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REVIEW

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Improvement of Surimi Gel Quality Using Protein Cross-Linker, Hydrocolloids and Protease Inhibitor

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Abstract

Surimi and its products have been consumed widely, especially in Japan and Southeast Asia. Market value and consumer acceptability are mainly determined by the textural properties of surimi gel. Textural and gelling properties are governed by the type of fish, lipid content, processing conditions, and presence of endogenous protease and transglutaminase. In addition, several additives such as protein cross-linkers, hydrocolloids, protease inhibitors, etc. have been used in surimi to improve their gelling properties. Cross-linking is the promising method for protein modification to obtain desirable surimi-based gel products possessing unique textural properties. Endogenous transglutaminase (TGase) plays a profound role in protein cross-linking during setting in surimi paste. For surimi from fish with low setting phenomenon, microbial TGase (MTGase) has been widely used. Plant polyphenols have also been employed for protein cross-linking to strengthen the surimi gel. Polysaccharides mainly the hydrocolloids also strengthen the gel network and increase water holding capacity (WHC) of gel. During heating, endogenous heat-activated proteases that are firmly attached to muscle proteins are active in hydrolyzing myofibrillar proteins. This results in a weaker gel with low WHC. Protease inhibitors form plants as well animals could impede the protease activity, thus maintaining the myofibrillar proteins responsible for the gelation. In addition to the additives, processing methods such as high-pressure technology or ohmic heating in combination with those additives have a positive impact on the gel properties. For this review, the role of various kinds of additives namely protein cross-linkers, hydrocolloids and protease inhibitors in surimi gel improvement was revisited.

Introduction

Surimi is the Japanese term used to call 'washed fish mince'. It can be utilized to produce numerous products such as fish balls, imitation crab sticks, fish tofu, etc. In general, surimi is manufactured in a frozen form and the proteins in concentrated washed fish mince are stabilized with cryoprotectant (sucrose or sorbitol) (Singh et al., 2021; Yingchutrakul et al., 2022). Furthermore, different additives with varied functions such as protease inhibition, water retention augmentation, and whiteness improvement have been used in surimi and surimi products (Bharane et al., 2020; Quan & Benjakul, 2019; Gani et al., 2018; Petcharat & Benjakul, 2018). Previously, lean fish, such as Alaska pollock, bigeye snapper, threadfin bream, etc. were the major raw material used for the preparation of surimi, which was associated with their white flesh, and excellent gelling property (Singh & Benjakul, 2018; Gani et al., 2018). Those characteristics yielded surimi products with superior quality and whiter color. However, due to their overexploitation, the surimi industry has inclined towards dark-fleshed fish, such as sardines, mackerel, etc. Those fish are rich in lipids and proteases, which could lower the gel properties and whiteness of gel products (Singh & Benjakul, 2017a; Singh & Benjakul, 2017b). The total global production of frozen surimi is approximately 850,000 tons, which is valued at around $2.6 billion (Buglak, 2023). In general, the Alaska pollock and Pacific whiting surimi have been

produced mainly by the United States. Southeast Asian countries use tropical or warm water fish, which includes threadfin bream, bigeye snapper, and red sea bream, to produce frozen fish surimi (Jiao et al., 2023). In addition,. China is producing freshwater fish surimi, especially Asian carp, have been employed for the production of surimi, which has already been used in Chinese traditional fish products such as fish balls and fish cakes (Jiao et al., 2023; Yingchutrakul et al., 2022; Li et al., 2021). Freshwater fish are gaining more attention to be an alternative source for the surimi industry, due to low-cost culturing and fish can attain optimum size in a short time. Asian carp, namely, bighead carp, grass carp, silver carp, and black carp, are freshwater fish, which can be commonly found in Chinese cuisine. Various recent research has been performed to improve the gel properties of surimi made from Asian carp (Yingchutrakul et al., 2022). In 2019, 5.7, 4.7, 3.1, and 0.7 million tons amount of grass carp, silver carp, bighead carp, and black carp, respectively produced worldwide. On the other hand, in the same year, 3.5 million tons of Alaska pollock, 0.4 million tons of pacific whiting, and 0.03 million tons of arrowtooth flounder were produced (FAO, 2020). However, their muddy flavor limits their use in the preparation of surimi products. Furthermore, due to the increasing interest in plant-based proteins, certain plant proteins have been also incorporated into surimi (Chen et al., 2023; Zhang et al., 2023).

Surimi or surimi gel properties have been improved using various additives such as protease inhibitors, protein cross-linkers, hydrocolloids, etc. (Singh et al., 2021; Singh & Benjakul, 2017b; Yingchutrakul et al., 2022; Quan & Benjakul, 2019; Jeyakumari et al., 2016; Gani et al., 2018; Buamard & Benjakul, 2015; Buamard & Benjakul, 2017a; Buamard & Benjakul, 2017b). The major objective of the current review article is to gather information related to the several additives, which have been used to improve the gel properties of the surimi from different fish.

Surimi Gelation

Surimi possessed superior gelling, binding, and emulsifying properties, mainly due to the presence of myofibrillar proteins (MPs) (Buamard & Benjakul, 2015; Benjakul et al., 2003a). In general, MPs consist of fibers, and bundles, which consist of contractile proteins arranged in repeating end-on-end units called sarcomeres. Those sarcomeres contain both thick and thin filaments, which are involved in the contraction (Carvajal et al., 2005). Those thick filaments contained myosin, which is the major MP responsible for the gelling properties of the surimi (Benjakul et al., 2010). Myosin is a long rod with two globular heads and a tail portion comprising two α-helical coil polypeptide chains (An et al., 1996). In addition, actin is the second major MP followed by tropomyosin, troponin, actinins, nebulin, C- and M-proteins (Park, 2005). At a high ionic strength, myosin is depolymerized, thus allowing counter-ions to neutralize each charge, and cancelling the attractive force. As a result, the myosin molecules are dissociated from one another and aid the dispersion of the protein molecules. When dispersed proteins were subjected to the setting (40°C), so-called suwari, cross-linking of proteins takes place, mediated by endogenous transglutaminase (TGase). Finally, the gel is subjected to heating at a high temperature (90-100°C) (Benjakul et al., 2003a; Singh & Benjakul, 2018). During heat induced gelation, myosin tail portions interacted with each other, thus enhancing the elasticity of the gel followed by interactions among hydrophobic residues of the head portion of the myosin molecule (Wang et al., 2023b; Yan et al., 2020). Normally, heating unfolds the protein molecules, in which those hydrophobic residues buried inside are exposed (An et al., 1996). In addition, disulfide bonds could be formed (Zhong et al., 2023; Zhao et al., 2023). Overall, covalent disulfide bonds and non-covalent interactions are involved in the development of rigid and strong gel structures (Wang et al., 2023b; Yan et al., 2020).

Thermal and High-Pressure Induced Gelation of Surimi

Structural-modifications of proteins induced by various treatments can affect the gelation of surimi (Zhi et al., 2015). Thermal-induced gelation is the common approach in the surimi industry due to its easy operation and low equipment requirements. Muscle proteins including myosin, actin, tropomyosin, and troponin are denatured and aggregated by heat, creating a 3-dimensional network of surimi gel. Li et al. (2022) suggested that surimi from *Harpadon nehereus* showed better gel properties when it was heated at 30°C for 120 min, followed by cooking at 90°C for 30 min as evidenced by the highest breaking force and hardness among all samples. Sardine surimi protein underwent cross-linking at 35-38°C, mainly via hydrogen bonds between protein molecules as well as non-disulfide covalent bonds mediated by endogenous TGase (Buamard and Benjakul, 2015). At higher temperatures (65-67°C), the creation of a thermo-irreversible gel network is attributed to an increase in cross-linking between unfolded protein molecules, mainly denatured myosin heavy chain. However, the slow heating may lead to poor gel formation associated with proteolysis induced by heat-activated proteases, especially when the gel is exposed to heat at 50-55 °C, known as 'modori' (Alvarez et al., 1999). The rapid heating process, especially ohmic heating, was implemented to provide instantaneous heat to form a gel, while rapidly inactivating endogenous proteases (Yongsawatdigul et al., 1995). It has been known that the heating method affects the gelling ability of surimi gels. Different heating processes, e.g., ohmic heating have been employed for surimi gel preparation. Ohmic heating has a fast-heating rate, generated via alternating current, which is passed through an electrically conducting material. This process can create uniform temperature distribution (Pataro et

al., 2011). In contrast to conventional heating (heated in a 90°C-water bath for 15 min), Yongsawatdigul et al. (1995) found that ohmic heating (90°C, voltage gradient of 13.3 V/cm, voltage 200 V) produced surimi gel from Pacific whiting with higher shear stress (from 14 to 30 kPa) and shear strain (from 1.4 to 2.9). Tadpitchayangkoon et al. (2012) also found that ohmic heating (90°C, voltage gradient of 6.7, voltage 200 V) upsurged breaking force and deformation of surimi from threadfin bream and bigeye snapper by 1.3 and 1.6-fold, respectively, in comparison to water bath-heating. The gel characteristics of Pacific whiting surimi combined with salmon blood plasma were further enhanced by ohmic setting (60°C for 30 min) and cooking (90°C) (Fowler and Park, 2015a, b).

High hydrostatic pressure (HPP) is one of the popular non-thermal technologies in the food industry. In general, pressure between 100 and 1,000 MPa has been employed (Muntean et al., 2016). HHP renders the products with good physical, chemical and sensory qualities. Thus, surimi gelation can be induced under high pressure, yielding a gel with high elasticity. (Chen et al., 2020). During the first stage of pressurization, volume reduction caused the breakdown of hydrophobic connections of protein molecules. Moreover, pressure decreased the distance between sulfhydryl groups and induced the disulfide bond formation (Hwang et al., 2007). During the pressure release, the unfolding of proteins occurred and caused the formation of hydrogen bonds and hydrophobic interactions (Buamard & Benjakul, 2018; Liang et al., 2017). Chen et al. (2021) discovered that *Nemipterus virgatus* surimi gel made by HPP at 100 or 200 MPa for 15 min, followed by a two-stage heating, namely 100-H and 200-H, had increased gel strength, water-holding capacity, and whiteness. Improvement of tilapia surimi gel property was also achieved by HPP application (0-400 MPa, 15 min) (Lu et al., 2021). Tilapia surimi gel treated with HPP 300 MPa for 15 min had the highest gel strength, which increased by about 226.4%, compared to the untreated sample. The pressurized gels are generally formed a dense and flexible structure, which is further stabilized by disulfide bonds and hydrophobic interactions during heat treatment (Buamard & Benjakul, 2018).

Role of Transglutaminase (TGase) in Surimi Gelation

Endogenous TGase (EC number: 2.3.2.13) initiates an acyl transfer reaction between primary amines and the ɤ-carboxamide group of peptides or protein-bound glutaminyl residues (Liang et al., 2020). During the gelation, the reaction between glutamine and lysine in MP is promoted and the formation of ε-(γ-glutamyl)-lysine cross-linkages is induced in the presence of Ca^{2+}. As a result, a firmer and more stable gel is developed (Buamard & Benjakul, 2017a; Chanarat & Benjakul, 2013b). Surimi from some fish species possesses an inferior setting phenomenon, microbial TGase (MTGase) has been introduced (Chanarat & Benjakul, 2013b).

Surimi Protein Cross-Linkers and Additives

Plant Extract Containing Phenolic Compounds

An approach to producing desirable properties of surimi gel is achieved by protein modification via cross-linking (Figure 1). Many cross-linkers from different

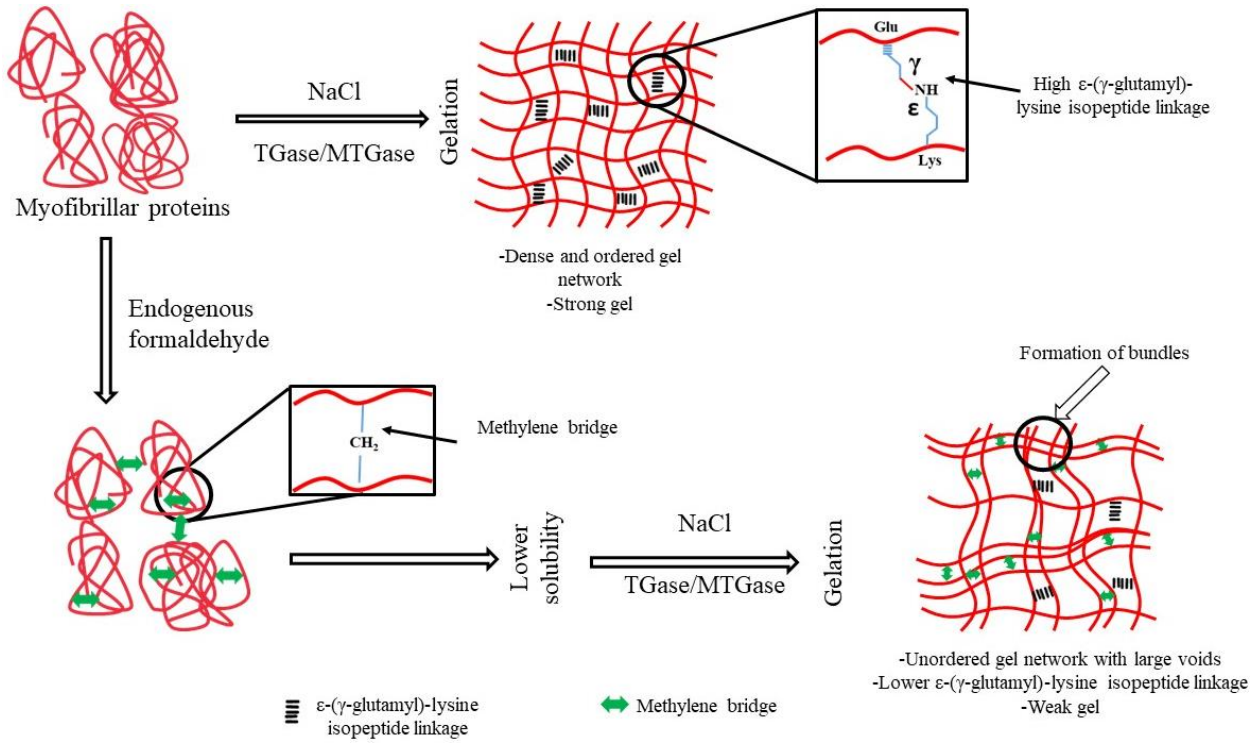

Figure 1. Role of plant polyphenols in surimi gelation

sources have been utilized in food proteins (Table 1). Some protein cross-linkers, nevertheless, cannot be used in foods because they are poisonous or cause allergies. Due to their safety and potential for application in food systems, natural protein cross-linkers, particularly those derived from plants, have drawn augmenting attention (Sharma et al., 2022; Wu et al., 2022). Phenolic compounds contain both polar (hydroxyl groups) and non-polar (phenol ring) groups, among which the hydroxyl group is a superior hydrogen donor as it reacts with the carbonyl group via hydrogen bonds. Phenolic compounds with high protein affinity must have an appropriate size to localize at inter-fibrillar regions of protein molecules and cross-link them (Welc et al., 2022). Ethanolic coconut husk rich in phenolics was added to strengthen surimi gel from sardine at varying levels (0.025-0.15 g/100 g protein). Surimi gel from sardine added with coconut husk extract (CHE), which contained 454 mg tannin/g dry powder, at 0.125 g/100 g, could increase gel strength by approximately 240%, compared to the gel without CHE added (Buamard & Benjakul, 2015). Arsyad et al. (2019) prepared surimi gel from red sea bream added with olive leaf powder (OLLP), containing 7 g/100 g polyphenol, at various amounts (0.05-4 g/100 g). The breaking stress and strain of surimi gel increased with augmenting OLLP levels. The addition of 0.3 g/100 g OLLP upsurged the breaking stress of surimi gel by 80% and breaking strain by 38%. The resulting gel also had finer/denser networks than that without OLLP. Wu et al. (2022) documented that the addition of tea polyphenols at 300 mg/kg improved water water-holding capacity and hardness of tilapia surimi.

Malondialdehyde (MDA), a reactively electrophilic aldehyde produced by the lipid oxidation during the freezing of surimi, has the potential to accelerate protein oxidation by producing protein adducts and releasing reactive oxygen species (ROS) into the food system (Benjakul et al., 2005). Oxidized proteins may bring about the formation of aggregates causing lower solubility, which might decrease the gelling property (Zhang et al., 2020). Polyphenols might be employed to prevent protein oxidation and degradation while being stored. They also lessen the damage to proteins' spatial structures, preserving the gelling quality of frozen surimi (Staszewski et al., 2011). Tea polyphenol significantly inhibited lipid and protein oxidation and protein degradation in tilapia surimi, yielding better gel properties of surimi from tilapia (Wu et al., 2022).

Furthermore, oxidized forms of polyphenols or quinone are effective protein cross-linkers. Yu et al. (2023) found that oxidized dihydromyricetin (DMC) at 0.4 g/100 g could interact with myofibrillar proteins in silver carp surimi through three kinds of covalent links including Lys-DMY-Lys, Lys-DMY-Cys and Cys-DMY-DMY-Cys. Such cross-links resulted in higher hardness

Table 1. Phenolic extracts from different natural sources and their uses in surimi.

Sources	Active compounds	Applications	References
Coconut husk (CHE)	Tannic acid, catechin, gallic acid, quercetin	Use of CHE at 0.125 g/100 g protein increased gel strength of sardine surimi ~240%	Buamard & Benjakul (2015)
Duea ching fruit (DC)	Naringenin-7-O-glucoside, Quercetin 3-galactoside, rutin, indole-4-carbaldehyde	Breaking force of sardine surimi gel was increased by 100% by adding DC at 0.05 g/100 g.	Buamard et al. (2023)
Kiam wood (KW)	Tannic acid, lignin	Oxidized KW at 0.15 g/100 g increased breaking force of sardine surimi gel up to 136.9-157.5%, compared to the control.	Balange & Benjakul (2011)
Lotus root knot (LRK)	B-type procyanidin dimer·H_2O, (−)-epicatechin, chlorogenic acid, propyl gallate, rutin	Gel strength, storage modulus, and disulfide bond of silver carp surimi gels were significantly increased with the addition of the LRK especially at 10 g/100 g, where gel strength and disulfide bond were enhanced by 14.7% and 41.6%, respectively.	Wang et al. (2023)
Olive leaf (OL)	Oleuropein, hydroxytyrosol, verbacoside	Addition of OL (0.3 g/100 g) resulted in 80% increase in the breaking stress of red sea bream surimi gel. The OL gels had finer and denser protein networks.	Arsyad et al. (2018)
Perilla leaf (PL)	Caffeic acid, ferulic acid, rosmarinic acid, quercetin, and apigenin	PL (0.03 g/100 g) retarded lipid and protein oxidation of surimi fish balls from white croaker during storage at 4 °C.	Zhao et al. (2019)
Pineapple peel (PP)	Gallic acid, epicatechin, catechin, ferulic acid	PP (1 g/100 g) demonstrated improved breaking force of silver carp surimi gel from 355.71 g to 511.64 g. PP also contributed to the formation of amide bonds with the protein of the gels	Sharma et al. (2022a)
Pomegranate peel (PoP)	Punicalagin, ellagic acid, gallic acid, ellagitannins	Silver carp surimi gel fortified with 0.45 g/100 g PoP exhibited higher gel strength than the gel without PoP by 101% (609.58 g cm).	Sharma et al. (2022b)
Seaweed (SW)	Phlorotannins, rosmarinic acid, quinic acid, rutin, quercitin, hesperidin	Lesser sardine surimi gel added with 2 g/100 g SW had the increases in gel strength by 76.27 %, compared with the control gel.	Shitole et al. (2014)
Tea polyphenols (TP)	(−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin gallate (EGCG)	TP at 300 mg/kg could maintain water holding capacity and hardness of tilapia surimi during refrigerated storage for 7 days.	Wu et al. (2022)

(from 1,603 to 2073 g) and chewiness (from 126.3 to 159.1 mJ). To increase the gel property of mackerel surimi, Balange and Benjakul (2009) evaluated the optimal concentration (0-0.60 g/100 g of protein content) of several oxidized phenolic compounds, including ferulic acid (OFA), tannic acid (OTA), caffeic acid (OCF), and catechin (OCT). In comparison to the control, a breaking force of gels containing 0.40 g/100 g OFA, 0.50 g/100 g OTA, 0.50 g/100 g OCF, or 0.10 g/100 g OCT increased by 45, 115, 46.1, and 70.3%, respectively. Via the regeneration of hydroquinone, the quinone either forms a dimer as a byproduct or combines with amino or sulfhydryl side chains of polypeptides to form the covalent C-N or C-S bonds with phenolic ring. The latter could re-oxidize and link with another polypeptide to form a cross-link. Additionally, two quinones could dimerize and create a cross-link (Strauss & Gibson, 2004). However, an excessive amount of extract may reduce gel strength, owing to the extravagant cross-linking and poor protein network alignment, as well as lower the whiteness of the resultant gel (Buamard et al., 2023).

In addition to the improvement of texture of surimi gel, plant phenolics also lowered the oxidation of proteins or lipids present in surimi or surimi products, thus enhancing their shelf-life. Recently, Wu et al., (2023) reported reduction of acid value, peroxide value, conjugated diene value and thiobarbituric acid reactive substances value (~30%) of surimi added with barley green powder in a dose-dependent manner during frozen storage. This could be associated with the ability of phenolic compounds to transfer hydrogen atom or single electron, sequential proton loss electron transfers to free-radical, as well as the chelation of transition metals (Zeb, 2020).

Microbial Transglutaminase (MTGase)

MTGase is produced by some microorganisms, e.g., *Streptoverticillium mobaraense*, *Streptomyces mobaraense, Corynebacterium glutamicum*, etc. (Zhang et al., 2009). It is a Ca^{2+} independent enzyme, which is different from indigenous TGase. Chanarat et al. (2012) investigated the impact of MTGase on the gel properties of surimi from threadfin bream, Indian mackerel and sardine. MTGase at all levels (0–0.6 U/g) augmented the breaking force of all gels, except for threadfin bream surimi gel, where the breaking force decreased at 0.6 U/g surimi. This might be associated with the aggregation of MP proteins due to the higher cross-linking associated with TGase and MTGase. In addition, the self-aggregation of MTGase at a higher level could be another possible reason (Jiang et al., 2000). Along with the MTGase, surimi gel has been incorporated with other several additives (such as plant phenolics, dietary fibers, pigments, etc.) which could produce the premium quality of surimi (Zhong et al., 2023; Huang et al., 2021; Singh et al., 2020). Nevertheless, higher cross-linking of surimi proteins might decrease their digestibility, which could lower the bioavailability of proteins or peptides (Fang et al., 2019). To increase the efficiency of cross-linking in proteins in surimi, other bioactive compounds possessing polymerization activity can be implemented along with MTGase. MTGase in combination with ethanolic coconut husk extract synergistically enhanced WHC and breaking force by 709% than that of control gel prepared from spotted golden goatfish surimi (Singh et al., 2020). In another study, dietary fiber, such as inulin was added to the gel prepared from silver carp surimi in combination with the MTGase (Huang et al., 2021). Recently, Jiang et al. (2023a) prepared shrimp surimi gel by adding L-arginine and MTGase, in which L-arginine replaced sodium chloride and MTGase improved the textural properties of in shrimp surimi gel. In addition to the positive impact of MTGase on textural properties, it also limits the production of biogenic amines. In general, biogenic amines are the product of the decarboxylation of amino acids and their production increased during storage owing to enzymatic and microbiological activity (Yerlikaya et al., 2015). Those biogenic amines could create health if exceeded to certain limits (1000 mg/kg total biogenic amines) in fish (Food & Administration, 2001). The production of biogenic amines could be lowered by the addition of TGase or MTGase, due to ϵ-(γ-glutamyl) lysine linkage. This causes the hindrance in the decarboxylation of lysine amino acid (Yerlikaya et al., 2015). Yerlikaya et al. (2015) observed a reduction in the formation of biogenic amines (putrescine, cadaverine and tyramine) when mackerel mince was incorporated with 2 g/kg MTGase during the storage of 8 days. Moreover, biogenic amines can serve as acyl acceptors. Hence, in the presence of biogenic amines, the breaking force could be decreased, regardless of the addition of MTGase. Chanarat et al. (2017) observed a reduction in the breaking force of Nile tilapia surimi gel when biogenic amines were added with the MTGase as compared to the gel added with only MTGase. This could be associated with the competition between proteins and biogenic amine for acyl transfer. Huang et al. (2023) studied the impact of commercial MTGase at the minimum possible concentrations (0–0.295 U/g of surimi) on tilapia surimi gel at varying incubation temperatures (20-50 °C). The resulting gel had the increases in hardness, elasticity, gel strength, and WHC. Although MTGase promotes the cross-linking of muscle proteins, in the presence of formaldehyde and dimethylamine, it cannot improve the textural properties of lizardfish surimi gel (Chanarat & Benjakul, 2013a). Formaldehyde and dimethylamine are the products of the reaction of TMAO demethylase (Leelapongwattana et al., 2008). TMAO is found in several marine fish such as cod, hake, lizardfish, pollack and whiting (Chanarat & Benjakul, 2013a). The cross-linking of muscle proteins in the presence of formaldehyde limits the acyl-transfer mediated by the MTGase. In general, formaldehyde could form a methylene bridge, which cross-links the proteins'

polypeptide chains. This led to the unavailability of substrate for MTGase (Figure 2) (Chanarat & Benjakul, 2013a).

During the surimi preparation or storage, oxidation of protein reduced the textural and sensorial properties. Moreover, lipid oxidation at higher rate can induce the formation of hydroxyl, peroxyl, and superoxide radicals, which further oxidize the proteins and enzyme (TGase) (Zhou et al., 2014). Qian et al. (2021) determines the effects of 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) on the crosslinking ability of MTGase against silver carp myofibril protein. They observed that addition of AAPH (5 mM) induced oxidation of proteins, which promoted the glutamine-lysine and disulfide cross-linking associated with the MTGase (10 U/g). This could be related to the exposure of acyl groups during unfolding of myofibrillar proteins caused by AAPH oxidation. However, when the AAPH concentration was increased further to 10 mM, suppression of MTGase activity was noticed. This was more likely due to the excessive unfolding, which might cause the aggregation of proteins, causing reduction in the active site for the MTGase. Therefore, the extent of oxidation determines the MTGase activity, thus gel properties.

Food Hydrocolloids

Polysaccharides are natural biocompatible polymers with no toxicity. Polysaccharides could be obtained from several sources including microorganisms (dextran), algae (carrageenan and alginate), plant (pectin, guar gum, cellulose), and animal (chondroitin, hyaluronan) (Wijesekara et al., 2011; Rahman et al., 2022, Zhang et al., 2021). Polysaccharides are widely used in the food industry, drug delivery, regenerative medicine, and other biomedical applications (Buamard et al., 2020; Rani et al., 2017; Zhang et al., 2021). For surimi industries, polysaccharides have an essential impact on the textural and functional properties of the gel (Petcharat & Benjakul, 2017; Zhang et al., 2019).

Several polysaccharides, such as curdlan, xanthan gum, glucomannan, carrageenan, etc. are commonly used in foods due to their gelling, emulsifying, thickening, and stabilizing properties (Table 2). The majority of polysaccharide hydrocolloids may absorb water and expand when heated, filling up the network of surimi gels and exerting strength in the protein matrix (Zhuang et al., 2018). When heated over 80 °C, curdlan, a linear glucan linked by β-(1→3)-d-glucans without branching, may produce a persistent and thermo-irreversible gel. Curdlan (0-8 g/100 g) might increase the gel strength of hairtail surimi from 135 to 260 g. (Hu et al., 2015). Chen et al. (2020) found that silver carp surimi had increased gel strength with the addition of curdlan or κ-carrageenan at 4 and 2 g/kg, respectively. Buda et al. (2021) stated that the addition of 0.025 g/100 g of pectin from apple or 2 g/100 g of konjac glucomannan also improved the gel-forming ability of silver carp surimi.

Pectin is primarily an acid heteropolysaccharide made up of D-galacturonic acids (D-Gal-A) linked by α-1,4-glycosidic bonds (Cai et al., 2023). Furthermore, pectin is β-(1 → 4) linking D-mannose and D-glucose (1.6:1 ratio) with roughly one in every nineteen sugar units being acetylated (Zhang et al., 2016). Pectin showed a higher potential to be a gel enhancer, while glucomannan-treated gel exhibited superior whiteness. Gellan, an anionic microbial polysaccharide, is secreted from *Sphingomonas elodea* and consists of repeat units

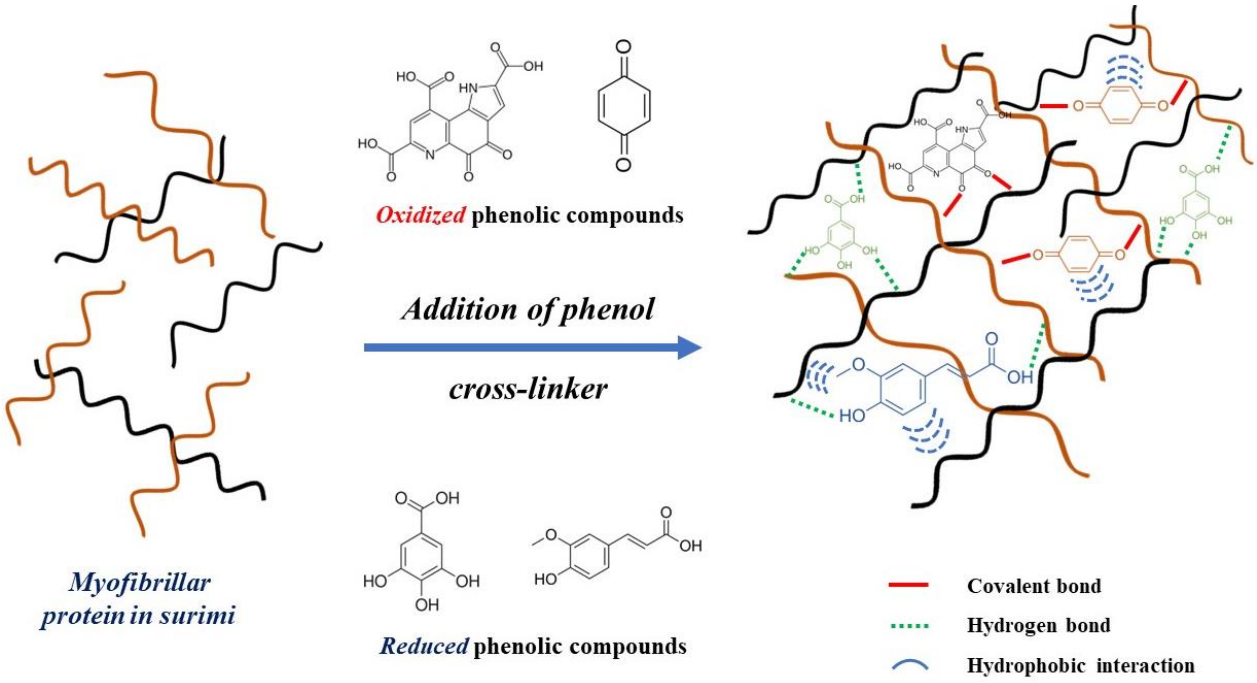

Figure 2. Role of TGase/MTGase and formaldehyde in surimi gelation

Table 2. Applications of food additive for improving surimi gel property

Additives	Applications	References
Protease inhibitor		
Egg white/Albumen	Salted albumen from duck egg (SADE), possessed trypsin inhibitory activity of 5,975 kunits/g powder. SADE at 8.61 g/100 g could increase breaking force of sardine surimi gel from 351.43 to 655.36 g, compare to the control.	Quan & Benjakul (2019)
	Addition of SADE at 2.5 g/100 g increased breaking force of threadfin bream surimi gel by 78%, compare to the control sample.	Wasinnitiwong et al. (2022b)
Yellow fin tuna roe (YFR)	Breaking force of bigeye snapper surimi gel increased as the amount of YFR (MW 70 kDa) increased from 520.88 to 803.00 g (YFR levels 0-3 g/100 g).	Klomklao et al. (2016)
Squid ovary (SO)	SO containing serine protease (MW 9.10 and 10.27 kDa) could enhance breaking force and deplete autolysis of bigeye snapper surimi in a dose dependent manner (0.5-2 g/100 g).	Singh & Benjakul (2017b)
Sarcoplasmic protein from tilapia dorsal muscle (SpC)	Lizardfish surimi added with 1 g/100 g SpC added and pre-incubated at 37 °C for 1 h exhibited 91.6% and 26.7% increases in breaking force and deformation, respectively, when compared to the control.	Yongsawatdigul & Piyadhammaviboon (2007)
Bambara groundnut protein isolate (BGPI)	BGPI prevented the degradation of myosin heavy chain in a dose-dependent manner in sardine surimi gel (0.25-1.5 g/100 g). At 1.5 g/100 g of BGPI, the gel showed the highest breaking force (~850 g).	Kudre & Benjakul (2013)
Soy protein isolate (SPI)	SPI at 10 g/100 g could deplete the autolysis during incubation at 60 °C for 1 h before cooking at 85°C for 30 min in grass carp surimi.	Luo et al. (2006)
Pea protein isolate (PPI)	Alaska pollock surimi gels were more flexible (higher γ_{max}), with greater energy stability (higher E parameter) and gel strength (higher G' and G" moduli) than the control gel when PPI was added at 1.41 g/100 g.	Moreno et al. (2021)
Polysaccharides		
Curdlan (CD)	Addition of CD at 0.4 g/100 g increased gel strength of silver carp surimi by 50.22%, compared to the gel without CD. CD also increased whiteness of surimi gel.	Chen et al. (2020)
	Use of curdlan of 4 g/100 g in combination with MTGase (0.4 units/g paste) could improve gel strength, water holding capacity and whiteness of hairtail surimi gel.	Hu et al. (2015)
Pectin (PT)	Surimi gel from silver carp added with PT at 0.025 g/100 g had higher breaking force (347.33 g) than that of without PT (302.57 g).	Buda et al. (2021)
Glucomannan (GMN)	GMN at 2 g/100 g with degree of acetylation of 34.13% enhanced gel strength of Alaska pollock surimi gel from 400 to 540 g·cm.	Zhang et al. (2015)
	Breaking force of gel from silver carp surimi was increased (100 g to 350 g, approximately) by adding GMN at 2 g/100 g.	Buda et al. (2021)
Gellan (GL)	Bigeye snapper surimi gel incorporated with 8 g/100 g GL suspension had the highest breaking force (637 g) with the increase by 99%, compared to that of control.	Petcharat & Benjakul (2018)
Cassava starch (CS)	Addition of Native CS at 1 g/100 g could increase gel strength and water holding capacity of golden threadfin bream surimi by 21.6 and13.3%, respectively, compared to those of control.	Mi et al. (2019)
Acetic acid esterification starch (AES)	Golden threadfin bream surimi had the increased gel strength by 42.9% compared to that of control when AES at 2 g/100 g was fortified.	Mi et al. (2019)
β-glucan (BG)	The addition of BG from yeast at 2 g/100 g reinforced hardness (from 1403.27 to 1763.99 g) and increased springiness (from 0.9 to 0.92) of gel from silver carp surimi.	Zhang et al. (2019)
	Under low salt condition (1 g/100 g), the addition of BG from oat at 1 g/100 g promoted hardness of silver carp surimi from 1,144.54 g to 1,249.08 g. There was no difference in springiness.	He et al. (2023)

of β-D-glucose (DGlc), β-D-glucuronic acid (D-GlcA) and α-L-rhamnose (L-Rha) at a molar ratio of 2:1:1. Gellan improved breaking force of bigeye snapper surimi gel when incorporated in surimi at a level of 6 g/100 g (Petcharat & Benjakul, 2018). Generally, gellan at a high level is more likely to form very hard or brittle gels, especially in the presence of cations such as calcium ions, resulting in the loss of elasticity or springiness (Leone et al., 2020). Petcharat and Benjakul (2017) documented that the penetration distance and springiness of surimi gel containing gellan at 6 g/100 g were decreased when the concentrations of $CaCl_2$ increased up to 75 mmol/kg.

Starch and modified starch have been also reported to improve the textural properties of surimi gel. Gel strength and textural profiles of golden threadfin bream could be promoted by the addition of native cassava starch and acetic acid esterification starch at levels of 10 and 20 g/kg, respectively (Mi et al., 2019). The major chemical force of the surimi gel network was hydrophobic interaction. In the surimi-starch system, the amounts and types of starch had a substantial impact on hydrogen bonding, ionic bonding, and hydrophobic interaction (Liu et al., 2014). Recently, β-glucan from oat or yeast has been introduced to improve the gel properties of surimi. This is a naturally occurring polysaccharide that is created from the monomer -D-glucopyranose by β-(1 → 3) and β-(1 → 4) glycosidic bonds. Due to numerous hydroxyl groups, hydrogen bonds can be formed with water molecules

and aid in the unfolding and cross-linking of proteins to produce a strong gel network with better water-holding capacity. Glucan has an excellent ability to absorb water (He et al., 2023). Proteins were unfolded more easily when oat-glucan was added at 1 g/100 g, in which changes from α-helix to β-sheet and β-turn occurred. As a result, the network of surimi-glucan gels was denser, and the voids were smaller and became uniform. Zhang et al. (2019) also reported that the addition of β-glucan (2 g/100 g) sharply improved gelling and textural properties of surimi gel from silver carp. Moreover, β-glucan has various medicinal properties, thereby enhancing overall human health (Daou & Zhang, 2012). Thus, β-glucan not only enhances the gelation of surimi but also renders health benefits.

Those advantages can be gained from polysaccharides when the optimal level is used. Conversely, an excessive level of polysaccharides may undergo self-aggregation and disturb the formation of an ordered network structure, resulting in a decrease in gel strength (Zhang et al., 2019). In addition, this can cause the dilution effect of myofibrillar proteins in surimi as a result of polysaccharides addition (Petcharat & Benjakul, 2018). Moreover, in the case of a small amount of water, polysaccharide-based hydrocolloids tend to aggregate and coil upon themselves, thereby occupying a large void and resulting in the distortion of the protein gel network (Ramírez et al., 2002). Thus, the type of polysaccharides and the level used have to be considered before being used as a gel strengthener in surimi.

Proteolysis in Surimi and Surimi Gel

After harvesting fish, protein degradation takes place during handling and storage. Digestive proteases can be released and contaminated in the muscle, causing hydrolysis of myofibrillar proteins (Singh & Benjakul, 2018). Along with those digestive enzymes, fish muscle also consists of proteases namely cathepsins and calpains, playing a profound role in the hydrolysis of myosin heavy chain (MHC) (Singh & Benjakul, 2018; Yan et al., 2023; Kwon & Chang, 2021). In general, cathepsins are more responsible for the hydrolysis of myofibrillar proteins than calpains (Benjakul et al., 1997; Singh & Benjakul, 2018). These proteases are known to promote the softening or mushiness as well as the gapping of the fish muscles, which leads to rejection by consumers. (Bremner & Hallett, 1985; Singh & Benjakul, 2018). Liu et al. (2020) extracted the sarcoplasmic serine protease (SSP) from the belly muscle of the threadfin bream. SSP considered a modori-inducing protease was cloned for the full-length cDNA (ORF 726 bp). A highly homologous amino acid sequence to trypsinogen from fish was observed. The SSP was mainly involved in the modori phenomena as indicated by the presence of mRNA of SSP and protease activity in the muscle tissue. Under physiological circumstances, those SSP were mostly produced and present in digestive system. Apart from the softening of the fish muscle tissues, weakening of fish meat or surimi gels also raised serious concern in the surimi industry. Although washing process removes most of non-myofibrillar components in fish muscle tissue, endogenous proteases remain tightly bound to the muscles (Yingchutrakul et al., 2022; Singh & Benjakul, 2018). During the preparation of surimi gel via setting/cooking, endogenous proteases get activated, resulting in the formation of a weakened or softened gel. The phenomenon of the formation of weak gel during cooking is called “modori” (Figure 3) (Yu et al., 2023; Singh & Benjakul, 2018). Heat-activated proteases become more active and severely degrade MPs, especially MHC when heated between 50 and 60°C. Nonetheless, actin is comparatively resistant to proteolysis (Benjakul et al., 2003b). In general, modori phenomenon is highly species-specific and is mainly attributed to cathepsins and heat-stable alkaline proteases (Jiang & Yin, 2004). In addition to those endogenous proteases, proteolysis or gel weakening depends on several factors including spawning period, parasitic association with fish (myoliquefaction), processing conditions, etc. (Singh & Benjakul, 2018; Shi et al., 2023). To combat proteolysis, many protease inhibitors of plant or animal origins have been introduced into surimi and surimi products.

Protease Inhibitors

Protease inhibitors are compounds that either reversibly or irreversibly block proteases and are crucial in the control of proteolysis (Yaiche Achour & Saadi, 2023). A wide range of protease inhibitors from various sources have been employed in surimi for the improvement of gel properties via inhibiting protease (Table 1). Plant seeds and tubers are rich in protease inhibitors, especially serine protease inhibitors, which can inhibit trypsin and chymotrypsin-like enzymes (Bijina et al., 2011; Singh & Benjakul, 2018; Divekar et al., 2022). Among the plants, legumes are particularly rich in inhibitors, which are divided into Bowman-Birk trypsin and chymotrypsin inhibitors and Kunitz trypsin inhibitors (Norioka et al., 1988). Several legume seeds, such as soybean and pigeon pea, tepary bean, cowpea and Bambara groundnuts, mung bean seed, velvet bean, jack bean, adzuki bean, Thai mung bean, and horse gram have been used to extract trypsin-like or serine protease inhibitors (Singh & Benjakul, 2018; Priyadarshini et al., 2022; Singh & Singh, 2020). In addition to legumes, protease inhibitors from wheat, barley, potato, and tomato showed inhibition towards trypsin and chymotrypsin (Habib & Fazili, 2007; Singh & Benjakul, 2018). For animal sources, egg white is one of the popular protease inhibitors used in surimi, while whey protein concentrate, by-product from dairy industry, has been used in surimi (Rawdkuen & Benjakul, 2008; Wang et al., 2023c). Furthermore, protease inhibitors are generally isolated from byproducts from agricultural or food processing industries such as bovine, porcine,

and chicken blood plasma and viscera (Walayat et al., 2022; Yamada et al., 2020; Singh & Benjakul, 2018). In addition, tuna roe (Klomklao et al., 2016) and squid ovary (Singh & Benjakul, 2017b), the leftover from seafood processing, were used for extraction of protease inhibitors, which could be used to lower proteolysis in surimi gel.

Applications of Protease Inhibitors in Surimi

Animal-based Protease Inhibitors

To preserve its commercial value and customer acceptance, protease inhibitors have been added to surimi and surimi products to suppress proteolysis mediated by natural proteases. In general, hydrolyzed myofibrillar proteins with short chains cannot form a strong gel network (Figure 3). Hence, the incorporation of protease inhibitors could help to maintain the chain length of proteins responsible for gelation. Whey protein isolate (WPC) and gg white powder (EWP) and inhibitors from legumes have been used widely in surimi (Choi et al., 2005; Singh & Benjakul, 2018; Wasinnitiwong et al., 2022a; Wasinnitiwong et al., 2022b; Wang et al., 2023c; Munawaroh et al., 2024; Priyadarshini et al., 2022). In general, EWP or albumen consists of several protease inhibitors, among which ovastatin (780 kDa) has strong trypsin-like protease inhibitory activity. Ovastatin shows similarity to α_2-macroglobulin in molecular structure, function, and inhibition mechanism (Nagase et al., 1983). Quan and Benjakul (2017) reported the proteolytic inhibitory activity of the salted duck egg albumins as witnessed by retarded autolysis. Salted duck egg albumin at all levels (0.5–2.5 g/100 g) inhibited the proteolytic activity in sardine surimi gel. In another study, Quan and Benjakul (2017) also performed a comparative study between duck egg albumen and hen egg albumen. Increases in breaking force and deformation were attained by the addition of both albumens to surimi, particularly as the amounts added rose. According to Jitesh et al. (2011), egg albumen often contains several protease inhibitors, including ovoinhibitor, ovomucoid, ovamacroglobulin, etc. that are effective in inhibiting serine proteases. However, both serine protease and metalloprotease can be inhibited by duck egg ovostatin (ovomacroglobulin) (Hu et al., 2016). Recently, Wasinnitiwong et al. (2022b) observed that the breaking force of surimi gel increased by 78% with the addition of 2.5 g/100 g salted duck EWP as compared to the control sample (without additive). Similarly, deformation was increased by 13.8% and the expressible moisture content was reduced by 38.5% with the addition of 1.5 g/100 g salted duck EWP. In addition, EWP is also known as gel filler or binder, which can strengthen the gel network and enhance water holding capacity (Singh & Benjakul, 2018). Salted duck EWP along with 0.5 g/100 g κ-carrageenan also improved the gelling properties of threadfin bream surimi (Wasinnitiwong et al., 2022a). In addition to EWP and WPC, the surimi is also added with chicken, porcine, and cow plasma proteins. WPC boosted the shear strain of surimi gels made from Pacific whiting and Alaska pollock, goatfish, bigeye snapper, threadfin bream, lizardfish, arabesque greenling, walleye pollock, and other species (Park et al., 1994; Piyachomkwan & Penner, 1994; Rawdkuen & Benjakul, 2008; Singh & Benjakul, 2018; Morrissey et al., 1993; Rawdkuen et al., 2008). Along with the protease inhibitors, other

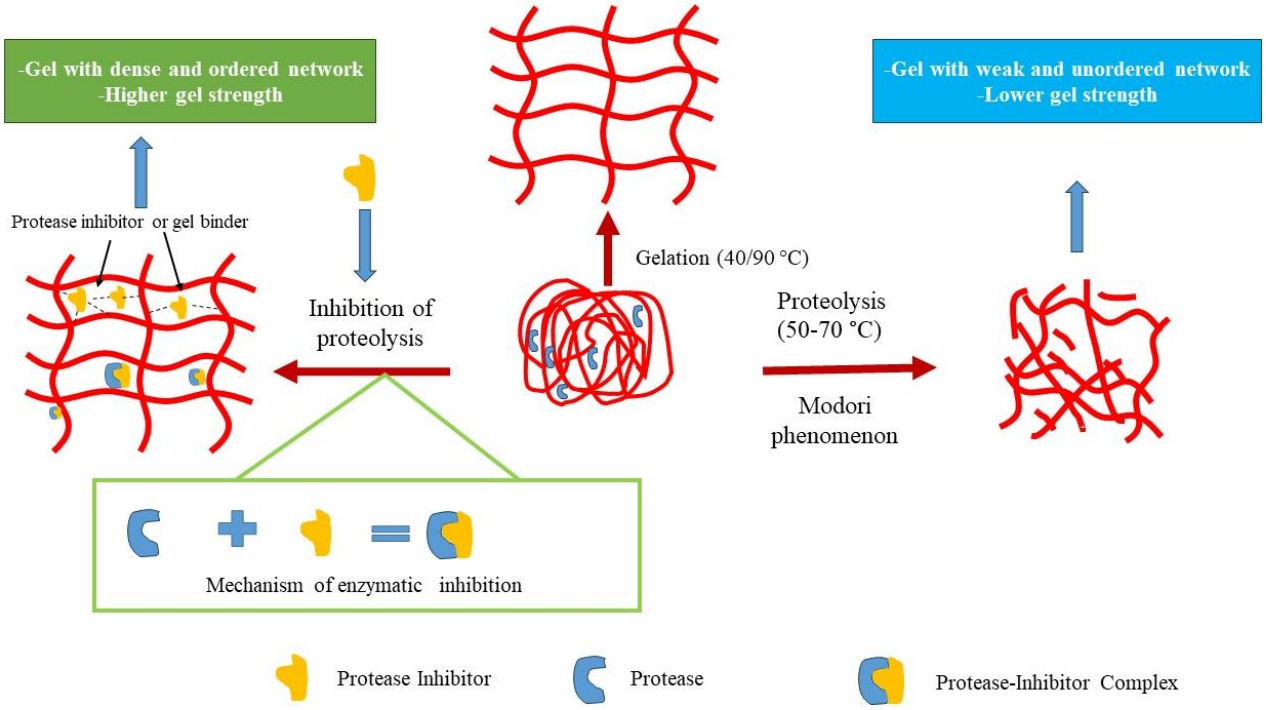

Figure 3. Gelation and proteolysis in surimi without and with protease inhibitors or binder

compounds such as MTGase, hydrocolloids, etc. were added to surimi gel, which synergistically increased the gelling properties. Recently, Zhao et al. (2023) used WPC in combination with the Ca^{2+} and MTGase (3.5, 5 and 1 g/100 g, respectively), which significantly increased hardness and water holding capacity by 156% and 25%, respectively. Cross-linked proteins might be more resistant to proteolysis. Cleavage site of proteins might be masked, in which hydrolysis could not take place, thus lowering proteolysis in the gel.

Fish roe protease inhibitors also inhibit pancreatic serine proteinases. When trypsin inhibitors from yellowfin tuna roe were incorporated in kamaboko and modori gels from bigeye snapper surimi, breaking force and deformation augmented (Klomklao et al., 2016). Apart from tuna roe, squid ovary proteins also showed inhibitory activity against serine protease. Furthermore, protease inhibitors derived from the squid ovary prevented autolysis of bigeye snapper surimi, as evidenced by preserved MHC and a decreased TCA-soluble peptide content (Singh & Benjakul, 2017b). The sarcoplasmic proteins concentrated from tilapia showed inhibitory activity, along with TGase activity (Yongsawatdigul & Piyadhammaviboon, 2007). The crude plasma and ethanol-extracted fraction from swamp eel plasma were added to the tilapia surimi. Ethanolic extract (1.5 mg/g) improved gel properties more effectively than the crude one (Nopianti et al., 2023). Salmon blood plasma also slows the autolysis of Pacific whiting and salmon mince surimi (Fowler and Park, 2015b). Salmon blood plasma inhibited cysteine and serine proteases strongly. Furthermore, the gel characteristics of Pacific whiting surimi were improved when it was mixed with salmon blood plasma and heated ohmically at 60°C for 30 min before further heating ohmically to 90°C. Salmon blood plasma at 1 g/100 g could augment the strength of gels held at 25°C for 2 h prior to ohmic heating (Fowler & Park, 2015a). The inhibitory effect of the plasma could be associated with the presence of alpha-2-macroglobulin (Li et al., 2008). Recently, tissue inhibitor of metalloproteinase-2 (TIMP-2; 18 kDa) partially purified from the soluble fraction of yellowtail muscle, was cloned to two isoforms (TIMP-2a and TIMP-2b) (Jiang et al., 2023b). The protease inhibitory activity of TIMP-2 was determined via human TIMP-2, which suggested that TIMP-2 from yellowtail muscle could be an alternative candidate to inhibit the modori phenomenon (Jiang et al., 2023b).

Plant-Based Protease Inhibitors

Although animal-based proteins possess excellent inhibitory activity against surimi protease, religious constraints, allergies, etc. limit their application in the surimi industry. Hence, alternative protease inhibitors, mainly from plants, could be used as alternative sources. Protein isolates or protein concentrates or extracts of plant origin, such as legume seed, etc. have been known to possess protease inhibitory activity (Singh & Benjakul, 2018; Wang et al., 2023a). Legumes, such as Bambara groundnut, black bean, and mung bean protein isolates have been documented to increase the breaking force and deformation of kamaboko as well as suwari gels from sardine surimi (Kudre et al., 2013; Kudre & Benjakul, 2013). This could be associated with the presence of protease inhibitors (Benjakul et al., 2000). Benjakul et al. (2000) extracted and partially purified proteinase inhibitors from Thai legumes such as cowpeas, pigeon peas, and Bambara groundnut. Those protease inhibitors inhibited sarcoplasmic modori-inducing proteinase from threadfin bream muscle, especially as the concentration upsurged (Kudre et al., 2013). However, when soy protein isolate (SPI) levels were increased to 400 g/kg of total proteins, breaking force and other gel properties of grass carp surimi were decreased (Luo et al., 2006). Although protease inhibitors in SPI enhanced gel properties, an excessive amount might cause dilution of myofibrillar proteins. This led to the lower interconnection among the surimi proteins (Singh et al., 2021). Moreno et al. (2021) replaced myofibrillar proteins with 5 and 8 g/100 g of pea flour and 1.41 g/100 g of pea protein isolate for gel preparation. Thereafter, frozen-stored gels were kept for one year under vacuum. The gel containing pea protein isolate-surimi was more flexible with higher gel strength (higher G' and G" moduli) than the control gel. Moreover, the properties of surimi gels added with pea flour or protein isolate were retained during the one year of frozen storage. In addition to the legumes, 2% of potato proteins isolate showed the highest inhibitory activity against autolysis of Pacific whiting surimi (Yoon et al., 2022). The result was also supported by the improved breaking force, penetration distance, and water retention ability of surimi gel during the ohmic heating at 60°C for 30 min prior to heating up to 90°C (Yoon et al., 2022).

Conclusion

Gel properties of surimi vary from species to species, habitat, processing conditions, and additives used. The gel property of surimi can be improved by the addition of protein cross-linker, especially phenolics from the plants as well as MTGase. In addition, WHC and gel strength of gel can be enhanced with the addition of hydrocolloids when an appropriate amount is added. Proteolysis occurring in surimi known as 'modori' could be retarded using natural food-grade protease inhibitors. Thus, novel, and safe additives, especially those derived from natural sources or food processing byproducts, should be recovered for improvement of surimi gel quality, in that their products can meet the consumer and market requirements.

Ethical Statement

Ethics approval was not required for this research.

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Author Contribution

Natchaphol Buamard: Conceptualization and Writing-original draft; Avtar Singh: Conceptualization, Writing-original draft and Writing-review and editing; and Soottawat Benjakul: Conceptualization, Supervision and Writing-review and editing. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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