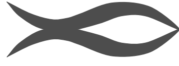

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# The Influence of Additives and Frozen Storage on Functional Properties and Flow Behaviour of Fish Protein Isolated from Haddock (*Melanogrammus aeglefinus*)

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**Abstract**

Fish protein isolates (FPI) were extracted from haddock (*Melanogrammus aeglefinus*) cut-offs using the pH-shift method. Flow behaviour and some functional properties of FPI containing 19% protein (pH 6.4) and different amounts of salt, sucrose and also polyphosphate stored 12 weeks at -18°C were studied. Additives influenced viscosity, but not flow behaviour. Adding salt and sucrose increased water holding capacity (WHC), but significantly decreased viscosity (Brabender Unit) and whiteness. Using polyphosphate and sucrose did not affect WHC, and whiteness of FPI but it decreased viscosity. Different amounts of additives and frozen storage time changed functional attributes of FPI significantly. The results suggest that the isolated proteins obtained through the pH-shift also need to be preserved against denaturation during frozen storage like surimi

*Keywords*: Functional properties, flow behavior, haddock, fish protein isolates, additives.

**Katkı Maddeleri ve Dondurarak Saklamanın Mezgitten (*Melanogrammus aeglefinus*) İzole Edilen Balık Proteininin Fonksiyonel Özellikleri ve Akış Davranışı Üzerine Etkisi**

**Özet**

pH–shift yöntemi kullanılarak mezgit (*Melanogrammus aeglefinus*) cut-off'larından balık proteini izolatları (FPI) alınmıştır. 12 hafta -18°C'de saklanan, %19 protein (pH 6,4), farklı miktarda tuz, sakaroz ve polifosfonat içeren FPI'a ait akış davranışı ve bazı fonksiyonel özellikler incelenmiştir. Katkı maddeleri, akışkanlığı (viskoziteyi) etkilemiş fakat akış davranışını etkilememiştir. Tuz ve sakarozun eklenmesi, su tutma kapasitesini (WHC) arttırmış, anlamlı bir şekilde akışkanlığı (Brabender Birimi) ve beyazlığı azaltmıştır. Polifosfonat ve sakaroz; WHC'yi ve FPI beyazlığını etkilememiş fakat akışkanlığı azaltmıştır. Farklı miktarda katkı maddeleri ve dondurarak saklama süresi, FPI'a ait fonksiyonel özellikleri anlamlı bir şekilde değişmiştir. Sonuçlar; pH- shift ile elde edilen izole proteinleri denaturasyona karşı korumak için surimi gibi soğukta saklanması gerektiğini öne sürmektedir.

*Anahtar Kelimeler*: Fonksiyonel özellikler, akış davranışı, mezgit, balık proteini izolasyonu, katkı maddesi.

## Introduction

The most important fish species in Icelandic waters belong to the gadoids namely cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), pollock (*Theragra chalcogramma*), and blue whiting (*Micromesistius poutassou*). They are mainly processed as fresh fillets, due to market preferences over other fish products, providing a considerable amount of by-products annually. The volume of cut-offs -a by-product from filleting process- is about 3-4% of gutted fish (Arason, 2003) and can be used as a good source of raw material for production of fish protein products. Thus, it seems applying pH-shift process which has been developed to increase the recovery of proteins from different fish sources (Hultin and Kelleher, 2000, 2001) is a good method for utilizing rest raw material of seafood industry to develop food for human consumption.

In the pH-shift process proteins of the muscle tissue are first solubilized. The solubilization can be accomplished in 5-10 volumes of water with alkali added to obtain approximately pH 10.5 or higher, or with acid added to about pH 3.5 or lower. The mixture is then centrifuged. This allows the light oil fraction to rise to the top of the suspension. At the

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same time, the lipids of the membrane are removed due to density differences compared to the main protein solution. Other insoluble impurities, such as bone or skin, are also sedimented at this stage. The muscle proteins are then precipitated by adjusting the pH to a value near the isoelectric point and collected by a process such as centrifugation (Hultin *et al*., 2005). Fish protein isolate (FPI) obtained from this process can be frozen like surimi or used as mince further processed products.

Denaturation and subsequent loss of functional properties is an important technical problem in manufacturing frozen fish proteins (surimi) (Lee 1984). Therefore, the addition of additives (cryoprotectants) is required to ensure long-term frozen stability of these products (Tanikawa, 1985; Yoon and Lee, 1990; McDonald and Lanier, 1991; Yataka and Harohiko 1992; McDonald *et al*., 1990; Park and Lin, 2005).

From additive adding point of view, there are two types of frozen surimi; salt-added and salt-free. In salt-added surimi 2.5% salt and 10% sugar (sucrose, glucose and sorbitol) are added to dewatered washed mince. While in salt-free surimi 0.2-0.3% of polyphosphate and 5-8% sugar are added (Tanikawa, 1985; Yataka and Harohiko, 1992). According to McDonalds *et al*. (1990) cryoprotectants are compounds that extend the shelf-life of frozen foods. They include all compounds that aid in preventing changes induced in foods by freezing, frozen storage, or thawing and may be added either during processing or formulation. The cryoprotectant effect of sugar is enhanced by adding polyphosphates, perhaps by buffering effect of polyphosphates on muscle pH and/or the chelating of metal ions (Park and Lin, 2005; Matsomuto and Noguchi, 1992). Arakawa and Timasheff (1982) reported that cryoprotectants increase the surface tension of water as well as the binding energy, preventing withdrawal of water molecules from the protein, thus stabilizing the protein. Thawornchinsombut and Park (2006) studied frozen stability of fish protein isolate (FPI) under various conditions. They recommended using cryoprotectant in FPI to prevent freeze-induced aggregation during frozen storage. However, using a mixture of trehalose and polyphosphate as protein preservative of frozen squid PI was also suggested by Campo-Deano *et al.* (2010).

On the other hand the rheological behavior of fish proteins (surimi and FPI) is important because of its effect on conditions during processing, texture and sensory quality of the final product. From a technological point of view flow properties may give a quantitative contribution to texture characterization and process control when using different formulations (Steffe, 1996). The flow property of fish protein determines the ability to pump the material within the manufacturing plant and affects the extrusion properties of the material during forming operations (Kim *et al*., 2005).

Many of the classical methods to measure flow behaviour of material are not easily applicable to fish due to the morphological specifications of fish mainly because of low amount of connective tissue which is easily disintegrated upon heating and muscle fibres dominating the microstructure (Dunajski 1979). Apparent viscosity of protein solutions has been suggested by Borderias *et al*. (1985) as a quality index for frozen fish, based on work on filtered protein solutions from cod mince. Venugopal *et al*. (1994) applied Brabender viscograph-E as a torsion viscometer for measuring viscosity changes and to monitor gel formation in shark myofibrillar protein.

However, Kristbergsson and Sigfusson (2002) used Brabender viscograph-E to study the rheological behaviour of cod (*Gadus morhua*), halibut (*Hippoglossus hippoglossus*), pollock (*Pollachius virens*) and ocean perch (*Sebastes marinus*). They recommended applying this instrument to analyze the effect of additives on viscosity and muscle protein denaturation. Based on Kristbergsson and Sigfusson's work (2002) several researches have been done on viscosity behaviour of fish protein hydrolysates (Gunnarsson *et al*., 2005), viscosity changes of saithe (*Pollachius virens)* powder during storage (Bragadottir *et al*., 2007) and flow behaviour of fish protein isolate products (Shaviklo, 2008). The overall objectives of this study were: to investigate whether flow and functional properties of FPI, which contain chemically recovered unfolded/refolded proteins, were affected by various additives, freezing and frozen storage time; and to determine the appropriate amount of additives (cryoprotectants) to protect functional properties of FPI.

## Material and Methods

### Fish Protein Isolate

FPI were extracted from cut-offs of haddock (*Melanogrammus aeglefinus*) using the pH-shift method (Hultin and Kelleher, 2000, 2001). FPI (50 kg) was obtained and transferred from MPF fish processing company in Grindavik to the processing lab at Matis (Icelandic Food Research) in Reykjavik (Iceland) under chilled conditions (<+4°C). FPIs were squeezed manually to decrease water content to 80% by using cheese cloths. It was stored at +2°C until preparation of test samples. All test samples were prepared within six hours after receiving FPIs.

### Preparation of Test Samples

The type and amount of additives used for frozen surimi have been suggested by many authors and seafood research institutes (Yataka and Harohiko, 1992; Min *et al*., 1988). Based on these information 36 samples of fish protein isolates were prepared as follows;

- Nine samples free of any additive (control sample),
- Nine samples containing 0.8% salt and 3% sucrose,
- Nine samples containing 1.3% salt, and 5% sucrose,
- Nine samples containing 4% sucrose and 0.1% polyphosphate.

FPI and additives were weighed separately and mixed completely for 3 minutes using a high speed vertical cutter (VCB-62, HILDE® Sweden). Samples were air packed immediately after mixing. Each polythene bag containing 1kg sample, was blast frozen and stored at temperature of -18°C except samples that were evaluated in week 0 and stored at +2°C.

### Methods

Dry matter was calculated as the loss in weight during drying at 105°C for 4 hours (ISO, 1983). pH of FPI was measured using pH meter, Knick Portamess®913 (Electronishe Megerate Gmb H&Co. Germany). All samples were measured at room temperature.

### Viscosity (Brabender® viscograph-E)

The Brabender viscosity of FPI samples was determined using a Brabender® Viscograph-E coaxial viscometer (Brabender® OHG, Duisburg, Germany) based on Kristbergsson and Sigufsson work's (2002). The Viscograph-E is a rotational viscometer designed to measure viscosity as a time and temperature dependent parameter. This instrument measures the resistance of the sample against flow. It is assumed that this resistance is proportional to the viscosity of the system. The term "Brabender viscosity" is used for describing the resistance. The measuring bowl containing the sample is placed in a temperature-controlled holder and rotates on a vertical axis forcing the sample to be pressed against the pin-style stirrer. Torque on the pins is measured and expressed as viscosity in Brabender units (BU). The force exerted on the stirrer depends on the resistance of the sample (Kristbergsson and Sigufsson, 2002).

The measurement of flow and viscosity of samples were done with following modification. Starting temperature was 5°C, heating rate 1.5°C/min, and maximum temperature 45°C with a holding time 3 minutes, then cooling rate of 1.5°C/min to 5°C. Measuring cartridge was 700 cmg (0.7 Nm) and speed of bowl 75 rpm. Sample quantity was 450 g (ratio of FPI to water 1:2). Temperature of sample was 0-2°C at the beginning of measurement. Samples viscosities were recorded from 5°C to 45°C and again to 15°C during cooling. Measurements were done in duplicate.

### Water Holding Capacity

Water-holding capacity (WHC) was determined by centrifugation following the method described by Shahidi *et al.* (1995). Two grams of FPI were placed in a plastic cylinder in a plastic holder cup. The cylinder had a fine mesh at the other end (diameter of sample cylinder 2.5 cm). This mesh had the purpose of holding the sample and also to allow liquid to pass through it since it was porous. The sample cylinder was then placed in a Biofuge Stratos; Heraeus Instruments (Hanau, Germany). Temperature interval was set at 5°C, speed 1350 rpm and the time was 5 minutes. After the centrifugation was completed, the sample cylinder was weighed and the difference in weight of the sample before and after was noted. WHC was expressed as the amount of water retained after centrifugation per gram of dry weight of the product.

### Color Evaluation

Color of fish protein isolate was measured by a Minolta CR-400 Chroma Meter (Minolta Camera Co. LTD. Osaka, Japan) in L*, a*, b* measuring mode with CIE Illuminant C. Color of fish protein isolate was measured in a test tube (25mm in diameter) which was placed in a side stand (Minolta CRA 70, Minolta Camera Co. LTD. Osaka, Japan). The color was measured three times turning the test tube 120° between measurements. Results were given as lightness (L*), redness (a*) and yellowness (b*). Whiteness was calculated by the equation: L*-3b* such as referred by Codex Alimentarius (Park, 2005).

### Statistical Analysis

Analysis of variance (ANOVA) was carried out on the viscosity, WHC, whiteness and L*, a*, b* results of all treatments in the statistical program NCSS 200 (NCSS, UT, USA). The program calculates multiple comparisons using Duncan's test to determine if sample groups are different. Significance of difference was defined at the 5% level.

## Results and Discussion

Dry matter of received FPI was 14.1±0.6. After dewatering it increased to 19±0.2. The pH of FPI free of additives was 6.4±0.1. The pH of salt-added samples and cryoprotectant added samples were 6.3±0.1 and 6.5±0.1, respectively.

### Viscosity (Brabender Unit)

Adding salt, sucrose and polyphosphate to FPI affected viscosity significantly ($P<0.05$) as shown in Table 1. The group without additives had the highest viscosity in week 0, followed by the salt added groups. Viscosity of the group with sucrose and phosphate was the lowest. The group without additives had the highest viscosity after 12 weeks of storage at -18°C ($P<0.05$) giving the greatest

**Table 1.** Viscosity, water holding capacity (WHC) and whiteness of FPI within 12 weeks of storage at -18°C

| Attributes | Week(s) | | A | B | C | D |
|---|---|---|---|---|---|---|
| Viscosity | 0 | * | 81.5±3.46 $^{aB}$ | 53.5±3.51 $^{b}$ | 52.0±2.83 $^{bC}$ | 47.0±1.42 $^{c}$ |
| | 4 | * | 120.5±2.95 $^{aB}$ | 65.0±4.33 $^{b}$ | 68.5±2.14 $^{bB}$ | 46.5±0.74 $^{c}$ |
| | 8 | * | 117.0±4.24 $^{aB}$ | 57.5±2.14 $^{c}$ | 64.0±4.22 $^{bB}$ | 42.5±2.11 $^{d}$ |
| | 12 | * | 137.0±3.82 $^{aA}$ | 67.0±2.81 $^{c}$ | 82.0±3.82 $^{bA}$ | 50.5±1.91 $^{d}$ |
| | | | * | NS | * | NS |
| WHC | 0 | * | 84.8±0.43 $^{cA}$ | 92.3±0.83 $^{bA}$ | 95.1±0.78 $^{a}$ | 84.7±0.85 $^{cA}$ |
| | 4 | * | 66.7±2.25 $^{cB}$ | 83.0±0.92 $^{bA}$ | 91.1±0.61 $^{a}$ | 63.7±2.53 $^{cB}$ |
| | 8 | * | 62.1±2.79 $^{cB}$ | 78.5±3.04 $^{bB}$ | 89.7±0.92 $^{a}$ | 63.5±2.62 $^{cB}$ |
| | 12 | * | 59.2±2.34 $^{cB}$ | 76.1±2.33 $^{bB}$ | 90.2±0.54 $^{a}$ | 60.1±2.74 $^{cB}$ |
| | | | * | * | NS | * |
| Whiteness | 0 | * | 54.2±0.83 $^{aA}$ | 50.4±1.15 $^{bA}$ | 47.2±0.55 $^{bA}$ | 55.2±1.61 $^{aA}$ |
| | 4 | * | 49.9±1.14 $^{aA}$ | 52.4±0.42 $^{aA}$ | 50.4±1.24 $^{aA}$ | 53.6±1.72 $^{aA}$ |
| | 8 | * | 47.4±2.23 $^{cB}$ | 49.3±1.63 $^{bA}$ | 48.4±1.91 $^{bA}$ | 52.4±1.89 $^{aA}$ |
| | 12 | * | 34.5±0.35 $^{bC}$ | 37.0±2.10 $^{aB}$ | 37.3±0.53 $^{aB}$ | 38.1±1.25 $^{aB}$ |
| | | | * | * | * | * |

Values are means of 2 evaluations for viscosity and 3 evaluations for WHC and whiteness.
Different lowercase superscript letters in the same column indicate significant differences among products.
Different uppercase superscript letters in the same row indicate significant differences among month of storage ($^{*}$ P<0.05).
(A) FPI without additives (control), (B) FPI with 0.8% salt and 3% sucrose, (C) FPI with 1.3% salt and 5% sucrose, (D) FPI with 4% sucrose and 0.1% sodium polyphosphate. NS=Not significant.

resistance to flow which may suggest a firmer protein structure than in the other groups. The addition of salt significantly reduced the viscosity, possibly due to a liquefying effect on the muscle myofibrillar structure. This has been attributed to a decreased interaction between proteins and the surrounding medium due to aggregation of proteins (Borderias *et al.*, 1985; Sadawsk and Sikoroski, 1977) and decrease in pH. Razavi-Shirazi (2002) reported that addition of salt to fish meat can decrease pH in the range of 0.1 to 0.2 units because of replacing $Na^{+}$ at the surface of proteins and releasing $H^{+}$. Kristbergsson and Sigfusson (2002) also reported that the addition of salt to fish mince decreased Brabender viscosity. The viscosity of the group without additives and the groups containing salt increased significantly (P<0.05) after 12 weeks of storage at -18°C.

The low viscosity of the group containing sucrose and polyphosphate may possibly be explained by decreasing interaction between proteins and surrounding medium. Contrary to the results of Kristbergsson and Sigfusson (2002) that frozen storage decreased Brabender viscosity of fish mince, the findings in this study was that it increased viscosity significantly (P<0.05) in the control group and samples containing 1.3% salt and 5% sucrose after 12 weeks of storage at -18°C. Viscosity of groups containing sucrose and phosphate and samples with 0.8% salt and 3% sucrose did not change during frozen storage.

## Flow Behavior of Frozen FPI

Effect of additives on flow behavior of fish protein isolate was studied within 12 weeks of storage at -18°C (Figures 1). During flow behaviour measurements, viscosity of all sample groups decreased by increasing temperature and the lowest viscosity was observed at 45°C. Viscosity gradually increased as temperature was reduced. Although the samples had different viscosity, their flow behavior always looked like thixotrophic, even after 12 weeks of frozen storage showing that flow behavior is independent from viscosity. A thixotropic fluid decreases in viscosity with time at a fixed (constant) rate of force (stress) (Steffe, 1996). It may be irreversible, reversible or partially reversible. There is general agreement that the term thixotropy refers to the time-dependent decrease in viscosity, due to shearing, and the subsequent recovery of viscosity when shearing is removed (Mewis, 1979).

There were significant differences (P<0.05) in flow behavior between the group without additives and the groups containing additives. From the results it can be concluded that frozen storage changed flow behavior of control group during the period of study (P<0.05) but not of the groups containing additives suggesting that they preserved proteins against denaturation.

## Water Holding Capacity

Water holding capacity of samples without additives and samples containing sucrose and polyphosphate was the same in week 0 (Table 2). Sample with 1.3% salt and 5% sucrose had the highest WHC followed by sample containing 0.8% salt and 3% sucrose (P<0.05). From the results it can be concluded that using salt and sucrose together will increase water holding capacity in fish protein isolate significantly (P<0.05). Added sucrose together with polyphosphate did not have any significant effects on

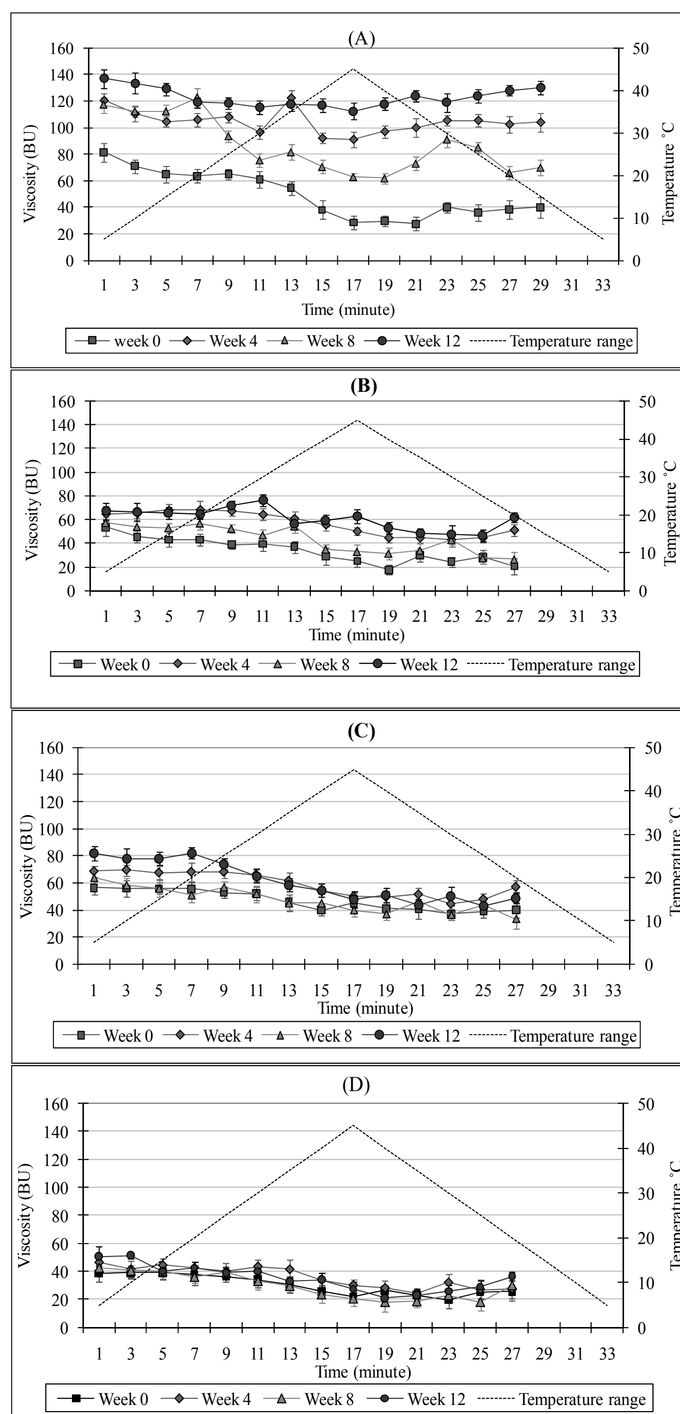


**Figure 1.** The influence of additives, storage time and temperature on time-dependent flow behaviors of frozen FPI stored at -18°C. A: FPI free of additives (control sample); B: FPI with 0.8% salt and 3% sucrose; C: FPI with 1.3% salt and 5% sucrose; D: FPI with 4% sucrose and 0.1% phosphate.

water holding capacity.

Samples containing salt and sucrose had the highest water holding capacity during 12 weeks storage at -18°C ($P<0.05$). There was not a significant difference between the group without additives and the group with sucrose and phosphate after 12 weeks of storage at -18°C. Samples containing 1.3% salt and 5% sucrose were stable during frozen storage. The WHC of these samples did not change significantly during 12 weeks storage at -18°C.

Water holding capacity (WHC) is an important factor for muscle protein gels as it not only affects the economics of their production but also their quality. Adding alkaline phosphates to fish meat can increase pH (Yapar *et al.*, 2006) and can increase WHC in fish proteins (Hunt *et al.*, 2004). Increasing pH can also increase water holding capacity in fish protein isolate (Kristinsson and Hultin 2003) but in this study FPI

**Table 2.** Color measurements (L*, a*, b*) of FPI within 12 weeks of storage at -18˚C

| L*a*b* | | Week(s) | A | B | C | D |
|---|---|---|---|---|---|---|
| Lightness (L*) | * | 0 | $70.6 \pm 1.75^{a}$ | $63.7 \pm 1.43^{b}$ | $62.1 \pm 1.52^{b}$ | $68.5 \pm 1.23^{a}$ |
| | * | 4 | $71.5 \pm 1.32^{a}$ | $64.1 \pm 1.11^{b}$ | $63.8 \pm 1.64^{b}$ | $68.2 \pm 1.54^{a}$ |
| | * | 8 | $71.9 \pm 1.14^{a}$ | $64.9 \pm 1.56^{b}$ | $65.2 \pm 1.33^{b}$ | $67.5 \pm 1.67^{a}$ |
| | * | 12 | $73.3 \pm 2.43^{a}$ | $65.8 \pm 2.25^{c}$ | $68.8 \pm 2.04^{b}$ | $67.0 \pm 2.84^{b}$ |
| | | | NS | NS | NS | NS |
| Redness (a*) | * | 0 | $-1.91 \pm 0.15^{aA}$ | $-2.34 \pm 0.22^{bA}$ | $-2.04 \pm 0.21^{bA}$ | $-1.93 \pm 0.14^{aA}$ |
| | * | 4 | $-2.31 \pm 0.24^{aA}$ | $-3.56 \pm 0.13^{bB}$ | $-3.41 \pm 0.17^{bB}$ | $-2.8 \pm 0.23^{aA}$ |
| | * | 8 | $-3.78 \pm 0.24^{aB}$ | $-4.22 \pm 0.34^{bC}$ | $-4.9 \pm 0.23^{bC}$ | $-5.3 \pm 0.17^{cB}$ |
| | * | 12 | $-5.22 \pm 0.11^{aC}$ | $-5.81 \pm 0.14^{bD}$ | $-6.0 \pm 0.15^{cD}$ | $-6.1 \pm 0.32^{cC}$ |
| | | | * | * | * | * |
| Yellowness (b*) | * | 0 | $5.5 \pm 0.34^{aC}$ | $4.4 \pm 0.13^{cD}$ | $5.0 \pm 0.45^{bC}$ | $4.4 \pm 0.64^{cC}$ |
| | * | 4 | $6.3 \pm 0.61^{aC}$ | $5.9 \pm 0.56^{aC}$ | $6.1 \pm 0.83^{aBC}$ | $5.1 \pm 1.12^{bBC}$ |
| | * | 8 | $8.9 \pm 1.1^{aB}$ | $7.8 \pm 1.34^{bB}$ | $7.2 \pm 1.12^{bB}$ | $6.2 \pm 0.89^{cB}$ |
| | * | 12 | $12.6 \pm 0.82^{aA}$ | $9.5 \pm 0.81^{cA}$ | $10.5 \pm 0.8^{bA}$ | $9.5 \pm 1.15^{cA}$ |
| | | | * | * | * | * |

Values are means of 3 evaluations. Different lowercase superscript letters in the same column indicate significant differences among products. Different uppercase superscript letters in the same row indicate significant differences among month of storage (* $p<0.05$). (A) FPI without additives (control), (B) FPI with 0.8% salt and 3% sucrose, (C) FPI with 1.3% salt and 5% sucrose, (D) FPI with 4% sucrose and 0.1% sodium polyphosphate. NS=Not significant.

containing phosphate and sucrose had the lowest value of WHC among other test samples suggesting that this material may not increase WHC in FPI.

The effect of salt on WHC, and thereby yield, has been described by many authors (Hamm, 1960; Warrier *et al*., 1975; Offere and Knight 1988; Fennema 1990). It is known that WHC increases with increasing salt concentration up to 6%. In this study increasing amount of salt and sucrose increased WHC significantly ($P<0.05$) and it was highest in the group with 1.3% salt and 5% sucrose within 12 weeks storage at -18°C.

**Whiteness (L*-3b*)**

As shown in Table 1, the highest value for whiteness in week 0, was for control sample and the group containing sucrose and polyphosphate which is significantly different from samples with salt and sucrose ($P<0.05$). No significant difference was seen in whiteness between samples containing 0.8% salt and 3% sucrose and samples containing 1.3% salt and 5% sucrose in week 0. All groups had the same value of whiteness after 4 weeks of storage at -18°C. However, after 12 weeks of storage at -18°C, whiteness of all groups significantly decreased ($P<0.05$).

Lightness (L*), redness (a*) and yellowness (b*) values of all fresh samples are given in Table 2. Adding salt and sucrose to FPI affected lightness, redness and yellowness of sample groups significantly ($P<0.05$). Frozen storage time did not influence lightness significantly but affected a* and b* values.

According to Kristinsson *et al*. (2005) the color and whiteness of FPI can in part depend on connective tissue that can increase the lightness; the retention of lipids that can influence yellowness values; co-precipitation of heme proteins which affect redness and denaturation and oxidation of hemoglobin that causes a yellow-brownish color in products. Meanwhile high redness values could be attributed to heme proteins in the final product.

Like surimi L*, a*, and b* values of haddock protein isolate were affected by moisture content and additives. L*, a*, and b* values were similar to the values which were reported by Lanier *et al*. (1992) for surimi indicating that pH-shift process can produce products with high lightness similar to surimi.

Myoglobin and hemoglobin, which are responsible for color characteristics of fish flesh, (Park, 1995) may remain in the fish protein isolated by pH-shift process. Oxidation of these proteins in haddock protein isolate, possibly, decreased a* values and increased b* values in all samples after 12 weeks of frozen storage. The highest a* and b* values after 12 weeks of frozen storage were for control sample indicating that adding salt, sucrose, and polyphosphate may possibly decrease oxidation of the proteins.

## Conclusions

Different amounts of additives and frozen storage time changed functional attributes of FPI significantly. Adding salt to fresh haddock protein isolate increased WHC, but decreased viscosity (BU) and whiteness. Using polyphosphate and sucrose together did not affect WHC, whiteness of FPI, but it decreased viscosity (BU). Storage time of frozen isolates decreased WHC and whiteness of all groups. Apart from the viscosity, samples with different amount of additives had the same flow behavior

(thixotropic) which it appeared to be reversible. Isolates with salt and sucrose had higher viscosity than isolate containing sucrose and polyphosphate.

It is obvious that that the addition of additives is required to improve long-term stability of fish protein during frozen storage (Yoon and Lee, 1990; McDonald and Lanier, 1991; Park and Lin, 2005; Park, 1995). On the other hand the factors responsible for decreasing texture quality in FPI are freeze/thaw cycles and/ the absence of cryoprotectants (Thawornchinsombut and Park, 2006). So, like conventional surimi the results suggest that the isolated proteins processed with the pH-shift method also need additives to preserve them against denaturation during frozen storage. Thus adding salt and sucrose as a protein preservative to FPI is recommended.

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