Freezing of meat and aquatic food : Underlying mechanisms and implications on protein oxidation

Bao, Yulong

2021-11

Bao , Y , Ertbjerg , P , Estévez , M , Yuan , L & Gao , R 2021 , ' Freezing of meat and aquatic food : Underlying mechanisms and implications on protein oxidation ' , Comprehensive Reviews in Food Science and Food Safety , vol. 20 , no. 6 , ARTN 1-22 , pp. 5548-5569 . https://doi.org/10.1111/1541-4337.12841

http://hdl.handle.net/10138/348351
https://doi.org/10.1111/1541-4337.12841

unspecified
acceptedVersion

Comprehensive REVIEWS in Food Science and Food Safety

Freezing of meat and aquatic food: Underlying mechanisms and implications on protein oxidation

Journal:	*Comprehensive Reviews in Food Science and Food Safety*
Manuscript ID	Draft
Manuscript Type:	Comprehensive Review
Date Submitted by the Author:	n/a
Complete List of Authors:	Bao, Yulong; Jiangsu University, School of Food and Biological Engineering Ertbjerg, Per; University of Helsinki, Food and Nutrition Estévez, Mario; Faculty of Veterinary (University of Extremadura), Food Technology Yuan, Li; Jiangsu University, School of food science and biological engineering Gao, Ruichang; Jiangsu University, School of food science and biological engineering
Keywords:	

School of Food and Biological Engineering
No.301 Xuefu Road, P.O. Box 212013
Zhenjiang, Jiangsu, China
Email: spxy@ujs.edu.cn
Tel (fax): +86 0511-88780201

March 27th 2021

Dear Editor

Please find the attached manuscript entitled: **Freezing of meat and aquatic food: Underlying mechanisms and implications on protein oxidation**. We would like to have this manuscript considered for publication in Comprehensive Reviews in Food Science and Food Safety.

Over the recent decades, protein oxidation in muscle foods has gained increasing research interests. Protein oxidation occurs during freezing/thawing and frozen storage of muscle foods, leading to irreversible physicochemical changes and impaired quality traits. The submitted review paper focus on key physicochemical factors in freezing/thawing and frozen storage of muscle foods, such as formation of ice crystals, freeze concentrating and macromolecular crowding effect, instability of proteins at the ice-water interface, freezer burn, lipid oxidation, etc. Possible relationships between these physicochemical factors and protein oxidation are thoroughly discussed. This review will shed light on the complicated mechanism of protein oxidation in frozen muscle foods.

We confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. All authors have approved the publication of this manuscript and, if accepted, it will not be published elsewhere including electronically in the same form, in English or in any other language, without the written consent of the copyright-holder.

We upload in separate files:
Manuscript
Tables 1 to 2
Figures 1 to 5

Yours sincerely

Ruichang Gao, Professor
School of Food and Biological Engineering
Jiangsu University

Freezing of meat and aquatic food: Underlying mechanisms and implications on protein oxidation

Yulong Bao[1], Per Ertbjerg[2], Mario Estévez[3], Li Yuan[1], Ruichang Gao[1]*

1. School of Food and Biological Engineering, Jiangsu University, 212013 Zhenjiang, China

2. Department of Food and Nutrition, University of Helsinki, 00014 Helsinki, Finland

3. Meat and Meat Products Research Institute, University of Extremadura, 10003 Cáceres, Spain

* Corresponding author: Dr. Ruichang Gao, School of Food and Biological Engineering, Jiangsu University, 212013 Zhenjiang, China

Email: 1000003432@ujs.edu.cn

Tel./fax: +86 0511-88780174

Abstract

Over the recent decades, protein oxidation in muscle foods has gained increasing research interests as it is known that protein oxidation can affect eating quality and nutritional value of meat and aquatic products. Protein oxidation occurs during freezing/thawing and frozen storage of muscle foods, leading to irreversible physico-chemical changes and impaired quality traits. Controlling oxidative damage to muscle foods during such technological processes requires a deeper understanding of the mechanisms of freezing-induced protein oxidation. This review focus on key physicochemical factors in freezing/thawing and frozen storage of muscle foods, such as formation of ice crystals, freeze concentrating and macromolecular crowding effect, instability of proteins at the ice-water interface, freezer burn, lipid oxidation, etc. Possible relationships between these physicochemical factors and protein oxidation are thoroughly discussed. In addition, the occurrence of protein oxidation, the impact on eating quality and nutrition, and controlling methods are also briefly reviewed. This review will shed light on the complicated mechanism of protein oxidation in frozen muscle foods.

Keywords: Protein oxidation, protein denaturation, ice-water interface, freezing potential, freezer burn, macromolecular crowding

1. Introduction

Meat and aquatic products are, in a global perspective, among the most important foods consumed. Muscle foods provide high-value animal proteins and a wide range of essential micronutrients to a large majority of people in the world. Muscle-based foods are nutritious and highly appreciated by their sensory properties. However, fresh meat and aquatic foods are perishable and freezing is widely used for preservation purposes. The effects of freezing and thawing on muscle foods have been extensively studied and many excellent reviews have been available on this topic over the years. Mazur (1970) thoroughly reviewed the major effects of freezing on biological systems, and emphasized that the properties of cell membrane were closely related to the responses of cells to freezing. Many of the theories/hypothesis discussed in that paper benefited the research area of meat freezing. In due course, Mackie (1993) reviewed the effects of freezing on muscle proteins and focused on similarities and differences between proteins from different animals. Possible mechanisms of protein denaturation in muscle during freezing and frozen storage were introduced. More recently, Leygonie et al. (2012) reviewed the effects of freezing and thawing on major quality parameters of meat, such as moisture loss, color, pH, protein denaturation, lipid oxidation, tenderness and microbial spoilage, while Nakazawa & Okazaki (2020) introduced various factors that influence quality of frozen seafoods. In recent years, advanced tools such as proteomics (Men et al., 2020; Shi et al., 2018) and molecular dynamic simulations (Zhang et al., 2018) have been used to provide further insights into the underlying biochemical processes occurred during frozen storage of muscle foods.

It is well known that textural properties of frozen foods will generally be compromised after thawing, and ice crystals play a key role. The biochemical components are much less affected by frozen storage though no foods could remain unmodified during long-term storage. Native proteins are just marginally stable as the thermodynamic stability of folded proteins is often 1 to 4 kcal/mol higher than that of the corresponding unfolded ones (Sanfelice & Temussi, 2016). Freezing leads to a series of physicochemical changes and therefore pronounced denaturation of muscle proteins are often observed after frozen storage. In addition to protein denaturation, proteins can be oxidized during frozen storage as well. Increased protein oxidation with frozen storage has been ascribed to catalytic iron release after membranes being disrupted, and freeze-concentrating of pro-oxidants and protein molecules (Estévez et al., 2011; Leygonie et al. 2012; Soladoye et al., 2015). Unlike protein denaturation, protein oxidation has been less studied in frozen meat, but it is receiving increased research interest. So far, the mechanisms of freezing-induced protein oxidation are not well-understood. Therefore, in this paper, we focus on the physiochemical changes occurring in frozen muscle foods and discuss their implications on protein oxidation.

2. Major physicochemical changes in frozen muscle foods

2.1 Physicochemical environment in muscle foods prior to freezing

Muscle foods are derived from animal flesh. Fundamental aspects such as chemical composition and structural organization of meat is well known. In brief, lean

meat is mainly composed of water and proteins and both pro-oxidant (myoglobin, lipids, etc.) and antioxidant components (reducing enzymes, glutathione, etc.) naturally occur in muscle tissue (Lawrie & Ledward, 2006). The final pH value of postmortem muscle is of critical importance to reactions in meat and thus affects the final quality of muscle foods. Ions can be bound to or tightly associated with proteins, or they can loosely associate to proteins or exist as free ions (Hamm, 1986). Like many other cellular tissues, muscle contains a very high concentration of proteins (~ 20%), along with other components, such as nucleic acids, lipids and sugars. They cumulatively occupy a large fraction of the total cell volume since macromolecules generally cannot interpenetrate and this is called macromolecular crowding. This crowding effect could affect many reactions, yet many biochemical studies of macromolecular properties are carried out in dilute solutions in which crowding do not occur (Minton, 2006). Much of the cellular water exists between a variety of fibrous and membranous structures. Between 10% and 100% of the fluid lies within one macromolecular diameter of the surface of these fibrous or membranous structures. As these structures do not move, this leads to a situation named as macromolecular confinement. Both crowding and confinement are expected to increase the stability of folded proteins. When reactants bear net charges opposite to that of the surface of nearby fibers or membranes, adsorption may occur and thus change the reaction kinetics. The abovementioned macromolecular crowding, confinement, and adsorption can greatly influence the equilibria and reaction rates.

2.2 Decrease in temperature

Temperature is one of the most important factors in determining the rates of chemical reactions (Boekel, 2008). Frozen storage of muscle foods usually takes place at temperatures ranging from -10 ℃ to -30 ℃, while storage of some precious muscle foods such as tuna often requires -60 ℃ or lower. Frozen temperatures from -20 to -30 ℃ are generally satisfactory in maintaining quality of muscle foods. The marginal gain in quality of storage at -60 ℃ may be small, but can be critical for demanding consumers (Ji et al., 2021). During freezing (and thawing), if heat removal (supply) is slow, muscle foods will stay at temperatures around -1 ℃ for quite long time. During frozen storage, the temperature fluctuations in the freezer will lead to fluctuations in the muscle foods as well, but to a lesser extent. A storage temperature well below the freezing point of muscle is expected to slow down chemical reactions, as the migration of molecules is largely suppressed in the solid phase. However, some reactions may be greatly enhanced by freezing or a lower storage temperature. For example, freezing was found to accelerate the reaction between nitrite and dissolved oxygen by a factor of 10^5 (Takenaka et al., 1992).

2.3 Formation of ice crystals

Upon freezing, the extracellular solution first crystallizes as it has a higher freezing point due to a lower content of solutes. The extracellular water in muscle has a freezing point around -1.2 ℃, while the intracellular water starts to freeze around -1.6 ℃. Once ice crystals form in the extracellular space, solutes get concentrated and therefore there will be osmotic pressure across the sarcolemma (i.e. cell membrane). To achieve a

130 balanced state again, cells can response in two ways: cellular dehydration or
131 intracellular ice formation (Fig. 1a). These subsequent events during cooling depend
132 mainly on the cooling rate and membrane permeability to water (Mazur, 1970). The
133 cooling rate can be assessed by the characteristic freezing time (t_c), and t_c is defined as
134 time needed for temperature to decrease from -1℃ (beginning of freezing) to -7℃
135 (freezing of 80% of the water present). Bevilacqua & Zaritzky (1980) found that
136 intracellular ice formation in beef only happened when t_c was less than 5 min when
137 freezing is transverse to the fibers. When freezing is longitudinal to the fibers,
138 intracellular ice crystals can advance in the direction of heat flow. Therefore,
139 intracellular ice can be found with a t_c up to 20 min in this situation. The longer the t_c,
140 the larger the size of the crystals formed, and larger extracellular ice crystals lead to
141 increased distortion of the muscle microstructure. As for membrane permeability, not
142 enough information is available on how the low temperature and high electrolyte
143 concentrations in frozen condition will affect the permeability. It has been suggested
144 that a high concentration of electrolytes makes the sarcolemma leaky. According to
145 Mazur (1970), the surface membranes of cells prevent extracellular ice from entering
146 cells at temperatures about -10 ℃ to -15 ℃. One possible explanation is based on the
147 assumption that the membranes contain water-filled pores. At temperatures of -10 ℃ to
148 -15 ℃, ice crystals are so large that they cannot pass through pores in the membranes.
149 Cooling may result in a transition of membranes from the liquid crystalline to gel phase
150 (Crowe et al., 1989), which may lead to leak of cell contents.

During storage, ice crystals grow larger at the expense of smaller ones (Fig. 1b), and increased ice crystals may lead to increased physical damage of the muscle structure. The driving force for the process of recrystallization is that small crystals have higher vapor pressure and water diffuse down the pressure gradient. Temperature fluctuation tend to accelerate the recrystallization process and hence need to be controlled during frozen storage. Recently, Nakazawa & Okazaki (2020) summarized factors influencing the quality of frozen seafood and they argued that the formation of ice itself may not be detrimental to muscle structure. In the case of low level of protein denaturation, thawing-induced free water is absorbed and the muscle cell can be largely recovered. If the denaturation level is high, then the released water is not absorbed and the muscle cell is not recovered (Fig. 1c). If the thawing rate is high, the melted water does not have enough time to be re-absorbed by the muscle fibers and part of it is eliminated as exudate.

As volume change occurs along with the formation of ice, muscle structure may be distorted by ice crystals. It is speculated that the rigor bond and myosin filament are among the sites of distortion (Fig. 2). It is known that myosin is prone to denaturation during frozen storage. And structure distortion of muscle by changing the sarcomere length was found to influence protein denaturation during heating (Findlay & Stanley, 1984). Therefore, freezing-induced distortion of muscle structure may affect protein stability. Mechanisms of ice formation and controlling methods of ice crystallization has been studied extensively. Regarding the freezing of food, modelling approaches of water crystallization, evaluation methods for the process and different novel methods

for improving water crystallization were presented in an excellent review by Kiani & Sun (2011). Antifreeze proteins (AFPs) can protect living organisms from freezing-induced damage via tuning ice nucleation, shaping ice crystals, and inhibiting ice growth and recrystallization (He et al., 2018).

2.4 Concentrating of solutes

During freezing, a large proportion of water turns into ice and thereby reduce the amount of available liquid water. At the same time, the remaining solutes (including soluble proteins, pro-oxidants, and anti-oxidants) get concentrated in the unfrozen fraction of water. Freezing has been found to accelerate oxidation of some small molecules, such as nitrite, sulfite, and ascorbic acid (Hambly & Gross, 2009). However, the extent of acceleration was much higher than what would be expected from the concentrating effect only. Takenaka et al. (1996) proposed a mechanism for the accelerated reaction between nitrous acid and oxygen (Fig. 3). With the formation of ice crystals, dissolved oxygen and nitrite are excluded from ice and concentrated at the interface of each ice crystal (Fig. 3A). As ice crystals continue to grow, concentrated solutions are confined in the unfrozen solution surrounded by walls of ice crystals (Fig. 3B). When crystals grow further, solutes become extremely high in the solutions (Fig. 3C) and therefore the reactions can be accelerated.

2.5 Ice-water interface

193 The presence of ice is critical for the instability of proteins subjected to freezing
194 treatment. For example, lactate dehydrogenase lost its activity in frozen state, however,
195 concentrated solutions at the same temperature and composition maintained its activity
196 (Bhatnagar et al., 2008). Both water and ice are composed of a tetrahedral network of
197 hydrogen bonds, and the structural difference are minimal. The ice-water interface has
198 been suggested as a major source of protein denaturation during freezing (Arsiccio &
199 Pisano, 2020). Interestingly, the nature of a growing ice surface can be different from a
200 melting ice surface (Takenaka et al., 1996). Possible mechanisms of the ice-water
201 interface-induced protein denaturation can be summarized as follows:

202 *2.5.1 Changes in microenvironment*

203 Generally, solutes cannot be incorporated into the crystal lattice of ice and the
204 expelled solutes thus get concentrated in a quasi-liquid layer. A quasi-liquid layer
205 represents an intermediate state between the solid ice crystal and bulk liquid.
206 Concentrated protons and ions would change the pH and ionic strength and likely to
207 affect protein stability (Arsiccio & Pisano, 2020) (Fig. 4a). Zhang & Ertbjerg (2019)
208 suggested that pH of pork meat decreased due to freeze-induced concentrating of
209 protons. Difference in freezing rate may resulted in different degree of proton
210 entrapment by ice crystals, and therefore the difference in pH values of the remaining
211 unfrozen liquid. It is well-known that pH is an important factor for protein stability.

212 *2.5.2 Interface adsorption*

As a biological material is frozen, formation of ice crystals leads to an increase in the concentration of all solutes, including dissolved air, which is mainly composed of nitrogen and oxygen. In addition, the solubility of oxygen increases at lower temperatures. The increase in oxygen concentration can be hypothesized to accelerate oxidation during freezing as follows. Once the effect of freeze concentrating on air exceeds its solubility, air bubbles are formed (Authelin et al., 2020). Air bubbles trapped between ice crystals and the hydrophobic air-water interface denature proteins (Fig. 4b). In addition to the adsorption to the air-water interface, proteins can be adsorbed directly to the ice surface and unfold (Fig. 4c), such as antifreeze proteins (He et al., 2018)

2.5.3 Mechanical stress

Volume expansion occurs during growth of ice crystals. Water-to-ice transformation leads to approximately a 9% increase in volume, and such expansion results in great shear stress and local pressure (Fig. 4d). It has been estimated that freeze-induced local pressure can reach 200 MPa (Authelin et al., 2020). It is known that high pressure treatment led to protein modifications in muscle foods, which mainly occur in the tertiary and quaternary structures (Cruz-Romero et al., 2004; Shi et al., 2020; Xue et al., 2018).

2.5.4 Cold denaturation

Cold denaturation of proteins is linked to specific and strongly temperature-dependent interaction of protein nonpolar groups with water. There are many nonpolar

groups in proteins and the hydrophobic forces contribute to stabilization of the native protein structure. At sufficiently low temperature, the interaction between nonpolar residues with water becomes less unfavorable (Fig. 4e). This may promote exposure of the hydrophobic core of the protein, and this phenomenon is called as cold unfolding or cold denaturation. For example, Arsiccio et al. (2020) found that protein L was destabilized in the presence of ice, and they explained that ice slowed down nearby water molecules, and theses water molecules could form a larger number of hydrogen bonds with the protein, thus promoting hydration of nonpolar patches and ultimately leading to exposure of the hydrophobic core.

2.6 pH change

As aforementioned, pH might change in the quasi-liquid layer under frozen conditions (section 2.5.1). One hypothetical explanation is related to the freezing potential. When a dilute electrolyte solution is frozen, an electric potential is generated between the ice and the solution. This freezing potential is also known as the Workman-Reynolds Effect (Workman & Reynolds, 1950). The sign and value of the freezing potential mainly depend on the kinds of solutes and the freezing rate, which lead to selective segregation of ions with opposite sign into ice phase and the quasi-liquid layer (Levi & Milman, 1966). In extensively frozen solutions, the quasi-liquid layer may act as a microscopic film wetting polycrystalline ice. Ions are marginally, but selectively, incorporated in ice as substitutional dopants during the freezing of electrolyte solutions.

For example, more Cl^- is incorporated into the ice than Na^+ during freezing of aqueous solution of NaCl, the charge of the ice will be negative; while in the case of NH_4Cl, more NH_4^+ is incorporated than Cl^- and therefore produces positive potential (Takenaka et al., 1996). The potential is then neutralized by highly mobile H_3O^+ or OH^-, therefore pH in the quasi-liquid layer is varied (Fig. 5). For solutions of 2-(N-morpholino) ethanesulfonic acid zwitterion, the acidity barely changes upon freezing. This is because the covalently linked cationic and anionic centers cannot be separated across the ice/solution interface (Robinson et al., 2006). Freezing potential may affect chemical reactions. In the reaction of nitrite with oxygen, addition of a stainless-steel wire depressed the oxidation rate, suggesting the acceleration of the reaction by freezing is an electrostatic effect rather than an electrochemical reaction (Takenaka et al., 1996). Those authors further investigated the electrostatic effect in the freezing process on the reaction. Different salts were added to the reaction mixture, and results showed that the formation of nitrate is affected by the sign of the freezing potential, rather than by the kind of ions.

Besides the above-mentioned mechanisms, pH change in frozen meat may be affected by other factors. At the generally used frozen storage temperature of -20 ℃, around 90% water are frozen in beef and haddock muscle (Mackie, 1993). If protons remained in the liquid water during slow freezing, 90% frozen water would theoretically cause a 10-fold increase in proton concentration which equals to a drop of 1 pH unit. Although the buffering capacity of muscle would counteract the increase of protons, freezing could plausibly lead to a lower pH in vicinity of the protein surfaces

277 of frozen meat. Zhang & Ertbjerg (2019) suggested that fast freezing entraps more
278 protons in ice crystals, therefore less proton will remain in solution which leads to a
279 higher pH. At certain temperatures, solutes may exceed their solubilities and precipitate.
280 Precipitation of solutes may shift pH during freezing (Pikal-Cleland et al., 2000).

281

282 **2.7 Occurrence of 'Freezer burn'**

283 'Freezer burn' describes the dehydration and associated changes in color, texture
284 and flavor that may occur on the surface of foods during frozen storage. The
285 dehydration is caused by sublimation of ice when the vapor pressure of ice at the surface
286 is greater that the vapor pressure of water in the air (Schmidt & Lee, 2010). A lower
287 storage temperature leads to lower vapor pressure, for example the vapor pressure of
288 ice is about 100 Pa at -20 ℃, but reduces to around 4 Pa at -50 ℃. Surface dehydration
289 leads to a local concentrating effect, where endogenous pro-oxidants and soluble
290 proteins get concentrated, and the oxidation process may be enhanced. Surface color of
291 frozen beef gradually change from red to brown due to the oxidation of myoglobin to
292 the brown colored metmyoglobin. Sublimation from the surface of frozen meat may
293 cause small air pockets to develop, forming a honeycomb structure. Modern self-
294 defrosting freezers may contribute to an increase in freezer burn. In a traditional manual
295 defrost freezer, accumulation of ice in the freezer compartment serves to increase the
296 vapor pressure of the air in the freezer, and thereby decreases the sublimation. However,

297 in self-defrost freezers, the removal of frost keeps the vapor pressure in the air at a low
298 level and therefore promotes sublimation from the food surface.

299 2.8 Lipid oxidation and hydrolysis

300 Lipids are chemically unstable food components and readily undergo oxidative
301 reactions. Intramuscular fat, especially the lipids in fish tissues, are of high unsaturation
302 level. Therefore, lipid oxidation progresses to a noticeable extent during long-term
303 frozen storage. According to Leygonie et al. (2012), lipid oxidation mainly occurs at
304 the cellular membranes, therefore, lipid oxidation was observed both in lean and fatty
305 meats. In addition to oxidation, lipids may undergo hydrolysis during frozen storage
306 due to enzymatic hydrolysis, and free fatty acids will create more hydrophobic regions
307 in protein and promote denaturation (Mackie, 1993).

308

309 3. Protein oxidation in frozen muscle foods

310 3.1 Protein oxidation: chemistry and consequences

311 Meat, being a protein-rich food and containing pro-oxidants, such as lipids and
312 myoglobin, is susceptible to oxidation. One pathway of protein oxidation is via the
313 abstraction of a hydrogen atom in the protein by reactive oxygen species (ROS), which
314 is a collective term that includes oxygen-containing radicals ($O_2^{\bullet-}$, $^{\bullet}OH$, $RO^{\bullet}$, $RO_2^{\bullet}$…)
315 and also some non-radical derivatives of oxygen (H_2O_2, HClO, O_3…) (Bao & Ertbjerg,
316 2019). In addition, reactive nitrogen species may also induce oxidative stress in muscle
317 proteins (Skibsted, 2011). The detailed aspects of the chemistry of protein oxidation are

318 out of the scope of this paper, and they have been covered in a number of previous
319 review papers (Hawkins & Davies, 2001; Hellwig, 2019, 2020). Oxidative conditions
320 readily occur in post-mortem muscle and protein oxidation products may accumulate
321 in food during processing, storage and subsequent food intake (Estévez & Luna, 2017).
322 Oxidative modifications of muscle proteins include carbonylation, depletion of thiols
323 and tryptophan, formation of crosslinks, etc. (Estévez, 2011; Lund et al., 2011). Various
324 methods can be applied to the quantification of protein oxidation in biological systems.
325 Those methods are generally based on monitoring changes in parent amino acid
326 residues (Cys, Met, His, Lys etc.), detection of radical and non-radical intermediates,
327 or formation of products (e.g. formation of carbonyls, protein cross-linking).

328 Physicochemical properties of proteins are greatly affected by mobile electrolyte
329 ions and by ionized amino acid residues attached to the backbones of protein molecules.
330 Oxidative modifications of those ionized amino acids are expected to affect the protein
331 functionality in muscle food. Histidine, for example, mainly exits as a positively
332 charged amino acid when pH is lower than its pKa (~ 6.5). And it has been suggested
333 to be oxidized into 2-oxo-histidine, which carries no charges. This loss of charge will
334 subsequently affect protein net charges and ultimately the filament net charges (Bao et
335 al., 2018). The oxidation-induced modification of protein net charges offered a novel
336 perspective in understanding oxidation-induced functionalities change of muscle
337 proteins. Protein oxidation also affects eating quality of muscle foods, such as color,
338 water-holding and texture. Many reviews have covered those topics in great depth (Bao

& Ertbjerg, 2019; Estévez, 2011; Hematyar et al., 2019; Lund et al., 2011; Zhang et al., 2013). In general, protein oxidation has a negative impact on the eating quality of meat.

Relationship between dietary protein oxidation and human health has received great interest. As pointed out by Hellwig (2019), several aspects need to be considered on the relation between protein oxidation and human health. Those aspects include the loss of essential amino acids, the influence on protein digestibility, the formation of potentially toxic compounds and possible transfer of oxidative damage to body proteins. Oxidative modification of essential amino acids limits their bioavailability. The mechanism by which oxidation modulates digestibility is complex. Mild oxidation of many proteins increases their susceptibility to proteolysis. When oxidation proceeds to a certain degree, excessive protein polymerization and aggregation occur, which subsequently impairs protein digestibility (Li et al., 2017; Santé-Lhoutellier et al., 2007; Santé-Lhoutellier et al., 2008). Other than lowered nutritional value, protein oxidation may be potentially toxic as the gastrointestinal tract and internal organs are exposed to oxidation products. Oxidatively modified amino acids and peptides which resist digestion may enter the large intestine and be utilized by microbiota and turn into mutagenic compounds such as biogenic amines, ammonia, cresol and indole (Hellwig, 2019). Some pathologies are accompanied by the presence of oxidized amino acids at levels beyond the physiological situation, indicating a toxic effect of oxidized proteins. Oxidative damage may transfer from proteins to other targets such as DNA and lead to malfunction of gene expression. Furthermore, there is a strong link between protein oxidation and aging (Davies & Dean, 1997)

3.2 Possible links between physicochemical changes and protein oxidation in frozen muscle foods

As discussed in section 2, freezing leads to a series of physicochemical changes in muscle foods, including temperature drop, formation of ice, concentrating of solutes, alteration of ionic strength and pH, freezer burn, lipid oxidation, protein denaturation, etc. These physicochemical changes may have significant impacts on protein oxidation.

Environmental factors such as pH and ionic strength are expected to affect many chemical reactions, including protein oxidation. Bertram et al. (2007) investigated the oxidation of myofibrillar proteins by hemoglobin and H_2O_2 under different combinations of pH and ionic strength, the results showed that pH had a great impact on the formation di-tyrosine, a general maker of protein oxidation. The formation of di-tyrosine was much greater in pH 5.4, as compared to pH 6.2 or pH 7.0, and di-tyrosine appeared to increase with increased ionic strength. Several possible explanations were proposed for the pH-dependent formation of di-tyrosine including i) The activation and pro-oxidant actions of hemoglobin is facilitated at acidic pH ii) lower pH induces denaturation of hemoglobin, leading to iron release which, in turn, initiates a Fenton reaction and iii) elevated pH leads to longer distances between tyrosine residues and limits the effective cross-linking reaction. Other than directly affecting protein oxidation, changes in pH and ionic strength may lead to protein denaturation (Zhang et al., 2021). Denatured proteins may expose the previously buried oxidation-susceptible amino acid residues, and thereby enhance protein oxidation. Other factors, such as the formation of ice-water interface, distortion of myofibrillar proteins caused by ice

crystals, and generation of free fatty acids, all lead protein denaturation in frozen meat. Therefore, those factors may promote protein oxidation as well. In the study of Zhang et al. (2021), they used different combinations of pH and ionic strength to mimic the condition of frozen-thawed meat. Results showed that both a lower pH and a higher concentration of KCl increased the surface hydrophobicity of myofibrillar proteins, which indicated a greater protein denaturation. It should be noted that both Bertram et al. (2007) and Zhang et al. (2021) used model systems, where extracted myofibrillar proteins were incubated with solutions containing different concentrations of salt and protons. The reactions in the meat matrix can differ significantly, due to reasons like macromolecular crowding, confinement or adsorption as discussed in section 2.1.

A portion of water remains as liquid in frozen muscle foods and it is concentrated with solutes (most of them prooxidants). Such unfrozen water is normally surrounding muscle proteins and therefore molecular crowing takes place during frozen storage may promote the contact between pro-oxidants and susceptible protein molecules, making of frozen meat a pro-oxidative environment despite of the reduced movement of molecules due to low temperatures. For example, freezing-induced dehydration caused close approach of the protoplasmic proteins in leaves, which promoted the formation of disulfide bonds (Levitt, 1962), a general marker of protein oxidation. Oxygen exposure is obviously one of the important factors for the development of protein oxidation. Therefore, membrane rupture caused by ice crystals can accelerate oxidation via allowing more diffusion of oxygen into close contact with cell interior. In the case of severe freezer burn, sublimation-induced honeycomb structures in the surface of

meat will greatly facilitate the penetration of oxygen into a deeper position, and thereby accelerate oxygen-induced protein oxidation.

Hambly & Gross (2009) aimed to address the question of whether protein oxidation occurs primarily when its solution is frozen or during freezing, and they found that freezing of apomyoglobin in peroxide solution led to protein oxidation. Furthermore, the oxidation was dependent on incubation temperature and time, but not as a result of freeze or thaw. After 2 hour, protein oxidation is less at 4 ℃ or 22 ℃, as compared to freezing at -15 ℃ or -80 ℃. At temperatures above the freezing point, H_2O_2 molecules are moving in three dimensions and they are constantly moving away from the protein. Therefore, the oxidation reaction proceeds slowly. In contrast, when the solution is frozen, the few H_2O_2 molecules are trapped and cannot diffuse away from the protein. Oxidant diffuses over some portion of the protein surface in the solid state and oxidize the most reactive amino acid residues. Buried residues are also oxidized suggesting electron transfer between the solvent-exposed residues and the interior of the protein in the solid state.

Theoretically, oxidative reactions could be transferred between lipids and proteins. This was supported by Burcham, & Kuhan (1996) who showed that incubation with MDA introduced carbonyl groups into BSA. In agreement, Zhang et al. (2011) found that consumption of oxidized oil was related to higher protein carbonyl content in breast meat of broiler chickens. There were also evidences of lipid-induced protein oxidation in frozen meat. For example, Utrera et al. (2014a) observed more intense protein oxidation in frozen beef patties with higher fat content. Many studies found that protein

427 oxidation was accompanied with lipid oxidation in frozen muscle foods. However,
428 other than lipid oxidation, the relationships between physicochemical factors (muscle
429 structure distortion, macromolecular crowding, generation of freezing potential, altered
430 pH, etc.) and protein oxidation in frozen muscle foods require further deep
431 investigations.

432

433 **3.3 Factors affecting protein oxidation in frozen muscle foods**

434 Protein oxidation occurs readily in frozen muscle foods. Studies on protein
435 oxidation in frozen muscle foods are summarized in Table 1 (livestock meat) and in
436 Table 2 (aquatic products) with a focus on recent progress. Various factors may affect
437 protein oxidation in frozen muscle foods as outlined below.

438 3.3.1 *Types of muscle foods*

439 Major categories of muscle foods include livestock animals and aquatic animals.
440 Chemical composition of muscles from mammals, domestic birds and aquatic animals
441 can be greatly influenced by type of animal, feeding, physiological status, slaughter
442 process, muscle cuts, etc. (Lawrie & Ledward, 2006). Such factors are expected to
443 influence protein oxidation as they determine the concentration of pro- (heme iron,
444 unsaturated lipids, etc.) and antioxidant (tocopherols, carotenoids, etc.) components in
445 muscle tissue. For example, Benjakul et al. (2003) compared the stability of muscle
446 proteins from some tropical fish during frozen storage, and they found that sulfhydryl
447 content in lizardfish decreased quickly and was lower compared to other species studied.

448 This was attributed to a higher level of formaldehyde formed in lizardfish. Estévez et
449 al. (2011) found a significant effect of muscle type on the formation of specific
450 carbonyls (α-aminoadipic and γ-glutamic semialdehydes) in pork subjected to frozen
451 storage. And the different susceptibility of muscle cuts to oxidative reactions during
452 frozen storage was ascribed to the variations between muscle fibers (oxidative vs.
453 glycolytic), which largely determines the overall chemical composition of the muscles.
454 Utrera et al. (2014b) observed the oxidative stability of patties made from different
455 muscle cuts, and the heme-iron content, antioxidant enzyme activity and PUFA content
456 were suggested to play a major role. Myofibrillar proteins are susceptible to oxidation
457 with myosin being the most sensitive (Lund et al., 2011). Liu et al. (2011) compared
458 the stability of fish actomyosin and pork actomyosin, and they found that pork
459 actomyosin had higher stability (less exposed thiols and hydrophobic groups) during
460 cold storage. Differences in physicochemical properties of muscle proteins and the
461 antioxidative defense systems of the muscle are likely to affect the process of oxidation
462 during frozen storage.

463

464 3.3.2 *Freezing rate, and frozen storage and thawing conditions*

465 In general, longer storage time leads to increased protein oxidation in frozen
466 muscle foods. However, the carbonyl content of amino acid side chains, a generally
467 used marker of protein oxidation, may decrease with prolonged storage (Estévez et al.,
468 2011; Holman et al., 2018). The decrease may be caused by cross-linking between

469 protein semialdehydes and other amine-containing amino acid residues, as suggested
470 by Estévez et al. (2011). As for storage temperature, Baron et al. (2007) found that
471 carbonyls of rainbow trout fillets increased with storage time at -20 ℃, but not at -30
472 or -80 ℃, indicating that temperatures lower than -30 ℃ can inhibit protein oxidation.
473 To similar conclusions came Utrera et al. (2014), who reported that frozen storage had
474 a remarkable impact on specific oxidative changes in meat proteins (carbonylation, and
475 tryptophan depletion) and such modifications were temperature-dependent. According
476 to these authors, the protein oxidation occurred during frozen storage at -8ºC and -18ºC
477 contributed to impaired quality traits in patties cooked upon thawing. Similarly, Li et
478 al. (2020a) reported that pork patties stored at -18 or -25 ℃ had lower content of
479 carbonyls than at -8 ℃, but no difference was found between -18 and -25 ℃. Qian et al.
480 (2021) investigated protein oxidation in beef stored at temperatures ranged from -1 ℃
481 to -18 ℃, and they found that myofibrillar protein oxidation decreased when the
482 temperature decreased from -1 to -12 ℃, but there was no clear difference between -12
483 ℃ and -18 ℃. Therefore, it appears that protein oxidation in frozen muscle foods can
484 be inhibited when the storage temperature is lower than a critical point. However, this
485 critical temperature varies between studies, and the mechanism remains to be
486 investigated. Szymczak et al. (2020) compared the effect of constant and fluctuating
487 temperatures during frozen storage on quality of herrings, and they found that herrings
488 stored at constant temperatures had lower peroxide values, suggesting constant storage
489 temperature may be beneficial in maintaining the oxidative stability.

Other than frozen storage, the freezing and thawing process may also affect protein oxidation in muscle foods. Increased freeze-thaw cycles have been demonstrated to increase protein oxidation in a variety of muscle foods (Pan et al., 2021; Shao et al., 2018; Zheng et al., 2020). Kim et al. (2018) found that the freezing rate had an impact on the oxidative stability of muscle proteins, however, the aging combination had overriding impacts over freezing rate. Hou et al. (2020) observed no difference in total and free sulfhydryl groups of pork between immersion solution freezing and air blast freezing, although they found that immersion solution freezing reduced lipid oxidation. Different thawing methods have been studied in meat and meat products, including conventional thawing by water or air, or combined with microwave, ultrasound, electrostatic field, etc. Cai et al. (2020b) compared effects of different thawing methods on the myofibrillar protein oxidation in large-mouth bass. The results showed that microwave thawing in combination with either vacuum or magnetic nanoparticles resulted in a lower degree of protein oxidation as compared to microwave thawing alone. Combination of far-infrared thawing with magnetic nanoparticles was found to be the most effective methods in controlling protein oxidation. Protein oxidation did not receive much research attention during the freezing and thawing of meat, but existing literature clearly show that the freezing and thawing processes have significant impact on protein oxidation.

3.3.3 *Processing of the muscle foods*

Processed meat constitutes a large percentage of muscle foods. Various processing technologies affect the physicochemical properties of meat and they potentially affect protein oxidation. Soladoye et al. (2015) thoroughly reviewed the impacts of processing on protein oxidation in meat and meat products. It is worth to point out that frozen storage may increase the susceptibility towards oxidation during successive technological processes, and processing frozen/thawed meat compared to unfrozen one has been reported to influence protein oxidation and quality traits in dry-cured and cooked meat products (Utrera et al., 2012; Utrera et al., 2015; Lorido et al., 2016). Some processing technology can be applied to meats in combination with freezing. The impacts of processing on protein oxidation in frozen muscle foods, with a focus on recent studies, are briefly summarized as below.

Packaging is widely used for meat and meat products and oxygen in the package is expected to induce protein oxidation. Vacuum packaging was found to inhibit the formation of protein carbonyls in frozen pork as compared to oxygen permeable bags (Estévez et al., 2011). Similarly, it was observed that both light exposure and increased oxygen concentration in the package could significantly accelerate protein oxidation in the frozen obscure pufferfish (Zheng et al., 2021). Non-thermal processing technologies can be applied in muscle foods to extend shelf life. It has been reported that treatment with high pressure (Prego et al., 2021), pulsed electric field (Kantono et al., 2021; Li et al., 2020b), and irradiation (Arshad et al., 2020) promoted lipid oxidation in frozen muscle foods. Since protein oxidation and lipid oxidation are closely related, it is expected that these non-thermal processes can enhance protein oxidation as well. For

example, Cropotova et al. (2019) found that high-pressure treated haddock and mackerel minces had higher content of carbonyls in sarcoplasmic and myofibrillar proteins. The relationship between lipid oxidation and protein oxidation in high pressure treated meat has been thoroughly discussed by Guyon et al. (2016). During the production of meat products, non-meat ingredients may be added. It has been shown that the incorporation of plant extracts (Botsoglou et al., 2014; Huang et al., 2019; Ozen & Soyer, 2018; Turgut et al., 2017; Wang et al., 2017), hydrolysates from surimi process byproducts (Zhang et al., 2020), and antifreeze protein (Nian et al., 2020; Wang et al., 2021) inhibited protein oxidation in frozen muscle foods.

3.4 Control of protein oxidation in frozen muscle foods

Inhibition of protein oxidation in muscle foods can be achieved through animal feeding or processing (Estévez, 2011; Lund et al., 2011). Feeding can modify the fatty acid composition of muscle tissue and also deposit anti-oxidants such as vitamin E in muscle tissue, and thereby regulate the oxidative stability of raw meat. Liu et al. (2018) investigated the effects of saturation level of added lipids on protein oxidation in minced pork, and the results showed that less saturated group had higher oxidative stability in relation to both lipid and protein oxidation. This is in contrast to general belief and may be caused by the higher amount of antioxidant (vitamin E) in less saturated lipid fractions obtained from pork back fat. Incorporation of antioxidants into muscle foods is an effective way of controlling protein oxidation. There is a growing

554 interest in using natural antioxidants in meats (for generally used natural antioxidants
555 in meat and products, see Ribeiro et al., 2019). In frozen muscle foods, it has been
556 demonstrated that extracts of olive leaf (Botsoglou et al., 2014), dog rose (Utrera et al.,
557 2015), pomegranate peel (Turgut et al., 2017), green tea, grape seed, and pomegranate
558 rind (Ozen & Soyer, 2018), clove and rosemary (Huang et al., 2019), nutmeg (Zhu et
559 al. 2020) were effective antioxidants towards protein oxidation.

560 Freezing, storage and thawing are known to affect protein oxidation, optimization
561 of these processes may bring benefits. For examples, Hou et al. (2020) found that
562 immersion solution freezing produced smaller ice crystals in pork, and reduced lipid
563 oxidation was observed as compared to air blast freezing. The addition of ice structuring
564 protein (Wang et al., 2021) or herring antifreeze protein (Nian et al., 2020) were found
565 to inhibit protein oxidation in frozen meats. As we discussed in this paper, a series of
566 physicochemical factors may affect protein oxidation throughout the whole process of
567 frozen muscle foods. A better understanding of the possible mechanism of frozen-
568 induced protein oxidation would allow to achieve a better control of protein oxidation,
569 and ultimately improve the quality of frozen muscle foods.

570

571 **4. Concluding remarks**

572 The present review thoroughly discussed key physicochemical changes in frozen
573 muscle foods, including the formation of ice crystals and the ice-water interface,
574 concentrating of proteins and pro-oxidants, altered pH and ionic strength, freezer burn,

575 etc. These changes may contribute to the occurrence of protein oxidation in frozen
576 muscle foods. Of these key physicochemical changes in frozen muscle foods, the ice-
577 water interface is believed to be a main source of protein instability. Based on the
578 assumption that protein denaturation would expose previously buried residues which
579 are susceptible to oxidation, we suggest that freezing-induced oxidation may be
580 mediated by protein denaturation in frozen muscle foods. In muscle foods, protein
581 oxidation can proceed to a significant extent, which often negatively affects the eating
582 quality and nutritional value. A deeper understanding of the mechanism for freezing-
583 induced protein oxidation may lead to a better control of oxidative damage to muscle
584 foods.

585

586 **Acknowledgements**

587 Yulong Bao would like to acknowledge the financial support from the National
588 Natural Science Foundation of China (31901758) and the Natural Science Foundation
589 of Jiangsu Province (BK20190591). Mario Estévez would like to acknowledge the
590 financial support from the Spanish Ministry of Economy and Competitiveness through
591 the project AGL2017-84586R.

592

593 **Conflicts of Interest**

594 The authors confirm that there are no conflicts of interest to declare.

595

596 **References**

597 Ali, S., Zhang, W., Rajput, N., Khan, M. A., Li, C., & Zhou, G. (2015). Effect of
598 multiple freeze–thaw cycles on the quality of chicken breast meat. *Food*
599 *Chemistry, 173*, 808-814. https://doi.org/10.1016/j.foodchem.2014.09.095

600 Arshad, M. S., Kwon, J.-H., Ahmad, R. S., Ameer, K., Ahmad, S., & Jo, Y. (2020).
601 Influence of E-beam irradiation on microbiological and physicochemical
602 properties and fatty acid profile of frozen duck meat. *Food Science & Nutrition,*
603 *8*, 1020-1029. https://doi.org/10.1002/fsn3.1386

604 Arsiccio, A., & Pisano, R. (2020). The ice-water interface and protein stability: A
605 review. *Journal of Pharmaceutical Sciences, 109*, 2116-2130.
606 https://doi.org/10.1016/j.xphs.2020.03.022

607 Arsiccio, A., Mccarty, J., Pisano, R., & Shea, J. E. (2020). Heightened cold-
608 denaturation of proteins at the ice-water interface. *Journal of the American*
609 *Chemical Society, 142,* 5722-5730. https://doi.org/10.1021/jacs.9b13454

610 Authelin, J. R., Rodrigues, M. A., Tchessalov, S., Singh, S. K., Mccoy, T., Wang, S.,
611 & Shalaev, E. (2020). Freezing of biologicals revisited: scale, stability,
612 excipients, and degradation stresses. *Journal of Pharmaceutical Sciences, 109*,
613 44-61. https://doi.org/10.1016/j.xphs.2019.10.062

614 Bao, Y., & Ertbjerg, P. (2019). Effects of protein oxidation on the texture and water-
615 holding of meat: A review. *Critical Reviews in Food Science and Nutrition, 59*,
616 3564-3578. https://doi.org/10.1080/10408398.2018.1498444

617 Bao, Y., Boeren, S., & Ertbjerg, P. (2018). Myofibrillar protein oxidation affects
618 filament charges, aggregation and water-holding. *Meat Science, 135*, 102-108.
619 https://doi.org/10.1016/j.meatsci.2017.09.011

620 Baron, C. P., Kjaersgard, I. V. H., Jessen, F., & Jacobsen, C. (2007). Protein and lipid
621 oxidation during frozen storage of rainbow trout (Oncorhynchus mykiss).
622 *Journal of Agricultural and Food Chemistry, 55*, 8118-8125.
623 https://doi.org/10.1021/jf070686f

624 Benjakul, S., Visessanguan, W., Thongkaew, C., & Tanaka, M. (2003). Comparative
625 study on physicochemical changes of muscle proteins from some tropical fish
626 during frozen storage. *Food Research International*, *36*, 787-795.
627 https://doi.org/10.1016/S0963-9969(03)00073-5

628 Bertram, H. C., Kristensen, M., Østdal, H., Baron, C. P., Young, J. F., & Andersen, H.
629 J. (2007). Does oxidation affect the water functionality of myofibrillar proteins?
630 *Journal of Agricultural and Food Chemistry, 55*, 2342-2348.
631 https://doi.org/10.1021/jf0625353

Bevilacqua, A. E., & Zaritzky, N. E. (1980). Ice morphology in frozen beef. *Journal of Food Science & Technology*. 15, 589–597. https://doi.org/10.1111/j.1365-2621.1980.tb00978.x

Bhatnagar, B. S., Pikal, M. J., & Bogner, R. H. (2008). Study of the individual contributions of ice formation and freeze-concentration on isothermal stability of lactate dehydrogenase during freezing. *Journal of Pharmaceutical Sciences, 97*, 798-814. https://doi.org/10.1002/jps.21017

Boekel, M. A. J. S. van. (2008). *Kinetic modeling of reactions in Foods*. Boca Raton: CRC Press.

Botsoglou, E., Govaris, A., Arnbrosiadis, I., Fletouris, O., & Botsoglou, N. (2014). Effect of olive leaf (*Olea europea L.*) extracts on protein and lipid oxidation of long-term frozen n-3 fatty acids-enriched pork patties. *Meat Science, 98*, 150-157. https://doi.org/10.1016/j.meatsci.2014.05.015

Burcham, P. C., & Kuhan, Y. T. (1996). Introduction of carbonyl groups into proteins by the lipid peroxidation product, malondialdehyde. *Biochemical and Biophysical Research Communications, 220*, 996-1001. https://doi.org/10.1006/bbrc.1996.0521

Cai, L., Nian, L., Cao, A., Zhang, Y., & Li, X. (2020a). Effect of carboxymethyl chitosan magnetic nanoparticles plus herring antifreeze protein on conformation and oxidation of myofibrillar protein from red sea bream (*Pagrosomus major*) after freeze-thaw treatment. *Food and Bioprocess Technology, 13*, 355-366. https://doi.org/10.1007/s11947-019-02384-x

Cai, L., Wan, J., Li, X., & Li, J. (2020b). Effects of different thawing methods on conformation and oxidation of myofibrillar protein from largemouth bass (*Micropterus salmoides*). *Journal of Food Science, 85*, 2470-2480. https://doi.org/10.1111/1750-3841.15336

Coombs, C. E. O., Holman, B. W. B., Collins, D., Kerr, M. J., Friend, M. A., & Hopkins, D. L. (2018). Effects of chilled-then-frozen storage (up to 52 weeks) on an indicator of protein oxidation and indices of protein degradation in lamb M. longissimus lumborurn. *Meat Science, 135*, 134-141. https://doi.org/10.1016/j.meatsci.2017.09.013

Cropotova, J., Mozuraityte, R., Standal, I. B., Grovlen, M. S., & Rustad, T. (2019). Superchilled, chilled and frozen storage of Atlantic mackerel (Scomber scombrus) fillets - changes in texture, drip loss, protein solubility and oxidation. *International Journal of Food Science and Technology, 54*, 2228-2235. https://doi.org/10.1111/ijfs.14136

Cropotova, J., Mozuraityte, R., Standal, I. B., Ojha, S., Rustad, T., & Tiwari, B. (2020). Influence of high-pressure processing on quality attributes of haddock and mackerel minces during frozen storage, and fishcakes prepared thereof.

Innovative Food Science & Emerging Technologies, 59, 102236. https://doi.org/10.1016/j.ifset.2019.102236

Crowe, J. H., Hoekstra, F. A., Crowe, L. M., Anchordoguy, T. J., & Drobnis, E. (1989). Lipid phase transitions measured in intact cells with Fourier transform infrared spectroscopy. *Cryobiology, 26*, 76-84. https://doi.org/10.1016/0011-2240(89)90035-7

Cruz-Romero, M., Smiddy, M., Hill, C., Kerry, J. P., & Kelly, A. L. (2004). Effects of high pressure treatment on physicochemical characteristics of fresh oysters (*Crassostrea gigas*). *Innovative Food Science & Emerging Technologies, 5*, 161-169. https://doi.org/10.1016/j.ifset.2004.01.002

Guyon, C., Meynier, A., & De Lamballerie, M. (2016). Protein and lipid oxidation in meat: A review with emphasis on high-pressure treatments. *Trends in Food Science &Technology, 50*, 131-143. https://doi.org/10.1016/j.tifs.2016.01.026

Davies, M. J., & Dean, R. T. (1997). Radical-mediated protein oxidation: from chemistry to medicine: Oxford University Press.

Estévez, M. (2011). Protein carbonyls in meat systems: A review. *Meat Science, 89*, 259-279. https://doi.org/10.1016/j.meatsci.2011.04.025

Estévez, M., & Luna, C. (2017). Dietary protein oxidation: A silent threat to human health? *Critical Reviews in Food Science and Nutrition, 57*, 3781-3793. https://doi.org/10.1080/10408398.2016.1165182

Estévez, M., Ventanas, S., Heinonen, M., & Puolanne, E. (2011). Protein carbonylation and water-holding capacity of pork subjected to frozen storage: Effect of muscle type, premincing, and packaging. *Journal of Agricultural and Food Chemistry, 59*, 5435-5443. https://doi.org/10.1021/jf104995j

Findlay, C.J. And Stanley, D.W. (1984), Differential scanning calorimetry of beef muscle: Influence of sarcomere length. *Journal of Food Science, 49*, 1529-1531. https://doi.org/10.1111/j.1365-2621.1984.tb12837.x

Hambly, D. M., & Gross, M. L. (2009). Cold chemical oxidation of proteins. *Analytical Chemistry, 81*, 7235-7242. https://doi.org/10.1021/ac900855f

Hamm, R. (1986). Functional properties of the myofibrillar system and their measurements. In P. J. Bechtel (Ed.). Muscle as food. Food Science and Technology (pp. 135-191). London: Academic Press Inc.

Hawkins, C. L., & Davies, M. J. (2001). Generation and propagation of radical reactions on proteins. *Biochimica et Biophysica Acta (BBA) - Bioenergetics, 1504*, 196-219. https://doi.org/10.1016/S0005-2728(00)00252-8

He, Z., Liu, K., & Wang, J. (2018). Bioinspired materials for controlling ice nucleation, growth, and recrystallization. *Accounts of Chemical Research, 51*, 1082-1091. https://doi.org/10.1021/acs.accounts.7b00528

Hellwig, M. (2019). The chemistry of protein oxidation in food. *Angewandte Chemie International Edition, 58*, 16742-16763. https://doi.org/10.1002/anie.201814144

Hellwig, M. (2020). Analysis of Protein Oxidation in Food and Feed Products. *Journal of Agricultural and Food Chemistry, 6*, 12870-12885. https://doi.org/10.1021/acs.jafc.0c00711

Hematyar, N., Rustad, T., Sampels, S., & Kastrup Dalsgaard, T. (2019). Relationship between lipid and protein oxidation in fish. *Aquaculture Research, 50*, 1393-1403. https://doi.org/10.1111/are.14012

Holman, B. W. B., Coombs, C. E. O., Morris, S., Kerr, M. J., & Hopkins, D. L. (2018). Effect of long term chilled (up to 5 weeks) then frozen (up to 12 months) storage at two different sub-zero holding temperatures on beef: 3. Protein structure degradation and a marker of protein oxidation. *Meat Science, 139*, 171-178. https://doi.org/10.1016/j.meatsci.2018.01.028

Hou, Q., Cheng, Y., Kang, D., Zhang, W., & Zhou, G. (2020). Quality changes of pork during frozen storage: comparison of immersion solution freezing and air blast freezing. *International Journal of Food Science & Technology, 55*, 109-118. https://doi.org/10.1111/ijfs.14257

Huang, L., Ding, B., Zhang, H., Kong, B., & Xiong, Y. L. (2019). Textural and sensorial quality protection in frozen dumplings through the inhibition of lipid and protein oxidation with clove and rosemary extracts. *Journal of the Science of Food and Agriculture, 99*, 4739-4747. https://doi.org/10.1002/jsfa.9716

Huang, L., Xiong, Y. L., Kong, B., Huang, X., & Li, J. (2013). Influence of storage temperature and duration on lipid and protein oxidation and flavour changes in frozen pork dumpling filler. *Meat Science, 95*, 295-301. https://doi.org/10.1016/j.meatsci.2013.04.034

Ji, W., Bao, Y., Wang, K., Yin, L. and Zhou, P. (2021), Protein changes in shrimp (Metapenaeus ensis) frozen stored at different temperatures and the relation to water-holding capacity. *International Journal of Food Science & Technology*. IJFS15009. https://doi.org/10.1111/ijfs.15009

Kantono, K., Hamid, N., Ma, Q., Oey, I., & Farouk, M. (2021). Changes in the physicochemical properties of chilled and frozen-thawed lamb cuts subjected to pulsed electric field processing. *Food Research International, 141*, 110092. https://doi.org/10.1016/j.foodres.2020.110092

Kiani, H., & Sun, D. W. (2011). Water crystallization and its importance to freezing of foods: A review. *Trends in Food Science & Technology, 22*, 407-426. https://doi.org/10.1016/j.tifs.2011.04.011

Kim, H.-W., Kim, J.-H., Seo, J.-K., Setyabrata, D., & Kim, Y. H. B. (2018). Effects of aging/freezing sequence and freezing rate on meat quality and oxidative

stability of pork loins. *Meat Science, 139*, 162-170. https://doi.org/10.1016/j.meatsci.2018.01.024

Kjaersgard, I. V. H., Norrelykke, M. R., Baron, C. P., & Jessen, F. (2006). Identification of carbonylated protein in frozen rainbow trout (*Oncorhynchus mykiss*) fillets and development of protein oxidation during frozen storage. *Journal of Agricultural and Food Chemistry, 54*, 9437-9446. https://doi.org/10.1021/jf061885m

Larsson, K. J., & Undeland, I. K. (2010). Effect of caffeic acid on haemoglobin-mediated lipid and protein oxidation in washed cod mince during ice and frozen storage. *Journal of the Science of Food and Agriculture, 90*, 2531-2540. https://doi.org/10.1002/jsfa.4121

Lawrie, R.A., & Ledward, D. A. (2006). *Lawrie's Meat Science (Seventh Edition)*. Cambridge: Woodhead Publishing Limited

Levi, L., & Milman, O. (1966). Freezing Potential of Electrolytic Solutions. *Journal of the Atmospheric Sciences, 23*, 182-186. https://doi.org/10.1175/1520-0469(1966)023<0182:FPOES>2.0.CO;2

Levitt, J. (1962). A sulfhydryl-disulfide hypothesis of frost injury and resistance in plants. *Journal of Theoretical Biology, 3*, 355-391. https://doi.org/10.1016/S0022-5193(62)80032-0

Leygonie, C., Britz, T. J., & Hoffman, L. C. (2012). Impact of freezing and thawing on the quality of meat: Review. *Meat Science, 91*, 93-98. https://doi.org/10.1016/j.meatsci.2012.01.013

Li, F., Zhong, Q., Kong, B., Wang, B., Pan, N., & Xia, X. (2020a). Deterioration in quality of quick-frozen pork patties induced by changes in protein structure and lipid and protein oxidation during frozen storage. *Food Research International, 133*. Article 109142. https://doi.org/10.1016/j.foodres.2020.109142

Li, J., Shi, J., Huang, X., Zou, X., Li, Z., Zhang, D., . . . Xu, Y. (2020b). Effects of pulsed electric field on freeze-thaw quality of Atlantic salmon. *Innovative Food Science & Emerging Technologies, 65,* 102454. https://doi.org/10.1016/j.ifset.2020.102454

Li, L., Liu, Y., Zou, X., He, J., Xu, X., Zhou, G., & Li, C. (2017). In vitro protein digestibility of pork products is affected by the method of processing. *Food Research International, 92*, 88-94. https://doi.org/10.1016/j.foodres.2016.12.024

Liu, M., Lampi, A.-M., & Ertbjerg, P. (2018). Unsaturated fat fraction from lard increases the oxidative stability of minced pork. *Meat Science, 143*, 87-92. https://doi.org/10.1016/j.meatsci.2018.04.028

Liu, R., Zhao, S., Yang, H., Li, D., Xiong, S., & Xie, B. (2011). Comparative study on the stability of fish actomyosin and pork actomyosin. *Meat Science, 88*, 234-240. https://doi.org/10.1016/j.meatsci.2010.12.026

Lorido, L., Ventanas, S., Akcan, T., & Estévez, M. (2016). Effect of protein oxidation on the impaired quality of dry-cured loins produced from frozen pork meat. *Food Chemistry, 196*, 1310-1314. https://doi.org/10.1016/j.foodchem.2015.10.092

Lund, M. N., Heinonen, M., Baron, C. P., & Estevez, M. (2011). Protein oxidation in muscle foods: A review. *Molecular Nutrition & Food Research, 55*, 83-95. https://doi.org/10.1002/mnfr.201000453

Mackie, I. M. (1993). The effects of freezing on flesh proteins. *Food Reviews International, 9*, 575-610. https://doi.org/10.1080/87559129309540979

Mazur, P. (1970). Cryobiology: The Freezing of Biological Systems. *Science, 168*, 939-949. https://doi.org/10.1126/science.168.3934.939

Men, L., Li, Y., Wang, X., Li, R., Zhang, T., Meng, X., Liu, S., Gong, X., & Gou, M. (2020). Protein biomarkers associated with frozen Japanese puffer fish (*Takifugu rubripes*) quality traits. *Food Chemistry, 327*, 127002. https://doi.org/10.1016/j.foodchem.2020.127002

Minton, A. P. (2006). How can biochemical reactions within cells differ from those in test tubes? *Journal of Cell Science, 119*, 2863-2869. https://doi.org/10.1242/jcs.03063

Nakazawa, N., & Okazaki, E. (2020). Recent research on factors influencing the quality of frozen seafood. *Fisheries Science, 86*, 231-244. https://doi.org/10.1007/s12562-020-01402-8

Nian, L., Cao, A., & Cai, L. (2020). Investigation of the antifreeze mechanism and effect on quality characteristics of largemouth bass (*Micropterus salmoides*) during F-T cycles by hAFP. *Food Chemistry, 325*. Article 126918. https://doi.org/10.1016/j.foodchem.2020.126918

Ozen, B. O., & Soyer, A. (2018). Effect of plant extracts on lipid and protein oxidation of mackerel (*Scomber scombrus*) mince during frozen storage. *Journal of Food Science and Technology-Mysore, 55*, 120-127. https://doi.org/10.1007/s13197-017-2847-6

Pan, N., Dong, C., Du, X., Kong, B., Sun, J., & Xia, X. (2021). Effect of freeze-thaw cycles on the quality of quick-frozen pork patty with different fat content by consumer assessment and instrument-based detection. *Meat Science, 172*, 108313. https://doi.org/10.1016/j.meatsci.2020.108313

Pikal-Cleland, K. A., Rodríguez-Hornedo, N., Amidon, G. L., & Carpenter, J. F. (2000). Protein denaturation during freezing and thawing in phosphate buffer systems:

monomeric and tetrameric β-galactosidase. *Archives of Biochemistry and Biophysics, 384*, 398-406. https://doi.org/10.1006/abbi.2000.2088

Prego, R., Fidalgo, L. G., Saraiva, J. A., Vázquez, M., & Aubourg, S. P. (2021). Impact of prior high-pressure processing on lipid damage and volatile amines formation in mackerel muscle subjected to frozen storage and canning. *LWT-Food Science and Technology, 135*, 109957. https://doi.org/10.1016/j.lwt.2020.109957

Qian, S., Li, X., Wang, H., Mehmood, W., Zhang, C., & Blecker, C. (2021). Effects of frozen storage temperature and duration on changes in physicochemical properties of beef myofibrillar protein. *Journal of Food Quality*, *2021*, 8836749. https://doi.org/10.1155/2021/8836749

Ribeiro, J. S., Santos, M. J. M. C., Silva, L. K. R., Pereira, L. C. L., Santos, I. A., da Silva Lannes, S. C., & da Silva, M. V. (2019). Natural antioxidants used in meat products: A brief review. *Meat Science, 148*, 181-188. https://doi.org/10.1016/j.meatsci.2018.10.016

Robinson, C., Boxe, C. S., Guzmán, M. I., Colussi, A. J., & Hoffmann, M. R. (2006). Acidity of frozen electrolyte solutions. *The Journal of Physical Chemistry B, 110*(15), 7613-7616. https://doi.org/10.1021/jp061169n

Sanfelice, D., & Temussi, P. A. (2016). Cold denaturation as a tool to measure protein stability. *Biophysical Chemistry*. 208, 4-8. https://doi.org/10.1016/j.bpc.2015.05.007

Santé-Lhoutellier, V., Astruc, T., Marinova, P., Greve, E., & Gatellier, P. (2008). Effect of meat cooking on physicochemical state and in vitro digestibility of myofibrillar proteins. *Journal of Agricultural and Food Chemistry, 56*, 1488-1494. https://doi.org/10.1021/jf072999g

Santé-Lhoutellier, V., Aubry, L., & Gatellier, P. (2007). Effect of oxidation on in vitro digestibility of skeletal muscle myofibrillar proteins. *Journal of Agricultural and Food Chemistry, 55*, 5343-5348. https://doi.org/10.1021/jf070252k

Santos, M. M. F., Lima, D. A. S., Madruga, M. S., & Silva, F. A. P. (2020). Lipid and protein oxidation of emulsified chicken patties prepared using abdominal fat and skin. *Poultry Science, 99*, 1777-1787. https://doi.org/10.1016/j.psj.2019.11.027

Schmidt, S. J., & Lee, J. W. (2010). How does the freezer burn our food? *Journal of Food Science Education, 8*, 45-52. https://doi.org/10.1111/j.1541-4329.2009.00072.x

Shao, Y., Wang, L., Chen, C., Xiong, G., Hu, Y., Qiao, Y., . . . Ding, A. (2018). Antioxidant capacity of fermented soybeans and their protective effect on protein oxidation in largemouth bass (*Micropterus salmoides*) during repeated freezing-thawing (FT) treatments. *LWT-Food Science and Technology, 91*, 213-221. https://doi.org/10.1016/j.lwt.2018.01.048

Shi, J., Zhang, L., Lei, Y., Shen, H., Yu, X., & Luo, Y. (2018). Differential proteomic analysis to identify proteins associated with quality traits of frozen mud shrimp (*Solenocera melantho*) using an iTRAQ-based strategy. *Food Chemistry, 251*, 25-32. https://doi.org/10.1016/j.foodchem.2018.01.046

Shi, L., Xiong, G., Yin, T., Ding, A., & Wang, L. (2020). Effects of ultra-high pressure treatment on the protein denaturation and water properties of red swamp crayfish (*Procambarus clarkia*). *LWT- Food Science and Technology, 133*, Article 110124. https://doi.org/10.1016/j.lwt.2020.110124

Skibsted, L. H. (2011). Nitric oxide and quality and safety of muscle based foods. *Nitric Oxide, 24*, 176-183. https://doi.org/10.1016/j.niox.2011.03.307

Soladoye, O., Juárez, M., Aalhus, J., Shand, P., & Estévez, M. (2015). Protein oxidation in processed meat: Mechanisms and potential implications on human health. *Comprehensive Reviews in Food Science and Food Safety, 14*, 106-122. https://doi.org/10.1111/1541-4337.12127

Soyer, A., Ozalp, B., Dalmis, U., & Bilgin, V. (2010). Effects of freezing temperature and duration of frozen storage on lipid and protein oxidation in chicken meat. *Food Chemistry, 120*, 1025-1030. https://doi.org/10.1016/j.foodchem.2009.11.042

Szymczak, M., Kamiński, P., Felisiak, K., Szymczak, B., Dmytrów, I., & Sawicki, T. (2020). Effect of constant and fluctuating temperatures during frozen storage on quality of marinated fillets from Atlantic and Baltic herrings (*Clupea harengus*). *LWT-Food Science and Technology*, *133*, 109961. https://doi.org/10.1016/j.lwt.2020.109961

Takenaka, N., Ueda, A., & Maeda, Y. (1992). Acceleration of the rate of nitrite oxidation by freezing in aqueous solution. *Nature, 358*, 736-738. https://doi.org/10.1038/358736a0

Takenaka, N., Ueda, A., Daimon, T., Bandow, H., Dohmaru, T., & Maeda, Y. (1996). Acceleration mechanism of chemical reaction by freezing: The reaction of nitrous acid with dissolved oxygen. *The Journal of Physical Chemistry B*. 100, 13874-13884. https://doi.org/10.1021/jp9525806

Turgut, S. S., Isikci, F., & Soyer, A. (2017). Antioxidant activity of pomegranate peel extract on lipid and protein oxidation in beef meatballs during frozen storage. *Meat Science, 129*, 111-119. https://doi.org/10.1016/j.meatsci.2017.02.019

Utrera, M., Armenteros, M., Ventanas, S., Solano, F., & Estévez, M. (2012). Pre-freezing raw hams affects quality traits in cooked hams: Potential influence of protein oxidation. *Meat Science, 92*, 596-603. https://doi.org/10.1016/j.meatsci.2012.06.005

Utrera, M., Morcuende, D., & Estévez, M. (2014a). Fat content has a significant impact on protein oxidation occurred during frozen storage of beef patties. *LWT-Food Science and Technology, 56*, 62-68. https://doi.org/10.1016/j.lwt.2013.10.040

Utrera, M., Morcuende, D., Ganhao, R., & Estevez, M. (2015). Role of Phenolics Extracting from Rosa canina L. on Meat Protein Oxidation During Frozen Storage and Beef Patties Processing. *Food and Bioprocess Technology, 8*, 854-864. https://doi.org/10.1007/s11947-014-1450-3

Utrera, M., Parra, V., & Estévez, M. (2014b). Protein oxidation during frozen storage and subsequent processing of different beef muscles. *Meat Science, 96*, 812-820. https://doi.org/10.1016/j.meatsci.2013.09.006

Wang, B., Li, F., Pan, N., Kong, B., & Xia, X. (2021). Effect of ice structuring protein on the quality of quick-frozen patties subjected to multiple freeze-thaw cycles. *Meat Science, 172*, 108335. https://doi.org/10.1016/j.meatsci.2020.108335

Wang, T., Li, Z., Yuan, F., Lin, H., & Pavase, T. R. (2017). Effects of brown seaweed polyphenols, alpha-tocopherol, and ascorbic acid on protein oxidation and textural properties of fish mince (*Pagrosomus major*) during frozen storage. *Journal of the Science of Food and Agriculture, 97*, 1102-1107. https://doi.org/10.1002/jsfa.7835

Workman, E. J., & Reynolds, S. E. (1950). Electrical Phenomena Occurring during the Freezing of Dilute Aqueous Solutions and Their Possible Relationship to Thunderstorm Electricity. *Physical Review, 78*, 254-259. https://doi.org/10.1103/PhysRev.78.254

Xia, X., Kong, B., Liu, Q., & Liu, J. (2009). Physicochemical change and protein oxidation in porcine longissimus dorsi as influenced by different freeze-thaw cycles. *Meat Science, 83*, 239-245. https://doi.org/10.1016/j.meatsci.2009.05.003

Xue, S., Yang, H., Yu, X., Qian, C., & Zhou, G. (2018). Applications of high pressure to pre-rigor rabbit muscles affect the water characteristics of myosin gels. *Food Chemistry, 240*, 59-66. https://doi.org/10.1016/j.foodchem.2017.07.096

Zhang, B., Zhang, X., Shen, C., & Deng, S. (2018). Understanding the influence of carrageenan oligosaccharides and xylooligosaccharides on ice-crystal growth in peeled shrimp (*Litopenaeus vannamei*) during frozen storage. *Food & Function, 9*, 4394-4403. https://doi.org/10.1039/C8FO00364E

Zhang, L., Li, Q., Hong, H., & Luo, Y. (2020). Prevention of protein oxidation and enhancement of gel properties of silver carp (Hypophthalmichthys molitrix) surimi by addition of protein hydrolysates derived from surimi processing by-products. *Food Chemistry, 316*, Article 126343. https://doi.org/10.1016/j.foodchem.2020.126343

Zhang, W., Xiao, S., & Ahn, D. U. (2013). Protein oxidation: basic principles and implications for meat quality. *Critical Reviews in Food Science and Nutrition, 53*, 1191-1201. https://doi.org/10.1080/10408398.2011.577540

Zhang, W., Xiao, S., Lee, E. J., & Ahn, D. U. (2011). Consumption of oxidized oil increases oxidative stress in broilers and affects the quality of breast meat. *Journal of Agricultural and Food Chemistry, 59*, 969-974. https://doi.org/10.1021/jf102918z

Zhang, Y., & Ertbjerg, P. (2019). On the origin of thaw loss: Relationship between freezing rate and protein denaturation. *Food Chemistry, 299*, Article 125104. https://doi.org/10.1016/j.foodchem.2019.125104

Zhang, Y., Puolanne, E., & Ertbjerg, P. (2021). Mimicking myofibrillar protein denaturation in frozen-thawed meat: Effect of pH at high ionic strength. *Food Chemistry, 338*, Article 128017. https://doi.org/10.1016/j.foodchem.2020.128017

Zheng, Y, Qiu, Z, Wang, X. (2020). Protein oxidation and tandem mass tag-based proteomic analysis in the dorsal muscle of farmed obscure pufferfish subjected to multiple freeze–thaw cycles. *Journal of Food Processing and Preservation, 44*, e14721. https://doi.org/10.1111/jfpp.14721

Zheng, Y., Zhou, F., Zhang, L., Wang, H., & Wang, X. (2021). Effect of different extent of protein oxidation on the frozen storage stability of muscle protein in obscure pufferfish (*Takifugu obscurus*). *LWT-Food Science and Technology, 137*, 110416. doi: https://doi.org/10.1016/j.lwt.2020.110416

Zhu, Y., Li, C., Cui, H., & Lin, L. (2020). Plasma enhanced-nutmeg essential oil solid liposome treatment on the gelling and storage properties of pork meat batters. *Journal of Food Engineering, 266*, 109696. https://doi.org/10.1016/j.jfoodeng.2019.109696

973 **Figure legends**

974

975 Fig.1. Schematic illustration of ice crystals in frozen muscle foods. (a) Formation of
976 ice crystals during freezing (Mazur 1970); (b) Ice recrystallization during frozen
977 storage; (c) Effect of ice crystals on muscle structure after thawing (Nakazawa &
978 Okazaki, 2020).

979

980 Fig.2. Illustration of mechanical distortion of muscle structure in frozen meat. (A)
981 Under unfrozen condition, water stays in liquid form and myosin heads are bound to
982 actin filament to form rigor bonds; (B) Under normal frozen condition, a major part of
983 the water exists in the form of solid ice. Due to the formation of extra-cellular ice
984 crystals, muscle fibers are severely compressed. The fiber compression leads to
985 distortion of rigor bonds and ultimately to distortion of myosin heads.

986

987 Fig.3. Schematic illustration of the change in reaction conditions caused by formation
988 of ice crystals (Adapted from Takenaka et al. 1996)

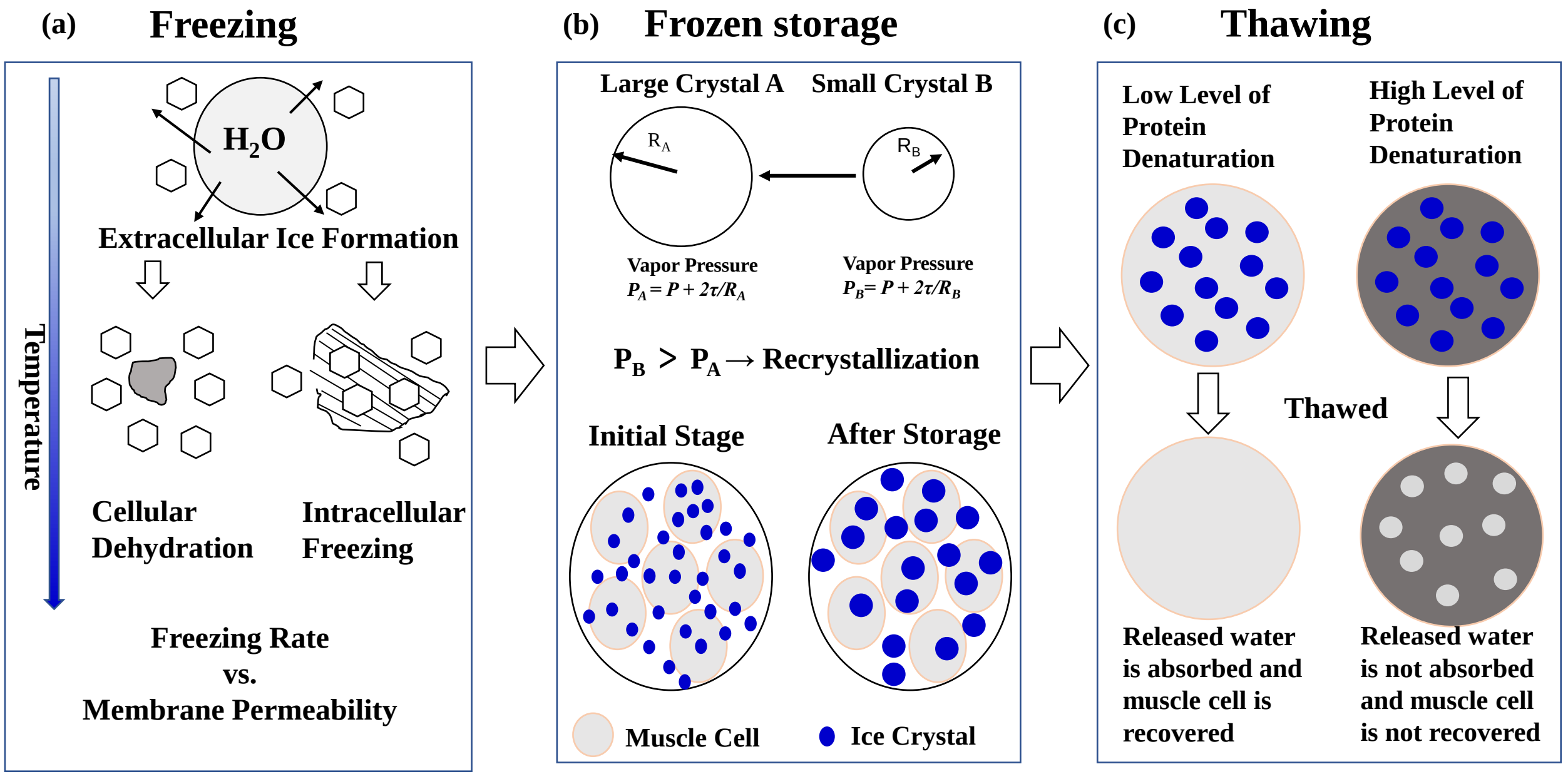
989

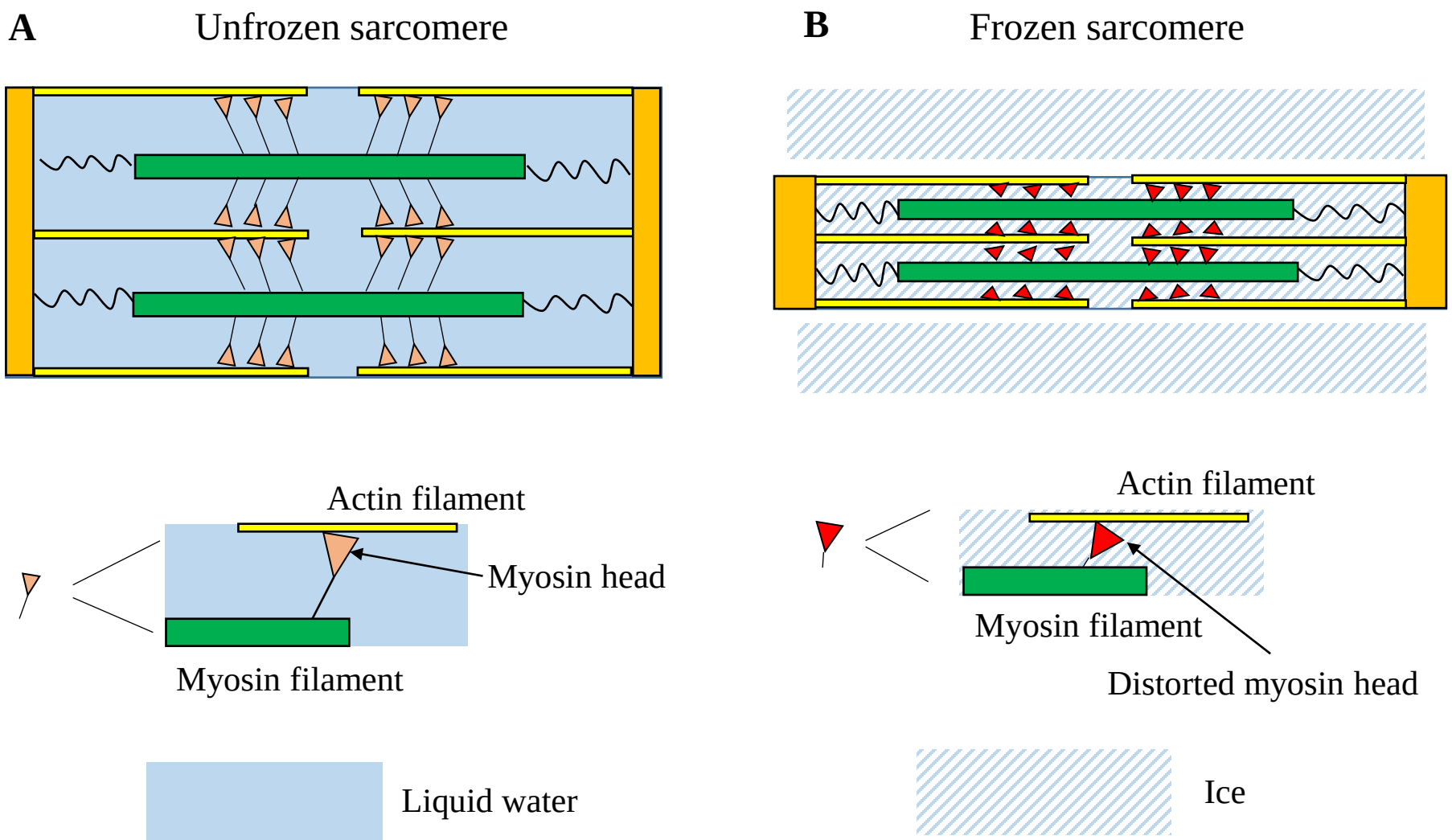
990 Fig.4. Possible mechanisms of ice-induced protein denaturation. (a) change of pH and
991 ionic strength; (b) accumulation of air bubbles at the ice interface; (c) adsorption at
992 the ice interface; (d) Pressure due to ice growth; (e), enhance cold denaturation.
993 Modified from Arsiccio & Pisano 2020.

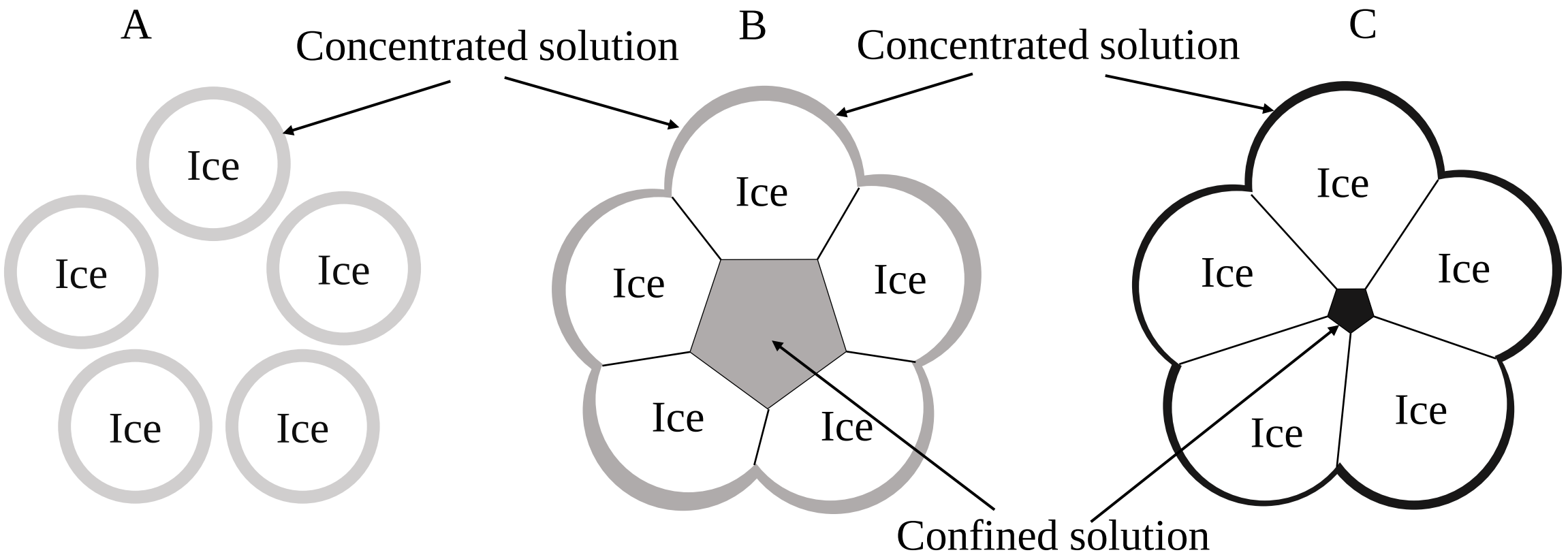
994

995 Fig.5. Schematic illustration of pH changes in frozen electrolyte solutions mediated
996 by freezing potential, based on Carmen et al., 2006.

997

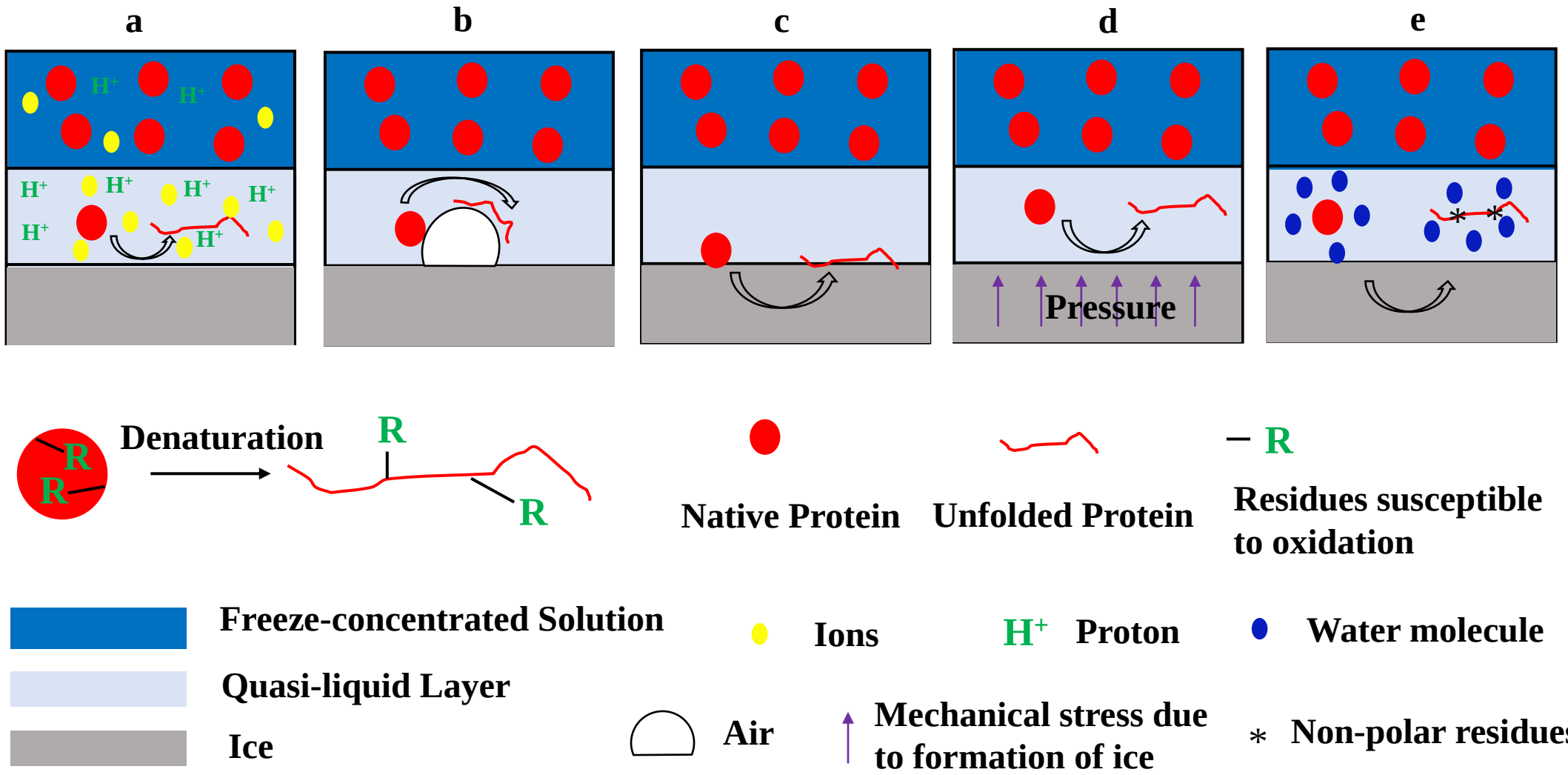

(a) Freezing
H_2O
Extracellular Ice Formation
Temperature
Cellular Dehydration
Intracellular Freezing
Freezing Rate vs. Membrane Permeability
(b) Frozen storage
Large Crystal A
Small Crystal B
R_A
R_B
Vapor Pressure $P_A = P + 2\tau/R_A$
Vapor Pressure $P_B = P + 2\tau/R_B$
$P_B > P_A \rightarrow$ Recrystallization
Initial Stage
After Storage
Muscle Cell
Ice Crystal
(c) Thawing
Low Level of Protein Denaturation
High Level of Protein Denaturation
Thawed
Released water is absorbed and muscle cell is recovered
Released water is not absorbed and muscle cell is not recovered


A
Unfrozen sarcomere
B
Frozen sarcomere
Actin filament
Myosin head
Myosin filament
Actin filament
Myosin filament
Distorted myosin head
Liquid water
Ice



Concentrated &
Confined solution

Dissolved oxygen ↑ Pro-oxidants ↑
Protein substrates ↑ Ionic strength ↑
pH may change


a
b
c
d
e
H+
Pressure
Denaturation
R
Native Protein
Unfolded Protein
Residues susceptible to oxidation
Freeze-concentrated Solution
Quasi-liquid Layer
Ice
Ions
Proton
Water molecule
Air
Mechanical stress due to formation of ice
Non-polar residues

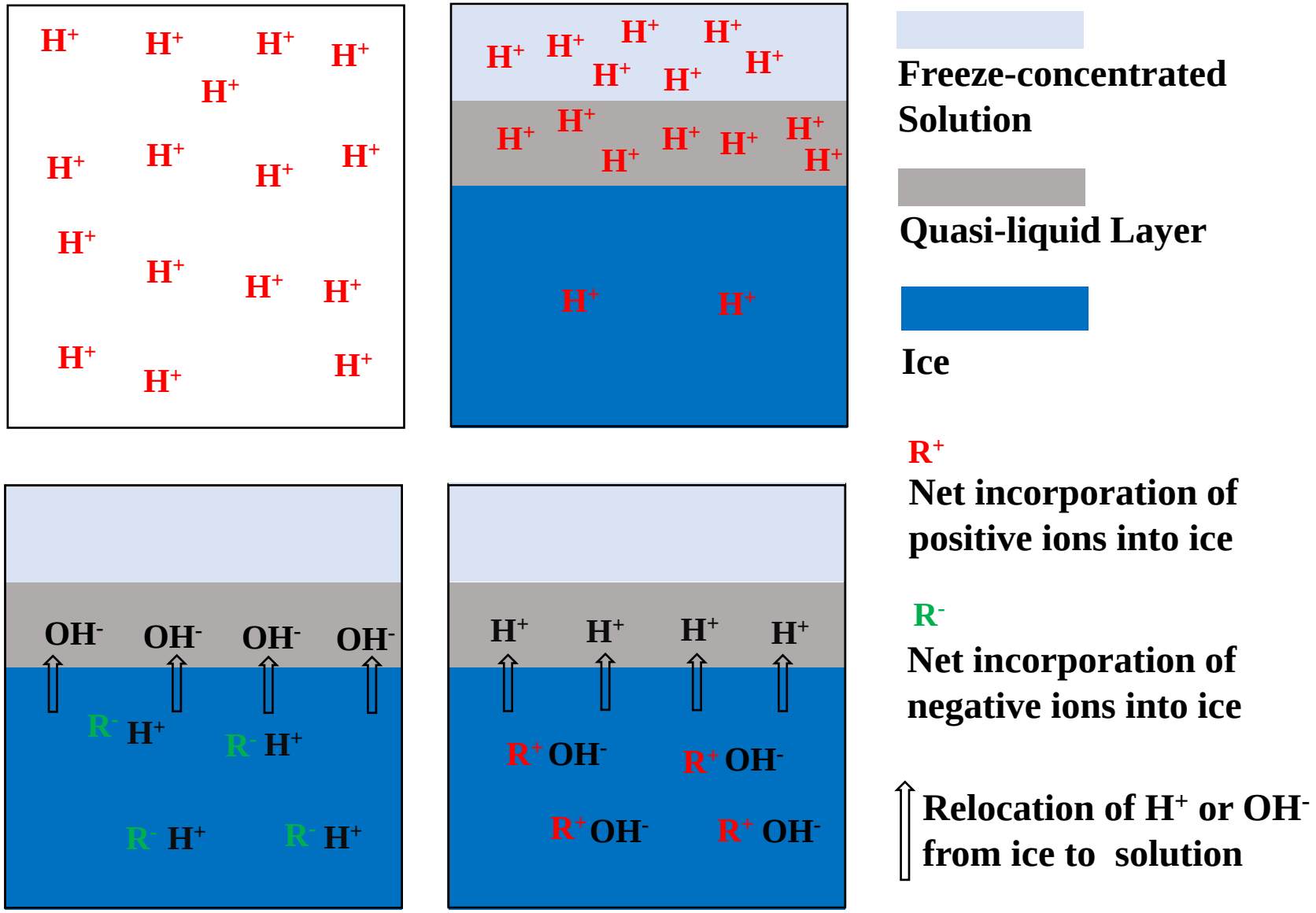

H+
OH-
R- H+
R+ OH-
Freeze-concentrated Solution
Quasi-liquid Layer
Ice
R+
Net incorporation of positive ions into ice
R-
Net incorporation of negative ions into ice
Relocation of H+ or OH- from ice to solution

Table 1. Summary of literature on the effects of freezing on protein oxidation in livestock muscle foods

Frozen Muscle foods	Main findings on protein oxidation	Physicochemical changes relevant to protein oxidation	References
Pork patties	Carbonyls increased with increased freeze-thaw cycles and fat content of patties	Lipid oxidation increased with increased freeze-thaw cycles and fat content of patties	Pan et al., 2021
Pork patties	Addition of ice structuring protein to patties reduced protein carbonyls	Ice structuring protein limited growth of ice crystals and inhibited lipid oxidation	Wang et al., 2021
Pork patties	Storage at -18, -25, -18/-25 ℃ led to lower content of carbonyls than at -8 ℃ or -8/-18 ℃; generally, no difference among -18, -25, -18/-25 ℃	Frozen storage led to unfolding and aggregation of myofibrillar proteins	Li et al., 2020a
Pork dumplings	Carbonyls increased with storage time; incorporation of extracts of clove and rosemary led to lower carbonyls and less protein cross-linking	Incorporation of extracts of clove and rosemary led to lower lipid oxidation	Huang et al., 2019
Pork	No difference in total and free sulfhydryl groups between Immersion solution freezing (ISF) and air blast freezing (AF); sulfhydryl groups decreased with time	ISF produced smaller ice crystals and reduced lipid oxidation compared to AF	Hou et al., 2020
Pork	Freezing rate had an impact on oxidative stability of muscle proteins, however, the aging process had overriding impacts over freezing rate	Aging prior or after freezing increased lipid oxidation	Kim et al., 2018
Pork patties	With increased time, carbonyls increased and sulfhydryl decreased; addition of oilve leaf extract inhibited protein oxidation	Addition of olive leaf extract inhibited lipid oxidation	Botsoglou et al., 2014
Pork dumplings	Carbonyls increased with storage time; lower temperature had lower carbonyls	Free fatty acids increased with frozen storage time; lipid oxidation increased with storage time and temperature	Huang et al., 2013
Pork	Specific carbonyls AAS and GGS first increase and then decrease with time; oxygen permeable bag vs. vacuum pack, minced vs. intact, *longissumus dorsi* vs. *psoas major* generally led to greater AAS and GGS	-	Estévez et al., 2011
Pork loin	Carbonyls increased and sulfhydryl decreased with increased freeze-thaw cycles	Lipid oxidation and protein denaturation increased with freeze-thaw cycles	Xia et al., 2009

Beef	Myofibrillar protein oxidation decreased when storage temperature decreased from -1 to -12 ℃, but there was no clear difference between -12 ℃ and -18 ℃	Protein denaturation was generally greater at higher temperatures, ionic- and hydrogen bonds were greater at lower temperatures	Qian et al., 2021
Beef loin	Frozen storage first led to increased carbonyls and then decreased	-	Holman et al., 2018
Beef meatballs	With increased time, carbonyls increased and sulfhydryl decreased; addition of pomegranate peel extract greatly inhibited the change in oxidation marker	Pomegranate peel extract reduced lipid oxidation	Turgut et al., 2017
Beef patties	Frozen storage increased carbonyl compound (AAA and AAS), and also increased the susceptibility towards oxidation during successive technological process; addition of rose extract inhibited the formation of AAS and AAA,	Rose extract inhibited lipid oxidation	Utrera et al., 2015
Beef patties	Frozen storage increased the specific carbonyl (AAS) and Schiff base, higher fat content in patties led to increased AAS and Schiff base	-	Utrera et al., 2014a
Beef patties	Different muscle cuts had different oxidative stability	Heme-iron content, antioxidant enzyme activity and PUFA content differ	Utrera et al., 2014b
Lamb loin	Frozen storage did not affect carbonyls	-	Coombs et al., 2018
Emulsified chicken patties	Carbonyls increased with storage time; no difference due to types of used fat (skin and abdominal fat)	Use of skin fat lower lipid oxidation	Santos et al., 2020
Chicken breast	After 3 freeze-thaw cycles, carbonyls increased and sulfhydryl decreased	Lipid oxidation increased after 3 cycles	Ali et al., 2015
Chicken	Carbonyls in chicken leg and breast increased with time; lower freezing temperature led to lower carbonyls; sulfhydryl decreased with time; lower freezing temperature led to higher content of sulfhydryl in leg meat	Lipid oxidation increased with time	Soyer et al., 2010

Table 2. Summary of literature on the effects of freezing on protein oxidation in aquatic muscle foods

Frozen **Muscle foods**	**Main findings on protein oxidation**	**Physicochemical changes relevant to protein oxidation**	**References**
Puffer fish	Light exposure, oxygen concentration or increased freeze-thaw cycles accelerated protein oxidation	Changes of protein structure were in line with the extent of protein oxidation	Zheng et al., 2021
Shrimp	Protein oxidation increased with time and storage temperature, and temperature had a greater impact	Protein denaturation followed similar pattern with protein oxidation	Ji et al., 2021
Puffer fish	Increased freeze-thaw cycles resulted decrease in sulfhydryl content and antioxidant enzyme activity, and increase in carbonyls and protein cross-linking	Increased freeze-thaw cycles led to increased lipid oxidation	Zheng et al., 2020
Largemouth bass	Fish samples soaked in solution containing herring antifreeze protein (hAFP) had lower content of dityrosine, carbonyls, and higher total sulfhydryl	hAFP inhibited recrystallization and decreased freezing point	Nian et al., 2020
Red sea bream	Fish samples soaked in solution containing herring antifreeze protein had lower content of dityrosine, carbonyls, and disulfide	hAFP stabilized secondary and tertiary conformation of myofibrillar proteins	Cai et al., 2020a
Largemouth bass	Microwave thawing in combination with either vacuum or magnetic nanoparticles, and far-infrared thawing with magnetic nanoparticles had lower degree of protein oxidation	Protein denaturation level was similar among different thawing methods	Cai et al., 2020b
Silver carp surimi	Hydrolysates via trypsin- and alcalase-treated surimi process byproducts delayed the oxidation of cysteine, and lowered protein carbonylation	Hydrolysates delayed the destroy of myofibrillar protein structural integrity	Zhang et al., 2020
Mackerel fillets	Carbonyls of sarcoplasmic and myofibrillar proteins increased with storage time	-	Cropotova et al., 2019
Large-mouth bass	Total sulfhydryl decreased with freeze-thaw cycles; fermented soybean delayed the decline of reactive and total sulfhydryl	-	Shao et al., 2018
Mackerel mince	Carbonyls increased with time; addition of extracts from green tea,	Lipid oxidation increased with time; addition of	Ozen & Soyer, 2018

	grape seed, pomegranate rind delayed the increase of carbonyls, but no difference among antioxidant sources; similar trend for the decrease of sulfhydryl	extracts inhibited lipid oxidation	
Haddock and mackerel minces	High-pressure treated samples had higher content of carbonyls in sarcoplasmic and myofibrillar proteins; higher pressure led to higher carbonyls	Lipid oxidation was higher in 200 MPa treated mackerel minces, but lower in 300 MPa	Cropotova et al., 2020
Red sea bream mince	Total sulfhydryl decreased with time, and α-tocopherol, ascorbic acid, brown seaweed polyphenol inhibited the loss of sulfhydryl; carbonyls increased, and α-tocopherol inhibited the formation of carbonyl	All added antioxidants inhibited lipid oxidation	Wang et al., 2017
Peeled shrimp	Incorporation of carrageenan oligosaccharides prior to frozen storage decreased protein carbonyl and dityrosine, maintained a higher total sulfhydryl content when compared with control	-	Zhang et al., 2018
Cod mince	Frozen storage increased carbonyls, the addition of caffeic acid had no protective effect on protein carbonylation	caffeic acid reduced lipid oxidation	Larsson & Undeland, 2010
Rainbow trout fillets	Carbonyls increased with time at -20 ℃, but not at -30 or -80 ℃	Lipid oxidation and free fatty acids increased with time and frozen storage temperature	Baron et al., 2007
Rainbow trout fillets	Carbonyls were higher at -20 ℃ in the total meat homogenate and low-salt soluble fraction; carbonylated proteins include nucleoside diphosphate kinase, adenylate kinase, pyruvate kinase, actin, creatine kinase, tropomyosin, myosin light chains 1 and 2, and myosin heavy chain	-	Kjaersgard et al., 2006