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Effect of Soy Protein Isolate and Wheat Fiber on the Texture and Freeze-Thaw Stability of Lean Fish Mince

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Abstract: The effects of cyclic freezing and thawing upon the quality of frozen fish mince were studied. The effectiveness of combined use of Soy Protein Isolates (SPI) (2%) and Wheat Fiber (WF) (2%) were investigated with respect to cryoprotective properties and textural changes in fish frozen mince. The changes in texture and water holding capacity of fish mince after two freeze-thaw cycles were assessed using Texture Analyzer (TA.XT Plus) and sensory analysis. Cooking loss upon freeze-thaw cycles was also determined. No cryoprotectant and texture modification effect of the wheat fiber was observed. The addition of soy protein isolate and/or wheat fiber significantly reduced cooking loss after two freeze-thaw cycles ($p<0.05$). Combined use of soy protein isolate (2%) and wheat fiber (2%) showed added benefit in reducing expressible moisture compared to using SPI alone (2%) even the differences were not significant ($p>0.05$) and provided synergistic effect in texture modification ($p<0.05$) and also reduced freeze-contraction of myofibrils after two freeze-thaw cycles according to the results of scanning electron micrographs. This cryoprotectant combination can be applied to fish mince for the improvement of their texture and frozen storability.

Key words: Freeze-thaw stability, lean fish mince, Soy Protein Isolate (SPI), Wheat Fiber (WF), texture changes

INTRODUCTION

There is increased consumer demand for high-quality fish and seafood products because of reported beneficial health effects (Echarte *et al.*, 2001; Saeed and Howel, 2001; Hsieh *et al.*, 1988). Restructured fishery products made from minced muscle with or without ingredients are use to make variety products with a new appearance and texture (Sanchez-Alonso *et al.*, 2007). Development of highly acceptable mince-based products led to an idea of separating refined meat and freeze stabilizing the mechanically recovered mince without washing for the production of a variety of value-added convenient seafood products including products with a health concept. Freezing and frozen storage are important techniques for long term preservation of fish but several alterations still take place (Rodriguez-Herrera *et al.*, 2006). Deleterious texture changes in frozen fish mince causes a major problem in developing mince-based seafood products especially the mince produced from lean, white fish species (Lee, 2011). Lean fish mince develops textural hardness during frozen storage, causing poor dispersibility which makes it difficult to blend with added ingredients (Lian *et al.*, 2000). The poor dispersibility, along with unacceptable texture hardening, remains a major problem for the commercial application of frozen fish mince in formulated seafood products. Textural hardening of fish during frozen storage is attributed to a variety of mechanisms including damage due to ice crystal formation (Yoon *et al.*, 1991). Breakdown of tissue structure accelerates changes in texture, water holding capacity and flavor (Yoon *et al.*, 1991). Prolonged storage, especially at temperature around -18°C, brings about significant deterioration of texture of frozen fish described as increased toughness, chewiness or rubberiness. The potential advantages of the refining and cryostabilization process include no washing, high yield, retention of nutrients inventory control of frozen stable intermediate products to be used in formulated seafood products with desired flavor and texture (Lee, 2011). Dietary supplements and food fortification is a potential alternative route to the consumption of minor plant components and Dietary Fiber (DF) that may have health benefits. The foods fortified with fiber increase their dietary fiber content and result in healthy products, low in calories, cholesterol and fat (Elleuch *et al.*, 2010). Cryoprotectant properties are described for cellulose in the freeze-thaw stability of surimi-based shellfish analogue products (Yoon and Lee, 1990). Cereal fibers have advantageous technological properties

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such as a high water and fat binding capacity (Sanchez-Alonso *et al*., 2007). The water-binding proteins are found to have the ability to keep muscle fiber from shrinking during frozen storage as reported by Yoon *et al*. (1991). The addition of water-soluble non-fish proteins prevent textural hardening during frozen storage by acting similarly as water soluble sarcoplasmic proteins (Yoon *et al*., 1991). The addition of soy protein may improve textural properties by reducing freeze-induced shrinkage of myofibrils that results from protein intermolecular cross-linking (Yoon *et al*., 1991; Lian *et al*., 2000; Bigelow and Lee, 2007). It would be ideal to find natural ingredients which can retard texture changes and freeze-thaw abuse.

The objective of this study was to determine the effects of using soy protein isolate and/or wheat fiber on freeze-thaw stability and texture modification in lean fish mince.

MATERIALS AND METHODS

A total of about 6 kg fresh skinless sea bass (*D. labrax*) fillets >1 day old after harvest were obtained from a local fish market (Izmir, Turkey) and transported in ice to the laboratory. Soy protein isolate (90% protein, moisture max 7% max 5% ash, max 1%) supplied by Smart Chemical Ltd. (Pingdingshan, China). The fiber used was Wheat Fiber (WF) Vitacel was WF 200 with 250 μm long and 25 μm wide particles, obtained from Orean Chemical Ltd. (Barcelona, Spain). This fiber consists of 74% cellulose, 26% hemicellulose and max 0.5% lignin.

Sample preparation and storage condition: Sea bass (*D. labrax*) was chosen because white fish has a great susceptibility to texture toughening and have poor freeze-thaw stability during frozen storage. The market for the whole sea bass presents signs of saturation and there is a potential market for sea bass fillets and also farmed fish species present good quality protein and may yield good quality mince based product. The fillets were minced through 5 mm diameter holes using a kitchen aid meat grinder (Model KPM5, St. Joseph, Michigan, USA). The grinder was chilled by running crushed ice through prior to grinding fish fillets. The soy protein isolates and wheat fiber was immediately mixed with the fish mince singly (2% w/w) or combination (2+2% w/w) using a kitchen aid bowl mixer for 3 min at a low speed. Cold water was added to equalize the moisture content to the original muscle moisture (76%) throughout the samples. Each mixture containing about 700 g fish mince was formed by packing approximately 20 g of mince using round mold (4.5 cm diameter, 1.0 cm height). Approximately 700 g of fish mince was kept as a control group without added SPI and WF and subjected to same mixing condition and formed as same as other trials. Formed fish mince was packed in a nylon/polyethylene bag and subjected to two freeze-thaw cycles to determine the cryoprotective effectiveness of each combination. Each freeze-thaw cycle was carried out by storing the sample at -20°C for 4 days and thawing at 4°C over night. Such abusive treatment is perhaps not representative of conditions likely to be encountered during normal storage of fish mince but rapidly induce changes which could normally occur during a long period of storage and shipping.

Determination of cooking loss: To measure the cooking loss the fish mince samples at day 0 and after two freeze thaw cycles were completely thawed at 4°C and weighed then placed in a commercial plastic oven bag (Koroplast; Istanbul, Turkey) and cooked on baking sheets at 170°C for 15 min in a preheated conventional electric oven. After cooking, the sample was cooled at ambient temperature for 10 min and reweighed to determine the cooking loss. Analysis for each treatment was carried out in triplicate. The cooking loss was calculated as follows:

$$\text{Cooking loss (\%)} = \frac{\text{(Weight before cooking} - \text{Weight after cooking)} \times 100}{\text{Weight before cooking}}$$

Determination of water holding capacity: Water holding capacity was characterized by measuring EM which means the quantity of liquid squeezed from mince up a compression (Jonsson *et al*., 2001). EM was determined using a modification of the filter paper press method as described, elsewhere (Schubring *et al*., 2003). To measure the EM, the fish mince samples at day 0 and after two freeze thaw cycles were analyzed. To determine the freeze-thaw abuse the samples were completely thawed at 4°C and cooked by the same method as described for cooking loss while they were in the oven bag and cooled at ambient temperature for 10 min and put in a refrigerator overnight while they were in the bag. Samples were pressed between paired filter sheets (Schleicher and Schuell, 7×7 cm) and parallel plates using a texture analyser TA.XT Plus Texture Analyser (Stable Micro Systems, Godalming, United Kingdom). A 25 kg load cell and a crosshead speed of 1.7 mm sec^{-1} were used. Samples were pressed to 60% deformation and held at the point for 15 sec. All measurements were repeated 8 times. WHC was determined as the expressible moisture, calculated as:

$$\text{Expressible moisture}(\%)=100\times\left(\frac{(\text{Initial weight} - \text{Final weight}}{\text{Initial weight})}\right)$$

Instrumental Texture Profile Analysis (TPA): The fish mince samples at day 0 and after two freeze thaw cycles were analyzed. The samples were prepared by the same method as described for expressible moisture while they were in the oven bag. The term hardness was chosen over toughness since, hardness more closely reflects the texture changes in frozen fish mince. Texture was evaluated using a TA.XT Plus Texture Analyser (Stable Micro Systems, Godalming, United Kingdom). Samples were cut out using a 1.5 cm cork borer. The TPA measurements were performed at 65% compression. Compression head having 5.0 cm diameter was used at a crosshead speed of 0.8 mm sec^{-1}. All TPA measurements were repeated 10 times (Schubring and Oehlenschlager, 1997). Additionally to these texture measurements on fish mince the penetration force of mince was also determined using the TA.XT Plus. Penetration force at 85% deformation was measured as an index of rigidity by using plunger of 0.5 mm diameter (Lee and Chung, 1989).

Sensory evaluation: Sensory evaluation of texture was conducted by a panel of five people from the Department of Fish Processing Technology. At day 0 and after two freeze-thaw cycles the samples were analyzed. The samples were prepared by the same method as described for cooking loss; the samples were fully thawed at 4°C and cooked in an electric oven as described for cooking loss. After cooling for about 10 min at room temperature, the samples were served while they were warm. Mince samples (~20 g) were cut into four pieces, numerically coded and randomly served to panelists. Firmness, moistness and overall acceptability were scored for their intensity using a 9-point scale (1: Least, 5: Moderate, 9: Greatest).

Electron microscopy: Effects of SPI and wheat fiber on the microstructure of fish mince before and after two freeze-thaw cycles were evaluated using Scanning Electron Microscopy (SEM). To prepare specimens, cooked fish mince with and without SPI and WF with a thickness of 2-3 mm was fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2). The samples were than rinsed for 1 h in distilled water before being dehydrated in ethanol with a serial concentration of 50, 70, 80, 90 and 100% (v/v) as described by Rawdkuen *et al*. (2004). The specimens were observed with a scanning electron microscope (Phillips XL-30S FEG).

Statistical analysis: Statistical analysis was performed using SPSS 11.5 (SPSS inc., Chicago, IL, USA). Duncan's multiple range test by one-way analysis of variance (ANOVA) was carried out to determine the significant differences among the samples with and without additive. The significance level was set at 95% ($p<0.05$) for all analysis. The differences between before and after two freeze-thaw cycles were carried out using independent sample t-test. Data analysis for each treatment was carried out in triplicate.

RESULTS AND DISCUSSION

Effects of cryprotectants on cooking loss of cooked fish mince: Changes in cooking loss in the fish mince are shown in Fig. 1. The addition of wheat fiber alone at the 2% level was found to be ineffective in reducing cooking loss. At day 0 (Unfrozen cooked samples) in the mince having wheat fiber alone resulted significantly higher cooking loss (37.95±0.52%) compared to the rest ($p<0.05$) and combined use of soy protein isolate and wheat fiber were not significantly different than mince having only soy protein isolate ($p>0.05$). At day 0, there were significantly less cooking loss (30.98±0.48%) in the control group (without additive) compared to the other treatments ($p<0.05$) but after two freeze-thaw cycles significantly increasing (34.45±0.56%) was observed in the control group while significantly decreasing was determined in the rest ($p<0.05$). The possible explanation is that the water remained in the frozen-thawed tissue treated with cryoprotectants tended to be bound more water than untreated control. After two freeze-thaw cycles the highest cooking loss (35.65±0.60%) was observed in the mince having wheat fiber alone and it was not significantly different than control group ($p>0.05$). The addition of Soy Protein Isolate (SPI) with or without

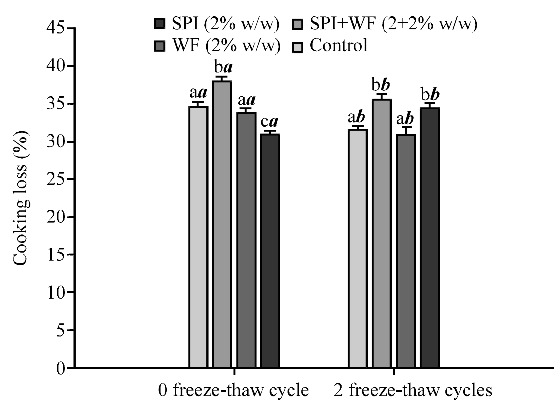

Fig. 1: Changes in cooking losses in cooked fish mince before and after two freeze-thaw cycles; Different letters denote significant differences among groups within the same freeze-thaw cycles and different italic bold letters denote significant differences between 0 and 2 freeze-thaw cycles for the same groups ($p<0.05$)

Wheat Fiber (WF) resulted in significantly less cooking loss after two freeze-thaw cycles ($p<0.05$). Bigelow and Lee (2007) reported that soy protein isolate was a significantly better water binder than other proteins (whey protein concentrate and caseinate at 2 and 5%). Yoon *et al.* (1991) were studied the changes in textural and microstructural properties of washed and unwashed frozen fish mince and they reported that the addition of soy protein isolate at the 6% level, significantly reduced the cooking loss in both washed and unwashed mince and they also demonstrated that the level of protein was increased, the effect became more pronounced.

The ineffectiveness of wheat fiber on cooking loss was observed by Sanchez-Alonso *et al.* (2007). They reported that the addition of 3% of fiber to hake and minced horse mackerel muscle did not lend to binding of significantly more water than the control when the moisture in both samples was adjusted to the original muscle moisture. They also reported that the addition of 3% of fiber without adjusting the moisture was effective to bind the cooking drip. On the other hand the ineffectiveness of fiber in reducing cooking loss could be also explaining by heating effects on cook losses. Skipnes *et al.* (2007) reported that the cook loss is increasing with increasing temperature. It is varied greatly with the fish species, the method of heating and the heating regime (i.e., sterilization, pasteurization, etc.) (Aitken and Conell, 1979). On the other hand Sanchez-Alonso *et al.* (2007) reported that the increase in water retention during cooking could be due to the hydrogen bonds forming between the water molecules and cellulose fibers. Thus, the hydrogen bonds weaken with the cooking temperatures and water can not be bound so easily (Ang, 1993). The apparent reason is that using fiber alone does not bind the water during cooking, unlike non-fish proteins which are able to bind water during thermal condition.

Effects of cryprotectants on expressible moisture of cooked fish mince: Measurement of expressible moisture in cooked fish mince was used to estimate the water holding capacity. Water holding capacity in meat tissue is strongly related to myofibril proteins. Increase of expressible moisture is a sign of reduction of water holding capacity due to denaturizing of proteins (Rostamzad *et al.*, 2011). At day 0 there were no significantly differences in water holding capacity between the all groups ($p>0.05$). The use of wheat fiber and soy protein isolate alone at the 2% level had no positive effect on water holding capacity of the cooked fish mince, they were not significantly different than control group after two freeze-thaw cycles ($p>0.05$) even the soy protein isolate added mince had lower expressible

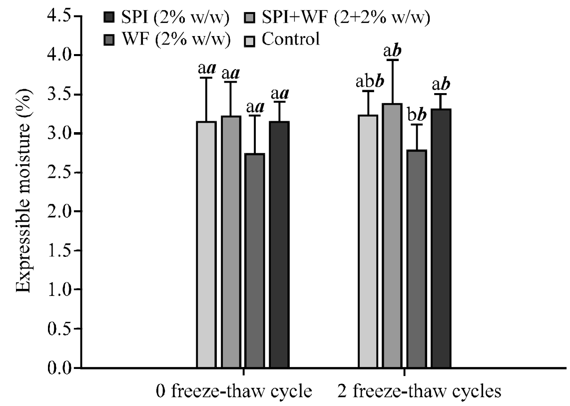

Fig. 2: Changes in expressible moisture in cooked fish mince before and after two freeze-thaw cycles. Different letters denote significant differences among groups within the same freeze-thaw cycles and different italic bold letters denote significant differences between 0 and 2 freeze-thaw cycles for the same groups ($p<0.05$)

moisture than control group. There were not significantly difference between soy protein isolate and wheat fiber added mince ($p>0.05$). The combined use of Soy Protein Isolate (SPI) and Wheat Fiber (WF) resulted significantly less expressible moisture compared to control group ($p<0.05$). Wheat fiber at the 2% level with 2% soy protein isolate showed added benefit in reducing expressible moisture compared to 2% SPI alone even the differences were not significant after two freeze-thaw cycles which expressible moisture values of the mince with SPI and WF was lower (2.78±0.33) than SPI alone (3.21±0.32) (Fig. 2). Sanchez-Alonso *et al.* (2007) found that there were very few significant differences in water holding capacity, when 3 or 6% fiber was added to minced hake and horse mackerel muscle. Significantly more water was observed, when 3% of fiber was added to minced hake muscle without adjusting the final moisture compared to water added mince. They also mentioned that the water is more firmly bound when the grain is shorter (80 μm). Yoon and Lee (1990) reported that fiber lengths ranging from 11-20 μm showed lower expressible moisture than those >20 μm. They also mentioned that their effect to lower expressible moisture within the range 11-20 μm was not significantly different from the control. In the present study, wheat fiber added mince was not significantly different than control group although, the size range used in the present research was different (250 μm). Cardoso *et al.* (2011) studied the production of high quality gel products from minced sea bass trimmings and change of their functional properties through incorporation of MTGase and dietary fiber. They reported

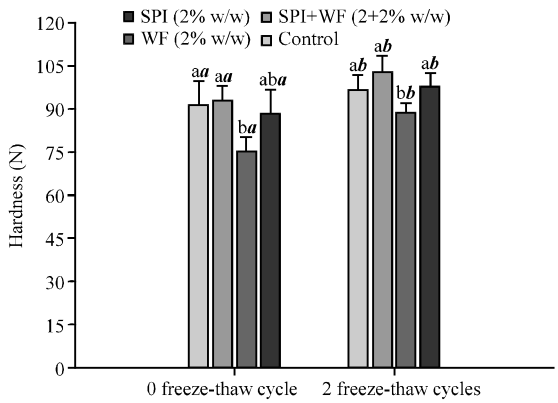

Fig. 3: Changes in textural hardness in cooked fish mince before and after two freeze-thaw cycles. Different letters denote significant differences among groups within the same freeze-thaw cycles and different italic bold letters denote significant differences between 0 and 2 freeze-thaw cycles for the same groups ($p<0.05$)

that inner pea fiber (40 g kg^{-1}, w/w) had a negative effect on texture which was quite similar to that of the control products (no DF) and reduced the water holding capacity. However, they also reported that the effectiveness of inner pea fiber on texture improvement is varied greatly with the fish species.

Effects of cryprotectants on textural properties of cooked fish mince: After two freeze-thaw cycle hardness values in all groups were significantly increased. As shown in Fig. 3, at day 0 combined use of Soy Protein Isolate (SPI) and Wheat Fiber (WF) significantly reduced the hardness compared to mince having SPI and WF alone ($p<0.05$) but it was not significantly different than control group ($p>0.05$). The mince having wheat fiber alone was higher than control group but the differences were not significant ($p>0.05$) at day 0 and after two freeze-thaw cycles not significantly differences were determined between mince having wheat fiber alone and control ($p>0.05$). After two freeze-thaw cycles, hardness values in the mince having soy protein isolate alone was lower than mince having wheat fiber alone and control group, even the differences were not significant ($p>0.05$). Combined use of SPI and WF significantly softened the texture compared to the rest ($p<0.05$) after two freeze-thaw cycles. Problems and disadvantageous characteristics of frozen fish mince are associated with texture hardening or toughening, poor dispersibility, poor functionality, discoloration, lack of flaky texture. Deleterious texture changes in frozen fish mince causes a major problem in developing mince-based seafood products, especially the mince produced from lean, white fish species which are known to have poor freeze-thaw storability as evidenced by a high freeze-thaw drip loss and texture hardening (Lee, 2011). In texture hardening of lean fish, calcium ion has been implicated since, it may promote the formation of ionic cross-linkages between polypeptide chains (Lee and Park, 1998). The mechanism for improvement of freeze-thaw stability by non-fish proteins was proposed by earlier. The addition of soy protein may improve textural properties by reducing freeze-induced shrinkage of myofibrils that results from protein intermolecular cross-linking (Yoon *et al.*, 1991; Lian *et al.*, 2000; Bigelow and Lee, 2007). Yoon and Lee (1990) reported that the addition of 0-2% cellulose in surimi products decreased firmness and cohesiveness although, >2% of cellulose increased firmness. They also mentioned that the improvement in the texture of the cellulose containing gel was probably due to the freeze-thaw stabilizing effect of cellulose. Sanchez-Alonso *et al.* (2006) reported that highly insoluble wheat fibre added to fish muscle gels can protect surimi from loss of gel strength and hardness during freezing but it can reduce the gel characteristics and cohesiveness throughout thermal gelling. Sanchez-Alonso *et al.* (2007) found that the hardness increased in the hake mince having 3% fiber, when the moisture was not adjusted to the original muscle moisture. They also reported that the effect of the texture parameters is different depending on the amount and type of insoluble fiber that is added to meat products (Cofrades *et al.*, 2000) and also on the water binding capacity and swelling properties of the fiber (Thebaudin *et al.*, 1997).

According to the present results, the combined use of soy protein isolate (2%) and wheat fiber (2%) provided synergistic effect in softening the texture compared to 2% soy protein isolate and 2% wheat fiber alone at which hardness values of the mince with soy protein isolate and wheat fiber was significantly lower ($p<0.05$) than SPI and WF alone which were not significantly different than control ($p>0.05$) after two freeze-thaw cycles. This suggests that some mechanism is implicated in the role of cryorotectants in the texture changes of frozen fish mince. The type of protein, mixing methods and cooking conditions greatly affected textural properties (Lee, 2011).

The dispersibility of fish mince is an important quality requirement for the ease of mixing with other ingredients during formulation (Lian *et al.*, 2000). The response of the material to penetration would be affected by the density and uniformity of the matrix (Lee and Chung, 1989). At day 0, the lowest penetration force (506.33 g) obtained in control group even the differences were not significant from the mince added soy protein isolate with or without wheat fiber ($p>0.05$). Wheat fiber added mince had higher penetration force (782.42 g) compared to combined use of

SPI and WF and control group ($p<0.05$) but it was not significantly different than mince added soy protein isolate alone ($p>0.05$) at 0 day. Significantly decreasing was observed in penetration force in the mince which was added wheat fiber alone after two freeze-thaw cycles but it was not significantly different than the rest ($p>0.05$) (Fig. 4). Yoon and Lee (1990) reported that the compressive force and penetration force of molded products gradually decreased with increased cellulose. Lian *et al*. (2000) studied the changes in penetration force of uncooked mackerel mince stored frozen for 6 months at varying levels (2, 4 and 6%) of added soy protein concentrate and found that at 0 day, mince having soy protein concentrate had higher penetration force than control group (without additive), after 6 month of frozen storage the addition of soy protein concentrate lowered the penetration force compared to control and this reduction was increased with an increased level of soy protein concentrate. The ability of non-fish proteins to prevent or minimize such textural hardening of fish mince during frozen storage is related to the reduced freeze-contraction of myofibrils through water binding and uniform dispersion (Yoon *et al*., 1991).

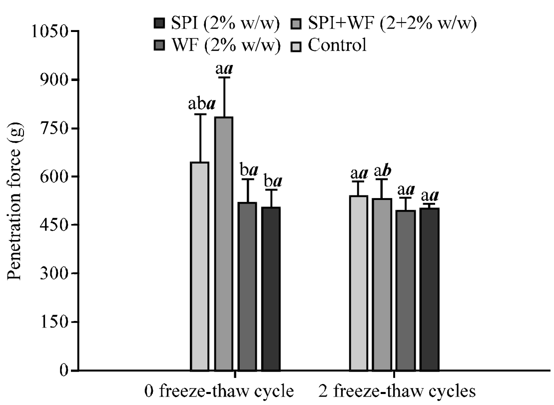

Fig. 4: Changes in penetration force in cooked fish mince before and after two freeze-thaw cycles. Different letters denote significant differences among groups within the same freeze-thaw cycles and different italic bold letters denote significant differences between 0 and 2 freeze-thaw cycles for the same groups ($p<0.05$)

Sensory evaluation: In addition to textural properties, mouthfeel and moistness are other important sensory attributes which determine the acceptability of the formulated composite products (Lee, 2011). Soy protein isolate added mince and control had highest overall acceptability score at the beginning of the study however, the differences were not significant between all groups. Lowest hardness and higher moistness score were observed in the control group but the differences were not significant compared to the rest at day 0 ($p>0.05$). After two freeze-thaw cycles combined use of soy protein isolate and wheat fiber received the highest overall acceptability score of 7.8±0.44, like very much on a 9-point hedonic scale (Table 1). This was followed closely by soy protein isolate added mince (7.6±0.55) but again there were no significant differences between all treatments ($p>0.05$) even the wheat fiber added mince and control group scored 6.8±0.84 and 6.8±0.44 like moderately, respectively. Lower hardness score was observed in combined use of soy protein isolate and wheat fiber added mince while the lowest moistness scored observed in the mince having wheat fiber alone, however there were no significantly differences determined between all groups ($p>0.05$). The panelist did not detect any unusual flavors among the samples. Yoon and Lee (1990) reported that in fiberized products, adding cellulose to surimi at 2% during chopping made fiberized products significantly firmer and chewier than adding to the mince at a 1% level prior to surimi preparation while the other rest of 1% during chopping and also 2% prior to surimi preparation according to the sensory results. They also reported that the addition of 3% cellulose to the mince yielded a significantly less cohesive gel, less firm and chewy fiberized product and received the lowest desirability score. Sanchez-Alonso *et al*. (2007) studied the technological effect of wheat dietary fibre in surimi gel products obtained from giant squid (*Dosidicus gigas*) and detected no significant differences in appearance between the control and samples with 3 or 6% wheat dietary fibre, either raw or after cooking and irrespective of the dietary fibre size and they also reported that the samples with wheat dietary fibre were softer and more deformable. Lee and Toledo (1979) studied the development of smoked sausage links from Spanish

Table 1: Changes in sensory score of cooked fish mince before and after two freeze-thaw cycles

	Soy Protein Isolate (SPI) (2% w/w)			Wheat Fiber (WF) (2% w/w)			(SPI+WF) (2+2% w/w)			Control (without additive)		
Storage condition	Overall acceptability	Hardness	Moistness	Overall acceptability	Hardness	Moistness	Overall acceptability	Hardness	Moistness	Overall acceptability	Hardness	Moistness
0 freeze-thaw cycle												
	8.00 ± 0.00^{a}	7.00 ± 1.22^{a}	7.20 ± 0.44^{a}	7.80 ± 1.09^{a}	7.20 ± 1.00^{a}	6.80 ± 1.92^{a}	7.80 ± 1.14^{a}	7.00 ± 0.44^{a}	7.00 ± 1.00^{a}	8.00 ± 0.54^{a}	6.80 ± 1.14^{a}	7.40 ± 1.30^{a}
2 freeze-thaw cycles												
	7.6 ± 0.55^{a}	7.20 ± 0.44^{a}	7.00 ± 0.7^{a}	6.80 ± 0.84^{a}	7.40 ± 0.44^{a}	6.20 ± 0.83^{a}	7.80 ± 0.44^{a}	6.80 ± 0.84^{a}	7.00 ± 0.7^{a}	6.80 ± 0.44^{a}	7.20 ± 0.83^{a}	6.40 ± 0.89^{a}

Different letters between rows denote significant differences among the groups ($p<0.05$)

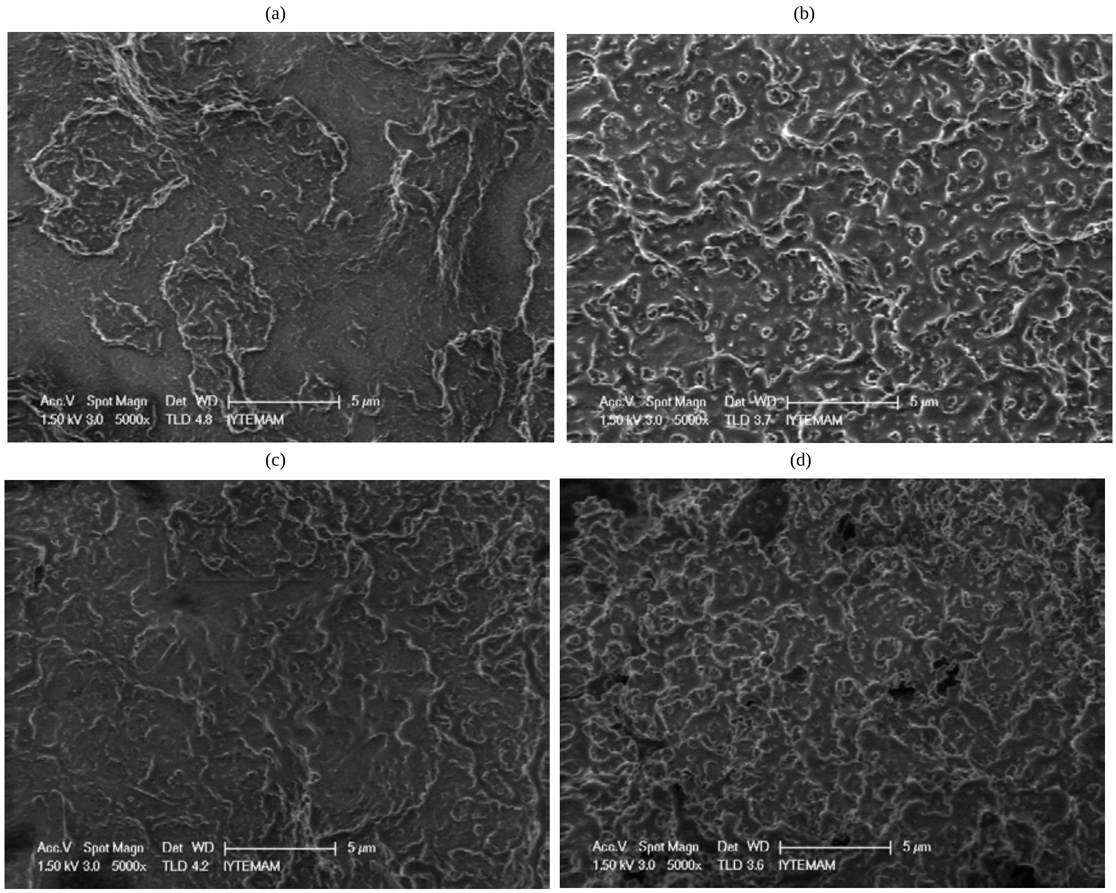

Fig. 5: Scanning electron micrographs of cooked fish mince. a) unfrozen cooked fish mince without additive; b) twice freeze-thawed cooked fish mince without additive; c) unfrozen cooked fish mince prepared combined use of Soy Protein Isolate (SPI) and Wheat Fiber (WF); d) twice freeze-thawed cooked fish mince prepared combined use of SPI and WF

mackerel (*Scomberomorous maculatus*) and reported that the use of Soy Protein Fiber (SPF) resulted in the most desirable texture with adequate firmness, chewiness and juiciness.

Electron microscopic observations: The mince prepared combined use of soy protein isolate and wheat fiber had a more uniform matrix distribution (Fig. 5c) of the randomly dispersed phase than control (Fig. 5a). For the twice freeze-thawed sample, there is an indication of ice crystal growth resulting in formation of disrupted matrix. The twice freeze-thawed fish mince show the reduced freeze-contraction of myofibrils when mince prepared combined use of soy protein isolate and wheat fiber (Fig. 5d). Such reduced freeze-contraction after addition of non-fish proteins appears to be related to the proteins' water binding measured by centrifugation (Chung and Lee, 1990) and dispersibility (Chung and Lee, 1991). It is suggested that the good water binding and dispersibility of non-fish proteins reduced the amount of free water available for ice crystallization and allowed uniform ice crystal formation (Yoon *et al*., 1991).

CONCLUSION

The addition of wheat fiber alone was not effective in texture and moisture control in the sea bass mince after two freeze-thaw cycles. The ineffectiveness of fiber could be due to the hydrogen bonds forming between the water molecules and cellulose fibers during cooking. The addition of soy protein isolate with or without wheat fiber was effective in reducing cooking loss after two freeze-thaw cycles. Combined use of soy protein isolate and wheat fiber showed significantly less expressible moisture than using wheat fiber and soy protein isolate alone. It was not as effective as combined use of SPI and WF in reducing expressible moisture. Combined use of SPI and WF provided synergistic effect in reducing texture hardening after two freeze-thaw cycles ($p<0.05$). SEM clearly reflected freeze-induced texture changes in cooked fish mince as affected by the combined use of soy-protein isolate and wheat fiber. Cryostabilization and texture improvement of fish mince will enable the development of a variety of functional mince based seafood products with a good understanding of physical characteristics of meat and ingredient functions in the food matrix.

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