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Review

The Formation and Control of Ice Crystal and Its Impact on the Quality of Frozen Aquatic Products: A Review

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Abstract: Although freezing has been used to delay the deterioration of product quality and extend its shelf life, the formation of ice crystals inevitably destroys product quality. This comprehensive review describes detailed information on the effects of ice crystals on aquatic products during freezing storage. The affecting factors (including nucleation temperature, freezing point, freezing rate, and temperature fluctuation) on the size, number, distribution, and shape of ice crystals are also elaborated in detail. Meanwhile, the corresponding technologies to control ice crystals have been developed based on these affecting factors to control the formation of ice crystals by inhibiting or inducing ice crystallization. In addition, the effects of ice crystals on the water, texture, and protein of aquatic products are comprehensively discussed, and the paper tries to describe their underlying mechanisms. This review can provide an understanding of ice crystallization in the aquatic products during freezing and contribute more clues for maintaining frozen food quality.

Keywords: ice crystal; aquatic products; control technology; quality; freezing

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1. Introduction

The quality of aquatic products will affect consumers' preferences and acceptability directly or indirectly [1]. Water is the main component in almost all fresh food, and it is also actively participated and accelerated in spoilage of foods, such as texture, appearance, and protein [2]. In order to minimize quality loss and preserve the safety of products, several techniques including cooling [1,3–5], freezing [6–8], and freeze-drying [9–11], etc. are used in the preservation of aquatic products. Among them, the most commonly used technique in aquatic product processing is freezing [12].

During the freezing process, the water of aquatic products is converted into ice crystals, inhibiting microorganism growth, slowing enzyme activities that degrade proteins or fats, and helping to extend the shelf life [13]. However, the formation of ice crystals may seriously impact the integrity of cells and muscle fibers, resulting in deterioration of quality [14]. It is generally believed that the freezing rate mainly affects the growth of ice crystals and then impacts the size and morphology of the ice crystals and their distribution inside the foods [12]. The formation and uneven distribution of large ice crystals in aquatic products are subjected to irreversible damage of cellular and tissue structure and lead to quality deteriorations of products, such as drip loss increasing after thawing, dehydration, tissue softening, discoloration, protein denaturation, mechanical damage, and so on [12,15–18]. On the contrary, the ice crystals generated by the high freezing rate have less damage on the quality of aquatic products. During subsequent freezing storage, the size and distribution of ice crystals in food change due to the occurrence of ice crystals

recrystallization [19]. In addition, the temperature fluctuation that occurs during freezing storage and transport is often unpredictable and inevitable. Ice crystals melt, as well as recrystallization, which adversely affect cryopreserved foods [20,21].

Nucleation has a crucial influence on the size of ice crystals and their distribution inside the products [22]. In addition to controlling the freezing rate, another important factors are the supercooling degree of ice crystals during the nucleation process [2,12]. In industrial production, the more traditional freezing methods are used, such as air blast freezing, immersion freezing, plate contact freezing, and so on [23,24]. Although rapid freezing like air blast freezing that requires very low temperatures generates smaller and more numerous ice crystals, it also means more energy and cooling costs [17,25]. Therefore, it is a challenge to reduce ice crystal size without increasing the costs and the energy consumption to freeze aquatic product. In recent decades, several assistance technologies for the freezing method to control ice nucleation and ice crystals growth have been introduced, including high pressure, ultrasounds, magnetic fields, electric fields, and the use of the inhibitors of ice recrystallization [2,20].

Therefore, the current review aims to provide detailed information on the effects of ice crystals on the quality of aquatic products. For this, the principle of ice crystals during the growth process is presented, and then the novel controlling techniques are summarized based on the factors that affect the ice crystal. Finally, the effects of ice crystals on the moisture, texture, protein, and colour of aquatic products are reviewed, and this paper tries to describe their underlying mechanisms. It is hoped that this review could provide more valuable clues on the ice crystal formation process of frozen aquatic products, thereby improving food quality.

2. Principles of Crystallization

During the freezing process of water, the crystallization goes through the following stages (Figure 1A): firstly, the specimen is rapidly precooled to remove sensible heat from the initial temperature to the initial freezing point. Continue to cool, when the temperature is low enough to surpass the phase change temperature of specimen and cross the energy barrier of nucleation, the nucleation begins to form and then the temperature suddenly increases to the freezing point of ice crystals. Next, latent heat in the specimen is removed and this crystal growth and new nucleation occur together. Finally, ice crystals recrystallization occurs in the last period of freezing and subsequent storage stage [26]. The rate of passing through the zone of maximum ice crystal formation (−1 to −5 °C) is slowest in actual aquatic products during freezing (Figure 1B).

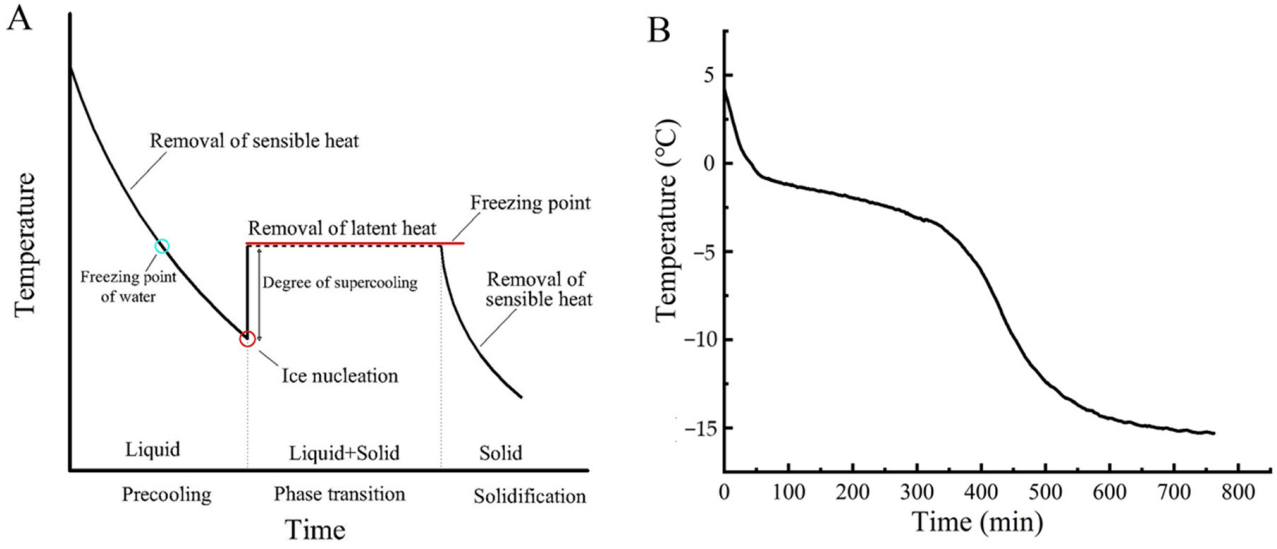

Figure 1. Typical time-temperature curve (**A**) of water during freezing processes; Freezing curves (**B**) for the centers of large yellow croakers with −20 °C refrigerator, and the Figure 1B was created from own data by the authors.

2.1. Ice Crystal Nucleation and Growth

The essence of nucleation is that the molecules of the liquid phase collide with each other and aggregate to form an amorphous cluster. After the further collision and aggregation of the amorphous cluster and reaching the critical volume, the molecules of the amorphous cluster gradually convert to a lattice and form stable nuclei [27]. Nucleation, consisting of primary nucleation and secondary nucleation, is an important affecting factor when the freezing process or other processes like freeze drying need to be considered for optimization [22]. For primary nucleation, the new crystals are formed in a solution without existing crystals, while secondary nucleation formed the new crystals in the solution where the pre-existing crystals exist [28].In food, the primary nucleation is mainly heterogeneous (with the presence of solid impurities) [29]. The formation of ice nucleation is quite random and influenced by many factors, such as impurities, sample volume, surface area, and roughness. etc, so it is difficult to monitor and control the ice nucleation [30].

After the formation of ice nucleation, the ice crystals began to grow rapidly under the driving force of supercooling. As we all know, since crystal growth occurs readily at temperatures close to the freezing point it has a high rate, which will cause a small number of nuclei to grow and a small number of large ice crystals to form [2]. The essence used to explain the crystal growth is the incorporation of molecules and growth units into the crystal lattice, which is controlled by two factors during the phase change: heat transfer and mass transfer [27]. Therefore, a key variable that affects the growth rate of the crystals is the degree of supercooling which is defined as $\Delta T_s = T_f - T_n$, where T_f is the freezing point temperature and T_n is the nucleation temperature [31]. During the crystal growth process, the molecules will diffuse and accumulate toward the ice-water interface. In other words, the water molecules move to the surface of the critical crystal nucleus, and the solute molecules diffuse to other places simultaneously [32]. This is why mass transfer is also an important factor affecting the growth of ice crystals. The nucleation and growth of ice crystals have a high effect on the quality of frozen aquatic products, so many technical research works aiming to control ice crystal nucleation and growth have been published, which will be described later.

2.2. Recrystallization

After the crystal is formed, the change process of shape, size, and number of ice crystals can be called recrystallization [33]. The recrystallization of ice in aquatic products is as follows: the average size of ice crystals increases, the number of crystals decreases, and the surface free energy of the entire crystal system decrease [34]. There are various recrystallization mechanisms during frozen storage, which can be described as isotonicity, accretive, migratory, pressure-induced, and irruptive. Among which, isotonicity, migration, and accretion may be the main types of recrystallization in frozen aquatic products [35]. More attention currently has been paid to the Ostwald ripening mechanism. Compared with large ice crystals, the water molecules on the surface of small ice crystals have higher free energy due to the high curvature and are more thermodynamically unstable. Consequently, the liquid of small ice crystals, melted by a migrating recrystallization (also known as Ostwald ripening) process during the process migrates to the surface of the large ice crystal, eventually resulting in the growth of the large crystal along with the vanishing of the small crystal [34]. The predecessors developed the Ostwald ripening equation (Equation (1)) to describe the mean crystal size at any time $(\overline{L})$ during the recrystallization process [36]:

$$\overline{L}(t) = \overline{L}_0 + kt^{\frac{1}{n}}$$

where $\overline{L}_0$. the initial mean crystal size, *k* and *n* representative the rate of recrystallization and a mechanism parameter of this process, respectively. In general, the process is always that free energy is minimized and the chemical potential between all phases is equalized, when the system tends toward a state of equilibrium, the recrystallization occurs [37].

3. Affecting Factors and Control Technology of Ice Crystal

It is generally accepted that nucleation is critical to the size, number, distribution, and shape of ice crystals [22]. The two important factors controlling the morphological characteristics of ice crystals are the freezing rate and degree of supercooling [38]. The nucleation temperature and solidification point, which are directly related to the degree of supercooling, are important concerns for controlling ice crystals. In addition, the recrystallization of ice crystals after nucleation that changes the morphological characteristics of ice crystals is exacerbated by temperature fluctuations [39]. Hereunder, the affecting factors on the morphological characteristics of ice crystals and the method used to control the ice crystals are discussed. The aggregated results are shown in Table 1.

3.1. Nucleation Temperature

As the temperature at which the first ice crystal appears during the nucleation process, the nucleation temperature is closely related to the size and distribution of the ice crystals [40]. Nevertheless, the nucleation temperature of the same type of aquatic products is not fixed and is easily affected by many factors, such as impurities, sample volume, and surface area, etc [30]. Thus, the morphological characteristics of ice crystals can be changed by increasing or decreasing the nucleation temperature of ice crystals.

More recently, electric field (EF) assisted freezing, as a technique for controlling ice crystals, has been used in the freezing process of aquatic products. When the external EF acts on the supercooled water, water molecules will be redirected and polarized in the direction of the EF, resulting in the reduction of the Gibbs free energy of the entire system [41]. This transformation is directly reflected in the increase of nucleation temperature, the decrease of supercooling degree, and the enhancement of ice nucleation [42]. For a long time, direct current (DC) voltage assisted freezing has been studied, including charge flow (CF) and static electrostatic field (SEF) [43]. Charge flow (CF) can induce a higher nucleation temperature and reduce the degree of supercooling, which will generate larger ice crystals [2]. This can be used in the freeze-drying of aquatic products to obtain a shorter drying time in the follow-up. Numerous ice nuclei are formed and the growth of ice crystals is interfered, due to the reduction of the energy barrier of the phase transition and the promotion of ice nucleation under the action of the SEF [41,44,45]. Wang et al. [46] developed an improved high voltage electrostatic field system on the effect of agarose gel, and the results indicated that the system could significantly reduce the degree of supercooling and increase the nucleation rate. Moreover, the increase in the magnitude of the electrostatic field could reduce the size of ice crystals. In recent years, the application of SEF in aquatic products has been studied in cold storage [47] and thawing [48], but the related report has not been found on freezing. Nevertheless, the high voltage electrostatic field is used for freezing other meats. Dalvi-Isfahan et al. [38] applied a high voltage electrostatic field (0–66.77 kV/mm) to freeze lamb. The result showed that the use of SEF was able to effectively reduce the ice crystal size in lamb meat during freezing and reduce the extent of damage to the meat. In addition, the ice crystal size decreased with increasing voltage. For this reason, the size of the voltage should be considered when assisting the freezing of aquatic products with SEF in the future.

Both microwave (MW) and radiofrequency (RF) belong to electromagnetic waves, their influence on ice crystals are similar to that of the electric field, all of which affect ice nucleation by affecting water dipole moment [49]. Xanthakis et al. [50] found that freezing food under MW assistance technology can effectively reduce the mean ice crystal size, while the degree of supercooling was decreased. Sadot et al. [25] also discovered that MW assistance during freezing can significantly reduce the size of ice crystals (−25%), and the amount of energy provided by microwaves had a negative correlation with the size of ice crystals. The mechanism of MW radiation on ice crystal size reduction during freezing can be explained by considering its influence on the hydrogen bonds between water molecules and can also be explained by nucleation caused by temperature oscillations induced by MW [51]. Most recently, Hafezparast-Moadab et al. [52] applied RF(27.12 MHz) assisted air

blast freezing in rainbow trout and revealed that the size of ice crystals in trout frozen by air freezing under RF assisted, was reduced by 75% compared to control samples without RF, also with lesser damage to the cellular structure and close to fresh sample. However, the underlying mechanisms of the influence of MW and RF on ice crystal formation remain unknown and need further investigations.

Since 1990, the ice fog technique has been used to control the formation of ice nuclei during freezing [53].The vials containing the solution are cooled to the desired nucleation temperature and then placed in a closed chamber, after which a cold nitrogen gas stream is released into the chamber. At this point, an ice fog is first generated due to the high humidity of the chamber, which then penetrates all vials and initiates ice nucleation on the solution surface [54]. Currently, this promising technique is mainly used in lyophilizers [55]. It is not yet reported in the freezing of aquatic products. In the future, it is possible to study the formation of uniform and fine ice crystals by putting aquatic products in vials filled with coolant and using ice fog technology to control the formation of ice nuclei in the products.

3.2. Freezing Point

The formation of ice nuclei is promoted or inhibited by adjusting the freezing point (FP), aiming to meet the different requirements of ice crystals during freezing [56]. Traditional methods of regulating FP, such as ionic solutions [57,58], alcohol solutions [59], and chemical reagents [60–62], alter FP by changing the solute concentration and hydrogen bonding environment [33]. It is necessary to develop naturally sourced biosubstances that can regulate freezing points, as these methods require the use of chemical reagents in the process of use, which is harmful to food safety. Besides, high pressure shift freezing (HPSF) technology is also related to FP regulation.

These biosubstances include ice binding proteins (IBPs) and natural deep eutectic solvents (NADES) [23]. Both ice nucleating proteins (INPs) and antifreeze proteins (AFPs) belong to IBPs which act by affixing to ice surfaces, but there is the opposite effect on freezing point [63]. AFPs reduce the freezing point by binding to the surface of ice crystals and inhibiting the formation of ice crystals [56]. Unlike AFPs that can inhibit ice nucleation, INPs can effectively align water molecules at the interface, elevate the FP of water, and promote ice formation [64]. Jin et al. [9] added INPs to the sucrose solution during the freezing stage, which aimed to improve the efficiency of freeze drying. According to the result of ice morphology, INPs could effectively increase the size of ice crystals and form a lamellar ice structure that favored for the sublimation process and that improves the efficiency of freeze drying with significant energy savings. Through inhibiting the growth of ice crystals, AFP which can reduce FP of water but without altering melting temperature keeps the better quality of frozen product [65]. Thus, it has broad application prospects in frozen aquatic products.

In recent years, scientists have discovered the existence of NADES from some cold-tolerant animal species, which are a mixture of two or more transparent liquids [66]. NADES makes it difficult for free water to achieve molecular movement and reorientation by affecting the hydrogen bonding of water molecules, resulting in the significant depression of freezing points. At the same time, the viscosity of NADES increase significantly, due to NADES is provided with a supramolecular network structure by hydrogen bonding [23]. The temperature during freezing also affects the properties of NADES, such as viscosity and density [67]. Castro et al. [68] reported the utilize of NADES based on glycerol and trehalose in cryopreservation, showed that NADES can decrease the amount of ice crystals, thereby reduced the ice crystals damage in cells and compared with dimethyl sulfoxide, reduce the toxic effect on cell viability. Consequently, these results indicate that NADES is a promising FP regulator for food studies. However, to date, very few studies have been reported regarding the use of NADES in the frozen aquatic products and hence many works remain to be conducted in the future.

In HPSF, the freezing point of water is reduced from 0 °C to −21 °C under high pressure (210 MPa), the supercooling degree increases, and leads to extensive nuclei form instantaneously when the pressure is released, thus the ice crystals appear to be smaller and uniform granule [12,69]. In recent years, HPSF has been used to freeze aquatic products, such as turbot [70], Atlantic salmon [71], sea bass [72], and abalone [73]. Su et al. [74] compared the changes in shrimp muscle ice crystals between HPSF and traditional freezing methods (air freezing and immersion freezing), and the results showed that the phase transition times of air freezing and immersion freezing were 148 min and 5.9 min, respectively, while the HPSF at 100 MPa was only 2.97 min. In addition, compared with the HPSF at 100 MPa, the ice crystals formed were smaller under 200MPa, and the cross-sectional area and equivalent diameter of ice crystals are reduced by 54% and 79%, respectively. The quality of the sample inevitably deteriorates under high pressure, such as protein denaturation [75], even with the ice crystals formed by HPSF that reduce the damages to the quality of the sample. Cheng et al. [69] explored the crucial factors (pressure and freezing) that induce protein deterioration during high pressure freezing process, and found that when the pressure is 100–200 MPa and 300–500 MPa, the main factors for protein denaturation are freezing-factors and pressure-factors, respectively. Therefore, the lowest total negative impact of freezing and pressure on protein denaturation was observed in HPSF at a pressure of 200 MPa, while 300 MPa can be used as a critical point for high-pressure freezing treatment to reduce the effect of pressure on protein denaturation.

3.3. Freezing Rate

The level of freezing rate affects the size of ice crystals. At low freezing rates, larger ice crystals can be obtained and on the contrary, a large number of small ice crystals are formed [76]. The growth of ice crystals is a function of the freezing rate [34,77]. During the freezing process, ice crystals occur outside the cell first, and the change of solute concentration causes the intracellular water to pass through the cell membrane and deposit on the extracellular ice crystals [34]. Therefore, if the freezing rate is low, the intracellular water continuously migrates outward, causing the continuous increase of extracellular ice crystals and cell dehydration [78]. For several decades, the thickness of the frozen product, the surface heat transfer coefficient, and the refrigerating medium temperature have been widely explored to increase the freezing rate [43]. Some studies indicated that the thinner samples had a faster heat transfer from the surface to the inside, and water transformed into ice quickly [79]. Yang et al. [17] found that compared with air freezing, immersion freezing had a higher heat transfer coefficient (i.e., faster freezing) and smaller ice crystals when freezing obscure pufferfish fillets. At the same time, the lower freezing medium temperature which could increase the temperature gradient has a better ability to improve the heat transfer. Although liquid nitrogen freezing has a high heat transfer coefficient and a significant advantage in freezing efficiency, it is significantly expensive and not in cyclic utilization. In addition, the extremely high freezing rate also cracks the frozen aquatic products [80,81]. In short, it is necessary to pay attention to cracking phenomenon of product surface to maintaining the quality of the aquatic product in the process of pursuing small ice crystals. In recent years, freezing assist technology has been successfully used to manipulate the freezing rate to control the size of ice crystals.

The cavitation and microstreaming effects generated by ultrasound effectively shorten the freezing time of aquatic products. Whether in a liquid medium or at a solid–liquid interface, cavitation bubbles contribute to the enhancement of heat and mass transfer [82,83]. Furthermore, the microstreaming caused by the motion of cavitation bubbles further strengthen heat and mass transfer [84]. The increase of heat and mass transfer efficiency in turn promotes ice crystallization, resulting in the improvement of freezing rate [85]. In recent years, the effects of ultrasound immersion freezing (UIF) on ice crystals and the quality of aquatic products have been fully probed. Sun et al. [86] applied UIF to evaluate the quality of frozen common carp and observed the microstructure of ice crystals of muscle tissue captured by a light microscope (Figure 2). Results indicated that the average ice

crystal diameters of the air freezing (AF) and immersion freezing (IF) samples after freezing were 37.73 and 26.55 µm, while the UIF was 21.47 µm. In addition to the higher heat transfer coefficient of the liquid than that of the air, the cavitation and microfluidics effects generated by ultrasound increase the heat and mass transfer efficiency. Furthermore, large ice crystals are broken into small ice crystals under the application of ultrasound which can be acted as primary nuclei, resulting in more ice nuclei and smaller size of ice crystals [87]. With the augment of ultrasonic power, the phase transition time tends to increase first and then decrease. Although higher ultrasonic power can accelerate nucleation and the rate of heat transfer, it also produces more heat at the same time [13]. Shi et al. [88] found that the control of ultrasonic power and duration played an important role in maintaining the quality of the sample when freezing grass carp with ultrasonic assisted freezing. In this case, the proper ultrasound parameters like ultrasonic power (0.38 W/cm^2) and duration (60 min) should be selected with the application of ultrasound assisted freezing in the future in order to increase the freezing rate.

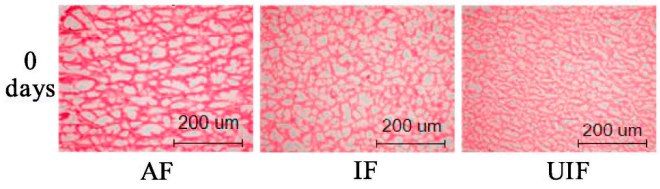

Figure 2. Microstructure changes of frozen carp under air freezing (AF), immersion freezing (IF), and ultrasound immersion freezing (UIF) during freezing. Where white is the hole left by ice crystals and pink corresponds to muscle fiber [86].

3.4. Temperature Fluctuations

The occurrence of temperature fluctuations during freezing storage, transportation, and sale has always been an important concern of the frozen aquatic products. Temperature fluctuations can lead to the change of size and distribution of ice crystals causing further mechanical damage of aquatic products, which degrades frozen sample quality [20,21]. The greater the fluctuation, the more serious the recrystallization, which aggravates the damage to the muscle structure and reduces the quality of the food [35]. Significantly, temperature fluctuations are inevitable even when samples are stored at −18 °C commercial refrigerator. In that case, the importance of how to delay the effect of temperature fluctuations on ice crystals is self-evident. In order to suppress temperature fluctuations, further than the control of the temperature stability during storage, cryoprotectants have been studied [34].

Recently, carbohydrates as antifreeze agents have been widely used in frozen aquatic products. Zhang et al. [89] investigated the effect of using carrageenan oligosaccharide (CO) and xylooligosaccharide (XO) on the ice crystals growth and recrystallization in frozen peeled shrimp during the temperature fluctuation, showed that compared with shrimp treated with $Na_4P_2O_7$, the CO and XO treatment were significantly inhibited the increase in the area values of ice crystals and the decrease in the roundness values of ice crystals formed in the tissue by temperature fluctuations. Similarly, trehalose and alginate oligosaccharides could suppress the growth and recrystallization of ice crystals and slow down the damage of large ice crystals to muscle tissue [20]. In order to investigate the ice growth inhibition mechanism of carbohydrates, Zhang et al. [90] used molecular dynamics (MD) simulations to prove that saccharides are embedded into the ice layer through hydrogen bonding or hydrophobic or electrostatic interactions during the binding process. As a result, the incorporated carbohydrates inhibit the ice crystals growth and recrystallization, thereby protecting them from temperature fluctuation damage. In that case, it would be a good choice to apply sugars to frozen aquatic products to avoid the influence of temperature fluctuations on ice crystals. In that case, it would be a good choice to apply carbohydrates in frozen aquatic products to avoid the influence of temperature fluctuations on ice crystals.

In addition to the ability of thermal hysteresis (the temperature gap between the melting point and the FP), which inhibits the formation of ice crystals, the ice recrystallization inhibition (IRI) is another ability for AFP [91]. Nevertheless, the correlation between thermal hysteresis (TH) and IRI in the same species is not obvious [92]. Numerous research works on the use of AFP to inhibit ice recrystallization in aquatic products, such as mirror carp [93], largemouth bass (Micropterus salmoides) [94], and red sea bream (Pagrosomus Major) [95], etc. have been published. These results revealed that temperature fluctuations caused the ice recrystallization, the shape of the ice crystals is affected by the fluctuations, and AFP reduces their mechanical damage caused by large ice crystals. However, AFP is difficult to use on a large scale in industrial production due to the high cost associated with extraction, purification, and synthesis. Thus, researchers began to explore chemical materials with IRI activity, such as synthetic high molecular polymers and nanomaterials [56].

Polyvinyl alcohol (PVA), as an FDA approved food additive, is a widely used IRI active polymer [96]. According to the molecular dynamics simulation studies, PVA binds to ice via the tight hydrogen bond between the water molecule and the hydroxyl group of PVA [97]. It is also found that PVA can inhibit the crystallization of ice crystals by reducing the hydrogen bonds between water molecules, and thus reducing the nucleation temperature [98]. Recently, Li et al. [99] also found IRI activity on nanocellulose for the first time. However, Velásquez-Cock et al. [100] found that cellulose nanofibers (CNF) did not affect the growth of ice cream crystals. This may be due to the low IRI activity of CNF, which is not significant for inhibiting the growth of ice crystals. In order to enhance the IRI activity, the two important structural parameters of nanocellulose: surface charge density [101] and fibril length [102] were studied. Although PVA and nanocellulose have been proven to have IRI activity, they still require a depth exploration on the frozen storage of aquatic products.

Table 1. Affecting factors and control technology of ice crystal.

Affecting Factors	Principles	Methods	References
Nucleation temperature	Altering supercooling degree and nucleation process	Electric field, electromagnetic waves, ice fog technique	Rainbow trout [52];
Freezing point	Decreasing freezing point, facilitating nucleation	Antifreeze proteins, natural deep eutectic solvents, high pressure shift freezing	Turbot [70], Atlantic salmon [71], sea bass [72], abalone [73], shrimp [74,95];
Freezing rate	Enhancing heat and mass transfer	Conventional air freezing, immersion freezing, liquid nitrogen freezing, ultrasonic assisted freezing	Pufferfish [17], perch [80], red swamp crayfish [81], common carp [86], grass carp [88];
Temperature fluctuations	The growth of the large crystal and the vanishing of the small crystal	Carbohydrates, antifreeze proteins, polyvinyl alcohol, cellulose nanofibers	Shrimp [20,89], mirror carp [93], largemouth bass [94], red sea bream [95,103];

4. Effect of Ice Crystals on the Quality of Aquatic Products

4.1. Water

The water in the muscle is composed of three distinct populations: bound water, immobilized water, and free water [104]. The free water of the product becomes ice crystals firstly, followed by the immobilized water, and the bound water is basically unchanged during the freezing process [105]. With the extension of freezing time, the bound water which is tightly bound to proteins migrates to the immobilized water and its content is reduced, especially for the protein denaturation under large ice crystals [86]. Meanwhile, the immobilized water migrates to the free water, and hence freezing at lower temperatures can produce smaller ice crystals and inhibit this migration [106]. Thawing loss is mainly determined by the content of free water [7,107]. Yu et al. [108] found that small ice crystals formed by rapid freezing could better suppress the thaw loss of giant freshwater prawns,

compared with the formation of large ice crystals in slow freezing. Generally, the thaw loss is simply attributed to the difference in the mechanical damage of muscle fibers caused by the size and location of ice crystals, and there is not further explanation [109]. Zhang et al. [78] proposed a model to explain thaw loss that water migrated from the inside of the muscle fiber to the outside during growth of ice crystals, and the slow freezing rate increased the mechanical damage caused by transverse shrinkage and deformation of the muscle fiber. Additionally, slow freezing also caused an increase in concentration of protons (lower pH and higher ionic strength) which leads to protein denaturation and the structural protein reabsorbs less water after thawing; nevertheless, some protons are trapped inside small ice crystals formed by rapid freezing. When the aquatic product is exposed to temperature fluctuations, the growth in size of ice crystals leads to an increase of thaw loss and weight loss of the product muscle.

The water transfer during freezing occurs between the product and its surrounding environment, resulting in dehydration which implies a weight loss while the product releases energy to the surrounding environment [110]. Mulot et al. [16] studied the effect of freezing temperature (from room temperature to −100 °C) and gas flow velocity (from 0 to 9 ms^{-1}) during freezing on food dehydration, and the results indicated that there was a close relationship between freezing temperature and weight loss: lowering the temperature decreases weight loss. For lower freezing temperatures, the increase in flow velocity can reduce weight loss, whereas, for high freezing temperatures, the influence of the gas flow velocity is less pronounced on the weight loss of the product. In order to limit the weight loss of the product, it is essential to reduce the total freezing time (reduction of initial product temperature, for instance). Additionally, Jin et al. [9] found that larger crystal size induced by ice nucleation proteins could facilitate the water vapor flow and thus increase the dehydration rate of the sucrose solution, which was conducive to improve the efficiency of the freeze drying process. The product frozen at a high freezing rate or low freezing temperature is left with small pore sizes resulting in a low void connectivity after freeze drying, which increases the resistance to water vapor flow and has low freeze drying efficiency [111]. However, it is also believed that large ice crystals melt and freeze dry more easily. The dehydration of frozen products inevitably occurs during frozen storage, especially affected by temperature fluctuations. According to Dalvi-Isfahan et al. [34], since the water vapor pressure difference between the surface of frozen products and the water in the air during frozen storage, ice sublimate and dehydrate to equilibrate with the vapor pressure of both sides. The dehydration of frozen products which cause the weight loss irreversibly affects other qualities of products, such as texture, color and protein, etc., resulting in a serious deterioration in the quality and value of frozen products [112,113]. Various techniques can be used to avoid the dehydration of aquatic products during freezing storage, such as glazing [8,114], vacuum packaging [21], and temperature control [34], etc.

4.2. Texture

The texture is directly influenced by the characteristics (shape and position) of ice crystals during the freezing of aquatic products. Under the influence of ice crystals, the myofibrils and the connective tissue around fibers of the sample thawed before or after was significantly fragmented (Figure 3). It is well documented that ice crystals cause the deterioration of aquatic products, especially the accelerated decrease of hardness caused by large ice crystals, such as common carp [115], horse mackerel [18], and prawns [108]. According to Yang et al. [17], ice crystals played an important role in the microstructure destruction and texture softening of pufferfish, and the even-distributed ice crystals contributed to maintaining the texture characteristics of frozen pufferfish. However, the softening of frozen aquatic products cannot be attributed only to the formation of ice crystals, but also to endogenous proteolytic activity [116] and lipid and protein oxidation [1]. In order to compare the main factors of these three reasons for softening, Yang et al. [15] compared and indicated that fish softening contributed the most was ice crystals during frozen storage,

and the fish softening during frozen storage mainly occurred in the initial stage. What is more, the evolution of ice crystals to large ice crystals due to temperature fluctuations aggravate the deterioration of texture, which was likely a result of the decrease in the mechanical strength of connective tissue, water loss, and protein aggregation [89]. Yet, the smaller ice crystals formed through the lower temperature is not necessarily better for the texture of aquatic products. Shi et al. [80] indicated that the perch frozen by liquid nitrogen (−196 °C) had an obvious crack on the muscle surface, which was not suitable for long-term storage of fish. It was suggesting that −85.0 °C should be regarded as the limit temperature for the industrial freezing of fish [117]. Thus, it was not simply considered the effect of freezing temperature on ice crystals for freezing aquatic products but also on the texture and energy consumption to select the appropriate freezing temperature.

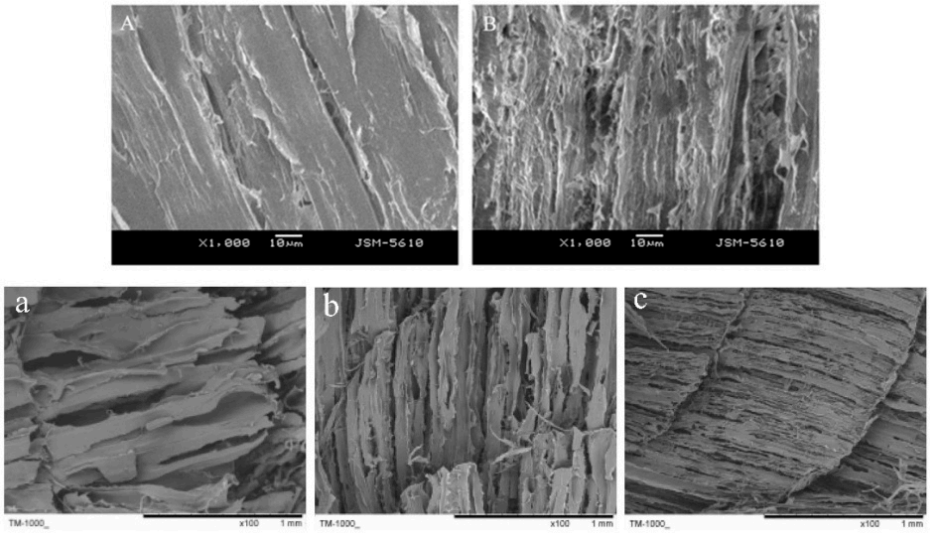

Figure 3. Effects of ice crystals on the structure of aquatic products. The SEM micrographs of peeled shrimp(**A**: fresh and **B**: freezing in a freezer at −30 °C) and hairtail (**a**: conventional air freezing at −20 °C, **b**: refrigerator cryogenic freezing at −80 °C, and **c**: liquid nitrogen immersion freezing at −196 °C) are adapted from Zhang et al. [118] and Luan et al. [119], respectively.

4.3. Protein

Protein molecules are substances that have a spatial conformation (including primary, secondary, tertiary, and quaternary structures) under the combined action of covalent and noncovalent bonds. In general, the mechanism of protein denaturation caused by freezing is due to the increased concentration of solutes in the unfrozen water phase of muscle tissue during ice crystal formation [78,120]. In other words, solutes are gradually concentrated in the unfrozen water phase outside the ice crystals during liquid nitrogen immersion freezing (LIF) at −196 °C freezing, which causes the concentration of protons in the unfrozen water around the protein to increase (lower pH and higher ionic strength) also resulting in protein denaturation, especially for slow freezing. However, several studies over the years have other opinions on this, such as the direct adsorption of proteins onto ice crystals [121], the accumulation of air bubbles at the ice-freeze concentrate interface [122], the mechanical stress associated with ice crystal growth that may result in pressure-induced protein unfolding [123], and the foster of cold denaturation in the presence of ice crystals [124]. Since water molecules that play an important role in maintaining the structure stability of secondary and tertiary of proteins participate in crystallization, led to the changes in the spatial conformation of protein molecules and then protein denaturation [125]. The formation of ice crystals during the freezing of snakehead destroyed the protein secondary structure conformations and compared with small ice crystals, samples under large ice crystals have higher thermal stability [126]. Similarly, the results of Sun et al. [87] showed a decrease in the α-helix proportion of the common carp after freezing and an increase

in random coil, indicated that the protein secondary structure changed. In the meantime, the formation of ice crystals leads to the unfolding of the protein tertiary structure and the reduction of thermal stability, but the protein primary structure (including sulfhydryl, carbonyl group, free amino group, dityrosine content, and surface hydrophobicity) of the frozen sample was no significant difference between and the unfrozen sample. The muscle cells are destroyed by ice crystals during freezing, and protein molecules are attacked and oxidized by some preoxidation factors. After thawing, the degree of protein oxidation is increased due to the release of mitochondria, lysosomal enzymes, and other pro-oxidation factors into the sarcoplasm [127,128]. Further, prolonged cryopreservation and temperature fluctuations might cause the growth of ice crystals in the sample, resulting in more serious damage to the protein structure [20].

4.4. Colour

Colour is the most direct indicator for consumers to evaluate the quality of food and is an important factor in influencing their purchases [13]. Frozen channel catfish have a significant increase in whiteness values after thawing, which is not only associated with the decomposition of myoglobin but also due to the generation of ice crystals during freezing, the increase of free water on their surface after thawing, and the enhanced reflection of light on the surface of the fish resulting in an increase in whiteness values [129]. In addition, as the freezing rate increases, the surface ice crystal size of frozen salmon fillet decreases, L* values (represent the degree of lightness to darkness) increase, a* (represent the degree of redness to greenness) and b* values (range from yellowness to blueness) decrease, and whiteness values increase, which means that less colour change [130]. After a long period of frozen storage, the surface of the common carp fillets gradually tended to be yellow [131]. The L* value of precooked Chinese shrimp decreased from 54.52 to 44.28 at the end of storage (180 d), which was probably due to astaxanthin degradation and lipid oxidation, and the same change trend was observed for b* values [132]. Temperature fluctuations accelerate the myoglobin oxidation in the meat and accelerate the colour change of the product [35]. From Figure 4, it is obvious that the surface color of large yellow croakers became dull and lusterless due to dehydration. However, the mechanisms by which ice crystals affect the colour of aquatic products remain to be explored in depth.

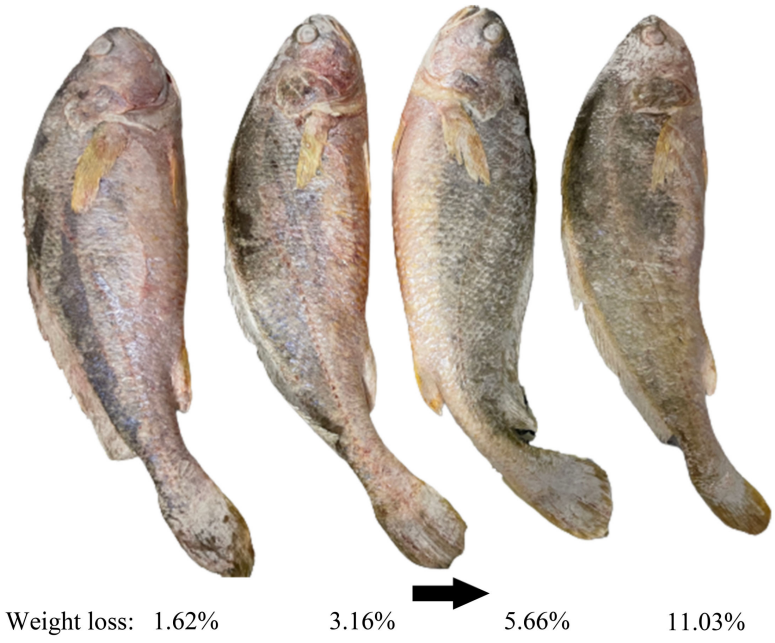

Figure 4. Effect of water sublimation on the surface color of large yellow croakers during frozen storage. The figure was created from own experimental images by the authors.

5. Conclusions and Future Trends

The frozen aquatic products of high quality are vital to consumers and producers and hence it is of great practical significance to study the impact of ice crystals on the quality of aquatic products during freezing. The most obvious damage is ice crystals to muscle fibers. Even though the crystallization is a process that spontaneous and difficult to control, it is still possible to develop corresponding technologies based on affecting factors (nucleation temperature, freezing point, freezing rate, and temperature fluctuations) to control the microstructure and morphology of the crystal.

The current freezing research is mainly focused on obtaining small and uniformly distributed ice crystals to realize better frozen product quality. Although some new freezing technologies assisted by physical fields, such as electric and magnetic fields, high pressure, and ultrasound have been studied in depth, these technologies are still in the experimental stage and their mechanisms are not well established. A great deal of work is still needed to do, such as optimizing the process parameters, exploring the mechanism, and developing simple and safe equipment. In addition, the model of the effects of heat and mass transfer on the freezing process have been studied, but other physical freeze damage phenomena accompanying the freezing process including mechanical damage and cryoconcentration are ignored. Therefore, in the future, it is necessary to establish heat and mass transfer models combining these phenomena so that this model can predict not only the size of ice crystals but also the product quality. The ice crystal recrystallization has a greater impact on product quality, so the empirical model can be constructed to predict the equivalent diameter of ice crystals in the recrystallization process of frozen products. It is well documented that the size of ice crystals has a great influence on the dehydration of aquatic products during freezing process, but the mechanism of their influence still needs to be studied in depth. Although there are many areas to be optimized yet in freezing, there is no doubt that it is a promising technology for the preservation of aquatic products.

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