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# Freezing and frozen storage of aquatic products: mechanism and regulation of protein oxidation

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**Abstract**

Freezing technology is currently an important preservation method for aquatic products. However, during the long-term freezing process proteins, which are the main components of muscle tissue, are inevitably exposed to an environment containing oxidative stress inducing changes in muscle protein structure. It leads to irreversible physical and chemical changes causing deterioration in the quality of aquatic products and reducing their nutritional value. Therefore, it is necessary clarify in depth to control oxidative damage of muscle during freezing and storage to gain more insight into the mechanisms of freezing-induced protein oxidation. The mechanism of protein oxidation in fish freezing, the effect of protein oxidation on fish quality, and the current regulatory methods for delaying protein oxidation were summarized in this paper review. The purpose is to provide a new research direction for the research and application of quality control in the process of fish frozen storage.

**Keywords:** frozen storage; aquatic products; protein oxidation; nutritional quality; control measures.

**Practical Application:** Aquatic food processing and regulation.

## 1 Introduction

China is a large producer and consumer of aquatic products. The protein content of aquatic products is high. The protein content of fish in general can reach 15% to 20%, and the protein content of shellfish is about 10%. It also contains various essential amino acids required by the human body, and is a high-quality food resource for animal protein (Li, 2018). Fish and other aquatic products are even healthier alternatives to red meat and fast-food products today (Tacon et al., 2020).

Protein is the main component in aquatic products, and it determines the quality characteristics and processing suitability of aquatic products. The quality of frozen aquatic products changes during processing, storage, transportation, and sales. The formation of ice crystals, protein denaturation and microbial action would lead to the deterioration of the quality of frozen aquatic products (Hu et al., 2018). Lipids and protein oxidation are two important influencing factors. Lipids oxidation lead to discoloration of aquatic products and produce off-flavors and toxic substances (Bhat et al., 2018); Protein oxidation causes protein cross-linking and aggregation, resulting in an increase in carbonyl and disulfide bonds, a decrease in active sulfhydryl groups, and a loss of functionality such as protein solubility. The changes in the spatial configuration and groups of these proteins affect the edible quality of aquatic products and the water-holding capacity of muscles (Zhu et al., 2022).

Based on databases such as Pub Med, Science Direct and Web of Science, the literature on protein oxidation research in the food field was mainly counted during the period from 2012 to 2022. The results showed that the research on protein oxidation was relatively weak compared with lipid oxidation, which has been intensively studied during the same period. At present, the research in the field of food protein oxidation is mainly focused on meat and meat products. In recent years, the related research on protein oxidation in aquatic products (fish, shrimp, crab, shellfish, etc.) tends to increase (Maeda et al., 2022). In view of this, this paper reviews the latest research progress on protein oxidation in aquatic products in terms of protein oxidation characterization and occurrence mechanism, quality regulation, etc., in order to provide theoretical support for the research and regulation of protein oxidation in aquatic products during freezing and frozen storage.

## 2 Mechanism of protein oxidation

Protein oxidation is a covalent modification change of proteins initiated directly by reactive free radicals or induced indirectly by oxidation by-products and belongs to the free radical chain reaction (Li et al., 2021a). Like the mechanism of lipid oxidation, protein oxidation includes three stages (chain initiation, transmission, and termination) (Hellwig, 2019). Protein oxidation has little influence on the appearance and taste of food compared with lipid oxidation, leading to incomprehension and

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its main influencing factors are metal, oxygen and light induction, etc. Among them, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are important factors causing oxidative damage to proteins, and lipid hydro peroxidation intermediates could also indirectly induce oxidation (Chen et al., 2022).

### *2.1 Mechanisms of protein oxidation*

The process of protein oxidation begins with the production of $O_2$ and hydroperoxides formed by free radicals such as hydroxyl radical OH • and superoxide anion radical (Zheng et al., 2022). Therefore, the occurrence of protein oxidation requires reactive intermediates such as ROS and RNS, which have high reactivity and could be generated through various metabolic pathways, such as chemical toxicant and drug metabolism, cellular respiration, radiation, and light (Li et al., 2022). It has been pointed out that the oxidative modification of the biochemical and functional properties of rhubarb myofibrillar proteins by hydroxyl radicals generated from $H_2O_2$ oxidized solutions and hydroxyl radical-mediated protein oxidation is one of the most critical causes of the quality of rhubarb fish during processing and frozen storage (Priyadarshini et al., 2021).

Lipid peroxidation is inevitable in the processing and storage of aquatic products, which will lead to the formation of many intermediates, such as alkyl radicals, alkyl peroxyls, active carbonyl compounds, hydroperoxides (Douny et al., 2015). Meanwhile, hydroperoxides can react with proteins via the ε-amino pathway to form amide adducts. The reactive aldehydes produced by lipid peroxidation reactions are mainly α, β-unsaturated aldehydes, which have a strong ability to induce proteins. The following is a respectively description of photoinduced, metal, enzyme, and ice catalyzed protein oxidation.

### *2.2 Induction mechanism of protein oxidation*

*Light-induced protein oxidation*

When the aquatic products are directly exposed to light during the sales process, consumers can see all their external appearance and features, which increases their desire to consume. This type of retailing can induce photo-oxidation of proteins, yet. Two main reactions are involved in the occurrence of protein photosensitized oxidation (Schoneich, 2020). The one is that proteins can be directly oxidized by UV radiation due to the absorption of color groups. Direct photochemistry is dominated by amino acid side chains. In addition, this mechanism can lead to electron transfer and hydrogen uptake in proteins, formatting of molecules in the excited state or radicals due to photoionization, which in turn can cause protein damage and changes in molecular characteristics. the other one is the single-linear oxygen-induced photooxidation. The reactive intermediates originating from single-linear oxygen interact with each other. Photosensitized oxidation induces protein modification, leading to solubility loss and discoloration. If the relative humidity is low, it promotes glycosylation of β-lactoglobulin and induces non-disulfide covalent cross-linking causing aggregation of insoluble fractions such as casein.

*Metal-catalyzed protein oxidation*

In addition to light-induced protein oxidation, aquatic products are also susceptible to the transition metal ions (Akagawa, 2021). Superoxide anions generated by various pro-oxidation reactions are readily changed to $H_2O_2$ by different methods, including spontaneous reactions (Trnkova et al., 2015). In addition, the generation of chelated compounds is attributed to the binding of metal ions in the reduced state to amino acid residues at the metal binding sites of proteins and enzymes. The generated chelated compounds react with $H_2O_2$ to form highly reactive hydroxyl radicals. Subsequently, hydroxyl radicals are particularly prone to attack amino acids located at (or near) metal binding sites and further lead to the formation of carbonyl derivatives (Halliwell et al., 2021).

In the case of iron-catalyzed oxidation of lysine residues, for example, after $Fe^{3+}$ reduction to $Fe^{2+}$, iron binds to proteins to form complexes. Hydrogen peroxide produced by oxygen reduction can bind to $Fe^{2+}$ complexes. Meantime, electrons bind to $Fe^{3+}$, leading to the regeneration of $Fe^{2+}$. In addition, the ε-amino is converted to an imine, degradation of imide derivatives and the release of ammonia and $Fe^{2+}$ subsequently produces an aldehyde derivative. Amino acids located in the myosin tail of silver carp fillets were found to be highly susceptible to oxidation, while the gill-cutting halo method was found to be more susceptible to oxidation in vitro due to structural disruptions caused by the stress response (Zhang et al., 2021). Moreover, it was also demonstrated that the addition of hydrogen peroxide and iron to chopped rainbow trout heads induced protein oxidation (Kvangarsnes et al., 2021).

*Enzyme-catalyzed protein oxidation*

To exclude non-enzymatic oxidation, the other is an enzyme-induced pathway. Typically, this process is divided into two parts, one is the production of reactive free radicals and the other is the action on proteins (Wu et al., 2022). In addition, Liu et al. (2022a) applied proteomic analysis to demonstrate that enzymes in exudate are important for protein oxidation in thawed bighead carp fillets. The results showed that many enzymes were present in the exudate, but there were few enzymes with antioxidant capacity.

*Determination of lipid oxidation in emulsions*

On the other hand, there is ice induced protein oxidation, which generates ice crystals due to temperature changes during frozen storage. The effect will be different at different temperatures and times, and will vary with the characteristics of the raw material itself. Aquatic products are usually placed in frozen storage at temperatures of -10 °C to -30 °C, while some of the more valuable foods, such as tuna, usually require storage temperatures of -60°C or lower. After freezing, the extracellular solution of the muscle cell crystallizes first, and once ice crystals form outside the cell, there is an osmotic pressure across the cell membrane, and in order to reach equilibrium again, the cell becomes dehydrated. The solute is concentrated or the cell freezes, and these changes during freezing depend mainly on the freezing rate and the permeability of the membrane to water. Ice crystal growth during storage can lead to physical damage, while temperature fluctuations also accelerate the recrystallization process. Figure 1 shows a schematic diagram of ice crystals in muscle foods during freezing.

Ice crystal formation itself may not cause serious damage to the thawed muscle structure, but it can increase the volume of the muscle cells (myofibrils), resulting in distortion. After thawing, water is present in liquid form and myosin heads are bound to actin filaments, forming rigid bonds. After freezing, if the freezing rate is too low, most of the water forms ice crystals outside the cell and the muscle fibers are compressed, leading to distortion of the myosin heads (Bao et al., 2021). Figure 2 shows a schematic diagram of the mechanical deformation of the muscle protein structure in the frozen state.

In general, solutes are not incorporated into the ice crystals, they are concentrated in representative crystals and liquids. Differences in freezing rates result in different degrees of proton trapping by ice crystals. Hence, the ambient pH may affect protein denaturation to a greater extent during slow freezing. At the same time, the formation of ice crystals increases the concentration of solutes and dissolved air. At low temperatures, the solubility of oxygen increases, at which point it can be assumed that the freezing process accelerates oxidation. When the ability to freeze exceeds its solubility, bubbles form at the interface of the ice and quasi-liquid layers. Water to ice generates local pressure due to volume increase due to density reduction. There are many non-polar groups in proteins and hydrophobic forces make the natural protein structure more stable. As the temperature drops, the conformational entropy of the protein exceeds the stable hydrophobic effect and therefore unfolds at low temperatures. The freezing denaturation of proteins is related to nonpolar groups, water specificity and strongly temperature dependent interactions (Domínguez et al., 2021).

## 3 The effect of protein oxidation on the quality of aquatic products

Protein oxidation during storage of aquatic products is one of the main causes of reduced freshness of raw materials and spoilage of products, which affects the quality of aquatic foods in several ways, adversely affecting both color stability and textural properties during refrigeration. The metabolism of amino acids may be altered when amino acid side chains are modified by reactive oxygen species (Soladoye et al., 2015). In addition, oxidized proteins and protein-derived end products contribute to disease production, potential induction, carcinogenic, and neurotoxic activities that impair human health (Ahmad et al., 2020).

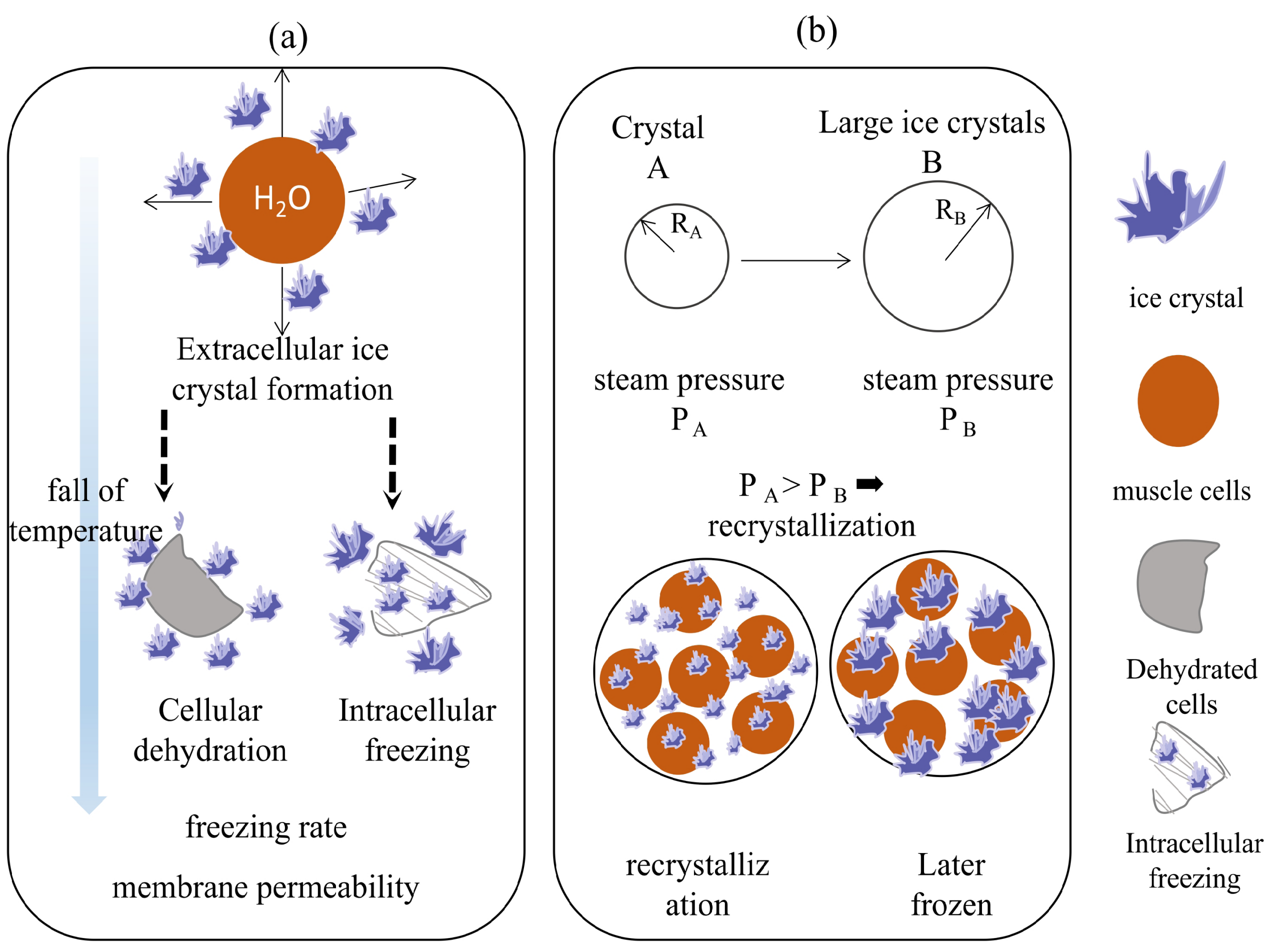


**Figure 1.** Schematic diagram of ice crystals of muscle food during frozen storage (a) ice crystal formation during freezing; (b) recrystallization during frozen storage (Bao et al., 2021).

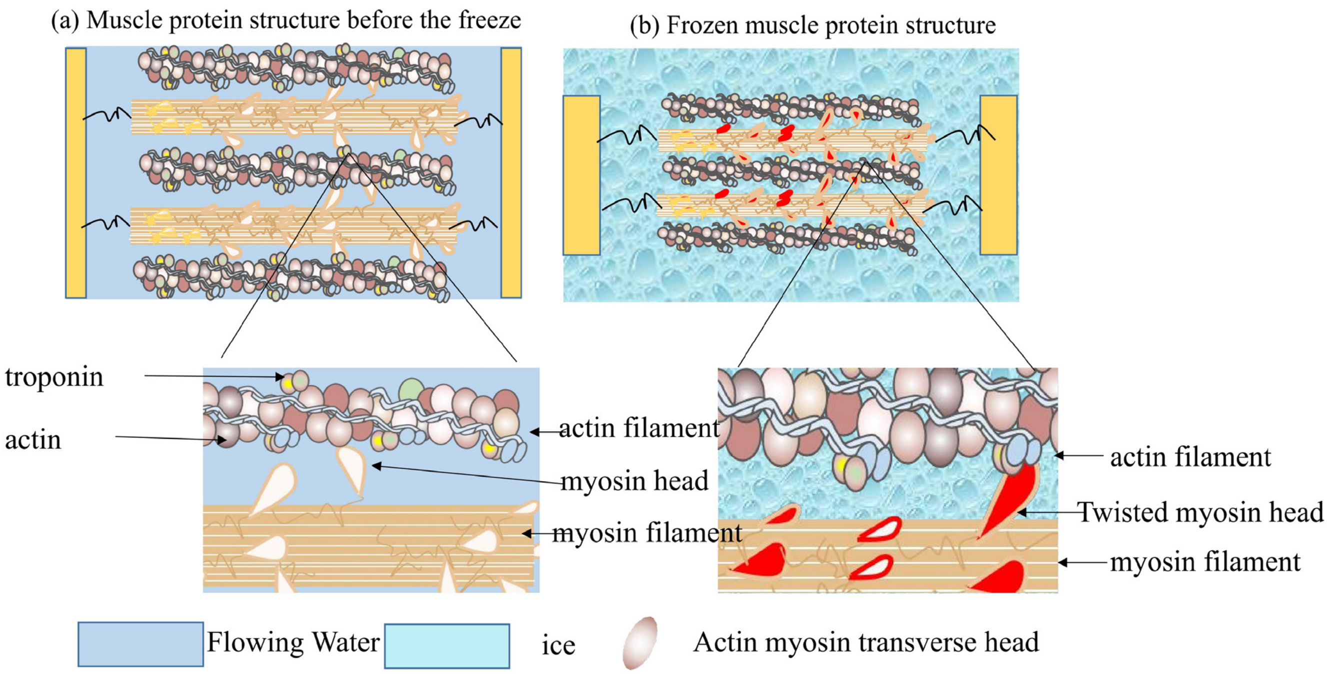


**Figure 2.** Schematic diagram of mechanical deformation of muscle protein structure under freezing condition (Bao et al., 2021).

### *3.1 Color*

Protein oxidation is generally considered to have a direct effect on the color of aquatic products (Wang et al., 2021a). Myoglobin is a globular protein that is highly susceptible to oxidation showing a brown oxidized state. The oxidation of ferrous myoglobin ($Fe^{2+}$) to high iron myoglobin ($Fe^{3+}$) also causes discoloration of livestock meat, aquatic muscle, and their products. Myoglobin in fish is more susceptible to oxidation than myoglobin in mammals, and this oxidation process causes the aquatic products to change from bright red to dark brown, with a decrease in the color indicator red ($a^*$ value) and an increase in yellow ($b^*$ value) (Wang et al., 2021b; Xia et al., 2021).

Lin et al. (2021) investigated the oxidative of hydroxyl radicals muscle proteins in peeled South American white shrimp. Higher concentrations of hydroxyl radicals had a negative impact on color and elasticity compared to the control group, due to the attack of active hydroxyl radicals on fragile amino acids, protein structures and conformations, which were responsible for the reduction of oxidative-treated shrimp and the stability of proteins. The oxidation state of myoglobin (protein oxidation measured as total carbonyl group) under the myoglobin oxidation is consistent with the artificial sensory and electronic sensory of surimi products (Carvalho et al., 2019). In addition, oxidation of myofibrillar proteins also causes color changes in protein gels and reduces the whiteness of the gel, which is related to the carbonylation process.

### *3.2 Texture*

Textural changes in aquatic products are directly related to protein oxidation and degradation. Postmortem tissue protein degradation is an important process in meat tenderization and is the main mechanism for becoming tenderized after aging at refrigeration temperatures (4-8 °C). Protein hydrolytic enzymes tenderize muscle tissue, especially calpain (Hematyar et al., 2019). The relationship between product tenderness and proteolytic enzyme activity depends on the degree of oxidation, with excessive oxidation resulting in a more compact protein structure; moderate oxidation results in protein unfolding and easier enzymatic breakdown of structural proteins. The increase in the hardness and elasticity of aquatic products is usually due to increased protein cross-linked structures and decreased protein hydrolase activity (Bao & Ertbjerg, 2019).

Protein oxidation (significant increase in free radical and carbonyl content) and degradation (increase in TCA soluble peptides and myogenic fiber fragmentation index) contribute to the reduction of firmness, elasticity, chewiness, and recoverability of ready-to-eat shrimp during storage, reducing their quality. (Li et al., 2020a). To assess the effect of protein oxidation on fish texture during frozen storage, Lu et al. (2021) found that there was a linear relationship between protein oxidation (salt-soluble protein, total sulfhydryl, disulfide, carbonyl content and Ca2+-ATPase activity) and texture (firmness) of bighead carp fillets.

### *3.3 Water holding capacity*

Another important property of proteins in aquatic products is the water holding capacity, a parameter that is related to qualitative changes. Most of the water in aquatic products is held in the myogenic fibril matrix (composed of actin and myosin), therefore, changes in myogenic fibril volume were proposed to explain the relationship between water holding capacity and protein oxidation (Wang et al., 2018). Due to the carbonylation process, oxidative modifications can lead to changes in protein charge involving histidine, lysine, and arginine residues (positively charged forms), which lose their positive charge upon oxidation and lead to an increase in net negative charge. This in turn increases the electrostatic repulsion between myofilaments, the swelling pressure and volume of myogenic fibers, and contributes to an increase in the water retention capacity of the muscular system (Wanzhu et al., 2018). Similar to protein hydrolysis, moderate oxidation facilitates ordered protein interactions and thus enhances protein function, whereas excessive oxidation promotes protein aggregation and reduces functional properties of proteins (Jiang et al., 2019). Liu et al. (2022b) investigated the effect of protein oxidation on the water holding capacity of frozen bighead carp fillets, and the centrifugal loss of fillets increased from 13.17% to 26.50% with increasing freezing time, and the decrease in water holding capacity could be explained by the following reasons.: crystallization, recrystallization-induced deformation of myofibrils (see 1.1) and protein oxidation (increase in carbonyl content, decrease in maximum fluorescence intensity and sulfhydryl content) changing the conformation and polarity of the protein.

### *3.4 Texture*

Protein oxidation also affects protein solubility, gelation, and emulsification properties (Zhang et al., 2020). Excessive oxidation leads to a decrease in protein solubility, while moderate oxidation facilitates a uniformly distributed dense network and improves protein gelation and emulsification properties. Bao & Ertbjerg (2019) investigated that the dynamic rheological properties of myogenic fibrin change with protein oxidation and that physicochemical changes (mainly disulfide bond formation) contribute to the enhancement of the gel network structure during thermal gelation. The effect of protein oxidation on the quality of aquatic products such as color, texture, water-holding capacity, and digestibility is beneficial and detrimental, depending on the degree of protein oxidation. Table 1 collected the effects on the quality of aquatic products under different oxidation systems in recent years at home and abroad.

## 4 Regulatory measures for protein oxidation of frozen aquatic products

In recent years, research on aquatic product quality improvement and its applications have been continuously reported, especially measures to regulate the freshness and improve the shelf life. Active coating technologies, antioxidants, packaging methods and high-pressure radio frequency have been used to improve the quality and shelf life of aquatic products (Lira et al., 2021).

### *4.1 Addition of antioxidants*

Yu et al. (2022c) investigated the effect of oxidation on protein digestion and transport in cooked abalone muscle, using for the first time a combination of simulated digestion and an Evert-rat-intestinal-cyst model, where the addition of bamboo leaf antioxidants attenuated heat treatment-induced increases in protein oxidation, aggregation, and hydrophobicity, thereby improving the digestibility and transport levels of abalone muscle proteins. The addition of different concentrations and types of antioxidants would have different effects. Li et al. (2020b) investigated the oxidative modification of MPs in grass carp, and the addition of low concentrations (5 and 10 μ mol/g protein) of tea polyphenols (TP) effectively inhibited carbonyl formation, loss of sulfhydryl and α-helix conformations, and hydroxyl radical-induced changes in the tertiary structure of myofibrillar The addition of high concentrations (10 μ mol/g protein) of TP was more effective in preventing oxidation-induced cross-linking

**Table 1.** Effect of protein oxidation on the quality of aquatic products.

| Category | Research Subjects | Oxidation system | Quality change | References |
|---|---|---|---|---|
| Fish | Coregonus Peled | Hydroxyl radical oxidation system | The carbonyl group, surface hydrophobicity increased with increasing oxidation concentration and time, and the total sulfhydryl group, free amino group and enzyme activity decreased. | (Deng et al., 2019) |
| | pseudo Sciaena crocea | Hydroxyl radical oxidation system | Reduced solubility, gelling and emulsification properties. Mild oxidation modestly improves gel strength and water retention. | (Li et al., 2020c) |
| Shrimp | South American White Prawns | Hydroxyl radical oxidation system | Moderate oxidation will increase water retention, reduce water migration, increase hardness and elasticity, while excessive oxidation will reduce mechanical properties leading to a decrease in water retention. | (Li et al., 2021a) |
| | Metapenaeus ensis | - | Compared to -60°C, shrimp thawing losses and the ability of myogenic fibers to hold water were poor at -18°C. | (Ji et al., 2021) |
| Shellfish and others | Peruvian Squid | Hydroxyl radical oxidation system | Carbonyl, hydrophobicity increases and fluorescence intensity decreases. Protein cross-linking or degradation occurs, water holding capacity decreases | (Zhu et al., 2019) |
| | Sea Cucumber | - | Protein aggregation or degradation caused during heat treatment | (Xin et al., 2021) |
| | Abalone | - | Oxidation leads to degradation of proteins, especially collagen-linked proteins, resulting in a decrease in water holding capacity, which leads to a decrease in shear and hardness | (Yu et al., 2022b) |

Note: "-" is not expressed in the text; "*a** value" represents red and green values.

or aggregation of myosin heavy chains and actin, and improved gel hardness and gel strength.

### *4.2 Plant extract addition*

Plant extract is a product formed by selecting a suitable extractant and combining physical and chemical extraction methods to obtain and concentrate one or more active ingredients in plants. (Ferraz et al., 2022). It can be widely used in pharmaceutical, food, daily chemical, and other industries (Al-Hijazeen, 2022).

The alcoholic extract of lime peel can significantly reduce peroxides, total volatile basic nitrogen, and free fatty acids in frozen rainbow trout in vacuum packaging (Mayeli et al., 2019). The use of different processing methods (e.g., edible coatings and plant extracts) and appropriate combinations of these agents can extend shelf life. Addition of plant extracts can improve sensory attributes (e.g., flavor, taste, and color), as well as antioxidant and antimicrobial properties. The solubility, sulfhydryl content and Ca2+-ATPase activity of MPs in fish fillet were increased, carbonyl content and surface hydrophobicity were significantly reduced after the compound coating treatment, and the secondary structure of proteins was more complete (Damerau et al., 2020). The sodium metabisulfite and plant extract group was more effective than the single plant extract group in retarding melanin in shrimp, inhibiting microbial spoilage and available to act as a natural alternative to synthetic anti-melanin agents for controlling shrimp melanosis (Priyadarshini et al., 2021). Table 2 summarized the domestic and international studies on the application of plant extracts in aquatic products.

### *4.3 Application of other methods*

Fish pretreated during cold chain transportation has a longer shelf life than freshly slaughtered fish. Insufficient slaughter and bleeding can lead to blood accumulation in the fillet, affecting the organoleptic quality and shelf life of the fish. Therefore, bleeding treatment after slaughter can be an important component of pretreated fish to prolong the freshness of the fish (Huang et al., 2021). The Ultra High Pressure (UHP) technique also inhibits lipid oxidation, protein oxidation and degradation in blackfish fish during refrigeration and maintains fish and myogenic fibrous protein structure and improves surimi gel properties (Qiaoyu et al., 2022). Studies on cod protein by comparing with conventional heat treatments (baking and steaming) have shown that UHP can improve the quality of fish products such as cod by increasing the content of soluble protein nitrogen and avoiding quality deterioration and protein oxidation during heat treatment (Zhang et al., 2019). Different packaging materials and methods have impact on the quality of fish products; the smoking process and proper packaging methods can protect fish lipids and polyunsaturated fatty acids from oxidation, and vacuum packaging and gas conditioning ensure microbiological quality and protein and lipid stability to extend the shelf life of smoked fish (Popelka et al., 2021). The use of biodegradable packaging materials for the preservation of aquatic products improves product shelf life by reducing lipid oxidation and protein oxidation and inhibiting microorganisms through oxygen isolation. Freezing as a common means of preserving aquatic products can be combined with different packaging methods and different materials for product freshness and quality preservation in the future (Hojatoleslami et al., 2022).

**Table 2.** Application of plant extracts in aquatic products.

| Category | Research Subjects | Extracts | Mode of action | Effectiveness | References |
|---|---|---|---|---|---|
| Fish | Carp | Robinia pseudoacacia | Feeding | Reduces nephrotoxicity, inhibits catalase, and increases antioxidant enzyme activity | (Dzydzan et al., 2022) |
| | Grass carp | Pomegranate peel | Coating | Inhibits spoilage microbiota such as Shigella and extends shelf life | (Yu et al., 2022a) |
| | Sturgeon | Volvoxia | Feeding | Inhibits Escherichia coli and Listeria monocytogenes, and has an immunomodulatory effect | (Shekarabi et al., 2022) |
| | Rainbow Trout | Pomegranate peel | Feeding | The strongest immunity of rainbow trout at a daily feeding rate of 500 mg/kg | (Sonmez et al., 2022) |
| Shrimp | South American white shrimp | Rosemary and green tea | Feeding | Inhibition of microbial spoilage, phenolic oxidative enzyme activity | (Yatmaz & Gokoglu, 2016) |
| | South American white shrimp | Portulaca oleracea | Feeding | Effective in delaying melanin in shrimp, used as a natural substitute for inhibiting melanin increase | (Calvo et al., 2021) |
| | Vannabei shrimp | Nine-mile fragrance | Coating | A natural alternative to synthetic antimelanocytes that can act to control melanosis in shrimp | (Soni et al., 2021) |
| | Rochester marsh shrimp | Banana peel | Feeding | As a potential immunostimulant in giant freshwater shrimp culture to reduce organic contaminants | (Naksing et al., 2022) |
| Other Squid | Squid | Blueberry Leaf | Add under heat treatment | Inhibits the formation of dimethylamine, trimethylamine and formaldehyde in aquatic products | (Li et al., 2021b) |

## 5 Conclusions

During the freezing process of aquatic products, ice crystal growth and protein denaturation would induce protein oxidation, which cause physical and chemical properties, functional properties, and nutritional quality. At present, there are three ways to improve product quality: first, pretreatment and storage, second, adding necessary antioxidants, and third, using effective packaging materials and methods, which can improve the oxidation stability of aquatic products, improve product quality, and prolong their shelf life.

The conversion of sulfhydryl groups into disulfide bonds during protein oxidation can be used as one of the indicators of protein oxidation. For this reason, it is worthwhile to investigate whether there is a certain protein change that can be used to indicate freshness of aquatic products. By exploring the interactions and molecular consequences of lipid and protein oxidation products, applying new analytical techniques to dig deeper into the extent of protein oxidation from amino acid study sites, and assisting new physical techniques such as microwave technology, irradiation technology, ultrasonic impregnation thawing, and plant composite film packaging to improve the shelf life of aquatic foods. In summary, an in-depth description of the protein oxidation mechanism during freezing will help to better control the oxidative damage of aquatic products and improve product quality.

## Conflicts of interest

The authors declare no conflict of interest.

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