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Quality Changes of Fresh and Frozen Protein Solutions Extracted from Atlantic Cod (*Gadus morhua*) Trim as Affected by Salt, Cryoprotectants and Storage Time

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Abstract

Fish protein solutions were extracted from cod (*Gadusmorhua*) trim using the pH-shift process. Fresh and frozen stability of cod protein solutions (CPS) [at 3% protein, pH 7.9, and different levels of salt, with/without cryoprotectants (sucrose, sorbitol and polyphosphate)] stored for different times and temperatures were studied. The results indicated that the fresh CPS was spoiled microbiologically after 3 days of storage at +2 °C. Viscosity (Brabander Unit) decreased in samples containing 3 and 5% salt and increased in samples containing 10 and 15% salt after 2 days of storage at +2 °C. Viscosity of fresh samples containing 10 and 15% salt decreased with storage time. The expressible moisture and whiteness of fresh samples were not influenced by the storage time. Adding cryoprotectants to CPS containing 1.2, 3, 5, and 15% salt increased the water holding capacity (WHC) and decreased the expressible moisture. It also increased viscosity (BU) in samples containing 3 and 5% salt significantly after 14 weeks of storage at -24 °C. The cryoprotectants had no significant effect on improving the whiteness of the samples.The storage time also increased the expressible moistureand decreased WHC in frozen samples. The most stable frozen samples were those with 5% salt and cryoprotectants followed by the samples with 3% salt and cryoprotectants.

Keywords: Fish protein solution, pH-shift process, salt, cryoprotectants, viscosity, water holding capacity, cod, *Gadusmorhua.*

Introduction

A fish protein solution (FPS) is a semi-solid protein-in-water colloid which is prepared by using the acid or alkaline aided process or diluted fish protein isolate (FPI) with brine or homogenized fish mince with water and it may contain saltand other ingredients such as cryoprotectants depending on the final use (Shaviklo, 2008). From an economical point of view fish by-products and underutilized fish are the best raw materials for producing FPS and this can improve the use of fish resources (Arason *et al.*, 2009). Annually a large amount of fish by-products (the rest raw materials) are obtained when developing fishery products (Batista *et al.*, 2007; Arason *et al.*, 2009).Utilization of such materials for human consumption has been an important issue in fish industry (Arason *et al.*, 2009).Thus, a new process was developed to increase the recovery of proteins from different sources of raw materials (Batista, 1999; Hultin and Kelleher, 2000, 2001; Hultin *et al.*, 2005).This process can be potentially used with any kind of fish or fish by-products as it presumably removes approximately all lipids and provide san 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; Arason *et al.*, 2009).

The pH-shift process for separating fish proteins involves the solubilization of minced and homogenized fish flesh either in an aqueous acidic (pH $\leq$ 3.5) or alkaline (pH $\geq$ 10.5) solution. The protein rich 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 to 5.5 and the precipitated proteins are removed by centrifugation (Hultin *et al.*, 2005; Batista *et al.*, 2007). FPI and FPS made by this method can be kept chilled or frozen (Shaviklo, 2008).

FPS can be used for fish fillet fortification through brine injection (Thorkelsson *et al.*, 2008; Arason *et al.*, 2009). The injection of a solution containing FPI or homogenised fish flesh from the same species could increase the weight (yield) of cod and haddock fillets and increased cooking yield by 5-20% (Thorkelsson *et al.*, 2008). Fish protein injection can improve frozen stability (Kim and Park, 2006)

and water holding capacity (Nolsoe and Undeland, 2009) of fish fillets. FPS can be applied as an ingredient in batter mix (Shaviklo, 2008) or as a fat blocker in fish fingers and patties by preventing fat absorption (Kim and Park, 2006; Einarsdottir *et al*., 2007). However, functional properties and stability of isolated fish protein and its applications are influenced by other ingredients "i.e." salt, sugar, phosphate and storage time (Thawornchinsombut and Park 2006; Campo-Deano *et al*. 2010; Shaviklo *et al*. 2010). So, the objective of this study was to investigate quality changes of fresh and frozen FPS made from cod trim and containing different levels of salt and cryoprotectants and thereby providing practical information for fish processors who want to improve the use of their raw materials.

Materials and Methods

The raw material was from Atlantic cod (*Gadusmorhua*) that was caught in south east of Iceland in January. Its average weight and size were 7 kg and 80 cm respectively at 4-5 years of age. The cod was gutted on board and kept chilled in a polyethylene tub (<+4 °C). It was 3 days old when it arrived to the processing plant. Cod protein solutions (CPS) were extracted from cod filleting by-products (trim) using alkaline aided process at Matís pilot plant in Saudárkrókurr, Iceland. It was then transferred to Matís processing laboratory in Reykjavik, Iceland in 4 polyethylene (PE) buckets (each 20 l) under refrigeration conditions (<+4 °C). All test samples were prepared within 4 h after receiving the shipment. The pH and dry matter of fresh samples were 7.9 ± 0.1 and 4.2 ± 0.1% respectively. Salt content and protein percentage of CPS were 1.2 and 3.0% respectively. The pH of samples containing cryoprotectants was 8.1 ± 0.1.

Preparation of Test Samples

Two experimental designs were separately used for fresh and frozen CPS treatments. A 5×2 factorial design was employed for fresh samples. The factors used were in 5 levels of salt (1, 2, 3, 5, 10 and 15%) and 2 time points (day 2 and day 5). The experimental design for frozen CPS was based on a 2×5×3 factorial design containing 2 sample groups (with/without cryoprotectants "i.e." sucrose, sorbitol and polyphosphate (Merck KGaA, Darmstadt, Germany) at 1, 1, and 0.1% respectively), 5 levels of salt content (1.2, 3, 5, 10, and 15%) and 3 time points (2, 8 and 14 weeks). The entire experiment was replicated.

An appropriate amount of salt, cryoprotectants and CPS containing 1.2% salt were separately weighted and mixed in a plastic jar to make 1 l of each test sample. Solutions were mixed by using a kitchen KHB300ER immersion hand blender (alaTest, London, UK) while the plastic jar was immersed in a mixture of chilled water and crushed ice to control the temperature. The temperature of the solutions during sample preparation was <4 °C. Samples were packed in 15×25cm polyethylene bags (Plastprent Co., Reykjavik, Iceland) and sealed by hand operated thermal sealing machine (400 mm Heat Sealing Machine, cmr-catering-equipment Co., Ashford,UK). Fresh and frozen samples were stored at +2 and -24 °C respectively.

Microbiological Analysis

Aerobic plate counts were conducted according to the methods for the microbiological examination of foods (APHA, 1992).

Physicochemical Analysis

Protein content of CPS was determined by the Kjeldahl method and dry matter was calculated by ashing the sample at 105 °C for 4 h (AOAC, 1990). Total volatile basic nitrogen (TVB-N) was determined in triplicate by the method described by Malle and Poumeyrol (1989). The TVB-N measurement was performed by direct distillation into boric acid using a Kjeldahl-type distillatory (Struer TVN distillatory, STRUERS, Copenhagen, Denmark). The acid was back-titrated with diluted H_2SO_4 solution. The TVB-N was expressed in mg N/100 g cod tissue.

The pH of CPS was measured using a pH meter, Radiometer PHM80 (Radiometer Analytical A/S, Copenhagen, Denmark). The pH meter was previously calibrated with buffer solutions of pH 7.00±0.01 and 4.00±0.01 at 20 ºC.The pH of CPS were the average of 3 measurements.

The water holding capacity (WHC) and the expressible moisture (EM) were determined by centrifugation following the method described by Shahidi *et al.* (1995) with some modifications (Shaviklo *et al.*, 2010). A plastic cylinder was put in a plastic holder cup, and then 2 g of CPS were placed in a plastic cylinder which had a fine mesh at the other end (diameter of sample cylinder 2.5 cm). The cylinder was placed in aBiofugeStratos (Heraeus Instruments, Hanau, Germany) at 5 °C, speed 1350 rpm for 5 min. After centrifugation, the cylinder was weighted and the difference in weight of the sample before and after was noted.The WHC was calculated according to the following Formula (Shaviklo *et al.*, 2010):

$$\text{WHC (\%)} = \frac{(A \times B) - C}{A \times B} \times 100 \qquad (1)$$

Where:

A= Moisture content of sample before centrifugation (g),

B= Sample weight (g),

C= Sample weight after centrifugation (g)

The results of WHC are expressed as the amount of water retained after centrifugation per g of dry

weight of the product.

The viscosity of the CPS samples was determined using a Brabender® Viscograph-E coaxial viscometer (Brabender® OHG, Duisburg, Germany) based on Kristbergsson and Sigfusson work's (2002). The Viscograph-E is a rotational viscometer designed to measure viscosity as a time and temperature dependent parameter. This instrument measures the resistance of the sample against flow. It is assumed that this resistance is proportional to the viscosity of the system. The term "Brabender viscosity" is used for describing the resistance. The measuring bowl containing the sample is placed in a temperature-controlled holder and rotates on a vertical axis forcing the sample to be pressed against the pin-style stirrer. Torque on the pins is measured and expressed asviscosity in Brabender units (BU). The force exerted on the stirrer depends on the resistance of the sample (Kristbergsson and Sigfusson, 2002). The measurement of viscosity of 450 g CPS samples was done with the following modification (Shaviklo*et al.*, 2010). Starting temperature was 5 °C, heating rate 1.5 °C/min, and maximum temperature 45 °C with a holding time of 3 min, then a cooling rate of 1.5 °C/min to 15 °°C. The measuring cartridge was 700 cmg (0.7 Nm) and the speed of the bowl was 75 rpm.

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

$$\text{Whiteness} = L^* - 3b^* \qquad (2)$$

Statistical Analysis

The statistical program NCSS 2007 (NCSS, UT, USA) was implemented to analyze the results of all treatments. Student's *t*-test was used to determine whether there was a difference between treatments of fresh CPS. Analysis of variance (ANOVA) was carried out on the results of frozen samples. The program calculated multiple comparisons using Duncan's test to determine if frozen sample groups were different (Duncan, 1955). Significance of difference was defined as the 5% level.

Results

Fresh CPS

The CPS samples were spoiled after 3 days of storage at +2 °C. Total count of bacteria at 22 °C at day 1 and day 3 were $6.1\pm0.5\times 10^4$ to $8.4\pm0.8\times 10^4$ and $2.2\pm0.3\times10^6$ to $3.4\pm0.5\times10^6$ cfu/g respectively. TVB-N value of the fresh protein solution after 1 and 3 days were 2.6-3.2 and 4.6-5.4 (mg N/100 g) respectively.

Fresh CPS samples containing 15% salt had the highest viscosity (BU) after 2 days of storage followed by those with 1.2 and 10% salt ($P<0.05$). The viscosity of samples containing 10 and 15% salt decreased significantly ($P<0.05$) after 5 days (Figure 1). The EM in fresh CPS samples with 1.2, 3, 5, 10, and 15% salt content was at the same level ($P>0.05$) after 2 days of storage (Figure 2) and it did not change after 5 days of storage ($P>0.05$). Fresh CPS samples with 1.2-15% salt content had the same values of whiteness ($P>0.05$) on day 2 and day 5 (Figure 3). Lightness (L*), redness (a*) and yellowness (b*) values of all samples were at the same level ($P>0.05$) and did not change during the storage time (Table 1).

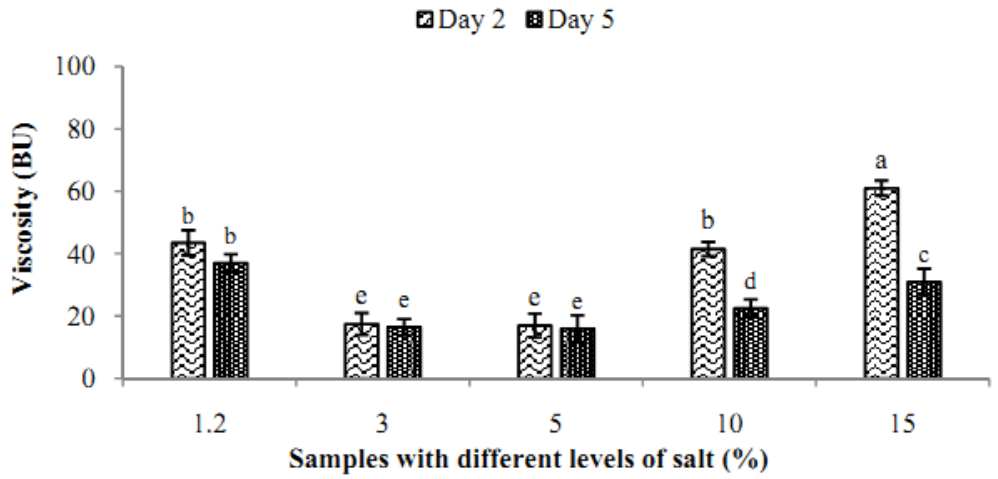

Figure 1. Viscosity of fresh CPS during 5 days storage at +2 ºC. Values are means of 3 analyses with ± standard deviation lines. Different small letters on the top of the bars indicate significant differences ($P<0.05$).

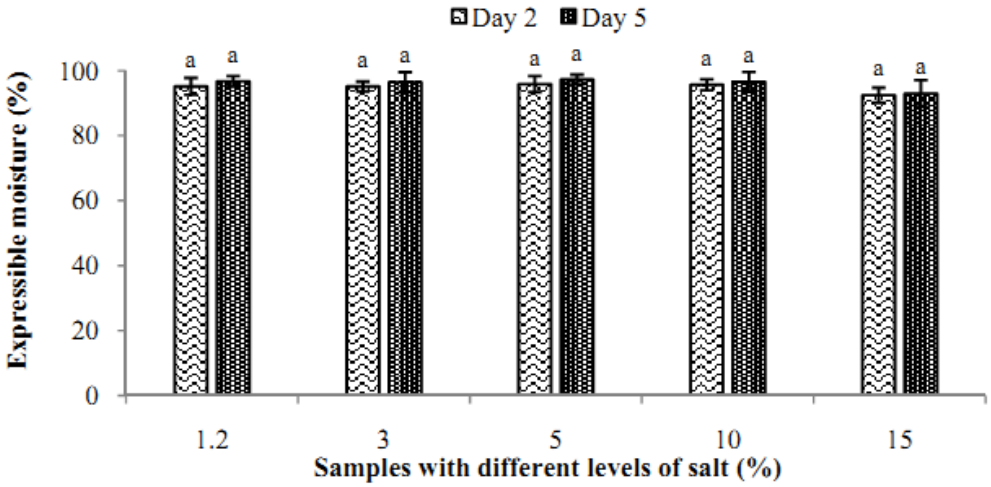

Figure 2. EM (%) of fresh CPS during 5 days storage at +2 ºC.Values are means of 3 analyses with ± standard deviation lines. The same small letters on the top of the bars indicate that there were no significant differences among samples (P>0.05).

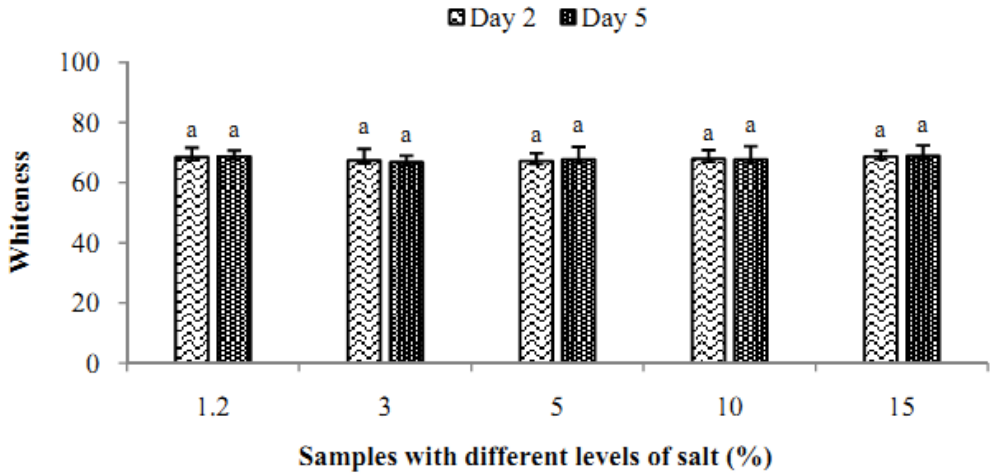

Figure 3. Whiteness of fresh CPS during 5 days storage at +2 ºC. Values are means of 3 analyses with ± standard deviation lines. The samesmall letters on the top of the bars indicate that there were no significant differences among samples (P>0.05).

Frozen CPS

The viscosity (BU) of frozen CPSs was influenced by salt content and cryoprotectants (Figures 4-5). Among the samples containing cryoprotectants, those with 15% salt had the highest viscosity after 14 weeks of storage at -24 °C, followed by the samples with 3% and 5% salt (P<0.05).The lowest viscosity was found for the samples with 1.2% salt at the same period of time (P<0.05). The viscosity (BU) of the sampleswith 3 and 15% salt and without cryoprotectants and those with 15% salt and with cryoprotectants did not change (P>0.05) during frozen storage. The increasing of the salt levels increased the viscosity (P<0.05). In both groups, the samples with 15% salt had the highest value of viscosity (BU) after 14 weeks of storage (P<0.05).

Generally the samples with cryoprotectants had higher WHC and lower EM than the samples without cryoprotectants (Figures 6-9). Among the samples with cryoprotectants, those with 5% salt had the highest WHC after 14 weeks of storage at -24 °C, followedby the sample with 3% salt (P<0.05). The lowest WHC was found for the sample with 1.2% salt followed by the sample with 15% salt (P<0.05) at the same period of time. The WHC of samples containing 1.2% and without cryoprotectants and those with 5% salt with/without cryoprotectants did not change significantly (P>0.05) during frozen storage (Figures 6-7).

The samples containing 1.2 and 15% salt and without cryoprotectants had the same level of EM after 14 weeks of storage (P>0.05).The EM of all samples without cryoprotectants except the sample with 15% salt contentdid not change (P>0.05)during storage. Among the samples with cryoprotectants the highest EM was found for samples with 1.2% salt content (P<0.05), followed by the samples with 15%

Table 1. Lightness (L*), redness (a*) and yellowness (b*) of fresh CPS samples during 5 days of storage at +2 ˚C

Characteristics	Age (days)		Cod protein solutions containing different levels of salt (%)				
			1.2	3	5	10	15
L*	2	NS	49.2 ± 0.4	48.7 ± 0.4	47.8 ± 0.4	48.7 ± 0.4	48.0 ± 0.2
	5	NS	49.6 ± 0.8	48.8 ± 1.0	48.3 ± 0.3	47.5 ± 0.2	47.8 ± 0.8
			NS	NS	NS	NS	NS
a*	2	NS	-2.3 ± 0.0	-2.1± 0.1	-2.2 ± 0.0	-2.1 ± 0.4	-2.1± 0.0
	5	NS	-2.3 ± 0.1	-2.3 ± 0.0	-2.3 ± 0.0	-2.3 ± 0.0	-2.2 ± 0.0
			NS	NS	NS	NS	NS
b*	2	NS	-6.4 ± 0.1	-6.2 ± 0.8	-6.5 ± 0.4	-6.4 ± 0.0	-6.9 ± 0.0
	5	NS	-6.4 ± 0.3	-6.0 ± 0.2	-6.4 ± 0.1	-6.7 ± 0.0	-7.0 ± 0.2
			NS	NS	NS	NS	NS

Each value is expressed as means ± S.D (*n=3*).Values in the same raw/ column are equal. NS=Not significant (P>0.05).

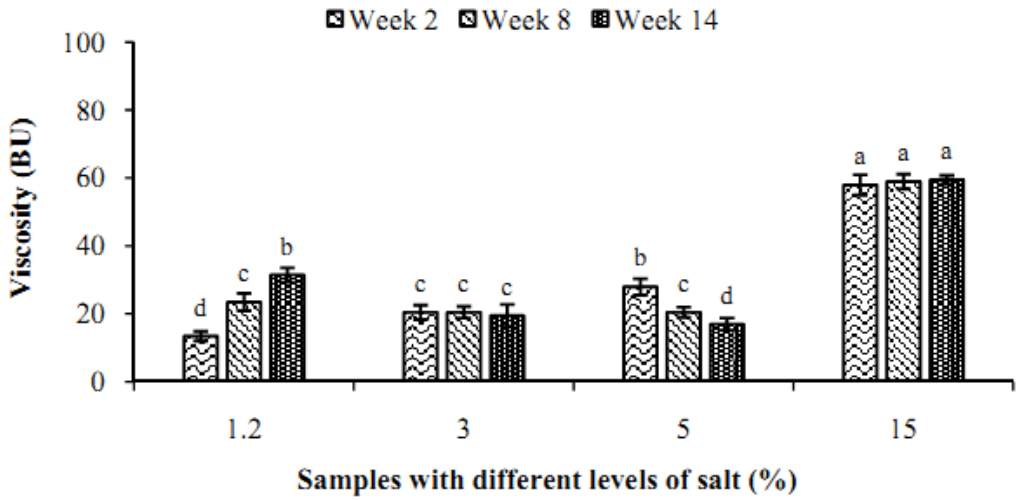

Figure 4. Viscosity of frozen CPS without cryoprotectants during 14 weeks of storage at -24 ºC. Values are means of 3 analyses with ± standard deviation lines. Different small letters on the top of the bars indicate significant differences (P<0.05).

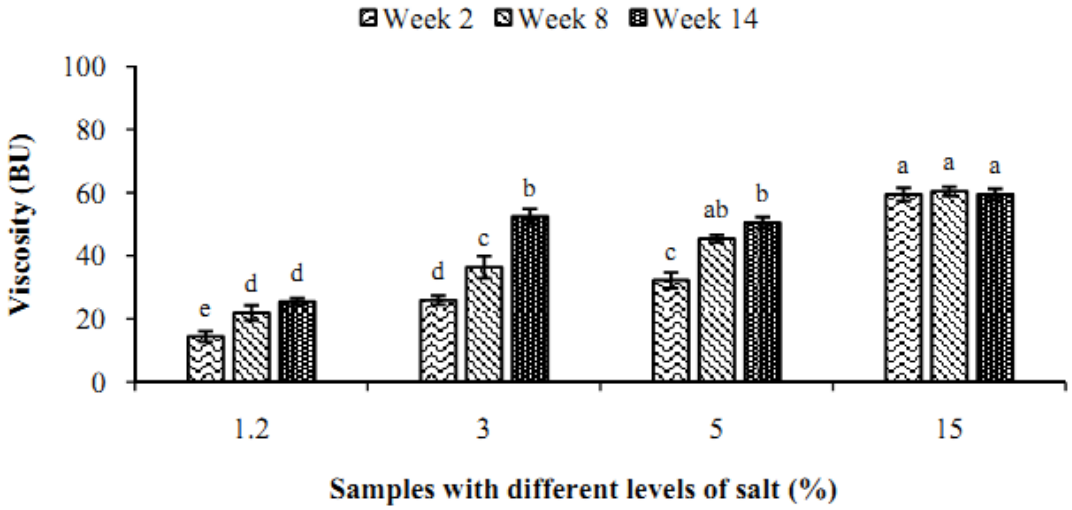

Figure 5. Viscosity of frozen CPS with cryoprotectants (sugar 1%, sorbitol 1% and sodium tripolyphosphate 0.1) during 14 weeks of storage at -24 ºC. Values are means of 3 analyses with ± standard deviation lines. Different small letters on the top of the bars indicate significant differences (P<0.05).

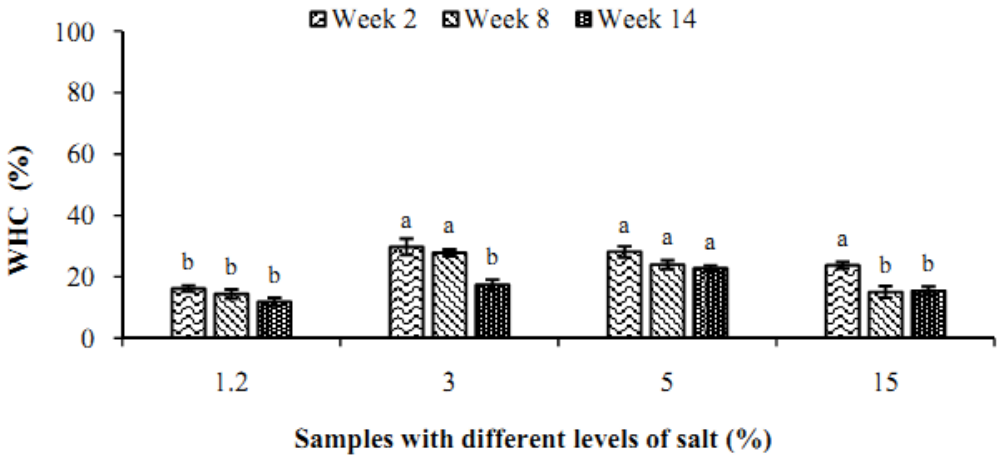

Figure 6. WHC of frozen CPS without cryoprotectants during 14 weeks of storage at -24 ºC. Values are means of 3 analyses with ± standard deviation lines. Different small letters on the top of the bars indicate significant differences (P<0.05).

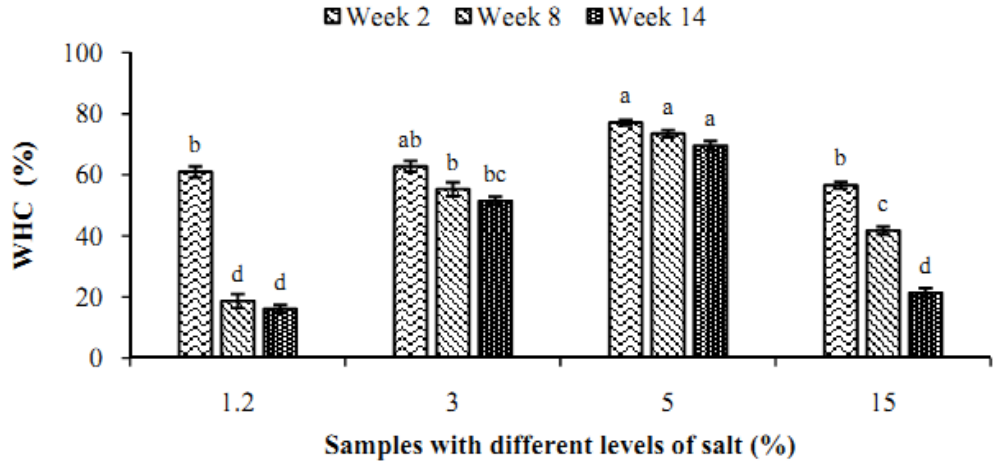

Figure 7. WHC of frozen CPS with cryoprotectants (sugar 1%, sorbitol 1% and sodium tripolyphosphate 0.1%)during 14 weeks of storage at -24 ºC. Values are means of 3 analyses with ± standard deviation lines. Different small letters on the top of the bars indicate significant differences (P<0.05).

salt content (P<0.05) after 14 weeks of storage. The EM of samples containing 5% salt and with cryoprotectants did not change significantly (P>0.05) during frozen storage (Figures 8-9).

The whiteness was not affected by the cryoprotectants. All samples with/without cryoprotectants had the same values of whiteness after 14 weeks of storage (P>0.05). The whiteness values of the frozen CPS and without cryoprotectants except the sample with 15% salt did not change (P>0.05) within the 14 weeks of storage time. The whiteness of the samples containing cryoprotectants and 5% salt did not change significantly (P>0.05) during 14 weeks of storage at -24 °C (Figures 10-11).

All samples with and without cryoprotectants had the same values of L*, a* and b* after 2 weeks of storage but cryoprotectants added samples had higher L* and b* values than the samples without cryoprotectants after 14 weeks of storage (P<0.05). L*, a* and b* values of the samples with cryoprotectants were not influenced by frozen storage (Tables 2-4).

Discussion

The spoilage of the fresh CPS samples may be associated with growth of halophobic bacteria (Horner, 1997; Kolodziejska *et al.*, 2002). The total volatile basic nitrogen (TVB-N) of fresh CPSs increased during storage probably due to increased enzyme activities. TVB-N is an important parameter for determining the freshness of fish products (Huss, 1995; Gökoğlu *et al.*, 1998). It is a general term which includes the trimethylamine, dimethylamine, ammonia and other volatile basic nitrogenous compounds associated with seafood spoilage (Huss,

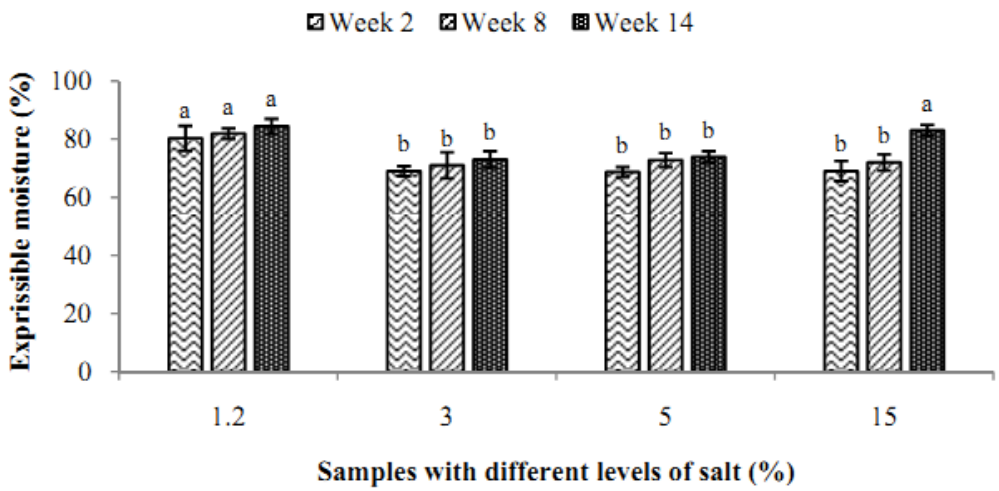

Figure 8. EM of frozen CPS without cryoprotectants during 14 weeks of storage at -24 ºC. Values are means of 3 analyses with ± standard deviation lines. Different small letters on the top of the bars indicate significant differences (P<0.05).

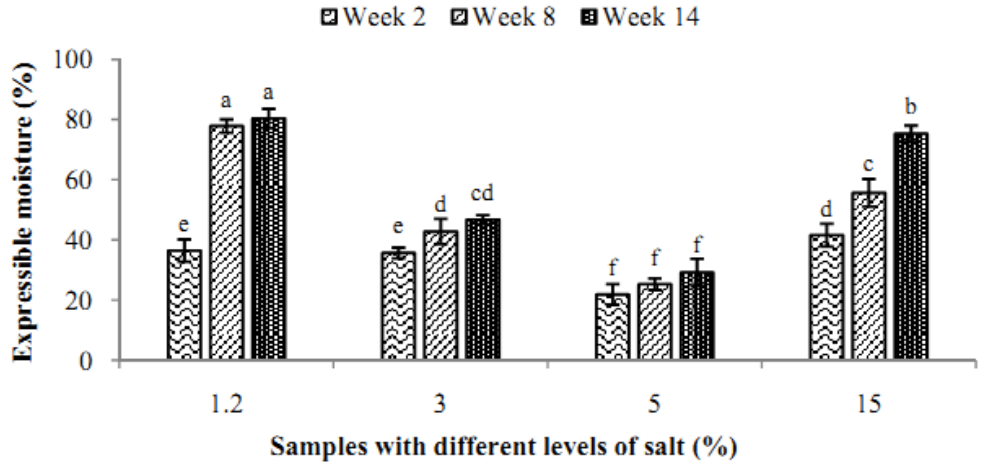

Figure 9. EM of frozen CPS with cryoprotectants (sugar 1%, sorbitol 1% and sodium tripolyphosphate 0.1%) during 14 weeks of storage at -24 ºC. Values are means of 3 analyses with ± standard deviation lines. Different small letters on the top of the bars indicate significant differences (P<0.05).

1995). The raw material was 4 days old when it was processed into CPS. This may have influenced the quality of CPS samples.

Partial denaturation and/or heat induced polymerization increase the hydrodynamic size of proteins and thus increase the viscosity (Damodaran, 1997). But other factors like temperature, shear rate, and induced force may also affect viscosity. Denaturation may explain the differences between treatments in this study.

The EM and the WHC of the protein isolates are affected by the storage time and temperature (Kristensen and Purslow, 2001) by levels of salt and cryoprotectants (Shaviklo *et al.*, 2010), and by muscle protein denaturation (Sigurgisladottir *et al.*, 2000, Thorarinsdottir *et al.*, 2002).WHC is increased in fish protein if phosphates are properly added (Hunt *et al.*, 2004). The activity of phosphates may be due to the effects on pH and ionic strength and specific interactions of phosphate anions with divalent cations and myofibrillar proteins. However, the effectiveness of the phosphates on WHC of fish products depends on the type and quality of the phosphate. The effect of phosphate to change the pH is in descending order of: pyrophosphates, tripolyphosphates, and hexametaphosphates. The extremes of pH can cause proteins to unfold and to increase their water binding (Feng and Hultin, 2001). This may indicate why CPS containing cyoprotectantshad a higher WHC than the control sample in this study. Similar results were reported for fish protein isolated from cod cut-offs (Shaviklo *et al.*, 2010).

The effect of salt on the WHC, and thereby the yield, has been reported by many authors (Hamm, 1960; Warrier *et al.*, 1975; Offer and Knight, 1988; Fennema, 1990). It is known that the WHC of fish flesh increases with increasing salt concentration up to 6%. When the salt concentration reaches levels

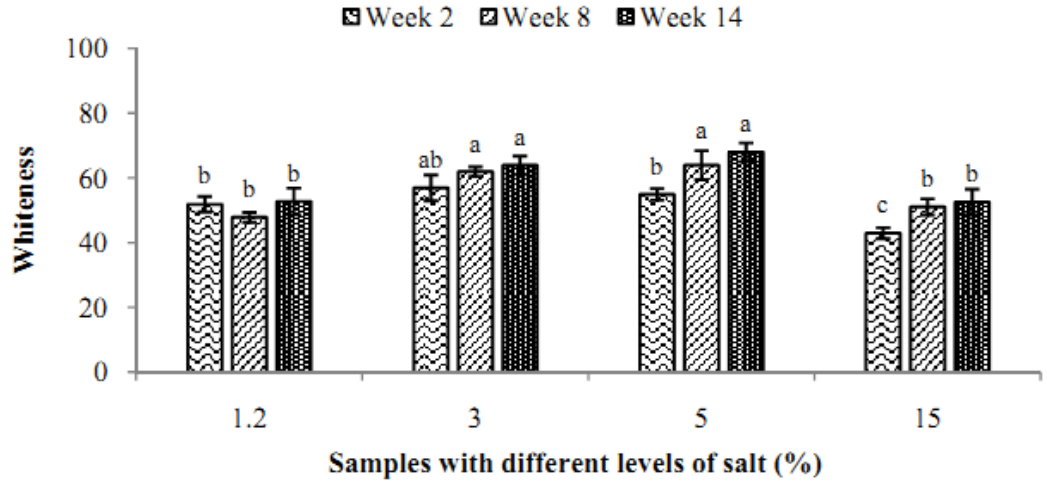

Figure 10. Whiteness of frozen CPS without cryoprotectants during 14 weeks of storage at -24 ºC. Values are means of 3 analyses. Values are means of 3 analyses with ± standard deviation lines. Different small letters on the top of the bars indicate significant differences (P<0.05).

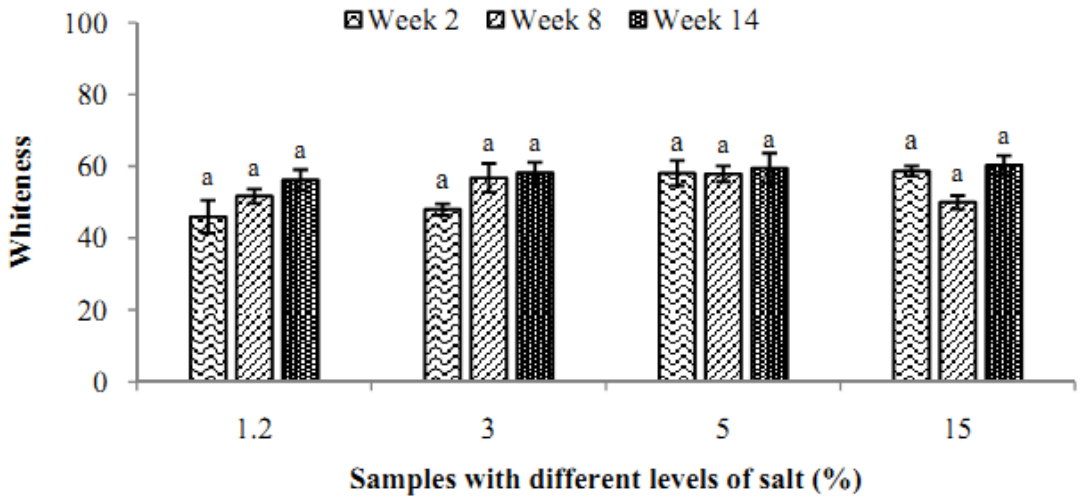

Figure 11. Whiteness of frozen CPS with cryoprotectants (sugar 1%, sorbitol 1% and sodium tripolyphosphate 0.1%)during 14 weeks of storage at -24 ºC. Values are means of 3 analyses with ± standard deviation lines. The same small letters on the top of the bars indicate that there were no significant differences among samples (P>0.05).

Table 2. Lightness (L*) values of CPS samples during 14 weeks of storage at -24 ˚C

Samples	Age (Weeks)		Cod protein solutions containing different levels of salt (%)			
			1.2	3	5	15
CPS without	2	NS	41.3 ± 0.3	42.2 ± 0.7	42.7 ± 0.5	44.1 ± 1.0^{a}
cryoprotectants	8	*	37.4 ± 2.0^{B}	41.9 ± 0.5^{AB}	48.8 ± 1.3^{A}	41.9 ± 3.0^{aAB}
	14	*	34.4 ± 1.0^{B}	41.7 ± 2.0^{A}	44.8 ± 0.4^{A}	36.4 ± 2.7^{bB}
			NS	NS	NS	*
CPS with	2	NS	43.2 ± 1.8	44.8 ± 1.3	49.0 ± 0.8	47.9 ± 0.2
cryoprotectants	8	NS	42.3 ± 2.5	44.9 ± 3.2	48.1 ± 2.0	46.2 ± 1.4
	14	NS	46.6 ± 1.2	40.2 ± 0.9	46.9 ± 0.8	41.6 ± 2.0
			NS	NS	NS	NS

Cryoprotectants: Sugar 1%, sorbitol 1% and sodium tripolyphosphate 0.1%.
Each value is expressed as means ± S.D (*n=3*). Different lowercase superscript letters in the same column indicate significant differences among products. Different uppercase superscript letters in the same row indicate significant differences for month of storage (* p<0.05). NS=Not significant (P>0.05).

Table 3. Redness (a*) values of CPS samples during 14 weeks of storage at -24 ˚C

Samples	Age (Weeks)		Cod protein solutions containing different levels of salt (%)			
			1.2	3	5	15
CPS without cryoprotectants	2	NS	-1.9 ± 0.4	-2.0 ± 0.2	-1.8 ± 0.2	-1.9 ± 0.2
	8	NS	-1.7 ± 0.2	-1.4 ± 0.0	-1.3 ± 0.1	-1.5 ± 0.1
	14	NS	-1.5 ± 0.3	-1.6 ± 0.3	-1.7 ± 0.2	-1.4 ± 0.3
			NS	NS	NS	NS
CPS with cryoprotectants	2	NS	-2.0 ± 0.5	-2.1 ± 0.4	-2.3 ± 0.4	-2.3 ± 0.1
	8	NS	-1.4 ± 0.4	-1.3 ± 0.4	-1.9 ± 0.4	-1.8 ± 0.2
	14	NS	-0.9 ± 0.1	-1.2 ± 0.2	1.6 ± 0.1	-1.5 ± 0.3
			NS	NS	NS	NS

Cryoprotectants: Sugar 1%, sorbitol 1% and sodium tripolyphosphate 0.1%
Each value is expressed as means ± S.D (*n=3*).Values in the same raw/ column are equal. NS=Not significant (P>0.05).

Table 4. Yellowness (b*) values of CPS samples during 14 weeks of storage at -24 ˚C

Samples	Age (Weeks)		Cod protein solutions containing different levels of salt (%)			
			1.2	3	5	15
CPS without cryoprotectants	2	NS	-3.5 ± 0.7^{a}	-3.5 ± 0.2^{a}	-3.6 ± 0.3^{a}	-3.3 ± 0.5^{a}
	8	*	-3.5 ± 0.4^{aA}	-5.8 ± 0.2^{bB}	-5.8 ± 0.4^{aB}	-3.6 ± 0.4^{aA}
	14	*	-6.1 ± 0.8^{bB}	-7.4 ± 0.1^{cA}	-6.7 ± 0.3^{bA}	-5.4 ± 0.6^{bB}
			*	*	*	*
CPS with cryoprotectants	2	NS	-3.2 ± 0.6	-3.2 ± 0.2	-3.0 ± 0.5	-3.6 ± 0.5
	8	NS	-3.8 ± 0.7	-3.7 ± 0.4	-2.9 ± 0.8	-3.4 ± 0.3
	14	NS	-2.9 ± 0.7	-3.5 ± 0.1	-3.6 ± 0.1	-3.8 ± 0.4
			NS	NS	NS	NS

Cryoprotectants: Sugar 1%, sorbitol 1% and sodium tripolyphosphate 0.1%.
Each value is expressed as means ± S.D (*n=3*). Different lowercase superscript letters in the same column indicate significant differences among products. Different uppercase superscript letters in the same row indicate significant differences for month of storage (* P<0.05). NS=Not significant (P>0.05).

above 10%, denaturation of proteins leads to decreased WHC of the muscle. This may explain why the CPS with 15% salt had the lowest WHC among the cryoprotectants added samples. In this study, increasing of the amount of salt added to the CPS increased the WHC, but the highest WHC was seen in the samples containing cryoprotectants, probably because of a combination of salt and sodium tripolyphosphate.The WHC of isolated fish protein by the pH-shift method is influenced by the pH (Shaviklo *et al.,* 2010). Kristinsson and Hultin (2003) reported that increasing pH from 6.4 to 7.4 increased WHC in fish protein isolate. So, adding cryoprotectants to CPS and increasing the pH could possibly increase the WHC in cryoprotectants added samples.

The whiteness of some samples was affected by the storage time. The whiteness increased significantly (P<0.05) in the samples containing 3 and 5% salt and without cryoprotectants after 14 weeks of storage at -24 °C.This is probably due to decreasing of b* value in all samples because of more coagulation and thus more opacity.The color and the whiteness of fish protein isolated by the pH-shift method depend on the content of connective tissue, lipids, co-precipitation of hem proteins and denaturation and oxidization of hemoglobin (Kristinsson *et al.*, 2005). It can be concluded that the protein and water contents and also the frozen storage time have important roles in the color and the whiteness of CPS.

Conclusions

The fresh CPS was very sensitive to spoilage. If the CPS is going to be used fresh it should be processed from a very fresh and well chilled (≤0 °C) raw material and kept at the same temperature until used for processing and used within 3 days. The frozen CPS was much more stable. But salt level, cryoprotectants and storage time influenced its properties. CPS with 5% salt and cryoprotectants was the most stable frozen product followed by the product with 3% salt and cryoprotectants. Like frozen FPI, fish protein solution should be protected during storage. Hence, applying 3-5% salt and a small amount of cryoprotectants is recommended for developing frozen stable FPS. Further research is needed for improving quality and how to apply frozen CPS as an ingredient to other products like fillets and formulated seafood.

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