroxide free radicals or Fe^{2+} to generate alkoxy free radicals and their hydroxyl derivatives, promoting the chain reaction of protein oxidation. It is worth noting that in an anaerobic environment, two alkyl free radicals can combine with each other to form carbon-carbon cross-linked derivatives. Studies have shown that when ice crystals pierce the cell membrane during frozen storage, they release a large amount of catalytic metal ions, increasing the concentration of pro-oxidants in the non-frozen phase around protein molecules, which promotes the generation of alkyl peroxides, alkoxy free radicals, and hydroxyl derivatives[37]. At the same time, peroxides formed during lipid oxidation contribute to the generation of alkoxy free radicals and hydroxyl derivatives[38]. The formation of the final oxidation product leads to the termination of the protein oxidation chain reaction.

4.2 Damage caused by endogenous enzyme activity

The quality changes of fish meat are regulated by a variety of biochemical reactions, among which enzymatic oxidation is very critical. This process mainly involves three types of enzymes: phospholipases, lipases, and lipoxygenases, which act on phospholipids and triacylglycerols, respectively, to promote the release of free fatty acids. These released fatty acids form a variety of volatile oxidation products including carbonyl compounds, alcohols, and alkanes during further oxidation. These oxidation products provide fish meat with unique odor characteristics in terms of sensory perception (Figure 4). During continuous freeze-thaw cycles, the cell structure suffers mechanical damage, resulting in the overflow of oxidases. The fat in the fish body undergoes decomposition reactions under the catalysis of oxidases and is converted into low molecular weight aldehyde and ketone compounds. The gradual accumulation of these derivative compounds triggers an increase in acid value[38].

In addition, endogenous proteases in fish meat will promote the protein degradation process and change the texture of fish meat, thus affecting the quality of fish meat[39]. Calpain, matrix metalloproteinase and cathepsin are the main endogenous

proteases that proteolyze muscle food after freezing[40]. Changes in protease activity of fish may occur throughout the entire process of frozen storage. At the beginning of freezing, enzyme activity is limited due to the inhibitory effect of low temperature, so there is no significant protein degradation. However, this inhibitory effect gradually diminishes over time. After the ice crystals are formed, they will pierce the lysosomal membrane and promote the release of proteolytic enzymes from lysosomes into the muscle fibers and sarcoplasm, thus accelerating the process of protein decomposition by proteolytic enzymes. According to research by Subbaiah et al.[41], tilapia was frozen and stored at –18 °C for 150 d and found that the freezing process significantly reduced the solubility of its protein. Through sodium dodecylsulfate-polyacrylamide gel electrophoresis detection, the myosin heavy chain did not change significantly during the first three months of storage, but began to degrade on the 90th day. In addition, temperature is also critical to protease activity. Lu et al.[42] revealed that the activities of cathepsin B and B + L in bighead carp frozen at –12 °C were higher than that at –28 °C, proving that freezing has the effect of inhibiting these enzymes. Studies have also pointed out that the lower the freezing temperature, the stronger the inhibitory effect on enzyme activity[43]. Activity has a greater impact, but this impact gradually weakens with the increase of freezing time. During the freezing process, the changes in protease activity are influenced by temperature and ice crystal formation, which are important factors contributing to the deterioration of fish meat quality.

5 Novel technology for quality control

In order to reduce ice damage to fish during freezing and maintain the quality of its products, more and more scholars have begun to explore effective strategies to regulate ice formation. Common new freezing technologies are shown in Figure 6. The following discusses new technologies for regulating ice crystal nucleation, formation, recrystallization behavior and increasing freezing speed.

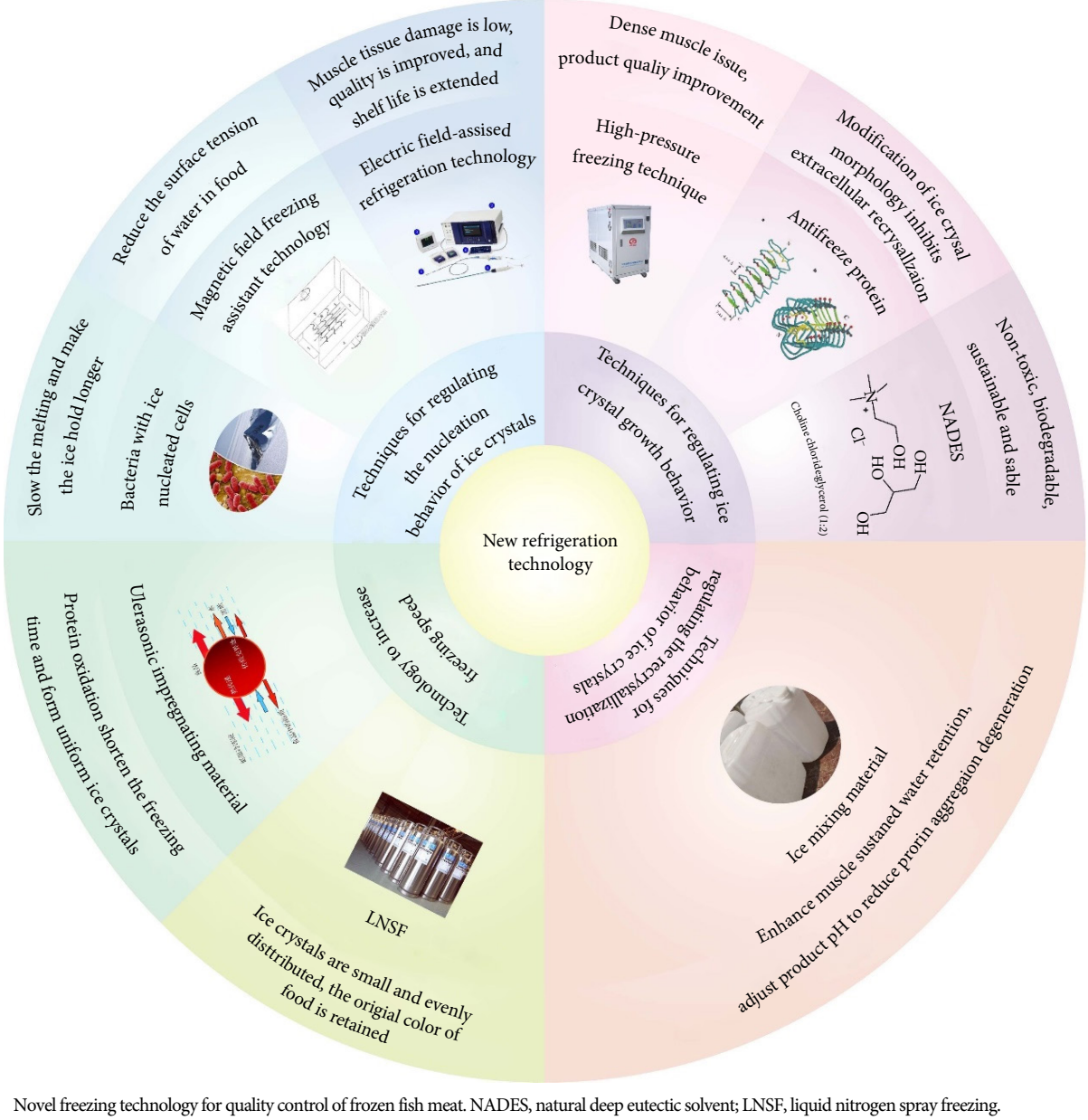

Figure 6 Novel freezing technology for quality control of frozen fish meat. NADES, natural deep eutectic solvent; LNSF, liquid nitrogen spray freezing.

5.1 Techniques to regulate ice crystal nucleation behavior

Electric field-assisted freezing technology is based on in-depth exploration of the polarization effect of water molecules and regulates the growth process of ice crystals by reducing the free energy of water molecules. Specifically, the regulation of ice crystal nucleation, growth, and morphological distribution by electric fields can be divided into three aspects: firstly, the electric field can promote the generation of ice crystal nuclei and improve the nucleation efficiency; secondly, the electric field effectively inhibits the formation and growth of macro-scale ice crystals, avoiding excessive increase in the size of ice crystals; finally, the electric field optimizes the morphology and spatial distribution of ice crystals, helping to form a more uniform ice crystal structure that is conducive to food texture[43]. Zhang et al.[44] conducted micro-freezing experiments on hairtail samples at −4 °C under a low-voltage electrostatic field environment of 3 kV and 50 Hz, which showed that this electric field treatment had relatively fresh-keeping effect on hairtail and could effectively reduce the degree of destruction of the organization, thereby comprehensively improving its quality and greatly extending the shelf life of hairtail to more than 7 d. At present, electrostatic field-assisted freezing technology shows significant preservation effects in the refrigeration and freezing process of marine aquatic products. However, the research on the application of electric field-assisted freezing of aquatic products is still in the development stage, and its in-depth mechanism and optimal application strategy still need to be explored in depth[45]. Hafezparast-Moadab et al.[46] comparatively studied the effects of different radio frequency modes and different electrode gaps (2, 3, 4 cm) on rainbow trout. The results showed that when the electrode gap was 2 cm, compared with the conventional air freezing method, it could significantly reduce ice crystal size in rainbow trout mince by 75%. This phenomenon is mainly due to the fact that radio frequency technology promotes the movement of water molecules and increases the number of nucleation points.

Magnetic field-assisted freezing technology has attracted much attention as an innovative quick freezing method. This technology applies a magnetic field during the food freezing process to improve freezing efficiency. Although there is currently no consensus on the specific mechanism of how magnetic fields accelerate the freezing rate, one of several hypotheses is that magnetic fields can reduce the surface tension of the water inside the food, thereby affecting the rate of ice crystal formation[47]. In addition, magnetic fields can affect the hydrogen bonding in water, changing the friction coefficient of water, affecting the mass and heat transfer speed during the freezing process, thereby changing the freezing rate. Okuda et al.[48] used a pulsed magnetic field with a strength of 0.1 to 0.8 mT to conduct freezing experiments on mackerel fillets. The research results showed that this technical method significantly shortened the freezing time during the rapid freezing stage. More importantly, the application of oscillating magnetic fields effectively limits the damage of ice crystals to muscle tissue, thus improving the quality of frozen food. As a new technology, electric field and magnetic field technology provide a new technical route for maintaining the freshness of food and extending the frozen storage period of food.

In addition, bacteria with ice nucleation activity can compactly arrange water molecules in a specific order under the action of ice nucleation proteins, thereby forming regular ice nuclei. Currently, the research and application of ice-nucleating proteins have received widespread attention in the fields of improving insect frost resistance, plant protection, crop cold tolerance, food industry processing, and artificial snowmaking. At different temperatures, different concentrations of ice nucleation proteins were added to perform freeze-thaw experiments. Test results show that adding this kind of ice-nucleating protein could affect the phase change speed: adding an ice-nucleating protein solution with a concentration of 2.3×10^{-4} or 1.15×10^{-4} mg/mL at −5 °C could effectively accelerate the freezing speed and make the ice more transparent; adding ice-nucleating protein obviously helped to slow down the melting and kept the ice longer[49]. Applying ice-nucleating active bacteria to the freeze-concentration of food can not only promote the generation of ice crystals, but also solve technical problems such as low solid-liquid separation efficiency when food viscosity is high[50].

5.2 Technology to regulate ice crystal growth behavior

High-pressure freezing technology is an important means to improve the quality of aquatic products and has shown significant results in fish processing. The technology has been widely used in commercial fish such as turbot, Atlantic salmon, and large yellow croaker. According to the experimental results of researchers such as Li et al.[51], high-pressure treatment and then freezing of *Penaeus vannamei* could significantly improve its post-frozen quality. During the freezing process at −18 °C, *P. vannamei* was placed under a pressure of 500 MPa and maintained for 5, 10, and 20 min, respectively. The results showed that high-pressure treatment of 5 and 10 min had better results, which could effectively slow down the blackening of the shrimp body and significantly improve the color and appearance quality of the shrimp body. Additionally, the density of the muscle tissue was enhanced, thereby improving the overall quality of the frozen shrimp. In the current field of frozen aquatic products processing, high-pressure freezing technology has been widely recognized as an advanced and effective technical means, which can significantly optimize the ice crystal formation process, promote its size miniaturization and uniform distribution under pressure conditions of no more than 200 MPa, thereby avoiding excessive damage to the protein structure and ensuring the quality stability of aquatic products during frozen storage. Moreover, high-pressure freezing technology can be well used for fixing samples. Freezing fixation has two significant advantages over chemical fixation: it takes only a few milliseconds to complete the freezing fixation process, and it also ensures the simultaneous fixation of all macromolecular complexes. The interaction structure of many proteins is extremely unstable, and even the slightest osmosis or temperature change will lead to structural separation, and freezing fixation can minimize the above adverse effects. In the study of biological samples, these techniques can maintain the good shape of ultrastructure and facilitate the study of dynamic processes. Currently, the only way to achieve glass freezing fixation of thicker samples (up to 200 μm) is through high-pressure freezing.

Ice structuring proteins (ISPs), also known as antifreeze proteins or thermal hysteresis proteins, are a class of stress-resistant proteins induced when organisms are exposed to low-temperature environments. These proteins protect organisms from damage caused by low temperatures by regulating the formation of ice crystals inside and outside cells. Studies have shown that certain substances can lower the freezing point of aqueous solutions in an unconventional colligative manner. This process involves the substance acting directly on the surface of ice crystals through the synergistic effect of hydrogen bonds and hydrophobic groups, thereby modifying the morphology of ice crystals, controlling the

growth process of ice crystals, and effectively inhibiting the phenomenon of extracellular recrystallization[52–54]. Studies have shown that the use of ISPs extracted from wheat as antifreeze protectants can effectively improve the quality of frozen meat and maintain the structural characteristics of proteins, and provide scientific evidence for the use of ISPs in frozen fish[55]. Firstly, ISPs can be used in production of ice cream as a stabilizer and emulsifier, improve the stability and anti-aging of ice cream, prevent ice crystal and hardening phenomenon; secondly, ISPs can be used as a water retaining agent and adhesive, improve the water retention and adhesion of meat products, prevent water loss and separation phenomenon; finally, ISPs can be used as an improver and moisturizer in baked goods, improve the elasticity and toughness of baked goods to prevent hardening and drying. NADESs are a special type of liquid composed of primary metabolites including sugars, polyols, and amino acids in a specific molar ratio, which enable organisms to survive at extremely low temperatures by lowering the freezing point of water[56]. Overall, NADESs lower the freezing point of the system and restrict the mobility of water, showing promising potential for regulating ice formation. Benefiting from their non-toxicity, biodegradability, solute stability, sustainability, and low cost, they have become an available option for ice regulation.

5.3 Technology to increase freezing rate

Ultrasonic-assisted immersion freezing (UIF) is a kind of cryogenic treatment of food that uses secondary refrigeration technology. It is an effective means to significantly improve the quality of frozen food. This method mainly involves placing food in a lower temperature environment (such as −25 °C) and utilizing the cooling efficiency of the secondary refrigerant to optimize the food freezing process[57], which mainly involves increasing the heat transfer rate and enhancing ice crystal formation. Nuclear probability can be used to improve the quality of frozen food, thereby shortening the freezing time and causing the frozen food to form small and uniform ice crystals[58]. However, the local high temperature caused by ultrasonic cavitation and thermal effects can cause cell damage, thereby causing problems such as protein oxidation[58–59]. In the study by Sun et al.[60], ultrasonic technology was used to assist the freezing process of carp, and the results showed that this method can promote freezing efficiency at different levels. In particular, when the ultrasonic power reached 175 W, the size of the formed ice crystals could be significantly reduced, thereby reducing the dynamic activity between bound water and free water molecules. This discovery effectively alleviates the problem of carp quality decline during the thawing stage. At the same time, Sun et al.[61] has conducted an in-depth discussion on the impact of ultrasound-assisted freezing technology on the functional properties of carp myofibrils, which shows that the use of ultrasonic-assisted freezing can effectively slow down the decrease in carp muscle gel properties caused by the freezing process. Specifically, the intervention of ultrasound plays a role in stabilizing the myofibril structure at the molecular level, thus maintaining the functional properties of fish meat during frozen storage. Based on UIF, combined with other technologies, it can more effectively reduce the formation of large ice crystals in solid foods, improve the loss of protein content and the retention capacity of cells. Ultrasound is a potential assistive technology that can improve the performance of food during freezing and thawing. The application of power ultrasound in the freezing process can better maintain the microstructure of food, reduce drip loss, color and texture changes, and retain some natural nutrients in food.

LNSF utilizes the large amount of heat absorbed during the vaporization process to realize the fast heat transfer or freezing rate, the food in the LNSF process is in the maximum crystallization temperature range for a short time, resulting in small and uniformly distributed ice crystals. Therefore, the original color, aroma, and taste of the food are retained to the maximum extent after thawing. Mao et al.[62] used LNSF, liquid immersion freezing, and air freezing to freeze large yellow croaker. The results showed that LNSF was a promising freezing technology. Under the conditions of −90 °C freezing temperature, −30 °C final temperature and 4 m/s wind speed, LNSF frozen large yellow croaker had the best ice crystal size and uniformity, the qualities of samples frozen by LNSF with the aforementioned parameter values were well maintained and met the industrial standard. Luo et al.[63] studied and revealed the changes in the odor characteristics and potential mechanisms of different cross-linked fish paste gels under LNSF. The results showed that LNSF had an essential effect on the odor of the gel, LNSF at −80 °C could reduce the texture properties of the gel, enhance lipid and protein oxidation, change protein conformation, and increase surface hydrophobicity and hydrophobic interaction. These changes affected the flavor characteristics of the gel, resulting in a decrease in fish aroma and an increase in fishy and oily odors after freezing, these tendencies were more pronounced at −35 °C LNSF with cross-linking degree was lower, which alleviated the deterioration of the flavor quality of the gel.

5.4 Technology to regulate ice crystal recrystallization behavior

Ice regulation in biomaterials, whether promoting or inhibiting ice formation, can be achieved by a variety of materials[64]. Different types of ice-regulating materials have been shown to regulate ice formation. Traditional ingredients such as phosphates, glycerol, sucrose, and polyphenols have effective ice-regulating abilities and can be used as food ingredients at permissible concentrations. Several sugars, including glucose, sucrose, and mannitol, accumulate in cold-adapted organisms when exposed to low temperatures, demonstrating their ice-regulating properties. Some sugars can become osmolytes to relieve osmotic pressure[65]. They can bind to water molecules through hydroxyl groups, increasing the content of bound water, thereby slowing water migration and stabilizing food preservation. In addition, they approach the ice surface and embed into the ice plane through hydrogen bonding, hydrophobic, or electrostatic forces, inhibiting ice growth and recrystallization. Phosphates include tripolyphosphate, hexametaphosphate, sodium triphosphate, tetrasodium pyrophosphate, and salt of pyrophosphate, which play an important role in improving the quality of frozen meat, especially aquatic products. These compounds can enhance the water-holding capacity of muscle protein, adjust the pH of the product, and reduce protein aggregation and denaturation[66]. As an alternative sugar component, fish protein hydrolysate showed better stabilization effects on myosin and taste improvement compared to sucrose-sorbitol mixture[67].

6 Conclusion

Based on the characteristics and mechanisms of quality deterioration during frozen storage of fish, mechanical damage caused by large and uneven ice crystals is the root cause of quality

deterioration. The freezing process can be dynamically adjusted to reduce protein oxidation, lipid oxidation, and endogenous enzyme action. Some novel technologies that regulate ice crystal nucleation, growth behavior, recrystallization, and increase freezing rate have played a positive role in maintaining and regulating fish quality. In conclusion, emerging freezing technologies have great potential, and the combination of multiple technologies may be more effective in maintaining the quality of frozen fish. In the future, we must further improve and optimize low-temperature freezing technology, strive to reduce the adverse effects of ice crystals generated during frozen storage on fish meat quality; strive to avoid temperature fluctuations as much as possible during storage and transportation; develop new barrier technologies, and explore more effective protease inhibition methods and antioxidant formulations. At the same time, ice film technology should be combined to maintain the quality of frozen fish during a longer storage period, thereby extending its market sales period and enhancing its commercial value. The optimization of the freezing environment, combined with antioxidant additives, modified atmosphere packaging, ultra-high pressure and other technologies, is expected to solve the problems of microbial corruption and oxidation of fish meat during low-temperature storage, which is conducive to maintaining the color, taste, flavor, and nutrition of fish meat. In addition, the development of stable temperature-controlled storage equipment is also an effective way to solve the problem of deterioration of fish meat storage quality.

Conflicts of interest

All authors declare that they have no conflicts of interest.

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