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Evaluation and Utilisation of Fish Protein Isolate Products

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By

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Evaluation and Utilisation of Fish Protein Isolate Products

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Declaration

I hereby declare that this thesis is based on my own experiments and observations. It is written by me and has not been in a part or as a whole submitted before to a higher academic degree.

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Abstract

The aims of the thesis were to evaluate functional properties of fish protein solutions and fish protein isolates and develop a ready to eat food product containing fish protein isolate. The objectives were as follows:

1. to study the effects of salt concentration, cryoprotectants and chill and frozen storages on viscosity, colour and water holding capacity/weight loss of solutions of cod protein isolate;
2. to determine the effects of cryoprotectants on the functional properties of the haddock protein isolates;
3. to study if Brabender® viscograph E can be useful in studying rheological behaviour of fish protein solutions and fish protein isolates, and
4. to develop cooked fish balls based on mince and isolate.

Added 3 and 5% salt to cod protein solutions with 3% protein decreased Brabender viscosity but adding 10 and 15% salt decreased it significantly ($P < 0.05$). Five days storage at $+2^{\circ}\text{C}$ decreased Brabender viscosity of samples with 1,2,3,5,10 and 15% salt ($P < 0.05$) and had no significant effect on weight loss of cod protein solutions with different amount of salt ($P > 0.05$). Storage time also increased whiteness in chilled sample with 5% salt ($P < 0.05$). Applying cryoprotectants to cod protein solutions increased water holding capacity (%) and Brabender viscosity in samples containing 3 and 5% salt ($P < 0.05$) and had no significant effect on whiteness ($P > 0.05$) after 14 weeks of storage at -24°C . Added cryoprotectants changed rheological flow behavior in all solutions except in solutions with 5% salt. WHC decreased during frozen storage. The solution with 5% salt and cryoprotectant was the most frozen stable solution followed by solution with 3% salt and cryoprotectant. The conclusion is that a cryoprotectant and 3-5% salt are needed to make a frozen stable fish protein solution.

Adding salt to fresh haddock protein isolate (HPI) with 20% protein, pH 6.4, different amounts of sucrose and also polyphosphate and stored at $+2$, -18 and -24°C increased WHC, but significantly ($P < 0.05$) decreased viscosity (BU and Pa) and whiteness. Using polyphosphate and sucrose as a cryoprotectant did not affect WHC, viscosity (Pa) of HPI ($P > 0.05$) but it decreased Brabender viscosity ($P < 0.05$). Apart from the viscosity fresh samples with different amount of additives had the same flow behavior (thixotropic). Different amount of additives and also frozen storage time changed under study attributes of HPI significantly ($p < 0.05$). Like the conventional surimi results suggested that the isolated proteins obtained through the pH-shift also need cryoprotectants to preserve them against denaturation during frozen storage. Thus adding 1.3% salt and 5% sucrose as an additive to HPI is recommended.

Haddock protein isolate with 20% protein and pH 7.4 was added to haddock mince in different proportions (50:50, 25:75) in manufacturing two types of fried fish balls. A mince fish ball product was also prepared as a control. The products were assessed for physical properties and sensory changes within a period of 8 weeks of frozen storage at -18°C . The Brabender viscosity of mince decreased significantly ($P < 0.05$) as added haddock protein isolates to mince increased. Control sample and fish balls containing isolate had the same cooking loss after two thermal settings ($P > 0.05$). Significant differences were seen for grainy and softness texture, colour and frozen storage flavour during sensory evaluation of fish balls that had been stored for 8 weeks at -18°C ($P < 0.05$). These attributes depended on the proportion of isolate to mince and also freshness level of the mince. This study shows good potential for HPI to be used as an ingredient in mince-based product development.

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Chapter 1

Literature Review

1. Introduction
 2. pH-shift process for isolating fish protein
 3. FPI vs. surimi
 4. Utilisation of fish protein isolate products
 5. Conclusion
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Literature Review

1. INTRODUCTION

Many efforts have done both in academy and in industry during recent decades to recover or isolate protein from fish by-products and underutilised fish species (Kristinsson *et al.*, 2006). A new technology has been developed to increase fish-based protein food for human consumption. According to Thorkelsson *et al.*, (2008) this innovation which is called the acid and alkali processes or pH-shift method has been documented and developed by Batista (1999); Hultin and Kelleher (1999); and Hultin *et al.*, (2005). This technology also has been introduced and described in three recently published books (Park JW, 2005; Shahidi F, 2006; and Borresen T, 2008).

Much research has been done using this method to isolate protein from fish by-products and under-utilised fish species. Since introducing pH-shift processes to the seafood industry more than 90 scientific papers, patents and MSc. /PhD thesis have been published. Most of these documents describe the acid-aided or alkali-aided processes to recover protein from fish by-products and underutilised fish or describing the specifications of isolated proteins. Only little information has been published on utilization of fish protein isolate products (Thorkelsson *et al.*, 2008, and Nolsoe and Undeland 2008). This chapter describes the pH-shift process at a glance, its advantages *vs.* surimi and the literature on the utilisation of isolated fish protein products

2. pH-SHIFT PROCESS FOR ISOLATING FISH PROTEINS

The basic outline of pH-shift method is simple. According to Hultin *et al.*, (2005) the most important steps of this method are as follows:

- A-Solubilisation of proteins at low or high pH;
- B-Removing fat and impurities by using high-speed centrifuge; and finally
- C- Precipitation of the proteins at their isoelectric point.

In this process as shown in figure 1, fish flesh is first solubilised in alkali or acid after the raw material has been mechanically deboned and minced or chopped to a small particle size. Solubilisation is done by using 5 to 10 volumes of water with alkali or acid added to obtain approximately pH 2.5 or 11 for example. The mixture is then centrifuged to remove oil and other insoluble material. Precipitation of proteins is accomplished by adjusting pH to 5.2 to 5.5. Protein isolate is sedimented by using a high speed centrifuge (Hultin *et al.*, 2005). FPI can be frozen like surimi or mince for further use. Figure 2 presents a frozen block of fish protein isolate.

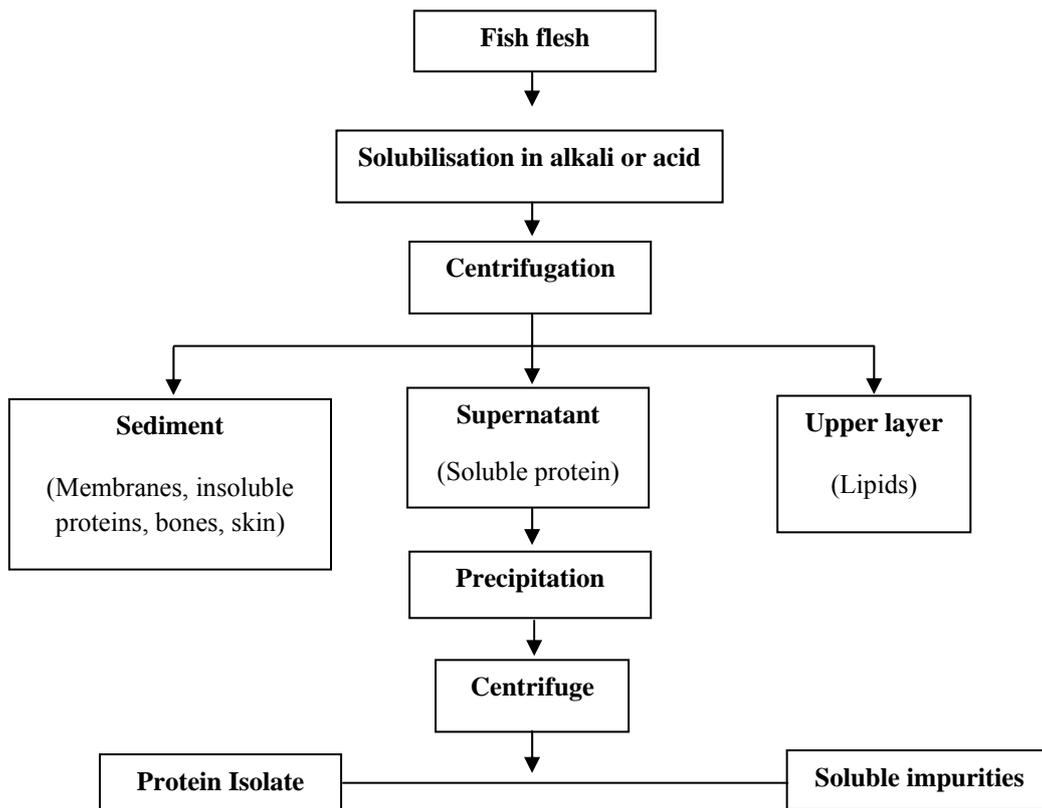


Figure 1: pH-shifts process for production fish protein isolate (Adopted from Hultin *et al.* 2005).



Figure 2: A frozen block of fish protein isolate (Shaviklo 2007)

One of the most important technical issues in pH-shift method according to Kristinsson *et al.*, (2006) is that undesirable materials like skin, bones, microorganisms, cholesterol, membrane lipids, and other contaminants are removed during the first centrifugation stage, although the bones may be removed during mechanical deboning.

Protein isolate made from an acid-aided process shows differs from a protein isolated with the alkali-aided process. A comparison between the acid and alkaline methods and surimi processing for recovery of proteins from channel catfish muscles which was done by Kristinsson *et al.*, (2005) indicates that the alkali process give better attributes such gel strength to FPI. However, the acid-aided method generally results higher protein yields compared to the alkali-aided process (Kristinsson *et al.*, 2006)

Thorkelsson *et al.*, (2008) reported that the alkali-aided process provides a more oxidative stable protein isolate than the acid-aided process and sometimes it is more stable than surimi (Krisstinsson and Demir 2003, Petty and Kristinsson, 2004). According to him, heme proteins are denatured and co-precipitated in the acid process and make the product less stable and darker (Kristinsson and Hultin, 2004; Kristinsson *et al.*, 2005; Choi and Park, 2002).

According to Kristinsson *et al.*, (2005) the alkali-aided process recover proteins of higher whiteness than acid-aided process. The acid-aided process also results a higher yellowness than the alkali-aided protein isolate and surimi made from catfish muscle.

3. FPI VS. SURIMI

Much research has been carried out comparing the properties of various FPI made from different raw materials and surimi. The comparison can be summarized as follows:

3.1 Raw material

Fish by-products and under-utilised fish species that usually are not used directly for human food can be utilized in pH-shift process (Hultin *et al.*, 2005). On the other hand whole fish with skin and bones and also fatty fish can be used in this technology, because proteins are selectively separated and isolated from undesirable materials (Kristinsson *et al.*, 2006). Fish flesh after filleting and mincing is used for surimi manufacturing. Applying other sources of raw material for processing affects the quality of surimi dramatically.

3.2 Machinery

Machinery used in surimi processing such as fish washing tanks and conveyors, heading, gutting, filleting and deboning machines, leaching tanks and conveyors and strainers are not used in pH-shift process. Therefore there is less investment cost although other machines are added to processing lines of isolate processing (Shaviklo 2007). However, using high-speed centrifugation step during the pH-shift methods for removing lipids makes these processes more complex to work with than the surimi process (Nolsoe and Undeland 2008).

3.3 Man power

Ingadottir (2004) reported that the acid and alkali-aided processes require less labor than surimi processes. For example, liquefaction of the material makes it easier to move it

around a processing plant compared to a more solid material such as in surimi processing. According to Hultin *et al.*, (2005) the pH-shift process is also faster than the conventional surimi processing because it does not depend on diffusion processes to extract the water-soluble materials from broken muscles.

3.4 Volume of used water and effluent

The leaching process and the volume of water used for surimi manufacturing depend on fish species, fish freshness, the scale of production and the final quality of the surimi. Park and Lin (2005) reported that 29.1 liters of water is used for manufacturing 1 kg surimi. This amount of water generates a large amount of effluent which is a main environmental problem of this industry. Hultin *et al.*, (2005) reported that 5-10 liters of water is used for production 1 kg fish protein isolate. According to him treatment of isolate effluent is much easier and cheaper than surimi effluent because of having a low biological oxygen demand and low salt content. Compared to industrial surimi process processing FPI leads to less protein in the waste water and a decrease in environmental pollution (Batista *et al.*, 2007).

3.5 Chemical treatments

No chemicals are used for surimi manufacturing, although sodium bicarbonate is used in leaching steps to increase the pH for improving gel forming ability when processing red (dark) meat fishes (Min TS, 1988). However pH-shift is a chemical-based method. According to (Nolsoe and Undeland 2008) the use of acid and alkali in the pH-shift processes needs stronger safety requirements. For example HCl, is a strong oxidizing agent for metals that can cause corrosion on processing machineries.

3.6 Protein recovery

Parke and Lin (2005) reported that protein recovery in surimi processing depends on fish freshness, the water/meat ratio, washing time, washing cycle, and pH of the washing solution. While according to Torres *et al.*, (2006) protein recovery in pH-shift process depends on pH of both solubilisation and precipitation steps. He studied protein recovery from processing trout by-products by pH-shift technology. As shown in table 1 the highest values for protein recovery were seen when pH of solubilisation and precipitation were 2 and 5.5 respectively. Hultin *et al.*, (2005) also reported that more than 85% yields are generally obtained from fillets in pH-shift process compared to 55-70% from the surimi process.

Kristinsson *et al.*, (2005) reported 62.5% protein recovery for channel catfish surimi and 71.5% and 70.3% protein recovery for cat-fish protein isolates made from acid-aided and alkali-aided processes in lab scale respectively. In another work he and his co-workers reported 57.7% protein recovery for Atlantic Croaker surimi and 78.7% and 65.0% protein recovery for Croaker protein isolate made from acid-aided and alkali-aided process respectively (Kristinsson and Liang 2006).

Table 1: Protein recovery from processing trout by-products by pH-shift technology (Adapted from, Torres *et al.*, 2006)

pH (Solubilisation / precipitation)	% Protein recovery
2.5 / 5.5	89.0
2.5 / 5.0	81.9
2.5 / 6.0	85.9
2.0 / 5.5	91.3
3.0 / 5.5	86.2
12.5 / 5.5	84.4
12.5 / 5.0	77.7
12.5 / 6.0	83.4
12.5 / 5.5	82.9
13.0 / 5.5	88.1

3.7 Gel strength

The gel strength is one of the most important attributes of FPI and surimi. Hultin *et al.*, (2005) compared gel strength of three different forms of fish proteins prepared from pH-shift processes and surimi. In his report gel strength of surimi was weaker than FPI from alkali-aided process and both of them had a stronger gel than protein isolated from the acid-aided process. Table 2 shows the gel strength of different FPI.

Table2: Puncture test and fold test values of different types of alkali aided FPI

Source	Types of FPI		Punch test values (g×cm)	Fold Test value
1	Cod	Fillet	478	5
1	Cod	Minced	304	2
1	Cod	Frame	277	5
1	Herring	Fresh	801	5
1	Herring	Aged 6 days	287.5	3
1	Herring	Frozen	668	5
1	Farmed catfish	Fillet	850	5
1	Pacific whiting	Fillet	248	-
2	Cod	cut off	274	3
2	Saithe	cut off	199	2
2	Arctic charr	fish frame	30	1

1-Hultin *et al.*,2005; 2- Shaviklo *et al.*,2008

3.8 Whiteness

Whiteness is another of the most important attributes of surimi and FPI and the market is in general most interested in products having high value of whiteness. Kristinsson *et al.*, (2005) reported higher values of whiteness for FPI made from both acid and alkali process than surimi for cat-fish. Table 3 shows whiteness and lightness values of different FPI and surimi.

Table 3: Colour coordinates and whiteness of cooked fish protein isolates and surimi

Source	Sample	Raw material	Colour coordinates			Whiteness (L*-3b*)
			L*	a*	b*	
1	Surimi	Cat fish	70.4 ± 1.1	-0.9 ± 0.2	0.7 ± .4	68.3 ± 0.1
1	FPI (alkali process)	Cat fish	73.8 ± 0.4	-3.6 ± 0.2	5.7 ± 0.3	72.9
1	FPI (acid process)	Cat fish	75.0 ± 0.7	-3.0 ± 0.2	0.2 ± 0.4	74.8
2	FPI (alkali process)	Cod (cut offs)	71.1 ± 0.2	-3.9 ± 0.2	-0.9 ± 0.0	73.5 ± 0.1
2	FPI (alkali process)	Saithe (cut offs)	61.7 ± 0.1	-2.7 ± 0.1	7.7 ± 0.5	38.3 ± 0.2
2	FPI (alkali process)	Arctic charr (fish frame)	76.2 ± 0.1	3.3 ± 0.4	14.8 ± 0.5	32.3 ± 0.5
3	FPI (alkali process)	Rockfish (fish fillet)	76.2	-0.04	5.67	59.2
4	FPI(acid process)	Pacific whiting (fish fillet)	70.1± 0.3	1.6 ± 0.0	4.6 ± 0.1	60.7 ± 4.5

1- Kristinsson *et al.*, 2005; 2- Shaviklo *et al.*, 2008; 3- Yongsawatdigul and Park 2004; 4- Choi and Park 2002

3.9 Removing of lipids

Removal of lipids form surimi depends on fish species and number of washing cycles (Park and Lin 2005); however fat content is less than 1% in Alaska pollock surimi. According to Hultin *et al.*, (2005) lipids can be effectively removed in pH-shift processes. Lipid-soluble toxins such polychlorinated biphenyls are removed and cholesterol levels are also reduced.

Kristinsson *et al.*, (2005) reported 58.3% lipid reduction for catfish surimi and 70.3% and 71.5% for alkali-aided catfish protein isolate respectively. In another work he and his colleagues reported 16.7% lipid reduction for Atlantic Croaker surimi and 68.4% and 38.1% for alkali-aided and acid-aided Croaker protein isolate respectively (Kristinsson and Liang 2006).

3.10 Frozen storage stability

A mixture of alt and sucrose or cryoprotectans are added to the surimi at the end of processing to make surimi stable during freezing and frozen storage(Yataka and Harohiko, 1992). Few studies have been done for frozen stability of FPI. Thawornchinsombut and Park (2006) studied frozen stability of FPI. They reported that like conventional surimi, alkali-treated protein isolate also needs cryoprotectants to prevent freeze-induced aggregation during frozen storage. Table 4 presents the additives used for frozen surimi.

Table 4: Additives used for frozen surimi (Yataka and Harohiko, 1992)

Kind of surimi	Sucrose (%)	Sorbitol (%)	Polyphosphat (%)	Salt (%)
Salt-free	4	4	0.3	0
Salt-added	5	5	0	2.5

3.11 Sensorial attributes

Surimi is primarily developed to have little or no flavor at all (Manley and Dubosc 2005). Texture of this product is soft, similar to comminuted fish flesh and it mixes completely with ingredients during the manufacturing products, but fish protein isolate has not the

same attributes of surimi. The results of different trials indicated that FPI has more or less chemical (soapy) flavor and grainy texture. These attributes were seen in cod, saithe and arctic charr protein isolated by pH-shift process but not at the same level (Shaviklo 2007). Improving the sensory quality of FPI is a vital issue for developing FPI-based products.

4. UTILISATION OF FPI PRODUCTS

A fish protein solution (FPS) is a kind of semi-solid protein-in-water colloid which is prepared by using the acid/alkaline aided process. It may also include salt, and other optional ingredients (cryoprotectants, etc.) based on the final use.

Fish protein solutions can be made in two ways. One is by resolubilising proteins isolated by the acid/alkali-aided methods, and the other is to directly use solubilised ground fish muscle, in the acid or alkaline state, for injection into fillets without using any precipitation step (Nolsoe and Undeland 2008).

The fish protein isolated made by the pH-shift process looks like surimi when it is dewatered and packed. It may contain about 14-20% protein and 80-86% water. The quality of this product depends on several factors such as source of raw material, method of processing, pH, amount of protein and water content etc. Different attempts have also been made to utilise fish protein isolate in cooked products. Little information has been published for applying FPI in seafood products.

4.1 Fortification of fish fillet

Fortification of fish fillet by multineedle injection of fish proteins, static soaking, or vacuum tumbling have been reported by Thorkelsson *et al.*, (2008). He reported that injection of brine containing fish protein isolated solution or homogenised fish flesh increase the weight (yield) in cod and haddock fillets by 5-20% and increase cooking yield. Fish protein isolates also provided a higher cooking yield and microbiologically more stable products than those injected with fish mince (Thorkelsson *et al.*, (2008). Kim and Park (2006) reported that fish protein injection can enhance the yield and improve the frozen stability of fish fillet. Improvements in water holding capacity, by re-solubilisation of FPI powder and inject into fish fillets has been reported by Nolsoe and Undeland (2008).

4.2 Using as a batter mixture

FPS can be applied as an ingredient for preparing batter mixtures used in battering and breading processes. It seems FPI can improve the viscosity of batter solution. During experiments the author noticed that FPS with 3% protein when mixed with batter powder (50:50, 60:40 w/w) could increase the viscosity of the solution and its stability against sedimentation after 48 hours of storage at refrigerated temperature.

4.3 Using as a dipping solution

FPS can be used as a dipping solution in battering and breading process to reduce absorption of oil in fried products. Kim and Park (2006) reported that when protein solutions [mixture of homogenized isolated fish protein and water (1:3)] were applied as a dipping solution for fish finger and patties before battering or breading, the quantity of oil absorbed in fried products was significantly reduced. Fish protein may form a protein film and act as a fat blocker. Therefore, Thorkelsson *et al.*, (2008) reported that applying fish protein isolates to reduce fat in deep-fried battered and breaded cod and saithe did not change the fat content of the finished product in the set-up and conditions used in the tests (Einarsdottir *et al.*, 2007). Using re-solubilised FPI powder as a batter to create low-fat fried seafood products has been reported by Nolsoe and Undeland (2008).

4.4 Manufacturing of ready to eat products

Ready to eat fish products are processed by mixing fish protein (surimi/ minced fish) with different ingredients such as vegetable proteins, starches, wheat flour, spices etc. and forming fish paste into the intended shape of the product. Fish protein isolate can be used in this case as a fish protein ingredient or even replacer of whole/or a part of mince and surimi in the formula. It seems a variety of ready to eat fish products can be processed by using fish protein isolate, but few reports have been published in this field.

Shaviklo (2007) produced fish balls and fish burgers from arctic charr protein isolate and reported good organoleptic properties for the products. Pires *et al.*, (2007a, 2007 b) reported producing of frankfurter type sausages from Cape hake protein isolates. Finally, the use of acid produced cod proteins as emulsifiers has also been reported (Nolsoe and Undeland 2008).

5. CONCLUSIONS

The utilization of fish by-products and underutilised fish species has been one of the most important challenges in the seafood industry in recent years. The pH-shift process is more suitable than surimi for these complex raw materials and can serve an alternative to surimi (Thorkelsson *et al.*, 2008). This technology also has various advantages over surimi production (Hultin *et al.*, 2005). The future aim of FPI processing is to apply this new source of protein to human food products. Therefore, it could be concluded that this technology is going to be commercialized for processing a large volume of by-products and underutilized fish species and producing a variety of seafood products.

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Chapter 2

Effects of Salt and Cryoprotectants on the Functional Properties of Fresh and Frozen Protein Solution Made from Atlantic Cod (*Gadus morhua*) Cut-offs

Abstract

1. Introduction

2. Material and methods

3. Results and discussion

3.1 Chemical and microbial results

3.2 Whiteness

3.3 WHC and WL

3.4 Viscosity

3.5 Rheological behaviour of cod protein solutions

4. Conclusions

References

Effects of Salt and Cryoprotectants on the Functional Properties of Fresh and Frozen Protein Solution Made from Atlantic Cod (*Gadus morhua*) Cut-offs

Abstract

*Fish protein solutions were extracted from cut-offs of cod (*Gadus morhua*) using the pH-shift process. Fresh and frozen stability and rheological behaviour of cod protein solutions (CPS) at 3% protein, pH 7.9, and different amounts of salt, with or without cryoprotectants stored at different times and temperatures were studied. The results indicated that the fresh fish protein solution was spoiled after 3 days of storage at +2°C. Increasing salt level of cod protein solutions decreased Brabender viscosity in samples containing 3 and 5 % salt and increased it in samples containing 10 and 15% salt after 2 days of storage at +2°C ($p < 0.05$). Added 20% salt to cod protein solution possibly caused protein denaturation as protein clots were separated from water. Rheological behaviour of samples containing 10 and 15 % salt was changed after 2 days of storage at +2°C. Storage time decreased Brabender viscosity of fresh samples containing 10 and 15% salt ($P < 0.05$). It appears storage time did not change weight loss of samples significantly ($P > 0.05$). Storage time also increased whiteness of sample containing 5% salt ($P < 0.05$) and decreased viscosity (Pa) of all samples ($P < 0.05$). From a rheological point of view fresh samples containing 1.2, 3 and 5% salt were stable within 5 days of storage at +2°C. They had thixotropic flow behaviour which was reversible. Adding cryoprotectants to CPS containing 1.2, 3, 5, and 15% salt increased water holding capacity (%) and decreased weight loss ($P < 0.05$). It increased viscosity (BU and Pascal) in samples containing 3 and 5% salt significantly ($P < 0.05$) after 14 weeks of storage at -24°C. Cryoprotectants had no significant effect to improve whiteness in samples ($P > 0.05$). Cryoprotectants changed rheological flow behaviours in all samples except samples with 5% salt. Storage time also increased weight loss and decreased WHC in frozen samples ($P < 0.05$). Among frozen trials, sample with 5% salt and containing cryoprotectant was the most stable followed by sample with 3% salt and containing cryoprotectants. These samples had thixotropic flow behaviour which was reversible after 14 weeks of storage at -24°C. To make stable fish protein solutions (preferability containing 3-5% salt) during frozen storage it was recommended to add cryoprotectants to the products at the end of the processing.*

Key Words: cod, fish protein solution, storage time, temperature, stability, rheological behaviour.

1. INTRODUCTION

A fish protein solution (FPS) is a kind of semi-solid protein-in-water colloid which is prepared by using acid/alkaline aided process or diluting FPI with brine. The solution may include salt, and other optional ingredients (cryoprotectants, etc.) based on the final use. From an economical point of view fish by-products are the best raw material for producing FPS and this can improve the utilisation of fish resources.

According to Batista *et al.*, (2007) the yields of conventional technologies to manufacture value-added fishery products are relatively low and large amounts of by-products are lost. This is a big issue in fish filleting processes where less than 40% of fish tissue is recovered for human consumption. Thus, a new process was developed to increase the recovery of proteins from different sources of raw material (Batista 1999, Hultin and Kelleher, 2000, 2001, Hultin *et al.*, 2005). This process can be potentially used for any kind of fish and fish by-products as it presumably removes essentially all the membrane lipids and provides an increased yield of protein. The improved yield results in less protein in the wastewater during industrial processing and less environmental pollution (Batista *et al.*, 2007).

This process for isolating fish proteins involves the solubilisation of minced and homogenized fish tissue either in an acidic ($\text{pH} \leq 3.5$) or in an alkaline ($\text{pH} \geq 10.5$) aqueous solution. The protein rich aqueous solution is separated from solids (insoluble proteins, skin, bones, and scales) and neutral lipids by centrifugation. The soluble proteins are then recovered by isoelectric precipitation by adjusting the pH at 5.5 and the precipitated proteins are removed by centrifugation (Batista, *et al.*, 2007). Fish protein isolate and fish protein solutions made by this method can be kept chilled or be frozen for future utilization.

Fish protein solutions can be used for fortification of fish fillets (Thorkelsson *et al.*, (2008). FPS also was applied as a fat blocker in fish fingers and patties (Kim and Park 2006).

1.1 Effects of phosphate and pH on functional properties of FPS

Phosphate has been added to meat and seafood products to induce water holding capacity during processing, which has maintained or even increased weight of these products. The effectiveness of phosphates on water holding capacity of fish meat products depends on the type and quality of phosphate. Hunt *et al.*, (2004) reported that phosphates can increase WHC in fish protein if they are properly added. The activity may be because of effects on pH and ionic strength and specific interactions of phosphate anions with divalent cations and myofibrillar proteins. Factors such as the concentration of ions (Mg^{2+} , Ca^{2+} , Cl^-), temperature, and pH are believed to affect how phosphates interact with muscle proteins (Trout and Schmit 1984, Offer and Knight 1988, Lindsay 1996, Chang and Regenstein 1997).

Knipe (1992) reported that alkaline phosphates increase meat pH in the range of 0.1 to 0.6 units, depending on the phosphate (0.1-0.3%) used. The effect of phosphate to change the pH is in descending order of: pyrophosphates, tripolyphosphates, and hexametaphosphates.

Feng and Hultin (2001) also reported that extremes of pH can cause proteins to unfold and to increase their water binding.

1.2 Rheological behaviour of FPS

The rheology of fish protein is evaluated because of its importance for the choice of formulation, process condition and quality control. From a technological point of view rheological properties may give a quantitative contribution to texture characterisation and process control when using different formulation. Viscosity which is one of the most common contributors to the texture of a semi-solid food or beverage measures a fluid or material's resistance to flow. According to Steffe (1996) fluids are classified into 2 groups; Newtonian and Non-Newtonian fluids. Newtonian fluids are those whose viscosity is not affected by shear rate or time. Non-Newtonian fluids exhibit various degree of change with varying levels of shear. They may also be time-dependent and are divided into two groups; thixotropic and rheopectic. A thixotropic (work softening) fluid decreases in viscosity as time is increased at a fixed (constant) rate of force or stress (Figure 1). A rheopectic (work hardening) fluid increases in viscosity as time is increased at a fixed (constant) rate of force (stress).

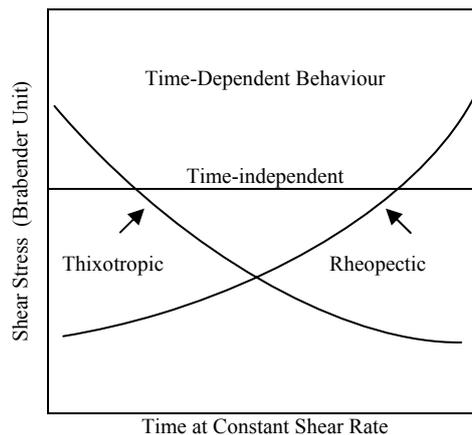


Figure 1 .Time-dependent behaviour of fluids (Adapted from: Steffe JF, 1996)

Mewis (1979) reported that both phenomena 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.

Steffe (1996) reported that thixotropy in many fluid foods may be described in term of sol-gel transition phenomenon and it could, apply for example, to starch-thickened baby food or yogurt. After being manufactured and placed in a container, these foods slowly develop a three dimensional network and may be described as a gel. When subjected to shear, structure is broken down and the materials reach a minimum thickness where they exist in the sol state. In foods that show reversibility, the network is rebuilt and the gel state re-obtained. Irreversible materials remain in the sol state. When shear is removed, during the

rest period the material, thixotropic fluids may completely recover, partially recover or not recover any of its original structure leading to a high, medium, or low torque response in the sample. Rotational viscometers [like Brabender viscometer] have proven to be very useful in evaluating time-dependent fluid behaviour because they easily allow materials to be subjected to alternate periods of shear and rest.

1.3 Objectives:

Experiments were carried out to study:

- changes in viscosity, colour and water holding capacity / weight loss of cod protein solutions (CPS) containing different proportion of salt and cryoprotectants during fresh and frozen storages at different temperatures, and
- rheological behaviour of test samples using a Brabender[®] viscograph E.

2. MATERIAL AND METHODS

2.1 Materials

Atlantic cod (*Gadus morhua*) protein solutions (CPS) were extracted from cut-offs using pH-shift process at Iceprotein ehf in. It was transferred from Iceprotein to Matis in Reykjavik in three polyethylene (PE) tubs under chilled conditions ($<+4^{\circ}\text{C}$). It was stored at $+2^{\circ}\text{C}$ until used. All test samples were prepared within 4 hours after receiving samples. Three samples were taken for microbial tests (total count) under hygienic conditions.

2.1.1 Preparation of test samples

Based on a work plan (annex 1), 36 samples of cod protein solutions were prepared as follows:

- 12 samples containing 1.2, 3, 5, 10, 15, and 20% salt;
- 12 samples containing 1.2, 3, 5, and 15% salt;
- 12 samples were prepared by adding 1.2, 3, 5, and 15 % salt, and cryoprotectants (sucrose, sorbitol and polyphosphate with 1, 1, and 0.1% respectively).

According to the work plan an appropriate amount of salt, cryoprotectants and CPS containing 1.2% salt were weighted separately and mixed in a plastic jar to make 1 litter of each test sample. Solutions were mixed by using an immersion hand blender while the plastic jar was immersing into a mixture of chilled water and crushed ice to control the temperature. The solutions temperature during sample preparation was less than 4°C . Samples were packed in polyethylene bags and sealed by thermal sealing machine. Fresh and frozen samples were stored in 2 cold storages with temperature $+2^{\circ}\text{C}$ and -24°C respectively. Fresh samples were evaluated after 2 and 5 days of storage and frozen samples were assessed after 2, 8 and 14 weeks of storage.

2.2 Methods

2.2.1 Microbiological analysis

Aerobic plate count was conducted according the procedures of the Compendium of Methods for the Microbiological Examination of Foods (APHA 1992).

2.2.2 Dry Matter

Dry matter was calculated as the loss in weight during drying at 105 °C for 4 hours (ISO 1983).

2.2.3 TVB-N

TVB-N content of CPS sample was measured using direct distillation into boric acid (based on AOAC 1990). The acid was then titrated with a diluted sodium hydroxide solution. The unbound ammonia was calculated as g/16gN.

2.2.4 pH

pH of CPS was measured using a pH meter, Knick Portamess®913 (Electronische Meßgeräte GmbH H&Co. Germany). All samples were measured at room temperature. pH values were the average value of three readings for cod protein solutions.

2.2.5 Water holding capacity and weight loss

Water-holding capacity (WHC) and weight loss (WL) was determined by centrifugation following the method described by Gunnarsson *et al.*, (2005) with some modification. A plastic cylinder was put in a plastic holder cup, and then 2 grams of CPS were placed in a plastic cylinder which 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 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 had completed, the sample cylinder was weighted and the difference in weight of the sample before and after was noted. The water holding capacity was calculated according to the following formula:

$$\text{WHC (\%)} = \frac{[\% \text{ water in sample (before centrifugation)} \times \text{sample weight (g)}] - [\text{weight after centrifugation (g)}]}{[\text{Water in sample (before centrifugation)} \times \text{sample weight}]} \times 100$$

The results of WHC are expressed as the amount of water retained after centrifugation per gram of dry weight of the product.

2.2.6 Viscosity (Bohlin BV88)

The viscosity of CPS was analysed using a Bohlin BV88 viscometer (Bohlin Instruments, England). Each chilled sample was mixed by an immersion hand blender to make a homogenized solution. A beaker containing 200 mm of sample was put inside a 500 mm beaker containing crushed ice to control temperature (Photo 2.4 (L), Annex 2). The instrument cylinder was immersed into the solution. The viscosity of sample was recorded

after 20 seconds of operating instrument at 5-7°C, speed setting 6, system switch 6. Measurements were done in triplicate.

2.2.7 Viscosity (Brabender® viscograph E)

The Brabender viscosity of CPS samples was determined using a Brabender® Viscograph E coaxial viscometer (Brabender® OHG, Duisburg, Germany) based on Gunnarsson *et al.*, (2005) but with some modification. The Viscograph E is a rotational viscometer designed to measure “viscosity” as a time and temperature dependent parameters. This instrument measures a 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 (Kristbergsson and Sigfusson 2002). The instrument offers the ability to measure Brabender-viscosity of viscous materials during a heating cycle. It consists of an electronic measuring system, a measuring bowl (100 mm height, 90 mm diameter) containing eight (8) protruding pins, and a seven (7) pin stirrer (Fig. 2). The measuring bowl, containing the sample (approximately 450 g) is placed in a temperature-controlled holder and rotates on a vertical axis forcing the sample to be pressed against the pin-style stirrer (Photo 2.4 (R), Annex 2). 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. The instrument was designed for carbohydrate-based materials, but has also been used to study “viscosity” in other food materials such as chocolate, juice and other liquid, and semi-liquid foods and for testing the “viscosity” of materials in the chemical-cosmetics and textile industries (Kristbergsson and Sigfusson 2002).

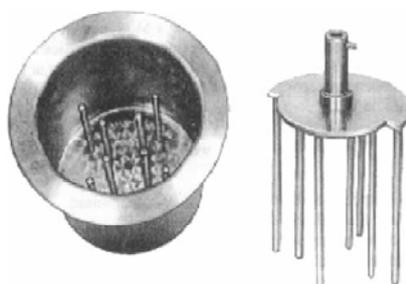


Figure 2. Measuring bowl and the pin-style stirred used for the Brabender® viscograph E, (From: Kristbergsson and Sigfusson 2002).

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 the bowl 7 rpm. Sample quantity was 450 g of CPS without addition of water or other additives. The measurements were done in duplicate. Different measuring rates (cmg) and speed (revolutions per minute) [350 and 65, 1000 and 75, 1000 and 85, 250 and 55, 700 and 55] were tested to measure different protein solutions having different pH. The best measuring range and speed to study viscosity changes of protein samples were 700 cmg and 75 rpm respectively which were used during measuring all test samples. The temperature of the sample should be 0 to 2°C at the

beginning of measurement. Samples viscosities were recorded from 5°C to 45°C and again after cooling to 15°C.

2.2.8 Colour measurement

Colour of cod protein solutions were measured at room temperature using a Chroma Meter CR-400 (Minolta Co. LTD. Japan). Colour analysis was done by putting the device on the PE bags containing cod protein solutions at least in triplicates. Colour of solution was measured as the average of 3 readings for each sample after calibrating the instrument. Colour was measured as lightness (L*), redness (a*) and yellowness (b*). Whiteness was calculated by the equation: $L^* - 3b^*$ as referred by Codex Alimentarius (WHO/FAO 2005).

2.2.9 Statistical analysis

Statistical analysis was performed by Microsoft Excel 2007 (Microsoft Inc, Redmond USA) and Minitab® 15, Statistical Software. Data were subjected to analysis of variance (ANOVA). Tukeys comparison test was used to determine significance between samples ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1 Observations

After mixing of samples with different proportions of salt and cryoprotectants colour of solutions changed a little and looked lighter. Proteins were possibly denatured and coagulated clots were seen after mixing of sample containing 20% salt.

Sample with 20% salt had two separated phases; a phase of proteinaceous clots and a liquid phase, after 2 and 5 days of storage at +2°C. These clots were not seen in other samples after 2 days of storage, but they were few in 10%, and 15% salt containing samples after 5 days (Figures 2.2 and 2.3 annex2).

Big clots of denatured protein were observed in all samples free from cryoprotectants after defrosting samples within 14 weeks of storage at -24°C. The more salt content and the more storage time the more clots. In samples containing 5 and 10% salt, denatured proteins were separated from water phase. There were not any denatured protein clots or separation in cryoprotectant added samples within the storage time. Tested all samples had a slight off-odour after 14 weeks of storage at -24°C. Off-flavour in cryoprotectant added samples was perceived to be less than in salt added samples.

3.2 Chemical and microbial results

pH and dry matter of fresh samples were 7.9 ± 0.1 and $4.17 \pm 0.0\%$. Salt content and protein percentages of cod protein solutions were 1.2% and 2.97%, respectively. pH of samples containing cryoprotectants were 8.1 ± 0.1 . The cod protein solution was spoiled after 3 days of storage at +2°C. Total count of bacteria at 22°C day 1 and day 3 were $8.4 \pm 0.8 \times 10^4$ and $3 \pm 0.5 \times 10^6$ respectively. TVB-N value of the fresh protein solution after

1 and 3 days of storage at +2°C were 3.22 (mg N/100 g) and 4.69 (mg N/100 g) respectively (Table 1).

Table 1: Results of microbial and chemical tests for cod protein solution

Age	Total platecount of bacteria at 22°C (cfu/g)	TVB-N (mg N/100 g)
Day 1	8.4±0.8 × 10 ⁴ a	3.2 ± 0.2 d
Day 3	3 ± 0.5×10 ⁶ b	4.9 ± 0.1 e
Day 5	6.3 ± 0.4×10 ⁶ c	Not measured

Values are means of 2 analyses. Means within column followed by different letters are significantly different (P<0.05).

3.3 Whiteness (L*-3b*)

3.3.1 Fresh CPS

As shown in Table 2, samples with different percentages of salt content had approximately the same values of whiteness (P>0.05) at day 2 and 5 of storage at +2°C except for samples with 20% salt which had the lowest value among samples (P<0.05). In samples containing 20% salt, proteinous clots had been separated from the aqueous phase and whiteness of this liquid was measured during experiments. Whiteness of samples had not been significantly affected by salt level and storage time after the 5 days of storage at +2°C (P>0.05).

Table 2: Whiteness values of fresh CPS trails during 5 days of storage at +2°C

Age (days)	Cod Protein solutions containing different levels of salt (%)					
	1.2	3	5	10	15	20
2	68.5 ± 0.2 a	67.4±0.8 b	67.2 ± 0.1c	67.9 ± 0.3 e	68.7 ± 0.3 f	32.2 ± 1.1 g
5	68.7 ± 0.4 a	66.8±1.4 b	67.7 ± 0.1d	67.7 ± 0.1 e	68.9 ± 0.2 f	31.7 ± 0.9 g

Values are means of 3 analyses. Means in the same column followed with the same letter are equals (P>0.05).

Lightness (L*), redness (a*) and yellowness (b*) attributes of all samples were approximately in the same range (P>0.05) except for sample with 20% salt which had been separated in 2 phases (P<0.05). Colour measurements of CPS within 5 days of storage at +2°C are presented in table 3-5. During the storage time, CPS with 1.2% salt had the highest value of lightness (L*) and it was lowest for CPS with 20% salt. The latter had the highest values of redness and yellowness among all samples stored 5 days at +2°C.

Table 3: L* values of CPS during 5 days of storage at +2°C.

Time (day)	Cod Protein solutions containing different levels of salt (%)					
	1.2	3	5	10	15	20
2	49.2 ± 0.4 a	48.7 ± 0.4 b	47.8 ± 0.4 c	48.7 ± 0.4 d	48.0 ± 0.2 e	34.4 ± 2.1 f
5	49.6 ± 0.8 a	48.8 ± 1.0 b	48.3 ± 0.3 c	47.5 ± 0.2 d	47.8 ± 0.8 e	27.4 ± 0.7 f

Values are means of 3 analyses. Means in the same column followed with the same letter are equals (P>0.05).

Table 4: a* values of CPS during 5 days of storage at +2°C.

Time (day)	Cod Protein solutions containing different levels of salt (%)					
	1.2	3	5	10	15	20
2	-2.3 ± 0.0 a	-2.1± 0.1 b	-2.2 ± 0.0	-2.1 ± 0.4	-2.1± 0.0	-0.4 ± 0.2 c
5	-2.3 ± 0.1 a	-2.3 ± 0.0 b	-2.3 ± 0.0	-2.3 ± 0.0	-2.2 ± 0.0	-1.4 ± 0.2 c

Values are mean of 3 analyses. Means in the same column followed with the same letter are equals (P>0.05).

Table 5: b* values of CPS during 5 days of storage at +2°C

Time (day)	Cod Protein solutions containing different levels of salt (%)					
	1.2	3	5	10	15	20
2	-6.4 ± 0.1 a	-6.2 ± 0.8 b	-6.5 ± 0.4 c	-6.4 ± 0.0 d	-6.9 ± 0.0 f	0.7 ± 0.4 g
5	-6.4 ± 0.3 a	-6.0 ± 0.2 b	-6.4 ± 0.1 c	-6.7 ± 0.0 e	-7.0 ± 0.2 f	-1.4 ± 0.2 h

Values are means of 3 analyses. Means in the same column followed with the same letter are equals (P>0.05).

3.3.2 Frozen CPS

Whiteness increased significantly (P<0.05) in samples containing 3 and 5% salt and free of cryoprotectants after 14 weeks of storage at -24°C. No significant difference was seen for whiteness in cryoprotectant added samples and the group free of cryoprotectants during the 14 weeks of storage (P>0.05). However, whiteness of samples free of cryoprotectants, except samples containing 1.2 and 15% salt, changed significantly (P<0.05) during frozen storage (Table 6). Whiteness of samples containing cryoprotectants did not change significantly (P>0.05) during 14 weeks of storage at -24 °C (Table 7).

Table 6: Whiteness values of frozen CPS trails during 14 weeks of storage at -24 °C

Age (W)	Cod Protein solutions containing different levels of salt (%)			
	1.2	3	5	15
2	51.9 ± 2.5 a	52.6 ± 0.3	53.6 ± 1.2	54.0 ± 1.3 b
8	37.9 ± 0.9	59.3 ± 0.4	60.9 ± 0.9	61.1 ± 2.3 b
14	52.8 ± 3.5 a	63.9 ± 2.3	64.8 ± 0.8	52.6 ± 4.6 b

Values are means of 3 analyses. Means in the same column followed with the same letter are equals (P>0.05).

Table 7: Whiteness values of frozen CPS trails with cryoprotectants during 14 weeks of storage at -24 °C

Age (W)	Cod Protein solutions containing different levels of salt with cryoprotectants*			
	1.2	3	5	15
2	52.9 ± 0.2 a	54.5 ± 1.6 b	58.1 ± 1.0 c	58.7 ± 1.4 d
8	61.7 ± 2.6 a	66.8 ± 3.6 b	67.9 ± 1.9	66.2 ± 1.9 d
14	56.2 ± 3.3 a	56.2 ± 1.3 b	59.5 ± 1.1 c	62.3 ± 2.8 d

*: Sugar 1%, sorbitol 1% and sodium tripolyphosphate 0.1%.

Values are mean of 3 analyses. Means in the same column followed with the same letter are equals (P>0.05).

Lightness (L*), and redness (a*) and yellowness (b*) attributes in all samples are given in table 8-10. Samples with 5 and 1.2% salt and free of cryoprotectants had the highest and the lowest values of lightness (L*) respectively after 14 weeks of storage at -24°C.

Table 8: L* values of CPS trails during 2 weeks of storage at -24°C

Week	Cod Protein solutions containing different levels of salt (%)				Cod Protein solutions containing different levels of salt (%) with cryoprotectants*			
	1.2	3	5	15	1.2	3	5	15
2	41.3 ± 0.3	42.2 ± 0.7 a	42.7 ± 0.5	44.1 ± 1.0	43.2 ± 1.8 b	44.8 ± 1.3 c	49.0 ± 0.8	47.9 ± 0.2 d
8	27.4 ± 2.0	41.9 ± 0.5 a	58.8 ± 1.3	71.9 ± 3.0	42.3 ± 2.5 b	44.9 ± 3.2 c	46.1 ± 2.0	46.2 ± 1.4 d
14	34.4 ± 1.0	41.7 ± 2.0 a	44.8 ± 0.4	36.4 ± 2.7	36.6 ± 1.2 b	40.2 ± 0.9 c	39.6 ± 0.8	41.6 ± 2.0 d

*: Sugar 1%, sorbitol 1% and sodium tripolyphosphate 0.1.

Values are means of 3 analyses. Means in the same column followed with the same letter are equals (P>0.05).

Table 9: a* values of CPS trails during 2 weeks of storage at -24°C

Week	Cod Protein solutions containing different levels of salt (%)				Cod Protein solutions containing different levels of salt (%) with cryoprotectants			
	1.2	3	5	15	1.2	3	5	15
2	-2.1 ± 0.4	-2.0 ± 0.2 a	-1.8 ± 0.2 b	-1.9 ± 0.2 c	-2.0 ± 0.5 d	-2.1 ± 0.4 e	-2.7 ± 0.4	-2.3 ± 0.1 f
8	-0.9 ± 0.3	-1.1 ± 0.0 a	-1.3 ± 0.1 b	-0.9 ± 0.1 c	-1.4 ± 0.4 d	-1.3 ± 0.4 e	-1.6 ± 0.4	-1.8 ± 0.2 f
14	-1.0 ± 0.3	-1.3 ± 0.3 a	-1.7 ± 0.2 b	-0.9 ± 0.3 c	-0.9 ± 0.1 d	-1.2 ± 0.2 e	1.0 ± 0.1	-1.2 ± 0.3 f

*: Sugar 1%, sorbitol 1% and sodium tripolyphosphate 0.1%.

Values are means of 3 analyses. Means in the same column followed with the same letter are equals (P>0.05).

Table 10: b* values of CPS trails during 2 weeks of storage at -24°C

Week	Cod Protein solutions containing different levels of salt (%)				Cod Protein solutions containing different levels of salt (%) with cryoprotectants			
	1.2	3	5	15	1.2	3	5	15
2	-3.5 ± 0.7	-3.5 ± 0.2 a	-3.6 ± 0.3 b	-3.3 ± 0.5 c	-3.2 ± 0.6	-3.2 ± 0.2 d	-3.0 ± 0.5 e	-3.6 ± 0.5 f
8	-3.5 ± 0.4	-5.8 ± 0.2 a	-0.8 ± 0.4 b	-3.6 ± 0.4 c	-6.4 ± 0.7	-7.3 ± 0.4 d	-7.3 ± 0.8 e	-6.7 ± 0.3 f
14	-6.1 ± 0.8	-7.4 ± 0.1 a	-6.7 ± 0.3 b	-5.4 ± 0.6 c	-6.5 ± 0.7	-5.3 ± 0.1 d	-6.6 ± 0.1 e	-6.9 ± 0.4 f

*: Sugar 1%, sorbitol 1% and sodium tripolyphosphate 0.1%.

Values are means of 3 analyses. Means in the same column followed with the same letter are equals (P>0.05).

Storage time appeared to make some of the samples whiter. Whiteness increased significantly (P<0.05) in samples containing 3 and 5% salt and free of cryoprotectants after 14 weeks of storage at -24°C. This is probably due to decreasing of b* value in all samples probably because of more coagulation and thus more opacity.

The colour and whiteness of FPI depend on connective tissue, lipids, co-precipitation of heme proteins and denaturation and oxidization of haemoglobin (Kristinsson *et al.*, 2005). In fish protein solutions it seems protein and water content and also frozen storage have important roles in colour and whiteness of products.

3.4 Weight and WHC loss in cod protein solutions

3.4.1 Fresh CPS

Weight loss in samples containing 1.2, 3, 5, 10, 15 and 20% salt was the same value (P>0.05) after 2 days of storage at +2°C. It did not change after 5 days of storage and all samples had approximately the same value (P>0.05). Weight loss (%) attributes of CPS within 5 days of storage at +2°C are given in table 11. From the results it can be concluded that weight loss of fresh CPS samples was not affected by salt levels and storage time.

Table 11: Weight loss values (%) of fresh CPS trails during 5 days of storage at +2°C

Age (days)	Cod Protein solutions containing different levels of salt (%)					
	1.2	3	5	10	15	20
2	95.2 ± 0.8 a	95.2 ± 1.5 b	95.9 ± 0.5 c	95.7 ± 0.4 d	92.5 ± 1.3 e	97.4 ± 1.7 f
5	96.8 ± 0.6 a	96.5 ± 0.1 b	97.3 ± 0.2 c	96.6 ± 0.6 d	93.0 1.4 e	97.6 ± 0.7 f

Values are means of 4 analyses. Means in the same column followed with the same letter are equals (P>0.05).

3.4.2 Frozen CPS

Samples containing cryoprotectant and samples free from cryoprotectant had different WHC and WL ($P < 0.05$). Among samples free of cryoprotectant, samples with 5% salt had the highest value of WHC ($P < 0.05$) after 14 weeks of storage at -24°C , followed by samples with 3% salt ($P < 0.05$). The lowest WHC was in samples with 1.2% salt followed by sample with 15% salt after 14 weeks of storage ($P < 0.05$). The highest WL was for samples with 1.2% salt ($P < 0.05$), followed by samples with 15% salt ($P < 0.05$) after 14 weeks of storage at -24°C . WHC and WL of samples containing 1.2 and 5% salt and free of cryoprotectant did not change significantly ($P > 0.05$) during 14 weeks of frozen storage (Tables 12 and 13).

Table 12: WHC values (%) of frozen CPS trails during 14 weeks of storage at -24°C

Age (W)	Cod Protein solutions containing different levels of salt (%)			
	1.2	3	5	15
2	16.2 ± 1.4 a	29.8 ± 2.7	28.2 ± 1.8 b	23.8 ± 1.1
8	14.5 ± 1.5 a	27.9 ± 1.1	24.0 ± 0.5 b	15.1 ± 1.9
14	11.9 ± 1.6 a	17.5 ± 0.4	22.8 ± 0.3 b	15.5 ± 0.2

Values are mean s of 4 analyses. Means in the same column followed with the same letter are equals ($P > 0.05$).

Table 13: WL values (%) of frozen CPS trails during 14 weeks of storage at -24°C

Age (W)	Cod Protein solutions containing different levels of salt (%)			
	1.2	3	5	15
2	80.3 ± 1.4 a	67.3 ± 2.6	68.7 ± 1.7 b	73.0 ± 1.0
8	81.9 ± 1.5 a	69.1 ± 1.1	72.8 ± 0.5 b	76.0 ± 1.8
14	84.4 ± 1.6 a	78.1 ± 0.4	73.9 ± 0.3 b	80.9 ± 0.2

Values are means of 4 analyses. Means in the same column followed with the same letter are equals ($P > 0.05$).

Among samples with cryoprotectant, the sample with 5% salt had the highest WHC after 14 weeks of storage at -24°C , followed by sample with 3% salt ($P < 0.05$). The lowest WHC was for sample with 1.2% salt followed by sample with 15% salt ($P < 0.05$) at the same period of time. The highest WL was for sample with 1.2% salt followed by the sample containing 15% salt ($P < 0.05$) after 14 weeks of storage at -24°C . Tables 14 and 15 are given WHC and WL (%) values cryoprotectant added samples within 14 weeks of storage at -24°C . WHC and WL of samples containing 3 and 5% salt and cryoprotectants did not change significantly ($P > 0.05$) during 14 weeks of frozen storage.

Table 14: WHC values (%) of frozen CPS trails containing cryoprotectan during 14 weeks of storage at -24°C

Age (W)	Cod Protein solutions containing different levels of salt (%) with cryoprotectant*			
	1.2	3	5	15
2	61.9 ± 1.8	62.8 ± 1.8 a	77.1 ± 0.5 b	56.6 ± 0.8
8	18.7 ± 2.3	55.3 ± 2.3 a	73.5 ± 1.5 b	41.8 ± 0.8
14	16.1 ± 1.3	51.3 ± 3.5 a	69.6 ± 2.5 b	21.4 ± 1.5

*: Sugar 1%, sorbitol 1% and sodium tripolyphosphate 0.1%.

Values are mean s of 4 analyses. Means in the same column followed with the same letter are equals ($P > 0.05$).

Table 15: WL values (%) of frozen CPS trails containing cryoprotectant during 14 weeks of storage at -24 °C

Age (W)	Cod Protein solutions containing different levels of salt (%) with cryoprotectant*			
	1.2	3	5	15
2	36.5 ± 1.7	35.7 ± 1.8 a	21.9 ± 0.4 b	41.6 ± 0.8
8	77.9 ± 2.2	42.8 ± 2.2 a	25.3 ± 1.4 b	55.7 ± 0.7
14	80.4 ± 1.2	46.7 ± 3.4 a	29.2 ± 2.4 b	75.3 ± 1.4

*: Sugar 1%, sorbitol 1% and sodium tripolyphosphate 0.1%.

Values are means of 4 analyses. Means in the same column followed with the same letter are equals (P>0.05).

Samples containing 3 and 5% salt with cryoprotectant had higher WHC and lower WL than samples free of cryoprotectant (P>0.05). pH and protein content can affect WHC. Kristinsson and Hultin (2003) reported that increasing pH from 6.4 to 7.4 increased WHC in fish protein isolate. So adding cryoprotectant to CPS and increasing pH could possibly increase WHC in cryoprotectant added samples.

The effect of salt on WHC, and thereby yield, has been reported by many authors (Hamm 1960, Warriar *et al.*, 1975, Offer and Knight 1988, Fennema 1990). It is known that WHC of fish muscle increases with increasing salt concentration up to 6%. When the salt concentration reaches levels above 10%, denaturation of proteins leads to decreased WHC of muscle. This may explain why protein solution with 15% salt had the least value of WHC among cryoprotectant added samples. In this study increasing amount of salt to CPS increased WHC, but the highest WHC was seen in samples containing cryoprotectants, probably because of combination of salt and sodium tripolyphosphate and increasing water retention in fish protein.

3.5 Viscosity

3.5.1 Brabender Units

3.5.1.1 Fresh CPS

After 2 days of storage, samples containing 15% salt had the highest Brabender units at +5°C followed by samples containing 1.2 % salt (P<0.05). Viscosity of sample with 20% salt was 0 (BU) possibly because of protein denaturation. Samples containing 3 and 5% salt had the lowest viscosity (BU) at +5°C during 5 days storage at +2°C (P<0.05). Brabender viscosity of samples containing 10 and 15% salt at +5°C after 5 days of storage decreased significantly (P<0.05). Brabender attributes of CPS within 5 days of storage at +2°C is presented in table 16.

Table 16: Viscosity (BU) values of fresh CPS trails during 5 days of storage at +2°C

Age (days)	Cod Protein solutions containing different levels of salt (%)					
	1.2	3	5	10	15	20
2	43.5 ± 2.1 a	17.5 ± 0.7 b	17.0 ± 0.0 c	41.5 ± 0.7	61.0 ± 4.2	0.0 ± 0.0 d
5	37.0 ± 2.8 a	16.5 ± 0.7 b	16.0 ± 1.4 c	22.5 ± 3.5	31.0 ± 0.0	0.0 ± 0.0 d

Values are means of 2 analyses. Means in the same column followed with the same letter are equals (P>0.05).

Results demonstrated that storage time did not change viscosity of samples containing 1.2, 3 and 5% salt ($P>0.05$). The highest viscosity after 5 days of storage at $+2^{\circ}\text{C}$ was for sample with 1.2% salt and the lowest for samples with 3, 5 and 20% salt ($P<0.05$).

3.5.1.2 Frozen CPS

Samples containing cryoprotectant and samples free from cryoprotectants had different Brabender units (Table 17 and 18). Samples containing 3 and 5% salt and cryoprotectant had higher Brabender viscosity than the samples with the same level of salt and free from cryoprotectant ($P<0.05$). Among samples free of cryoprotectant, samples with 15% salt had the highest viscosity after 14 weeks of storage at -24°C , followed by sample with 1.2% salt ($P<0.05$). The lowest viscosity was for sample with 5% salt followed by samples with 3% salt at the same period of time ($P<0.05$). Storage time had not significant effect on viscosity (BU) in all samples free of cryoprotectant ($P>0.05$).

For samples containing cryoprotectants samples with 15% salt had the highest viscosity after 14 weeks of storage at -24°C , followed by samples with 5% salt ($P<0.05$). The lowest viscosity was for samples with 1.2% salt followed by sample with 3% salt at the same period of time ($P<0.05$). Storage time increased viscosity (BU) in samples containing 3, 5 and 15% salt ($P<0.05$). It did not affect on viscosity of samples with 1.2% salt ($P>0.05$). In samples with or without cryoprotectants, increasing salt levels increased viscosity ($P<0.05$). In both groups, samples with 15% salt had the highest value of viscosity (BU) after 14 weeks of storage ($P<0.05$).

Table 17: Viscosity (BU) values of frozen CPS trails during 14 weeks of storage at -24°C

Age (W)	Cod Protein solutions containing different levels of salt (%)			
	1.2	3	5	15
2	13.5 ± 3.5 a	20.5 ± 2.1 b	28.0 ± 2.8 c	58.0 ± 1.4 d
8	23.5 ± 3.5 a	20.5 ± 0.7 b	20.5 ± 0.7 c	59.0 ± 2.8 d
14	31.5 ± 3.5 a	19.5 ± 3.5 b	17.0 ± 2.8 c	59.5 ± 3.5 d

Values are mean s of 2 analyses. Means in the same column followed with the same letter are equals ($P>0.05$).

Table 18: Viscosity (BU) values of frozen CPS trails containing cryoprotectant within 14 weeks of storage at -24°C

Age (W)	Cod Protein solutions containing different levels of salt (%) with cryoprotectants*			
	1.2	3	5	15
2	14.5 ± 0.7 a	26.0 ± 1.4	32.3 ± 4.9	59.5 ± 0.7
8	25.0 ± 2.8 a	52.5 ± 3.5	45.5 ± 2.1	70.5 ± 2.1
14	22.5 ± 3.5 a	36.5 ± 3.5	50.5 ± 3.5	59.5 ± 0.7

*: Sugar 1%, sorbitol 1% and sodium tripolyphosphate 0.1%.

Values are means of 2 analyses. Means in the same column followed with the same letter are equals ($P>0.05$).

3.5.2 Bohlin viscosity

3.5.2.1 Fresh CPS

After 2 days of storage at +2°C (Table 19) sample with 15% salt had the highest viscosity (Pa) among all samples followed by sample with 10% salt (P<0.05). Viscosity (Pa) of all samples except samples containing 20% salt after 5 days of storage decreased significantly (P<0.05).

Table 19: Viscosity (Pa) values of fresh CPS trails during 5 days of storage at +2°C

Age (days)	Cod Protein solutions containing different levels of salt (%)					
	1.2	3	5	10	15	20
2	0.15±0.0 a	0.148±0.0 b	0.146±0.0 c	0.152±0.0 d	0.228±0.0 e	0.079±0.0
5	0.143±0.0 a	0.119±0.0 b	0.117±0.0 c	0.123±0.0 d	0.129±0.0 e	0.026±0.0

Values are means of 3 analyses. Means in the same column followed with the same letter are equals (P>0.05).

3.5.2.2 Frozen CPS

Samples containing cryoprotectant and samples free from cryoprotectants had different values of viscosity in Pascal units (Pa). Samples without cryoprotectants with 15, and 3% salt had the highest viscosity (Tables 20 and 21) respectively after 2 weeks of storage at -24°C (P<0.05). The lowest viscosity was in samples with 5% salt followed by sample containing 1.2% salt after 2 weeks of storage at -24°C (P<0.05). Viscosity of all samples free of cryoprotectants increased after 14 weeks of storage at -24°C significantly (P<0.05). Among samples free from cryoprotectant samples with 15% salt had the highest viscosity after 14 weeks of storage at -24°C, followed by samples with 1.2% salt (P<0.05). The lowest viscosity was in samples with 5% salt followed by sample with 3% salt during the same period of time (P<0.05).

Samples with cryoprotectants and 5, and 15% salt had the highest viscosity respectively after 2 weeks of storage at -24°C (P<0.05). Samples containing 1.2, and 3% salt had the lowest viscosity respectively during the same period of time (P<0.05). Storage time increased viscosity of samples containing 3% salt and decreased viscosity of samples with 1.2, and 5% salt significantly (P<0.05) but did not have a significant effect on sample with 15% salt (P>0.05). Samples with cryoprotectants and 3% salt had the highest value of viscosity after 14 weeks of storage at -24°C, followed by samples with 5% salt (P<0.05). The lowest viscosity was in samples with 1.2% salt followed by samples with 15% salt during the same period of time (P<0.05).

Table 20: Viscosity (Pa) values of frozen CPS trails during 14 weeks of storage at -24 °C

Age (W)	Cod Protein solutions containing different levels of salt (%)			
	1.2	3	5	15
2	0.09±0.0	0.105±0.0	0.087±0.0	0.133±0.0
8	0.126±0.0	0.095±0.0	0.11±0.0	0.164±0.0
14	0.174±0.0	0.127±0.0	0.107±0.0	0.177±0.0

Values are means of 3 analyses. Means in the same column are equals (P>0.05).

Table 21: Viscosity (Pa) values of frozen CPS trails containing cryoprotectant during 14 weeks of storage at -24 °C

Age (W)	Cod Protein solutions containing different levels of salt (%) with cryoprotectant*			
	1.2	3	5	15
2	0.116±0.0	0.127±0.0	0.262±0.0	0.164±0.0
8	0.093±0.0	0.139±0.0	0.141±0.0	0.16±0.0
14	0.094±0.0	0.23±0.0	0.209±0.0	0.165±0.0

*: Sugar 1%, sorbitol 1% and sodium tripolyphosphate 0.1.

Values are means of 3 analyses. Means in the same column are equals ($P>0.05$).

According to Damodaran (1997) partial denaturation and/or heat induced polymerization, increases hydrodynamic size of proteins and thus increases viscosity. It seems other factors like temperature, shear rate, and shape of induced force can affect viscosity especially when viscosity of the same sample is measured by different methods.

3.6 Rheological behaviour of CPS

In this study CPS samples had thixotropic and rheopectic behaviour and time-dependent characteristics. Figures 3-17 demonstrate flow curves of fresh and frozen CPS samples containing different amounts of salt and cryoprotectants.

3.6.1 Fresh CPS

As shown in Figures 3-9 fresh CPS containing 1.2, 3 and 5% salt demonstrated thixotropic flow behaviour within 5 days of storage at +2°C. Viscosity of these samples decreased as time increased at a fixed force. Viscosity reached to 0 when temperature was at 35-40 °C, then it increased again. Viscosity of these samples increased again by decreasing temperature.

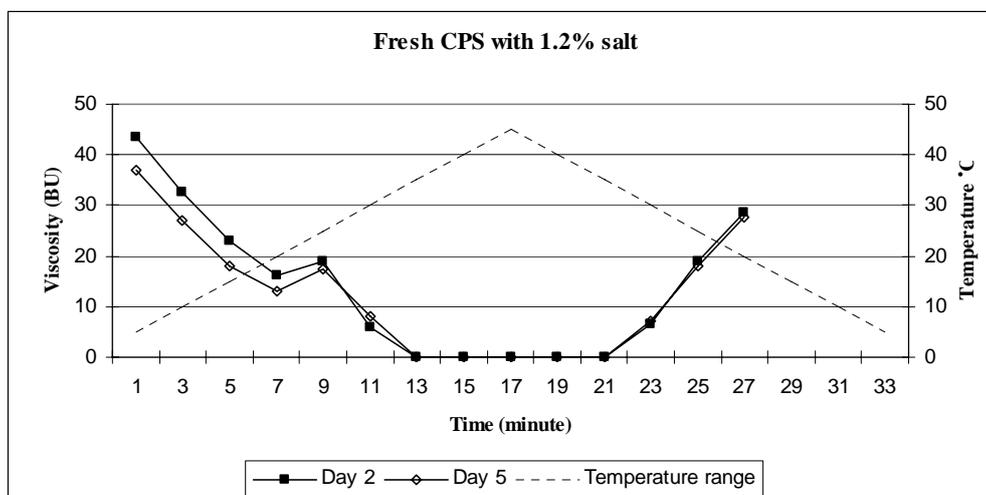


Figure 3: Time-dependent flow behaviour viscosity of CPS containing 1.2% salt during 5 days of storage at +2 °C

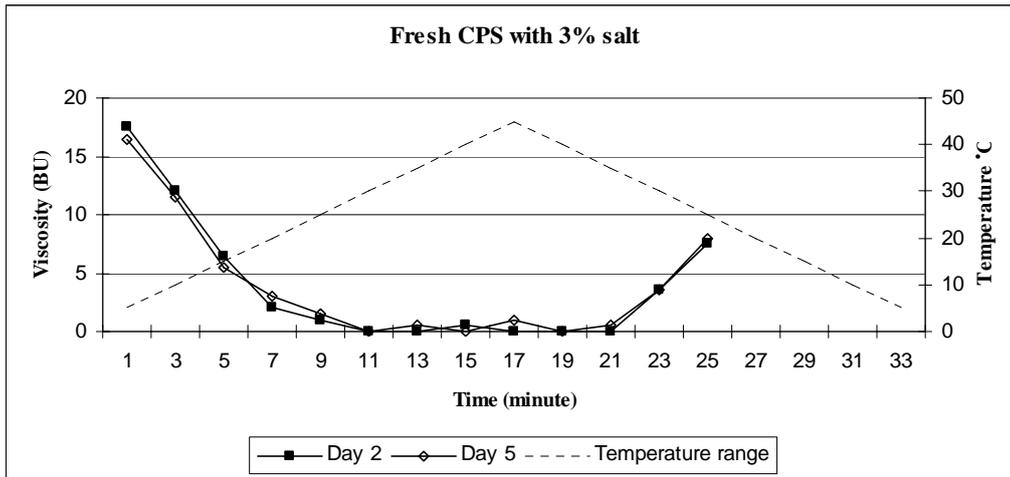


Figure 4: Time-dependent flow behaviour viscosity of CPS containing 3% salt during 5 days of storage at +2 °C

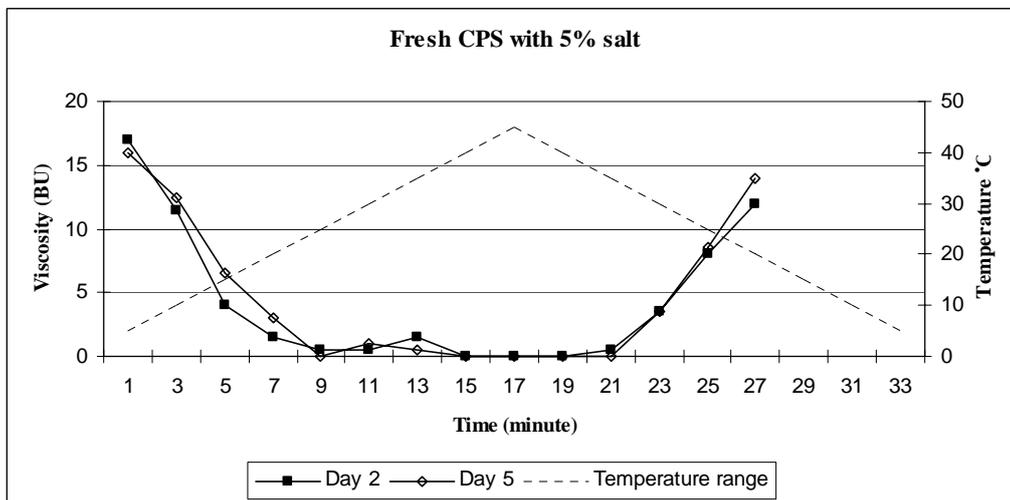


Figure 5: Time-dependent flow behaviour viscosity of CPS containing 5% salt during 5 days of storage at +2 °C

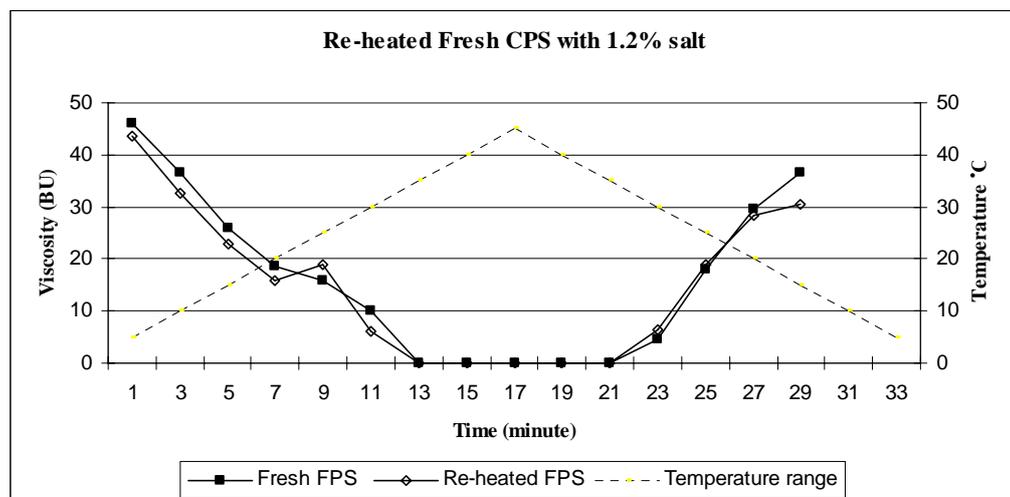


Figure 6: Temperature-dependent flow behaviour viscosity of CPS containing 1.2% salt before and after heating

To study reversible phenomenon, samples containing 1.2% salt were reheated at 80°C for 5 minutes and chilled to +2°C after measuring viscosity by the Brabender machine. Surprisingly, flow behaviour of this reheated sample was approximately the same as for first measurement (Figure 6). This measurement showed that CPS may have thixotropic behaviour and its viscosity can be reversible.

Sample containing 10% salt had rheopectic behaviour and viscosity increased as time increased at a constant stress. After 5 days of storage at +2°C, this sample had the same flow behaviour with a significant decrease ($P>0.05$) in viscosity (Figure 7).

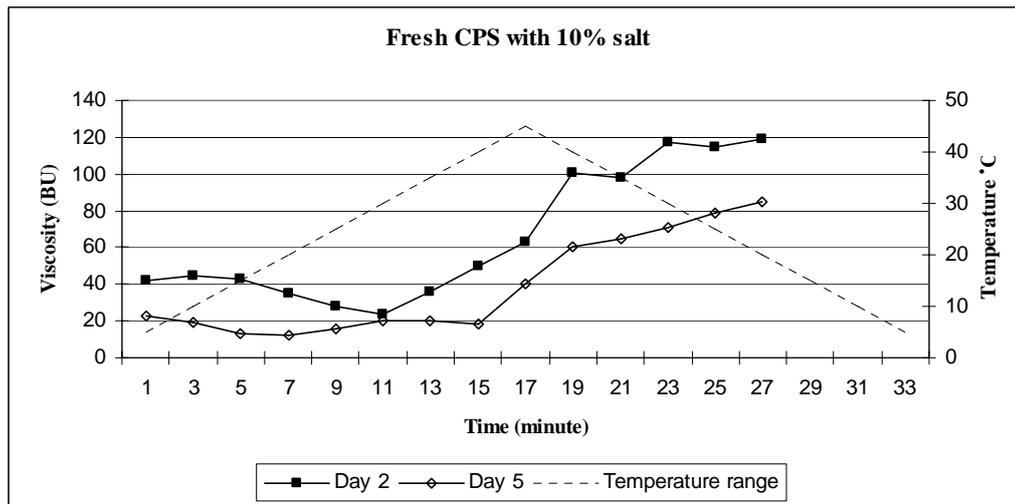


Figure 7: Temperature-dependent flow behaviour of CPS containing 10% salt during 5 days of storage at +2 °C

CPS with 15% salt had a strange flow behavior. It had constant flow in the beginning and in the end of operation time and increasing and decreasing flow at the middle of operation time possibly because of phase separation (Figure 8). When temperature increased viscosity increased and when it decreased viscosity decreased. The viscosity of samples containing 20% was not recorded because of presumed protein denaturation (Figure 9).

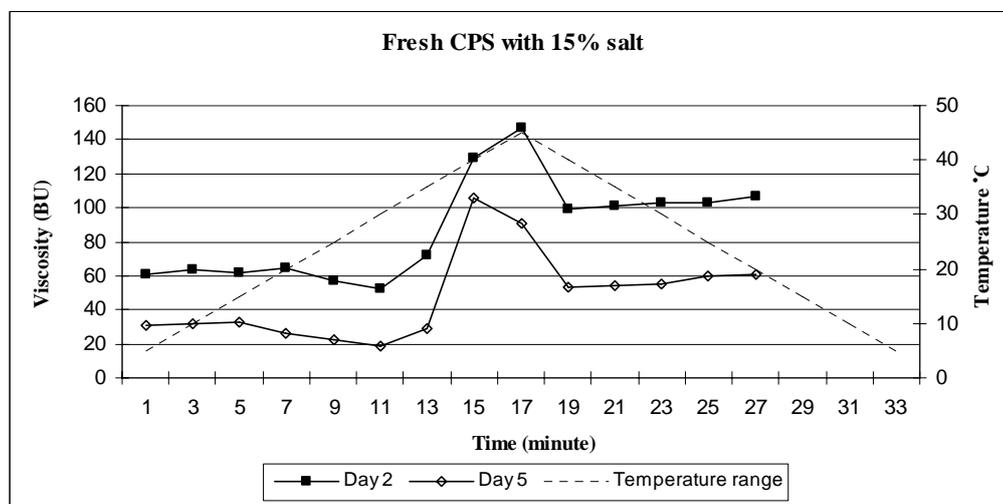


Figure 8: Temperature-dependent flow behaviour of CPS containing 15% salt during 5 days of storage at +2 °C

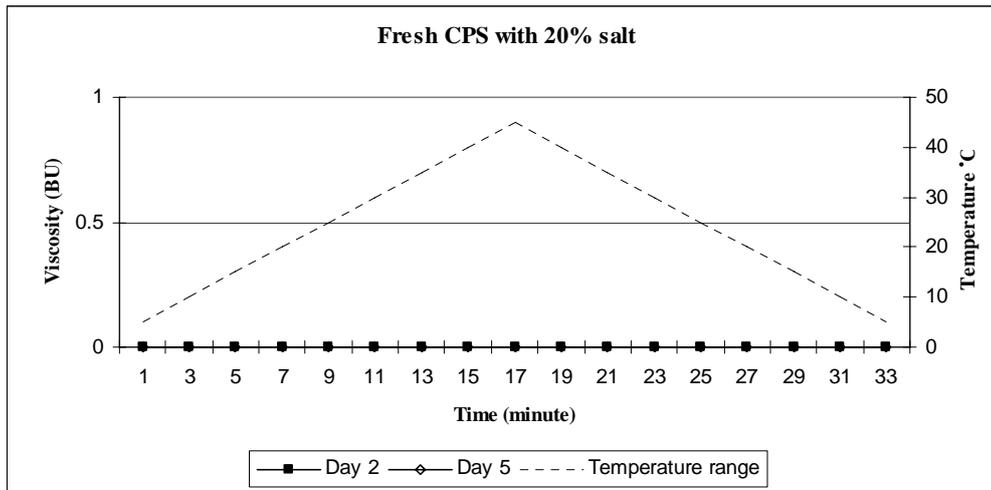


Figure 9: Temperature-dependent flow behaviour of CPS containing 15% salt during 5 days of storage at +2 °C

3.6.2 Frozen CPS

Samples containing salt and samples containing cryoprotectant had different flow behaviours during 14 weeks of storage at -24°C. There were few differences between flow behaviour of each sample containing the same amount of salt or cryoprotectants within 14 weeks of storage.

Samples containing 1.2% salt with or without cryoprotectants had rheopectic behaviour (Figures 10 and 11); but samples containing 3% salt had rheopectic flow. Surprisingly, this sample with cryoprotectants had thixotropic flow behaviour (Figures 12 and 13).

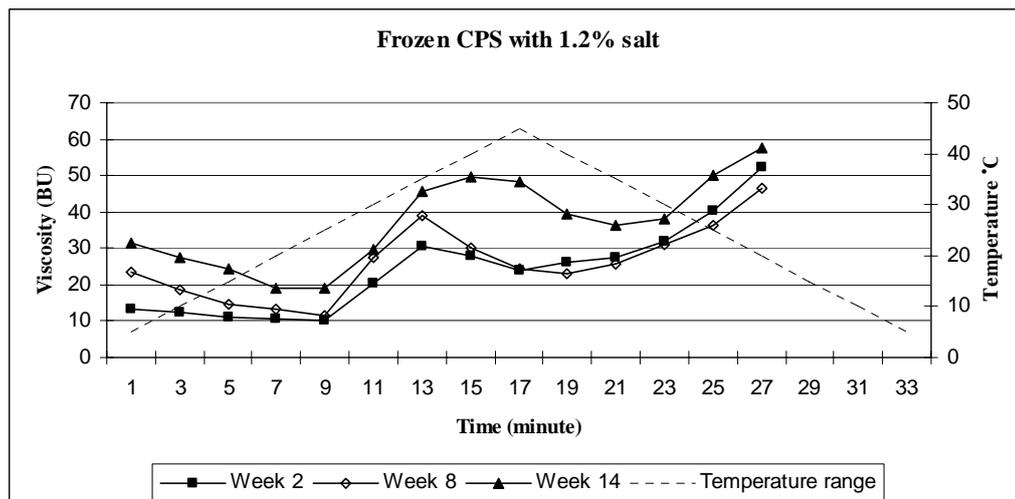


Figure 10: Flow behaviour of CPS containing 1.2% salt during 14 weeks of storage at -24 °C

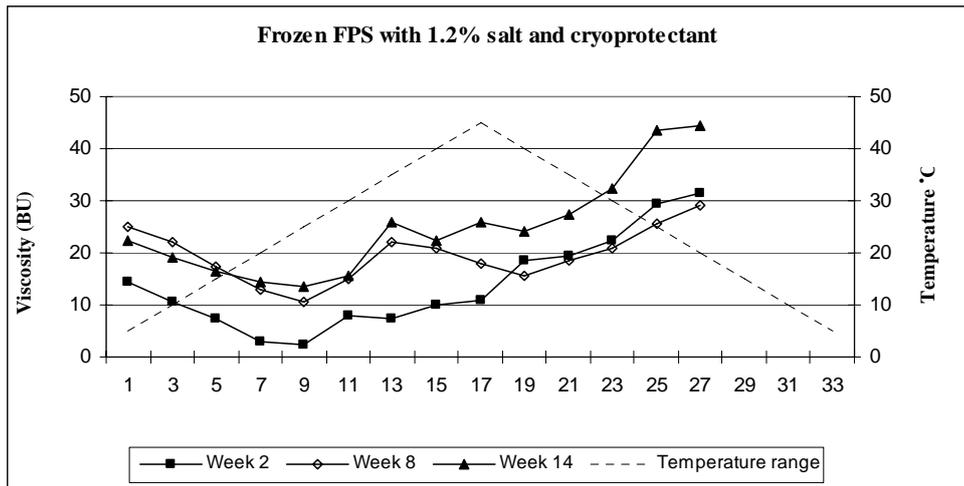


Figure 11: Flow behaviour of CPS containing 1.2% salt and cryoprotectant during 14 weeks of storage at -24 °C

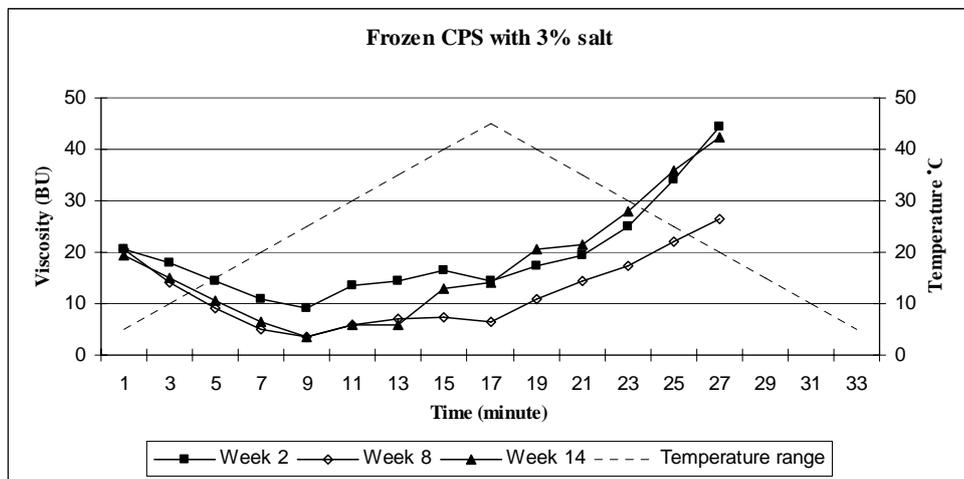


Figure 12: Flow behaviour of CPS containing 3% salt during 14 weeks of storage at -24 °C

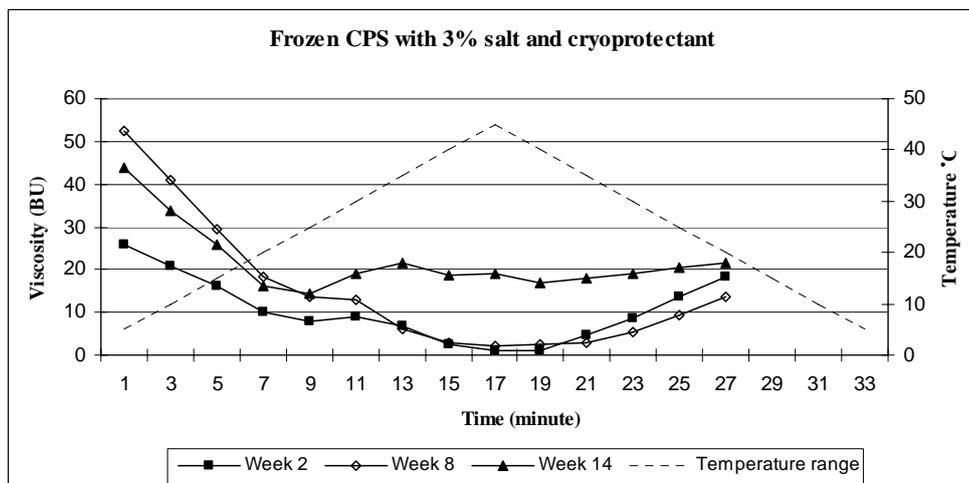


Figure 13: Flow behaviour of CPS containing 3% salt and cryoprotectant during 14 weeks of storage at -24 °C

Samples containing 5% salt with or without cryoprotectant had thixotropic flow behaviour although samples containing cryoprotectants had higher viscosity value than samples free from cryoprotectants (Figures 14 and 15).

Flow behaviour of samples containing 15% salt and free from cryoprotectant was thixotropic, while flow behaviour of cryoprotectant added sample with the same amount of salt was rheopectic (Figures 16 and 17).

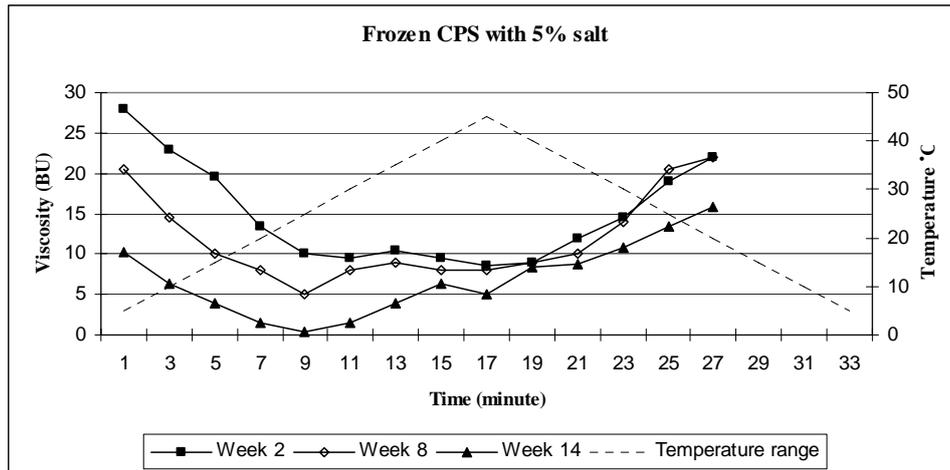


Figure 14: Flow behaviour of CPS containing 5% salt during 14 weeks of storage at -24 °C

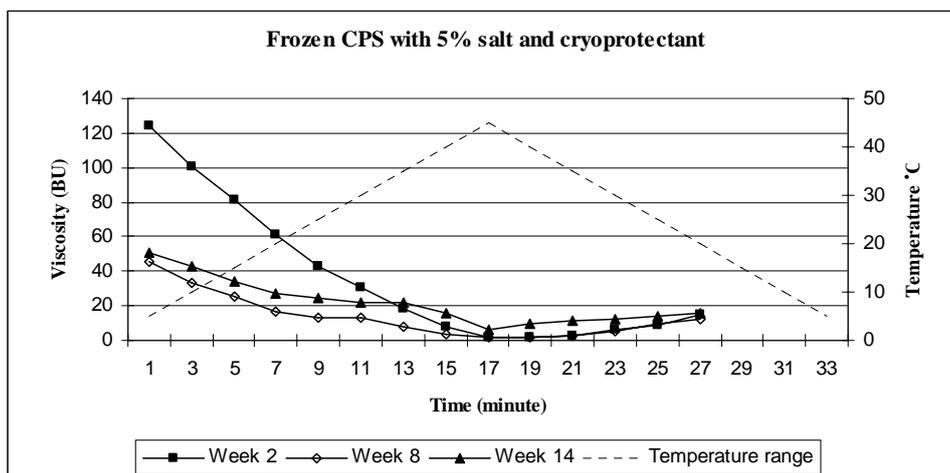


Figure 15: Flow behaviour of CPS containing 5% salt and cryoprotectant during 14 weeks of storage at -24 °C

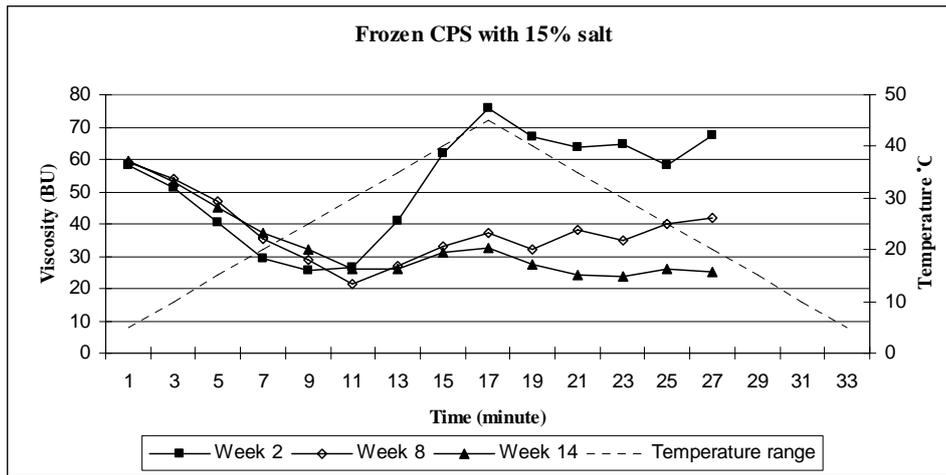


Figure 16: Flow behaviour of CPS containing 15% salt during 14 weeks storage at -24 °C

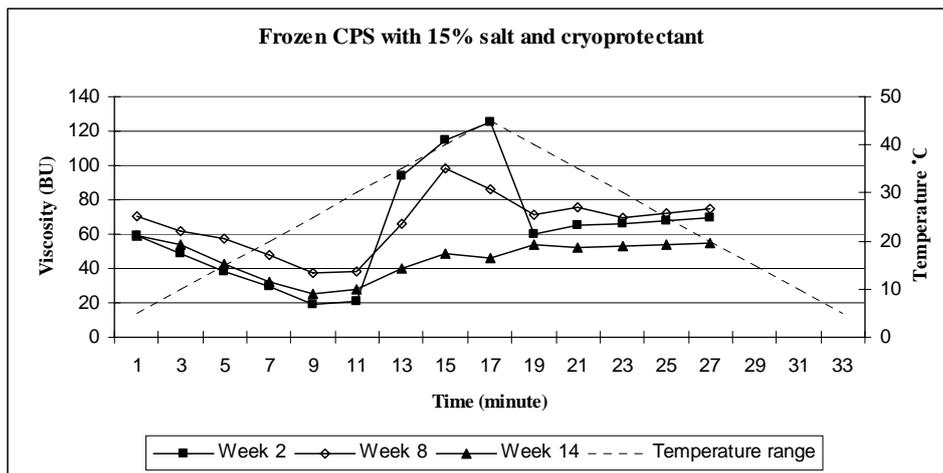


Figure 17: Flow behaviour of CPS containing 15% salt and cryoprotectant during 14 weeks of storage at -24

From the results it can be concluded that freezing changes flow behaviour of CPS unexpectedly. It changed flow behaviour of samples containing 1.2 and 3% to rheopectic which was thixotropic when samples were fresh. Flow behaviour of samples containing 15% salt was rheopectic for fresh CPS but changed to thixotropic after freezing. Although it changed again to rheopectic when cryoprotectants was added possibly because of preserving of proteins against denaturation.

4. CONCLUSIONS

If the CPS is going to be used fresh it should be kept chilled ($\leq 0^{\circ}\text{C}$) until processing and be used within 3 days. The test sample was spoiled after 3 days of storage at $+2^{\circ}\text{C}$. Based on the results of this study to extend shelf-life of fresh CPS containing 1-5 % salt, it is suggested to provide pasteurized CPS for injection operation. A further study in this regard is recommended. Increasing salt to fish protein solutions had the following effects on these products after 2 days of storage at $+2^{\circ}\text{C}$:

- Viscosity (BU) decreased in samples containing 3, 5, and 10% salt ($p < 0.05$); most for samples with 3 and 5%.
- Viscosity (BU) increased in samples containing 15% salt ($p < 0.05$); the viscosity of this sample was more viscous than samples with 1.2% salt.
- Proteins in samples containing 20% salt may have denatured;
- No significant effect was observed for weight loss ($p > 0.05$); except in samples with 20% salt;
- No significant effect was seen in whiteness ($p > 0.05$);
- Rheological behaviour of samples containing 10 and 15 % salt was changed.

Storage time did not change weight loss ($P > 0.05$) and decreased viscosity in fresh samples containing 10 and 15% salt ($P < 0.05$). It seems storage time did not change whiteness of samples significantly ($P > 0.05$). From a rheological point of view fresh samples containing 1.2, 3 and 5% salt were stable within 5 days of storage at $+2^{\circ}\text{C}$. They had thixotropic flow behaviour which was reversible.

Adding cryoprotectants to CPS containing 1.2, 3, 5, and 15% salt had the following significant effects ($p < 0.05$):

- Increased water holding capacity;
- Decreased weight loss;
- Increased viscosity (BU and Pa) in samples containing 3 and 5% salt ($P < 0.05$);
- Did not affect whiteness ($p > 0.05$);
- Changed rheological flow behavior, except for sample with 5% salt.

Storage time also increased weight loss and decreased WHC in frozen samples ($p < 0.05$). In the frozen treatment, samples with 5% salt and containing cryoprotectant were the most stable followed by sample with 3% salt and containing cryoprotectant. They had thixotropic flow behaviour which was reversible after 14 weeks of storage at -24°C . To make stable fish protein solutions during frozen storage it is recommended to add cryoprotectants to the products (preferably containing 3-5% salt) at the end of the processing.

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Chapter 3

Functional Properties and Rheological Behaviour of Haddock (*Melanogrammus aeglefinus*) Protein Isolates under Various Storage Conditions

Abstract

1. Introduction

2. Material and methods

3. Results and discussion

3.1 Chemical and microbial results

3.2 Whiteness

3.3 WHC and WL

3.4 Viscosity

3.5 Rheological behaviour of HPS

4. Conclusions

References

Functional Properties and Rheological Behaviour of Haddock (*Melanogrammus aeglefinus*) Protein Isolates under Various Storage Conditions

Abstract:

*Fish protein isolates (FPI) were extracted from haddock (*Melanogrammus aeglefinus*) cut-offs using the pH-shift method. Fresh and frozen stability and rheological behaviour of haddock protein isolates (HPI) containing 20% protein (pH 6.4) and different amounts of salt and sucrose and also polyphosphate stored at +2, -18 and -24°C were studied. The results indicated that in fresh FPIs added salt and sucrose to the test samples increased WHC, but significantly ($p < 0.05$) decreased viscosity (BU and P) and whiteness. Using polyphosphate and sucrose as a cryoprotectant did not affect WHC, viscosity (Pa) and whiteness of haddock protein isolate ($p > 0.05$) but it decreased Brabender viscosity ($P < 0.05$). Apart from the viscosity fresh samples with different amount of additives had the same flow behaviour (thixotropic). Different amount of additives and also frozen storage time changed attributes of HPI significantly ($p < 0.05$). Like conventional surimi the results suggested that the isolated proteins obtained through the pH-shift also need cryoprotectants to preserve them against denaturation during frozen storage. Thus adding 1.3% salt and 5% sucrose as a cryoprotectant to HPI is recommended.*

Key Words: haddock protein isolates (HPI), functional properties, and rheological behaviour.

1. INTRODUCTION

The most important fish species in Icelandic waters belong to the gadoids namely cod, haddock, pollack, and blue whiting. They are mainly processed as fillet, due to market preferences for fresh fillets 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 S. 2003) and can be used as a good source of raw material for production fish protein products. Thus, a new process was developed to increase the recovery of proteins from different sources (Batista 1999, Hultin and Kelleher, 2000, 2001). This process can be used for any kind of fish and fish by-products, as it removes essentially all the membrane lipids and provides an increased yield of protein. FPI can be frozen like surimi or minced fish for further utilization.

During frozen storage there are changes in fish muscle quality. Rapid texture deterioration occur during frozen storage and these changes are connected to protein changes, especially of the myofibrillar proteins, myosin and actin, which are responsible for the two-step process of gel formation (Madsen 1984).

Lee (1984) reported that structural changes occurring during frozen storage of surimi lead to protein denaturation and subsequent loss of functional properties. Therefore, the inclusion of cryoprotectants is required to ensure long-term frozen stability of conventional

surimi. Sucrose and sorbitol have been reported to protect against freeze denaturation of Alaska pollock surimi (Park *et al.*, 1988). Sucrose is usually combined with sorbitol to reduce sweetness. According to Matsumoto and Noguchi (1992) the cryoprotective effect of sugar is enhanced by adding polyphosphates, perhaps by buffering effect of polyphosphates on muscle pH and/or the chelation of metal ions. 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.

According to Park and Lin (2005) the addition of cryoprotectants is important to ensure maximum functionality of frozen surimi because freezing induces protein denaturation and aggregation. Sucrose and sorbitol, alone or mixed at approximately 9% w/w to dewatered fish meat, serve as the primary cryoprotectants in the manufacturing of surimi. In addition sodium tripolyphosphate and tetrasodium pyrophosphate at 0.2 to 0.3% are commonly used as both a chelating agent, which makes metal ions in surimi inactive and as a pH adjusting agent.

Few documents have been published regarding to frozen stability of FPI. Thawornchinsombut and Park (2006) studied frozen stability of FPI under various conditions. They recommended using cryoprotectant to FPI to prevent freeze-induced aggregation during frozen storage. However, the kind and amount of additives are unknown. In this work frozen stability of haddock protein isolate containing various additives have been investigated under different storage temperatures.

1.1 Objectives:

The overall objective was to investigate whether HPI, which contain chemically recovered unfolded/refolded proteins, were affected by freezing and frozen storage. Other objectives were:

- To determine the effect of cryoprotectants on the functional properties of HPI,
- To determine the rheological behaviour of test samples using Brabender® viscograph E during frozen storage.

2. MATERIAL AND METHODS

2.1 Haddock protein isolate

Haddock (*Melanogrammus aeglefinus*) proteins were extracted from cut-offs using the pH-shift method. Haddock protein isolate was obtained and transferred from MPF Iceland in Grindavik to Matis in Reykjavik in 12 polyethylene (PE) bags (each about 3 Kg.) under chilled conditions ($<+4^{\circ}\text{C}$). HPIs were squeezed to decrease water content from 86% to 80% by using a stainless steel sausage stuffer (Model TSM 15 Lb,USA) [photo 2.5 Annex 2]. HPI was stored at $+2^{\circ}\text{C}$ until making the trials were conducted. All test samples were prepared within six hours after receiving HPIs.

2.2 Preparation of test samples

The type and amount of additives used for frozen surimi have been suggested by many authors and seafood research companies (Yataka and Harohiko, 1992, Min *et al.*,1988). Based on this information and a work plan (Annex 1), 36 samples of fish protein isolates were prepared as follows;

- 9 samples free of any additive,
- 9 samples containing 0.8% salt and 3% sucrose,
- 9 samples containing 1.3% salt, and 5% sucrose,
- 9 samples containing 4% sucrose and 0.1% polyphosphate.

HPI and additives were weighed separately and mixed completely for 3 minutes using a silent cutter machine. The bowl and blades of the machine were chilled by leaving crushed ice on them for a few minutes to prevent of increasing temperature during operation. Samples were packed in polyethylene bags immediately after mixing. Each bag contained a 1 kg sample, was sealed by thermal sealing machine. Fresh and frozen samples were stored at three temperatures $+2^{\circ}\text{C}$, -18°C , and -24°C respectively. Fresh samples were evaluated after 2 days of storage and frozen samples were assessed after 2, 8 and 12 weeks of storage.

2.3 Methods

2.3.1 Dry matter

Dry matter was calculated as the loss in weight during drying at 105°C for 4 hours (ISO 1983).

2.3.2 pH

pH of HPI was measured using pH meter, Knick Portamess®913 (Electronische Megerate GmbH H&Co. Germany). All samples were measured at room temperature.

2.3.3 Water holding capacity and weight loss

Water-holding capacity (WHC) was determined by centrifugation following the method described by Gunnarsson *et al.*, (2005) with some modification. A plastic cylinder was put in a plastic holder cup, and then 2 grams of HPI were placed in a plastic cylinder which 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 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 had 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. Weight loss was expressed as the amount of water removed from sample after centrifugation per gram of sample weight of the product.

2.3.4 Viscosity (Bohlin BV88)

The viscosity of CPS was analysed using a Bohlin BV88 viscometr (Bohlin Instruments, England). Each chilled sample was mixed by an immersion hand blender to make a homogenized solution. A beaker containing 200 mm of sample was put inside a 500 mm beaker containing crashed ice to control temperature (Photo 2.4 (L), Annex 2). The instrument cylinder was immersed into the solution. The viscosity of sample was recorded after 20 seconds of operating instrument at 5-7°C, speed setting 6, system switch 6. Measurements were done in triplicate.

2.3.5 Viscosity (Brabender® viscograph E)

The Brabender viscosity of HPI samples was determined using a Brabender® Viscograph E coaxial viscometer (Brabender® OHG, Duisburg, Germany) based on the method by Kristbergsson and Sigfusson (2002) but with some modification. Measurements were done in duplicate. The Viscograph E is a rotational viscometer designed to measure “viscosity” as a time and temperature dependant 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 (approximately 450 g) 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 Sigfusson 2002). 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 revolutions per minute. Sample quantity was 450 g (ratio of HPI 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 during cooling to 15°C.

2.3.6 Colour measurements

Colour of fish protein solutions was measured at room temperature using a Chroma Meter CR-400 (Minolta Co. LTD. Japan). Colour analysis were done by putting the devise on the PE bags containing fish protein solutions at least in triplicates. Colour of solution was measured as the average of three reading for each sample. Colour was measured as lightness (L^*), redness (a^*) and yellowness (b^*). Whiteness was calculated by the equation: L^*-3b^* such as referred by Codex Alimentarius (WHO/FAO 2005).

2.3.7 Statistical analysis

Statistical analysis was performed by Microsoft Excel 2007 (Microsoft Inc, Redmond USA) and Minitab® 15, Statistical Software. Data were subjected to analysis of variance (ANOVA). Tukeys comparison test was used to determine significance between samples ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1 Dry Matter and pH

Dry matter of received HPI was 14.06 ± 0.61 . After dewatering it increased to 19.98 ± 0.17 . The pH of HPI free of additives was 6.4 ± 0.1 . The pH of salt-added samples and cryoprotectant added trails were 6.3 ± 0.1 and 6.5 ± 0.1 respectively.

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

3.2.1 Fresh HPI

As shown in table 1, the highest value for whiteness after 2 days of storage at $+2^\circ\text{C}$ was for sample containing sucrose and polyphosphate which is significantly different from samples with salt and sucrose ($P < 0.05$). No significant difference ($P > 0.05$) was seen for whiteness of sample containing 0.8% salt and 3% sucrose and sample containing 1.3% salt and 5% sucrose after 2 days of storage at $+2^\circ\text{C}$. Lightness (L^*), redness (a^*) and yellowness (b^*) of all fresh samples are given in table 3. Adding salt, sucrose and phosphate to HPI did not affect lightness, redness and yellowness of sample groups significantly ($P > 0.05$). The influence of cryoprotectants, storage time and temperature on colour attributes in haddock protein isolates is presented in table 3.

Table 1: Whiteness values of HPI trails after 2 days of storage $+2^\circ\text{C}$ and during 12 weeks of storage at -18°C .

Storage temperature ($^\circ\text{C}$)	Age	Haddock protein isolate containing following ingredients:			
		Free from any additives	0.8% salt and 3% sucrose	1.3% salt and 5% sucrose	4% sucrose and 0.1% sodium polyphosphate
+2	Day 2	54.2 ± 0.8 a	50.4 ± 1.1 a	47.2 ± 0.5 a	55.2 ± 1.6 a
-18	Week 2	50.7 ± 0.9 a	46.4 ± 1.7 a	49.7 ± 1.5 a	53.9 ± 1.4 a
-18	Week 4	49.9 ± 1.1 a	52.4 ± 0.4 a	50.4 ± 1.2 a	53.6 ± 1.7 a
-18	Week 8	47.4 ± 2.2 a	49.3 ± 1.6 a	48.4 ± 1.9 a	52.4 ± 1.9 a
-18	Week 12	34.5 ± 0.3 b	37.0 ± 2.1 b	37.4 ± 0.5 b	38.1 ± 1.2 b

Values are means of 3 analyses. Means in the same column followed with the same letter are equals ($P > 0.05$).

Table 2: Whiteness values of HPI trails after 2 days of storage +2°C and during 12 weeks of storage at -24°C.

Storage temperature (°C)	Age	Haddock protein isolate containing following ingredients:			
		Free from any additives	0.8% salt and 3% sucrose	1.3% salt and 5% sucrose	4% sucrose and 0.1% sodium polyphosphate
+2	Day 2	54.2 ± 0.8 a	50.4 ± 1.1 a	47.2 ± 0.5 a	55.2 ± 1.6 a
-24	Week 2	48.8 ± 1.6 a	49.3 ± 1.1 a	50.2 ± 1.3 a	52.0 ± 0.6 a
-24	Week 4	50.6 ± 1.6 a	51.5 ± 1.3 a	52.0 ± 1.9 a	54.6 ± 0.9 a
-24	Week 8	47.0 ± 1.3 a	52.9 ± 0.8 a	49.8 ± 1.2 a	52.2 ± 1.9 a
-24	Week 12	34.8 ± 1.1 b	38.5 ± 1.7 b	37.7 ± 1.0 b	40.1 ± 0.7 b

Values are means of 3 analyses. Means in the same column followed with the same letter are equals (P>0.05).

Table 3: Colour measurements (L*, a*, b*) of HPI trails after 2 days of storage +2°C and during 12 weeks of storage at -18° and -24°C.

Attribute	Storage temperature (°C)	Age	Haddock protein isolate containing following ingredients:			
			Free from any additives	0.8% salt and 3% sucrose	1.3% salt and 5% sucrose	4% sucrose and 0.1% sodium polyphosphate
L*	+2	Day 2	70.7 ± 1.7 a	63.8 ± 1.4 a	62.1 ± 1.5 a	68.6 ± 1.2 a
	-18	Week 12	73.3 ± 2.4 a	65.8 ± 2.2 a	68.8 ± 2.0 a	67.0 ± 2.8 a
	-24	Week 12	69.7 ± 3.9 a	65.7 ± 1.4 a	67.2 ± 1.6 a	66.8 ± 0.4 a
a*	+2	Day 2	-1.9 ± 0.1 b	-2.3 ± 0.2 b	-2.0 ± 0.2 b	-1.9 ± 0.1 b
	-18	Week 12	-5.2 ± 0.1 c	-5.8 ± 0.1 c	-6.0 ± 0.1 c	-6.1 ± 0.3 c
	-24	Week 12	-5.4 ± 0.2 d	-6.1 ± 0.1 d	-6.0 ± 0.1 d	-6.2 ± 0.2 d
b*	+2	Day 2	5.5 ± 0.3 e	4.4 ± 0.1 e	5.0 ± 0.4 e	4.4 ± 0.6 e
	-18	Week 12	12.6 ± 0.8 f	9.6 ± 0.8 f	10.5 ± 0.8 f	9.6 ± 1.1 f
	-24	Week 12	11.6 ± 1.5 g	8.9 ± 0.7 g	9.8 ± 0.8 g	8.9 ± 0.2 g

Values are means of 3 analyses. Means in the same column followed with the same letter are equals (P>0.05).

3.2.2 Frozen HPI

All groups had the same value of whiteness (P>0.05) after 2 weeks of storage at -18 and -24°C. The whiteness of all sample groups stored at -18°C and -24°C during 8 weeks did not change significantly (P>0.05). However, after 12 weeks of storage at -18° and -24°C, whiteness for all groups significantly decreased (P<0.05). Whiteness attributes of haddock protein isolates during 12 weeks of storage are given in tables 1 and 2.

Lightness (L*), and redness (a*) and yellowness (b*) attributes of samples after 12 weeks of frozen storage are presented in table 3. Frozen storage time did not influence lightness significantly (P>0.05). Lightness increased during 12 weeks storage in all treatments except the one with sucrose and polyphosphate (p<0.05). Thus lightness of all sample groups after 12 weeks of storage both at -18 and -24°C was equal (P>0.05).

According to Kristinsson *et al.* (2005) the colour 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 oxidisation of haemoglobin that causes a yellow-brownish colour 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 amount of moisture content and additives. L^* , a^* , and b^* values were similar to the values which were reported by Lanier *et al.* (1991) for surimi indicating that pH-shift process can produce products with high lightness similar to surimi.

Myoglobin and hemoglobin, which are responsible for the yellow and red colour of fish flesh, respectively (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 lowest a^* and the highest b^* values after 12 weeks of frozen storage were for control sample indicating added salt, sucrose, and polyphosphate may decrease oxidation of proteins in samples.

3.3 Water holding capacity and weight loss

3.3.1 Fresh HPI

Water holding capacity in samples without additives and samples containing sucrose and polyphosphate was the same ($P>0.05$) after 2 days of storage at $+2^\circ\text{C}$ (Table 4). Sample with 1.3% salt and 5% sucrose had the highest WHC followed by sample containing 0.8% salt and 3% sucrose ($P<0.05$).

Weight loss was also the same in sample without additives and samples containing sucrose and polyphosphate ($P>0.05$). Samples with 0.8% salt and 3% sucrose had more weight loss than samples containing 1.3% salt and 5% sucrose ($P<0.05$).

From the results it can be concluded that using salt and sucrose together will increase water holding capacity and decrease weight loss in haddock protein isolate significantly ($p<0.05$). Added sucrose together with polyphosphate did not have any significant effects on water holding capacity and weight loss ($P>0.05$).

3.3.2 Frozen HPI

Samples containing salt and sucrose had the highest water holding capacity and lowest weight loss during 12 weeks storage at -18 and -24°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 and -24°C ($P>0.05$). WHC values of frozen haddock protein isolate during 12 weeks storage at -18°C and -24°C are given in tables 4 and 5. Samples containing salt and sucrose were stable during frozen storage. The WHC of these samples did not change significantly ($P>0.05$) during 12 weeks storage at -18°C and -24°C .

Table 4: WHC values (%) of HPI trails after 2 days of storage +2°C and during 12 weeks of storage at -18°.

Storage temperature (°C)	Age	Haddock protein isolate containing following ingredients:			
		Free from any additives	0.8% salt and 3% sucrose	1.3% salt and 5% sucrose	4% sucrose and 0.1% sodium polyphosphate
+2	Day 2	84.8 ± 0.4 a	92.3 ± 0.8 d	95.1 ± 0.8 f	84.7 ± 0.8 h
-18	Week 2	67.9 ± 1.7 b	77.7 ± 1.5 e	89.9 ± 0.2 g	67.6 ± 1.2 i
-18	Week 4	66.8 ± 2.2 b	83.0 ± 0.9 e	91.1 ± 0.6 g	63.8 ± 2.5 i
-18	Week 8	62.1 ± 2.8 b	78.5 ± 3.0 e	89.7 ± 0.9 g	63.5 ± 2.6 i
-18	Week 12	52.9 ± 2.3 c	76.1 ± 2.3 e	90.2 ± 0.5 g	60.1 ± 2.7 i

Values are means of 4 analyses. Means in the same column followed with the same letter are equals (P>0.05).

Table 5: WHC values (%) of HPI trails after 2 days of storage +2°C and during 12 weeks of storage at -18° and -24°C.

Storage temperature (°C)	Age	Haddock protein isolate containing following ingredients:			
		Free from any additives	0.8% salt and 3% sucrose	1.3% salt and 5% sucrose	4% sucrose and 0.1% sodium polyphosphate
+2	Day 2	84.8 ± 0.4 a	92.3 ± 0.8 d	95.1 ± 0.8 f	84.7 ± 0.8 g
-24	Week 2	70.1 ± 0.7 b	81.8 ± 1.8 e	91.9 ± 1.0 f	70.1 ± 1.1 h
-24	Week 4	64.1 ± 2.8 b	83.9 ± 0.4 e	92.2 ± 0.61 f	70.6 ± 1.9 h
-24	Week 8	66.5 ± 2.0 b	82.4 ± 0.4 e	93.8 ± 0.5 f	66.5 ± 2.3 h
-24	Week 12	52.5 ± 2.8 c	78.6 ± 1.8 e	92.1 ± 0.5 f	60.5 ± 1.9 h

Values are means of 4 analyses. Means in the same column followed with the same letter are equals (P>0.05).

The difference between the group without additives and the group with sucrose and phosphate after 12 weeks of storage at -18 and -24°C was not significant (p>0.05). Weight loss of frozen haddock protein isolate during 12 weeks of storage at -18°C and -24°C is given in table 6 and 7. The WL (%) of samples containing salt and sucrose did not change during 12 weeks of storage at -18 and -24° C significantly (P>0.05).

Table 6: WL values (%) of HPI trails after 2 days of storage +2°C and during 12 weeks of storage at -18°C.

Storage temperature (°C)	Age	Haddock protein isolate containing following ingredients:			
		Free from any additives	0.8% salt and 3% sucrose	1.3% salt and 5% sucrose	4% sucrose and 0.1% sodium polyphosphate
+2	Day 2	11.7 ± 0.3 a	6.0 ± 0.6 d	3.8 ± 0.6 f	11.8 ± 0.7 h
-18	Week 2	24.8 ± 1.3 b	17.2 ± 1.2 e	7.8 ± 0.1 g	25.0 ± 1.0 i
-18	Week 4	25.6 ± 1.7 b	13.1 ± 0.7 e	6.9 ± 0.5 g	28.0 ± 1.9 i
-18	Week 8	29.2 ± 2.2 b	16.6 ± 2.3 e	8.0 ± 0.7 g	28.2 ± 2.0 i
-18	Week 12	36.4 ± 1.7 c	18.5 ± 1.8 e	7.6 ± 0.4 g	30.8 ± 2.0 i

Values are means of 4 analyses. Means in the same column followed with the same letter are equals (P>0.05).

Table 7: WL values (%) of HPI trails after 2 days of storage +2°C and during 12 weeks of storage at -24° C.

Storage temperature (°C)	Age	Haddock protein isolate containing following ingredients:			
		Free from any additives	0.8% salt and 3% sucrose	1.3% salt and 5% sucrose	4% sucrose and 0.1% sodium polyphosphate
+2	Day 2	11.7 ± 0.3 a	6.0 ± 0.6 d	3.8 ± 0.6 f	11.8 ± 0.7 g
-24	Week 2	23.1 ± 0.6 b	14.0 ± 0.8 e	6.2 ± 0.8 f	23.2 ± 0.9 h
-24	Week 4	27.7 ± 2.2 b	12.4 ± 0.3 e	6.0 ± 0.5 f	22.7 ± 1.5 h
-24	Week 8	25.8 ± 1.6 b	13.6 ± 0.3 e	4.8 ± 0.4 f	25.8 ± 1.8 h
-24	Week 12	36.6 ± 2.2 c	16.5 ± 1.4 e	6.1 ± 0.4 f	30.4 ± 1.5 h

Values are means of 4 analyses. Means in the same column followed with the same letter are equals (P>0.05).

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 (Knipe 1992) and can increase WHC in fish protein (Hunt *et al.*, 2004). Increasing pH can also increase water holding capacity in fish protein isolate (Kristinsson and Hultin, 2003) but in this study HPI containing phosphate and sucrose had the lowest value of WHC among other test samples suggesting that this material may not increase WHC in HPI.

The effect of salt on WHC, and thereby yield, has been described by many authors (Hamm 1960, Warriar *et al.* 1975, Offer 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 after 2 and 12 weeks storage at -18 and -24°C.

3.4 Viscosity

3.4.1 Brabender Unit

3.4.1.1 Fresh HPI

Brabender viscosity (BU) of HPI after 2 days of storage at +2°C is shown in table 8. Adding salt, sucrose and polyphosphate to HPI affected Brabender viscosity in sample groups significantly (p<0.05). The group without additives had the highest Brabender viscosity after 2 days of storage followed by the group with 0.8% salt and 3% sucrose and the group with 1.3% salt and 5% sucrose (P<0.05). Viscosity of the group with sucrose and phosphate was the lowest (P>0.05).

3.4.1.2 Frozen HPI

Viscosity (BU) of frozen haddock protein isolates during 12 weeks of storage at -18 and -24°C is shown in tables 8 and 9. The group without additives had the highest viscosity after 12 weeks of storage at -18°C and -24°C (P<0.05) giving the greatest resistance to flow which may suggest a firmer protein structure than in the other groups. The addition of salt significantly (p<0.05) reduced the Brabender viscosity, possibly due to a liquefying

effects 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, Sadowska and Sikorski 1977) and decrease of 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 additive and the group with 1.3% salt and 5% sucrose increased significantly ($P < 0.05$) after 12 weeks of storage at -18°C . storage time also increased Brabender viscosity of samples without additives and the groups containing salt and sucrose stored at -24°C significantly ($P < 0.05$).

Table 8: Viscosity values (BU) of HPI trails after 2 days of storage $+2^{\circ}\text{C}$ and during 12 weeks of storage at -18°C .

Storage temperature ($^{\circ}\text{C}$)	Age	Haddock protein isolate containing following ingredients:			
		Free from any additives	0.8% salt and 3% sucrose	1.3% salt and 5% sucrose	4% sucrose and 0.1% sodium polyphosphate
+2	Day 2	81.5 \pm 3.5 a	53.5 \pm 3.5 c	52.0 \pm 2.8 d	47.0 \pm 1.4 f
-18	Week 2	122.5 \pm 2.8 b	58.5 \pm 2.1 c	56.5 \pm 3.5 d	38.5 \pm 2.1 f
-18	Week 4	120.5 \pm 3.5 b	65.0 \pm 4.3 c	68.5 \pm 2.1 d	46.5 \pm 0.7 f
-18	Week 8	117.0 \pm 4.2 b	57.5 \pm 2.1 c	64.0 \pm 4.2 d	42.5 \pm 2.1 f
-18	Week 12	137.0 \pm 4.2 b	67.0 \pm 2.8 c	82.0 \pm 4.2 e	50.5 \pm 2.1 f

Values are means of 2 analyses. Means in the same column followed with the same letter are equals ($P > 0.05$).

Table 9: Viscosity values (BU) of HPI trails after 2 days of storage $+2^{\circ}\text{C}$ and during 12 weeks of storage at -24°C .

Storage temperature ($^{\circ}\text{C}$)	Age	Haddock protein isolate containing following ingredients:			
		Free from any additives	0.8% salt and 3% sucrose	1.3% salt and 5% sucrose	4% sucrose and 0.1% sodium polyphosphate
+2	Day 2	81.5 \pm 3.5 a	53.5 \pm 3.5 d	52.0 \pm 2.8 f	47.0 \pm 1.4 i
-24	Week 2	115.5 \pm 3.5 b	58.0 \pm 2.8 d	60.0 \pm 2.8 f	46.5 \pm 2.1 i
-24	Week 4	102.0 \pm 4.2 b	62.0 \pm 4.2 d	74.5 \pm 3.5 g	49.0 \pm 4.2 i
-24	Week 8	102.0 \pm 2.8 b	51.0 \pm 1.4 d	58.0 \pm 1.4 h	43.0 \pm 2.8 i
-24	Week 12	150.0 \pm 4.2 c	71.5 \pm 2.1 e	54.5 \pm 3.5 h	48.0 \pm 2.8 i

Values of means of 2 analyses. Means in the same column followed with the same letter are equals ($P > 0.05$).

The low Brabender 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 (BU) significantly ($p < 0.05$) in all test samples except the groups containing cryoprotectants after 12 weeks of storage at -18 and -24°C .

3.4.2 Bohlin viscosity (Pa)

3.4.2.1 Fresh HPI

The group without additives and the group with sucrose and polyphosphate had the highest and the same ($P>0.05$) viscosity (BU). The lowest viscosity (Pa) was for the group with 0.8% salt and 1.3% sucrose ($P<0.05$). The viscosity (Pa) of fresh haddock protein isolate is also shown in table 10, indicating that adding salt, sucrose and polyphosphate can affect viscosity of sample groups significantly ($p<0.05$).

3.4.2.2 Frozen HPI

Viscosity values (Pa) of frozen HPI during 12 weeks of storage at -18 and -24°C are presented in tables 10 and 11. The group without additives and samples with cryoprotectants had the highest viscosity (Pa) respectively after 12 weeks of storage at -18 and -24°C ($P<0.05$). The group with sucrose and polyphosphate had the highest viscosity after 12 weeks of storage at -24°C, followed by the group without additives ($P<0.05$). As shown in tables 10 and 11 storage time decreased viscosity in all test samples during 12 weeks of storage at -18 and -24°C significantly ($p<0.05$) indicating that freezer storage temperature can affect viscosity. The lowest viscosity was observed in the group with 1.3% salt and 5% sucrose and the group with 0.8% salt and 3% sucrose after 12 weeks of storage at -18 and -24°C respectively ($P<0.05$).

Table 10: Viscosity values (Pa) of HPI trails after 2 days of storage +2°C and during 12 weeks of storage at -18° C.

Storage temperature (°C)	Age	Haddock protein isolate containing following ingredients:			
		Free from any additives	0.8% salt and 3% sucrose	1.3% salt and 5% sucrose	4% sucrose and 0.1% sodium polyphosphate
+2	Day 2	0.076±0.0	0.055±0.0	0.057±0.0	0.076±0.0
-18	Week 2	0.056 ± 0.0	0.052 ± 0.0	0.058 ± 0.0	0.057 ± 0.0
-18	Week 4	0.047 ± 0.0	0.041 ± 0.0	0.049 ± 0.0	0.048 ± 0.0
-18	Week 8	0.049 ± 0.0	0.039 ± 0.0	0.040 ± 0.0	0.042 ± 0.0
-18	Week 12	0.050 ± 0.0	0.035 ± 0.0	0.039 ± 0.0	0.040 ± 0.0

Values are means of 2 analyses .Means in the same column are equals ($P>0.05$).

Table 11: Viscosity values (Pa) of HPI trails after 2 days of storage +2°C and during 12 weeks of storage at -24° C.

Storage temperature (°C)	Age	Haddock protein isolate containing following ingredients:			
		Free from any additives	0.8% salt and 3% sucrose	1.3% salt and 5% sucrose	4% sucrose and 0.1% sodium polyphosphate
+2	Day 2	0.076±0.0	0.055±0.0	0.057±0.0	0.076±0.0
-24	Week 2	0.054 ± 0.0	0.046 ± 0.0	0.048 ± 0.0	0.060 ± 0.0
-24	Week 4	0.057 ± 0.0	0.047 ± 0.0	0.040 ± 0.0	0.052 ± 0.0
-24	Week 8	0.037 ± 0.0	0.038 ± 0.0	0.042 ± 0.0	0.040 ± 0.0
-24	Week 12	0.047 ± 0.0	0.037 ± 0.0	0.041 ± 0.0	0.049 ± 0.0

Values are means of 2 analyses .Means in the same column are equals ($P>0.05$).

In this study no correlation was seen between Brabender viscosity and Bohlin viscosity results. For example, Brabender viscosity of samples containing salt and sucrose was increased after 12 weeks of frozen storage ($P < 0.05$), while Bohlin viscosity of all samples was decreased after 12 weeks of frozen storage ($P < 0.05$). Gunnarsson *et al.*, (2005) also reported that Brabender viscosity of fish hydrolysed samples did not correlate with results from Bohlin viscosity, although some of the results correlated, indicating that factors like shear rate, and shape of induced force can affect viscosity especially when viscosity of the same sample is measured by different methods.

3.5 Rheological behaviour

Rheology is the science of material deformation and flow, in which viscosity measures a fluid's resistance to flow. Fluids are classified into two groups; Newtonian and Non-Newtonian fluids. Newtonian fluids are those whose viscosity is not affected by shear rate or time. Non-Newtonian fluids exhibit various degree of change with varying levels of shear. They may also be time-dependent and are divided into two groups; thixotropic and rheopectic. A thixotropic (work softening) fluid decreases in viscosity as increasing the amount of time that a fixed (constant) rate of force (stress) has been applied. A rheopectic (work hardening) fluid increases in viscosity as increasing the amount of time that a fixed (constant) rate of force (stress) has been applied. (Steffe JF. 1996)

Both phenomena 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). 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).

The rheology of haddock protein isolate was studied in this work because of its importance for process condition and quality control. From a technological point of view rheological properties may give a quantitative contribution to texture characterisation and process control when using different formulation. In this study fish protein isolate samples had thixotropic behaviour and time-dependent characteristics. Figures 1-10 illustrate flow curves of HPI samples containing different amount of salt and cryoprotectants.

3.5.1 Effects of pH on viscosity and flow behavior of HPI

Viscosity of HPI is strongly dependent on pH of the product. In this study viscosity of 3 samples of haddock protein isolate with different pH was studied. Figure 1 illustrates flow behaviour of three HPI samples having thixotropic flow. The figure showed that the highest viscosity was at pH 9.3, followed by sample with pH 7.3 and the lowest viscosity was for sample with pH 5.3 ($P < 0.05$) indicating that there is a linear correlation between increasing of pH and increasing viscosity (BU). However, the 3 sample had approximately the same flow behaviour

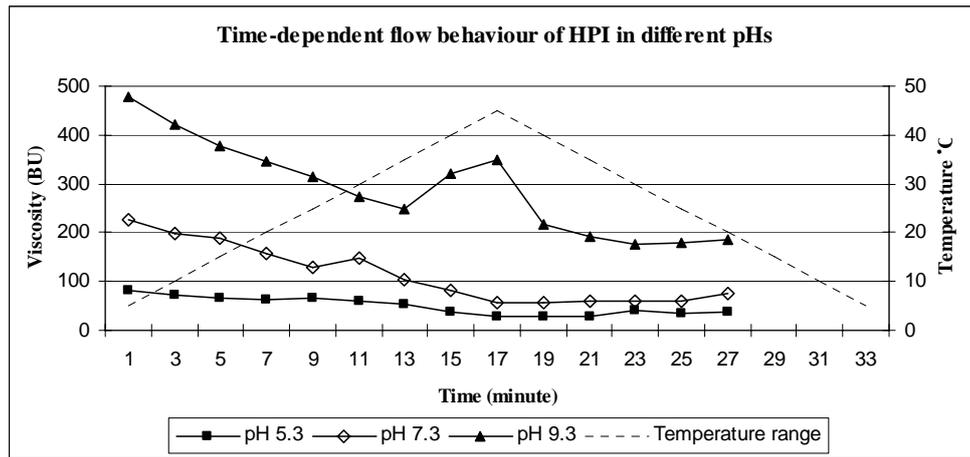


Figure 1: The influence of pH on time-dependent flow behaviour of haddock protein isolate.

3.5.2 Rheological behavior of fresh HPI

As shown in figure 12 viscosity of fresh HPIs decreased at the constant rate with time and then it increased again. This flow behavior looks like thixotropic behavior which is reversible. The figure also indicates that additives do not change the flow behaviour of fresh haddock protein isolate.

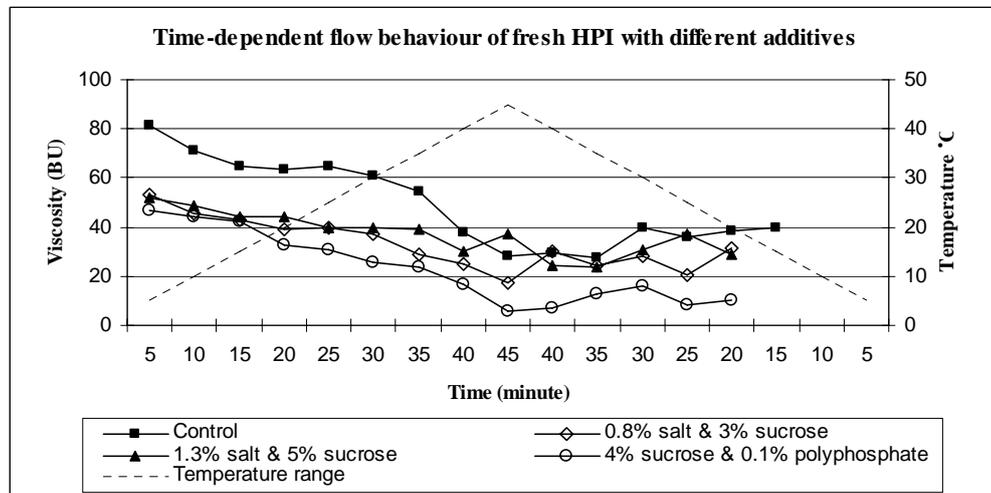


Figure 2: The influence of salt and cryoprotectants on time-dependent flow behaviour of fresh haddock protein isolate after 5 days of storage at +2°C.

3.5.3 Rheological behaviour of frozen HPI

Effect of cryoprotectants on flow behavior of haddock protein isolate was studied within 12 weeks of storage at -18°C and -24°C (Figures 3-10). Although the samples had different viscosity their flow behavior looked like thixotropic, showing that flow behavior is independent from viscosity. There were small flow behavior differences between the group

without additives and the group containing additives. It seems that storage temperature does not change flow behavior of samples. From the results it can be concluded that frozen storage did not change flow behavior of haddock protein isolate during the period of study.

3.5.3.1 Haddock protein isolate free from additives (control group)

Storage time increased viscosity. Increasing viscosity was greater when samples were storing at -24°C compare to storing them at -18°C (Figures 3 and 4). During flow behaviour measurement viscosity decreased by increasing temperature. The lowest viscosity was at 45°C . Viscosity gradually increased as temperature was reduced. The flow was thixotropic even after 12 weeks of frozen storage, indicating that freezing and frozen storage did not change flow behaviour of haddock protein isolates. Fluctuation of flow for samples stored at -24°C was lower than samples stored at -18°C .

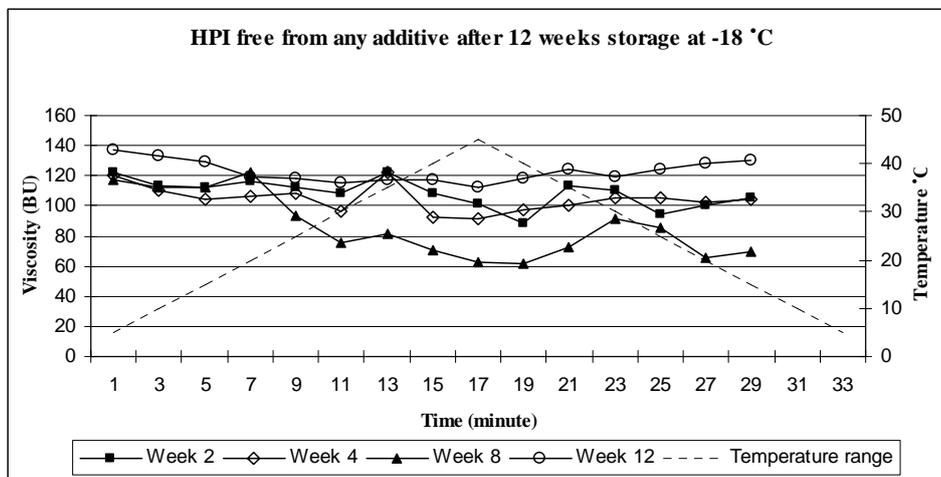


Figure 3: The influence of storage time and temperature on time-dependent flow behaviours of frozen haddock protein isolates free from additives stored at -18°C .

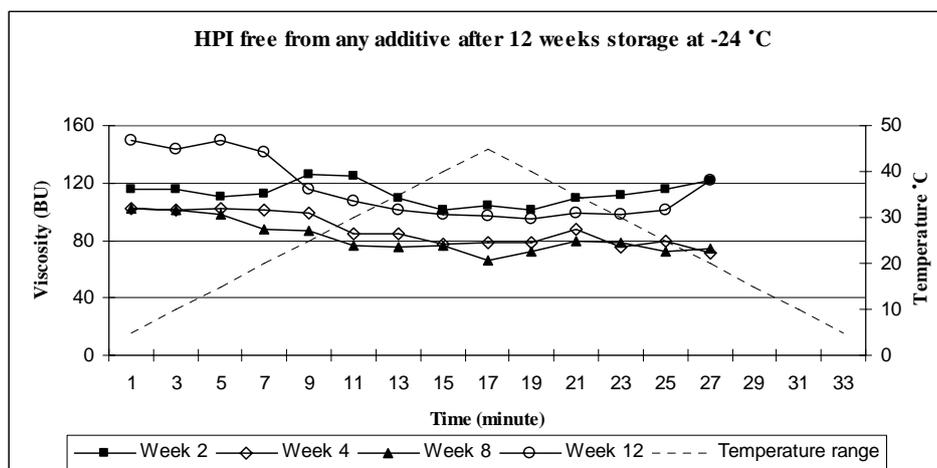


Figure 4: The influence of storage time and temperature on time-dependent flow behaviours of frozen haddock protein isolates free from additives stored at -24°C .

3.5.3.2 Haddock protein isolates containing 0.8% salt and 3% sucrose

As mentioned before added 0.8% salt and 3% sucrose decreased viscosity of haddock protein isolates, but storage time increased the viscosity within 12 weeks of frozen storage (Figures 5 and 6). The fluctuation of viscosity was greater when samples was stored at -24°C. The flow behaviour did not change still had thixotropic flow. It seems storing samples at -24°C had the lowest effect on flow behavior of haddock protein isolates.

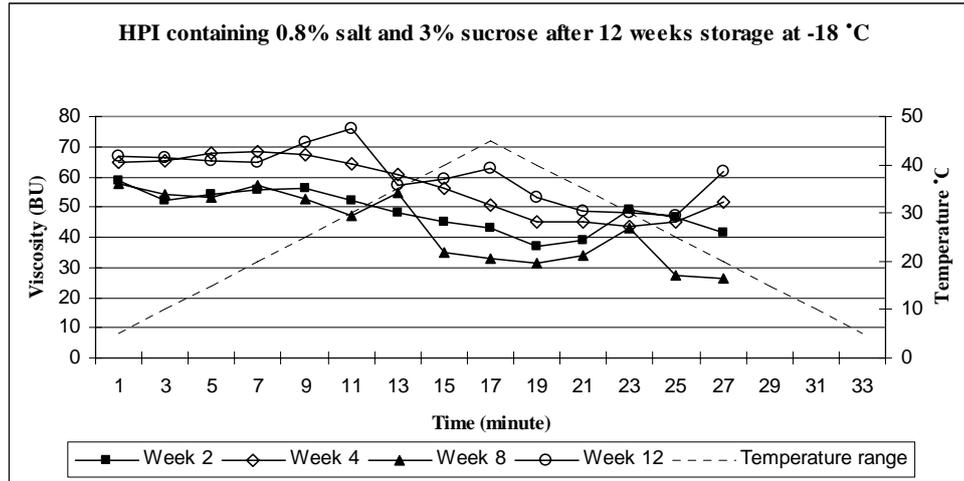


Figure 5: The influence of salt, cryoprotectants and storage time and temperature on time-dependent flow behaviours of frozen haddock protein isolates with 0.8% salt and 3% sucrose stored at -18 °C.

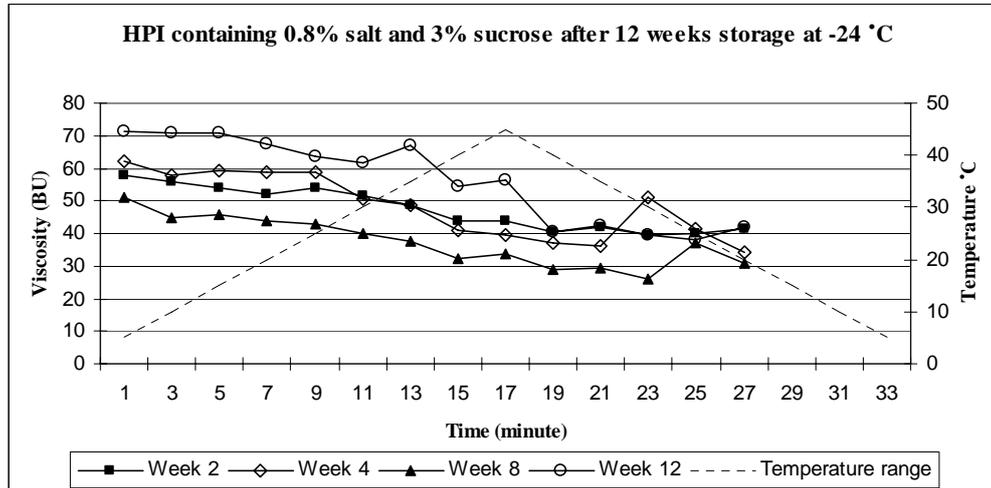


Figure 6: The influence of salt, cryoprotectants and storage time and temperature on time-dependent flow behaviours of frozen haddock protein isolates with 0.8% salt and 3% sucrose stored at -18 °C.

3.5.3.3 Haddock protein isolates containing 1.3% salt and 5% sucrose

Increasing amount of added salt and sucrose in haddock protein isolates decreased viscosity, but storage time increased viscosity (Figures 7 and 8). The fluctuation of viscosity was greater when samples were stored at -18°C . It seems storing samples at -18°C had the least effect on flow behavior of haddock protein isolates, although both flows appear thixotropic.

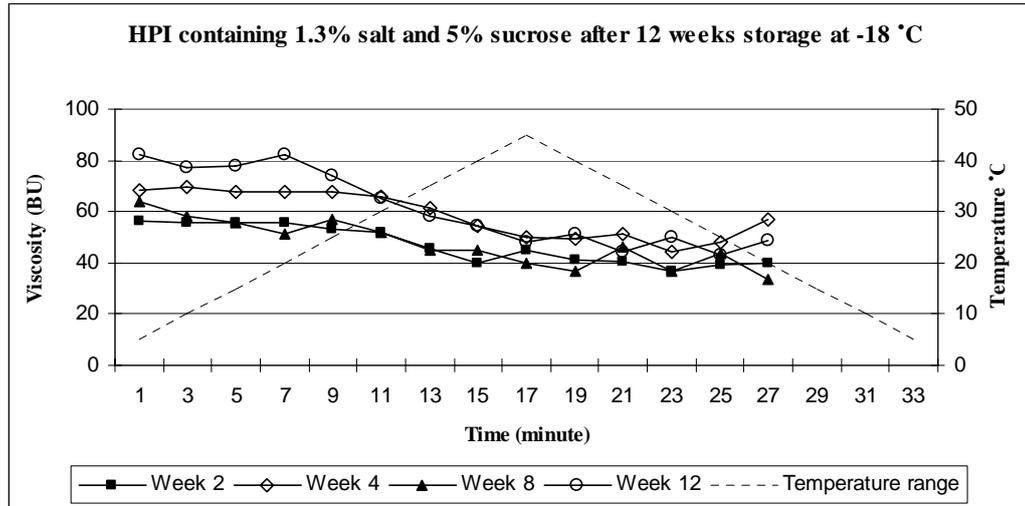


Figure 7: The influence of salt, sucrose and storage time and temperature on time-dependent flow behaviours of frozen haddock protein isolates with 1.3% salt and 5% sucrose stored at -18°C .

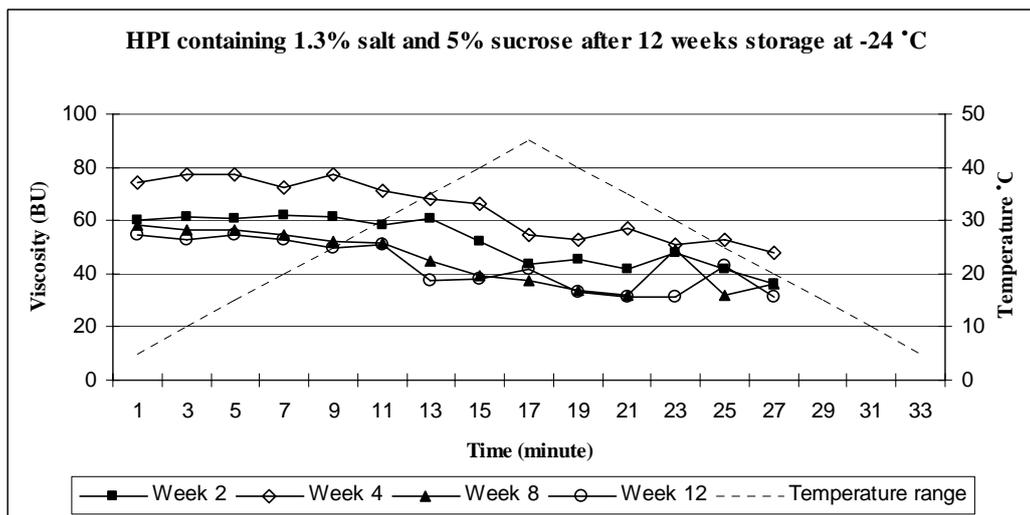


Figure 8: The influence of salt, sucrose and storage time and temperature on time-dependent flow behaviours of frozen haddock protein isolates with 1.3% salt and 5% sucrose stored at -24°C .

3.5.3.4 Haddock protein isolates containing 4% sucrose and 0.1% polyphosphates

As described before, added sucrose and polyphosphate to haddock protein isolate decreased viscosity. Frozen storage increased the viscosity and did not affect the flow behavior of haddock protein isolate. As shown in figures 9 and 10 storing samples at -24°C had the least effect on flow behavior of haddock protein isolates.

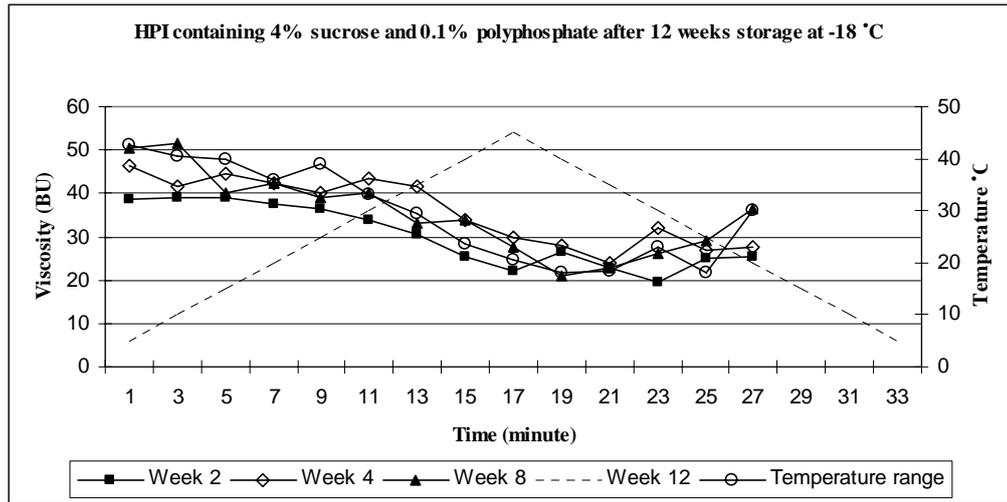


Figure 9: The influence of salt, cryoprotectants and storage time and temperature on time-dependent flow behaviours of frozen haddock protein isolates with 4% sucrose and 0.1% phosphate stored at -18°C .

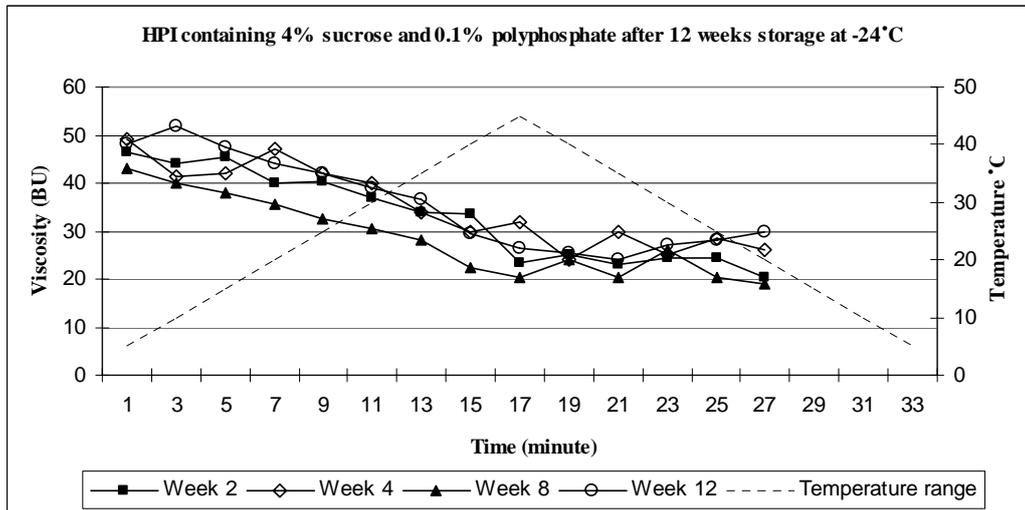


Figure 10: The influence of salt, cryoprotectants and storage time and temperature on time-dependent flow behaviours of frozen haddock protein isolates with 4% sucrose and 0.1% phosphate stored at -24°C .

4. CONCLUSIONS

Adding salt to fresh haddock protein isolate increased WHC, but decreased viscosity (BU and P) and whiteness significantly ($p < 0.05$). Using polyphosphate and sucrose together as cryoprotectant did not affect WHC, whiteness and viscosity (Pa) of HPI ($p > 0.05$), but it decreased viscosity measured by the Brabender instrument ($p < 0.05$). Apart from the viscosity, fresh samples with different amount of additives had the same flow behaviour (thixotropic). Different amounts of additives and also frozen storage time changed attributes of haddock protein isolate significantly ($p < 0.05$). Applying salt, sucrose and polyphosphate to haddock protein isolates had the following effects on functional and rheological properties after 12 weeks of storage at -18 and -24°C :

- Adding salt and sucrose increased WHC and decreased WL ($P < 0.05$) but adding sucrose and polyphosphate had no effects on these attributes ($P > 0.05$);
- Storage time of frozen isolate:
 - decreased WHC% and increased WL% in all treatments ($P < 0.05$);
 - decreased viscosity (Pa) of all treatments ($P < 0.05$);
 - increased Brabender viscosity (BU) of all samples except the group containing sucrose and polyphosphate;
 - decreased whiteness in all treatments ($P > 0.05$);
 - Viscosity was higher in samples without additives ($P > 0.05$);
 - Isolates with salt and sucrose had higher viscosity than isolate containing sucrose and polyphosphate ($P > 0.05$);
- Storage temperature:
 - did not affect WHC %, WL%, viscosity (BU and Pa) and whiteness significantly ($P > 0.05$);
- Frozen isolates with different amount of additives, had, apart from viscosity approximately the same flow behaviour. All samples showed thixotropic flow which it appeared to be reversible.

It is clear that that the addition of cryoprotectant is required to improve long-term stability of surimi during frozen storage (Takayoshi 1997; Yoon and Lee 1990; MacDonald and Lanier 1991, Park and Lin 2005). The factors responsible for decreasing texture quality in FPI are freeze/thaw cycles and/ the absence of cryoprotectants (Thawornchinsombut and Park 2006). Like conventional surimi the results suggested that the isolated proteins processed from the pH-shift also need cryoprotectants to preserve them against denaturation during frozen storage. Thus adding 1.3% salt and 5% sucrose as a cryoprotectant to HPI is recommended. This amount of sucrose does not give a sweet taste to the HPI which is a good technical issue for product development of HPI-based products.

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Chapter 4

Effects of Fish Protein Isolate on Physical and Sensorial Properties of Haddock (*Melanogrammus aeglefinus*) Mince Balls

Abstract

1. Introduction

2. Material and methods

3. Results and discussion

3.1 Effect of isolate on forming

3.2 Floatation of fish balls during setting

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3.4 Cook loss

3.5 Rheological effects of addition fish protein isolate to haddock mince

3.6 Sensory evaluation of fish balls

4. Conclusions

References

Effects of Fish Protein Isolate on Physical and Sensorial Properties of Haddock (*Melanogrammus aeglefinus*) Mince Balls

Abstract

Fish protein isolate (FPI) made from haddock (Melanogrammus aeglefinus) cut-offs by the pH-shift process was added to haddock mince in two different proportions (50:50, 25:75) with the goal to manufacture two types of fried fish balls. A minced fish ball product was also prepared as a control. The products were assessed for physical properties and sensory changes within a period of 8 weeks of freezer storage at -18°C. The rheological properties of fish minced with and without isolate were investigated by using a Brabender® Viscometer from 5° to 45° to 5°C in order to study viscosity and flow behaviour of minced and mixtures of minced and HPI. Cook loss of samples was measured and sensory analysis of fried products was carried out. The viscosity of mince decreased significantly ($P < 0.05$) as haddock protein isolate (HPI) added to mince increased. Control sample and fish balls containing isolate had the same cook loss after two thermal settings ($P > 0.05$). A significant difference ($P < 0.01$) was seen for grainy and soft texture, colour and frozen storage flavour during sensory evaluation of sample during 12 weeks of storage at -18 °C. Their characteristics depended on the proportion of isolate to mince and also frozen storage time. This study demonstrates good potential for HPI to be used as an ingredient in mince-based product development if fresh mince is used with appropriate additives.

Key words: mince, haddock, fish protein isolate, fish balls.

1. INTRODUCTION

Fish protein isolate (FPI) manufactured by the pH-shift technology from by-products can be used as an ingredient for production of ready to eat fish products based on mince or surimi. Little has been published on the functional characteristics of fish proteins added to food systems. However, Thorkelsson *et al.*, (2008) reported manufacturing fish sausages using FPI. Applying salmon protein hydrolysate reduced drip in salmon mince patties after freezing (Kristinsson and Rasco 2000). The addition of fish protein concentrate prepared from sardines has shown to improve cook yield of hamburgers (Vareltzis *et al.*, 1990). It can be concluded that, fish proteins may be used as functional ingredients. However, the particular process used for protein isolation affects their functional properties and must be controlled with respect to the desired characteristics of the isolated proteins.

Fish mince can be used for a variety of value-added formulated seafood products such as fish patties, balls, cakes, and nuggets (Lee 1997; Lee and Lian 2002). Developing new products from mince and isolate requires a comprehensive analysis of isolate characteristics with respect to the physical and sensory properties of the final product such as texture-forming properties, flavour, and colour characteristics. There have been several

studies on physicochemical stability of fish mince in relation to ingredient effects (Ponte *et al.*, 1985; Toro *et al* 1988; Yoon *et al* 1991; Huidobro *et al* 1998; Lian and others 2000) and some studies on characterization of fish mince in the finished product form with respect to physical and sensory properties (Lee and Toledo, 1979; Lee 1997, Lee *et al* 2007). However there is not published study on attributes of FPI products, or effects of this ingredient on sensorial attributes of final products. In developing isolate-based formulated seafood products, attention should be given to the moisture and pH of the isolate that can affect sensorial attributes of final products. Moisture loss is a critical factor that affects the quality and acceptability of precooked meat products. Since fish balls are usually subjected to precooking, freezing, and later reheating, it is desirable to have their tenderness and moistness retained through a proper formulation strategy. The present study was attempted to develop an innovative seafood product based on mince and isolate that is acceptable to domestic and overseas markets.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Fish mince

Frozen haddock mince was prepared by Isfiskur hf. in Kopavogur, Iceland using fresh haddock trimming within 48 hours of fishing. Haddock mince had the following chemical compositions presented by the producer company (Table 1).

Table 1. Chemical compositions of haddock mince

Compositions	Moisture	Protein	Fat	Minerals
%	82.9	16.5	0.4	0.2

2.1.2 Fish protein isolate

Haddock (*Melanogrammus aeglefinus*) protein isolates were extracted from cut-offs using the pH-shift method by MPF in Grindavik, Iceland and transferred to Matis in Reykjavik under chilled conditions. The pH of HPI was 6.4. The moisture was adjusted by removing excess water by using stainless steel sausage stuffer (Photo 2.5, Annex 2) . Since using isolate with pH 6.4 for fish balls manufacturing gave soft texture to the samples, it was adjusted to pH to 7.4 by adding NaOH before preparation of samples. No additive was added to HPI and it was stored at -24°C for one week.

2.1.3 Food ingredients

Food ingredients (Table 2) were bought from the local market, Reykjavik, Iceland, and were used in three different fish ball formulations; one control and two with mince and isolate.

Table 2: Composition (%) of the three fish balls formulas (adapted from Shaviklo 2007)

Ingredients	Formula 1	Formula 2	Formula 3
Haddock mince	70	52.5	35
Haddock protein isolate	0	17.5	35
Fresh onion	10	10	10
Bread crumbs	8.5	8.5	8.5
Wheat flour	4	4	4
Skim milk powder	3	3	3
Sunflower oil	2	2	2
Fresh garlic	1	1	1
Salt	1.5	1.5	1.5
Total	100	100	100

2.2 Methods

2.2.1 Fish balls preparation

A total of 15 Kg of fish balls of haddock mince and isolate were processed at the laboratory of Matis ohf, Reykjavik, Iceland, for training of panellist and the sensory evaluation study. Fish mince and isolate along with other ingredients were mixed in a food homogeniser (1094-Tecator, France). Forming was done manually under hygienic conditions. Fish balls were blast frozen at -18°C after setting and cooling. Figure 1 illustrates flow chart of the fish balls processing.

2.2.2 Thermal processing for setting

Thermal processing was a combination of boiling in hot water ($98.3 \pm 0.2^{\circ}\text{C}$) for 7 minutes followed by deep frying at $190 \pm 0.2^{\circ}\text{C}$ for 60 seconds. Core temperature of each fish ball was $74^{\circ}\text{C} \pm 1^{\circ}\text{C}$ immediately after boiling. It was $59^{\circ}\text{C} \pm 1^{\circ}\text{C}$ immediately after deep frying.

2.2.3 pH

The pH of HPI was measured using Knick Portamess®913 (Electronische Megetrate Gmb H&Co. Germany) a pH meter. All samples were measured at room temperature. The pH value was the average value of three readings.

2.2.4 Viscosity (Brabender® viscograph E)

The Brabender viscosity of mince and isolate was determined using a Brabender® Viscograph E coaxial viscometer (Brabender® OHG, Duisburg, Germany) based on Gunnarsson JR. *et al.*, (2005) but with some modification. Measurements were done in duplicate. The ratio of mince/mince-isolate to water was 1:2.

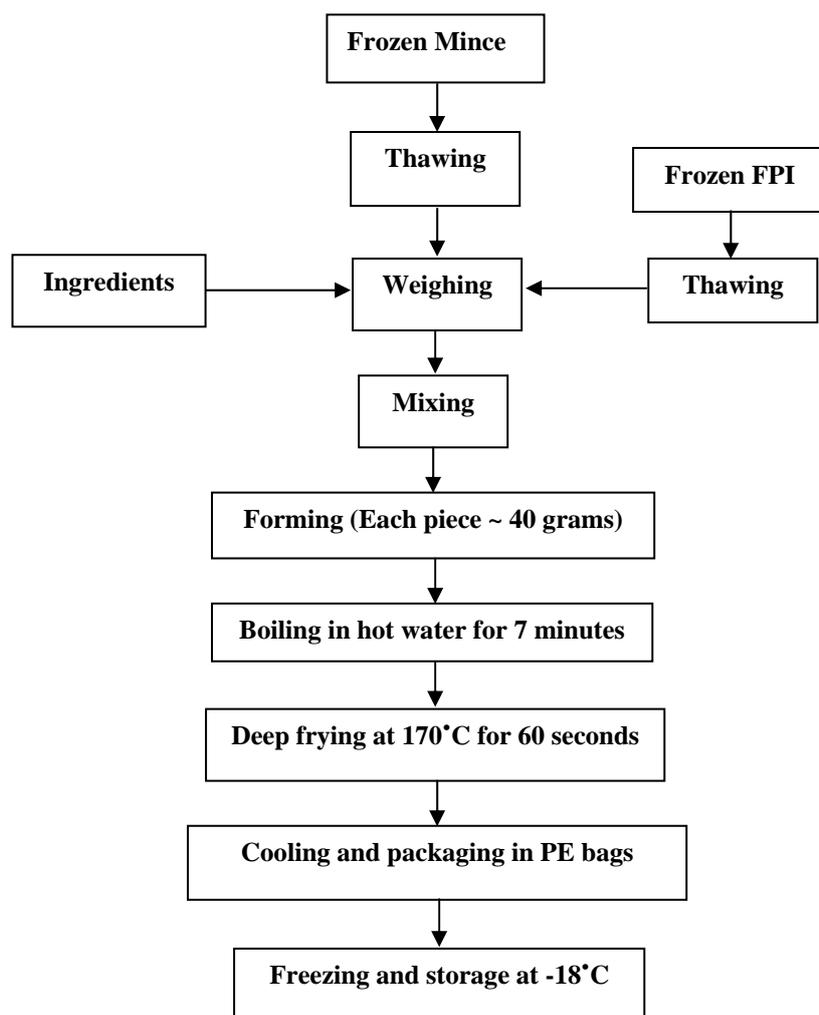


Figure 1: Flow chart of fish balls preparation

2.2.5 Weight loss after thermal processing (cook loss)

Following draining of fish balls after boiling/frying, samples were put on 1 layer of paper towels to remove the excess moisture/oil and equilibrated to room temperature. Then 5 fish balls were selected randomly and weighed directly. The cook loss was calculated as follows (Kim and Green 2007):

Weight loss (%) = $[(P1-P2)/P1] \times 100$, where:

P1=Fish ball initial weight (g) and P2=fish ball weight after boiling/frying (g).

2.2.6 Sample preparation for sensory evaluation

Frozen fish balls were removed from the freezer and were put into a refrigerator for thawing overnight. Fresh and thawed samples were cooked for sensory evaluation by

putting samples into a hot-air oven (Convotherm OEB/OGB, Germany) at 270 °C for 3 minutes.

2.2.7 Sensory analysis

Quantitative descriptive analysis (QDA) was used for evaluation of three fish ball samples. Two sessions were organised for training of panellist at Matis ohf, Reykjavik, Iceland for scaling procedures of sensory attributes of the fish balls under study one week before assessment of samples. Twenty sensory characteristics (Table 3) were evaluated by 8 trained panelists on a 0-100 point hedonic scale. All fish balls samples were coded with three-digit random numbers and presented to panelists on a tray in individual booths. Orders of serving were completely randomized. Water was provided between samples to cleanse the palate.

2.2.8 Statistical Analysis

For the sensory attributes a Panel Check statistical software version V1.3.2 was used to study significance between samples. For these analyses 192 observations (= 8 assessors × 4 time points × 3 treatments × 2 sessions of each assessment) were used.

Table 3: Selected attributes for sensory evaluation of fish balls after training of panellists

	Attributes	Definitions
Smell	Spicy	Inside balls: smell of spices, onion, etc.
	Fat	Inside balls: smell of fat from frying.
	Rancid	Inside balls: rancid smell can remind of cardboard, paints, nuts, etc.
	Fish	Inside balls: fish smell.
	Frozen storage	Inside balls: smell from frozen storage, old fish, etc.
Appearances	Wrinkle	Wrinkle on the surface of the balls.
	Uneven colour (outside)	Surface: Nothing (even colour), Much (uneven colour)
	Uneven colour (inside)	Inside balls: Nothing (even colour), Much (uneven colour)
	Colour (inside)	Inside balls: Is the colour light or dark?
Texture	Softness	Outside & inside: Softness in the first bite.
	Cohesiveness	Inside balls: Little (easy to take apart with a fork), Much (the inside of balls is firm).
	Juiciness	Outside & inside when chewing: Dry (sample draws liquid from mouth), Juicy (samples gives away liquid).
	Grainy	Inside balls: when rubbed against palate with tongue, grainy reminds of couscous or sand.
	Rubbery	Outside & inside: when chewing rubbery, springy.
Flavour	Spicy	Outside & inside: flavour of spices or onion.
	Fish	Outside & inside: fish flavour.
	Frozen storage	Outside & inside: flavour from long frozen storage.
	Rancid	Outside & inside: sign of decay.
	Fat	Outside & inside: fat flavour from frying.
	Soapy	Soapy, chemical flavour.

3. RESULTS AND DISCUSSION

3.1 Effect of isolate on forming

Added isolate to mince affected texture of the paste and its forming ability. Processed fish paste containing mince (control sample) was softer than other samples. Forming of this paste was not as easy as the other samples. Processed fish paste containing 50% mince and 50% isolate was stiffer than the other two samples. Forming of this paste was very easy. Processed fish paste containing 75% mince and 25% isolate was stiffer and formed more easily than control paste.

3.2 Floatation of fish balls during setting

Floatation of a fish ball is important especially when it floats on the water surface or sinks to the bottom of the setting tank. The former causes fish balls to stick to one another and the latter induces fish balls to flatten. Both cases result in deformed product (Kok and Park 2006). All three fish balls types sunk to the bottom of setting pot and did not stick to each and were suspended after 3 minutes of thermal setting without any deformation. At the end of thermal processing (after 7 minutes boiling) all set fish balls were floating on the water surface without any deformation.

3.3 Brabender viscosity of haddock mince and mixture of mince and isolate

Brabender viscosity of the three fish ball formulas at +5 °C are given in Table 4. Brabender viscosity of mince affected significantly ($p < 0.05$) by mixing with HPI. As shown the higher the amount of HPI the lower the Brabender viscosity. The highest value of Brabender viscosity was for haddock mince while the lowest value was for mixture of mince and isolate (50:50), followed by mixture of mince and isolate (75:25).

Table 4: The influence of adding HPI to haddock mince on Brabender viscosity and weight loss of cooked/deep fried fish balls

Sample	Viscosity at +5 °C (BU)	Weight loss (%) after boiling	Weight loss (%) after frying
Haddock mince (100%)	623.5 ± 2.1 a	1.2 ± 1.2 d	12.7 ± 0.8 e
Mince (75%) and isolate (25%)	514.5 ± 2.2 b	1.5 ± 0.1 d	10.1 ± 0.7 e
Mince (50%) and isolate (50%)	318.5 ± 1.4 c	1.5 ± 0.1 d	10.1 ± 0.7 e

Values are means of 2 analyses for measuring viscosity and means of 5 analyses for measuring weight loss. Means in the same column followed with the same letter are equals ($P > 0.05$).

3.4 Cook loss

No significant difference was seen for cook loss (%) of control sample and both trials containing HPI ($P > 0.05$) after two thermal setting, indicating that adding HPI did not change water holding capacity of the product.

3.5 Rheological effects of adding haddock protein isolate to haddock mince

The rheological properties of fish flesh (mince with and without isolate) were investigated by using a Brabender Viscometer in the range of 5° to 45° to 5°C in order to study flow behaviour and establishing the optimum temperature for setting of fish balls. Addition of

isolate, significantly affected the time dependency of the viscosity of the haddock mince balls ($p < 0.05$). In the case of haddock mince, a rheopectic behavior was observed at the beginning of the measuring process but it decreased because of increasing temperature to 45°C then increased by decreasing temperature (Figure 2). The haddock mince with 25% isolate exhibited a slight rheopectic behavior at the beginning of the operation then it increased like in the haddock mince. Haddock mince containing 50% isolate had a thixotropic flow behavior. Unlike other two samples Bartender viscosity of haddock mince containing 50% isolate increased at 45°C indicating that increased added isolate to mince can improve setting during food product processing. These results suggest that optimum temperatures for setting of mince and mince and isolate (75:25 and 50:50) are 45°C, 45°C, and 40°C respectively.

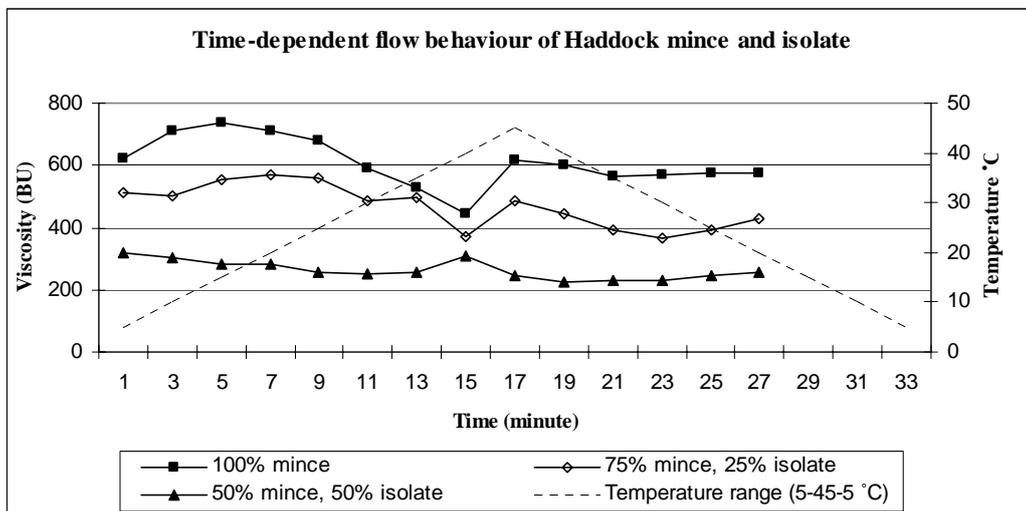


Figure 2: Influence of pH on flow behaviour of haddock mince and mixtures of mince and isolates

3.6 Sensory evaluation of fish balls

The sensory qualities of fish balls produced from haddock mince with/without haddock isolate were evaluated in terms of appearances, texture, odour, and flavour (Table 5). Significant differences ($p < 0.05$) were seen among products attributes such graininess and softness, colour, frozen storage flavor and juciness attributes during 12 weeks of storage at -18°C. Figures 3 and 4 present product effects and assessor to product interaction on sensorial attributes of three fish ball samples. Figures 5-8 illustrates the results of sensory evaluation of fish balls made from haddock mince and isolate.

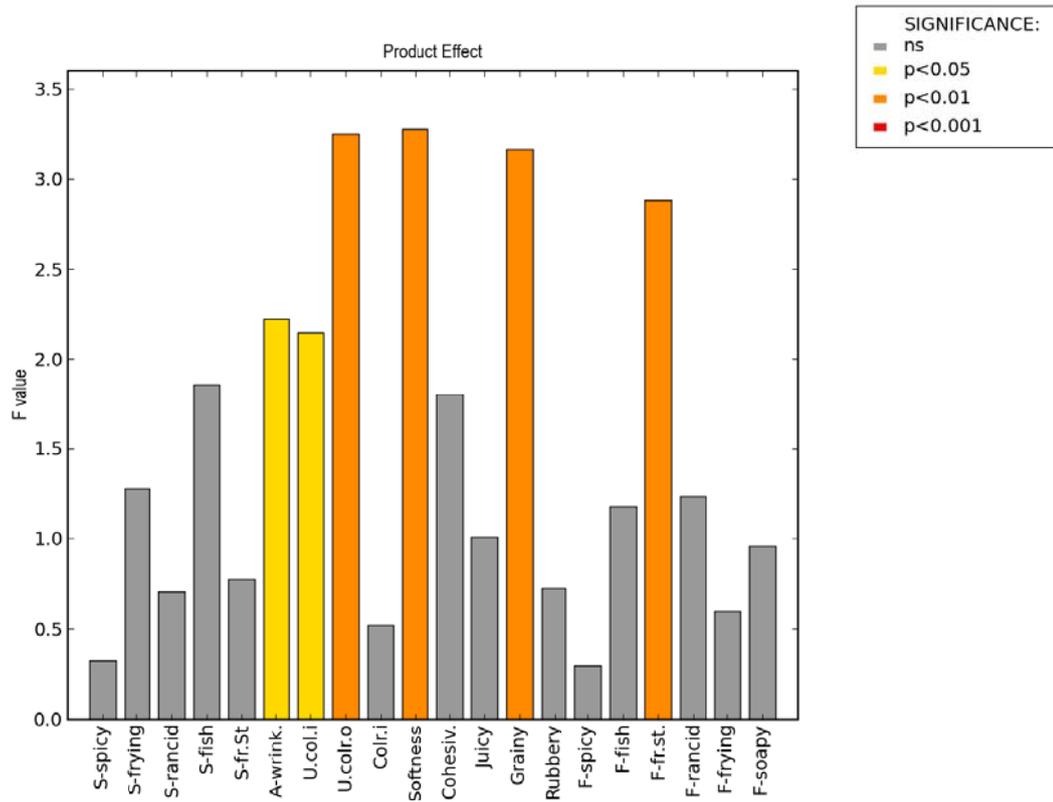


Figure 3: Product significantly effects on grainy and softness texture and uneven colour inside the fish balls

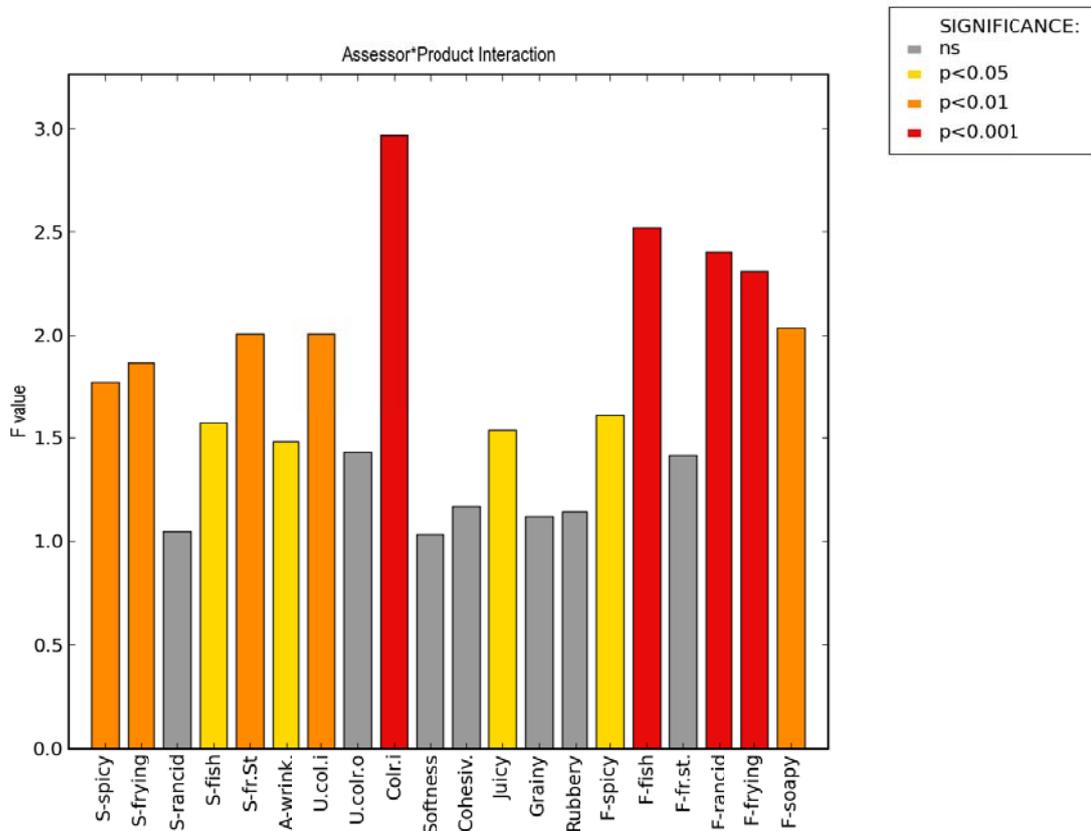


Figure 4: Assessor product interaction on fish ball attributes

3.6.1 Flavour

Soapy taste was detected in all samples. Fish balls containing isolate had the highest score of this attribute. The more isolate in the product the more soapy flavour was detected. This attribute decreased in all samples after 8 weeks of storage at -18°C.

Frying flavour was detected in all samples. Fish balls containing isolate had the highest score of this attribute. The highest score was for fish balls containing 25% isolate. This attribute decreased in all samples after 8 weeks of storage at -18°C.

Fish balls containing isolate had the highest score of *Rancidity flavour*. The more isolate the more rancidity flavour. This attribute decreased in all samples after 8 weeks of storage at -18°C.

The highest score of *Frozen storage flavour* in fresh fish balls was in samples containing isolates (the highest score was for fish balls containing 50% isolate); while after 8 weeks of storage at -18°C it increased in the control samples (containing only mince) and decreased in samples with isolates.

The highest score of *Fish flavor* in fresh fish balls was for the control; while after 8 weeks of storage at -18°C samples containing isolate had the highest fish flavour (the more isolate the more fish flavour in samples). This attribute increased in all samples after 8 weeks of storage at -18°C.

Frozen storage flavor was also detected in all samples. The highest score of this attribute in fresh fish balls was for samples containing isolates (the highest score was for fish balls containing 50% isolate); while after 8 weeks of storage at -18°C it increased in sample control sample (containing mince) and decreased in samples with isolates.

All samples had *fish flavour*. The highest score of this attribute in fresh fish balls was for control sample; while after 8 weeks of storage at -18°C samples containing isolates had the highest fish flavour (the more isolate the more fish flavour in samples). This attribute increased in all samples after 8 weeks of storage at -18°C.

In fresh fish balls the highest score of *Flavour from ingredients* (spices and onion) was for fish balls containing isolate. Fish balls with 25% isolate had the highest score. But after 8 weeks of storage at -18°C the highest score was for the control followed by the type with 50% isolate. This attribute increased the control and type with with 50% isolate and decreased in the type with 25% isolate after 8 weeks of storage at -18°C.

The most important of flavour attributes which can be considered a defect in fish balls samples were frozen storage flavour and soapy taste. Huss (1995) reported that post harvest activities can change sensory quality of fish. Meanwhile oxidation of free fatty acids may cause a "soapy" off-flavour. It seems using chemicals in FPI processing can affect flavour of FPI-based products. In this study fish balls containing 50% isolate had highest soapy score.

Table 5: Mean scores of sensory evaluation of fish balls made from haddock mince and isolate

	Smell					Appearance				Texture					Flavour					
Age	Spices	Fat	Rancid	Fish	Frozen storage	Wrinkle	Uneven colour <u>outside</u>	Uneven colour <u>inside</u>	Colour inside	Softness	Cohesiveness	Juicy	Grainy	Rubbery	Spices	Fish	Frozen storage	Rancid	Fat	Soapy
Fish balls made from 100% haddock mince (M100)																				
Day 1	41.2	30	12	26.2	8.8	31.3	34.3	23.7	22.8	51.4	56.3	44	32.5	30.4	44.8	37.5	7.6	11	28.2	16.4
Week 2	43	26.6	11.6	28.7	9.9	33.2	43	28	23	46.9	54.2	43.6	39	26.5	45	34.4	11.2	10.7	27.4	16.6
Week 4	37.4	21.6	9.4	29.8	10.9	29.6	40.8	34.6	26.8	48.3	42.9	49.5	42.1	33.3	48.6	37.7	10.9	6.3	25.4	16.4
Week 8	39.5	34.2	11.3	38.1	8.7	33.8	39.5	25.7	22.7	59.6	49.0	45.1	35.1	26.0	49.1	39.8	10.6	7.0	26.9	13.1
Fish balls made from 75% haddock mince and 25% haddock isolate (M75)																				
Day 1	42	31.7	13.4	28.2	9	23.8	37.4	19.4	19.5	50	53	42	45	26	46.5	39.7	14.2	13.3	33.3	18.8
Week 2	43	25	12.4	27.3	10	27	36	31	23.5	41	57.6	40	48.6	30.6	42.1	34.2	12.9	30	30	23.4
Week 4	40.1	20.1	8	33.6	10.2	29.5	28.7	34.8	30	49	52.3	48.4	40.1	28.6	50	38.4	9.8	6.9	22.6	16.2
Week 8	44.4	34.5	6.8	36.3	8.4	29.3	38.8	17.0	16.4	63.3	44.9	45.7	36.4	17.6	44.2	42.6	9.3	8.2	29.6	12.9
Fish balls made from 50% haddock mince and 50% haddock isolate (M50)																				
Day 1	41.6	27.8	12.8	30.6	9.6	16.7	34.6	21.7	19.9	41.7	59.2	37	54	24	45.4	39.3	15.8	13.4	29.7	23.5
Week 2	41.5	22	12.8	26	11.2	21.3	37	21.3	19	55.4	55.3	39.4	41	26.6	45	29	17.4	17.1	25	18
Week 4	42.3	21.9	8.9	38.6	9.1	35.4	46.6	20.2	24.9	59.7	48.1	47.9	42.7	23	47.8	41.5	14.5	14	20.4	15.2
Week 8	36.8	30.9	10.4	36.0	9.5	18.6	26.6	20.6	19.3	60.3	46.1	31.5	50.2	30.6	48.6	43.4	12.4	10.4	28.7	18.7

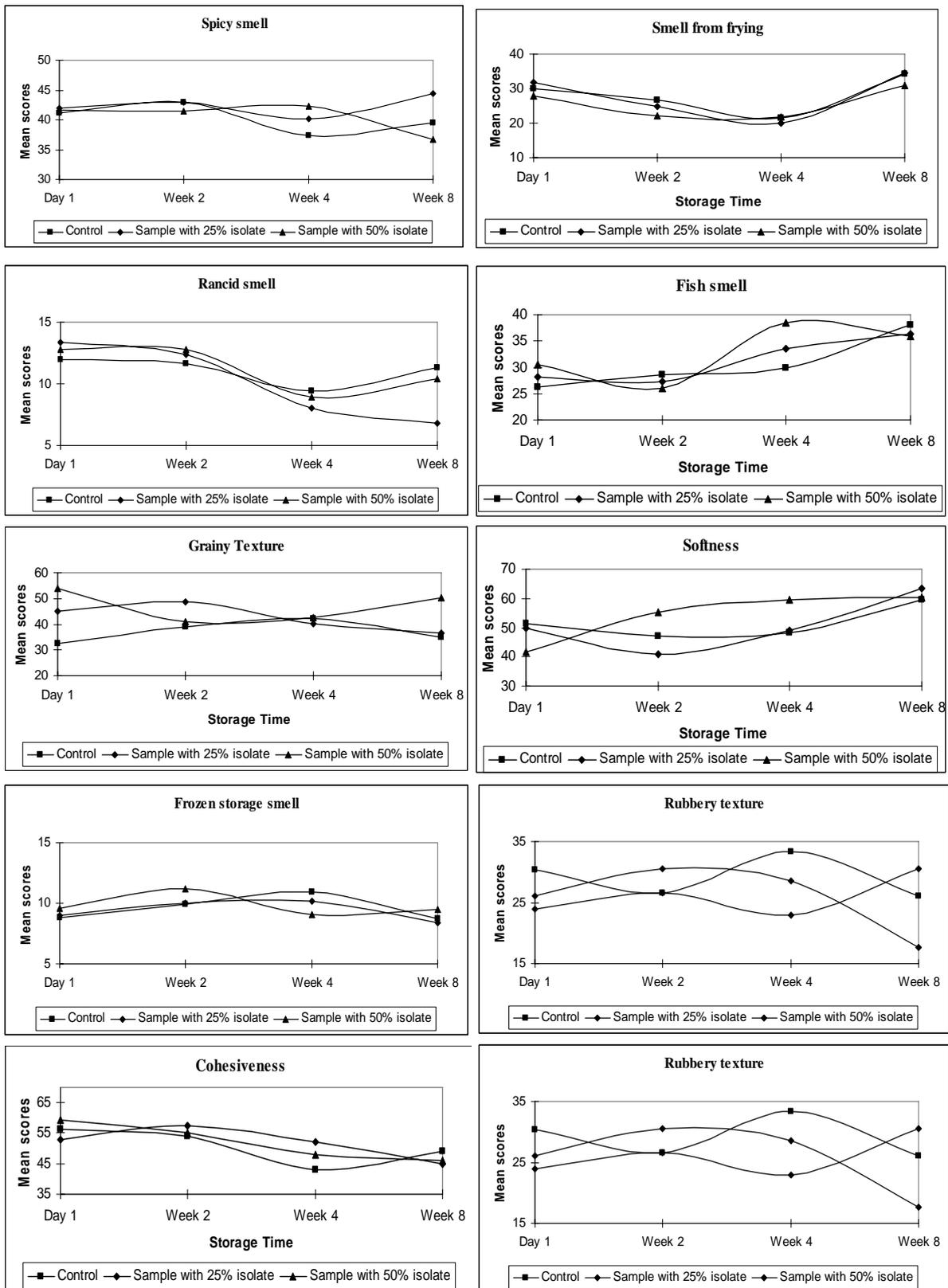


Figure 5: Influence of storage time at -18°C and addition of HPI on smell and textures of fish balls with haddock mince.

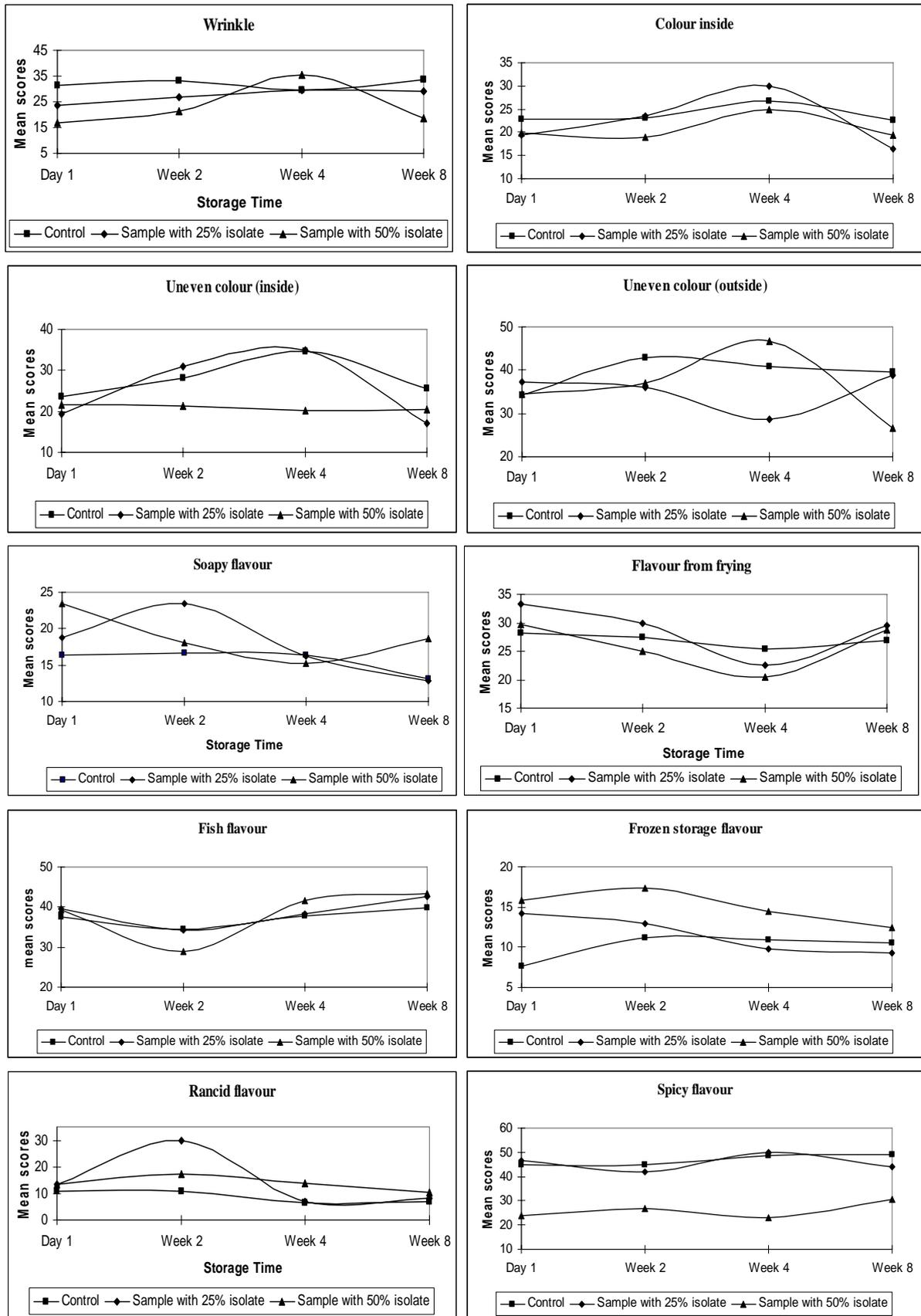


Figure 6: Influence of storage time at -18°C and addition of HPI on flavours and appearances of fish balls with haddock mince.

3.6.2 Texture

In fresh fish balls the highest score of *rubbery texture* was for the control followed by fish balls with 25% isolate. After 8 weeks of storage at -18°C the highest score was for samples containing 50% isolate followed by the control. This attribute decreased in the samples containing mince and with 25% isolate and increased in the samples containing 50% isolate.

Grainy texture was detected in all samples. In fresh and frozen fish balls the highest score of graininess was for the samples containing 50% isolate and 25% isolate respectively. Surprisingly this attribute increased in the control but decreased in isolate-added samples after 8 weeks of storage at -18°C.

In fresh and frozen samples the highest score of *juiciness* texture was for the control followed by samples containing 25% isolate. This attribute increased in both these samples but 25% isolate type and decreased in the samples containing 50% isolate after 8 weeks of storage at -18°C.

The highest score of *cohesiveness* in fresh fish balls was for the samples containing 50% isolate followed by the control; while it was highest in control after 8 weeks of storage at -18°C, followed by the samples containing 50% isolate. Cohesiveness of all samples decreased during freezer storage.

In fresh fish balls the control scored highest for *softness* followed by samples with 25% isolate; but in frozen fish balls the samples with 25% isolate had the highest score followed by the samples containing 50% isolate. Softness increased in all samples during frozen storage.

The most important of texture attribute which can be considered as a defect in fish balls samples is grainy texture, which was detected not only in isolate containing samples but also in control samples (mince balls). Mince usually has a grainy texture (Reppond and Babbitt 1995). Fabricant (1998) reported that overcooking of cod can cause unpleasantly grainy texture and make it lose its smoothness and delicate flavour. Grainy texture in soy protein products (Endres JG, 2001), cheese (Lee *et al.*, 2006) and other protein products have been reported. Using high pressure and high temperature during operation, and chemical processing of food are main reasons of this defect.

It seems pressure induced to fish flesh during mincing and also initial denaturation of proteins because of increasing temperature can form protein granules in mince which was detected by panelists. This defect is possibly developed in FPI during solubilisation and precipitation of proteins.

3.6.3 Appearance

Interior colour of fish balls was evaluated by panelist after cutting each fish ball into 2 parts. Out of the fresh samples the control had the lightest colour. Samples with isolate had approximately the same score of lightness. After 8 weeks of storage the interior colour scored highest followed by the samples containing 50% isolate. This attribute decreased in the 25% isolate containing samples but did not change in other samples.

The highest score of **uneven colour inside** fresh and frozen products was for the control followed by the samples containing 50% isolate. This attribute increased in the control sample and decreased in the isolate samples after 8 weeks of storage at -18°C.

The highest score of **uneven external colour fresh** products was for samples containing 25% isolate followed by the samples containing 50% isolate. After 8 weeks of storage the control scored highest followed by the samples containing 25% isolate. This attribute increased in the control and the samples containing 25% isolate and decreased in the samples containing 50% isolate after 8 weeks of storage at -18°.

Wrinkles were detected in all samples. In both fresh and frozen samples the highest score for wrinkles was for the control sample and samples containing 25% isolate. They increased in all samples after 8 weeks of storage at -18°C.

No important defects were detected for fish balls appearance. Colour of the products depends on the colour of raw material and ingredients. It can be formulated according to consumer's preferences. Thermal processing can affect the appearance of seafood products.

3.6.4 Smell

In fresh fish balls the highest score of **smell from frozen storage** was of fish balls with 50% isolate followed by fish balls with 25% isolate. After 8 weeks of frozen storage it was again highest of fish balls with 50% isolate followed by the control. This smell did not change during frozen storage in not changed the control sample and the fish balls with 50% isolate but it decreased for fish balls with 25% isolate.

Fish balls containing 50% isolate had the highest score of **Fish smell** followed by containing 25% isolate; but it was highest in the control followed by fish balls with 25% isolate after 8 weeks of freezer storage. This smell increased during freezer storage in all samples.

In fresh samples fish balls containing 25% isolate had the highest score of **Rancidity smell** followed by fish balls containing 50% isolate; but it was highest in the control followed by fish balls with 50% isolate after 8 weeks of freezer storage. This smell decreased during frozen storage in all samples.

Balls with 25% isolate scored highest in **smell from frying** both for fresh and frozen samples followed by the control. This smell decreased during freezer storage in all samples.

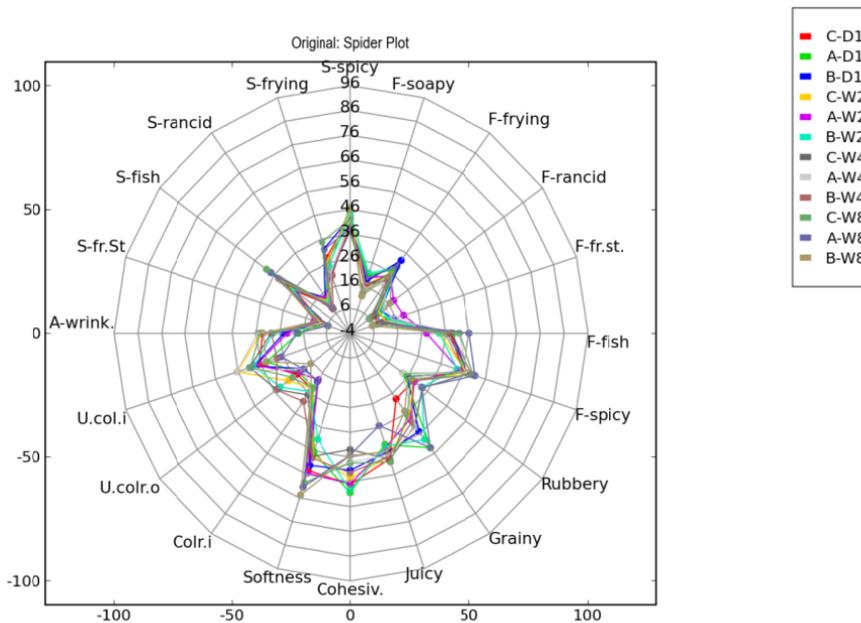


Figure 7: Spider plot of sensory attributes of fish balls showing changing of sensory scores during 12 weeks of frozen storage. [C: control, A: sample with 50% isolate, B: sample with 25% isolate, D: day, W: week]

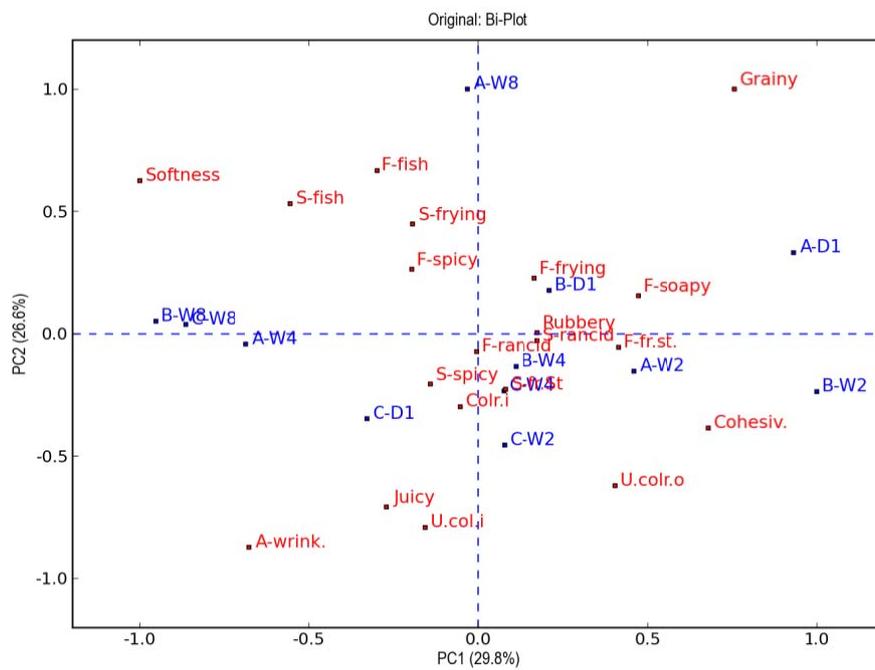


Figure 8: Bi-plot of sensory scores of fish balls indicating similarities and differences between fish ball samples and under study attributes. [C: control, A: sample with 50% isolate, B: sample with 25% isolate, D: day, W: week]

Smell from ingredients (spices and onion) was detected in all samples. In fresh fish balls the highest scores were for fish balls containing isolate. Fish balls with 25% isolate had the highest score. After 8 weeks of storage at -18°C the highest score was also for fish balls with 25% isolate followed by the control. This smell increased in the control and fish balls containing 25% isolate and decreased in fish balls with 50% isolate after 8 weeks of storage at -18°C.

No important smell defects were detected in the fish balls. Smell of the products depends on the freshness of raw material and ingredients. Sensory evaluation of fresh and frozen haddock balls with/without isolate during 8 weeks of frozen storage showed that the smell of haddock balls was not significantly affected by addition of isolate ($p > 0.05$).

The sensory scores, like graininess and softness were increased in all samples and other scores like rancidity smell and flavour, cohesiveness, fat flavour from frying and soapy taste declined significantly throughout the eight weeks of frozen storage ($p < 0.05$). However, each group remained quite fresh after the storage period.

Although applying HPI to mince affected mince balls attributes significantly ($p < 0.05$) it can be concluded from the sensory results that there is not a big difference between attributes of control sample and fish balls containing 25% isolate. This idea that soapy taste and grainy texture are exclusive attributes of FPI was rejected by sensory results of this study because they were detected also in control sample containing 100% mince. The range of these defects in all types is not considerable indicating the products had similarities which is positive point for applying FPI for developing mince-based products.

4. CONCLUSIONS

There were significant differences ($p < 0.01$) between the attributes of the three groups of fish balls. Although adding isolate to mince decreased viscosity it was still good for shaping and setting. The results show that viscosity of mince decreased significantly ($P < 0.05$) as HPI added to mince increased but forming ability improved. Both types of haddock balls containing isolate and also control sample had the same level of cook loss (%) after two thermal settings ($P > 0.05$). Data analysis of sensorial results indicates that FPI can affect graininess and softness, colour and frozen storage flavour significantly ($P < 0.01$). These attributes depend on and proportion of isolate to mince and probably frozen effects on texture of the products. It seems freshness level of mince, and kind of processing of isolate were the main reasons of detecting attributes like soapy and rancid taste in all samples. This study shows good potential for FPI to be used as an ingredient in mince-based product development provided that high quality mince and appropriate amount of isolate and additives specially spices, were used.

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Chapter 5

Overall Conclusions and Recommendations

1. Objectives
2. Main Results
3. Conclusions and Recommendations

OVERAL CONCLUSIONS AND RECOMMENDATIONS

1. OBJECTIVES OF THIS STUDY

This thesis was planned to evaluate functional properties of fish protein solutions and fish protein isolates and product development from FPI made by the pH-shift process. The objectives were as follows:

- to study the effects of salt concentration, cryoprotectants and chilled and frozen storage on viscosity, colour and water holding capacity / weight loss of cod protein solutions;
- to determine the effects of cryoprotectants on the functional properties of haddock protein isolates;
- to study if Brabender® viscograph E can be useful in studying rheological behaviour of fish protein solutions and fish protein isolates, and
- to develop cooked fish balls based on mince and isolate.

2. MAIN RESULTS

Both fish protein solutions and fish protein isolates are affected by different amounts of salt and cryoprotectants. The physicochemical and rheological properties of these products depend on the added additives and time and temperature of storage. On the other hand, fish protein isolate also can affect texture and sensory attributes of fish mince for product development. The main results of this thesis are listed below.

2.1 Shelf life of fresh cod protein solutions

The results show that the cod protein solution was spoiled after 3 days of storage at +2°C ($P < 0.05$). To extend the shelf life of fresh cod protein solutions containing 1-5 % salt, it is useful to pasteurize fish protein solutions for injection operation. A further study in this area is recommended.

2.2 Effects of salt on fresh cod protein solution

Increasing salt to fish protein solutions decreased viscosity (BU) in solutions containing 3 and 5% salt and increased it in samples containing 10 and 15% salt after 2 days of storage at +2°C ($P < 0.05$). Adding 20% salt to fish protein solution possibly caused protein denaturation in the solution with separated protein clots from water. Salt had not significant effect on whiteness of samples, except in sample with 20% salt. Salt affected weight loss in samples ($P < 0.05$).

2.3 Effects of storage time on fresh cod protein solution

Five days storage of CPS at +2°C had not significant effect on weight loss ($P>0.05$) and decreased viscosity in fresh samples containing 15% salt significantly ($P<0.05$). Storage time also increased whiteness of samples containing 5% salt ($P<0.05$).

2.4 Effect of cryoprotectants on frozen cod protein solutions

Adding cryoprotectants to cod protein solutions containing 1.2, 3, 5, and 15% salt increased water holding capacity (%) and decreased weight loss ($P<0.05$). It increased viscosity (BU and Pa) in samples containing 3 and 5% salt ($P<0.05$). Cryoprotectants also changed flow behaviour of samples except trails containing 5% salt.

2.5 Influence of storage time on frozen cod protein solution

14 weeks storage at -24°C increased weight loss and decreased WHC in frozen samples containing salt and cryoprotectant ($P<0.05$). The most stable frozen cod protein solution was with 5% salt and cryoprotectants followed by solutions with 3% salt and cryoprotectants. To make stable fish protein solutions during frozen storage it was recommended to add cryoprotectants to the products at the end of the processing.

2.6 Effects of salt and cryoprotectants on fresh haddock protein isolate

The results pointed out that the functional properties of fresh protein isolates are significantly affected by added salt and cryoprotectants. Added salt to the test samples increased WHC but decreased viscosity (BU and Pa) and whiteness in fresh haddock protein isolate ($P<0.05$). Applying cryoprotectants had not any positive effect on WHC and viscosity and whiteness of samples ($P>0.05$).

2.7 Effects of salt and cryoprotectants on frozen stability of haddock protein isolate

Adding salt and sucrose increased WHC and decreased WL ($P<0.05$) but adding cryoprotectants had no effects on these attributes ($P>0.05$). Added salt and sucrose increased WHC and decreased WL ($P<0.05$), but using polyphosphate and sucrose as cryoprotectants did not affect WHC and viscosity (P) of HPI ($P>0.05$). The highest value of WHC% and the lowest value of weight loss were seen in isolate with salt and sucrose ($P<0.05$).

2.8 Influence of storage time on frozen haddock protein isolate

12 weeks of storage at -18 and -24°C decreased WHC%, viscosity (Pa) and whiteness and increased WL% in all samples ($P<0.05$). Frozen storage increased Brabender viscosity whiteness in control samples and the groups containing salt and sucrose ($P<0.05$).

Like conventional surimi, the results suggest that the isolated proteins obtained through the pH-shift also need cryoprotectants to preserve them against denaturation during frozen storage. Thus adding 1.3% salt and 5% sucrose as a cryoprotectant to FPI is recommended.

2.9 Effect of frozen temperature on functional properties of HPI

There were no significant differences between groups stored at -18 and -24°C after 12 weeks of storage ($P>0.05$) suggesting that FPI can be stored commercially at -18°C.

2.10 Rheological behaviour of CPS and HPI

Cryoprotectants, frozen storage and storage time affected rheological behaviour of CPS. The most stable frozen cod protein solution was with 5% salt and cryoprotectants followed by solutions with 3% salt and cryoprotectants. They had thixotropic flow behaviour which was reversible after 14 weeks of storage at -24°C. In reversible food solutions, the network is rebuilt and the gel state re-obtained.

Apart from the viscosity, fresh and frozen haddock protein isolate with different amount of additives had the same flow behaviours. All isolates showed thixotropic flow which it seemed reversible.

From the results it can be concluded that Brabender Viscograph E could discriminate viscosity of protein solutions and protein isolates with different amount of protein and additives. It is very useful to study interaction of fish protein to additives and the effects of additives on flow behaviour of samples by Brabender viscograph because it easily allows the protein solution to be subjected to intended force and time. Studying the rheology of mixtures of fish proteins to discriminate similarities and differences between them for product manufacturing can be another application of this instrument.

2.11 Changing viscosity of isolated added haddock mince

Adding haddock protein isolate to haddock mince in different proportions (50:50, 25:75) decreased viscosity linearly ($P<0.05$). Using Brabender viscograph to study rheological behaviour of mince and mixture of isolate and mince indicated that the mince and mixture of 75% mince and 25% isolate had the same rheological behaviour suggesting textural similarities of mince and mixture of 75% mince and 25% isolate for product manufacturing.

2.12 Cook loss of haddock mince balls containing isolate

Control samples and fish balls containing 25% and 50% isolate had the same cook loss after two thermal settings ($p>0.05$) suggesting that addition of HPI did not affect water holding capacity of the products.

2.13 Effect of isolate on sensory attributes of haddock mince balls

Added isolate to mince influenced sensory attributes of final product. Significant difference was seen for graininess and softness, colour and frozen storage flavour during sensory evaluation of sample within 8 weeks of storage at -18 °C. They depend on proportion of isolate to mince and also freshness level of mince. Using high quality mince and applying an appropriate amount of isolate, soy proteins, spices and other ingredients is recommended for quality improvement of fish protein isolate-based products.

3. CONCLUSIONS AND RECOMMENDATIONS

Future aim of the pH-shift technology is applying isolated protein to food products. Better understanding of the quality properties of fish protein isolates made from different raw materials could contribute to the improvement of the products and optimal utilisation of fish protein resources. Hence this new industry needs more research in different fields of study. Based on the results of this work the following issues are recommended for future research and development.

1. Extending shelf life of fish protein solution containing 3-5% protein through thermal processing (pasteurization);
2. Process control for removing/decreasing soapy taste in FPI; washing precipitated proteins with potable water before final centrifugation;
3. Process improvement for eliminating/decreasing grainy texture in FPI;
4. Standardisation of process for adjusting water content, pH and cryoprotectants in final FPI product;
5. Studying effects of FPI on sensorial and textural properties of mince and surimi-based products (fish burgers, fish nuggets, etc.);
6. Introducing FPI-based products to food industry through product development;
7. Further study for frozen storage stability of fish protein isolate;
8. Shelf life study of FPI and FPI-based products.

Annexes

1. Work plan
2. Test samples photos
3. Temperature log plots
4. An introduction to fish species which were used as raw material in this work
 - 4.1 Haddock
 - 4.2 Atlantic cod

Annex 1. Work plan

Phase 1

1.1 Fresh Protein Solution variables and levels

Variables	Levels
Salt content	1.2-3-5-10-15-20%
Age	2-5 days
Protein content	~ 3%
Storage	+2°C
No. of trials	12
Intended tests	Colour, Viscosity, WHC, Dry matter, microbial tests, TVB

2.1 Fresh Protein Solutions trails (2°C)

Trail	Salt (%)	Age (days)
A1	1.2	2
A2	3	2
A3	5	2
A4	10	2
A5	15	2
A6	20	2
A7	1.2	5
A8	3	5
A9	5	5
A10	10	5
A11	15	5
A12	20	5

3.1 Frozen Protein Solutions variables and levels

Variables	Levels
Salt content	1.2-3-5-15%
Age	2-8-14 weeks
Storage	-24°C
No. of trials	24
Intended tests	Colour, Viscosity, WHC, Dry matter

2.3 Frozen Protein Solutions trails (-24°C)

Trail	Salt (%)	Crryoprotectants			Age (weeks)
		Sugar	Sorbitol	Phosphate	
B1	1.2	-	-	-	2
B2	3	-	-	-	2
B3	5	-	-	-	2
B4	15	-	-	-	2
B5	1.2	1	1	0.1	2
B6	3	1	1	0.1	2
B7	5	1	1	0.1	2
B8	15	1	1	0.1	2
B9	1.2	-	-	-	8
B10	3	-	-	-	8
B11	5	-	-	-	8
B12	15	-	-	-	8
B13	1.2	1	1	0.1	8
B14	3	1	1	0.1	8
B15	5	1	1	0.1	8
B16	15	1	1	0.1	8
B17	1.2	-	-	-	14
B18	3	-	-	-	14
B19	5	-	-	-	14
B20	15	-	-	-	14
B21	1.2	1	1	0.1	14
B22	3	1	1	0.1	14
B23	5	1	1	0.1	14
B24	15	1	1	0.1	14

Phase 2

1.2 Frozen stability of FPI; variables and levels

Variables	Levels
Salt content	0-0.8-1.25%
Sucrose	0-3-4-5 %
Polyphosphate	0.1%
Protein content	~ 20 %
Storage	-18°C, -24°C
No. of trials	36
Intended tests	Colour, Viscosity, WHC, Dry matter

2.2 Stability Assessment of Fresh and Frozen Fish Protein Isolate

Trials	FPI (%)	Salt (%)	Sucrose (%)	Polyphosphate (%)	Storage temperature	Age (weeks)
C1	100	0	0	0	2°C	2 days
C2	96.2	0.8	3	0	2°C	2 days
C3	93.7	1.3	5	0	2°C	2 days
C4	95.9	0	4	0.1	2°C	2 days
C5	100	0	0	0	-18°C	2
C6	96.2	0.8	3	0	-18°C	2
C7	93.7	1.3	5	0	-18°C	2
C8	95.9	0	4	0.1	-18°C	2
C9	100	0	0	0	-24°C	2
C10	96.2	0.8	3	0	-24°C	2
C11	93.7	1.3	5	0	-24°C	2
C12	95.9	0	4	0.1	-24°C	2
C13	100	0	0	0	-18°C	4
C14	96.2	0.8	3	0	-18°C	4
C15	93.7	1.3	5	0	-18°C	4
C16	95.9	0	4	0.1	-18°C	4
C17	100	0	0	0	-24°C	4
C18	96.2	0.8	3	0	-24°C	4
C19	93.7	1.3	5	0	-24°C	4
C20	95.9	0	4	0.1	-24°C	4
C21	100	0	0	0	-18°C	8
C22	96.2	0.8	3	0	-18°C	8
C23	93.7	1.3	5	0	-18°C	8
C24	95.9	0	4	0.1	-18°C	8
C25	100	0	0	0	-24°C	8
C26	96.2	0.8	3	0	-24°C	8
C27	93.7	1.3	5	0	-24°C	8
C28	95.9	0	4	0.1	-24°C	8
C29	100	0	0	0	-18°C	12
C30	96.2	0.8	3	0	-18°C	12
C31	93.7	1.3	5	0	-18°C	12
C32	95.9	0	4	0.1	-18°C	12
C33	100	0	0	0	-24°C	12
C34	96.2	0.8	3	0	-24°C	12
C35	93.7	1.3	5	0	-24°C	12
C36	95.9	0	4	0.1	-24°C	12

Phase 3

1.3 Utilisation of FPI; variables and levels

Variables	Levels
Fish Protein Isolate	0-25-50 %
Frozen mince	100-75-50%
Protein content	~ 20%
Additives	The same for all trails (except fish)
No. of trials	12
Intended tests	Sensorial test, cook loss, viscosity

2.3 Fish balls manufacturing trails

Sample	Fish Protein Isolate (%)	Frozen Mince (%)	Age	Storage Temperature
D1	0	100	Day1	0-4°C
D2	50	50	Day1	0-4°C
D3	25	75	Day1	0-4°C
D4	0	100	Week 2	-18°C
D5	50	50	Week 2	-18°C
D6	25	75	Week 2	-18°C
D7	0	100	Week 4	-18°C
D8	50	50	Week 4	-18°C
D9	25	75	Week 4	-18°C
D10	0	100	Week 8	-18°C
D11	50	50	Week 4	-18°C
D12	25	75	Week 4	-18°C

3.3 Fish balls recipe:

3.3.1 Recipe for all samples:

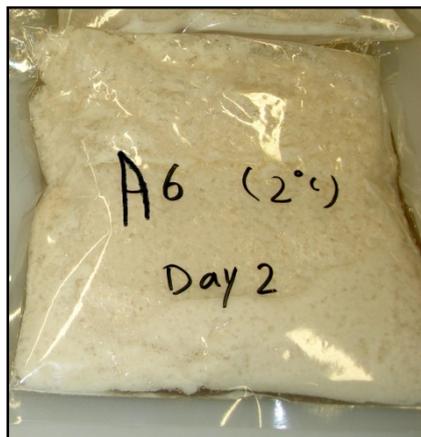
Recipe	%
Fish	70
Fresh onion	10
Bread crumbs	8.5
Wheat flour	4
Skim milk powder	3
Sunflower oil	2
Fresh garlic	1
Salt	1.5
Total	100

Annexe: 2

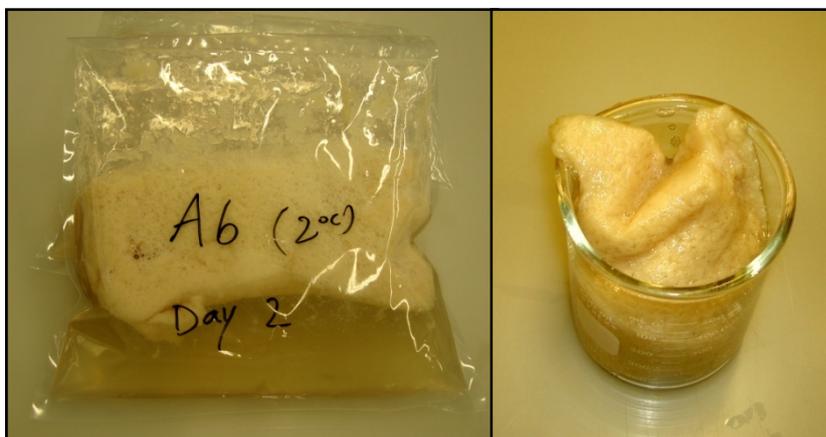
Test samples photos



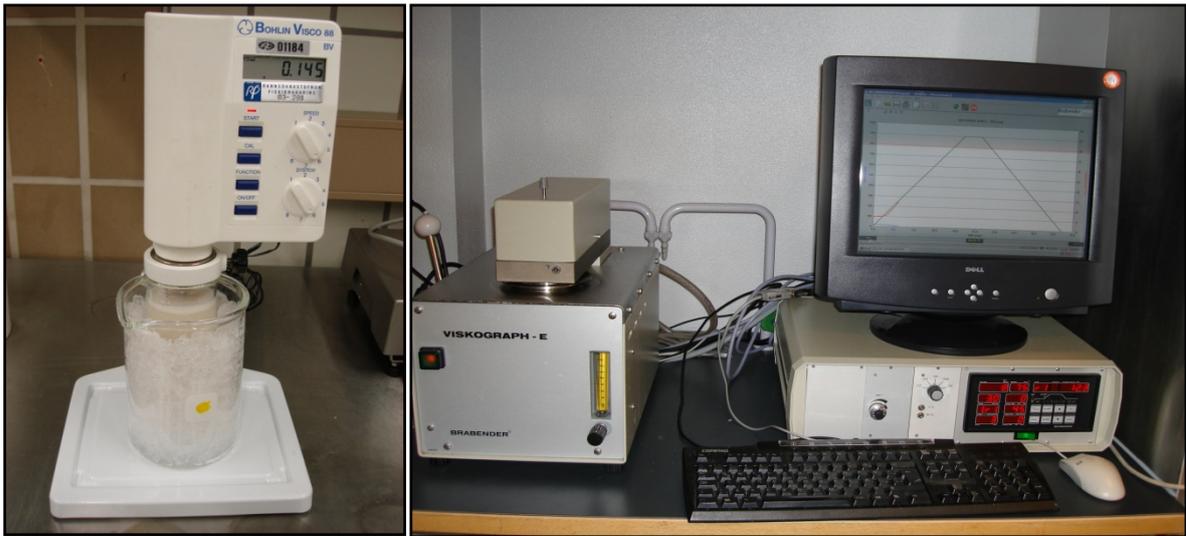
2.1 Fresh cod protein solution containing 1.2% salt and 2.97% protein



2.2 Protein clots after mixing fish protein solution with 20% salt



2.3 Separation of protein clots from solution containing 20% salt after 2 days of storage at +2°C



2.4 (L) Bohlin viscometer, (R) Brabender viscometer



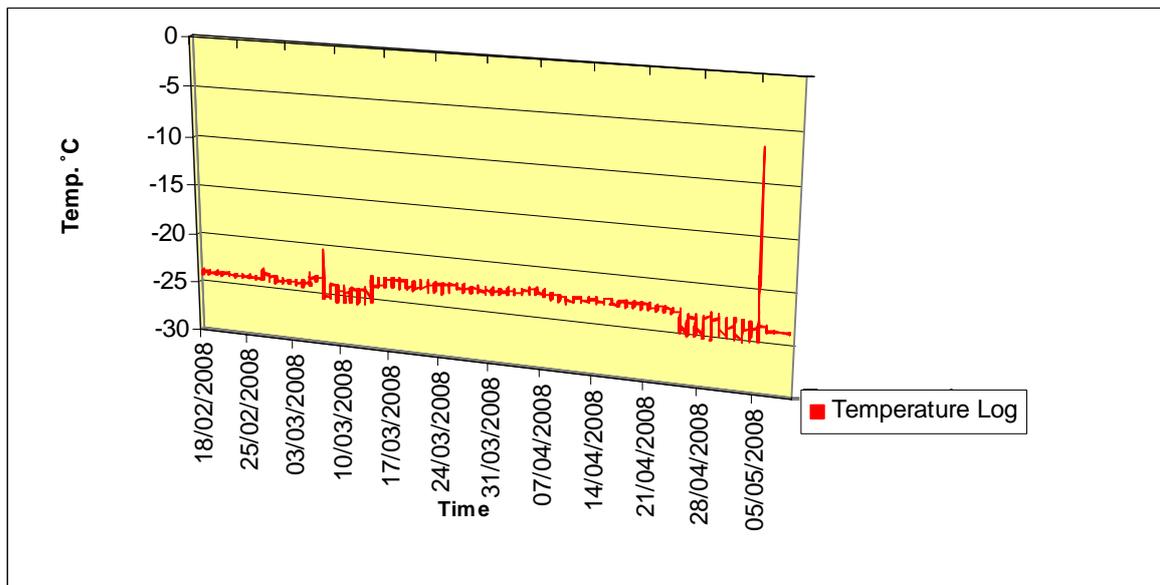
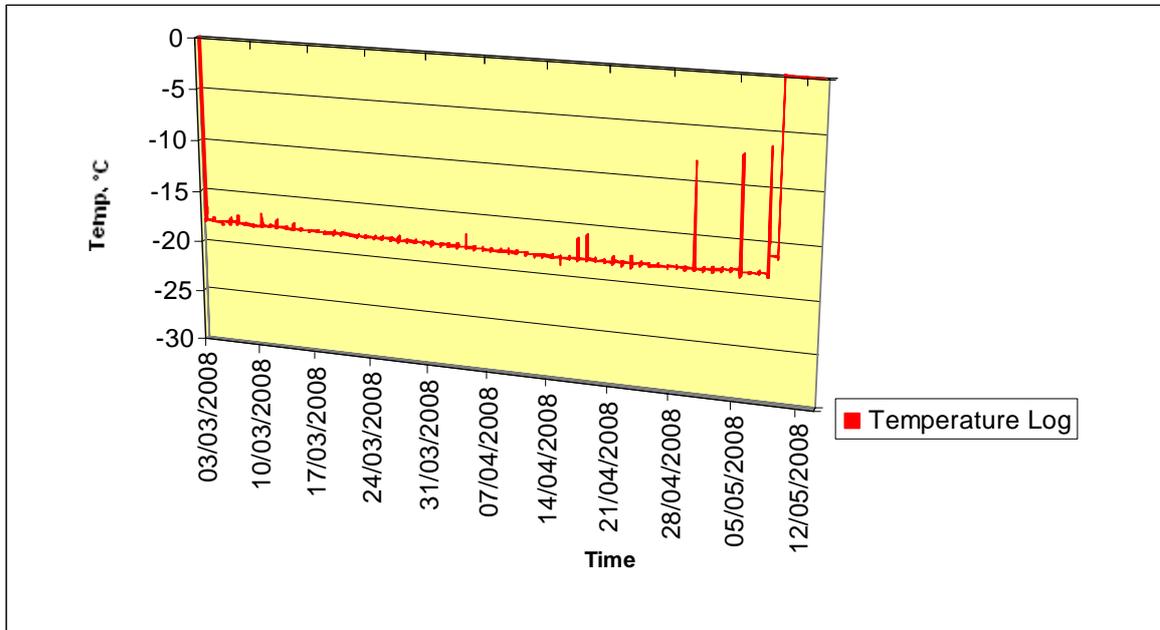
2.5 Receiving, dewatering, mixing with cryoprotectants and packing of haddock protein isolates



2.6 Fish balls preparation and steps and training session for sensory evaluations of samples.

Annex: 3

Temperature logs, indicating temperature fluctuations of freezers during storage of test samples at -18 and -24 °C



Annex: 4

An Introduction to fish species which were used as raw material in this work

4.1 HADDOCK

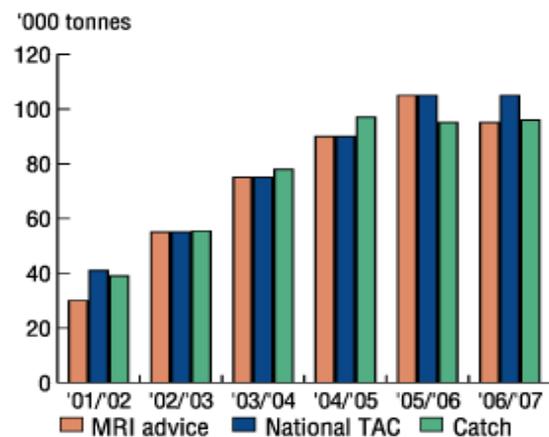


(Melanogrammus aeglefinus)

Haddock, a member of the Cod family is a marine fish found on both sides of the North Atlantic. Haddock is most commonly found at depths of 40m to 133m, but has a range as deep as 300m. It thrives in temperatures of 2°C to 10°C (36°F to 50°F). Juveniles prefer shallower waters and larger adults deeper water. The most important spawning grounds are in the waters off middle Norway near southwest Iceland, and Georges Bank. An average-sized female produces approximately 850,000 eggs, and larger females are capable of producing up to 3 million eggs each year. Haddock is not usually available beyond 3.5kg. The flesh is not as white as Cod, and is not as flaky, but has a slightly sweeter taste, which is why Haddock is the best whitefish for smoking.

Haddock is caught all around Iceland and throughout the year. The best grounds are off the west coast of Iceland and fishing is presently best in the winter months. Haddock spawns in April/May, mainly off the south, southwest and west coast after which the mature fish disperse for feeding. Haddock is mainly caught by bottom trawl, longline and gillnet. Haddock is a bottom feeder and is usually caught at depths of 10 - 200 m. The most common age of the catch is 4 - 6 years weighing 1 - 2 kg. The total fishable stock (3 years and older) was at the beginning of 2007 estimated at 300,000 tonnes and the spawning stock at 164,000 tonnes. Recruitment to the haddock stock tends to show great fluctuations but has been strong in most of the recent years.

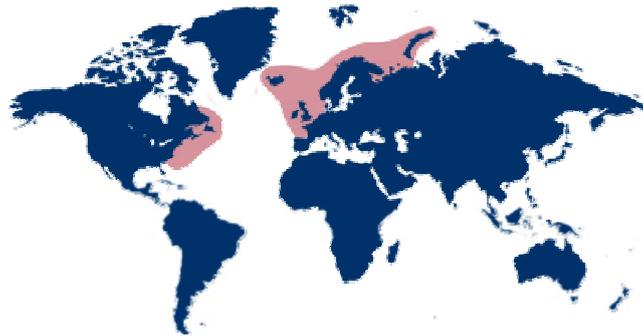
Historically, haddock catches have varied from 40,000 - 85,000 tonnes annually in the last 30 years, with an average of close to 50,000 tonnes. The total haddock catch by Icelandic vessels in Icelandic waters was 97,000 tonnes in the calendar year 2006, which was the highest for 40 years. TAC set for the fishing year 2006/2007 was 105,000 tonnes. For the 2007/08 fishing year the TAC is 100,000 tonnes, close to recommendations. The diagram shows the development of the annual haddock catch since 2001 together with the recommendations of the MRI and the set TAC.



Favorites of Icelanders

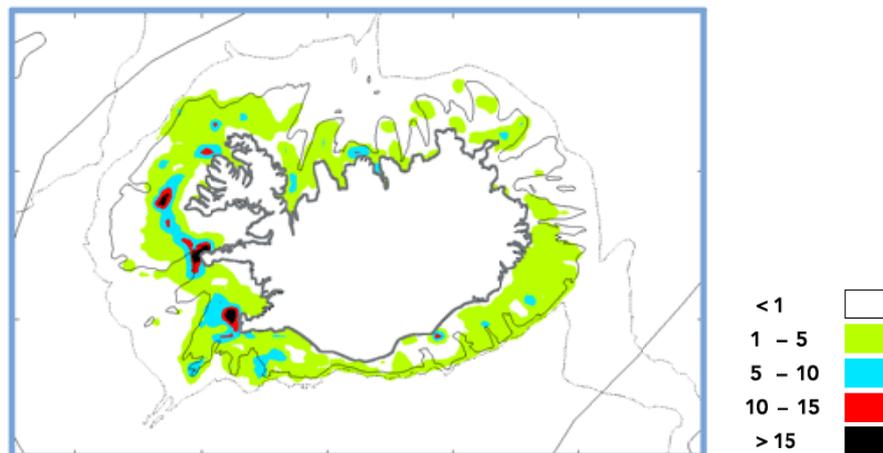
Haddock is the fish most often eaten fresh or bought frozen by the Icelanders. This may have historical origins since cod, the most abundant fish, was very often eaten salted. The annual domestic consumption of haddock is close to 5,000 tonnes based on catch weight, or 5% of landings. This equals an annual per capita consumption of 16 -17 kg of haddock.

Global Supply



Fishing grounds for haddock

The following figure shows the fishing grounds for haddock in 2005. The colour code shows the catch in tonnes per square mile. The haddock grounds are all around Iceland, but least in the northeast. The map shows some very rich grounds just off the southwest and west coast but also in the southeast.

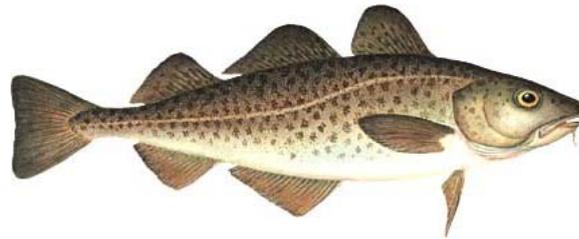


Sources:

<http://www.fisheries.is/stocks/haddock.htm>

http://www.youngsseafood.co.uk/web/fish_species_info.asp?sCode=00003

4.2 ATLANTIC COD

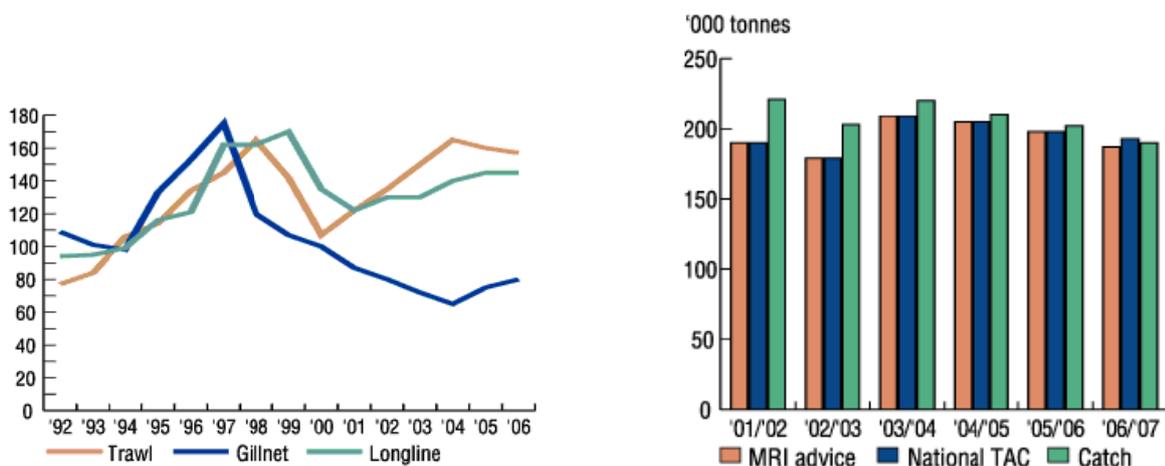


(Gadus morhua)

Cod is caught all around Iceland and throughout the year but fishing is often best in winter. Spawning takes place in late winter, mainly off the southwest coast of Iceland. The main feeding grounds are off the northwest coast where the warm Gulf stream of the Atlantic meets the cold Polar currents and also along the north and east coast of Iceland. Cod is mostly caught by bottom trawl, long line and gillnet and usually at depths of 100 - 250 m and ocean temperatures of 4 - 7 °C. The most common age of the landed catch is 4-7 years weighing 1-4 kg but larger fish are also caught.

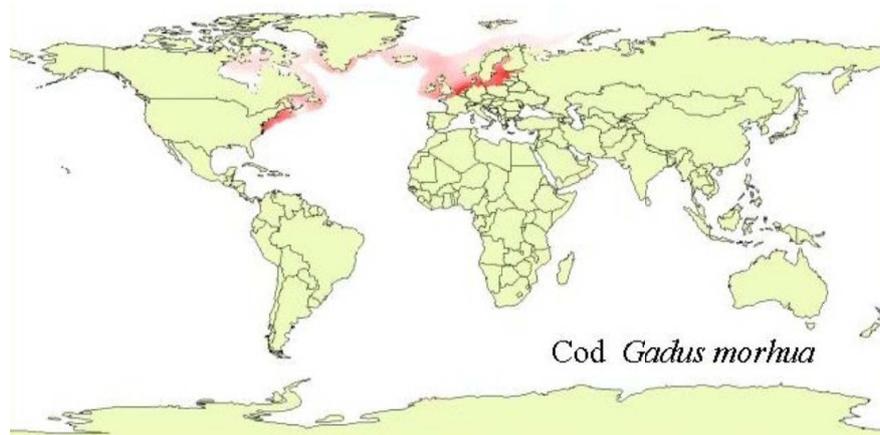
The total fishable stock (4 years and older) was at the beginning of 2007 estimated at close to 650,000 tonnes and the spawning stock at 180,000 tonnes. Long term landings of cod have varied from 180,000 tonnes to 470,000 tonnes per year in the last 30 years, with an average of close to 290,000 tonnes. The total catch of cod by Icelandic vessels in Icelandic waters was 194,000 tonnes in the calendar year 2006 and is expected to be 15-20,000 tonnes less in 2007. The TAC set for the fishing year 2006/07 was 193,000 tonnes.

The cod harvest rule employed since 1995 stated that the annual TAC for cod was to be set at 25% of the fishable biomass. Following recent recommendations by the Marine Research Institute, and based on recent poor recruitment to the cod stock, the government decided in July 2007 that the TAC for cod in the fishing year 2007/08 should be set at 20% of the fishable biomass. The TAC set for the fishing year 2007/08 is therefore 130,000 tonnes. Cod is by far the most economically important fish stock in Iceland. In recent years cod products have accounted for 35-40% of the total seafood export revenue.



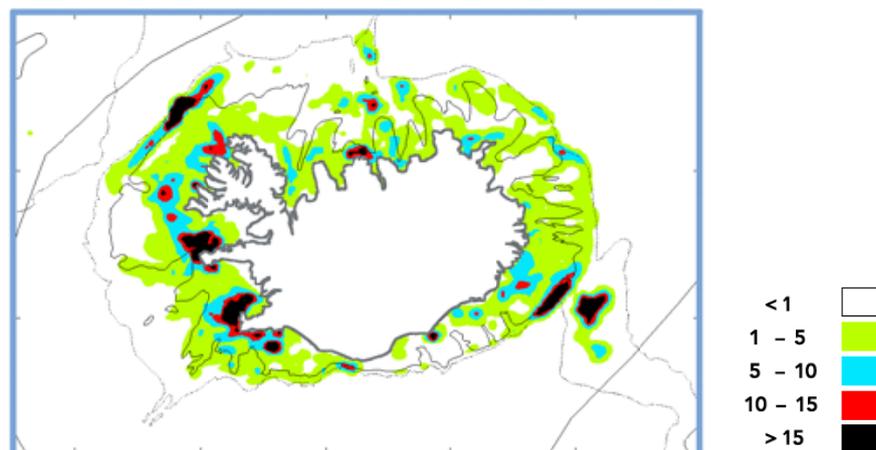
Trend in catch per unit of effort (CPUE) in cod fishing is an indication of fishing efficiency. With the standard for CPUE set at 100 for all types of gear in 1991, the CPUE for cod appeared to increase markedly in 1994-97 for all types of gear but after 1998 it seemed to fall significantly. In recent years the CPUE has increased again in fishing by trawl and long line.

Global Supply



Fishing grounds for cod

The following shows the fishing grounds for cod in 2005. The colour-coded scale on the right shows the catch in tonnes per square mile. The cod grounds are all around the country and some of them are legendary such as Halamid in the northwest that have traditionally been very rich grounds. Nearest to land the gear is hand-line, Danish seine, gillnets and long-line, while bottom trawling by regulation is further offshore.



Sources:

<http://www.fisheries.is/stocks/haddock.htm>

<http://www.searoundus.org/FAQ.htm>