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Effect of potato starch addition on gel-forming ability and autolytic inhibition of Rohu gels

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Abstract

This study investigated the effect of adding potato starch (PS) (1–4%) on the gel-forming ability and autolytic inhibition of rohu (*Labeo rohita*) surimi gels. Kamaboko gel (heating at 40 °C for 30 min, then 20 min at 90 °C) and modori gel (heated at 60 °C for 30 min, then 20 min at 90 °C) were made. In comparison to the control, adding 1% PS increased the gel strength and lowered the expressible water content of kamaboko considerably ($P < 0.05$). The textural qualities of the modori gel improved with the addition of 4% PS, and the expressible water content reduced to the same level as that of the kamaboko gel. The addition of PS was less efficient in suppressing autolytic activity, according to the TCA-soluble peptide content and SDS-PAGE profile. PS affected the whiteness of both surimi gels. After adding PS to surimi gels, the microstructure of the gel network became more compact, and voids within the fiber network reduced in size. PS added to the gel matrix was more effective as a filler than in preventing protein breakdown. Addition of 1% PS to kamaboko gels and 4% PS to modori gels improved their gel-forming ability.

Keywords: Proteolytic degradation, Gel properties, Potato starch, Rohu (*Labeo rohita*)

1. Introduction

The gel-forming ability of fish gel protein is negatively impacted by proteolytic degradation. In this process, endogenous protease plays a critical role. Myofibrillar proteins, particularly myosin, are degraded by the enzymes. This prevents the creation of a strong three-dimensional network, which weakens the gel [1, 2]. A number of studies have focused on the optimum temperature of proteolysis. Walleye pollack (*Theragra chalcogramma*) surimi, as well as yellow stripe trevally (*Selaroides leptolepis*) surimi [3], gels at 60 °C, according to Hu et al. [4]. At 65 °C, the surimi of red tilapia (*Oreochromis niloticus* × *O. placidus*) weakens [5]. Sutloet et al. investigated the effect of setting conditions on the gel-forming ability of unwashed rohu mince and reported that heating at 65 °C results in the least gel strength and the most protein breakdown [6]. The endogenous heat-stable protease becomes active around 50–70 °C [7, 8].

To prevent protease breakdown and enhance gel characteristics, egg white, whey protein concentrate, and potato starch (PS) have been used as food protease inhibitors [9, 10]. Starch is most commonly employed as a filler to boost the firmness and gel strength of surimi or fish-based products [11]. Our previous study found that cysteine protease is the most prominent endogenous protease in the autolysis of unwashed rohu gel, while serine protease is essential in the autolysis of washed rohu gel [12].

PS has been researched as a gel structure reinforcement component. The water-holding capacity of pressurized surimi gel from Pacific whiting (*Merluccius productus*) and Alaska pollock (*T. chalcogramma*) is increased by

the addition of 4% PS [13]. The gel strength of surimi gel treated with ohmic heating is slightly increased when PS is added [14]. Hunt et al. [15] revealed in a study of starch–protein systems that the starch granules, which expand when exposed to water during gelatinization, reinforce the gel structure. Many studies have also focused on the effect of potato powder and potato extract as a protease inhibitor [16–18]. Potato powder contains proteins with molecular weights of 67 kDa in cysteine inhibitory gels and 31 kDa in serine inhibitory gels [16]. The addition of potato powder at a concentration of 0.5% inhibits autolysis activity and myosin heavy chain (MHC) degradation [17]. Porter et al. [18] found that adding 0.1% potato extract to Pacific whiting and arrowtooth flounder substantially suppressed enzyme activity. They also found that at all concentrations, the strength of kamaboko gel containing dried potato extract and potato powder was greater than that of the control. In recently, Liu et al. [19] reported that potato protease inhibitors are one of components of potato protein. Its accounts for 50% of the protein with molecular weight of 5–25 kDa. PS, which contain protein content about 0.06% [20], might play a role in protease inhibitor in fish gel protein.

The purpose of this study was to investigate the optimum concentration of PS that affects the ability of rohu (*Labeo rohita*) surimi gels and inhibit autolysis in both of kamaboko and modori gels.

2. Materials and methods

2.1 Materials

Rohu (*Labeo rohita*) weighing 1000 ± 100 g each were obtained at Ying Charoen Market (Bangkok, Thailand). The fish were shipped to the lab in a container with ice within 90 min.

PS was obtained from Emsland Asia Food Innovation Corp Co., Ltd. Trichloroacetic acid (TCA) was purchased from QR&C (New Zealand). Folin–Ciocalteu phenol was supplied by Merck KGaA (Darmstadt, Germany). Bovine serum albumin (BSA) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Bio-Rad provided the reagents required for gel electrophoresis (Hercules, CA, USA).

2.2 Method

2.2.1 Preparation of surimi gels

The minced and surimi gels were prepared following the method described by Sutloet et al. [12]. After the addition of salt, PS was added at concentrations of 0%, 1%, 2%, 3%, and 4% (w/w) during grinding.

2.2.2 Folding test

The gel samples for folding test was prepared following the method described by Sutloet et al. [12] and were evaluated followed the standard 5-point grade system [21].

2.2.3 Determination of textural properties

Gel samples were left at room temperature (~ 30 °C). Five cylinder-shaped samples with a diameter and length of 2.5 cm were produced from each gel. The breaking force and breaking distance were multiplied to determine the gel strength (g.cm). The textural qualities were calculated using the approach published by Sutloet et al. [12] using a texture analyzer (TA – XTPlus, Stable Micro Systems, Godalming, Surrey, UK) with a spherical probe (5 mm diameter; 60 mm min⁻¹ test speed).

2.2.4 Determination of expressible water content

Gel samples were prepared and expressible water content was measured using the method described by Sutloet et al. [12].

2.2.5 Determination of whiteness

L*, a*, and b* values of gel samples were measured using a colorimeter (ColorFlex CX2687, HunterLab, USA) and a D65 illuminant as the light source, and the equation from Sutloet et al. [12] was used to compute whiteness.

2.2.6 Determination of TCA-soluble peptide content

The procedure described by Sutloet et al. [12] was used to determine the TCA-soluble peptide content.

2.2.7 Determination of protein pattern

The protein pattern of the samples was analyzed by slightly modifying the SDS–polyacrylamide gel electrophoresis (SDS-PAGE) method from Laemmli [22] reported by Sutloet et al. [12].

2.2.8 Microstructure of surimi gel

A scanning electron microscope (JSM-IT300, JEOL, Japan) was used to determine the microstructure of gel samples. Specimens were prepared following the method described by Sutloet et al. [12].

2.2.9 Statistical analysis

The experiment used a completely randomized design. Data were subjected to analysis of variance. At $p \leq 0.05$, the differences between sample means were analyzed using Duncan's new multiple range test. Each experiment was carried out in triplicate.

3. Results and discussion

3.1 Effect of PS on textural properties of rohu surimi gels

Figure 1 exhibits the results of the folding test as well as the gel strength of kamaboko and modori gels at different concentrations of PS. In the folding test, no significant change was found for either gel ($P > 0.05$), but the modori gel containing PS tended to have an increased degree of folding compared with the control ($P > 0.05$). However, the concentration of PS was significantly associated with gel strength in both gels ($P < 0.05$). As depicted in Figure 1, the modori gel had a lower gel strength than the kamaboko gel ($P < 0.05$). Setting at 40 °C causes non-disulfide linkages made by the endogenous transglutaminase (TGase) to crosslink ϵ -(γ -glutamyl) lysine in MHC [23, 24]. Modori gel weakening is carried out by endogenous protease activity. Sutloet et al. [6] reported that gels set at 60 °C show the highest levels of proteolytic activity and the lowest gel strength, supporting the findings of Kinoshita et al. and An et al. [7, 8]. The latter reported that gel weakening is caused by the activity of endogenous protease under heating at 50–70 °C.

Figure 1 illustrates this relationship, showing that the strength of both gels rose in proportion to the increase in PS ($P < 0.05$). Comparing the gel strength of modori gels with 1%, 2%, 3%, and 4% PS to that of the control, the corresponding increases were 14.4%, 25.1%, 29.0%, and 46.3%. The comparable increase in the kamaboko gels was 23.1%, 37.6%, 40.7%, and 60.4%. Kamaboko gel had the highest gel strength with 4% PS ($P < 0.05$). These results support the findings of Paker and Matak [25]. They studied the effect of PS concentration on protein gel from calcium-enhanced black bullhead catfish (*Ameiurus melas*) and used torsional shear stress and strain as the indicators of gel firmness and resistance to deformation, discovering that those indications increased as PS concentration did.

The starch in fish gel protein functioning as a filler has been attributed to the increase in gel strength when PS is added. Gelling of fish protein begins at 50 °C, preceding starch gelatinization [16, 26]. The protein gel network controls the amount that the starch granules can swell. A packing effect is produced by the starch granules that are packed in the network, which increases turgor pressure there [26, 27]. PS granules gelatinize at approximately 56–66 °C [16]. The strength of both surimi gels was therefore increased by the addition of PS. However, Jafarpour et al. [10], who investigated how PS affects the functional characteristics of common carp (*Cyprinus carpio*) surimi gel, found that as the PS concentration rose, the gel strength dropped ($P < 0.05$). This could be due to competition between the starch and protein. Chung and Lee [28] reported that the water-binding capacity of protein when forming a gel limits the availability of water for starch gelation. Protein and starch compete to bind water for gelatinization if insufficient water is introduced to the system [29]. According to Yang and Park [16], the molecular structure of fillers affects the textural characteristics of protein gels containing those fillers. They suggested that the primary gel structure is either weakened or reinforced by the addition of fillers. In addition, proteolytic degradation of some fish species is retarded by the addition of potato powder and potato extract [1, 17, 18]. This suggests that, in this study, the addition of PS improved the strength of both gels by acting as a filler and proteinase inhibitor.

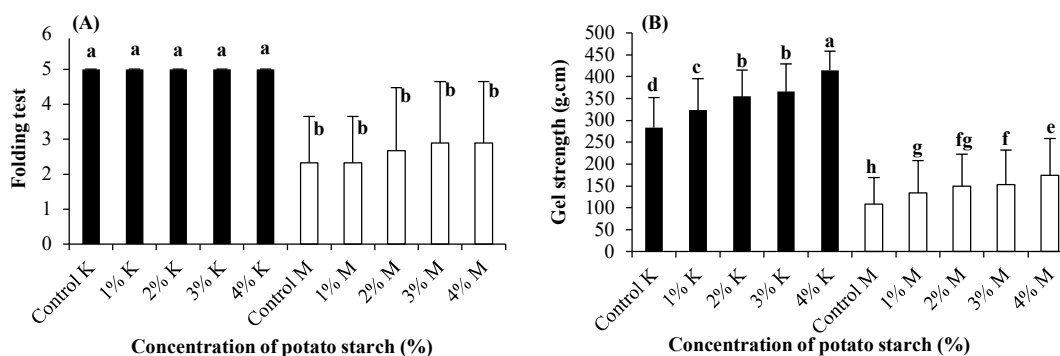


Figure 1 Folding test (A) and gel strength (B) of gels as affected by PS addition. K: kamaboko gel; M: modori gel. The mean and standard deviation are represented as bars (n = 5). A different letter appears on each bar to indicate significant differences (P < 0.05).

3.2 Effect of PS on expressible water content of rohu surimi gels

The expressible water content of surimi gels containing various levels of PS is shown in Figure 2. In comparison to the modori control, the expressible water content of the kamaboko control gel was 27.3% lower (P < 0.05). Rawdkaen and Benjakul [30] reported that a lower expressible water content is associated with a higher water-holding capacity. This suggests that the matrix of the kamaboko gel was better at retaining water than that of the modori gel. These results agree with those for the textural properties, suggesting that compared to the weaker network of modori, the stronger gel network of kamaboko is better at holding onto water.

The expressible water content of both gels reduced as the PS concentration rose (P > 0.05). The lowest expressible water content was found in kamaboko with 4% PS (P > 0.05), which decreased by 34.4%, compared with the control. In contrast, samples with 1%, 2%, and 3% PS decreased by 15.7%, 23.7%, and 24.8%, respectively (P < 0.05). The expressible water content of the gel containing 4% PS was the lowest in the modori gel, declining by 31.1% in comparison to the control (P < 0.05). At PS concentrations of 1%, 2%, and 3%, decreases of 4.25%, 12.28%, and 18.64% were observed. These findings suggest that adding PS can enhance a gel's ability to hold water, and confirmed those of Tabilo-Munizaga and Barbosa-Cánovas [13]. They found that adding 4% PS increased the water-holding capacity of pressurized surimi gels made from Pacific whiting (*M. productus*) and Alaska pollock (*T. chalcogramma*). The ability of the starch granules to bind water may be responsible for this. As the granule is heated, it starts to absorb water from its surroundings and swells. In the enlarged starch granule, the hydrogen bonds break down at the gelatinization temperature, releasing the hydroxyl groups, which then bind to the water molecules. The granules then continue to swell [16]. The starch granule both binds the water in the protein gel system and exerts turgor pressure on the gel matrix [15, 16]. A stronger gel therefore retains water within the structure more effectively than a weaker gel. Adding 4% PS to modori gel increased its capacity to hold water to a level comparable to that of kamaboko control gel, whereas adding 1% PS to kamaboko gel increased its capacity to hold water relative to control gel.

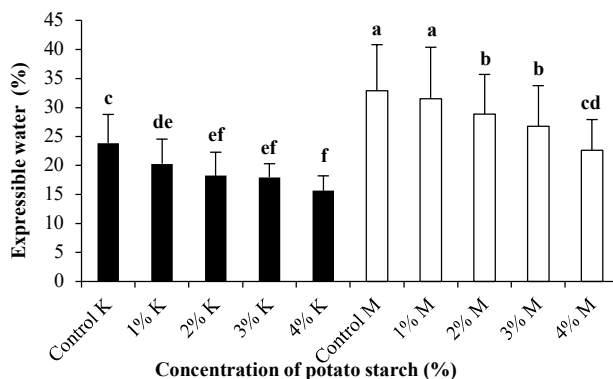


Figure 2 Expressible water content of gels as affected by PS addition. K: kamaboko gel; M: modori gel. The mean and standard deviation are represented as bars (n = 5). A different letter appears on each bar to indicate significant differences (P < 0.05).

3.3 Effect of PS on whiteness of rohu surimi gels

The whiteness of surimi gels containing 0–4% PS is depicted in Figure 3. The whiteness of both gels was considerably impacted by addition of PS ($P > 0.05$). As can be observed, as the PS concentration increased, both gels' whiteness diminished slightly. The L^* value of both gels behaved similarly, declining as the PS content rose (data not shown). This was possibly caused by swelling of the starch granules. As noted above, the gelatinization temperature of PS is approximately 56–66 °C [16]. Thus, the PS granules of both gels at 90 °C were swollen or gelatinized. Yang and Park [16] reported that starch granules in a gel absorb water, becoming fully swollen. A translucent gel forms, allowing more light to pass through. This could account for the decline in L^* value when the PS concentration rose ($P > 0.05$). Compared to kamaboko, modori gel had a higher level of whiteness. The modori gel network was weaker so it was unable to hold as much water inside its structure as the stronger kamaboko gel. The modori gel was whiter than the kamaboko gel because some of the water in it leaked out, preventing light from passing through.

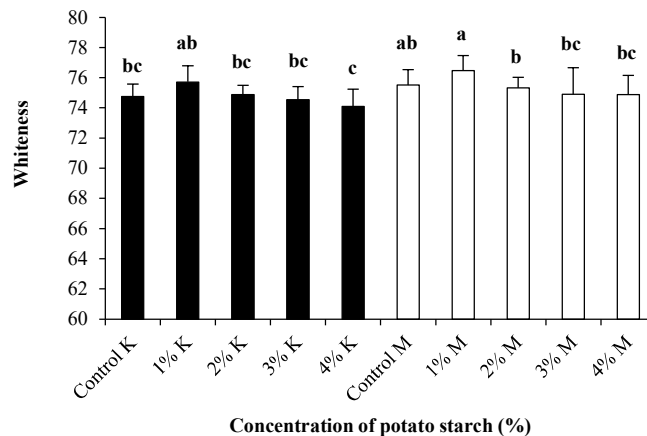


Figure 3 Whiteness of gels as affected by PS addition. K: kamaboko gel; M: modori gel. The mean and standard deviation are represented as bars ($n = 5$). A different letter appears on each bar to indicate significant differences ($P < 0.05$).

3.4 Effect of PS on TCA-soluble peptide content of rohu surimi gels

The TCA-soluble peptide content of the surimi gels with various PS levels is shown in Figure 4. In comparison to the modori control, the kamaboko control contained less TCA-soluble peptide ($P < 0.05$). The outcomes were consistent with those for expressible water content and textural properties, suggesting that the stronger kamaboko gel became less degraded than the weaker modori gel. As can be seen, both gels' TCA-soluble peptide content tended to fall as the PS concentration rose. This was only significant in the kamaboko gel with 1% PS, with a drop of 18.2% from the control ($P < 0.05$). Of the modori gels, only the one containing 4% PS showed a significant decline ($P = 0.05$), with a decrease of 24.3% in comparison to the control. The findings are in line with those of Akazawa et al. [17], who studied how the autolytic activity and myosin degradation of Pacific whiting (*M. productus*) are affected by potato powder. They reported that the addition of potato powder at a concentration of 0.5% inhibited both autolysis activity and MHC degradation, as observed from the SDS-PAGE results. Porter et al. [18] studied the effect of potato extract on the enzyme activity of Pacific whiting (*M. productus*) and arrowtooth flounder (*Atheresthes stomias*) muscle and surimi. They found that the addition of 0.1% potato extract inhibited enzyme activity in both species. This inhibition may be due to the protease inhibitors of the potato protein in PS [20]. Weerasinghe et al. [31] reported that potato powder contains serine and cysteine protease inhibitors. Some fish species, particularly Pacific whiting [1, 17], as well as arrowtooth flounder [18], have been shown to have slower proteolytic breakdown when potato powder and potato extract is added. However, 4% PS had to be used to bring the modori gel's TCA-soluble peptide content to the same level as the kamaboko control. These findings suggest that PS's role as a filler in the gel matrix is more effective than that as protease inhibitor.

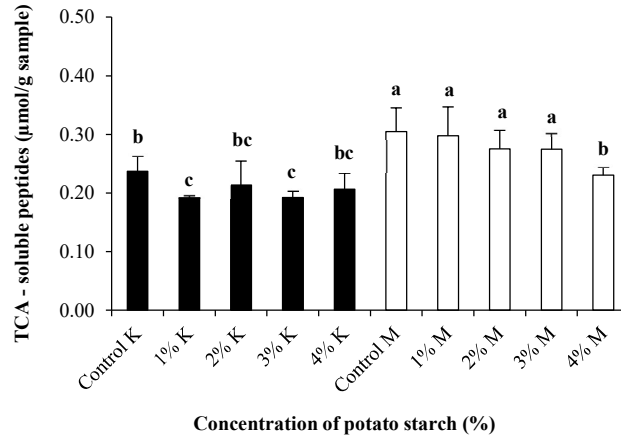


Figure 4 TCA-soluble peptide content of gels as affected by PS addition. K: kamaboko gel; M: modori gel. The mean and standard deviation are represented as bars ($n = 5$). A different letter appears on each bar to indicate significant differences ($P < 0.05$).

3.5 Effect of PS on SDS-PAGE pattern of rohu surimi gels

Figure 5 displays the protein patterns of surimi gels with PS at various doses. The addition of PS had no impact on the MHC intensity, which was somewhat lower in the kamaboko control than the unheated control mince (Lane 2, Figure 5A). This was observed in degraded protein from a gel containing PS, with molecular weights ranging from 97 to 116 kDa. In modori gel, the control's MHC intensity (Lane 3, Figure 5B) degraded more quickly than that of the unheated control mince (Lane 2, Figure 5B), and the addition of PS had no further impact on the intensity. This outcome was revealed by degraded modori protein, which had molecular weights between 97 and 116 kDa. There was no obvious change in the actin band in any gel. The protein pattern results are not in agreement with those for gel characteristics, expressible water content, or TCA-soluble peptide content. This suggests that the principal role of PS is not as a protease inhibitor but rather as a filler.

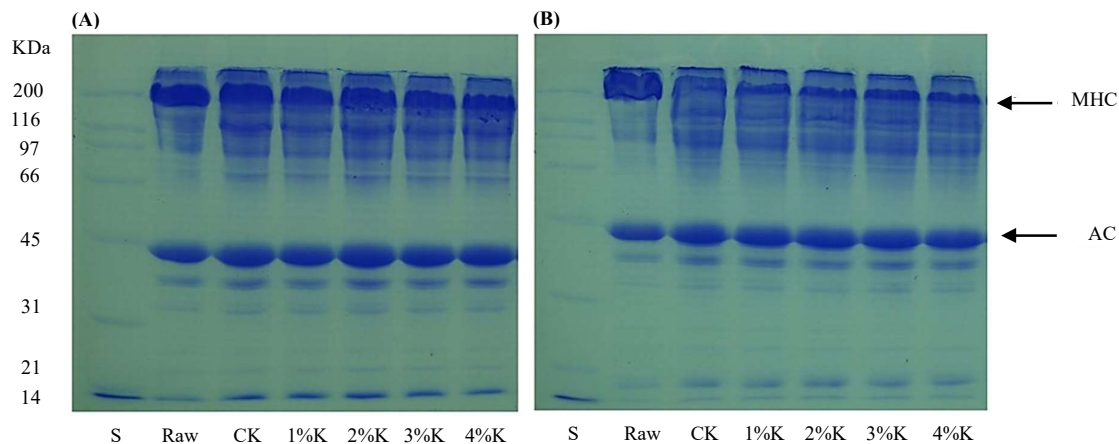


Figure 5 SDS-PAGE pattern of gels as affected by PS addition. Kamaboko gel (A), Modori gel (B). MHC: myosin heavy chain; AC: actin; S: standard protein; Raw: unheated control mince; CK: kamaboko control gel (0% PS); CM: modori control gel (0% PS); 1–4% K: kamaboko gel with 1%, 2%, 3%, and 4% PS; 1–4% M: modori gel with 1%, 2%, 3%, and 4% PS.

3.6 Effect of PS on microstructure of rohu surimi gels

Figure 6 shows the microstructure of surimi gels containing PS at various concentrations. The kamaboko control gel (Figure 6A) showed a large protein cluster associated with a fine fibrous network. The control gel structure contained a lot of large cavities. The addition of PS (Figure 6B and C) increased the density of the structure, compared with the control. As the process temperature (90 °C) was greater than the gelatinization temperature (56–66 °C), complete gelatinization of the PS granules took place [13, 16]. The modori control gel

also exhibited large protein clusters and cavities, as shown in Figure 6D. When PS was added, the gel had a denser structure and smaller cavities than the control (Figure 6E and F). No PS granules were visible in the gel structure. According to Tabilo-Munizaga and Barbosa-Cánovas [13], their gel samples from surimi (heated at 90 °C for 40 min) exhibited a fine fibrous network with small reticular zones scattered throughout the network. Under this heating condition, the PS seems to have fully gelatinized.

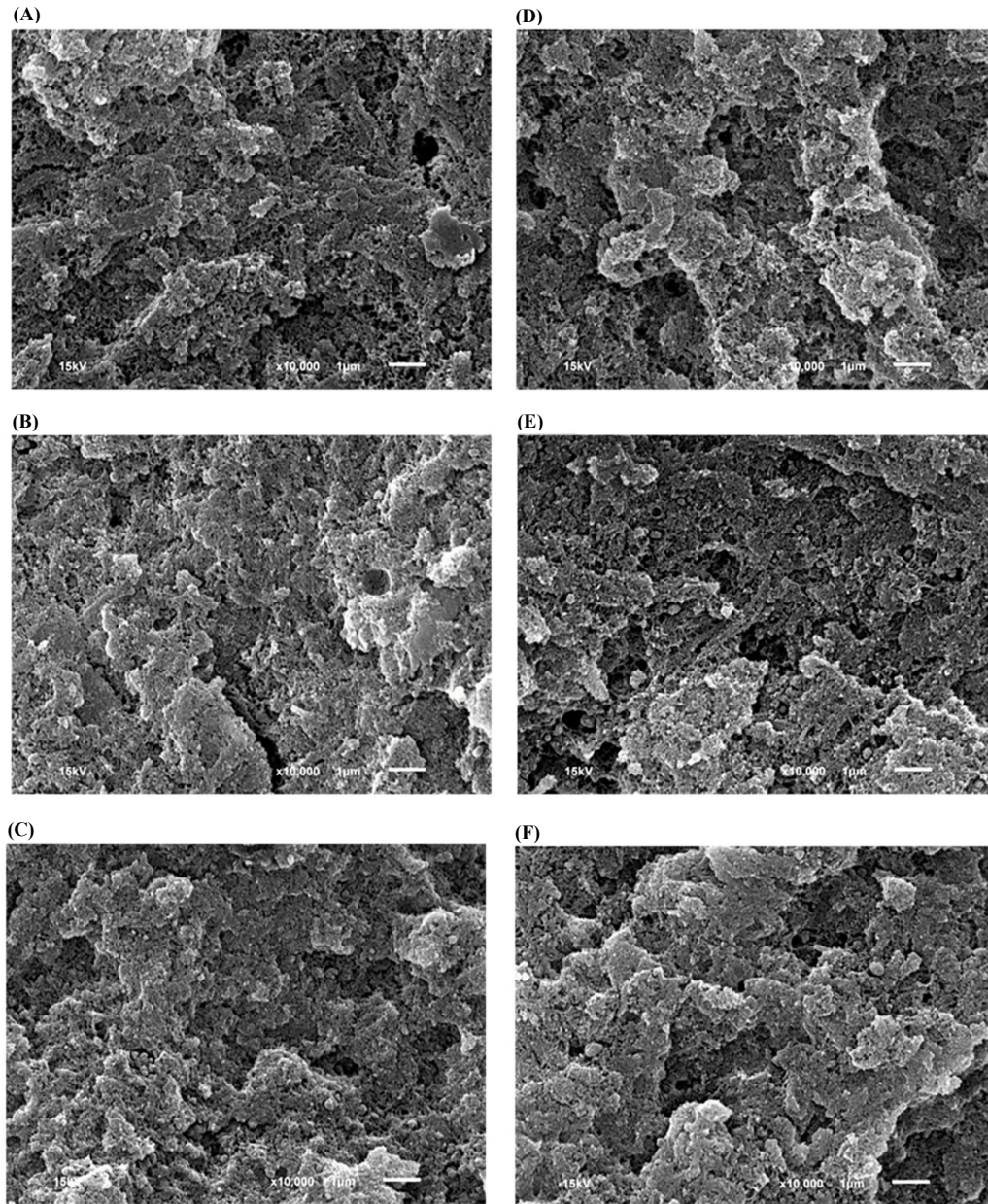


Figure 6 Microstructure of gels as affected by PS addition. Kamaboko gel (A–C) and modori gel (D–F). A and D: kamaboko and modori control gel (0% PS); B and E: gel with 2% PS; C and F: gel with 4% PS. Magnification: 10,000 \times .

4. Conclusion

Adding 1% PS to kamaboko gels and 4% PS to modori gels improved their gel-forming ability based on texture characteristics and water-holding capacity. PS was less effective as a protein protease inhibitor based on TCA-soluble peptide content and SDS-PAGE profile. The whiteness of both gels was impacted by the addition of PS. The cavities within the fibrous network of surimi gels became smaller. With the addition of PS, the gels'

microscopic structure became more tightly packed. The modori gels' ability to create gels was enhanced by the addition of PS, though not as significantly as it was for kamaboko gels.

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