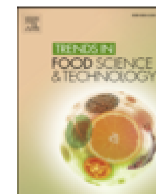
Trends in Food Science & Technology 57 (2016) 40–51

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: http://www.journals.elsevier.com/trends-in-food-science-and-technology



Review

# Hydrolysates from marine sources as cryoprotective substances in seafoods and seafood products

Mehdi Nikoo [a, *], Soottawat Benjakul [b], Kaveh Rahmanifarah [a]

[a] Department of Fisheries, Faculty of Natural Resources, Urmia University, Urmia, West Azerbaijan 5756151818, Iran
[b] Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

ARTICLE INFO

*Article history:*
Received 8 May 2016
Received in revised form
11 September 2016
Accepted 11 September 2016
Available online 14 September 2016

*Keywords:*
Marine hydrolysates
Cryoprotective effect
Myofibrillar proteins
Seafood products
Frozen storage

ABSTRACT

*Background:* During freezing or frozen storage, a number of chemical changes including protein denaturation and lipid oxidation inevitably occur in seafoods. Denaturation of muscle proteins induced by freezing process is associated with deterioration, especially the loss in functional properties and consumer acceptability. To mitigate such a problem the cryoprotectants have been employed widely. Some of the widely used cryoprotectants such as sucrose impart a sweet taste in the products which consumers often consider undesirable. As a consequence, it has led to a comprehensive investigation for alternative cryoprotective substances.
*Scope and approach:* Recently, hydrolysates from different marine sources such as fish, crustaceans and cephalopods have been produced and used to maintain the quality of seafood proteins during the extended frozen storage. This review covers the recent understanding and trends in the uses of marine hydrolysates as potential alternative cryoprotectants to prevent protein denaturation in seafoods and seafood products.
*Key findings and conclusions:* Hydrolysates from chitin or proteins have been demonstrated to retard the denaturation of myofibrillar proteins by lowering the modification of proteins structure or by inhibiting the formation of denaturation products such as carbonyls and protein cross-links. Therefore, functional properties of protein associated with quality of seafoods and seafood products can be maintained. As a consequence, the potential protein/chitin hydrolysate based cryoprotectant can be widely used and the drawback of carbohydrate based cryoprotectant can be tackled.
© 2016 Elsevier Ltd. All rights reserved.

## 1. Introduction

Freezing and frozen storage have been widely used to retain sensory quality and nutrients of seafoods and their products. Some 50% of the total processed seafoods consumed as well as 21% of total seafood production were offered to markets in the form of frozen products (Gonçalves, Nielsen, & Jessen, 2012). Although microbial spoilage is effectively terminated, quality deterioration, especially in texture, flavor and color, still take place during freezing and frozen storage due to the osmotic removal of water, myosin denaturation, mechanical damage, as well as cross-linking and aggregation of myofibrillar proteins (Leygonie, Britz, & Hoffman, 2012). Being main concern in seafood industry, temperature fluctuation and repeated freeze-thawing contribute to deterioration in physicochemical and textural changes in seafoods and their products (Andersen & Jørgensen, 2004).

Protein denaturation of seafoods and seafood products due to freezing or frozen storage occurs in the order of decreasing solubility of myofibrillar proteins (mainly myosin), disappearance of ATP-induced contraction of muscle fiber, and lowering of myosin ATPase activity (Benjakul & Visessanguan, 2011). To prevent changes in functionality and structure of myofibrillar proteins, cryoprotective substances are commonly added to ensure maximum functionality of frozen seafood products. Cryoprotectants are compounds that help in minimizing the denaturation and/or aggregation of myo brillar proteins during freezing or extended frozen storage (MacDonald & Lanier, 1997). They maintain functional properties, such as gel-forming ability, water holding capacity and solubility of proteins. Thus, the shelf life of frozen seafoods can be extended through the use of cryoprotective substances (Ma, Zhang, Deng, & Xie, 2015; Wu, Pan, & Wang, 2014; Zhou, Benjakul, Pan, Gong, & Liu, 2006). Sugars, sugar alcohols

* Corresponding author.
*E-mail address:* m.nikoo@urmia.ac.ir (M. Nikoo).

http://dx.doi.org/10.1016/j.tifs.2016.09.001
0924-2244/© 2016 Elsevier Ltd. All rights reserved.

and polyphosphates have been well known for their cryoprotective properties against the freezing-induced denaturation of seafood myofibrillar proteins (Herrera & Mackie, 2004; Kong et al., 2013; Okazaki & Kimura, 2014). These substances could prevent the withdrawal of water from the protein molecules, increase the surface tension of water as well as the amount of bound water and prevent the loss of protein solubility (Benjakul & Visessanguan, 2011). The cryoprotective activity of lactitol, litesse, sucrose and sorbitol (Herrera & Mackie, 2004) as well as trehalose and sodium lactate (Osako, Hossain, Kuwahara, & Nozaki, 2005; Wu et al., 2014; Zhou et al., 2006) have been reported. However, those carbohydrate-based cryoprotectants with their sweet taste would not be healthy by consumers suffering from diabetes or by other consumers (Cheung, Liceaga, & Li-Chan, 2009; Sych, Lacroix, Adambounou, & Castaigne, 1991). As a consequence, searching for alternative cryoprotective substances with low- or non-sweet taste has gained increasing attention in recent years.

Several studies have demonstrated that some natural compounds that derived from marine sources after acid or enzyme hydrolysis could inhibit the denaturation of seafood myofibrillar proteins during frozen storage and act as potential cryoprotectants. Those natural compounds which fall into two main groups i.e. oligosaccharides and protein hydrolysates have been extracted from different marine sources such as fish, crustaceans and cephalopods (Cheung et al., 2009; Hossain et al., 2004; Karnjanapratum & Benjakul, 2015; Kittiphattanabawon, Benjakul, Visessanguan, & Shahidi, 2012; Korzeniowska, Cheung, & Li-Chan, 2013; Limpisophon et al., 2014; Nikoo, Benjakul, Ehsani, et al., 2014; Somjit, Ruttanapornwareesakul, Hara, & Nozaki, 2005; Yamashita, Zhang, & Nozaki, 2003; Yanan, Li, Hua, Wei, & Meilan, 2012).

To monitor the changes in seafoods, particularly denaturation of muscle proteins, as well as to evaluate the cryoprotective effects of any compounds, $Ca^{2+}$-ATPase activity, surface hydrophobicity, total sulfhydryl group content, enthalpy of unfolding, thermal transition midpoint and denaturation products such as carbonyl derivatives and intra- and intermolecular cross-links are tested. Furthermore, changes in proteins functionality with regards to losses in protein solubility, gelling properties, water holding capacity, the amount of unfrozen water and water associated with myofibrils are also evaluated especially using some advanced devices. For better understanding on the use of marine hydrolysates as potential cryoprotective substances in seafoods and seafood products, the update information is revisited.

## 2. Mechanism of protein denaturation in seafoods and seafood products during frozen storage

Fish and shellfish muscle contains different types of proteins such as sarcoplasmic, myofibrillar and stroma proteins (Harnedy & FitzGerald, 2012). Myofibrillar proteins constitute 65–75% of the total proteins and are soluble in high salt solutions (Shahidi, 1994). The two major myofibrillar protein, myosin and actin, are responsible for muscle contraction-relaxation cycles. Myosin which is the most abundant fraction of these proteins constitutes 50–60% of its total amount and forms the thick myofilaments while actin, ranging from 15 to 20%, is the main component of the thin filaments (Gökoğlu & Yerlikaya, 2015). Myofibrillar proteins play a significant role in seafood products technological, nutritional and sensory properties. During freezing and frozen storage of seafoods, myofibrillar proteins undergo a number of changes including denaturation and aggregation. Being the main structural protein in seafoods, such changes have resulted into the modifications of myofibrillar proteins conformation, alterations in protein functionalities, such as viscosity, gelation, emulsification, solubility, and water-holding capacity occurs (Liu & Xiong, 2000).

The decrease in protein solubility is a primary criterion of protein denaturation during frozen storage. The alteration of protein extractability is a useful factor which may be used to determine the textural quality of seafood products, as protein aggregation is accompanied by a significant decrease in their solubility (Badii & Howell, 2002). A decrease in total sulphydryl group content is due to the formation of disulphide bonds through oxidation of sulphydryl groups or disulphide interchanges. The formation of disulphide bond which results in the aggregation of proteins might have contributed to low solubility of proteins (Cheung et al., 2009; Sriket, Benjakul, Visessanguan, & Kijroongrojana, 2007). White shrimp and black tiger shrimp had the decrease in solubility as the freeze-thaw cycles increased (Sriket et al., 2007). In frozen cod, protein solubility decreased significantly as the storage progressed, indicating the formation of protein aggregates (Badii & Howell, 2002). Lower loss of sulphydryl groups in seafood products or myofibrillar proteins containing marine hydrolysates might be due to lower oxidation. Seafoods and seafoods products without cryoprotective substances have shown a reduction in protein solubility and sulphydryl groups during frozen storage. Kittiphattanabawon et al. (2012) determined the cryoprotective effects of shark gelatin hydrolysate in surimi. In their study, the results of total sulphydryl group measurements correlated well with solubility of surimi proteins during freeze-thaw cycles.

The formation of ice crystals during freezing damages the ultrastructure of fish muscle which, in turn, leads to alterations in the biochemical reactions that occur at the cellular level and influence the physical quality parameters of the fish (Leygonie et al., 2012). As ice crystallization takes place, extracellular salt becomes concentrated, leading to differences in osmotic pressure gradient across cell membrane. Protein denaturation thus occurs due to dehydration of muscle cells and an increased intracellular ionic strength following the migration of intracellular water to the extracellular spaces (Gonçalves et al., 2012; Leygonie et al., 2012). Consequently, myofibrillar proteins losses the ability to hold water and thus exudate or free water is released (Andersen & Jørgensen, 2004), associated with losses of some nutrients and flavoring compounds. Therefore, to ensure high quality, it is necessary that no water in the seafoods or seafood products freezes during frozen storage (Gudjónsdóttir et al., 2011). Hydrolysates obtained from different marine proteins or chitin after enzymatic treatment stabilized water associated with myofibrils during frozen storage (Cheung et al., 2009; Hossain et al., 2004; Nikoo, Benjakul, & Xu, 2015). The amount of unfrozen water increased significantly during frozen storage of seafood products containing chitin hydrolysates or protein hydrolysates. This leads to structural stabilization of myofibrillar proteins and maintained their functionality (Yamashita, Zhang, & Nozaki, 2012).

During freezing or frozen storage of seafoods and seafood products, protein oxidation also occurs and this leads to a wide range of modifications, especially the formation of carbonyl compounds (Lund & Baron, 2010; Medina & Pazos, 2010). The formation of protein carbonyls from particular amino acid side chains is related with the conformational changes of myofibrillar proteins, leading to fragmentation, aggregation, loss of solubility and functionality. Estévez (2011) noted that muscle quality such as changes in water-holding capacity (WHC), texture, flavor and nutritional value could be influenced by the formation of protein carbonyls during frozen storage.

Fish myosin, the major myofibrillar protein, is particularly susceptible to denaturation during the extended frozen storage. Globular heads of this protein are responsible for ATPase activity and are sensitive to alteration in the configuration induced by freezing or thawing processes. During frozen storage, denaturation of myosin leads to the decrease in $Ca^{2+}$-ATPase activity mainly due

to the conformational changes of the myosin globular head as well as the aggregation of this portion. Additionally, the rearrangement of protein via protein–protein interactions induced by freezing or frozen storage also contributes to the loss in ATPase activity (Benjakul, Visessanguan, & Tueksuban, 2003). Thus, $Ca^{2+}$-ATPase activity is commonly used as an index to determine the quality of myofibrillar proteins during frozen storage or temperature fluctuations (Limpisophon et al., 2014). Sulfhydryl groups are considered the most reactive functional group in proteins, being oxidized to disulfide bond during the frozen storage of fish and fish products. The formation of disulfide bonds through oxidation of sulfhydryl groups or disulfide interchanges is coincidental with the decreases in total and surface sulfhydryl contents (Liu, Chen, Kong, Han, & He, 2014). The oxidation of sulfhydryl groups located in the head portion of myosin plays a role in the loss in $Ca^{2+}$-ATPase activity (Benjakul, Visessanguan, Thongkaew, & Tanaka, 2005). In frozen surimi, oxidation of sulfhydryl groups and the decrease in $Ca^{2+}$-ATPase activity was retarded by the addition of gelatin hydrolysate with cryoprotective effect and this was coincidental with the decreased disulfide bond formation (Kittiphattanabawon et al., 2012).

## 3. Development of cryoprotective hydrolysates from marine sources

During seafood processing, a considerable proportion of fish and shellfish is generated as leftovers, including head, fins, skin, roe, viscera and shell. Under-utilized or by-catch species can also serve as alternative proteinaceous or chitinous sources for development of marketable value-added substances. Olsen, Toppe, and Karunasagar (2014) reported that seafood by-products can constitute even 70% of fish and shellfish weight after industrial processing. Due to the increasing need for effective utilization of by-products from the seafood processing industries or under-utilized marine resource, many researchers emphasized, in particular the recovery or production of natural compounds from those resources to bring about the value-added products with market demand (Halim, Yusof, & Sarbon, 2016; Ordóñez-Del Pazo et al., 2014; Chalamaiah, Kumar, Hemalatha, & Jyothirmayi, 2012). Cryoprotective substances were extracted from different marine sources including fish (Cheung et al., 2009; Kittiphattanabawon et al., 2012; Yanan et al., 2012; Korzeniowska et al., 2013; Nikoo, Benjakul, Ehsani, et al., 2014; Limpisophon et al., 2014; Karnjanapratum & Benjakul, 2015; Nikoo, Benjakul, & Xu, 2015), shrimp (Dey, Dora, Raychaudhuri, & Ganguly, 2013; Ruttanapornvareesakul et al., 2006; Somjit et al., 2005), squid (Yamashita et al., 2003; Hossain et al., 2003; 2004), krill (Zhang, Yamashita, & Nozaki, 2002), lobster (Yamashita et al., 2003) and crab (Darmanto, 2003; Yamashita et al., 2003, 2012).

Several studies have shown that the skin of fish can be used for production of gelatin hydrolysates with cryoprotective activities in seafood products (Limpisophon et al., 2014). Gelatin is a heterogeneous mixture of peptides derived from the parent protein collagen (Boran & Regenstein, 2010; Nikoo, Benjakul, Bashari, et al., 2014). Heating collagen in water mainly cleaves hydrogen bonds; this destabilizes the triple helix by means of a helix-to-coil transition, leading to its conversion into soluble gelatin (Benjakul, Kittiphattanabawon, & Regenstein, 2012; Sai-Ut, ongjareonrak, & Rawdkuen, 2012). Thermal or enzymatic hydrolysis of gelatin can yield the hydrolysate containing peptides with cryoprotective activity. Composition and cryoprotective properties of gelatin hydrolysates can be governed by the processing parameters such as skin pretreatment, gelatin extraction, and hydrolysis conditions. The types of enzyme used in the hydrolysis of gelatin as well as hydrolysis parameters are very important, governing the size, composition and amino acid sequence of the peptides, which in turn influences their cryoprotective ability in frozen seafood (Karnjanapratum & Benjakul, 2015; Kittiphattanabawon et al., 2012; Nikoo, Benjakul, et al., 2015). Besides fish skin, under-utilized fish species have been used to produce protein hydrolysates with cryoprotective activity. Cheung et al. (2009) and Korzeniowska et al. (2013) hydrolyzed minced whole Pacific hake (*Merluccius productus*) using different commercial proteases and found their cryoprotective effects on natural actomyosin subjected to different freeze-thaw cycles. In their study, the types of proteases used and hydrolysates concentration influenced the efficacy of hydrolysates in preventing freeze-induced denaturation of seafood products.

The shells of crustaceans and cephalopods, which are generated during processing and could pose environmental burden, can serve as another marine source for production of compounds with cryoprotective function. Chitin is a natural linear polysaccharide that consists of β-(1,4)-linked *N*-acetyl glucosamine (GlcNAc) and is abundantly present as a structural compound in shells of crustaceans such as shrimp, crab, crayfish, lobster and cephalopods (Kazami et al., 2015). Due to the rigid crystal structure and water-insolubility of chitin which limited its potential utilization as cryoprotectant in seafoods and seafood products, the application of chitin hydrolysates which is water-soluble has been studied (Somjit et al., 2005; Yamashita et al., 2012). The oligosaccharides produced by acid hydrolysis showed advantages in retardation of freeze-induced denaturation of seafood proteins and increased the amount of unfrozen water in frozen/thawed myofibrils. Owing to the low sweetness, they have been demonstrated as alternative cryoprotectant in seafoods and seafood products. Table 1 shows the chemical composition and properties of hydrolysates produced from different marine raw materials.

## 4. Characteristics and roles of cryoprotective hydrolysates

Protein hydrolysates and chitin hydrolysates derived from various fish species, crustaceans and cephalopods have been shown to serve as potential alternative to carbohydrate-based cryoprotective compounds commonly used to stabilize frozen seafood products. The cryoprotective effects of protein hydrolysates and chitin hydrolysates have been influenced by several factors, associated with the differences in their interaction with myofibrillar proteins.

One of the most important characteristic of protein hydrolysates and chitin hydrolysates, influencing the cryoprotection in seafood products is the presence of hydrophilic amino acids in the sequence of peptides. During freezing or frozen storage, the formation of ice crystal can destruct the hydration layer around polar groups of the myofibrillar proteins and this leads to unfolding of protein molecule as well as hydrophobic interactions between unfolded domains. The interaction occurred between the hydrated water molecules of the myofibrillar protein and the polar groups of the hydrophilic peptides stabilized the bound water associated with myofibrillar protein resulting in more amounts of unfrozen water in products. This lowered structural alteration of these proteins. Hydrophilic amino acids such as proline, asparagine, glutamine, serine, histidine, arginine, glycine, threonine in the sequence of peptides showed the water binding property, resulting in lowering the migration of water to form ice crystals. This leads to stabilization of water molecules (Chen, Morikawa, & Hashimoto, 2005; Kittiphattanabawon et al., 2012). Types of proteases, proteinaceous/chitinous raw materials and hydrolysis conditions determine the size of peptides and their mechanism of action (Benajakul, Yarnpakdee, Senphan, Halldorsdottir, & Kristinsson, 2014). Besides the size of peptides, the composition of amino acids as well as

**Table 1**
Chemical composition and properties of hydrolysates from different marine raw materials.

| Cryoprotective substances | Raw material | Source | Molecular weight (Da) | Degree of polymerization | Sugar substances (g/100 g dry matter) | Protein (%) | Lipid (%) | Ash (%) | Sodium chloride (%) | References |
|---|---|---|---|---|---|---|---|---|---|---|
| Chitin hydrolysate | Shell | Black tiger prawn | 351.55 | 1.59 | 99.33 | <0.004 | 0.06 | 0.61 | <0.002 | Somjit et al. (2005) |
| | | Endeavour prawn | 383.03 | 1.73 | 99.13 | <0.004 | 0.06 | 0.81 | <0.002 | |
| | | Giant freshwater prawn | 420.01 | 1.90 | 99.23 | <0.004 | 0.07 | 0.70 | <0.002 | |
| | | King crab | 458.52 | 2.07 | 99.71 | <0.004 | 0.17 | 0.11 | _ | Yamashita et al. (2003) |
| | | Japanese fan lobster | 449.43 | 2.03 | 99.65 | <0.004 | 0.21 | 0.13 | _ | |
| | Cartilage | Spear squid | 450.23 | 2.04 | 99.42 | <0.004 | 0.39 | 0.18 | _ | |
| Protein hydrolysate | Scrap[a] | Horse mackerel | 1250 | _ | 0.01 | 85.83 | 0.23 | 8.70 | <0.01 | Khan et al. (2003) |
| | | Chub mackerel | 1000 | _ | 0.02 | 82.74 | 0.17 | 9.37 | <0.01 | |
| | | White croaker | 1250 | _ | 0.03 | 85.11 | 0.26 | 7.69 | <0.01 | |
| | | Sardine | 875 | _ | 0.01 | 83.05 | 0.17 | 8.67 | <0.01 | |
| | | Sardine | 875 | _ | 0.03 | 82.32 | 0.18 | 9.16 | <0.01 | |
| | Mince | Swordtip squid | 300–1400 | _ | 3.32 | 87.67 | 0.07 | 6.99 | <0.01 | Hossain et al. (2003) |
| | | Japanese flying squid | 300–1400 | _ | 3.40 | 87.72 | 0.26 | 6.07 | <0.01 | |
| | | Bigfin reef squid | 300–1400 | _ | 3.40 | 87.92 | 0.21 | 6.13 | <0.01 | |
| | Head | Northern pink shrimp | _ | _ | 4.58 | 89.79 | 0.02 | 4.72 | _ | Ruttanapornvareesakul et al. (2006) |
| | | Endeavour shrimp | _ | _ | 2.99 | 91.47 | 0.01 | 5.20 | _ | |
| | | Black tiger shrimp | _ | _ | 3.61 | 91.00 | 0.01 | 5.09 | _ | |

[a] Mixture of head, viscera, scale, skin, caudal fin and bone.

their positioning in the chain greatly determine in the mechanism of cryoprotection.

Gelatin hydrolysates with the unique motif have been considered as the potential cryoprotectant. Gelatin basically contains glycine, alanine, proline and hydroxyproline as the major amino acids (Karim & Bhat, 2009). The presence of glycine-X-Y (main constituent of collagen α-chain) is associated with high cryoprotective effect of hydrolysate and peptidic fractions (Damodaran, 2007). Peptides with such an amino acid composition or sequence were able to inhibit the formation of extracellular ice in the products during frozen storage and thus protected proteins against freeze denaturation (Cheung et al., 2009; Ruttanapornvareesakul et al., 2006). This was evidenced by the minimized water displacement between the different compartments (i.e. from the intra-to the inter-myofibrillar compartment) and higher amount of intra-myofibrillar water retained in the product containing peptides after several temperature fluctuations (Nikoo et al., 2015a). Several studies have demonstrated that the amino acid composition of gelatin was influenced by the type of species (Gomez-Guillen et al., 2002), the processing of raw material or the conditions used for gelatin extraction (i.e. time and temperature) (Nagarajan, Benjakul, Prodpran, Songtipya, & Kishimura, 2012). Amino acid composition of fish skin is influenced by the habitat temperature and hence gelatin hydrolysates from cold-water or warm-water fishes with differences in their collagen amino acid composition will exhibit different cryoprotective ability in the products. Flavourzyme hydrolysate derived from the skin of farmed Amur sturgeon with higher amounts of Asp, Glu, Ser, Gly, His, Arg, Thr, Pro, and Lys potentially protected myofibrillar proteins against frozen-induced denaturation and loss in functionality (Nikoo et al., 2015a). Differences in the cryoprotective ability of gelatin hydrolysates with different peptide size produced from the skin of different shark species were also reported (Kittiphattanabawon et al., 2012; Limpisophon et al., 2014).

When considering cryoprotective effect of chitin hydrolysate, it was suggested that the hydrophilic hydroxyl residues released from the main chain of chitins play the important role in the hydrolysates-protein–water interaction. Chitin hydrolysates from shrimp shell stabilized the water molecules surrounding myofibrillar proteins by bonding with them and convert free water to bound water (Somjit et al., 2005).

## 5. Use of hydrolysates as alternative cryoprotectants in seafoods and seafood products

Hydrolysates derived from marine sources including chitin-derived oligosaccharides and hydrolysates have been considered as potential alternatives to commercial cryoprotectants which add unwanted sweetness to seafood products. Hydrolysates from fish and shrimp leftovers have been employed to maintain the quality of frozen seafoods. In Table 2, marine sources used for production of cryoprotective compounds, production methods, seafood products and denaturation indices are summarized.

Yanan et al. (2012) investigated the effect of eel head protein hydrolysates prepared after single-step of two-step hydrolysis on the denaturation, gel strength and contents of the salt soluble protein in grass carp surimi stored at −20 °C for 80 days. Ca-ATPase activity in the control decreased drastically to 33.66% of the initial value right after freezing. The activity was then gradually decreased and reached 3.32% of the initial value, showing a biphasic denaturation pattern. Surimi with 10% of either hydrolysate had higher Ca-ATPase activity and higher amount of salt soluble proteins. The head of the three species of shrimp (northern pink shrimp, endeavour shrimp, black tiger shrimp) was hydrolyzed by various proteases and the cryoprotective effects of the resultant protein hydrolysates was investigated in surimi by measuring Ca-ATPase activity and the strength of the myofibrillar protein gel. At the end of frozen storage at −25 °C for 180 days, the residual Ca-ATPase activity in surimi without protein hydrolysates was 23.31% of the initial value while those with added protein hydrolysates the activity remained higher than 50% throughout the storage (Ruttanapornvareesakul et al., 2006). Additionally, Dey et al. (2013) reported the denaturation/aggregation inhibiting characteristics of shrimp waste protein hydrolysate on croaker fish surimi protein during frozen storage. Waste protein hydrolysate displayed protective effect through decreasing the loss of $Ca^{2+}$- ATPase activity in myofibrillar protein. The residual $Ca^{2+}$-ATPase activity of actomyosin from control, waste protein hydrolysate and sucrose + sorbitol blend surimi samples were 57.54, 99.15 and 100.84% respectively on the 20th day of storage and remained higher than 50% throughout the storage period except control in which only 10.24% of the activity was maintained. Surimi with 7.5 and 10% SWPH showed cryoprotective effect similar to sucrose-sorbitol mixture. The degradation of myosin heavy chain in surimi in the presence of

**Table 2**
Hydrolysates from different marine sources used as cryoprotective substances for seafoods and seafood products.

| Marine source | Species | Production methods | Cryoprotective substances | Seafood products | Storage condition | Denaturation indices | Reference cryoprotectant | References |
|---|---|---|---|---|---|---|---|---|
| Fish | | | | | | | | |
| | White croaker (*Argyrosomus argentatus*), horse mackerel (*Trachurus japonicas*), flying fish (*Cypselurus heterurus*), chub mackerel (*Scomber japonicas*), sardine (*Sardinops melanostictus*) | Scrap was mixed with double volumes of distilled water, boiled to inactivate endogenous enzymes, homogenized; pH was adjusted to 8.0 for hydrolysis by protease from *Bacillus subtilis* (0.1%) for 2 h and then adjusted to 6.0 for hydrolysis by protease from *Aspergillus oryzae* (0.1%, 2 h). | Protein hydrolysates | Lizardfish (*Saurida wanieso*) surimi | Frozen storage at −25 °C for 180 days | $Ca^{2+}$-ATPase activity, unfrozen water, water holding capacity (WHC) | – | Khan et al. (2003) |
| | Pacific hake (*Merluccius productus*) | Slurry of minced whole fish in water (1: 2 w/v) was heated to 50 °C, then 3% (v/w protein) of Alcalase or Flavourzyme was added and reaction was conducted for 1 h | Alcalase (FPH-A) and flavourzyme (FPH-F) hydrolysates | Pacific cod (*Gadus microcephalus*) mince | Freeze-thaw cycles (18 h freezing at −25 °C, 6 h thawing at 4 °C, 6 cycles) | Surface hydrophobicity, unfrozen water, expressible moisture (EM) | Sucrose + sorbitol (1:1, w/w) | Cheung et al. (2009) |
| | Eel | Heads were steamed (40 min) mixed with distilled water (1:4 w/v), crushed, pH was adjusted to 9.0 and hydrolyzed with Alcalase (48 AU/kg for 2 h, (single hydrolysis SH). SH was then hydrolyzed with papain at pH 6.5 for 4 h to obtain hydrolysate with two kinds of enzyme (TH). | Head protein hydrolysates (SH and TH) | Grass carp (*Ctenopharyngodon idella*) surimi | Frozen storage at −25 °C for 80 days | $Ca^{2+}$-ATPase activity, salt soluble protein | 4% sugar, 4% sorbitol and 0.3% compound phosphate | Yanan et al. (2012) |
| | Blacktip shark (*Carcharhinus limbatus*) | Gelatin (protein concentration of 30 g $L^{-1}$) was added with crude enzyme from papaya latex to obtain DH of 5, 10, 20 and 30%. Hydrolysis was taken place at 40 °C for 1 h, then enzyme was inactivated by heating at 90 °C for 15 min. | Gelatin hydrolysate with 10% DH | Threadfin bream (*Nemipterus spp.*) surimi | Freezing at −20 °C and thawing with tap water for 30 min; 6 cycles | $Ca^{2+}$-ATPase activity, protein solubility, Surface hydrophobicity, total sulfhydryl group content, disulfide bonds | 6% sucrose + 2% sorbitol | Kittiphattanabawon et al. (2012) |
| | Amur sturgeon (*Acipenser schrenckii*) | hydrolysate was fractionated using Sephadex G-15 column and fraction III was further separated with RP-HPLC | Tetrapeptide Pro-Ala-Gly-Tyr (MW = 405.99 Da) | Japanese sea bass (*Lateolabrax japonicas*) mince | Freeze-thaw cycles (18 h freezing at −18 °C, 6 h thawing at 4 °C; 6 cycles) | Thermal properties of proteins (DSC), Water populations (LF $^{1}H$ NMR) | – | Nikoo et al. (2014) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Blue shark (*Prionace glauca)* | Gelatin (2.25%; w/v) was hydrolyzed with 10,000-fold diluted Alcalase at a ratio of 13.31:1 (v/v) at 60 °C for 15–120 min. | Gelatin hydrolysates obtained after 90 min (SSGH-90) and 120 min (SSGH-120) hydrolysis time | Alaska Pollock (*Theragra chalcogramma*) surimi | Frozen at −25 °C for 135 days | Ca-ATPase activity, liquid water, gel strength | Bovine skin gelatin hydrolysate (BSGH), mixture of amino acids from shark gelatin (SA) and bovine skin (BA) | Limpisophon et al. (2014) |
| | Pacific hake (*Merluccius productus*) | Hake mince slurry (1:2 mince to water ratio) was added 1% Flavourzyme (w/w protein) and reacted for 1 h at 50 °C. The slurry was heated to 90 °C for 15 min then centrifuged at 3300 g. The supernatant was freeze-dried to obtain FPH. | Flavourzyme hydrolysate (FPH-F) | Pacific cod (*Gadus microcephalus*) natural actomyosin (NAM) | Freeze-thaw cycles (12 h at −25 °C, 12 h at 4 °C, 6 cycles) | Surface hydrophobicity, thermal properties, gel texture | Sucrose + sorbitol (1:1, w/w) | Korzeniowska et al. (2013) |
| | Unicorn leatherjacket (*A. monoceros*) | Autolysed non-swollen (NS) and swollen (0.1 M phosphoric acid/6 h) (SS) skins were prepared. NS and SS solutions (3%, w/v) were reacted with glycyl endopeptidase from *Papaya latex* (8%, at 40 °C for 60 min. | Non-swollen gelatin hydrolysate (NS-GH) | Short-bodied mackerel (*Rastrelliger brachysoma*) washed mince | Freezing at −20 °C and thawing with tap water for 30 min; 6 cycles | $Ca^{2+}$-ATPase activity, Surface hydrophobicity, disulfide bonds | Sucrose + sorbitol (1:1, w/w) | Karnjanapratum and Benjakul (2015) |
| | Amur sturgeon (*Acipenser schrenckii*) | Gelatin was hydrolyzed using Alcalase (pH 8.0 and 50 °C) or Flavourzyme (pH 7.0 and 50 °C) at an enzyme to substrate ratio of 1:20 for 3 h | Alcalase (PH-A) and flavourzyme (PH-F) hydrolysates | Japanese sea bass (*Lateolabrax japonicas*) mince | Freeze-thaw cycles (18 h freezing at −18 °C, 6 h thawing at 4 °C, 6 cycles) | Total sulfhydryl group content, protein carbonyls, thermal properties (DSC), Water populations (LF $^{1}$H NMR) | 4% sucrose + 4% sorbitol (w/w) | Nikoo et al. (2015b) |
| Shellfish | | | | | | | | |
| | *Penaeus monodon*, *Metapenaeus endeavouri*, *Macrobrachium rosenbergii* | Chitin was stirred with 12 N HCl for 7 h, hydrolyzed sample was centrifuged (20 min), supernatant was neutralized (25% NaOH), desalted sample was subjected to ultrafiltration using a MW cutoff at 1 × $10^4$ Da | Chitin hydrolysate | Lizardfish (*Saurida wanieso*) myofibrils | Frozen storage at −25 °C for 6 months | $Ca^{2+}$-ATPase activity, unfrozen water | Glucose (5%) or sucrose (5%) | Somjit et al. (2005) |
| | *Pandalus eous*, *Metapenaeus endeavouri*, *Penaeus monodon* | Heads were mixed with distilled water (1:2 w/v), heated at 90 °C for 30 min, homogenized, pH was adjusted to 8.0 for hydrolysis by protease from *Bacillus subtilis* (0.1%) for 2 h and then adjusted to 6.0 for hydrolysis by protease from *Aspergillus oryzae* (2 h, 0.1%) | Head protein hydrolysate (SHPH) | Lizardfish (*Saurida wanieso*) myofibrils | Frozen storage at −25 °C for 180 days | $Ca^{2+}$-ATPase activity, unfrozen water, gel strength | Sodium glutamate (Na-Glu) | Ruttanapornvareesakul et al. (2006) |

(*continued on next page*)

**Table 2** (*continued*)

| Marine source | Species | Production methods | Cryoprotective substances | Seafood products | Storage condition | Denaturation indices | Reference cryoprotectant | References |
|---|---|---|---|---|---|---|---|---|
| | *Fenneropenaeus monodon*, *F. indicus* | Freeze-dried waste powder was suspended (1:1, w/v) in distilled water and its pH was adjusted to 8.5. Hydrolysis was done using Alcalase at 60 °C for 2 h. | Shrimp waste protein hydrolysate | Croaker fish (*Johnius gangeticus*) surimi | Frozen storage at −25 °C for 120 days | Ca-ATPase activity, actin and myosin degradation, unfrozen water, gel texture | 4% sucrose + 4% sorbitol + 0.3% sodium tri-polyphosphate (STPP) | Dey et al. (2013) |
| | King crab (*Paralithodes camtschatica*), Japanese fan lobster, (*Ibacus ciliates* | Shell chitin powder was mixed with 12 N HCl and hydrolyzed for 195 min at 40 °C, reaction was neutralized by a 25% NaOH and subjected to ultrafiltration using a MW cutoff at 1 × $10^4$ Da, the filtrate was desalted and spray-dried. | Chitin hydrolysate | Lizard fish myofibrillar protein | Frozen storage at −25 °C for 120 days | $Ca^{2+}$-ATPase activity, unfrozen water | – | Yamashita et al. (2003) |
| Cephalopods | Spear squid (*Doritheus blekeri*) | Cartilage chitin powder was mixed with 12 N HCl and hydrolyzed for 195 min at 40 °C, reaction was neutralized by a 25% NaOH and subjected to ultrafiltration using a MW cutoff at 1 × $10^4$ Da, the filtrate was desalted and spray-dried. | Chitin hydrolysate | Lizard fish myofibrillar protein | Frozen storage at −25 °C for 120 days | $Ca^{2+}$-ATPase activity, unfrozen water | – | Yamashita et al. (2003) |
| | Japanese flying squid (*Todarodes pacificus*), swordtip squid (*Loligo Edulis*) | Squids mince was mixed with distilled water (1:2 w/v), heated at 90 °C for 30 min, homogenized, pH was adjusted to 8.0 (0.1 N NaOH) for hydrolysis by protease from *Bacillus subtilis* (0.2%) for 2 h and then adjusted to 6.0 for hydrolysis by protease from *Aspergillus oryzae*. Hydrolysate was subjected to ultrafiltration (30,000 Da MWCF), desalting and freeze-drying. | Squid protein hydrolysate (SPH) | Lizard fish myofibrillar protein | Frozen storage at −25 °C for 90 days | $Ca^{2+}$-ATPase activity, unfrozen water | Sodium glutamate (Na-Glu) | Hossain et al. (2004) |

hydrolysate was significantly reduced as evidenced by inhibiting the aggregation and subsequent insolubilization of myosin.

The potential of Pacific hake protein hydrolysates as alternative to sucrose–sorbitol blend (a commercial cryoprotectant) was studied in frozen cod mince. Myosin and actin became less extractable in salt solutions and formed high molecular weight aggregates during the storage. After 6 freeze–thaw cycles, the control sample showed >50% loss in natural actomyosin extractability and >50% increase in surface hydrophobicity, suggesting the enhanced freeze–denaturation and aggregation of proteins (Table 3). Minces incorporated with commercial cryoprotectant or protein hydrolysate produced using flavourzyme displayed a higher extractable actomyosin (0.72 and 0.68 g/g protein, respectively) than the control sample (0.40 g/g protein). No difference in surface hydrophobicity in actomyosin between two treatments was observed after freeze–thawing.

Limpisophon et al. (2014) assessed the protective effect of gelatin hydrolysates from blue shark skin on denaturation of surimi proteins during the 135 days of frozen storage at −25 °C. Gelatin hydrolysates, especially those hydrolyzed for 90 or 120 min could suppress the loss of Ca-ATPase activity induced by freezing. Ca-ATPase activity in surimi decreased to a range of 47.23–63.65% with either gelatin hydrolysate or amino acids; however a significant loss in activity was observed in surimi without added hydrolysates in which only 32.24% activity was retained. Surimi without the addition of hydrolysates also showed a significant loss in gel strength after 15 days of frozen storage while gel from surimi with additives exhibited stronger network throughout the entire storage.

Korzeniowska et al. (2013) investigated the effect of Pacific hake muscle hydrolysate on physicochemical properties of natural actomyosin from Pacific cod mince subjected to different freeze-thaw cycles Surface hydrophobicity of control and mince added with 2% protein hydrolysate increased after 6 freeze–thaw cycles which could be a result of unfolding of the proteins and exposure of hydrophobic residues. Surface hydrophobicity of protein with 8% protein hydrolysate did not change even after freeze–thaw process, demonstrating that the myofibrillar proteins were stabilized from conformational changes.

Apart from protein hydrolysate from fish and shrimp, protein hydrolysates prepared by hydrolysis of squid muscle using proteases from *Bacillus subtilis* and *Aspergillus oryzae* having molecular weight ranging from 300 to 1400 Da showed the prevention of freeze-induced denaturation of lizardfish myofibrillar proteins. In myofibrillar protein without the addition of squid hydrolysate, $Ca^{2+}$-ATPase activity decreased gradually and reached 15.8% at the end of the 90 days frozen storage while the inactivation markedly decreased throughout the freezing period by the addition of hydrolysate. The cryoprotective activity of swordtip squid hydrolysate was higher than that of Japanese flying squid more likely due to the structure and composition of the constituent peptides (Hossain et al., 2004).

Fish skin hydrolysates have gained increasing attention as the potential cryoprotectant which can exhibit the protective effect on protein denaturation during frozen storage (Table 3). Karnjanapratum and Benjakul (2015) observed the suppressive effects of unicorn leatherjacket skin gelatin hydrolysates on the inactivation of short-bodied mackerel natural actomyosin $Ca^{2+}$-ATPase activity. Only 10% of the initial activity was retained after 6 freeze-thaw cycles in mince without the addition of hydrolysate, whereas more than 60% residual activity was retained in the presence of gelatin hydrolysates after freeze-thawing process, demonstrating the higher preventive effect of peptides on myosin heavy chain. In addition, gelatin hydrolysate could lower the changes in surface hydrophobicity and disulfide bond formation in actomyosin, compared with the control.

The cryoprotective effect of gelatin hydrolysates from Amur sturgeon skin was determined in Japanese seabass minces stored at −18 °C and subjected to 6 freeze-thaw cycles (Nikoo, Benjakul, et al., 2015). Total sulfhydryl groups decreased with increasing freeze–thaw cycles. Addition of gelatin hydrolysates prepared using alcalase or flavourzyme decreased the changes in sulfhydryl groups induced by freeze–thawing, indicating the protective effect of gelatin hydrolysate toward the oxidation of the sulfhydryl groups. Protective effect of gelatin hydrolysates against protein oxidation was observed as evidenced by the lower protein carbonyls formed.

Kittiphattanabawon et al. (2012) investigated the cryoprotective properties of blacktip shark skin gelatin hydrolysates in threadfin bream surimi subjected to different freeze-thaw cycles. Only 22.9% of the initial $Ca^{2+}$-ATPase activity of natural actomyosin was retained after 6 freeze-thaw cycles, when gelatin hydrolysate was added, especially that with 10% degree of hydrolysis, residual activity higher than 50% at the end of storage. Surimi with this hydrolysate also had higher sulfhydryl groups and solubility concomitant with lower surface hydrophobicity and disulfide bonds than the other treatments.

Chitin hydrolysate has been demonstrated to act as the alternative cryoprotectant. Yamashita et al. (2012) investigated the effect of chitin hydrolysate prepared from shell of crustaceans and cartilage of cephalopods on the denaturation of lizard fish myofibrillar protein during frozen storage at − 25 °C for 120 days. A lower decrease of Ca-ATPase was found during frozen storage by addition of chitin hydrolysate (5.0%) into myofibrillar protein, signifying the cryoprotective effect by lowering protein denaturation.

The suppressive effect of shrimp chitin hydrolysates on freeze-induced denaturation of lizardfish surimi was shown by Somjit et al. (2005). Ca-ATPase activity of control and those with commercial cryoprotectant or the hydrolysates of endeavour, black tiger and giant freshwater shrimp chitin remained at 19.05%, 73.34%, 67.65%, 78.22% and 81.06%, respectively of the initial values after 6 months of storage at −25 °C. Surimi containing giant freshwater shrimp chitin hydrolysate showed the lowest rate constant of freeze-denaturation ($K_D$) ($1.17 \times 10^{-3}$/day); which was lower than those of sucrose ($1.57 \times 10^{-3}$/day) and glucose ($1.72 \times 10^{-3}$/day).

## 6. Advanced analyses of cryoprotective activity of hydrolysates from marine sources

There are several reports about the use of modern techniques for studying the cryoprotective effects of marine hydrolysates in different seafood systems. Those techniques have been successfully utilized as fast analytical methods for determination of denaturation stability of myofibrillar proteins during frozen storage. Differential scanning calorimetry (DSC) and low-field proton nuclear magnetic resonance (LF $^{1}H$ NMR) were the techniques that have been used for determination of cryoptotective effects of hydrolysates produced from different marine sources.

In recent years, differential scanning calorimetry (DSC) technique has been used to determine the thermal properties of seafood myofibrillar proteins during freezing and frozen storage (Beyrer & Klaas, 2007; Matos et al., 2011; Tironi, Tomás, & Añón, 2010). DSC is a thermal analysis devise that determines the temperature and heat flow associated with protein transitions as a function of time and temperature. With DSC, enthalpies ($\Delta H$) of protein unfolding as well as thermal transition midpoint ($T_m$) are measured as a result of heat denaturation. The transition midpoint $T_m$ is defined as the temperature, in which 50% of the proteins possess its native conformation, while the rest remains denatured. In general, there is equilibrium between native (folded) and denatured (unfolded)

**Table 3**
Changes in protein conformation or modification products in seafoods with added marine hydrolysates during the storage conditions.

| Substance | Marine source | Solubility | Ca-ATPase activity (%) | Surface hydrophobicity (%) | Total sulfhydryl groups | Disulfide bonds | Carbonyls | Unfrozen water (g/g) | Intra-myofibrillar water (%) | Thermal transition midpoint ($T_m$) (myosin) | Enthalpy (ΔH) of unfolding | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chitin hydrolysate | King crab, Japanese fan lobster, spear squid | | Inactivation completely suppressed at 5% | | | | | Higher than that of the control at 5.0−7.5% | | | | Yamashita et al. (2003) |
| Chitin hydrolysate | Endeavour shrimp, Black tiger shrimp, Giant freshwater shrimp | | 67.65−81.06% activity after 180 days frozen storage | | | | | Higher than control through storage | | | | Somjit et al. (2005) |
| Protein hydrolysate | Antarctic Krill | | Highest activity at 10% and 12.5% | | | | | Increased 1.3-fold at 5% hydrolysate | | | | Zhang et al. (2002) |
| Protein hydrolysate | Northern pink shrimp, endeavour shrimp, black tiger shrimp | | >50% after 180 days frozen storage | | | | | 1.29−1.36 fold higher than the control | | | | Ruttanapornvareesakul et al. (2006) |
| Protein hydrolysate | Pacific hake | | | 32%, no significant difference than unfrozen mince | | | | 0.36, higher than commercial cryoprotectant | | | | Cheung et al. (2009) |
| Protein hydrolysate | Pacific hake | | | No changes after 6 freeze-thaw cycles | | | | | | 51.1 °C | Greater total peak area than control and commercial cryoprotectant | Korzeniowska et al. (2013) |
| Protein hydrolysate | Indian shrimp, giant tiger prawn | 2.49% after 120 days of storage | >50% throughout frozen storage in contrast to control (10.24% at 120th day) | | | | | 1.38 fold higher than that of the control | | Myosin degradation was to a lesser extent (19.7%) than the control (48%) | | Dey et al. (2013) |
| Gelatin hydrolysate | Blacktip shark | Solubility was similar to commercial cryoprotectant | >50% activity retained after 6 freeze-thaw cycles | Lowest hydrophobicity | Only 14.24% loss after repeated freeze-thawing | Similar to commercial cryoprotectant | | | | | | Kittiphattanabawon et al. (2012) |
| Gelatin hydrolysate | Unicorn leatherjacket | | >60 activity after 6 freeze-thaw cycles | Similar in samples containing hydrolysate, Sucrose + sorbitol of their blend | | Lowest disulfide bonds | | | | | Higher residual ΔH (myosin, actin) compared with the control | Karnjanapratum and Benjakul (2015) |
| Gelatin hydrolysate | Amur sturgeon | | | | Retarded the loss induced by freeze−thawing | | The decrease in carbonyl formation was similar to sucrose-sorbitol | | 98.14%, no change after 6 freeze-thaw cycles | 52.4 °C | 1.70 mj/mg | Nikoo et al. (2015a) |

conformations of a protein molecule. Thus, thermodynamically a protein molecule is more stable when its thermal transition midpoint is higher (Gill, Tohidi Moghadam, & Ranjbar, 2010). Changes in the conformation of protein or its modification generally determine the position, sharpness, and shape of transition as evidenced in the resulting thermogram. During frozen storage, myofibrillar proteins are susceptible to denaturation as indicated by the decreased thermal transition midpoint and enthalpy of unfolding. In general, lower denaturation was obtained in seafood products with added marine hydrolysates.

Using DSC, Karnjanapratum and Benjakul (2015) reported that gelatin hydrolysate produced from the skin of unicorn leatherjacket decreased the denaturation of surimi actomyosin. Higher enthalpy was found in frozen/thawed surimi containing gelatin hydrolysate (8%) that prepared from non-swollen skin. Regarding actin, enthalpy of surimi with added gelatin hydrolysate was higher than that found for myosin, indicating that actin was more stable to frozen-induced denaturation than myosin. Similarly, Amur sturgeon skin gelatin hydrolysates that produced using alcalase and flavourzyme for 3 h had the ability to control myofibrillar protein denaturation as indicated by the higher remained enthalpy and the effect was comparable to commercial cryoprotectant (Nikoo, Benjakul, et al., 2015). In another study, Korzeniowska et al. (2013) investigated the effect of Pacific hake muscle mince protein hydrolysate at different concentrations (2 or 8%) on the thermal properties of natural actomyosin from Pacific cod mince subjected to 6 freeze-thaw cycles. Addition of 8.0% flavourzyme hydrolysate in natural actomyosin increased both the thermal transition midpoint and enthalpy of myosin when compared to freeze–thawed natural actomyosin without hydrolysate (control), indicating the mince proteins were more stable during frozen storage after addition of Pacific hake hydrolysate. When the effect of peptide Pro-Ala-Gly-Tyr on thermal stability of Japanese seabass white muscle mince that subjected to repeated freeze-thawing was studied it was observed that enthalpy of myosin in unfrozen minces ranged from 1.49 to 1.84 mJ $mg^{-1}$ and control mince had significantly lower values than minces incorporated with peptide (Nikoo, Benjakul, Ehsani, et al., 2014). After repeated freeze-thawing, enthalpy of myosin in all treated minces was higher (1.72–2.12 mJ $mg^{-1}$) than that of the control mince (1.55 mJ $mg^{-1}$). The protective effect of the combinations of this peptide at 12.5 ppm and caffeic acid at 50 ppm on mince samples was investigated. After repeated freeze-thawing (6 cycles), the enthalpy of myosin in minces incorporated with peptide and caffeic acid (1.49–1.74 mJ $mg^{-1}$) was higher than that of the control (1.21 mJ $mg^{-1}$) suggesting a higher stability of myosin. Furthermore, a lower enthalpy was found with lower concentration of peptide (i.e. 5 ppm) suggesting that peptide at higher concentration used had better denaturation-inhibiting property (Nikoo, Regenstein, et al., 2015).

Freezing or frozen storage is known to cause aggregation and destruction of myofibrillar proteins leading to loss in functional properties of proteins such as water binding capacity. Thus, to ensure high quality, it is necessary that no water in the seafoods or seafood products freezes during frozen storage (Gudjónsdóttir et al., 2011). With above background, in several studies the content of unfrozen water was considered indicative of the state of water in seafood myofibrils. To determine the changes in the amount of unfrozen water in seafoods and seafood products during the extended frozen storage, differential scanning calorimetry has been successfully applied. Several studies have demonstrated that water in myofibrils is stabilized by marine hydrolysates. Those studies have demonstrated that the amount of unfrozen water associated with myofibrillar proteins increased significantly during frozen storage of seafood products when incorporated with chitin hydrolysates or protein hydrolysates.

Protein hydrolysates prepared from muscle mince of underutilized Pacific hake using commercial enzymes (alcalase and flavourzyme) have been tested for their effect to increase the amount of unfrozen water in cod mince subjected to different freeze-thaw cycles. The mount of unfrozen water were determined to be 0.24, 0.29, 0.34, and 0.34 g/g for unfrozen control and those with added sucrose + sorbitol, alcalase hydrolysate or flavourzyme hydrolysate, respectively. At the end of storage, mince with added hake hydrolysates exhibited higher amount of unfrozen water (0.36 g/g) compared to mince that contained sucrose + sorbitol (0.33 g/g) and control (0.24 g/g) (Cheung et al., 2009). The increase in the amount of unfrozen water in frozen seafoods and seafood products with added chitin hydrolysates was reported by Somjit et al. (2005) and Ruttanapornvareesakul et al. (2006), respectively. Limpisophon et al. (2014) determined the effect of gelatin hydrolysates from the skin of blue shark in stabilizing water molecules that surround surimi proteins. Gelatin hydrolysates reduced the decline in the amount of unfrozen water in surimi stored at - 25 °C for 135 days compared with the control, indicating that peptides generated after hydrolysis of skin collagen stabilized bound water in myofibrils signifying their cryoprotective effects. In addition, Yamashita et al. (2012) investigated the effect of chitin hydrolysate from shell of crustaceans and cartilage of cephalopods on the state of water in lizard fish myofibrillar proteins during 120 days of storage at −25 °C. The amount of unfrozen water in the control was decreased suddenly during frozen storage and showed a biphasic pattern whereas the decrease was moderate in myofibrils containing the chitin hydrolysates. Myofibrils incorporated with chitin hydrolysate maintained a higher amount of unfrozen water compared to the control throughout the storage and could suppress the freeze-induced denaturation by stabilizing water molecules.

In contrast to conventional methods that used to elucidate the relationship between protein conformational changes and their functionality such as water holding capacity, recently low-field proton nuclear magnetic resonance have been successfully applied to seafood products. This technique is less time consuming and costly. In food industry, a common application of this device is the assessment of proton relaxation behavior. In fish and shellfish muscle, two types of relaxations have been recognized. These are longitudinal (spin–lattice or $T_1$) relaxation and transversal (spin–spin or $T_2$) relaxation. Because transversal relaxation component is more sensitive to protein unfolding, it has been widely used to monitor the mobility and distribution of water molecules in different compartments of fish and shellfish muscle (Erikson, Standal, Aursand, Veliyulin, & Aursand, 2012). Several studies have shown how transversal relaxation components were affected by initial quality of seafood raw materials and frozen storage (Carneiro et al., 2013; Gudjónsdóttir et al., 2011; Sánchez-Valencia, Sánchez-Alonso, Martinez, & Careche, 2015).

In fish and shellfish muscle at least two relaxation components are detected. Those components are generally referred to as $T_{21}$ with relaxation times in the range of 40–60 ms and $T_{22}$ with relaxation times in the range of 150–400 ms. A minor relaxation component at about 1–10 ms, referred to as $T_{2b}$ was also observed in fish and shellfish muscle (Erikson et al., 2012). $T_{2b}$ has been ascribed to water tightly associated with macromolecule such proteins. The shorter relaxation time $T_{21}$ relates to water located within organized protein structures (intra-myofibrillar water) and the longer relaxation time $T_{22}$, which cannot be seen in prerigor state, represents water in the space between the myofibrils (or free water). The more mobile $T_{22}$ can be developed during or after rigor mortis and finally can be lost as drip, especially when seafoods or their products have been kept frozen for a period of time (Aursand, Gallart-Jornet, Erikson, Axelson, & Rustad, 2008; Sánchez-Alonso,

Martinez, Sánchez-Valencia, & Careche, 2012).

Low-field $^1$H NMR measurements have shown a significant correlation between the $T_2$ component and water holding capacity (WHC) in fish and meat. A reduction in WHC has been reported to be directly linked to the denaturation of proteins in the muscle fibre structure (Andersen & Jørgensen, 2004). In this regard, Bertram et al. (2007) indicated that $T_2$ relaxation times were reduced when porcine myofibrillar proteins reacted with oxidants (hemoglobin and hydrogen peroxide) and this leads to the reduction in WHC of oxidized myofibrils. A close relationship between degree of Japanese seabass mince oxidation (peroxides and TBARS) and the loss in WHC of myofibrillar proteins as a result of temperature abuse during frozen storage was reported (Nikoo, Regenstein, et al., 2015).

Based on low-field nuclear magnetic resonance analysis, it was revealed that peptide Pro-Ala-Gly-Tyr showing the typical gelatin tripeptide repeating sequence of G-X-Y influenced water distribution in fish mince when being subjected to repeated freeze-thawing process (Nikoo, Benjakul, Ehsani, et al., 2014). A significant decrease in $T_{21}$ population was observed in samples without peptides and this was coincidental with the increase in more mobile $T_{22}$ water pools. The content of intra-myofibrillar water in mince with this peptide at 25 ppm was significantly higher than that of the other treatments, reflecting its cryoprotective effect on muscle proteins by lowering the water mobility induced by freezing and thawing. Furthermore, effect of freeze-thaw cycles on the distribution of water in *Lateolabrax japonicus* white muscle mince was studied. In minces subjected to different freeze–thaw cycles, a significant decrease in the content of intra-myofibrillar water and a simultaneous increase in the content of free water were observed. This indicated the movement of water molecules from the second to third compartment (i.e. from intra-to inter-myofibrillar compartment) induced by repeated freeze–thawing process and was associated with the highest amplitude of free water signal in the relaxation time spectra and the loss of water binding capacity of myofibrillar proteins. In contrast, when gelatin hydrolysates containing di-/tri-peptides and oligopeptides in varying concentrations were added into mince, the amount of both intra-myofibrillar and free water populations did not change. This was further demonstrated that water molecules associated with mince myofibrils were effectively stabilized and this maintained the conformation of proteins and their functionalities (Nikoo, Benjakul, et al., 2015).

## 7. Conclusion

Hydrolysates prepared from enzymatic or chemical hydrolysis of marine proteins and chitins can be used as the alternatives for carbohydrate-based cryoprotectants, which are mostly sweet in taste. Those protein or chitin hydrolysates have been shown to retard the denaturation of seafood myofibrillar proteins during frozen storage or freeze-thawing. Therefore, functional properties of protein associated with quality of seafoods and seafood products were maintained. As a consequence, the potential protein/chitin hydrolysate based cryoprotectant can be widely used and the drawback of carbohydrate based cryoprotectant can be minimized. In spite of preserving myofibrillar proteins conformation and functionality during frozen storage, scanty information is available on effect of marine hydrolysates on sensory property (such as color and taste) of seafood products. Thus, future studies need to focus more on sensory property of products containing marine hydrolysates. The proteinaceous raw material, especially under-utilized fish species, as a cheap source for the production of hydrolysates should be better utilized. Several marine hydrolysates with cryoprotective effect also showed antioxidant activity. Thus, hydrolysates with bifunctional properties i.e. cryoprotective-antioxidative activities can be further implemented as the alternative natural additive for maintaining the quality of seafoods and their products.

## References


Andersen, C. M., & Jørgensen, B. (2004). On the relation between water pools and water holding capacity in cod muscle. *Journal of Aquatic Food Product Technology, 13*, 13–23.

Aursand, I. G., Gallart-Jornet, L., Erikson, U., Axelson, D. E., & Rustad, T. (2008). Water distribution in brine salted cod (*Gadus morhua*) and salmon (*Salmo salar*): A low-field $^1$H NMR study. *Journal of Agricultural and Food Chemistry, 56*, 6252–6260.

Badii, F., & Howell, N. K. (2002). Effect of antioxidants, citrate, and cryoprotectants on protein denaturation and texture of frozen cod (*Gadus morhua*). *Journal of Agricultural Food Chemistry, 50*, 2053–2061.

Benjakul, S., Kittiphattanabawon, P., & Regenstein, J. M. (2012). Fish gelatin. In B. K. Simpson, L. M. L. Nollet, F. Toldrá, S. Benjakul, G. Paliyath, & Y. H. Hui (Eds.), *Food biochemistry and food processing* (pp. 388–405). West Sussex: John Wiley & Sons, Inc.

Benjakul, S., & Visessanguan, W. (2011). Impacts of freezing and frozen storage on quality changes of seafoods. In S. Devahastin (Ed.), *Physicochemical aspects of food engineering and processing* (pp. 283–306). New York: CRC Press.

Benjakul, S., Visessanguan, W., Thongkaew, C., & Tanaka, M. (2005). Effect of frozen storage on chemical and gel-forming properties of fish commonly used for surimi production in Thailand. *Food Hydrocolloids, 19*, 197–207.

Benjakul, S., Visessanguan, W., & Tueksuban, J. (2003). Changes in physico-chemical properties and gel-forming ability of lizardfish (*Saurida tumbil*) during post-mortem storage in ice. *Food Chemistry, 80*, 535–544.

Benajakul, S., Yarnpakdee, S., Senphan, T., Halldorsdottir, S. M., & Kristinsson, H. G. (2014). Fish protein hydrolysates: production, bioactivities, and applications. In H. G. Kristinsson (Ed.), *Antioxidants and functional components in aquatic foods* (pp. 237–282). West Sussex: John Wiley & Sons, Ltd.

Bertram, H. C., Kristensen, M., Østdal, H., Baron, C. P., Young, J. F., & Andersen, H. J. (2007). Does oxidation affect the water functionality of myofibrillar proteins? *Journal of Agricultural and Food Chemistry, 55*, 2342–2348.

Beyrer, M., & Klaas, M. R. (2007). Influence of freezing and of frozen storage on the specific heat capacity of trout and herring fillet. *European Food Research Technology, 224*, 349–353.

Boran, G., & Regenstein, J. M. (2010). Fish gelatin. *Advances in Food and Nutrition Research, 60*, 119–143.

Carneiro, C. S., Mársico, E. T., Ribeiro, R. O. R., Júnior, C. A. C., Álvares, T. S., & Jesus, E. F. O. (2013). Studies of the effect of sodium tripolyphosphate on frozen shrimp by physicochemical analytical methods and Low Field Nuclear Magnetic Resonance (LF $^1$H NMR). *LWT - Food Science and Technology, 50*, 401–407.

Chalamaiah, M., Kumar, B., Hemalatha, R., & Jyothirmayi, T. (2012). Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review. *Food Chemistry, 135*, 3020–3038.

Chen, N. J., Morikawa, J., & Hashimoto, T. (2005). Effect of amino acids on the eutectic behavior of NaCl solutions studied by DSC. *Cryobiology, 50*, 264–272.

Cheung, I. W. Y., Liceaga, A. M., & Li-Chan, E. C. Y. (2009). Pacific hake (*Merluccius productus*) hydrolysates as cryoprotective agents in frozen Pacific cod fillet mince. *Journal of Food Science, 74*, 588–594.

Damodaran, S. (2007). Inhibition of ice crystal growth in ice cream mix by gelatin hydrolysate. *Journal of Agricultural and Food Chemistry, 55*, 10918–10923.

Darmanto, Y. S. (2003). Effect of chitin and chitosan derived from crab shell and shrimp head on the unfrozen water and denaturation of lizard fish myofibrils during frozen storage. *Journal of Coastal Development, 6*, 97–105.

Dey, S. S., Dora, K. C., Raychaudhuri, U., & Ganguly, S. (2013). Cryoprotective effect of shrimp waste protein hydrolysate on croaker surimi protein and gel characteristics during frozen storage. *Fish Technology, 50*, 50–59.

Erikson, U., Standal, I. B., Aursand, I. G., Veliyulin, E., & Aursand, M. (2012). Use of NMR in fish processing optimization: A review of recent progress. *Magnetic Resonance in Chemistry, 50*, 471–480.

Estévez, M. (2011). Protein carbonyls in meat systems. *Meat Science, 89*, 259–279.

Gill, P., Tohidi Moghadam, T., & Ranjbar, B. (2010). Differential scanning calorimetry techniques: Applications in biology and nanoscience. *Journal of Bimolecular Techniques, 21*, 167–193.

Gökoğlu, N., & Yerlikaya, P. (2015). Chemical composition of fish. In N. Gökoğlu, & P. Yerlikaya (Eds.), *Seafood chilling, refrigeration and freezing: Science and technology*. West Sussex: John Wiley & Sons, Ltd.

Gomez-Guillen, M. C., Turnay, J., Fernandez-Diaz, M. D., Ulmo, N., Lizarbe, M. A., & Montero, P. (2002). Structural and physical properties of gelatin extracted from different marine species: A comparative study. *Food Hydrocolloids, 16*, 25–34.

Gonçalves, A. A., Nielsen, J., & Jessen, F. (2012). Quality of frozen fish. In L. M. L. Nollet (Ed.), *Handbook of meat, poultry and seafood quality* (pp. 479–509). Ames: John Wiley & Sons, Inc.

Gudjónsdóttir, M., Lauzon, H. L., Magnússon, H., Sveinsdóttir, K., Arason, S., Martinsdóttir, E., et al. (2011). Low field nuclear magnetic resonance on the effect of salt and modified atmosphere packaging on cod (*Gadus morhua*) during superchilled storage. *Food Research International, 44*, 241–249.

Halim, N. R. A., Yusof, H. M., & Sarbon, N. M. (2016). Functional and bioactive

properties of fish protein hydrolysates and peptides: A comprehensive review. *Trends in Food Science & Technology, 51*, 24–33.

Harnedy, P. A., & FitzGerald, R. J. (2012). Bioactive peptides from marine processing waste and shellfish: A review. *Journal of Functional Foods, 4*, 6–24.

Herrera, J. R., & Mackie, I. M. (2004). Cryoprotection of frozen-stored actomyosin of farmed rainbow trout (*Oncorhynchus mykiss*) by some sugars and polyols. *Food Chemistry, 84*, 91–97.

Hossain, M. A., Abu Alikhan, M., Ishihara, T., Hara, K., Osatomi, K., Osaka, K., et al. (2004). Effect of proteolytic squid protein hydrolysate on the state of water and denaturation of lizardfish (*Saurida wanieso*) myofibrillar protein during freezing. *Innovative Food Science and Emerging Technologies, 5*, 73–79.

Hossain, M. A., Ishihara, T., Hara, K., Osatomi, K., Abu Alikhan, M., & Nazaki, Y (2003). Effect of proteolytic squid protein hydrolysate on the state of water and dehydration-induced denaturation of lizard fish myofibrillar protein. *Journal of Agricultural and Food Chemistry, 51*, 4769–4774.

Karim, A. A., & Bhat, R. (2009). Fish gelatin: Properties, challenges, and prospects as an alternative to mammalian gelatins. *Food Hydrocolloids, 23*, 563–576.

Karnjanapratum, S., & Benjakul, S. (2015). Cryoprotective and antioxidative effects of gelatin hydrolysate from unicorn leatherjacket skin. *International Journal of Refrigeration, 49*, 69–78.

Kazami, N., Sakaguchi, M., Mizutani, D., Masuda, T., Wakita, S., Oyama, F., et al. (2015). A simple procedure for preparing chitin oligomers through acetone precipitation after hydrolysis in concentrated hydrochloric acid. *Carbohydrate Polymers, 132*, 304–310.

Khan, M. A. A., Hossain, M. A., Hara, K., Osatomi, K., Ishihara, T., & Nozaki, Y. (2003). Effect of enzymatic fish-scrap protein hydrolysate on gel-forming ability and denaturation of lizard fish *Saurida wanieso* surimi during frozen storage. *Fisheries Science, 69*, 1271–1280.

Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., & Shahidi, F. (2012). Cryoprotective effect of gelatin hydrolysate from blacktip shark skin on surimi subjected to different freeze-thaw cycles. *LWT – Food Science and Technology, 47*, 437–442.

Kong, B., Guo, Y., Xia, X., Liu, Q., Li, Y., & Chen, H. (2013). Cryoprotectants reduce protein oxidation and structure deterioration induced by freeze-thaw cycles in common carp (*Cyprinus carpio*) surimi. *Food Biophysics, 8*, 104–111.

Korzeniowska, M., Cheung, I. W. Y., & Li-Chan, E. C. Y. (2013). Effects of fish protein hydrolysate and freeze–thaw treatment on physicochemical and gel properties of natural actomyosin from Pacific cod. *Food Chemistry, 138*, 1967–1975.

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.

Limpisophon, K., Iguchi, H., Tanaka, M., Suzuki, T., Okazaki, E., Saito, T., et al. (2014). Cryoprotective effect of gelatin hydrolysate from shark skin on denaturation of frozen surimi compared with that from bovine skin. *Fisheries Science*. http://dx.doi.org/10.1007/s12562-014-0844-5.

Liu, Q., Chen, Q., Kong, B., Han, J., & He, X. (2014). The influence of superchilling and cryoprotectants on protein oxidation and structural changes in the myofibrillar proteins of common carp (*Cyprinus carpio*) surimi. *LWT - Food Science and Technology, 57*, 603–611.

Liu, G., & Xiong, Y. L. (2000). Electrophoretic pattern, thermal denaturation, and in vitro digestibility of oxidized myosin. *Journal of Agricultural and Food Chemistry, 48*, 624–630.

Lund, M. N., & Baron, C. P. (2010). Protein oxidation in foods and food quality. In L. H. Skibsted, J. Risbo, & M. L. Andersen (Eds.), *Chemical deterioration and physical instability of food and beverages* (pp. 33–69). Cambridge: Woodhead Publishing Limited.

MacDonald, G. A., & Lanier, T. C. (1997). Cryoprotectants for improving frozen food quality. In M. C. Erikson, & Y. C. Hung (Eds.), *Quality in frozen food* (pp. 197–232). New York: Chapman & Hall.

Matos, E., Silva, T. S., Tiago, T., Aureliano, M., Dinis, M. T., & Dias, J. (2011). Effect of harvesting stress and storage conditions on protein degradation in fillets of farmed gilthead seabream (*Sparus aurata*): A differential scanning calorimetry study. *Food Chemistry, 126*, 270–276.

Ma, L. K., Zhang, B., Deng, S. G., & Xie, C. (2015). Comparison of the cryoprotective effects of trehalose, alginate, and its oligosaccharides on peeled shrimp (*Litopenaeus Vannamei*) during frozen storage. *Journal of Food Science*. http://dx.doi.org/10.1111/1750-3841.12793.

Medina, I., & Pazos, M. (2010). Oxidation and protection of fish. In E. Decker, R. Elias, & D. J. McClements (Eds.), *Oxidation in foods and beverages and antioxidant applications* (pp. 91–120). Cambridge: Woodhead Publishing Limited.

Nagarajan, M., Benjakul, S., Prodpran, P., Songtipya, P., & Kishimura, H. (2012). Characteristics and functional properties of gelatin from splendid squid (*Loligo formosana*) skin as affected by extraction temperatures. *Food Hydrocolloids*. http://dx.doi.org/10.1016/j.foodhyd.2012.04.001.

Nikoo, M., Benjakul, S., Bashari, M., Alekhorshied, M., Idrissa Cissouma, A., Yang, N., et al. (2014b). Physicochemical properties of skin gelatin from farmed Amur sturgeon (*Acipenser schrenckii*) as influenced by acid pretreatment. *Food Bioscience, 5*, 19–26.

Nikoo, M., Benjakul, S., Ehsani, A., Jing, L., Wu, F. F., Yang, N., et al. (2014a). Antioxidant and cryoprotective effects of a tetrapeptide isolated from Amur sturgeon skin gelatin. *Journal of Functional Foods, 7*, 609–620.

Nikoo, M., Benjakul, S., & Xu, X. (2015a). Antioxidant and cryoprotective effects of Amur sturgeon skin gelatin hydrolysate in unwashed fish mince. *Food Chemistry, 181*, 295–303.

Nikoo, M., Regenstein, J. M., Ghomi, M. R., Benjakul, S., Yang, N., & Xu, X. (2015b). Study of the combined effects of a gelatin-derived cryoprotective peptide and a non-peptide antioxidant in a fish mince model system. *LWT - Food Science and Technology, 60*, 358–364.

Okazaki, E., & Kimura, I. (2014). Frozen surimi and surimi-based products. In I. S. Boziaris (Ed.), *Seafood processing: Technology, quality and safety* (pp. 209–235). West Sussex: John Wiley & Sons, Ltd.

Olsen, R. L., Toppe, J., & Karunasagar, I. (2014). Challenges and realistic opportunities in the use of by-products from processing of fish and shellfish. *Trends in Food Science & Technology, 36*, 144–151.

Ordóñez-Del Pazo, T., Antelo, L. T., Franco-Uría, A., Pérez-Martín, R. I., Sotelo, C. G., & Alonso, A. A. (2014). Fish discards management in selected Spanish and Portuguese métiers: Identification and potential valorization. *Trends in Food Science & Technology, 36*, 29–43.

Osako, K., Hossain, M. A., Kuwahara, K., & Nozaki, Y. (2005). Effect of trehalose on the gel-forming ability, state of water and myofibril denaturation of horse mackerel *Trachurus japonicas* surimi during frozen storage. *Fisheries Science, 71*, 367–373.

Ruttanapornvareesakul, Y., Somjit, K., Otsuka, A., Hara, K., Osatomi, K., Kongpun, O., et al. (2006). Cryoprotective effects of shrimp head protein hydrolysate on gel forming ability and protein denaturation of lizardfish surimi during frozen storage. *Fisheries Science, 72*, 421–428.

Sai-Ut, S., ongjareonrak, A., & Rawdkuen, S. (2012). Re-extraction, recovery, and characteristics of skin gelatin from farmed giant catfish. *Food Bioprocess Technology, 5*, 1197–1205.

Sánchez-Alonso, I., Martinez, I., Sánchez-Valencia, J., & Careche, M. (2012). Estimation of freezing storage time and quality changes in hake (*Merluccius merluccius*, L.) by low field NMR. *Food Chemistry, 135*, 1626–1634.

Sánchez-Valencia, J., Sánchez-Alonso, T., Martinez, I., & Careche, M. (2015). Low-field nuclear magnetic resonance of proton ($^1$H LF NMR) relaxometry for monitoring the time and temperature history of frozen hake (*Merluccius merluccius* L.) muscle. *Food and Bioprocess Technology, 8*, 2137–2145.

Shahidi, F. (1994). Seafood proteins and preparation of protein concentrates. In F. Shahidi, & J. R. Boota (Eds.), *Seafoods: Chemistry, processing technology and quality*. Springer Science+Business Media Dordrecht.

Somjit, K., Ruttanapornwareesakul, Y., Hara, K., & Nozaki, Y. (2005). The cryoprotectant effect of shrimp chitin and shrimp chitin hydrolysate on denaturation and unfrozen water of lizardfish surimi during frozen storage. *Food Research International, 38*, 345–355.

Sriket, P., Benjakul, S., Visessanguan, W., & Kijroongrojana, K. (2007). Comparative studies on the effect of the freeze–thawing process on the physicochemical properties and microstructures of black tiger shrimp (*Penaeus monodon*) and white shrimp (*Penaeus vannamei*) muscle. *Food Chemistry, 104*, 113–121.

Sych, J., Lacroix, C., Adambounou, L. T., & Castaigne, F. (1991). The effect of low- or non-sweet additives on the stability of protein functional properties of frozen cod surimi. *International Journal of Food Science and Technology, 26*, 185–197.

Tironi, V. A., Tomás, M. C., & Añón, M. C. (2010). Quality loss during the frozen storage of sea salmon (*Pseudopercis semifasciata*). Effect of rosemary (*Rosmarinus officinalis* L.) extract. *LWT - Food Science and Technology, 43*, 263–272.

Wu, S., Pan, S., & Wang, H. (2014). Effect of trehalose on *Lateolabrax japonicus* myofibrillar protein during frozen storage. *Food Chemistry, 160*, 281–285.

Yamashita, Y., Zhang, N., & Nozaki, Y. (2003). Effect of chitin hydrolysate on the denaturation of lizard fish myofibrillar protein and the state of water during frozen storage. *Food Hydrocolloids, 17*, 569–576.

Yamashita, Y., Zhang, N., & Nozaki, Y. (2012). Effect of chitin isolated from crustaceans and cephalopods on denaturation of fish myofibrillar protein and the state of water during frozen storage. *Transactions of the Japan Society of Refrigerating and Air Conditioning Engineers, 18*, 455–462.

Yanan, Z., Li, Z., Hua, L., Wei, S., & Meilan, Y. (2012). Effect of eel head protein hydrolysates on the denaturation of grass carp surimi during frozen storage. *Procedia Engineering, 37*, 223–228.

Zhang, N., Yamashita, Y., & Nozaki, Y. (2002). Effect of protein hydrolysate from Antarctic krill on the state of water and denaturation of lizard fish myofibrils during frozen storage. *Food Science and Technology Research, 8*, 200–206.

Zhou, A., Benjakul, S., Pan, K., Gong, J., & Liu, X. (2006). Cryoprotective effects of trehalose and sodium lactate on tilapia (*Sarotherodon nilotica*) surimi during frozen storage. *Food Chemistry, 96*, 96–103.