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Review

Role of freezing-induced myofibrillar protein denaturation in the generation of thaw loss: A review

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ABSTRACT

Formation of thaw loss cannot generally be avoided when meat is frozen and then thawed. Explanations have mainly focused on the damage to muscle fibers resulting from ice crystallization and the freezing-induced denaturation of myofibrillar proteins, the latter of which has, however, not received much research focus. This review discusses the relationship between myofibrillar protein denaturation and water-holding capacity of meat in freezing-thawing with the aim to improve the understanding the relative importance of protein denaturation in the formation of thaw loss. The contribution of decreased pH and high ionic strength in the unfrozen water in freezing is emphasized and we hypothesize that these two factors are causing protein denaturation and conformational changes within muscle fibers, and consequently loss of water-holding capacity. Slow freezing produces more thaw loss than fast freezing, and this is discussed here in relation to the impacts on myofibrillar protein denaturation induced by the freezing rate.

1. Introduction

Meat and meat products are generally believed to be one of the most important protein sources in the human diet. However, due to the existence of around 75% water in the muscle mass, meat-based foods are highly prone to bacterial spoilage and chemical deterioration during long-time storage. Thus, maintaining quality and safety of meat products are of critical importance for the meat industry.

Freezing and frozen storage have been widely applied to preserve freshness and quality as well as to extend the shelf life of meat products (Coombs, Holman, Friend, & Hopkins, 2017; Li, Zhu, & Sun, 2018). During freezing, water in muscle tissue starts to crystalize below −1 °C, and below −20 °C more than 90% of the muscle water is present in the frozen state (Calvelo, 1981). However, due to altered water distribution within the meat tissue that occurs during freezing and thawing, a substantial loss of muscle water is generally not avoided in frozen-thawed compared to unfrozen meat (Leygonie, Britz, & Hoffman, 2012). The water loss during thawing accompanied with significant losses of weight and valuable nutrients in the exudate will consequently result in decreases in economic and nutritional values. As the myowater freeze out, the meat proteins will be progressively exposed to concentrating solutes in the unfrozen water phase. This could accelerate physicochemical deterioration in frozen-thawed meat, i.e. protein denaturation, lipid and protein oxidation, and discoloration, thus affecting meat quality (Coombs et al., 2017; Coombs et al., 2018; Kantono, Hamid, Ma, Oey, & Farouk, 2021; Leygonie et al., 2012; Utrera, Parra, & Estévez, 2014). Therefore, when frozen-thawed meat is used to prepare meat products, inferior water-holding capacity (WHC) and meat protein properties would then negatively affect the juiciness, tenderness and natural yield after processing, which consequently becomes a significant problem for either the industry or consumers.

The current accepted theory for the driving force of thaw loss is mainly based on the ice crystallization, and in this context the size and distribution of intra- and extracellular ice crystals have been regarded as the decisive factors (Añón & Calvelo, 1980; Hamm, 1986; Li et al., 2018). Fast freezing produces small ice crystals uniformly distributed within and between the muscle fibers, which is causing less mechanical damage to the muscle integrity and consequently less thaw loss when compared to slow freezing in which large and uneven extracellular ice crystals are often generated (Hamm, 1961; Kim, Kim, Seo, Setyabrata, & Kim, 2018). Protein is, besides water, the most abundant component within the meat tissue and the myofibrillar protein comprises by far the largest part. A major part of water in the meat tissue is located within the myofibrils, and the WHC of meat is to a large extent influenced by the

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physiochemical changes occurring at the myofibrillar protein level (Huff-Lonergan & Lonergan, 2005). Any myofibrillar protein denaturation during freezing and thawing would thus be expected to negatively affect WHC of meat and thus contribute to the generation of thaw loss (Petrović, Grujić, & Petrović, 1993; Zhang & Ertbjerg, 2019; Zhang, Puolanne, & Ertbjerg, 2021). However, earlier literature reported only little evidence for the contribution of freezing-induced protein denaturation to thaw loss formation as reviewed by Hamm (1986) and Leygonie et al. (2012). Some studies on freezing and thawing did not find any significant effects on myofibrillar protein denaturation caused by different freezing rates (Farouk, Wieliczko, & Merts, 2003; Ngapo, Babare, Reynolds, & Mawson, 1999; Yu et al., 2010). The role of myofibrillar protein denaturation in the generation of thaw loss is thus currently not well understood, and agreement on the importance is still lacking in the scientific literature. This review aims to investigate in detail the relationship between protein denaturation and WHC of meat in freezing and thawing, and to expand our understanding on the relative importance of myofibrillar protein denaturation. The underlying mechanism for the formation of thaw loss is here comprehensively discussed from the perspective of myofibrillar protein denaturation.

2. Changes of water within the meat structure in freezing-thawing

2.1. Effect of freezing-thawing on water distribution within the meat tissue

Water and protein are the two most important compounds in meat, and lean meat at slaughter contains around 75% water (Offer & Knight, 1988a). The content of water and its distribution within the meat tissue can greatly influence the properties of meat and meat products, i.e. juiciness, toughness, appearance and natural yield after cooking. Generally, within the meat tissue, three water compartments are present, recognized as bound water, immobilized water and free water in terms of the degree of immobilization (Bertram et al., 2001; Hamm, 1972; Offer & Knight, 1988a; Pearce, Rosenvold, Andersen, & Hopkins, 2011). The location of these three water populations and their relevant properties in relation to freezing are summarized below in Table 1. Bound water (up to 8%) usually exists in the vicinity of protein side chains through hydrogen bonding and does not easily move to other compartments (Huff-Lonergan & Lonergan, 2005; Offer & Knight, 1988a). This water population is highly resistant to the application of physical force such as pressure exerted by extracellular ice in freezing. Some studies on freezing found that bound water is not easily freezable even at subzero temperatures below −40 °C (Aktas, Tulek, & Gokalp, 1997). It is generally believed that up to 85% of the muscle water is trapped between the myofilaments, mainly dependent on a balance between the electrostatic forces within the charged filaments and the osmotic pressure, as well as the structural constraint forces e.g. exerted by *Z*-disks and cross-bridges (Huff-Lonergan & Lonergan, 2005; Offer & Knight, 1988a; Puolanne & Halonen, 2010). This water population is referred to as immobilized (or entrapped; Hamm, 1986) water, and it would not flow freely from the postmortem meat tissue, yet it is vulnerable to temperature and pressure. A small fraction of water can be found in the extra-myofibrillar space, i.e. between the myofibrils, between the fibers and between the fiber bundles (Offer & Knight, 1988a). These water molecules are termed as free water and they are mainly restricted by capillary forces within the muscle structure (Offer & Knight, 1988a), and part of this water is easily lost by gravity as drip or purge. Immobilized and free water are often believed to be the freezable water. Xanthakis, Havet, Chevallier, Abadie, and Le-Bail (2013) reported the freezable water amount to be about 88% of the total moisture in pork when frozen at −20 °C.

Within the meat tissue, the initial freezing point of water located in intra- and extracellular spaces differ. As concluded by Hamm (1986), pure extracellular ice occurs when the temperature reaches around −1.2 °C, and intracellular ice formation often requires a lower freezing temperature of around −1.6 °C. This effect is likely attributable to the existence of a higher solute concentration inside the muscle fiber compared to outside. However, accompanied with ice formation in the extracellular space, solutes would gradually become more concentrated in the surrounding unfrozen water (Ohta & Tanaka, 1978). Thus, the ionic strength at −5 °C has by Finn (1932) been reported to correspond to 1.6 M potassium chloride. This would create an osmotic pressure for immobilized water in the intracellular area to migrate into the extracellular area becoming the extracellular ice during freezing (Offer & Knight, 1988a, 1988b). The heat conductivity of the pure ice is much higher than the other cell materials, so the ice formed extracellularly will conduct the heat fast which means that the extracellular ice will increase in size on the cost of the immobilized water that runs out of fibers. Part of the extracellular ice would then stay as extracellular free water and not be reabsorbed by the muscle fibers upon thawing (Huff-Lonergan & Lonergan, 2005; Offer & Cousins, 1992). Low-field NMR relaxation measurement has been applied as a technique to monitor the migration of water compartments within the meat tissue that occurs during freezing-thawing (Mortensen, Andersen, Engelsen, & Bertram, 2006). The mobility and population of the immobilized water have been reported to significantly decrease with freeze-thaw cycles in beef *semimembranous* muscle (Cheng et al., 2019) and in chicken breast meat (Ali et al., 2015), indicating that a shift of immobilized water to free water occurs in freezing-thawing.

Table 1
Summary of water populations and relevant properties in meat.

	Bound water	Immobilized water	Free water
Amount	Up to 8%	Up to 85%	Up to 10%
Compartment[1]	Bound to protein side chains	Within the myofibrils	Between the myofibrils Extracellular spaces
Main Forces[2]	Hydrogen bond	Electrostatic forces Osmotic pressure Structural constraint forces	Capillary force Structural constraint forces
Resistant to freezing	Stronger	Weaker	Weaker
Contributing to thaw loss	Nearly none	Part of fraction	Largest fraction
Factors affecting mobilization[2]	Not easily mobilized	Rigor process Protein denaturation	Gravity

Superscript 1, 2: references: 1. Pearce et al., 2011; Offer & Cousins, 1992; 2. Offer & Knight, 1988a; Huff-Lonergan & Lonergan, 2005.

2.2. Evidence of structural alterations within the meat tissue accompanied with water transfer in freezing-thawing

Ice crystallization during freezing of muscle usually begins in the extracellular area and consequently water moves osmotically from inside to outside of muscle fibers. This water migration results in transversal shrinkage of the muscle fibers and a subsequent dehydration. Martino and Zaritzky (1988) observed reduced fiber diameter in frozen-thawed beef. Tippala, Koomkrong, and Kayan (2021) reported a decrease by around 50% of cross-sectional area within the muscle fibers in pork during freezing and thawing cycles. Increased gaps between the fiber bundles have been reported to be accompanied with freezing and thawing in beef (Grujić, Petrović, Pikula, & Amidžić, 1993), chicken (Ishiguro & Horimizu, 2008; Oliveira, Gubert, Roman, Kempka, & Prestes, 2015), and pork (Hansen, Trinderup, Hviid, Darré, & Skibsted, 2003).

2.3. Effect of freezing-thawing on WHC of meat

WHC is the term to describe the property of meat in which it retains

its own or added water during subsequent processing (Wierbicki & Deatherage, 1958; Wierbicki, Kunkle, & Deatherage, 1957). WHC is of great importance when considering the weight and consequently the financial value of meat. Freezing is one of the most important methods that has been applied to preserve meat and meat products. However, freezing of meat often produces a negative influence on WHC of meat upon thawing in terms of increased thaw loss, drip loss and cooking loss (Hong et al., 2005; Hou, Cheng, Kang, Zhang, & Zhou, 2020; Sakata, Oshida, Morita, & Nagata, 1995; Tuell, Seo, & Kim, 2020). Freezing of beef or pork muscle has been observed to result in additional water loss being around 6–10% as compared to non-frozen slices (Kim & Kim, 2016; Setyabrata & Kim, 2019; Tuell et al., 2020; Zhang, Niu, Chen, Xia, & Kong, 2018). Bogdanowicz, Cierach, and Żmijewski (2018) has compared the effect of freezing and thawing on beef quality between two different crossbreeds and found lower water loss upon thawing in Hereford x Holstein-Friesian compared to Limousin x Holstein-Friesian. Hergenreder et al. (2013) has reported the influence of muscle types on the formation of thaw loss and that beef *gluteus medius* showed higher amount of thaw loss than *longissimus thoracis* and *longissimus lumborum*. The probiotic supplementation on the diet of broilers has been found by Kim et al. (2017) to increase phospholipid content and to further inhibit thaw loss formation and lipid oxidation during fast freezing of chicken breast muscle. Setyabrata and Kim (2019) has observed a higher cooking loss in frozen-thawed beef *Semitendinosus* muscle compared to *longissimus lumborum* and they attributed it to a larger shrinkage from connective tissue in *Semitendinosus* during process of freezing-thawing. Increased cooking loss by around 7–10% in frozen-thawed muscle has by Grayson, King, Shackelford, Koohmaraie, and Wheeler (2014) been found in beef, and by Choi et al. (2017) in pork. Also, However, some authors did not find any differences of cooking loss between fresh and frozen-thawed meat samples (Kim, Kim, et al., 2018; Kim, Meyers, Kim, Liceaga, & Lemenager, 2017), and these authors attributed it to the substantial increase of water loss upon thawing. The WHC of myofibrils has been used to estimate the WHC of the meat, and a distinct decrease in WHC of myofibrils was observed in the frozen-thawed pork as compared to fresh (Zhang & Ertbjerg, 2018; Zhang & Ertbjerg, 2019). Increased number of freezing-thawing cycles would inevitably cause more water to be released from the muscle structure (Chen et al., 2017; Tippala et al., 2021), reaching a limit of around 30% loss after 5 cycles (Cheng et al., 2019). It is generally accepted that the freezing rate plays an important role in the formation of thaw loss and that slow freezing often produces a higher amount of thaw loss than fast freezing (Añón & Calvelo, 1980; Hou et al., 2020; Kim, Liesse, Kemp, & Balan, 2015; Tuell et al., 2020).

3. Protein denaturation during freezing and thawing

3.1. Evidence of freezing-induced protein denaturation within the meat tissue

In general, the native protein conformation is stabilized dependent on covalent forces, e.g. disulfide linkages and non-covalent forces e.g. hydrogen bonds, hydrophobic interactions, electrostatic interactions, and van der Waals forces. Protein denaturation is therefore a complex phenomenon often describing changes of the secondary and tertiary structures principally resulting from rupture of the non-covalent forces (Tanford, 1968). Within the meat tissue protein accounts for around 20%, and meat proteins can be divided into myofibrillar proteins, sarcoplasmic proteins, and extracellular matrix proteins. Myofibrillar proteins comprise 60–70% of the total muscle proteins and build up a fibrous protein structure within the meat tissue (Offer & Knight, 1988a). The idea that meat proteins undergo denaturation during the process of freezing and thawing was proposed more than half a century ago (Hamm, 1961), however, some initial studies did not confirm irreversible denaturation of proteins due to the process itself. Nevertheless, scientists have over the years tried to prove the phenomenon of freezing-induced protein denaturation with various analytical parameters, and evidence from literature showing the relationship between freezing-thawing and denaturation of myofibrillar and sarcoplasmic proteins, respectively, is summarized in Table 2 and Table 3.

3.2. Solubility and extractability

In initial studies loss in the solubility or extractability of meat proteins has been used as the most popular measurement to estimate the freezing-induced protein changes. Many authors have over the years reported a significant decline in solubility or extractability of myofibrillar proteins in freezing-thawing (Chan, Omana, & Betti, 2011; Qi et al., 2012; Tuell et al., 2020). Sarcoplasmic protein solubility was observed to reduce in frozen-thawed lamb (Penny, 1975) and beef (Awad, Powrie, & Fennema, 1968) as compared to fresh. However, a distinct increase ($P < 0.05$) has been reported by Chan et al. (2011) in turkey breast meat after freezing and thawing.

3.3. Surface hydrophobicity

The surface of the myosin filaments is more hydrophilic, whereas the inner core is described as a hollow structure which contains more hydrophobic groups. Protein unfolding or rupture of the hydrophobic core in the myosin filaments often cause an exposure of inner hydrophobic groups to the surface, thus contributing to surface hydrophobicity (Lin & Park, 1998). Surface hydrophobicity has often been used to indicate protein denaturation induced by freezing and thawing of muscle food. Qian et al. (2019), Xia, Kong, Xiong, and Ren (2010) and Zhang and Ertbjerg (2019) have focused on myofibrillar proteins and found that freezing and thawing significantly increased surface hydrophobicity. Regarding sarcoplasmic proteins, however, Chan et al. (2011) found decreased surface hydrophobicity and they attributed it to reduced exposure of hydrophobic groups on the surface since soluble proteins possibly became more folded or aggregated during freezing and thawing.

3.4. Ca^{2+}-ATPase activity

Globular heads of myosin molecules harbor ATPase activity, and therefore any structural alterations in the myosin head due to external conditions could negatively influence ATPase activity. During freezing and thawing, myofibrillar proteins, especially myosin molecules, are susceptible to the freezing-induced denaturation as indicated by a decline in Ca^{2+}-ATPase activity (Khan & van den Berg, 1967; Xia, Kong, Liu, & Liu, 2009). For instance, Wagner and Añón (1985) found that Ca^{2+}-ATPase activity decreases by 47% in beef during the freezing-thawing process. Also Chan et al. (2011) reported a 35% loss of Ca^{2+}-ATPase activity in frozen-thawed turkey compared to fresh.

3.5. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) has been extensively used to investigate the thermal stability of protein structures in muscle foods, especially in cooked meat products (Martens, Stabursvik, & Martens, 1982). Three major endothermic peak transitions detected in the DSC thermogram indicate protein denaturation patterns (Fig. 1) in which the first peak has been ascribed to myosin denaturation, the second peak corresponds to sarcoplasmic protein and collagen denaturation, and the third peak to actin denaturation (Stabursvik & Martens, 1980). Freezing and thawing have been reported to reduce the thermal stability of the myosin structure as evidenced by reduced myosin peak temperature (Jia, Nirasawa, Ji, Luo, & Liu, 2018; Xia et al., 2010) or decreased denaturation enthalpy (Jia et al., 2017; Wagner & Añón, 1985). As compared to myosin, actin and sarcoplasmic proteins are less sensitive with respect to the freezing-induced thermal destabilization (Jia et al., 2017; Xia et al., 2010).

Table 2
Summary of evidence from literature indicating myofibrillar protein denaturation occurring during freezing and thawing.

Species	Freezing conditions	Denaturation evidence	Reference
Pork BF and SD	Frozen for three weeks then thawed at 4 °C	Protein solubility↓	Tuell et al. (2020)
Pork LTL	Frozen for two weeks then thawed overnight	Denaturation enthalpy↓ Peak temperature↓ Surface hydrophobicity↑ WHC capaciy of myofibrils↓	Zhang and Ertbjerg (2019)
Beef LTL	Frozen for 7 days then thawed at 4 °C	Protein solubility↓ Surface hydrophobicity↑ α-helix↓ Ionic and hydrogen bonds↓	Qian et al. (2019)
Pork tenderloin	Freezing then thawed immediately	Peak temperature↓ Particle sizes↑ Fluorescence intensity↓	Jia et al. (2018)
Pork LTL	Frozen for a week then thawed overnight	WHC capaciy of myofibrils↓	Zhang and Ertbjerg (2018)
Pork tenderloin	Freezing then thawed under 20 °C for 1 h	Peak temperature↓ Denaturation enthalpy↓	Jia et al. (2017)
Pork LTL	Frozen for 7 days then thawed at 4 °C for 12 h	Dityrosine↑ α-helix↓ Fluorescence emission wavelength↑ UV second derivative spectra↑	Zhang et al. (2017)
Chicken breast	Frozen for a week then thawed for 12 h	Denaturation enthalpy↓	Ali et al. (2015)
Lamb LTL	Frozen for 12 h then thawed for 12 h	Protein solubility↓	Qi et al. (2012)
Turkey breast	Frozen for 3 weeks then thawed overnight	Ca^{2+}-ATPase activity↓ Protein solubility↓ Total sulfhydryl content↓	Chan et al. (2011)
Pork LTL	Frozen for 4 days then thawed using running water	Denaturation enthalpy↓ Peak temperature↓ Surface hydrophobicity↑ Emulsifying activity↓	Xia et al. (2010)
Pork LTL	Frozen for 4 days then thawed using tap water	Total sulfhydryl content↓ ATPase activity↓	Xia et al. (2009)
Lamb LTL	Frozen for 1 day then thawed for 16 h	The SDS-PAGE bands (30- and 32-kDa) intensity↑	Ojeda, Wagner, and Crupkin (2001)
Beef LTL	Frozen for 2–3 days then thawed for 7 h	Water-binding capacity↓	Petrović et al. (1993)
Beef SD	Frozen until reaching −25 °C then thawed overnight	Denaturation enthalpy↓ ATPase activity↓	Wagner and Añón (1985)
Beef round	Frozen for 2 weeks then thawed	Actomyosin solubility↓	Awad et al. (1968)
Chicken PM and BF	Frozen then thawed immediately	ATPase activity↓ Sulfhydryl groups→	Khan and van den Berg (1967)

LTL *M. Longissimus thoracis et lumborum*; SD *M. semitendinosus*; PM *M. pectoralis major*; BF *M. biceps femoris*. '↑', increase; '↓', decrease; '→' no significant effect.

Table 3
Summary of evidence from literature indicating sarcoplasmic protein denaturation occurring during freezing and thawing.

Species	Freezing conditions	Denaturation evidence	Reference
Pork LTL	Frozen for two weeks then thawed overnight	Denaturation enthalpy↓ Fluorescence intensity↑	Zhang and Ertbjerg (2019)
Chicken breast	Frozen for a week then thawed for 12 h	Zeta-potential of exudate↓	Chen et al. (2017)
Lamb LTL	Frozen for 12 h then thawed for 12 h	Protein solubility↓	Qi et al. (2012)
Turkey breast	Frozen for 3 weeks then thawed overnight	Protein solubility↑ Surface hydrophobicity↓	Chan et al. (2011)
Pork LTL	Frozen for 3 days then thawed for 11 h	Protein band (97 kDa) intensity by electrophoresis↓	Hansen et al. (2003)
Pork LTL	Frozen then thawed within 7 days	Protein concentration in drip↓	Penny (1975)
Beef round	Frozen for 2 weeks then thawed	Protein solubility↓ Protein content in drip↑ Insolubilization in electrophoresis↓	Awad et al. (1968)

LTL *M. longissimus thoracis et lumborum.*
'↑', increase; '↓', decrease; '→' no significant effect.

3.6. Changes of the myofibrillar structure and intermolecular interactions

Freezing-induced denaturation often destabilizes the secondary structure of myofibrillar proteins. Decreased α-helix and increased β-sheet content following freezing-thawing have been observed by Qian et al. (2019) in beef and by Zhang, Li, Diao, Kong, and Xia (2017) in pork. Mohammed et al. (2021) found decreased concentrations of free amino acids in beef during frozen storage following inoculation of microorganisms. Increasing numbers of freezing and thawing cycles of pork reduced the content of free amino groups and sulfhydryl groups, and more dityrosine bonds were formed in the myofibrillar proteins (Xia et al., 2010; Zhang et al., 2017). This indicates that changes in the protein primary structure occurred during freezing-thawing. Furthermore, freezing and thawing have been reported to disrupt the intermolecular interactions within myofibrillar proteins as indicated by

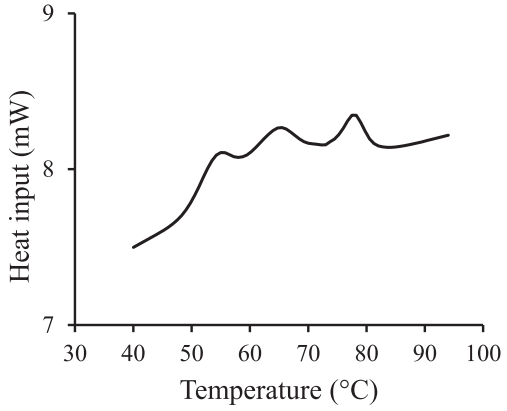

Fig. 1. Representative DSC thermogram for pork muscle showing three major endothermal peaks. Data from Zhang and Ertbjerg (2019).

decreased hydrogen bonds and ionic bonds (Qian et al., 2019). The relationship between protein oxidation and thaw loss during frozen storage of muscle foods has recently been reviewed (Bao, Ertbjerg, Estévez, Yuan, & Gao, 2021).

3.7. Spectroscopic indicators and other techniques

Protein often contains amino acid residues such as tryptophan, tyrosine, and phenylalanine which present intrinsic fluorescence attributes, and any conformational changes in the protein structure could alter the fluorescence intensity and local mobility of these aromatic amino acids (Royer, 2006). The process of freezing and thawing might induce myofibrillar protein unfolding and consequently an exposure of the partially buried tryptophan to a polar environment, which would then influence the fluorescence spectra of tryptophan residues as evidenced by a shift of emission wavelength (Zhang et al., 2017) and a decrease of fluorescence intensity (Jia et al., 2018) using intrinsic emission fluorescence spectroscopy. Some studies have also used UV derivative spectroscopy to monitor the changes of micro-environment within aromatic amino acids in relation to freezing and thawing (Cao et al., 2018; Zhang et al., 2017). New nondestructive techniques to evaluate the degree of freeze damage to meat proteins are constantly being developed. Egelandsdal et al. (2019) has suggested a role of microwave spectroscopy in detecting protein denaturation and aggregation in frozen-thawed pork, and these authors also observed different bioimpedance spectra and low-field nuclear magnetic resonance (NMR) spectra between fresh and frozen-thawed samples. Barbin, Sun, and Su (2013) has successfully applied NIR hyperspectral imaging to distinguish between fresh and frozen-thawed pork muscle. Raman spectroscopy has been reported to predict meat quality traits in lamb (Fowler, Schmidt, van de Ven, Wynn, & Hopkins, 2015). Also multispectral imaging is sensitive to the changes in minced beef when associated with freezing and thawing (Ropodi, Panagou, & Nychas, 2018).

3.8. Traditional theories to explain mechanisms behind freezing-induced protein denaturation

The conventional mechanisms to explain the freezing-induced protein denaturation were recognized in early reviews (Shenouda, 1980; Xiong, 1997) and were mainly based on the mechanical damage due to ice crystal formation and the effect of concentrating solutes in the unfrozen water.

3.8.1. Mechanical damage to protein structure due to ice crystal formation

It is well recognized that water molecules within the meat tissue often play an important role in maintaining hydrogen bonds and hydrophobic interactions in the three-dimensional protein conformation (Lewin, 1974; Puolanne & Halonen, 2010). Freezing usually brings about ice crystallization at the expense of water molecules within the meat tissue thus causing damage to muscle structures and dehydration of muscle fibers (Wang et al., 2020). In the dehydrated state, a rupture of the hydrogen bond network and an increased exposure of hydrophobic and hydrophilic groups on the protein surface are then expected to occur, thus leaving these regions more unprotected, consequently contributing to more hydrophobic interactions between protein molecules. These changes due to ice crystal formation in freezing would then potentially destabilize the protein three-dimensional structure, inducing denaturation and resultant aggregation. Qian et al. (2019) has reported myofibrillar protein denaturation in frozen-thawed beef as indicated by decreased hydrogen bonds coupled with increased surface hydrophobicity.

3.8.2. Increase in solute concentration in the unfrozen water

An increase in ionic strength has been recognized as the foundation of one of earliest theories to explain the freezing-induced protein denaturation. As freezing progresses, a high percentage of the tissue water will be crystallized, and simultaneously, solutes will become progressively more concentrated in the remaining unfrozen water (Calvelo, 1981; Li et al., 2018). Finn (1932) has reported a high ionic strength during freezing of beef juice at −15 °C which corresponded to around 3 M potassium chloride. This might influence the electrostatic interactions within the protein native structure, consequently inducing protein denaturation. Salts at comparatively low concentrations often have a solubilizing effect on protein molecules. For example, Wu et al. (2016) has reported increased solubilities of myofibrillar proteins in pork with increasing NaCl concentrations from 0.2 to 0.8 M. At very high ionic strength, above 1.5, salting-out may occur and further cause precipitation of proteins. Lin and Park (1998) has observed reduced protein solubility in salmon when being exposed to high ionic strength. Moreover, Thorarinsdottir, Arason, Geirsdottir, Bogason, and Kristbergsson (2002) has found decreased thermal stability of myosin and actin during salting of cod, indicating a role of salt in destabilizing protein conformation.

4. Protein denaturation in relation to WHC of meat

4.1. Role of structural alterations within the meat tissue related to denaturation

The WHC of meat is to a large extent dependent on the properties of the myofibrils, which is particularly shown in changes of the myofibrillar volume or more specifically, unit cell volume, i.e. sarcomere length × lattice area (Offer & Knight, 1988a). Factors that can induce lateral shrinkage within the myofibrillar structure often induce water loss from meat (Hughes, Oiseth, Purslow, & Warner, 2014). In the case of pale, soft and exudative (PSE) meat, the combination of temperature and pH i.e. high temperature and low pH induces denaturation of myofibrillar proteins, consequently contributing to myofibrillar shrinkage, and this has been believed as the main mechanism accounting for the extensive loss of WHC in PSE meat (Kim, Warner, & Rosenvold, 2014; Zhu, Ruusunen, Gusella, Zhou, & Puolanne, 2011). The alterations within the myosin structure in PSE meat, as indicated by reduced ATPase activity, could possibly change the shape of the cross-bridges resulting in a shortening in the length of myosin molecules, thus contributing to decreased distances between the filaments and a subsequent shrinkage within the whole myofibrillar structure (Liu, Arner, Puolanne, & Ertbjerg, 2016; Walker & Trinick, 1986). Consequently, water would then flow into the enlarged extracellular space between the fibers and the fiber bundles where it can easily be lost afterwards (Swatland, Irving, & Millman, 1989).

Accordingly, the observation in frozen-thawed meat of decreased distance between filaments (Martino & Zaritzky, 1988) and enlarged extracellular space (Ishiguro & Horimizu, 2008; Tippala et al., 2021) would be accompanied with shrinkage of the muscle fibers, which can be attributed to the migration of water from the intra- to the extracellular space due to freezing-induced protein denaturation. Freezing at −10 °C was thus found to cause a reduction in the length of attached myosin molecules in extracted actomyosin filaments (Jarenback & Liljemark, 1975). Also ATPase activity and DSC measurements (Section 3.1) have clearly suggested that conformational changes are occurring in the myosin area due to freezing-thawing. With regard to the characteristics of WHC of meat, the main explanation of PSE meat is that denaturation of myosin caused myofilamental lattice shrinkage and consequently reduced WHC of meat as discussed above. Likewise, the structural alterations within the myofibrillar structure occurring in freezing-thawing (Bao et al., 2021) thus potentially indicate a role of freezing-induced denaturation of myosin filaments causing reduced WHC of meat upon thawing.

4.2. Roles of pH and ionic strength

Myosin filaments are negatively charged at meat pH of around 5.5.

Myofibrillar proteins play an important role in WHC, and Hamm (1972) hypothesized that the electrostatic force between the myofilaments is influencing the myofibrillar volume and consequently affects the amount of water that can be held within the myofilaments. Offer and Knight (1988a), however, emphasized a role of osmotic force within the filament lattice in myofibrils. The osmotic force created due to the uneven distribution of ions is expected to pull water molecules into the filament lattice thus contributing to a better WHC. Both pH and ionic strength greatly contribute to WHC within the meat tissue (Offer & Knight, 1988a; Puolanne & Halonen, 2010), and a typical influence of pH and NaCl on the myofilament within the sarcomere is illustrated in Fig. 2. The average isoelectric point (pI) of myofibrillar proteins is close to pH 5.0, where the amounts of the positive charges are equal to that of the negative charges thus causing zero net charge on myofilaments and minimum myofilamental space. A shift of pH would then cause higher net charge of myofibrillar proteins, thereby causing greater myofilamental distances (Fig. 2) and better WHC. As reviewed by Puolanne and Halonen (2010), Na^+ often interacts with the negatively charged amino acids on the protein side chains, while Cl^- can easily be absorbed by the positively charged groups on the outer surface. Cl^- as anionic chaotrope could also be absorbed to the hollow and hydrophobic core within the myosin filaments, and therefore, Cl^- (rather than Na^+) has been proposed to be preferably bound to myofilaments. The contribution of ionic strength to the myofibrillar structure closely relates to pH. As shown in Fig. 2, the addition of NaCl increases the electrostatic repulsion within the myofilaments at pH above the pI, while it has a reducing effect at pH below the pI. In postmortem meat with an ultimate pH at around 5.5, added salts are thus expected to increase the negative charges within the myofilaments thereby introducing swelling of the myofibrillar systems and consequently better WHC (Knight & Parsons, 1988; Puolanne & Halonen, 2010).

Increased ionic strength would be expected to decrease interactions between myofibrillar proteins. As demonstrated by Puolanne and Halonen (2010), the absorption of Cl^- to the hydrophobic core of myosin filaments would potentially rupture the shaft inner core and then expose the hydrophobic groups to water thus inducing swelling and possibly even promoting the dissolution of myosin filaments into free myosin molecules. Simultaneously, a disruption of electrostatic linkages caused by the changes of the charges on the myofilaments would also lead to conformational changes possibly favoring hydrophobic interactions within protein molecules and consequently protein denaturation (Lin & Park, 1998).

The role of low pH and high ionic strength in meat tissue has been recognized by Puolanne and Peltonen (2013), and they observed a larger effect on WHC caused by changing the pH from 5.4 to 4.8 than the ionic strength from 0.5 to 1.5. The ultimate pH in postmortem pork muscle is generally around 5.5, and a shift towards the acidic side would reduce the number of negative charges of the myofilaments (Hamm, 1986). As reported by Sharedeh, Gatellier, Astruc, and Daudin (2015), reducing pH from 5.4 to 4.3 in beef tissue strongly denatured myofibrillar proteins as indicated by lower protein solubility and higher surface hydrophobicity, possibly affecting the myofilamental structure and the negative charges on the protein surface.

During freezing to below −20 °C more than 90% of the water will be crystallized, theoretically resulting in a > 10-fold increase of solute concentration in the unfrozen liquid. Increased solute concentration in the unfrozen water during freezing would possibly induce a higher exposure of hydrophobic groups than that occurred in nonfrozen meat in the myosin filaments, consequently supporting protein denaturation and the resultant decreased WHC (Section 3.2.2). However, elevated ionic strength would also increase the electrostatic repulsion within the myofilaments contributing to better WHC, as discussed above. As shown by Jiang et al. (2019), salting before freezing conversely reduced the microstructural damages during freezing as well as maintained better WHC and textural properties of thawed meat. The combinations of decreased pH and high ionic strength have been found to cause myofibrillar protein denaturation and consequently decreased WHC of meat tissue (Puolanne & Peltonen, 2013; Zhang, Sun, Chen, Liu, & Kong, 2021) and of the isolated myofibrils (Zhang & Ertbjerg, 2019). The decreased pH is thus expected to cause reduced negative charges within the myofilaments (Fig. 3) inducing protein denaturation and consequently loss of WHC. The protein changes observed in myofibrils isolated from frozen-thawed pork meat have been reproduced in nonfrozen meat by exposure to combinations of decreased pH and high

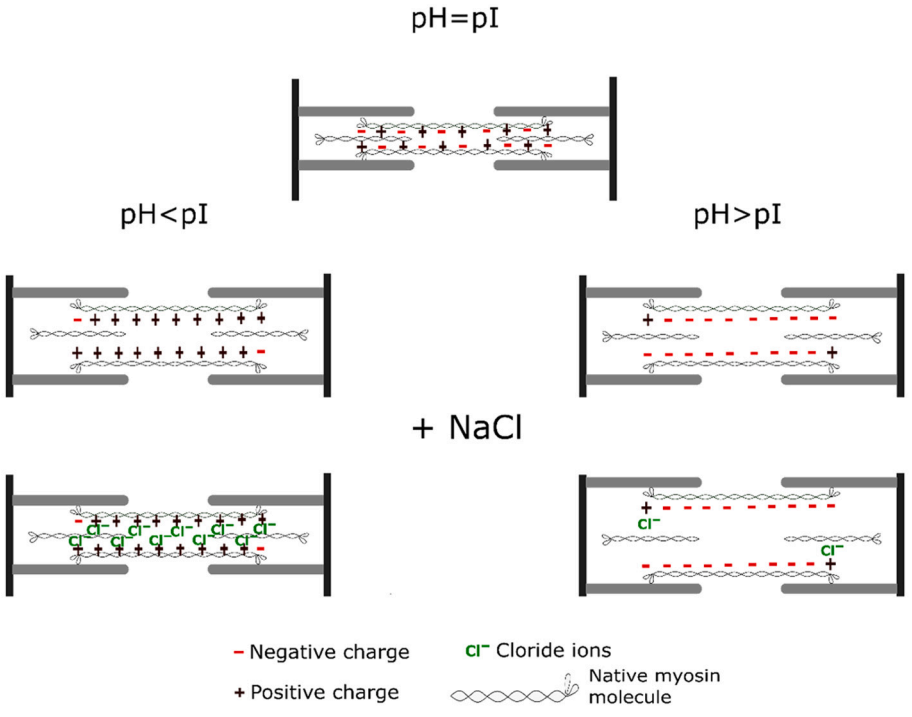

Fig. 2. Illustration of the effect of the combination of pH and NaCl on swelling of a myofilament within the sarcomere (pI, isoelectric point).

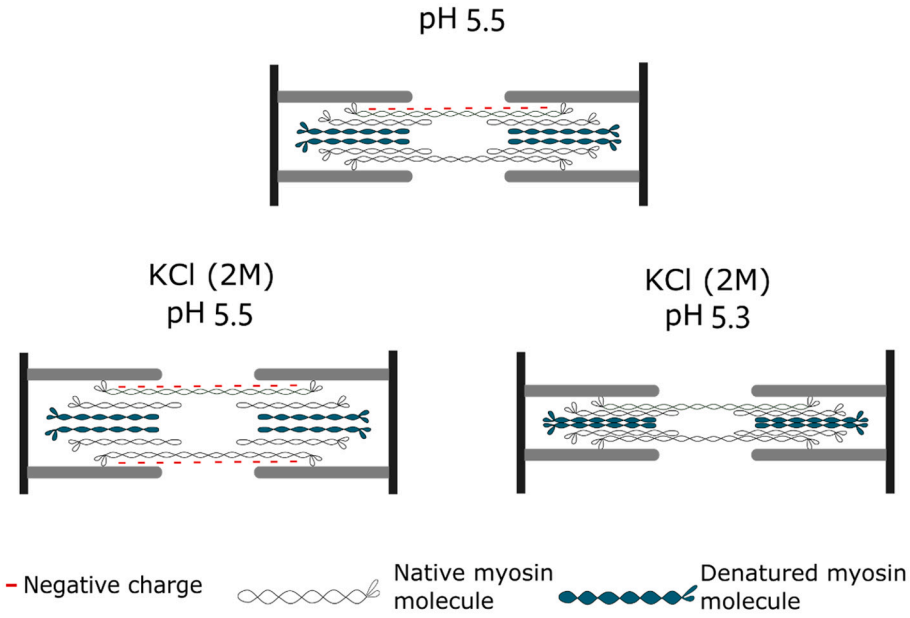

Fig. 3. Illustration of the exposure of a myofilament to the combination of high ionic strength (2 M KCl) and a lower pH (5.3) within the sarcomere as described by Zhang et al. (2021b). (A) Within a myofilament, myosin molecules are net negatively charged at pH 5.5 and low ionic strength. (B) The exposure to 2 M KCl increases negative charge density within a myofilament, therefore contributing to repulsion of myosin molecules and transverse swelling of the sarcomere. (C) The exposure to a pH-KCl combination reduces negative charge density (no negative charges are shown), resulting in transverse shrinkage within the myosin molecules and consequently the sarcomere.

ionic strength (Zhang, Sun, et al., 2021), and it was found a decreased WHC of myofibrils and increased surface hydrophobicity when exposure to high salt (2 M KCl) with lower pH (<5.5), which indicates more pronounced denaturation occurring within the myofibrillar proteins. As observed by Chen et al. (2017), frozen-thawed chicken breast showed decreased absolute Zeta potential values, indicating reduced negative charges in the proteins during freezing-thawing, possibly in relation to a decreased pH. Tan, Ye, and Xie (2021) has observed a decrease of pH in the unfrozen water when freezing myofibrillar protein solution from 4 to −20 °C. In addition, the structural alterations observed in frozen-thawed meat that muscle fibers shrunk accompanied with decreased spaces between thick filaments (Section 2.2) seem also to support reduced electrostatic repulsion in relation to pH decline.

5. Hypotheses for the formation of thaw loss

5.1. Current explanation based on ice crystal formation in relation to freezing rate

Freezing of meat and meat products inevitably leads to the formation of thaw loss upon thawing. It is often accepted that ice crystal formation during freezing and the resultant mechanical damage to the muscle fiber structure (Bao et al., 2021) induce the loss of WHC and consequently contribute to the formation of thaw loss (Añón & Calvelo, 1980; Hamm, 1986; Li et al., 2018). Freezing rate has been recognized as the decisive factor in the size and distribution of ice crystals that form inside or outside of the muscle fibers. The characteristic freezing time (t_c), defined as the time to pass through the critical temperature zone at a given spot (Añón & Calvelo, 1980), has been used to measure how the freezing rate affects ice crystal formation mainly based on histological analysis (Grujić et al., 1993; Kim, Kim, et al., 2018). Fast freezing, recognized as $t_c < 15$ min, forms numerous small ice crystals distributed uniformly in the intra- and extracellular spaces within the muscle structure. Slow freezing at $t_c > 23$ min, however, often produces large extracellular ice crystals located unevenly between the muscle fibers causing more migration of water from inside to outside of the muscle fibers (Grujić et al., 1993; Kim, Kim, et al., 2018). Consequently, slow freezing induced more evident structural damages to muscle fibers as compared to fast freezing (Hou et al., 2020; Ishiguro & Horimizu, 2008). This has been well recognized to explain the observation that slow freezing generated more thaw loss as compared to fast freezing (Farouk et al., 2003; Hou et al., 2020). Additionally, larger mechanical damage shown as a rupture of muscle fibers in slow freezing is also believed to negatively affect the reabsorption of water by muscle fibers upon thawing, as compared to smaller damage caused by fast freezing (Hamm, 1986). During freezing-thawing cycles, the muscle fiber structure would then repeatedly be exposed to the mechanical damage caused by ice crystallization, consequently causing a higher amount of thaw loss (Jiang, Jia, et al., 2019). Also temperature fluctuations during frozen storage has been found to increase the size of large ice crystals at the expense of small crystals (Bevilacqua & Zaritzky, 1982), leading to the recrystallization of ice crystals and further more damage to muscle structure (Wang et al., 2020).

5.2. Contribution of myofibrillar protein denaturation in relation to freezing rate

Freezing and thawing induces myofibrillar protein denaturation in muscle food (Table 2). Any physicochemical alteration of myofibrillar proteins causing a reduction in the amount of water held by the myofibrillar structure potentially contributes to the formation of thaw loss (Zhang & Ertbjerg, 2019). The rate of freezing strongly influences the amount of thaw drip and the current explanation for the different amounts of thaw drip in different freezing rates is primarily in relation to the size and location of ice crystals as discussed above. As reviewed by Hamm (1986) and Leygonie et al. (2012), the contribution of the freezing-induced myofibrillar protein denaturation to the decrease in WHC of meat upon thawing has been estimated as small, and several studies on freezing rate seem not to have observed a distinct influence on protein denaturation (Farouk et al., 2003; Ngapo et al., 1999). It was, however, noted by other studies that slow freezing resulted in a more pronounced denaturation of the myofibrillar proteins compared to fast freezing, and evidence investigated by different methodologies from different animals and muscles is summarized in Table 4. For instance, Tuell et al. (2020) and Petrović et al. (1993) both reported decreased values of myofibrillar protein solubility when pork or beef muscle was subjected to slow freezing as compared to fast freezing. Moreover, more severe myofibrillar protein denaturation in frozen-thawed meat has been reported in slow compared to fast freezing as indicated by an increased surface hydrophobicity, decreased ATPase activity and reduced denaturation enthalpy (Khan & van den Berg, 1967; Wagner & Añón, 1985; Zhang & Ertbjerg, 2019). Upon thawing, the more

Table 4
Summary of evidence from literature indicating more pronounced myofibrillar protein denaturation in slow than fast freezing.

Denaturation indicator	Species	Reference
Protein solubility	Pork BF and SD	Tuell et al. (2020)
	Beef LTL	Petrović et al. (1993)
ATPase activity	Beef SD	Wagner and Añón (1985)
	Chicken PM and BF	Khan and van den Berg (1967)
Surface hydrophobicity	Pork LTL	Zhang and Ertbjerg (2019)
Denaturation enthalpy and peak temperature on DSC	Pork LTL	Zhang and Ertbjerg (2019)
	Beef SD	Wagner and Añón (1985)
Water distribution on NMR	Pork LTL	Mortensen et al. (2006)
WHC of myofibrils	Pork LTL	Zhang and Ertbjerg (2019)
WHC of meat	Pork SM, SD, BF	Ku et al. (2014)
	Beef LTL	Petrović et al. (1993)
Protein content in thaw drip	Chicken PM and BF	Khan and van den Berg (1967)
	Chicken breast	Khan (1966)
Near-infrared spectroscopy	Pork LTL	Xie, Sun, Zhu, and Pu (2016)

LTL *M. longissimus thoracis et lumborum*; SD *M. semitendinosus*; SM *M. semimembranous*; PM *M. pectoralis major*; BF *M. biceps femoris.*

pronounced denatured structural proteins in slow freezing will reabsorb less water and consequently lead to a decreased WHC (Ku et al., 2014; Petrović et al., 1993), which could then potentially explain the observation of a larger amount of thaw loss in slow compared to fast freezing (Zhang & Ertbjerg, 2019). The amount of the thaw loss differs among animal species. Freezing of fish has been observed to produce thaw loss being less than 4% (Jiang, Nakazawa, Hu, Osako, & Okazaki, 2019; Li, Zhao, Muhammad, Song, & Liu, 2020; Samantaray et al., 2021), which is lower than that has been reported in beef or pork (Kim & Kim, 2016; Setyabrata & Kim, 2019). The post-rigor pH of fish is around 6.5–7.0, and thereby higher than pH 5.5–5.7 in beef or pork. Therefore, we expect that the pH in the non-frozen water fraction during freezing of fish will be higher and the freezing-induced protein denaturation would be less, consequently causing less amount of thaw loss. In agreement, Defreitas, Sebranek, Olson, and Carr (2010) has observed that pork sausages of high pH showed a lower amount of drip after freezing and thawing than sausages of low pH. The rigor state has been reported by Kim et al. (2012) to affect the formation of thaw loss and that freezing of pre-rigor duck breast showed a larger thaw loss as compared to post-rigor. PSE meat during frozen storage was more susceptible to the shift in the distribution of muscle water compared with dark, firm and dry (DFD) meat, as reported by Bertram, Andersen, and Andersen (2007). This could also be explained based on the influence of physiological conditions of meat before slaughter on the muscle pH after slaughter and the subsequent pH in the non-frozen water fraction during freezing.

5.3. Effects of aging prior to freezing and thawing rates on WHC of meat

Postmortem aging is one of the most common meat industry practices, where fresh meat is stored at chilling temperatures for a certain period of time (days to weeks). Considerable improvements in meat quality attributes, particularly, increased meat tenderness and WHC with extended aging have been reported by numerous studies. Proteolytic enzymes such as calpains, cathepsins, caspases and/or proteasome weaken the overall structure of the myofibril by degrading cytoskeletal myofibrillar proteins, subsequently increasing meat tenderization during aging (Kim et al., 2018). Furthermore, increased myofibrillar protein degradation can result in improving WHC by minimizing the rigor-induced lateral shrinkage of myofibrils associated with the formation of drip and also enable the inflow of previously expelled water (Huff-Lonergan & Lonergan, 2005; Zeng, Li, & Ertbjerg, 2017). In this regard, significant positive impacts of aging prior to freezing on WHC (by decreasing either purge or drip loss) of aged/frozen/thawed meat (beef, lamb, pork and venison) have been reported (Kim et al., 2015; Kim & Kim, 2016; Kim, Kim, et al., 2018; Kim, Ma, et al., 2018; Kim, Meyers, et al., 2017; Setyabrata & Kim, 2019). Increases in muscle fragmentation and protein solubility through the elevated proteolysis during aging could result in more flexible and tolerant properties of muscle cells to the cryo-damage induced by the ice crystal formation (Setyabrata, Tuell, & Kim, 2019), possibly leading to the decrease in the protein denaturation as well. In fact, improvements in protein functionality (e.g. total and myofibrillar protein solubility, emulsifying capacity, and gelling ability) of aged meat prior to freezing have been also reported (Choi et al., 2017).

Thawing rates can also affect meat quality attributes of aged/frozen meat. Although there are some inconsistent results, it has been suggested that fast thawing at low temperature would result in better frozen/thawed meat quality attributes by reducing mobility and loss of both immobilized and free water through the accelerated phase transition from ice to water, consequently minimizing the damage to the myofibrillar protein structure (Zhang, Sun, et al., 2021). However, in our preliminary study, we found a significant interaction between freezing rate and thawing rate, and thus the extent of thawing impact on meat quality can be vary depending upon the previous aging and/or freezing conditions (unpublished results). Given there are several new thawing techniques (e.g. high-pressure thawing, ultrasound-assisted immersion thawing, ohmic thawing, immersion solution thawing, microwave thawing etc.), further research looking into the effects of different thawing rates coupled with previous aging/freezing conditions on protein denaturation and WHC of meat would be beneficial to advance our understanding and develop practical post-handling strategies of frozen meat for the meat industry.

6. Conclusions

Freezing and thawing of meat inevitably induce formation of thaw loss. The present review summarizes studies about the process of freezing and thawing on WHC. The meat structure as well as myofibrillar protein characteristics are considered to explore the role of myofibrillar protein denaturation in causing the generation of thaw loss. In general, freezing and thawing induce myofibrillar shrinkage, decrease WHC of meat resulting in increased thaw loss, drip loss and cooking loss, as well as increased myofibrillar protein denaturation evidenced by changes in protein solubility, surface hydrophobicity, Ca^{2+}-ATPase activity or denaturation enthalpy. The relationship between WHC and protein denaturation may provide a novel perspective to understand the mechanism for the generation of thaw loss. The structural alterations within the meat tissue in relation to a decreased pH and increased ionic strength that occur in freezing-thawing potentially indicate the role of myofibrillar protein denaturation in reducing WHC of meat upon thawing. Slow freezing often generates more thaw loss than fast freezing, and previous explanations primarily focused on ice crystal formation in relation to the freezing rate. However, this paper emphasizes the effect of the freezing rate on myofibrillar protein denaturation in which slow freezing develops more severe protein denaturation than fast freezing. This also offers an explanation for why thaw loss is more in slow compared to fast freezing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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