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RESEARCH PAPER

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Gel-Forming Ability of Surimi from Aquacultured *Pagrus major* as Affected by Freeze-Thaw Cycle

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Abstract

This study focuses on the effect of freeze-thaw cycles on surimi and fish-meat gels prepared with *Pagrus major* (red sea bream, PM) compared to FA and RA grade Alaska pollock. Freeze-thaw cycling led to decreased values for water holding capacity (WHC), gel strength and gel texture with all surimi and fish-meat gels. Both PM surimi and fish-meat gels had greater WHC and whiteness than those of RA grade. PM was more comparable to FA following freeze-thaw abuse. Although still better than RA, PM fish-meat gels showed a loss of cutting strength, hardness, breaking force and deformation. Texture analysis of gels showed that FA gel had more favorable chewiness, springiness, cohesiveness and brittleness. Overall, PM fish-meat gels were comparable to Alaska pollock FA fish-meat gels with respect to freeze-thaw stability.

Introduction

Freezing has been reported to cause a decrease in product texture, flavor and color (Akamittath, Brekke, & Schanus, 1990; Zhuang & Savage, 2013) that depends on several such factors as storage temperature, freeze-thaw cycles, temperature fluctuations and storage time (Badii & Howell, 2002). Freezing causes changes in muscle tissue through formation of ice crystals and dehydration, while thawing of frozen products may cause fiber shrinkage and drip loss (Srinivasan, Xiong, Blanchard, & Tidwell, 1997). The shear force of thawed meat is higher than the non-frozen meat, which is attributed to myosin denaturation and myofibrillar protein crosslinking in seafood (Kim, Carpenter, Lanier, & Wicker, 1993). Freeze-thaw cycling leads to lipid oxidation which detrimentally changes texture in seafood, especially fish muscle (Thanonkaew, Benjakul, Visessanguan, & Decker, 2006).

Solubility and gel forming ability of fish muscle proteins are highest when un-denatured, i.e., at temperatures below 60°C (Takahashi, Kurose, Okazaki, & Osako, 2016). Compared to mammalian proteins, the gel forming ability of fish-meat proteins were more sensitive to fluctuating temperatures during frozen storage (Degner, Chung, Schlegel, Hutkins, & McClements, 2014).

Surimi obtained from seafood depends on a fish-meat gel as an intermediate product prepared from minced fish-meat, i.e., fish paste. Surimi seafood products remain in demand because of their low cost, savory taste, good nutrition, ease of production, storage and transportation (Guenneugues & Ianelli, 2013). Both raw materials and fish-meat gels are stored and distributed frozen and thus are subject to quality loss during frozen storage and thawing. Since white-

meat fish are generally preferred, Alaska pollock is the preferred raw material for fish-meat gel production (Asche, Roll, & Trollvik, 2009; Criddle & Strong, 2013) because of its texture, fat concentration and the hardness of its fish-meat gels. Its fish pastes are reasonably stable during frozen storage but are fairly susceptible to fluctuating temperatures (Kim, Hamann, Lanier, & Wu, 1986; Scott, Porter, Kudo, Miller, & Koury, 1988). Currently, high quality surimi is made using fresh unfrozen fish. Fish-meat gels prepared from frozen surimi often lack the quality of fresh fish products. Thus, other potential species might overcome some of the limitations of Alaska pollock.

In this context, *Pagrus major*, a widely aquacultured fish in Korea might be a candidate for producing fish-meat gel products. *P. major* is a white fleshed fish with a low fat content (Mustafa, Umino, & Nakagawa, 1994). In this study the durability and gel-forming ability of *P. major* surimi after 9 freeze-thaw cycles was compared with Alaska pollock surimi. In addition, fish-meat gels were prepared from surimi and subjected to freeze-thaw cycles.

Materials and Methods

Materials

Aquacultured red seabream (*Pagrus major*) were purchased dead (after 1-2 days of harvest) at a local market in Busan and used in preparing the conventional washed surimi. The length of the fish were approximately 42 cm and weighing between 1.9 kg and 2.5 kg at the time of purchase, and fillet lengths after removing head and tails were between 27 and 29 cm. For comparison, frozen surimi (washed minced fish-meat) prepared form Alaska pollock (*Theragra chalcogramma*) was obtained from Sungjin Fishery Co. Ltd. (Busan, Korea). FA grade Alaska pollock was a product of the USA, distributed and graded by American Seafoods Company (Seattle, WA, USA), and the listed ingredients were Alaska pollock, sorbitol, sugar, sodium tripolyphosphate and tetrasodium pyropohosphate (produced on February 6, 2015). RA grade Alaska pollock was also produced in the USA, and distributed and graded by Westward Seafoods, Inc. (Bellevue, WA, USA) Ingredients were Alaska pollock, sugar and tetrasodium pyrophosphate (produced on July 3, 2015). Polyvinylidene chloride casings (15 cm length, 2.5 cm diameter) were purchased from Ikjji Corp. (Seoul, Korea).

Surimi Preparation

Surimi was prepared using a traditional washing process. Fresh red seabream were decapitated, eviscerated, washed, skinned, and filleted manually, then deboned using a meat-bone separator (hole size: 2 mm) (YNS104-1, Young Nam, Busan, Korea). Fillets were minced using a silent cutter (Hanil Co. Ltd., Seoul, Korea) following the deboning. The mince was washed three times with cold salt water (0.05% NaCl) for 10 min at 0-4℃. After washing, the meat was dewatered using a centrifuge (Supra 21K, Hanil Co. Ltd., Seoul, Korea) at 8000 x g for 30 min at 4℃ and the remaining water removed by hand squeezing using four layers of cheesecloth. Then 4% sorbitol, 4% sucrose and 0.2% sodium triphosphate were added as cryoprotectants. The surimi was divided into approximately 200 g portions, sealed in a polyethylene vacuum package using a vacuum packing machine (IW-01, Eiffel Industrial Co., Ltd., Daegu, Korea) and subjected to 9 freeze-thaw cycles as were the FA and RA grade Alaska pollock surimi.

Freezing and Thawing

The samples were stored in a -30℃ freezer for 8 h and then in a 4℃ cooler for 16 h to thaw. They were frozen and thawed (1 freeze-thaw cycle) before making the fish-meat gel, which were then subjected to an additional 9 freeze-thaw cycles. Another set of fish-meat gels were prepared after the ninth cycle. The change of moisture content in surimi during the freeze-thaw cycles was measured according to AOAC method 950.46(AOAC, 1990).

Fish-Meat Gels Preparation

Fish-meat gels were prepared from the thawed frozen surimi in a chilled room (~17℃), cut manually into blocks (20×20×15 cm^3) with a knife and ground using a food cutter (Hanil Co. Ltd.) for 2 min while observing the sample temperature (<20℃). NaCl (2.0%, w/w) was added to the ground surimi and mixed with a blender (NFM-3561SN, NUC Co. Ltd., Seoul, Korea) for 3 min at high-speed grinding setting. Final moisture content was adjusted to 80% with ice water and samples were reblended for 5 min after resting the samples at room temperature (22ºC) for an h. The fish-meat sol was stuffed into a polyvinylidene chloride casing and heated in a hot water bath at 90℃ for 20 min (Figure 1). The gels were rapidly cooled in ice water for 10 min and stored in a refrigerator at 4.0±0.4℃ for 24 h prior to analysis.

Determination of Color

The fish-meat gels were sliced with a knife into ~20 mm thickness and color properties were measured using a Color Difference Meter (Lovibond Tintometer Model RT 300; Tintometer Ltd., Salisbury, UK) to obtain the Hunter color coordinates: the lightness (L), redness (+a) or greenness (-a), and yellowness (+b) or blueness (-b) values. Furthermore, whiteness was calculated using the Park equation (Park, 1994).

Whiteness = (L - 3b)

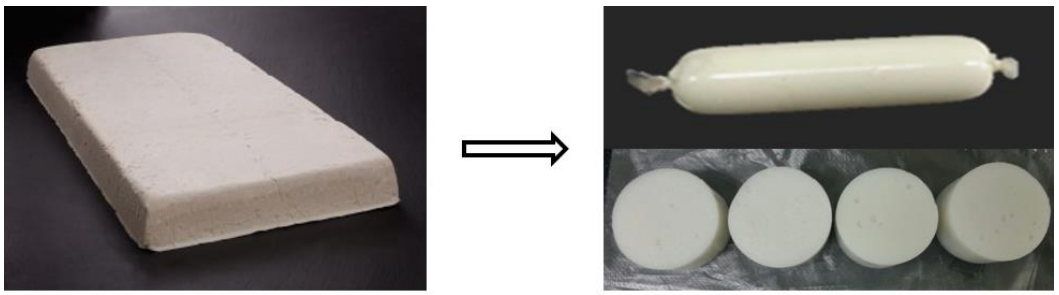

Figure 1. Images of frozen surimi blocks and fish-meat gel samples prepared from surimi following freeze-thaw cycles.

Table 1. Sample and texture analyzer settings for gel-forming ability analysis

	Gel-forming ability analysis setting
Sample	Diameter (Width): 25 mm Length (Height): 20 mm
Distance	10 mm
Entry distance (%)	50%
Table (head) speed	60 mm/min
Load cell shear stress (max.)	10 kg (20 kg)
Adapter number	No. 25ϕ25

Table 2. Sample and texture analyzer settings for gel texture and cutting strength analysis

	Gel texture setting	Cutting strength settings
Sample	Diameter (Width): 25 mm Length (Height): 20 mm	Width: 20 mm Height: 15 mm Length: 20 mm
Distance	10 mm	18 mm
Entry distance (%)	50%	120%
Table (head) speed	60 mm/min	60 mm/min
Load cell shear stress (max.)	10 kg (20 kg)	10 kg (20 kg)
Adapter number	No. 14ϕ50	No. 10 (wire cutter)

Determination of Expressible Moisture

The method of Benjakul and others (Benjakul, Visessanguan, & Srivilai, 2001) was used to determine the expressible moisture (EM) of the fish-meat gel. Gels were cut into a cylindrical shape with a thickness of ~5 mm, weighed, and placed on three sheets of filter paper (No. 1, Whatman International Ltd., Maidstone, UK) below and two sheets above the gel. Then, the gels were pressed with a 5 kg load for 2 min and the gel carefully removed from the filter paper and weighed. The expressible moisture was calculated as follows:

$$EM(\%) = \frac{(\text{gel weight before pressing (g)} - \text{gel weight after pressing (g)})}{\text{gel weight before pressing (g)}} \times 100$$

Determination of Gel-Forming Ability

Fish-meat gels were kept at room temperature, and sliced into ~20 mmthickness. Gel-forming ability was evaluated using a texture analyzer (Type COMPAC-100 Ⅱ; Sun Science Co., Tokyo, Japan) with a cylindrical plunger with the conditions shown in Table 1. The gel-forming ability was assessed by measuring the breaking force (g), deformation (mm), gel strength (g/cm^2) and hardness (g/cm^2) from the stress-strain curves developed by the computer provided with the instrument, during the analysis procedure.

Determination of Texture Properties

Texture properties were measured using the texture analyzer. Samples and analyzer settings were prepared as shown in Table 2 and analysis for the hardness (the force necessary to attain a given deformation, g/cm^2), springiness (height that the sample recovers between the end of the first compression cycle and the start of the second, % of the distance change), cohesiveness (the ratio of positive force during the second to that of the first compression

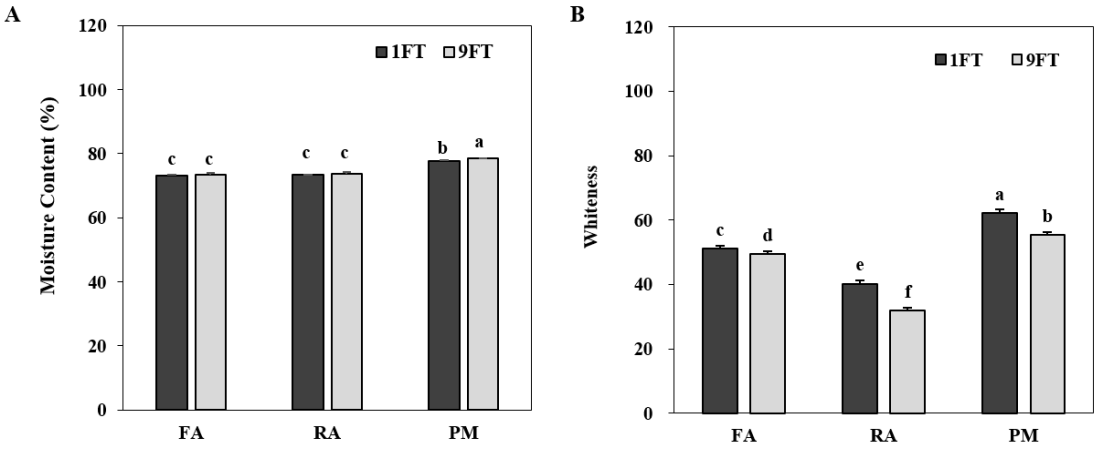

Figure 2. Moisture content (A) and whiteness (B) of surimi subjected to freeze-thaw cycles. FT, freeze-thaw; FA, FA grade Alaska pollock surimi; RA, RA grade Alaska pollock surimi; PM, *P. major* surimi. Values are means ± SD (*n=3*). [a-f] Means with different letters are significantly different (P<0.05).

cycle, % ratio of change in energy input), chewiness (maximum force that sample can withstand compression, g), brittleness (minimum force that results in fracture of samples, g) and cutting strength (ability to withstand the cutting force, g/cm^2) were carried out (Chang *et al*., 2015; Tahmasebi, Labbafi, Emam-Djomeh & Yarmand, 2016).

Statistical Analysis

The data were expressed as mean ± SD. Differences between the mean of the individual groups were analyzed using one-way ANOVA with the Statistical Analysis System, SPSS version 9.1 (SPSS Inc., Chicago, IL, USA). The differences between the means were evaluated using Duncan's multiple range test and the statistical significance of differences was set as P<0.05.

Results and Discussion

Effect of Freeze-Thaw Cycles on P. Major Surimi

Prior to observation of the effects of freeze-thaw cycles on fish-meat gels, moisture content and color values of surimi from *P. major* and the two grades of Alaska pollock were measured. Surimi color is one of the main aspects that is negatively affected by frozen storage and thawing (Scott *et al*., 1988). Color of surimi has a direct link to its water holding capacity. In surimi production, white color represents a higher moisture content. Desirable surimi products generally are opaque to translucent. Myosin denaturation and crosslinking of myofibrillar proteins significantly lowered the water holding ability of frozen surimi (Huff-Lonergan & Lonergan, 2005). Surimi made of *P. major* fish-meat (PM), and FA and RA grade Alaska pollock (FA and RA) were been subjected to 9 freeze-thaw cycles. Moisture content of the surimi samples were calculated after the first and ninth freeze thaw cycles and compared (Figure 2A). Both grades of Alaska pollock surimi retained the same amount of moisture after the first freeze-thaw cycle, 73.2±0.3 and 73.4±0.2% moisture for FA and RA grade, respectively. The moisture content did not change significantly after 9 cycles (P≥0.05) On the other hand, *P. major* surimi retained 77.7±0.2% moisture after the first cycle and 78.5±0.05% after the ninth. The moisture content of *P. major* surimi was higher than both grades of Alaska pollock surimi. Water holding ability of surimi is linked with the ability to withstand the harmful effects of frozen storage (Benjakul, Visessanguan, Thongkaew, & Tanaka, 2005). To confirm the changes in color, surimi from different sources were assessed for their Hunter color values (Figure 2B). Whiteness of the surimi is directly linked to its moisture content (Park, 1995). Surimi with less deformed protein structure have higher moisture content. Retained protein structure shows itself as stronger gels and greater stability against temperature fluctuations (Huff-Lonergan & Lonergan, 2005). Whiteness of the surimi were lower by the ninth freeze-thaw cycle. PM surimi whiteness values were higher than FA and RA surimi suggesting the stability of PM to protect against frozen storage and freeze-thaw cycles. Following 9 freeze-thaw cycles whiteness value of PM surimi was 55.3±0.7 while the FA and RA surimi values were 49.4±0.4 and 31.8±0.2, respectively. In terms of surimi, PM showed a significant protein and texture stability during frozen storage and freeze-thaw cycles comparable to that of both pollock surimi. Park (1995) showed a linear relation between moisture content and the quality of the surimi in terms of color values (Park, 1995). Alaska pollock was reported to be one of the species that retain moisture and yielded products with higher quality compared to other species such as Pacific

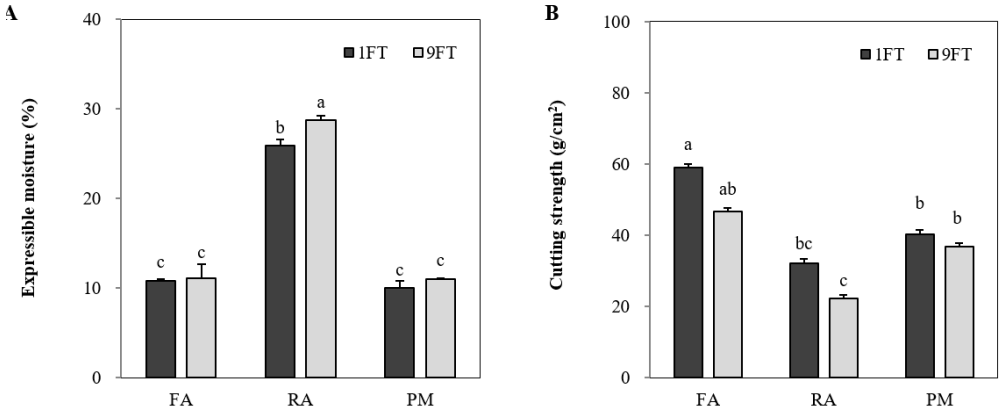

Figure 3. Expressible moisture (A) and cutting strength (B) of fish-meat gels subjected to freeze-thaw cycles. FT, freeze-thaw; FA, FA grade Alaska pollock fish-meat gel; RA, RA grade Alaska pollock fish-meat gel; PM, *P. major* fish-meat gel. Values are means ± SD (*n=3*). [a-c] Means with different letters are significantly different (P<0.05).

Table 3. Changes of Hunter color values in fish-meat gels that were subjected to 9 freeze-thaw cycles

Fish-meat gels		L	a	b	Whiteness
Alaska pollock (FA)	1FT	86.2±0.2^{c}	-3.70±0.05^{c}	2.8±0.1^{f}	77.7±0.3^{a}
	9FT	85.8±0.2^{d}	-3.83±0.02^{d}	3.6±0.1^{e}	74.3±0.03^{b}
Alaska pollock (RA)	1FT	81.3±0.2^{e}	-3.24±0.03^{b}	6.2±0.1^{d}	62.7±0.04^{e}
	9FT	81.1±0.2^{e}	-3.06±0.04^{a}	8.08±0.03^{a}	56.9±0.3^{f}
Pagrus major (PM)	1FT	89.6±0.1^{b}	-3.09±0.02^{a}	6.45±0.03^{c}	70.3±0.1^{c}
	9FT	90.0±0.2^{a}	-3.1±0.1^{a}	7.46±0.05^{b}	67.6±0.3^{d}

*FT, freeze-thaw; FA, FA grade Alaska pollock fish-meat gels; RA, RA grade Alaska pollock fish-meat gels; PM, *P. major* fish-meat gels. Values are means ± SD (*n=3*). [a-f] Means with different letters are significantly different (P<0.05).

whiting, herring and arrowtooth flounder (Reppond & Babbitt, 1997). *P. major* showed similar moisture content and whiteness to that of Alaska pollock suggesting a similar quality in final products as well.

Effects of Freeze-Thaw Cycles on PM Fish-Meat Gels

As a raw material for fish-meat gels, surimi is stored and distributed in a frozen form. During the shipment and further in the process line, surimi is often subjected to freeze-thaw cycles. Following the evaluation of the effects of freeze-thaw cycles on surimi, fish-meat gels that were prepared from PM, FA and RA surimi were evaluated for moisture content, color values, gel forming and texture properties, and cutting strength.

Darkened color is the first visible negative effect of frozen storage. Deformed proteins and lipid oxidation of frozen surimi causes unwanted changes to the translucent, white color of the fish-meat gels (Subbaiah *et al.*, 2015). Hunter color values of the PM, FA and RA fish-meat gels were measured and their whiteness values calculated. Whiteness of fish-meat gels prepared with surimi with nine freeze-thaw cycle decreased significantly (Table 3). Using surimi after one freeze-thaw cycle, FA fish-meat gel had a whiteness value of 77.7±0.3 while this value was 70.3±0.1 and 67.6±0.3 for PM and RA fish-meat gels, respectively. Using surimi after the ninth cycle, FA fish-meat gel whiteness value was 74.3±0.03 compared to PM fish-meat gel's value of 67.6±0.3. RA fish-met gel had the lowest whiteness value of 56.0±0.3. Production methods for fish-meat gels including further heating and cooling processes, were suggested to cause decreases in moisture holding capacity or texture of the PM fish-meat gel underlying the decreased whiteness of gels compared to surimi. Expressible moisture of fish-meat gels was calculated (Figure 3A). The expressible moisture of FA and PM gels were 10.8% and 10.0%, respectively using surimi after the first thawing. Using the surimi after nine freeze thaw-cycles did not increase the expressible moisture for FA and PM($P \geq 0.05$). On the other hand, RA fish-meat gel showed increased expressible moisture (9.8%, P<0.05) i which might be linked to darker color. On the other hand, FA and PM fish-meat gels' expressible moisture were the same (P<0.05) although PM showed slightly darker color. PM fish-meat gels suggested that its protein structure was preserved about as well as FA fish-meat gels and better than RA fish-meat gels.

The cutting strength of meat products is related to their chewiness which is related to gel forming abilities. Freeze-thaw cycles were reported to cause an undesirable softness in frozen-stored seafood mainly

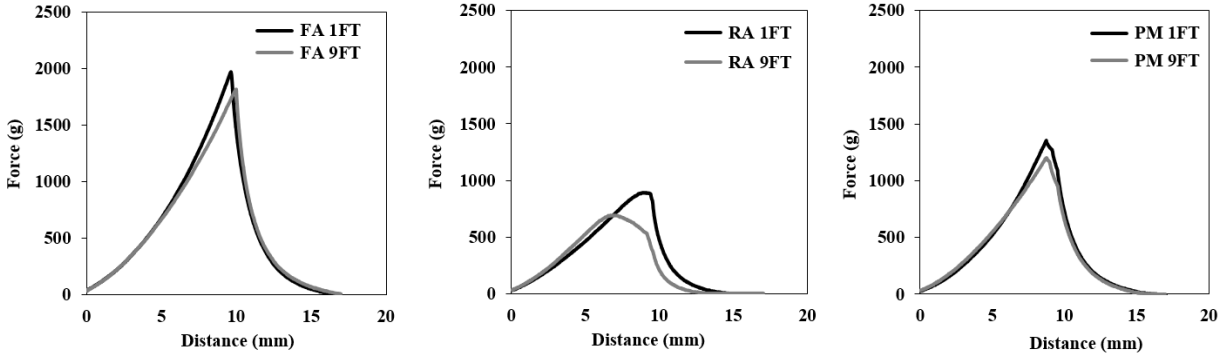

Figure 4. Changes in stress-strain of fish-meat gels subjected to freeze-thaw cycles. FT, freeze-thaw; FA, FA grade Alaska pollock fish-meat gels; RA, RA grade Alaska pollock fish-meat gels; PM, *P. major* fish-meat gels.

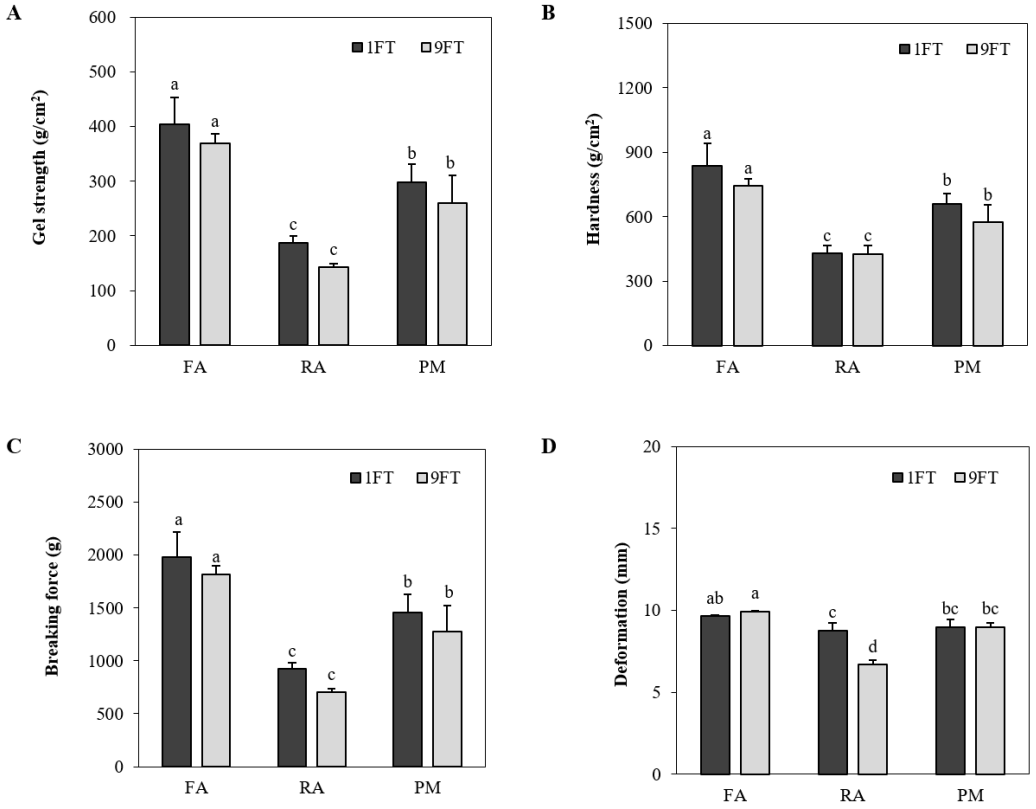

Figure 5. Changes of gel strength (A), hardness (B), breaking force (C), deformation (D) of fish-meat gels subjected to freeze-thaw cycles. FT, freeze-thaw; FA, FA grade Alaska pollock fish-meat gels; RA, RA grade Alaska pollock fish-meat gels; PM, *P. major* fish-meat gels. Values are means ± SD (*n=3*). [a-d] Means with different letters are significantly different (P<0.05).

due to protein changes (Scott *et al.*, 1988). FA fish-meat gel cutting strength was lowered to 47±3 from 59±2 g/cm^2 (Figure 3B). These values were 40±5 and 37±3 g/cm^2 for PM and 32±10 and 22±2 g/cm^2 for RA fish-meat gels with surimi after the first and nine freeze-thaw cycles, respectively. Stress-strain curves of the fish-meat gels were developed by gel-forming ability analysis values by the instrument software (Figure 4). The force needed for deformation was higher for FA fish-meat gels than PM and RA. Among the latter, PM fish-meat gels were more resistant to deformation than RA. PM fish-meat gels showed less change with freeze-thaw cycles as the difference between 1 and 9 cycles did not show any significant changes (P≥0.05) for PM and FA fish-met gels. Although FA was better than PM in strength, PM was better than RA in terms of resistance to strain.

To understand the extent of gel strength loss, the strength, hardness, breaking force, and deformation were measured for all fish-meat gels and compared (Figure 5). All tested criteria were negatively affected by frozen storage and further freeze-thaw cycles.

Table 4. Gel texture analysis of fish-meat gels following freeze-thaw cycles

Fish-meat gels	Freeze-thaw cycles	Hardness (g/cm^2)	Springiness (%)	Cohesiveness (%)	Chewiness (g)	Brittleness (g)
Alaska pollock (FA)	1FT	840±100^{a}	94±3^{a}	81±6^{a}	3200±370^{a}	298000±37000^{a}
	9FT	740±34^{a}	90±3^{a}	79±4^{ab}	1800±140^{b}	159000±37000^{bc}
Alaska pollock (RA)	1FT	430±35^{c}	85±4^{a}	52±8^{c}	540±70^{c}	46300±7800^{d}
	9FT	430±37^{c}	75±22^{b}	34±1^{d}	140±10^{c}	9700±3200^{d}
Pagrus major (PM)	1FT	660±47^{b}	97±0.2^{a}	75.9±0.5^{ab}	1800±140^{b}	181000±11000^{b}
	9FT	580±78^{b}	93±3^{a}	71±3^{b}	1500±250^{b}	137000±1900^{c}

*FT, freeze-thaw; FA, FA grade Alaska pollock fish-meat gels; RA, RA grade Alaska pollock fish-meat gels; PM, *P. major* fish-meat gels. Values are means ± SD (*n=3*). $^{a-d}$ Means with the different letters are significantly different (P<0.05) by Duncan's multiple range test. [please check the brittleness numbers]

Similar to cutting strength and stress-strain analyses, PM fish-meat gels kept these criteria better than RA fish-meat gels, however, but not as well as FA. To evaluate the effects of raw material freeze-thaw cycles on gel texture, fish-meat gels were also subjected to gel texture analysis and values of hardness, springiness, cohesiveness, chewiness, and brittleness were measured (Table 4). PM fish-meat gels maintained all of these criteria. Likewise, FA fish-meat gels maintained these criteria better than PM and even more than RA. Overall, PM fish-meat gels retained its moisture and desirable color but was behind FA in terms of gel forming strength and gel texture after freeze-thaw abuse. PM fish-meat gels had minimal lipid oxidation f similar to the FA fish-meat gels. However, it was not equal in gel forming strength and texture as FA. Other fish species, such as herring and Pacific whiting, were also used for producing surimi gels and tested for their gel texture profiles (Reppond & Babbitt, 1997; Park, 1995). Comparisons with Alaska pollock showed that most of the species were not comparable mostly due to their unstable gel strength, fluctuating moisture content, and color values (Yoon, Gunasekaran, & Park, 2004; Klesk *et al.*, 2000). Except for Pacific whiting, which showed promising results, all species had one or more problems compared to pollock. For instance, herring gave stronger but discolored and less chewy gels. Compared to whiting and herring, *P. major* showed a potential to be almost as good as high grade Alaska pollock surimi and gels and better than RA surimi. Thus, *P. major* meat may be as a substitute for mid-grade Alaska pollock.

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