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EFFECTS OF SALT AND PROTEIN INJECTION ON YIELD AND QUALITY CHANGES DURING STORAGE OF CHILLED AND FROZEN SAITHE FILLETS.

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ABSTRACT

Protein solutions prepared from saithe cut offs was used to inject saithe fillets. Weight gain, drip loss, fillet yield, cooking yield, colour, WHC, sensory evaluation, chemical and microbiological properties of chilled and frozen saithe fillets were investigated after chilled (2 and7 days) and frozen conditions (14 and 49 days). The results showed that injection of protein isolate resulted in higher drip loss but also higher fillet yield compared with the control. No difference was found in lightness (L value) and whiteness among the groups except that whiteness of frozen fillets injected with frozen isolate after 49 days was lower than that of the control. Frozen protein isolate injections resulted in a higher water holding capacity (WHC) but fresh protein injections in the lowest WHC of the frozen fillets. No difference was observed in sensory attributes, TVC, H2S, TVB-N, TMA and pH between the groups during chilled and frozen storage. Protein isolates showed positive effects on the sensory attributes such as texture attributes like tenderness and juiciness, and also on odour attributes like meat and vanilla. Based on these results, injection of protein isolate into saithe fillets is considered as an effective means to improve or stabilize the yield and the quality of saithe fillets.

Key words**:** *saithe, protein, injection, weight gain, drip, yield, WHC, sensory evaluation.*

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1. INTRODUCTION

1.1 Background

1.1.1 Fish fillets production and market

Fish fillets, especially from cod and pollock are very popular products that have been dominating the world market in fisheries. In recent years, the global annual harvesting of fish from the wild in oceans has been decreasing due to a big problem of overfishing in many fishing grounds (FAO, 2006). Market report from Global fish (2008) indicates that fillet prices have been increasing in recent years due to increasing demand and decreasing supply due to overfishing. According to FAO Fishstat data, the total world harvest of wild Atlantic and Pacific cod has declined dramatically from 1.8 million metric tons in 1997 to 1.2 million metric tons in 2005, while the total capture of Alaskan pollock has decreased remarkably from 6.7 million metric tons in 1986 to 3.8 million metric tons in 2003. Therefore, fullutilization of currently available fish fillets resources and searching for new fish fillets resources substitutes from other white fish is a must in order to meet the increasing market demand.

Saithe, a related species to cod, seems to be a good fish fillet substitute for cod. According to FISH INFO network market report (2006), saithe stock has been in a very good condition, and export of saithe fillets has increased, but the price has remained low. In fact, saithe has a good eating quality; however, it is not greatly prized as a good fish like cod. The colour of the fillets is not as white as that of cod, it is possible to remove the dark brown muscle from fillets, but it causes more fillet weight loss.

In recent years, the export of frozen fish fillets prepared from imported frozen fish has steadily increased in China due to the advantage of low labour cost, but the price is still lower compared to that of other countries due to inferior quality even if market demand has increased. Besides, large fish production from aquaculture makes it possible for China to export more fresh fillets by using various kinds of farmed fish to sell on the international market. Frozen products are susceptible to drying out during storage and lose their characteristic flavour; therefore, they must be well glazed and wrapped in either waxed paper or polyethylene film before storage to prevent dehydration.

1.1.2 Utilization of fish by-products

Fish processing has been growing very fast in recent decades, and a large amount of fish by-products from processing have been produced. For an example, in cod fillet processing 52-55% are by-products including head, backbone, skin and trimmings based on a gutted cod with head (Arason, 2003). Among them, 3% of skin and 10-18% flesh from the by-products can be expected (Kristbergsson and Arason 2007). However, the amount of products prepared from by-products is still limited and the value is low. Few products like protein isolate, surimi and gelatine are produced for human consumption from fish by-products, some of them go to animal feed, and the others have been thrown away. Lack of protein has been a big problem challenging

the world, and resources of both wild fish stocks and protein from fish are still limited. Besides, a dramatically decreased harvest of white fish for filleting undoubtedly has resulted in increasing fish material and production cost, therefore, full utilization of fish by-products is not only important but profitable as well.

1.2 Objective

In the present project, isolate protein and gelatine from saithe filleting by-products combined with brine will be injected into saithe fillets, and the influence of protein injection on the quality and yield of saithe fillets stored as chilled and frozen during different storage time will be studied. The variables evaluated are fillet yield, WHC, liquid loss, cooking yield, colour, pH, TVC, SSO, TVBN and TMA and sensory scores. The objective is to find a good method for reducing liquid loss and yield reduction to maintain the quality of saithe fillets, and a goal of value-added by-products by injecting proteins in fillets will hopefully be achieved.

2. LITERATURE REVIEW

2.1 Quality of fish fillets

Two very important quality indicators of fish fillets, shelf life and freshness have been well documented for the past decades. Nowadays, great attention has been paid to the fish fillet quality and consumers taste, by maintaining or improving, water holding capacity, juiciness, fillet yield and cooking yield of the products.

2.1.1 Water holding capacity and liquid loss of fish fillets

The water holding capacity (WHC), usually expressed as liquid loss of muscle and is regarded as an essential quality parameter and a high WHC is of great importance both to the industry and the consumer (Duun *et al*. 2007). It influences not only the appearance of the muscle before cooking, but also cooking yield and juiciness when consumed (Olssona, 2003c). The final weight of the fillets is determined by both process yield and liquid loss during storage, and thus, a liquid loss may lead to a reduction of price.

Considerable work has addressed liquid loss of fish muscle. Liquid loss is influenced by factors like rigor state, storage time, storage temperature, species, pH, and added salt content in the products. Pre-rigor-filleted cod muscle has a significantly higher liquid loss than post-rigor-filleted cod (Kristoffersen *et al.* 2006; Kristoffersen *et al*. 2007). Liquid loss increases with the time of storage (Kristoffersen *et al*. 2007). Duun *et al*.'s (2007) study showed that liquid loss was significantly higher in super chilled fillets compared with ice chilled and frozen fillets. Liquid loss was higher in the muscle of farmed fish than in the wild due to lower pH in farmed fish. It was observed that a low pH was associated with reduced water-holding capacity (Ofstad *et al*. 1996c), and a low ultimate pH would result in a higher liquid loss (Rustad 1992, Olsson *et al.* 2003c).

Brining has been well documented and widely used in the fillet industry. Brining significantly reduces the weight loss of fish fillets during storage (Esaiassen *et al*. 2008), and brining with low salt concentration also gives a higher process yield of smoked salmon (Gallart-Jornet *et al.* 2007). Lower salt concentrations are known to increase water holding capacity and less protein denaturation compared to high salt concentrations (>10-12%) (Barat *et al*. 2002).

In recent years, attention has been paid to protein in liquid loss. The total amount of proteins loss correlates to that of liquid loss (Olssona *et al*. 2003c, Kristoffersen *et al.* 2007), and proteins in drip have been found to be similar to the sarcoplasmic proteins extracted from the muscle (Kristoffersen *et al*. 2007). Liquid loss is closely related to the change of microstructure of the fish muscle. Depolymerisation of myofibrillar proteins results in the formation of a homogeneous protein matrix, where pores and gaps appear in the protein matrix, thus enhancing liquid loss (Ofstad *et al.* 1995, 1996a).

Effects of collagen on the quality of fish fillet have been studied. Melted collagen is seen either as a thin film or as an aggregated network structure, depending on the

temperature. It can fill the pores and gaps in the aqueous phase (Ofstad *et al.* 1996b). Interesting finding were observed in halibut that spoilage bacteria present had higher collagenolytic activities, which then contributed to the increased WHC (Olsson 2003b). Gelatine produced from collagen by hydrolysis has a gel forming ability and has been widely used in food as a binding agent. In the present project, fish skin gelatine will be used to maintain the gelling ability of fish fillet.

Fish sarcoplasmic proteins have been reported to enhance gelation of muscle proteins. Sarcoplasmic protein possess a cross-linking activity due to the contribution of the residual activity of transglutaminase contained in the sarcoplasmic protein fraction, thus resulting in enhancement of gel strength and breaking force (Ko and Hwang. 1995, Karthikeyan *et al.* 2004, Benjakula *et al.* 2004, Yongsawatdigul and Piyadhammaviboona 2007, Piyadhammaviboona 2009), Sarcoplasmic protein can be a good protein additive that maintains textural properties of fish muscle products like surimi and a new way of utilizating fish by-products.

2.1.2 Fish fillet quality improvement by addition of additives and methods

2.1.2.1 Addition of salt and other additives

The quality of fillets can be maintained by the application of additives or salt. It has been well documented by the studies that salt has positive effects on fish fillet quality. Brining of fish fillets can improve fillets palatability and water holding capacity, even increase consumers overall liking compared to non-brined fillets (Esaiassen *et al*. 2004, 2005). Studies have also shown that brining can reduce gaping and weight loss during storage and increase product yield (Barat *et al*. 2002, Esaiassen *et al*. 2008, Larsen *et al.* 2008). However, brining also results in the loss of soluble components within the fillets, such as free amino acids, vitamins and proteins (Martínez-Alvarez *et al.* 2005, Larsen *et al.* 2007, Rune Larsen *et al*. 2007) especially due to pH change and high salt contents in fillets (Martínez-Alvarez *et al.* 2005). Research demonstrated that relatively low salt levels have a more positive impact than higher salt levels on maintaining the quality of fish fillet final products (Thorarinsdottir *et al*. 2004a, Yasemen Yanar 2006, Larsen *et al.* 2008). Brines with small concentrations of salt can promote better yield and water holding capacity of salted fish fillets than saturated brines (Barat *et al.,* 2002).

In recent years, salt mixed with additives such as phosphate, glucose and ascorbate, have been used in fish fillet processing (Thorarinsdottir *et al*. 2001, 2004a; Esaiassen *et al.* 2004, 2005, Woyewoda and Bligh 2006. Studies show that the mixture of additives can decrease liquid loss, increase water holding capacity and enhance consumer preferences (Thorarinsdottir *et al.* 2004b, Esaiassen *et al.* 2004, 2005). SFK 428, a mixture of phosphates products, has been commonly adopted in improving the quality of frozen fish fillets, water holding capacity and product yield.

Frozen and thawed fish products are generally characterized by of having a lower quality than fresh ones. Especially products exposed to repeated freezing and thawing cycles (Mackie 1993, Nilsson and Ekstrand 1995, Hurling and McArthur 1996). However, recent studies have shown that it is possible to enhance the consumers liking of frozen and thawed cod fillets by brining with a commercially available brine

mixture consisting of salt, phosphates, sodium-ascorbate and glucose (Esaiassen *et al*. 2004, 2005). Improvement of quality like juiciness, liquid loss and texture by additives addition were also found in the muscle of other frozen products (Krivchenia and Fennema 1988, Dziezak 1990, Craig *et al.* 1991, MacDonald and Lanier 1997, Park *et al.* 1997, Badii and Howell 2002, Herrera *et al.* 2002, Qu *et al.* 2003).

Reducing dietary salt intake and increasing potassium intake has been recommended (Appel *et al.* 2006). A new study indicated that KCl could be used in fillet brining as asubstitute for NaCl (Larsen *et al*. 2008). Usually, naturally occurring salt content in fish raw material is quite low, approximately 0.3%, (van Klaveren and Legendre 1965, Thorarinsdottir *et al,* 2004b). The addition of proteins may compensate for reduced salt levels, in order to increase water holding capacity.

2.1.2.2 Addition of proteins

The addition of functional proteins to muscle food has been successfully practiced in the meat industry. It has been shown that soy protein isolates can improve water and fat binding properties of products (Cunningham *et al*. 1988). Functionality some fish proteins have been added to meat products has been done. Kristinsson *et al*. (2000a) proposed that salmon protein hydrolysates reduces drip of salmon mince patties after freezing. The addition of fish protein concentrates prepared from sardine has also shown to improve the cooking yield of hamburgers (Vareltzis *et al*. 1990). However, little literature on the addition of functional proteins by injection into fish products has been published. A patent (WO/2004/071202) describes a method to inject a high concentration of 8% to 20% protein hydrolysate from salmon bone off-cuts by enzyme with 18% brine to smoked salmon fillets and then to storage in cold and frozen conditions. It shows positive effects on lightness and rancidity. Sensory evaluation showed that smell and taste slightly changed. Thorarinsdottir *et al.* (2004a) indicated in their study that higher water holding capacity and less liquid loss could be achieved by injecting frozen cod fillets with either a combination of soy protein concentrate or fish hydrolysate with salt or phosphate.

2.1.2.3 Methods of adding salt, additives and proteins to fish fillets

Several methods are now available to be applied when incorporating additives to fish muscles. Brining is a widely adopted method for fish fillets. It is a process of immersing fish fillets in brine at a low temperature. Brining time depends on the salt content in brine and optimum salt content in products. Sometime it is accompanied by vacuum tumbling to lessen the time of adding ingredients to fish fillets (Esaiassen *et al.,* 2004, 2005). Another method is brine injection is a process of injecting brine directly into the fish products, it features not only a shortening time of processing, but also adds a mixture of additives into the fish fillets more easily and effectively, compared with brining. Injection was found to be a suitable method to supply salt into salted fish fillets (Rørå *et al*. 2004, Birkeland *et al*. 2007, Birkeland *et al*. 2007, Akse *et al.* 2008) because brine injection makes it easier to obtain a homogenous salt distribution in the muscle (Rørå *et al*. 2004) and salt content can be controlled at a required level (Birkeland *et al*. 2007). Phosphate, which is commonly used in fish processing, was also added to other meat products by injection (Wynveen *et al.* 2001).

2.1 Products and their properties from fish by-products

The main products prepared from the fish by-product are restructured products like mince block and surimi, protein isolates, protein hydrolysates and gelatine, etc. Protein isolates and hydrolysates are usually dried powders, and surimi and mince blocks are frozen products. The value of all the products is quite low compared to the fillet itself. However, the properties and composition of the products prepared or proteins recovered from the by-products can be changed. Some functionality of the protein is weakened or lost due to different methods or treatments. Surimi is produced by washing a few times with water, where a large amount of water soluble proteins and nutrients leach out. Protein isolates, common products of recovering proteins from fish by-products in the seafood industry, are usually prepared by shifting pH to extreme values. The aim is to solubilise and precipitate the proteins by adding acid and alkaline which inevitably result in a change of the protein properties and a loss of water soluble nutrients. Protein hydrolysates are usually made by enzymatic degradation of the muscle, followed by enzyme inactivation by high temperatures above 90 C. The process also results in changes of protein properties. The main functionality of protein lost in protein recovering from fish by-products is gel forming properties (Chen *et al.* 2007ab, Pe´rez *et al*. 2006). Practically, binding agents like transglutaminase are commonly used to improve the texture of protein-rich foods such as surimi or ham (Yokoyama *et al.* 2004). Especially gelling ability for fish minces (Benjakul *et al.* 2008) and restructured products Ramírez *et al*. 2007ab, Vácha 2006. In fact, properties and composition of flesh from the fish by-products of trims and frames are the similar to fillets. Therefore, it is promising work, to add mince or fresh fish fillet where the added proteins have a minimal change on the functional property of native fish protein.

Fish skin is a major by-product of the fish processing industry, and provides a valuable source of gelatine (Badii and Howell 2006). Interest in fish gelatine has been increasing because it has a good gel forming ability and can be used as a binding agent in food formulations. It has been considered an attractive alternative for mammalian gelatine where domestic animal products are not considered desirable. (Karim and Bhat 2008). However, cold-water fish gelatines have low gelling and melting temperatures compared to mammalian and warm-water fish gelatines (Gilsenan and Ross-Murphy 2000), gelatine from cod, haddock and pollock exhibited considerably lower gelling (4-5C) and melting temperature (12-13C) compared to mammalian gelatine gels, which makes these gelatines unsuited for mammalian gelatine replacements in food industry (Haug *et al*. 2004). On the other hand, the low melting point of coldwater fish gelatine enhances the release of flavour (Choi and Regenstein 2000), and products can be stored at refrigerated temperatures or frozen without negative effect on the functional properties of the gelatine (Gudmundsson and Hafsteinsson 1997). The functional properties of fish gelatines can be improved by the addition of salts and TGase (Ferna´dez-Dı´az *et al*. 2001, Sarabia *et al*. 2000). Applying the gelatine to fish fillets seems to be a possible way to reduce liquid loss from fish fillets.

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Fish

Saithe used for the project was caught from the north Atlantic Ocean, during a fishing trip between 28th of November and 3rd of December 2008. The saithe was separated from cod and slaughtered and gutted on board. It was then collected into tubs and kept on ice until processed. The fish was landed on 3rd of December. Part of the catch (280 kg) was selected for the trial.

3.1.2 Fillet processing

On 4th of December, the selected fishes (1.8-2.5 kg) were beheaded (Baader 434), filleted (Baader 252) and skinned (Baader 51, Nordischer Maschinenbau, Rud. Baader GmbH and Co, Lubeck Germany). The fillet size ranged from 500 to 700g. Most of the fillets were used for injection but part of it was minced for protein isolate production.

3.1.3 Mince processing

Saithe mince for injection was produced in a bone separator (SEPAmatic, Bergisch Gladbach, Germany) with a 2 mm drum. Then half of the fresh mince was kept on ice for 30 h, the other half was fast frozen (2 or 3 h to reach the target temperature) and stored frozen for 24 h at -24 °C.

3.1.4 Preparation of protein isolate

The frozen mince was thawed by adding 0-1 °C cold water for 6 h. Approximately 4 parts of cold water (0-1 °C) was added to 1 part of fresh or thawed frozen mince. Salt was added to reach 1.5 % of the final salt concentration in the solution used for injection. After that it was homogenized at about 7000 x g by a special homogenizer to get a solution with 3% protein content (referred to as protein isolate in this report). After homogenisation the solution was sieved to reach a partial size in the range of 0.5 to 1.0 mm, to make it suitable for injection.

3.1.4 Gelatine

The gelatine used was high molecular weight fish gelatine, dried (Norland Products Inc., Shelburne, Nova Scotia, Canada). It was a granulate product with 86.89% protein content (Table 1).

Table 1. Specifications for the gelatine (Norland Products Inc., Shelburne, Nova Scotia, Canada) used in the experiment.

	SPECIFICATIONS	BATCH NO. 8003 HMWD
APPEARANCE	GRANULATE	OK
COLOR	1-13	2.0
ODOR	NONE	OK
IDENTITY	CORRESPONDS	OK
pH OF 10% SOLUTION	5.0-7.0	5.92
VISC OF 10% SOLUTION @ 30C	25.0 - 35.0 CS	29.04 cs
LOSS ON DRYING	MAX. 13.5%	12.50%
PROTEIN	MIN. 80%	86.89%

3.1.5 Preparation of solutions for injection

All solutions were prepared by using tap water (approx. 5°C). Salt concentration was 1.5% salt (w/w). The protein concentrate of both fresh and frozen isolates solution was 3%, while the protein concentrate of gelatine was 2% and 1.5%. Viscosity of the solutions for injection was a limiting factor with regard to protein concentration. For comparison, untreated fillets and treated fillets were injected with solutions containing solely salt (1.5%).

3.1.6 Salt

Food grade pure dried vacuum salt (>99.9% NaCl) was used for preparation of the brine with a purity of 99.9%.

3.2 Injection

Injection was carried out, immediately after filleting by adopting a brine injector (Dorit INJECT-O-MAT, PSM-42F-30I, Dorit Fleischereimaschinen GmbH, Ellwangen, Germany) to obtain approximately 10% pick-up for injected fillets. The temperature of the solutions injected and the processing workshop where the injection took place was 5 °C and 16 °C, respectively. After injection, fillets were placed carefully on a grid for 10 min to guarantee that the solutions injectedeffectively spread inside the muscle before packaging and to drain off excess solution liquid (Akse *et al.*, 2008).

3.3 Experiment design and sampling

The experiment was conducted by injecting 6 different solutions (6 groups) into saithe fillets:

- Fresh protein isolate (3% protein+ 1.5% salt, FePI),
- Frozen protein isolate (3% protein+ 1.5 % salt, FoPI),
- Gelatine (1.5% protein+ 1.5% salt, G),
- Gelatine (2% protein+ 1.5 % salt, G1) – (used to evaluate uptake due to inj.)

- Salt (1.5 %) without protein (S) and
- Control (F).

After injection, half of the fillets were packed in Styrofoam boxes with filter paper on the bottom to absorb excess water, and stored in cooler at -1 °C for 4 h, the other half of fillets were frozen immediately at -24 °C for 3 h and packed in cartons lined with plastic bag. Each box or carton contained 12 pieces of fillets, and then all the fillets were transported to Matis, Reykjavik, within 5 h under the temperature of 0 °C and -18 °C, respectively. The fillets were stored chilled (0 °C) and frozen (-24 °C) until sampling and analyses took place (Table 2).

Sampling was done on day 2, day 5 and day 7 after the injection for the chilled fillets and on day 14 and day 45 for the frozen fillets (Tables 3 and 4). Tables 5 and 6 below illustrate the sampling and analysis of fresh and frozen mince, and the sampling and analysis of the solutions for injection, respectively.

Table 2. Experimental design

Injection	**Storage condition**	**No of Sample**	**Fillets /group**	**Total Fillets**	**Total Fillet Weight(kg)**
- (control)	Chilling	3	12	36	18
- (control)	Frozen	2	12	24	12
Salt 1.5%	Chilling	3	12	36	18
Salt 1.5%	Frozen	2	12	24	12
FePI 3.0% +salt 1.5%	Chilling	3	12	36	18
FePI 3.0% +salt 1.5%	Frozen	2	12	24	12
FoPI 3.0% +salt 1.5%	Chilling	3	12	36	18
FoPI 3.0% +salt 1.5%	Frozen	2	12	24	12
G 1.5% + salt 1.5%	Chilling	3	12	36	18
G 1.5% + salt 1.5%	Frozen	2	12	24	12
G1 2,0% + salt 1.5%	Chilling	3	12	36	18
G1 2,0% + salt 1.5%	Frozen	2	12	24	12

FePI = fresh protein mince; FoPI = frozen protein mince; G = gelatine used to prepare solution for injection

Table 3. Sampling and analysis of fresh fillets

Indicator	Storage time (d) 0	2	5	7
Weight gain	v			
Drip loss		V	v	v
Yield		V	v	v
Cooking yield		V	v	v
Colour		V	v	v
Salt			v	
pH		V	v	v
WHC		V	v	v
Sensory Evaluation		V		
TVB-N, TMA		V		v
TVC, H_2S		V		v

Table 4. Sampling and analysis of frozen fillets

Indicator	Storage time (d) 0	14	49
Weight gain	V		
Drip loss		v	v
Yield		v	v
Cooking yield		v	v
Colour		v	v
Salt		v	
pH		v	v
WHC		v	v
Sensory evaluation		v	v
TVB-N, TMA		v	v
TVC, H_2S		v	v

Table 5. Sampling and analysis of fresh and frozen mince

Colour		v
pH		v
Protein ontent		v
Water		v
Salt		v
TVC, H_2S		v
TVN/TMA		v

Table 6. Sampling and analysis of solutions for injection

Protein isolate /Gelatin solution	Colour	v
	Viscosity	v
	Solubility	v
	Viscosity	v
	TVC, H_2S	v
	Water	v
	Protein content	v
	Salt	v
	pH	v
	TVB-N/TMA	v
Salt	TVC and H_2S	v
	Salt	v
	pH	v

3.4 Determination of weight gain after injection

Injected fillets were weighed 5 minutes after injection before packaging into Styrofoam boxes (Birkeland *et al*, 2007). Fillet weight gain was determined based on fillet weight before injection as follows:

Weight gain (%) = 100 × (g injected fillet$_{\text{before storage}}$ − g fillet $_{\text{before injection}}$) / (g fillet $_{\text{before injection}}$).

3.5 Determination of drip loss during storage

Drip loss was expressed as weight reduction during storage. Drip loss of chilled fillets was determined according to Larsen *et al.* 2008. Fillets were removed from boxes and weighed excluding the drip. Drip loss was determined based on injected fillet weight as follows:

Drip loss (%) = 100 × (g $fillet_{before\ storage}$ – g $fillet_{after\ storage}$) / (g $fillet_{before\ storage}$).

Drip loss of frozen fillet stored in frozen condition was determined according to Bigelow and Lee (2007). Frozen fillets samples were put on plastic pellets with small size holes and thawed at 4 °C overnight (24 h). Thawed fillets were removed from the pellet, left to drip on the plastic film on the top of pallet, and the drip was left on the plastic film.

Drip loss (%) = 100 × (g $fillet_{before\ freezing}$ – g thawed $fillet_{after\ frozen\ storage}$) / (g $fillet_{\ before\ freezing}$).

3.6 Determination of fillet yield after storage

Fillet yield after storage was determined according to Thorarinsdottir *et al.* (2004a, 2002). The fillets were weighed as raw material and after storage; yield was determined by the observed changes in weight with respect to the weight of the raw fillets as follows:

Yield (%) = g $fillet_{after\ storage}$ / g $fillet_{before\ injection}$.* 100

3.7 Determination of cooking yield

Cooking yield was determined according to Bigelow *et al*. (2007). Each fillet (n=3) was cut into three parts for each fillet:

- ✓ Front part (85-90 g),
- ✓ Middle part (75-80 g)
- ✓ Tail part (65-70 g),

Then placed on baking paper on the grid and cooked at 95 - 100 °C for 15 min in a preheated conventional steam oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany). After cooking, samples were cooled down at room temperature for 8 min, and then reweighed. The cooking yield was calculated as follows:

Cooking yield (%) = g cooked fillet / g fillet before cooking.

Cooking yield of the whole fillet was the average of the three parts cut from the fillet.

3.8 Measurement of colour

Colour measurement of L (lightness), a (red-green colours) and b (yellow-blue colours) of the saithe fillets was performed by a Minolta Chroma Meter CR-300 (Minolta, Osaka, Japan) using light source D, CIE standard illuminates for daylight. W (whiteness) was calculated as W = L – 3b* according to Park (1994). Measurements of three parts (head, middle and tail) of each fillet was carried out and the average value of the three parts represented the value of each fillet. Three fillets (n = 3) for each group was done.

3.9 Determination of water-holding capacity

The WHC was determined by a centrifugation method (Eide *et al*. 1982). The saithe samples (n = 3) were coarsely minced in a mixer (Braun Electronic, Type 4262, Kronberg, Germany) for approximately 20 s at speed 4. Approximately 2 g of the minced saithe muscle was weighed accurately into a sample glass with membrane (100 µm) on the bottom (height 62 mm, inner diameter 19 mm and outer diameter 25 mm) and immediately centrifuged at 1350 x g for 5 min, with a temperature maintained at 2 °C to 5 °C in rotor SS-34 for Sorvall centrifuge type RC-5B (Dupoint, USA). The water remaining after centrifugation was divided by the water content of the fillet and expressed as % WHC (Thorarinsdottir, *et al*, 2004).

$$\text{WHC} = \frac{W1 - \Delta r}{W1} * 100(\%)$$

3.10 Water content determination

Water content of the fresh fish was determined according to ISO 6496:1999(E). About 5 g of homogenised fillets was mixed thoroughly on a dish with sand using a glass rod. The glass rod was kept on the dish and then left to dry for 4h ± 0,1h in the oven at 103°C. The dish was removed from the oven and allowed to cool to ambient temperature in the desiccators for about 15 minutes. The water content was calculated by formula as follows:

$$\frac{M1 - (M3 - M2)}{M1} \times 100(\%)$$

M1 is the mass, in grams, of the test portion.

M2 is the mass, in grams, of the dish, test portion, sand and glass rod.

M3 is the mass, in grams, of the dish, dried test portion, sand and glass rod.

3.11 Sensory evaluation

Quantitative Descriptive Analysis (QDA), introduced by Stone and Sidel (1985), was used to assess cooked samples of saithe fillets. An unstructured scale (0-100%) was used on a vocabulary with defined sensory attribute describing door, flavour, appearance and texture; fifteen panellists of Matis sensory panel participated in the QDA of the cooked saithe fillets. They were all trained according to international standards (ISO 8586-1 1993); including detection and recognition of tastes and doors,

training in the use of scales, and in the development and use of descriptors. The members of the panel were familiar and trained in applying the QDA method for cod and used a vocabulary previous developed for cod products (Wang *et al*, 2008; Sveinsdóttir *et al* 2009). Each panellist evaluated duplicates of each sample in a random order in eight sessions.

All sample observations were conducted according to international standards (ISO 8589 1988). Samples weighing 40–50 g with 2.5 x 2.5 cm size were taken from the loin part of the fillets and placed in aluminium boxes coded with three-digit random numbers. 3 pieces of fillets for each group were randomly selected. The samples were cooked at 95-100C for 7 min in a pre-warmed oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany) with air circulation and steam and then served to the panel. A computerized system (FIZZ, Version 2.0, 1994-2000, Biosystémes) was used for data recording.

QDA data was corrected for level effects (level effects caused by level differences between assessors and replicates removed) by the method of Thybo and Martens (2000). Multivariate comparison of samples and significant sensory attributes was done with principal component analysis (PCA) on mean level corrected values, using full cross validation. All multivariate analysis was conducted in the statistical program Unscrambler v9.7 (CAMO Software AS, OSLO, Norway). Analysis of variance (ANOVA) was carried out on a level corrected QDA data in the statistical program NCSS 2000 (NCSS, Utah, USA). Duncan´s Multiple-Comparison Test was used for stepwise comparison at the 95% significance level.

3.12 Microbiological analysis

Three pieces of fillets from each group were randomly selected and aseptically minced separately in the experiment and the basic methodology used in the laboratory was according to NMKL (Nordisk Metodikkomité for Næringsmidler) and Compendium of Methods for the Microbiological Examination of Foods published by the American Public Health Association *(APHA 1992).*

Twenty five g of minced fillet were weighed and homogenized in 225 mL of cooled Maximum Recovery Diluent (MRD, Oxoid) for 1 minute in a stomacher to make 1/10 dilution. Further decimal dilutions were made and then 0.1 ml of each dilution was transferred with pipettes onto the surface of Petri plates. Total viable psychotropic count (TVC) was evaluated by spread-plating aliquots on pre-chilled plates of Modified Long and Hammer's medium (LH) containing 1% NaCl (van Spreekens 1974) and incubated aerobically for five days at 17°C. TVC and selective counts of H_2S-producing bacteria were enumerated on iron agar (IA) as described by Gram *et al.* (1987) with the exception that 1% NaCl was used instead of 0.5%. Plates were surface-plated and incubated at 15°C for five days.

3.13 Determination of TVB-N and TMA

Total volatile basic nitrogen (TVB-N) and trimethylamine (TMA) were determined in triplicate by the methods 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 H2SO4 solution. To determine TMA, the same method was used as for TVB-N but by adding 20 mL of 35% formaldehyde to the distillation flask to block the primary and secondary amines. The TVB-N and TMA content were expressed in mg N/100 g saithe tissue.

3.14 Measurement of pH

The pH measurements were performed with a pH electrode (SE 104 Mettler Toledo GmbH. Greifensee, Switzerland) connected to a Knick pH meter (Portames 913 pH, Knick, Berlin, Germany). The electrode was immersed directly in the minced samples at 20 ± 2 °C. The pH meter was previously calibrated with buffer solutions of pH 7.00 ± 0.01 and 4.00 ± 0.01 at 20 °C. And the sample was done in triplicate.

3.15 Salt and protein content

The salt content (% w/w) was determined according to the AOAC Official Methods of analysis (AOAC 2000). The total protein content of the fish muscle was estimated with the Kjeldahl method (ISO-5983, 2005) and calculated using total nitrogen (N) * 6.25.

3.16 Statistical analysis

Analysis of variance (ANOVA) was carried out on all measured quality attributes in the statistical program NCSS 2000 (NCSS, Utah, U.S.A.). The program calculates multiple comparisons using Duncan's test to determine which sample groups are different. Significance of differences was defined at the 5% level ($P < 0.05$). Principal component analysis (PCA) was conducted on sensory data using Unscrambler (version 9.7, CAMO, Trondheim, Norway).

4. RESULTS

The mince produces contained 81% water and 17.5% protein, the TVN content was 21 mgN/100g and a total viable count of 5.1 (log number /g). Microbial counts were reduced by the freezing by 1 log value (Table 7).

Table 7. Chemical content and microbial count (Total viable count and H_2S producing bacterias) in mince and solutions used for injection of fillets.

	Water (%)	**Protein (%)**	**Salt (%)**	**TMA (mg N/100g)**	**TVN (mg N/100g)**	**TVC/g**	**H_2S/g**
Mince fresh	81.0	17.5	0.2	5.57	21.46	40,000	5,000
Mince frozen	80.3	18	0.2	5.43	19.92	16,000	600
Isolate - mince fresh in brine	96.2	2.1	1.4	0.42	4.04		
Isolate - frozen mince in brine	96.7	2.4	0.5	0	2.93		
Salt brine (1.5%)						20,000	700

Salt content in the solutions for injection was lower than planed in the one prepared from frozen mince (0.5%) but 1.4% in the solution prepared from fresh mince. This may have affected the uptake which was 15.1% in fillets injected with fresh mince solutions (4.1 Weight gain of fillets after injection, p. 20), which means that added salt was approximately 0.21g/100g of fillets. The uptake of fillets injected with frozen mince solution was 11.9%; yield a salt uptake of 0.06g /100g, assuming that salt and water were taken up in the same proportion as in brine.

4.1 Weight gain of fillets after injection

Weight gain of fillets after injection was quite different among fillets groups as showed in Figure 1. Fillets groups injected with protein isolates had much higher fillet weight gain compared with other groups, weight gain of fillets injected with 3% fresh protein isolate (FePI) and 3% frozen protein isolate (FoPI) was 15.1± 1.9% and 11.9% ± 1.7%, respectively, while weight gain of salt injection (S) was 4.1% ± 1.5%, fillet weight gain injected by 2% (G) gelatine and 1.5% gelatine(G1) was 3.6% ± 1.1%, and 3.9 ± 1.3%, respectively. It is obvious that weight gain injected with fresh isolate or frozen protein isolate was nearly 3 to 4 times as much as that of those injected with salt and gelatine, therefore, it is an effective way to attain a high weight gain by injection of protein isolates. One of the main reasons for the results was probably that protein isolates contained similar protein components to the fillets, especially, myofibrillar protein which was proved to be effective in cross linking ability between proteins and water holding capacity, therefore, more protein isolates could be easily kept in the fillets. The other main reason might be viscosity of the solution. Higher viscosity of solution like gelatine resulted in a lower weight gain because it was difficult to inject such high viscosity gelatine into the fillets, it could be also indicated by gelatine injection itself, namely, weight gain was lower in 2.0% gelatine injection

(3.6 ± 1.1%) than 1.5% gelatine injection (3.9 ± 1.3%) just because 2.0% gelatine had higher viscosity than 1.5% gelatine. On the other hand, lower viscosity of solution like salt usually gave a lower weight gain because the salt solution injected into the fillets could be easily come out along the holes made by needle due to the lack of components in the fillet connecting the salt solution.

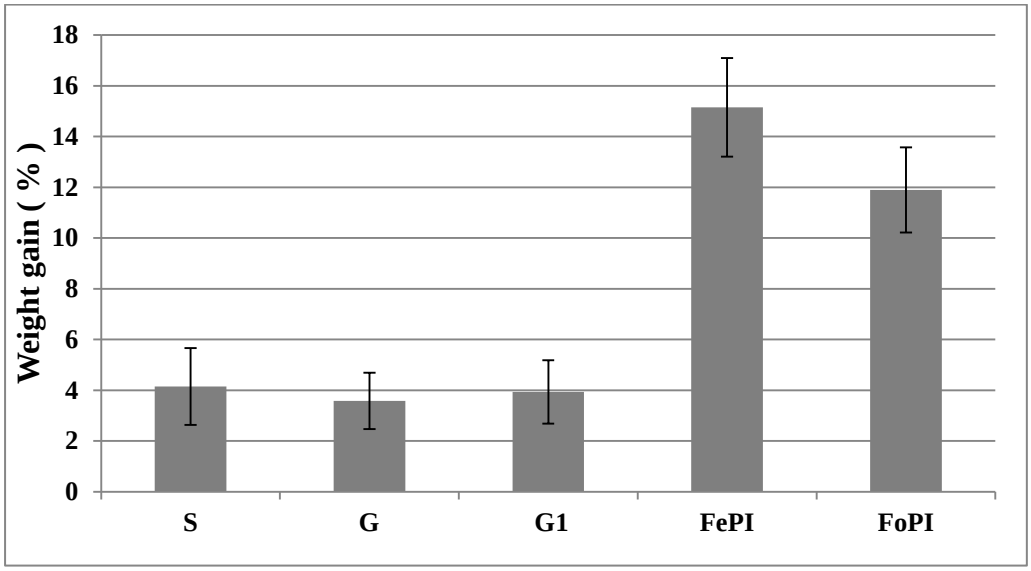

Figure 1. Weight gains of fillets injected by different solutions before storage

4.2 Drip loss of fillets after storage

Drip loss of all the fish fillets injected increased dramatically with storage time in chilling conditions, and drip loss was different among the fillets treated by different solutions (Figure 2). Fillets injected with salt (S) always demonstrated the highest drip loss during the same storage time, drip loss at day 2, day 5 and day 7 was 3.6% ± 0.9%, 5.4% ± 0.5% and 7.1% ± 2.3%, respectively, the lowest drip loss was found in gelatine injection (G) with 2.3% ± 1.0%, 2.8% ± 1.1% and 4.3% ± 2.5% during storage time of 2 d, 5 d and 7 d, respectively; Drip loss of both fresh protein isolate injection (FePI) and frozen protein isolate injection (FoPI) were higher than that of control (C), especially after 7 d storage: the drip loss for FePI was 3.1% ± 0.7%, 4.1% ± 0.6% and 6.9% ± 1.8% at 2 d, 5 d and 7 d, respectively, and the drip loss for frozen protein isolate (FoPI) injection was 3.2% ± 0.9% , 4.7% ± 1.3% and 6.4% ± 1.9% at day 2, day 5 and day 7, respectively, while the drip loss was 3.0% ± 1.8%, 4.1 ± 1.5% and 4.3 ± 1.4% at day 2, day 5 and day 7, respectively.

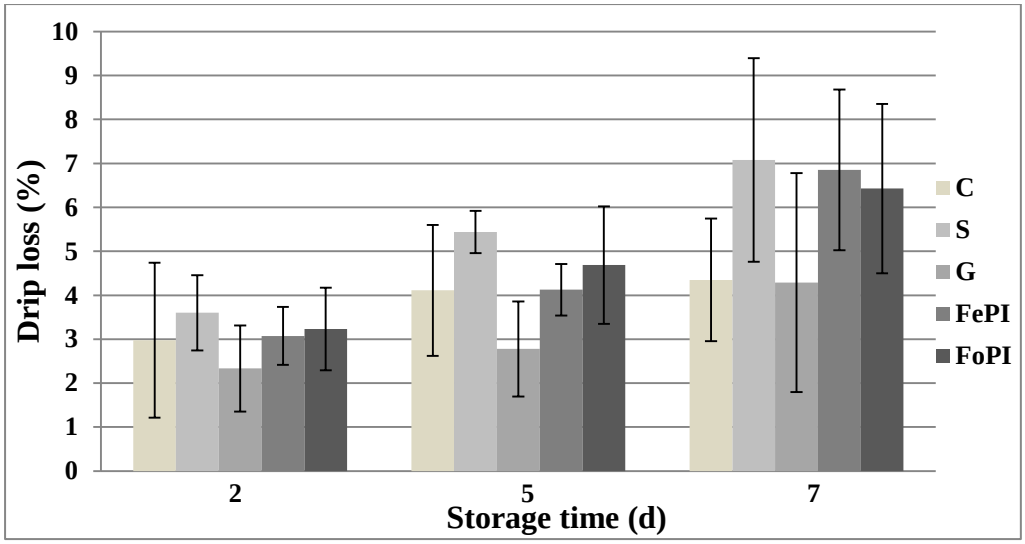

Figure 2. Drip losses of chilled fillets injected by different solutions during chilling storage

Drip loss of all fillets treated by different solutions during frozen storage increased except for control (C), and drip loss was quite different among the fillets groups (Figure 3). Fresh protein isolate (FePI) showed the highest drip loss during the same storage time, and the control (C) had the lowest drip loss during the same storage time and remained stable. After 14 d frozen storage, the drip loss of FePI and FoPI was 14.0% ± 3.6% and 11.8% ± 2.2%, 7.4% and 5.2% higher than control (C), respectively, after 49 d frozen storage, the drip loss of FePI and FoPI was 17.6% ± 1.4% and 14.8% ± 1.5%, 11.0% and 8.2% higher than control (C), respectively. The salt injection also showed a higher drip loss (15.5%) after 49 d frozen storage, while the gelatine injection (G) had a slight change and just 4% higher than the control during frozen storage. However, compared with the counterpart of chilled fillets groups, frozen fillets showed much higher drip loss than chilled fillets.

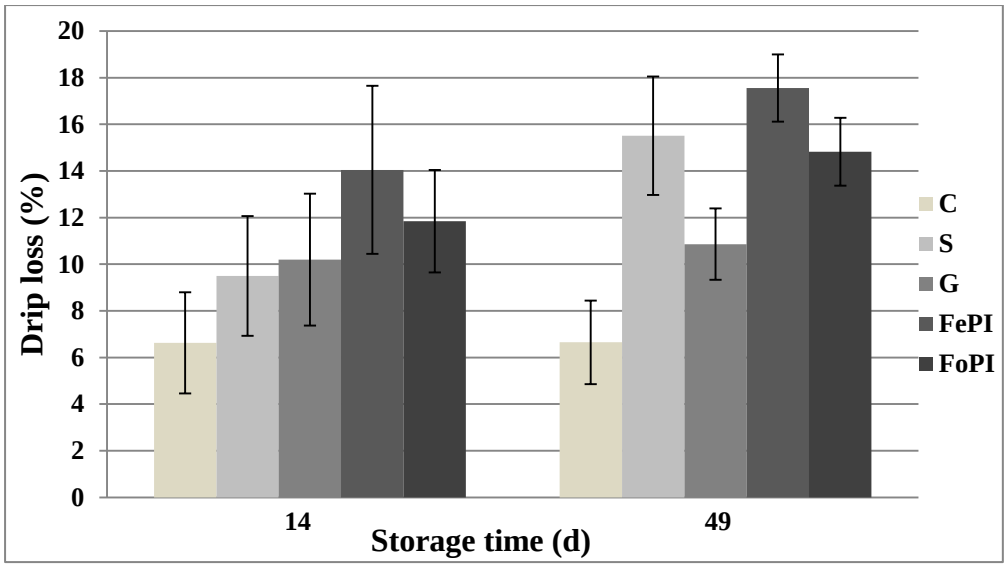

Figure 3. Drip losses of frozen fillets injected by different solutions during frozen storage

4.3 Fillet yield after storage

Yield of fillets injected by different solutions was quite different but in all groups fillet yield decreased with storage time in chilling condition (Figure 4). Fillet yield among the groups was different: fillets injected with fresh protein isolate (FePI) had the highest fillet yield during the same storage time, followed by frozen protein isolate (FoPI) injection; control and salt injection showed lower fillet yield. The yield of fillets injected by fresh protein isolate (FePI) and frozen protein isolate (FoPI) after 7 days storage was 107.7% ± 2.5% and 103.6% ± 2.1%, respectively, 11.8% and 8.3% higher than control (C). Gelatine injection (G) showed 3.7% higher compared with control (C), while no difference was found between the control (C) and salt injection (S). After 7 days storage, only the yield of protein isolate injection of both FePI and FoPI was still above 100% of original weight before injection.

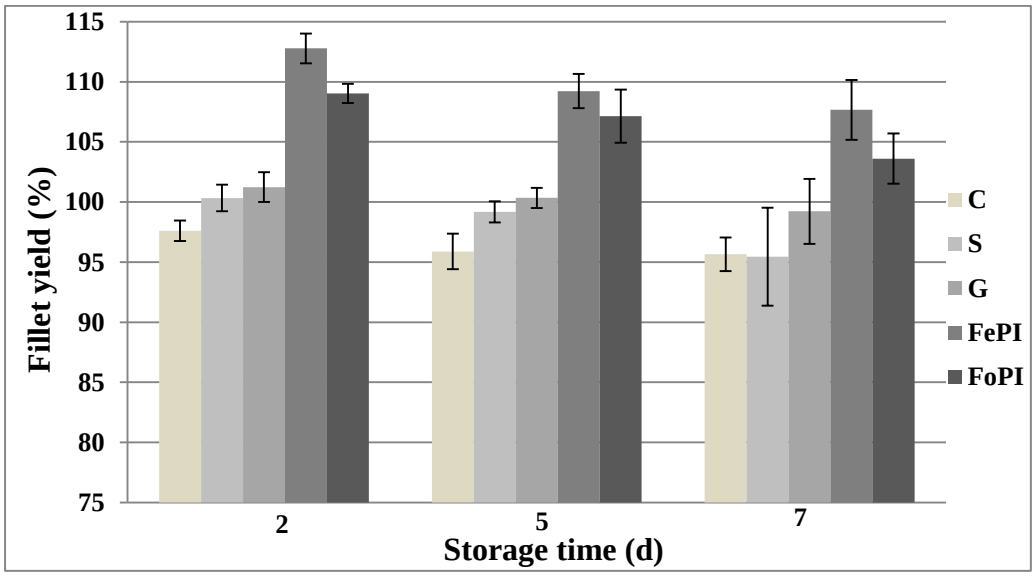

Figure 4. Yield of fillets injected by different solutions during chilling storage

Yield of frozen fillets injected by different solutions was different but in all groups fillet yield decreased with storage time in frozen condition (Figure 5). Fillets injected by fresh protein isolate (FePI) had the highest fillet yield after 14 d storage, followed by frozen protein isolate injection (FoPI), the lowest was found in gelatine injection (G). The fillet yield of FePI and FoPI was 98.7 ± 4.4 and 98.0 ± 3.2, respectively, 5.3% and 4.6% higher than control (C). After 49 d frozen storage, fillets injected with protein isolates and salt dropped dramatically, and gelatine injection showed a slight decrease, while the control (C) remained unchanged. Compared with the count part of chilled fillets, frozen fillet yields during 14 d and 49 d frozen storage were quite lower than chilled fillets during chilled storage from day 2 to day 7.

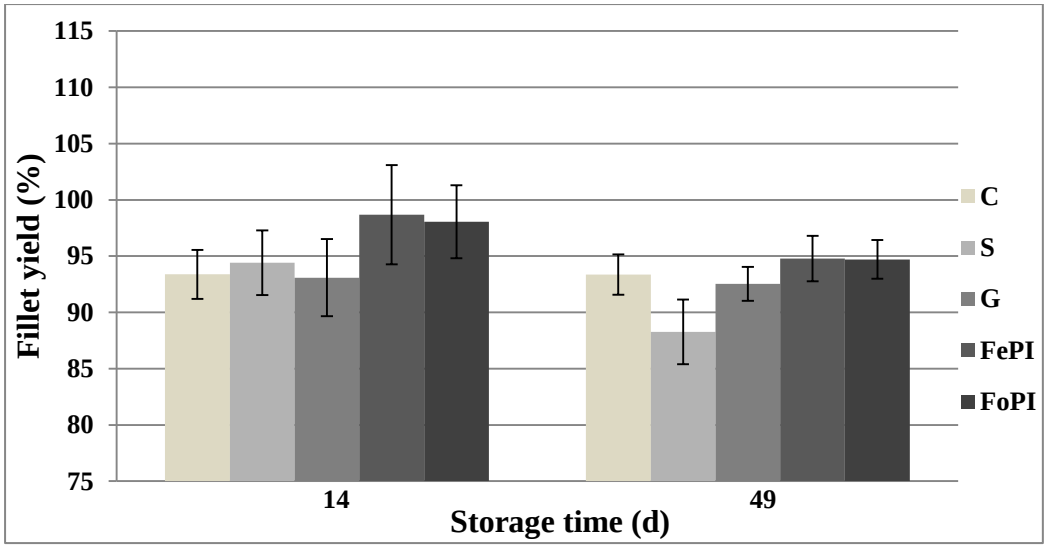

Figure 5. Yield of frozen fillet injected by different solutions during frozen storage

4.4 Cooking yield of fillet during storage

Cooking yield of all fillets injected by different solutions in chilling condition increased with storage time, but no difference was found among the fillets injected by different solution during the same storage time (Figure 6), this indicated that injection had no influence on cooking yield of fillets during chilling condition.

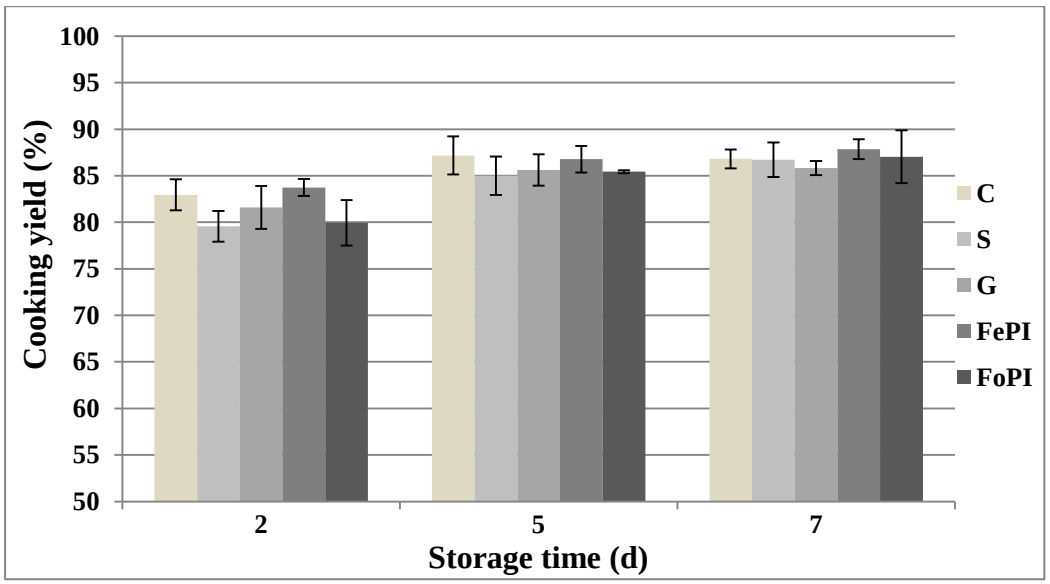

Figure 7. Cooking yields of chilled fillets injected by different solution during storage

Contrary to the chilling condition, cooking yield of all frozen fillets injected by different solutions in frozen condition decreased with storage time (Figure 6). Fresh protein isolate injection (FePI) and control (C) demonstrated higher cooking yield during the same storage time of 14 d and 49 d, the cooking yield was 89.9 ± 1.7 and 90.3 ± 0.5 d after 14 d, respectively, and 88.5 ± 1.0 and 87.3 ± 0.6; Frozen protein

isolate injection (FoPI) and gelatine injection (G) showed lower cooking yield, more than 3 % lower than FePI and control (C), this revealed that different solutions injection had influence on cooking yield of frozen fillets during storage. It was also interesting to see that cooking yield was higher in frozen fillets stored at 14 d than the counterpart of chilled fillets before 5 d storage.

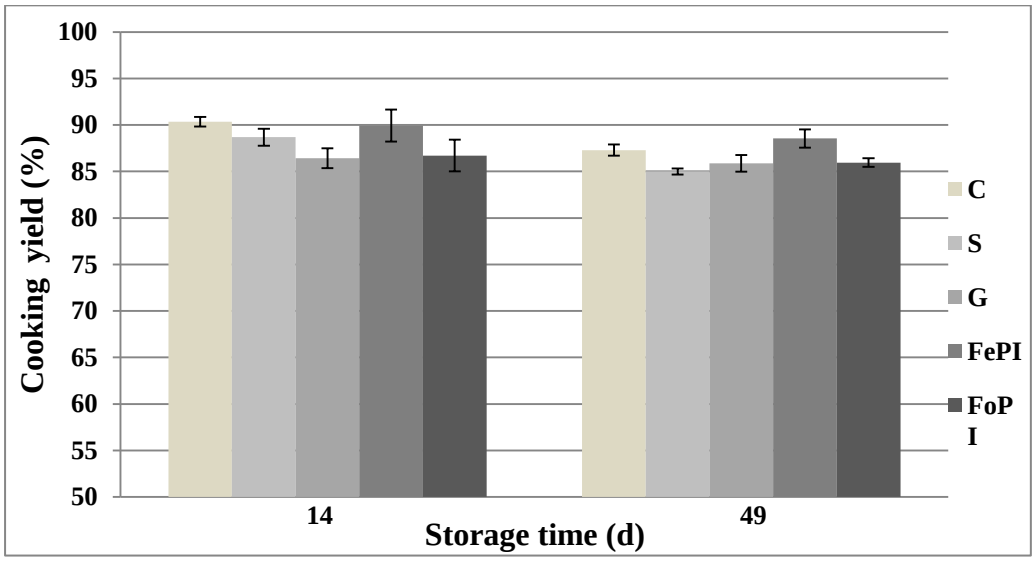

Figure 6. Cooking yields of frozen fillets injected by different solution during frozen storage

4.5 Colour change of fillets during storage

4.5.1 Colour change of chilled fillets during storage

All the colour parameters of fillets injected and control changed with chilling storage time, and the values of colour parameters among the fillets injected by different solutions were different (Table 8).

L value of all groups increased with storage time except for fillets injected with salt brine (S); Frozen protein isolate injection (FoPI) resulted in the highest L value (51.6 ± 1.1) at 2 d chilling storage, followed by salt (S, 49.2 ± 1.5), the lowest L value was observed in fillets injected with fresh protein isolate (FePI, 46.3 ± 2.1) and control (C, 46.8 ± 1.5). After 5 d, the highest L value was still found in fillets injected with frozen protein isolate (FoPI) with the L value of 51.3 ± 1.9, followed by fresh protein isolate (FePI) with the L value of 50.5 ± 1.7, and the lowest L value was 47.2 ± 1.2 in control (C). After 7 d storage, fresh protein isolate injection (FePI) showed the highest L value, followed by frozen protein isolate injection (FoPI), the L value was 53.7 ± 3.3 and 53.3 ± 2.7, respectively, 6.8% and 6.0% higher than control (C), the lowest L value was observed in salt injected fillets (S, 48.2 ± 1.0). It could be concluded that protein isolate injection enhanced the L value of the fillets, and the frozen protein injection (FoPI) always demonstrated highest L value during storage compared with groups except for fillets injected with fresh protein isolate (FePI) at 7 d storage. Change of a value of saithe fillets injected by different solutions during storage was different. The a value of fillets treated by fresh protein isolate (FePI) and control (C)

decreased with storage time from day 2 to day 7, decreased by 0.2 and 0.5, respectively. Salt injection (S) showed a reverse trend, a value increased by 0.4 from day 2 to day 7, while frozen protein isolate (FoPI) and gelatine injection (G) remained stable from day 2 to day 7. It was interesting to notice that the lowest a value of gelatine(G) and fresh protein isolate injection (FePI) was found in day 5, and a value of frozen protein isolate (FoPI) was similar at day 5 (0 ± 0.6) and day 7 (0 ± 0.3). Change of b value of saithe fillets during storage was quite different. Decrease of b value was observed in the fillets treated by salt (S), gelatine (G) and control (C) from day 2 to day 7 during chilling storage. The b value decreased by 1.4, 2.4 and 0.9 from 1.9 ± 0.6, 1.7 ± 0.5, and -0.1 ± 0.8, respectively; while the b value increased in the fillets injected by fresh protein isolate (FePI) and frozen protein isolate (FoPI), the b value was 0.1 ± 0.4 and -0.8 ± 0.9 at day 2, respectively, increased by 0.2 and 1.8 after 7 day chilling storage. It was also interesting to see that the lowest b value of all groups except frozen protein isolate injection (FoPI) was observed at day 5. However, the difference of b value of FoPI at day 5 (-0.5 ± 0.6) and day 2 (-0.8 ± 0.9) was not significant. From the above observation of a value and b value, conclusion could be made that day 5 was an important day to determine the change of a value and b value in chilling storage.

Table 8. Colour change of chilled fillet injected different solutions during storage

Colour parameter	**Storage time (d)**	**Different injection solutions**				
		C	**S**	**G**	**FePI**	**FoPI**
L value	2	46.8 ± 1.5	49.2 ± 1.5	48.5 ± 1.8	46.3 ± 2.1	51.6 ± 1.1
	5	47.2 ± 1.2	50.2 ±1.2	49.8 ±1.0	50.5 ± 1.7	51.3 ± 1.9
	7	50.3 ± 1.3	48.2 ± 1.0	52.6 ± 2.2	53.7 ± 3.3	53.3 ± 2.7
a value	2	-0.2 ± 0.7	-0.6 ± 0.4	0.1 ± 0.5	0.3 ± 0.6	-0.1 ± 0.2
	5	-0.4 ± 0.5	-0.4 ± 0.4	-0.5 ± 0.4	-0.1 ± 1.2	0 ± 0.6
	7	-0.7 ± 0.5	-0.2 ± 0.7	0.1 ± 0.8	0.1 ± 0.1	0 ± 0.3
b value	2	-0.1 ± 0.8	1.9 ± 0.6	1.7 ± 0.5	0.1 ± 0.4	-0.8 ± 0.9
	5	-1.1 ± 0.4	-1.0 ± 0.2	-0.9 ± 0.5	-0.6 ± 0.4	-0.5 ± 0.6
	7	-1.0 ± 0.4	0.5 ± 0.3	-0.7 ± 0.6	0.3 ± 0.6	0.8 ± 0.4
W value	2	47.0 ± 3.8	55.0 ± 2.0	53.7 ± 1.4	46.1 ± 3.1	53.9 ± 1.8
	5	50.5 ± 1.0	53.3 ± 1.1	52.4 ± 1.0	52.4 ± 2.3	52.7 ± 2.0
	7	53.2 ± 1.2	46.9 ± 1.9	54.7 ± 2.1	52.9 ± 3.8	50.8 ±3.5

The W value increased during day 2 to day 7 in fillets treated by gelatine injection (G), fresh protein isolate injection (FePI) and control (C). The W value was 54.7 ± 2.1, 52.9 ± 3.8 and 53.2 ± 1.2 at day 7, it had increased by 1, 6 and 6.8, respectively, compared with W value at day 2. The W value decreased in fillets injected by frozen protein isolate (FoPI) and salt (S) from day 2 to day 7, the W value was 50.8 ±3.5 and 46.9 ± 1.9 at day 7, it had decreased by 3.1 and 8.1 from day 2 to day 7, respectively.

Statistic analysis showed no significant ($p>0.05$) difference among the W value at day 7.

4.5.2 Colour change of frozen fillets during storage

All the value of colour parameters in fillets injected by both fresh proteins isolates (FePI) and frozen protein isolate (FoPI) almost remained unchangead during the frozen storage (Table 9). The L and W value were similar between fillets injected by fresh protein isolate (FePI) and the control fillets (C). After 14 d frozen storage, fresh protein isolate injection (FePI) showed the highest L value (54.5 ± 2.2), and control had the lowest L value (49.8 ± 1.8). The highest a value was found in control (C) with the value of -0.7 ± 0.7, the lowest a value was observed in gelatine injection (G), and followed by fresh protein isolate injection (FePI). Control (C) revealed the highest b value (3.0 ± 0.6), followed by FePI (2.6 ± 0.5). The biggest W value was found in gelatine injection (G) with the value of 47.6 ± 3.4, followed by FePI (46.6 ± 3.2), control (C) showed the lowest W value (40.9 ± 3.2). Compared with the count part in fillets during chilling storage, fillets during frozen storage showed higher L value and b value, and lower a value and W value.

Table 9. Colour change of frozen fillet injected by different solutions during storage

Colour parameter	**Storage time (d)**	**Different injection solutions**				
		C	**S**	**G**	**FePI**	**FoPI**
L value	14	49.8 ± 1.8	52.0 ± 2.2	54.3 ± 1.7	54.5 ± 2.2	51.9 ± 2.2
	49	51.3 ± 0.7	51.9 ± 2.2	51.8 ± 3.2	53.9 ± 1.3	51.7 ± 2.4
a value	14	-0.7 ± 0.7	-0.9 ± 0.4	-1.3 ± 0.3	-1.2 ± 0.3	-0.9 ± 0.5
	49	-1.3 ± 0.3	-1.4 ± 0.4	-0.9 ± 1.0	-1.1 ± 0.5	-0.5 ± 1.0
b value	14	3.0 ± 0.6	1.8 ± 0.9	2.2 ± 0.9	2.6 ± 0.5	2.2 ± 0.6
	49	1.6 ± 0.8	2.6 ± 1.3	3.0 ± 1.2	2.7 ± 1.0	3.4 ± 1.7
W value	14	40.9 ± 3.2	46.5 ± 2.4	47.6 ± 3.4	46.6 ± 3.2	45.4 ± 3.2
	49	46.6 ± 2.5	44.0 ± 4.9	42.8 ± 6.5	46.3 ± 0	41.6 ± 6.4

4.6 Water holding capacity (WHC)

The water holding capacity (WHC) of chilled fillet groups except for gelatine (G) increased slightly from 2 d to 7 d chilling storage (Table 10). Fillets injected with fresh protein isolate (FePI) had higher WHC than the other groups at 2 d, followed by control (C). The lowest WHC was observed in frozen protein isolate (FoPI). After 7 d storage, control (C) demonstrated the highest WHC, followed by fresh protein isolate (FePI), but the lowest WHC were found in fillets injected with frozen protein isolate (FoPI).

The WHC of all frozen fillets changed greatly from 14 d to 49 d. The WHC of all frozen fillets groups except for gelatine injection (G) was reduced. Fillets injected with fresh protein isolate (FePI) always showed the lowest WHC. On the contrary fillets injected with frozen protein isolate had the highest WHC after 14 d and the second highest after 49 d frozen storage. Freezing of the fillets reduced the WHC of frozen fillets compared with chilled fillets. It was also found that the WHC of fillets decreased greatly during frozen storage and increased slightly during chilled storage apart from the gelatine injection group (G).

Table 10. Water holding capacity (WHC, %) of fillets injected by different solutions during chilling and frozen storage

Storage		**Different injection solutions**				
	Time (d)	**C**	**S**	**G**	**FePI**	**FoPI**
Chilling	2	90.2 ± 2.7	88.6 ± 1.4	92.0 ± 1.1	92.7 ± 1.3	88.3 ± 0.7
	7	93.9 ± 1.2	91.4 ± 1.6	91.1 ± 2.0	93.0 ± 1.6	90.3 ± 1.0
Frozen	14	83.6 ± 1.4	81.8± 2.1	75.2 ± 2.8	74.2 ± 4.2	84.7 ± 2.8
	49	74.2 ± 4.5	78.6 ± 3.5	83.0 ± 1.1	72.5 ± 6.3	82.3 ± 7.2

4.6 Water and salt content

Water content was increased by the injection of protein solutions in chilled fillets. Freezing and thawing resulted in higher thaw drip of the injected fillets, resulting in similar values in all groups after frozen storage (but decreased again during thawing). No significant differences were observed between groups due to thaw drip (Table 11).

Table 11. Water content (%) of fillets injected by different solutions during chilling and frozen storage

Storage		**Different injection solutions**				
	Time (d)	**C**	**S**	**G**	**FePI**	**FoPI**
Chilling	2	78.7 ± 1.0	80.0 ± 0.6	80.6 ± 0.4	80.3 ± 0.8	81.3 ± 0.5
	7	80.7 ± 0.9	81.4 ± 0.5	80.9 ± 0.4	81.7 ± 0.2	81.2 ± 0.4
Frozen	14	79.0 ± 0.4	78.8± 0.4	80.2 ± 1.4	79.8 ± 0.4	79.6 ± 0.4
	49	78.6 ± 0.7	78.4 ± 1.0	77.4 ± 1.1	78.6 ± 0.9	78.8 ± 0.3

Injection with pure salt brine and brine containing proteins from fresh mince resulted in a higher salt content of fillets. The uptake of gelatine containing brine was low and the salt content of the brine containing frozen mince was lower than of fresh mince brine. These factors partly explain why the salt content of the injected fillets was lower (Table 12).

Table 12. Salt content (%) of fillets injected by different solutions during chilling and frozen storage

Storage		**Different injection solutions**				
	Time (d)	**C**	**S**	**G**	**FePI**	**FoPI**
Chilling	2	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.4 ± 0.1	0.2 ± 0.0
Frozen	14	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
	49	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0

4.10 Change of pH of fillets during storage

The pH of all the chilled fillets remained unchangeable from day 2 to day 7 as shown in Table 13, no difference was observed among the fillets, which indicated that solutions injection had no effect on the pH levels of fillets during chilling storage.

Table 13. pH of chilled fillets injected by different solutions during different storage time

Storage time (d)	**C**	**S**	**G**	**FePI**	**FoPI**
2 d	6.3 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	6.3 ± 0.0	6.3 ± 0.0
5 d	6.4± 0.0	6.4± 0.1	6.4± 0.0	6.5± 0.1	6.4± 0.1
7 d	6.5± 0.1	6.4± 0.1	6.3± 0.1	6.4± 0.0	6.3± 0.0

The pH level of all the frozen fillets was similar after 14 d storage as showed in **Table 14**; there was no difference among the groups, which indicated that the solutions injection had no effect on the pH level of fillets during frozen storage.

Table 14. pH of frozen fillets injected by different solutions during different storage time

Storage time (d)	**C**	**S**	**G**	**FePI**	**FoPI**
14 d	6.4 ± 0.1	6.4 ± 0.1	6.3 ± 0.1	6.3 ± 0.0	6.4 ± 0.1
49 d	6.3 ± 0.0	6.3 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	6.3 ± 0.0

4.9 TVB-N and TMA

Both TVB-N and TMA of all chilled fillets groups increased dramatically from 2 d to 7 d in chilling storage (Table 15), but statistical analysis showed no significant difference among the groups during the same storage time.

The TVB-N and TMA content of all frozen fillets groups increased from the fresh stage, most likely do to activation of spoilage organisms during thawing (Table 16). Storage time did affect TVB-N and TMA content and no significant differences were found between the groups. Compared with the chilled fillets, frozen fillets showed quite low TVB-N and TMA.

Table 15. TVBN and TMA change of chilled fillets injected by different solutions during chilling storage

Parameter	Storage time (d)	Different injection solutions				
		C	S	G	FePI	FoPI
TVBN	2	12.0 ± 2.0	13.2 ± 0.2		12.6 ± 0.7	11.0 ± 1.5
	7	69.5 ± 1.7	64.2 ± 2.5	61.6 ± 6.4	63.2 ± 0.6	58.2 ± 3.6
TMA	2	1.1 ± 0.6	1.3 ± 0.3		1.4 ± 0.7	1.3 ± 0.4
	7	55.2 ± 2.3	52.2 ± 0.8	47.1 ± 5.5	50.0 ± 2.0	47.1 ± 1.0

Table 16. TVBN and TMA change of frozen fillets injected by different solutions

Parameter	Storage time (d)	Different injection solutions			
		C	S	FePI	FoPI
TVBN	14	17.1 ± 0.6	16.4 ± 0.5	15.6 ± 1.3	16.3 ± 2.5
	49	18.1 ± 1.2	17.3 ± 1.5	16.5 ± 0.3	15.5 ± 0.2
TMA	14	2.3 ± 0.6	2.3 ± 0.5	2.2 ± 0.4	2.9 ± 1.1
	49	2.3 ± 0.2	2.2 ± 1.0	1.8 ± 0.6	1.5 ± 0.1

4.8 Microbiological analysis of chilled and frozen fillets

Both total viable psychotropic bacteria count (TVC) and H2S producer bacteria count (HC) increased with storage time in all groups (Table 17). At day 2, fillets treated by fresh protein isolate (FePI) and control (C) showed higher TVC (5.7 ± 0.4 and 5.8 ± 0.4, respectively) and HC (4.0 ± 0.3 and 3.8 ± 0.6, respectively) than fillets injected with frozen protein isolate (FoPI) and control (C). At day 7, salt injection demonstrated the lowest TVC (7.0 ± 0.7) and HC (5.2 ± 0.5), the highest TVC was observed in frozen protein isolate injection (FoPI) and the highest HC in control (C). Statistical analysis showed that there was no difference ($p>0.05$) among the TVC in

control (C), fresh protein isolate injection (FePI) and frozen protein isolate injection (FoPI) at day 7.

Table 17. Growth of total viable psychotropic bacteria and H2S- producer bacteria of chilled fillets

Microbial Count	**Storage Time(d)**	**Different injection solutions**			
		C	**S**	**FePI**	**FoPI**
TVC (log number/g)	2	5.3 ± 0.2	5.8 ± 0.4	5.7 ± 0.4	5.2 ± 0.8
	7	7.6 ± 0.1	7.0 ± 0.7	7.6 ± 0.1	7.7 ± 0.1
H_2S (log number/g)	2	3.3 ± 0.4	3.8 ± 0.6	4.0 ± 0.3	3.5 ± 0.5
	7	6.1 ± 0.3	5.2 ± 0.5	5.8± 0.1	5.4 ± 0.4

Both total viable psychotropic bacteria count (TVC) in all groups remained unchanged during frozen storage (Table 18) and statistical analysis showed that there was no difference ($p>0.05$) between groups. The H_2S producer bacteria count (HC) of all fillets decreased with frozen storage time. Compared with the counterpart of chilled fillets, TVC and HC of all frozen fillets were lower than in chilled fillets. Fillets treated by fresh protein isolate (FePI) always showed the highest TVC and HC compared with other groups. It illustrated that to keep a lower TVC of fresh protein isolate it is very important to guarantee the freshness of fillets and the mince used for injection.

Table 18. Growth of total viable psychotropic bacteria and H2S- producer bacteria of frozen fillets injected different solutions during storage

Microbial Count	**Storage time(d)**	**Different injection solutions**			
		C	**S**	**FePI**	**FoPI**
TVC (log number/g)	14	4.4 ± 0.1	4.5 ± 0.2	4.9 ± 0.1	4.6 ± 0.2
	49	4.5 ± 0.1	4.5 ± 0.3	4.8 ± 0.1	4.5 ± 0.1
H_2S (log number/g)	14	2.3 ± 0.6	2.1 ± 0.2	3.2 ± 0.2	2.3 ± 0.4
	49	1.3 ± 0.1	1.7 ± 0.4	2.9 ± 0.2	2.1 ± 0.4

4.7 Sensory evaluation

According to sensory evaluation, all groups were past their shelf life on day 7 (results are shown in appendix). Then the odour and flavour characteristic for fish at the end of their shelf life were very characteristic for the samples, such as table cloth, TMA and sour odour, sour, TMA and off-flavour (QDA score over 20, Magnússon *et al*, 2006). The variables characteristic for fresh products were meaty, sweet and metallic flavour, vanilla and sweet odour, juicy soft and tender texture (Figure 7). The first two principal components in PCA analysis explained 78% and 9% of the variation

between the samples. The samples are clearly different with regard to storage conditions (chilling/frozen) and storage time (14d and 49d) (Figure 8).

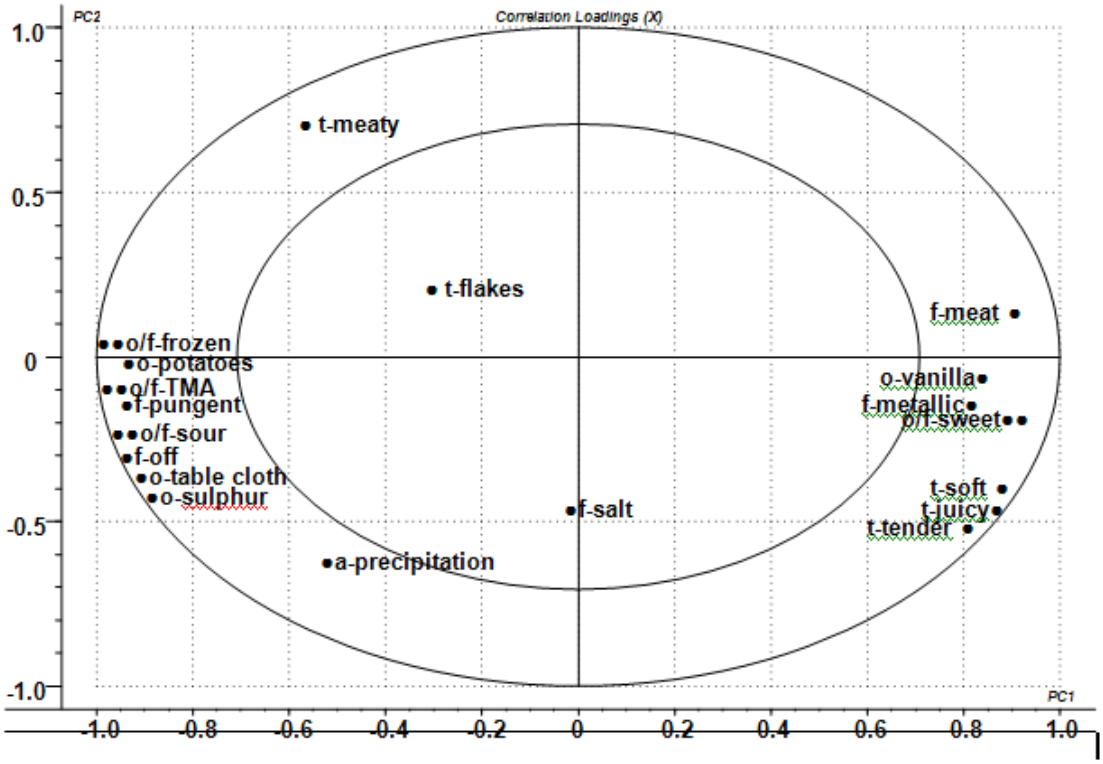

Figure 7. PCA loading plot of sensory attributes during storage of chilled and frozen saithe fillets

Chilled fillets injected with both fresh proteins isolate and frozen protein isolate had more juice and tenderness than control fillets. Freezing significantly reduced freshness of all groups and the effects of storage time could also be seen. Samples that were stored for a longer time had a more meaty texture and less precipitation of proteins ("cooked egg white") was seen on the surface of the fillets after cooking.

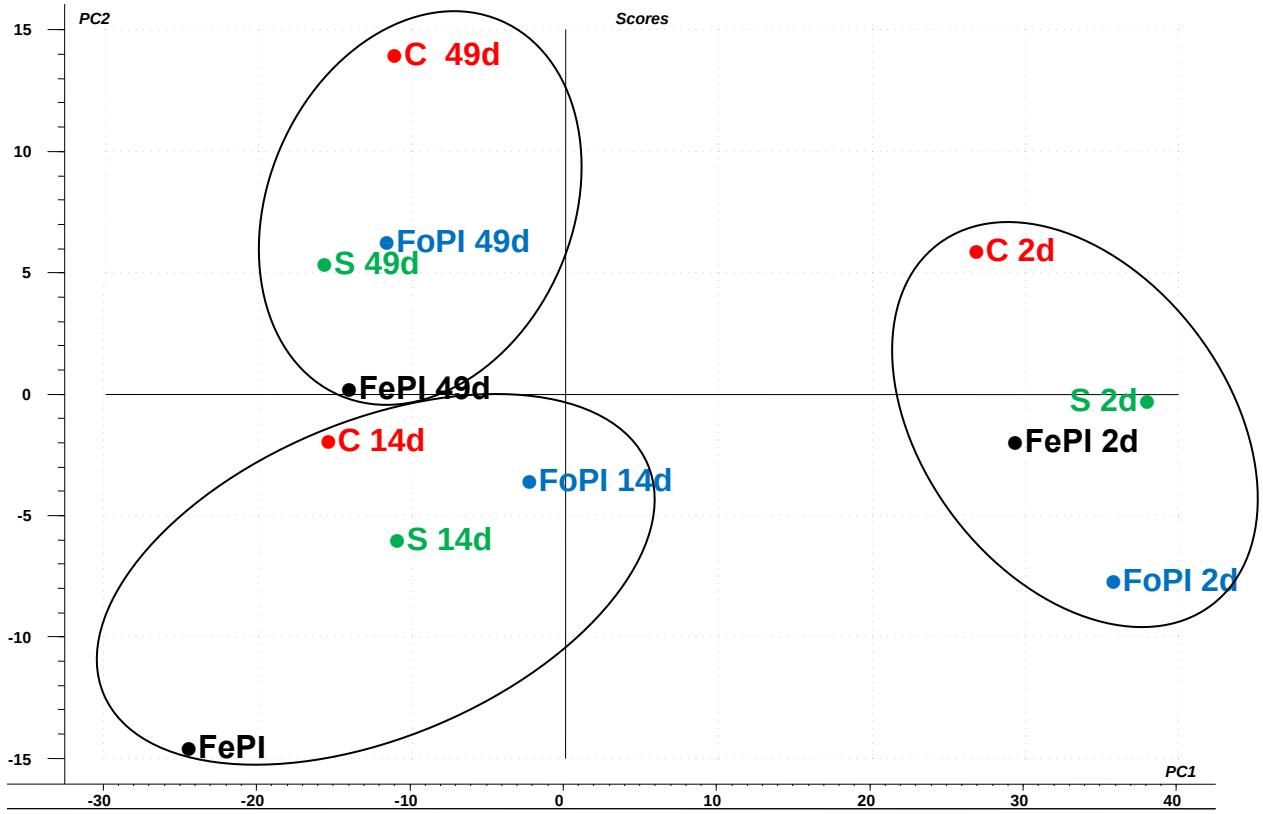

Figure 8. PCA scores plot of all the fillets groups during chilling and frozen storage conditions

ANOVAs analysis of data collected after each sampling time showed which variables were significant each time. The overall results can be found in the appendix but only the significant variables are shown in tables 18-20. After two days fillets that had been injected had a saltier flavour and less flaky texture (Table 19).

Table 19. Comparison of average QDA scores of cooked fillets by Duncan's test during 2d chilling storage (Different superscript letters showed that samples were different within a line).

Sensory attribute	*p-value*	C	S	FePI	FoPI
flavour-salt	*0.011*	17 ^{b}	27 ^{a}	28 ^{a}	20
texture-flakes	*0.019*	44	44 ^{a}	34 ^{b}	32 ^{b}

After frozen storage of 14 days, fillets injected with frozen protein isolate had better odour of meat and vanilla compared with other groups (Table 20).

Table 20. Comparison of average QDA scores of cooked fillets by Duncan's test during 14d frozen storage (Different superscript letters showed that samples were different within a line).

Sensory attribute	*p-value*	C	S	FePI	FoPI
odour-meat	*0.027*	24	27	22 [b]	30 [a]
odour-vanilla	*0.002*	17 [b]	21 [b]	17 [b]	28 [a]

After 49 days, control fillets had a significantly darker appearance than fillets injected with salt and proteins solution from fresh mince (Table 21).

Table 21. Comparison of average QDA scores of cooked fillets by Duncan's test during 49d frozen storage (Different superscript letters showed that samples were different within a line).

Sensory attribute	*p-value*	C	S	FePI	FoPI
a-dark	*0.007*	45 [b]	55 [a]	55 [a]	49

Based on the above mentioned results, a conclusion could be made that protein isolate injection had no bad impact on the sensory evaluation of saithe fillets during chilled and frozen storage.

5. DISCUSSIONS

5.1 Weight gain

In this experiment, the weight gain of fillets with salt injection (1.5% brine concentration) was relatively low (4.1% ± 1.5%). Thorarinsdottir *et al.* (2004b) observed that the weight gain of cod fillets injected with brine, protein, phosphate or a combination ranged from 4% to 7%. In this experiment, the weight gain of salt injection was similar (4.1%), but weight gain of protein injections in this experiment was much higher (15.1% and 11.9%), this might be caused by different viscosity in different protein concentrates, because higher protein concentrate showed higher viscosity and was difficult to inject into the fillets, protein concentrate in this experiment was lower (3%) than Thorarinsdottir's (10%).

In fact, the weight gain of fish fillets can be influenced by many factors. Increased injection volume, injection times and post rigor state significantly increased weight gain (Birkeland *et al.*, 2007). Higher pressure also remarkably raised the weight gain (Birkeland *et al.*, 2003), but it might damage muscle structure due to a significant increase of fillet gaping (Birkeland *et al.*, 2003). Slits and discoloured spots may appear on the surface of fillets (Freixenet 1993). Therefore, multineedle injectors, equipped with lower pressure of 4 bars (0.4 MPa) pumps propelling the brine as a continuous jet into the muscle through holes in the needle tip (Freixenet, 1993), are currently in commercial use in production of cold-smoked salmon. The injectors usually showed a high stability with respect to the amount of brine injected into the muscle (Freixenet, 1993) and the distribution of brine in muscle tissue (Birkeland *et al*. 2003).

5.2 Drip loss

In this experiment, fresh protein isolate injection showed the highest drip loss after 49d frozen storage, followed by salt injection and frozen protein isolate injection, but Thorarinsdottir *et al.* (2004a) found that salt injection had higher drip loss than fish protein hydrolysate injection. Control showed the lowest drip loss after injection with fish protein during frozen storage. In contrast to fish protein hydrolysate, Thorarinsdottir *et al.* (2004a) also found that soy protein injection and salt injection showed lower drip loss than the control.

In this experiment, drip loss was much higher than that of Thorarinsdottir *et al*. (2004a). This was probably due to different concentration and type of protein used. Previous studies claimed that drip loss could be reduced by using different protein materials (Porcella *et al*. 2001; Kristinsson *et al*. 2000b). Drip loss has been found to be linked to partial denaturation of proteins taking place during frozen storage, which leads to decreased water holding capacity (Mackie, 1993). Addition of salt to fish fillet before freezing increased water holding capacity and decreased drip loss (Woyewoda, 1986). Conclusions might be drawn that protein concentration and type determined drip loss.

5.3 Fillet yield

Effects of salt and protein injection on the yield of fillets during frozen storage were quite different in this experiment compared with Thorarinsdottir *et al.* (2004a). In this experiment, protein injection (3% protein isolate + 1.5% salt) had a higher fillet yield than control and salt (1.5%) injection during frozen storage. Fillet yield of both salt and protein injection was quite lower than that of Thorarinsdottir *et al*. (2004a). In this experiment, fillet yield of 1.5% salt, 3% fresh protein isolate with 1.5% salt and 3% frozen protein isolate with 1.5% salt was 94.4% ± 2.9%, 98.7% ± 4.4% and 98.0% ± 3.2% at 14 d frozen storage, and 88.3% ± 2.9%, 94.8% ± 2.0% and 94.7% ± 1.7% at 49 d frozen storage, respectively.

According to Thorarinsdottir *et al*. (2004b), fillet yield of 5% salt, 10% soy protein with 5% salt and 10% cod hydrolysate with 5% salt was 105.0% ± 1.5%, 105.1% ± 1.5% and 103.1% ± 1.9% after 3 months of frozen storage, respectively, but fillet yield of control in this experiment was slight lower (93.4% ± 1.8%) than Thorarinsdottir *et al*.' (2004a) results (94.9% ± 1.8%). Reasons were probably as follows: First of all, higher salt concentrate resulted in higher water holding capacity (Paterson *et al.,* 1988), which caused more retention of the solution injected; Secondly, lower protein concentrate, which means higher water content, resulted in less water or solution holding in the fillet and it could be explained by lower fillet yield in water injection compared with protein injection (Thorarinsdottir *et al*., 2004a). Thirdly, higher fillet yield might be obtained by immersing in the same solution (brine and/or protein) immediately after injection (Thorarinsdottir *et al*., 2004b). Therefore, conclusion could be drawn from the above results that fillet yield could be raised by injecting fish protein isolate when lower (1.5%) salt is applied.

5.4 Cooking yield

In this experiment, the injection of a combination of fresh protein isolate with salt into saithe fillet during frozen storage resulted in higher cooking yield than only a salt injection, and the mixture of fresh protein isolate with salt injection. On the other hand, there was no difference between control and the mixture of fresh protein isolate with salt injection. This result was different from the previous studies. Thorarinsdottir *et al*. (2004a) study showed that injection only with salt had higher cooking yield than the mixture of protein and salt injection into cod fillets during frozen storage. Jittinandana *et al*. (2002) also observed that the cooking yield increased with increased salt concentration (from 8.7% to 17.4%); Shahidi *et al*. (1995) suggested that the cooking yield of rainbow trout fillets increased with the increase of the concentration of capelin protein hydrolysate (from 0% to 3%). Differences may have resulted from the different salt concentration used in the above studies. In this experiment, 1.5% salt was used, but 5% or more salt was used in the previous studies. According to the above results, conclusions can be drawn that lower salt concentration had no impact on the cooking yield, but the cooking yield could be influenced by the protein properties and concentration.

5.5 Colour

In this experiment, protein isolate injection improved or retained the colour parameters such as L and W value, this was the same findings as Harald *et al.* (2004) that claimed that fish protein hydrolysate improved lightness of the salmon fillets, and that the colour remained unchangead during long time frozen storage.

5.6 Water Holding Capacity (WHC)

In this experiment, the frozen protein isolate injection had a much higher WHC than the control and salt injected fillets. Thorarinsdottir *et al*. (2004a) also found the same results in fish protein hydrolysate injection. The contrary was found in soy protein injection, which was the same as fresh protein isolate injection in this experiment. According to Thorarinsdottir *et al*. (2004a) findings, there was an interaction between protein and salt. It had been established that WHC increased with increased salt concentration up to 6% (Fenneema, 1990), but at higher salt concentration (over 9-10%), fish muscle lost water and resulted in decreased water holding capacity (Thorarinsdottir *et al.*, 2002). In fact, commercial brine injection for moisture enhancement of meat products was often made up of 4- 5% salt (Uttaro and Aalhus, 2007).

In this experiment, WHC decreased with increasing frozen storage time, this is probably explained by protein denaturation during freezing. While WHC increased with chilled storage, and WHC in chilled fillet was higher than frozen fillet, this result was similar with Erikson *et al.* (2004). Salt injections had higher WHC than control after 49 d frozen storage, which was in accordance with Erikson *et al*. (2004) and Regenstein *et al*. (1984). They claimed that about 1.8% salt concentration and above could improve WHC of fish muscle, but fresh protein isolate with salt injection showed quite lower WHC compared with frozen protein isolate injections, this is a reason for further studies. In this experiment, the value of WHC was lower compared with others (Rustad, 1992; Erikson *et al.,* 2004; Ofstad *et al.,* 1996c)

5.8 Microbiological analysis

In this experiment, after 7d chilling storage, TVC was higher,all fillets by sensory evaluation were rejected, and shelf life was short. This might be due to temperature fluctuation during transport on the way back to IFL and 4d old fish after catch. However no difference was found among fillets groups. Several studies suggested that injection leads to an increase of total bacterial count (Cannon *et al*., 1993; Dustin *et al.,* 2007).

5.9 TVB-N and TMA

In this experiment, both TVN-N and TMA were higher in all groups at 7d chilling storage and rejected by sensory evaluation, this was in accordance with the results of microbiological analysis and sensory evaluation.

5.10 pH

In this experiment, the pH of both fresh and frozen fillets remained stable although chilled fillet was rejected after 7 d storage; this might be due to the balancing stability of fish protein isolate, since that Harald *et al*. (2004) claimed that increasing concentrations of fish protein hydrolysate has a favourable effect on pH.

5.7 Sensory evaluation

In this experiment, results showed that there was no significant difference among the groups, but protein isolates showed the positive effects on sensory attributes such as texture attributes like tenderness, and also odour attributes like meat and vanilla. Dustin *et al.* (2007) found similar results. They claimed that beef injection with solubilised protein was comparable to phosphate-enhanced steaks for discoloration and overall acceptability. Harald *et al.* (2004) also declared that fish protein hydrolysate injection into salmon fillet showed no change in smell and taste.

Frozen and thawed fish products are in general characterized by having lower eating quality than fresh ones due to considerable freezing denaturation (Mackie, 1993; Nilsson and Ekstrand, 1995; Hurling and McArthur, 1996; Pham and Mawson, 1997). However, a wide variety of compounds have been shown to improve properties like juiciness and texture in different processed food and among these salt, starch, glucose, sodium ascorbate and phosphates (Krivchenia and Fennema, 1988; Dziezak, 1990; Craig *et al.* 1991; MacDonald and Lanier, 1997; Park *et al*., 1997; Zheng *et al*.,1999; Badii and Howell, 2002; Herrera *et al.*, 2002; Qu *et al*., 2003). Recent studies have shown that it is possible to enhance the consumers liking of frozen and thawed cod fillets by brining with a commercially available brine mixture consisting of salt, phosphates and glucose (Esaiassen, 2004, 2005).

6. CONCLUSIONS

Weight gain and fillet yield of saithe fillets could be effectively raised by the injection of protein isolate. However, fillets injected with protein isolate also showed higher drip loss. Colour and water holding capacity were improved and the injection had a positive effect on the sensory attributes compared with other groups. Besides, no difference was observed in sensory evaluation, TVC, H2S, TVB-N, TMA and pH among the groups during storage revealed that protein injection had no bad effects on fillet quality. Therefore, the injection of protein isolate into saithe fillets is an effective means to improve or stabilize the weight and quality of saithe fillets.

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9. APPENDIX

Following tables show the average QDA scores for all groups, and average QDA scores of the groups evaluated fresh (2 and 7 days) and frozen (14d and 49d), based on the evaluation of 15 assessors in replicate for each sample o= odour, a= appearance, f= flavour, t= texture.

Table 22. The average QDA scores for odour of cooked fillets injected with different solutions during chilling and frozen storage

Sensory	C				F				FePI				FoPI			
attribute	2d	7d	14d	49d	2d	7d	14d	49d	2d	7d	14d	49d	2d	7d	14d	49d
odour																
o-sweet	44	20	27	24	46	20	29	23	42	18	25	24	46	19	33	22
o-shellfish	39	21	34	25	37	23	30	28	32	21	32	23	36	22	34	23
o-meat	32	16	24	18	33	16	27	19	34	14	22	19	30	16	30	18
o-vanilla	29	15	17	19	33	14	21	21	25	10	17	21	26	15	28	18
o-potatoes	28	25	35	29	24	24	33	30	24	22	31	31	27	26	33	31
o-frozen	8	10	19	17	5	8	17	17	9	9	20	19	10	6	17	16
o-table cloth	7	24	20	19	9	25	20	21	9	30	28	19	8	19	19	18
o-TMA	5	33	18	13	5	30	18	15	3	35	23	16	5	25	15	11
o-sour	5	26	16	11	4	20	11	12	3	20	18	13	5	19	11	10
o-sulphur	2	14	9	4	2	9	10	7	2	11	12	5	4	7	9	6

Table 23. The average QDA scores for flavour of cooked fillets injected with different solutions during chilling and frozen storage

Sensory	C				F				FePI				FoPI			
attribute	2d	7d	14d	49d	2d	7d	14d	49d	2d	7d	14d	49d	2d	7d	14d	49d
flavour																
f-salt	17	14	18	12	27	16	24	15	28	19	21	15	20	15	19	12
f-metallic	44	13	24	23	43	16	26	19	38	15	22	21	44	16	27	19
f-sweet	37	15	24	20	38	15	26	23	30	16	23	26	35	17	25	21
f-meat	37	19	23	24	35	14	27	21	41	14	22	21	34	20	27	21
f-frozen	9	11	18	16	8	8	18	17	11	10	19	21	8	9	13	18
f-pungent	14	22	18	9	10	14	18	7	13	19	17	9	11	13	16	8
f-sour	11	20	14	9	6	16	13	14	7	16	18	10	6	15	11	8
f-TMA	7	28	14	13	5	22	17	16	7	18	21	17	5	18	12	13
f-off	11	30	16	12	7	29	16	19	9	23	24	19	8	26	15	17

Table 24. The average QDA scores for appearance of cooked fillets injected with different solutions during chilling and frozen storage

Sensory	C				F				FePI				FoPI			
attribute	2d	7d	14d	49d	2d	7d	14d	49d	2d	7d	14d	49d	2d	7d	14d	49d
appearance																
a-dark	50	58	52	45	47	59	50	55	47	50	51	55	46	55	49	49
a-heterogeneous	44	57	50	42	44	52	45	50	43	50	47	49	44	56	45	45
a-precipitation	38	44	46	34	36	48	48	35	41	55	51	41	35	53	45	42

Table 25. The average QDA scores for texture of cooked fillets injected with different solutions during chilling and frozen storage

Sensory	C				F				FePI				FoPI			
attribute	2d	7d	14d	49d	2d	7d	14d	49d	2d	7d	14d	49d	2d	7d	14d	49d
texture																
t-flakes	44	52	47	36	44	46	42	35	34	44	42	29	32	47	42	30
t-soft	59	53	43	37	64	49	42	40	61	48	42	40	65	53	46	40
t-juicy	50	50	37	33	54	48	40	35	56	46	41	37	61	49	41	38
t-tender	50	61	44	44	53	57	43	45	58	58	46	52	60	60	47	48
t-mushy	37	45	33	30	44	46	40	33	48	46	39	30	40	49	38	32
t-meaty	45	31	41	46	39	25	36	44	43	24	34	44	37	26	40	47
t-clammy	27	15	25	37	18	18	26	30	24	11	26	32	20	18	26	33
t-rubbery	17	12	19	20	20	10	17	21	19	10	20	18	18	10	20	19

Statistical comparison of average QDA scores among the fillets groups without day 7 can be found in following tables. Day 7 was kept out of multivariate and anaova analysis because it was beond the shelf life - evaluated spoiled, and some panelists could not taste due to spoilage. Different superscript letters show that samples were different within a line.

Table 26. Comparision of average QDA scores of cooked fillets by Duncans test after 2d

Sensory attribute	*p-value*	C	S	FePI	FoPI
odour					
o-sweet	*0.920*	44	46	42	46
o-shellfish	*0.769*	39	37	32	36
o-meat	*0.866*	32	33	34	30
o-vanilla	*0.521*	29	33	25	26
o-potatoes	*0.809*	28	24	24	27
o-frozen	*0.551*	8	5	9	10
o-table cloth	*0.969*	7	9	9	8
o-TMA	*0.957*	5	5	3	5
o-sour	*0.932*	5	4	3	5
o-sulphur	*0.905*	2	2	2	4
appearance					
a-dark	*0.771*	50	47	47	46
a-heterogeneous	*0.999*	44	44	43	44
a-precipitation	*0.667*	38	36	41	35
flavour					
f-salt	*0.011*	17 [b]	27 [a]	28 [a]	20
f-metallic	*0.572*	44	43	38	44
f-sweet	*0.447*	37	38	30	35
f-meat	*0.493*	37	35	41	34
f-frozen	*0.887*	9	8	11	8
f-pungent	*0.659*	14	10	13	11
f-sour	*0.370*	11	6	7	6
f-TMA	*0.875*	7	5	7	5
f-off	*0.788*	11	7	9	8
texture					
t-flakes	*0.019*	44	44 [a]	34 [b]	32 [b]
t-soft	*0.391*	59	64	61	65
t-juicy	*0.078*	50	54	56	61
t-tender	*0.107*	50	53	58	60
t-mushy	*0.326*	37	44	48	40
t-meaty	*0.309*	45	39	43	37
t-clammy	*0.148*	27	18	24	20
t-rubbery	*0.925*	17	20	19	18

Table 27. Comparision of average QDA scores of cooked fillets by Duncans test after 14d

Sensory attribute	*p-value*	C	S	FePI	FoPI
odour					
o-sweet	*0.132*	27	29	25	33
o-shellfish	*0.567*	34	30	32	34
o-meat	*0.027*	24	27	22 [b]	30 [a]
o-vanilla	*0.002*	17 [b]	21 [b]	17 [b]	28 [a]
o-potatoes	*0.830*	35	33	31	33
o-frozen	*0.654*	19	17	20	17
o-table cloth	*0.150*	20	20	28	19
o-TMA	*0.342*	18	18	23	15
o-sour	*0.194*	16	11	18	11
o-sulphur	*0.768*	9	10	12	9
appearance					
a-dark	*0.831*	52	50	51	49
a-heterogeneous	*0.470*	50	45	47	45
a-precipitation	*0.250*	46	48	51	45
flavour					
f-salt	*0.083*	18	24	21	19
f-metallic	*0.353*	24	26	22	27
f-sweet	*0.673*	24	26	23	25
f-meat	*0.120*	23	27	22	27
f-frozen	*0.077*	18	18	19	13
f-pungent	*0.982*	18	18	17	16
f-sour	*0.084*	14	13	18	11
f-TMA	*0.140*	14	17	21	12
f-off	*0.142*	16	16	24	15
texture					
t-flakes	*0.294*	47	42	42	42
t-soft	*0.706*	43	42	42	46
t-juicy	*0.865*	37	40	41	41
t-tender	*0.775*	44	43	46	47
t-mushy	*0.367*	33	40	39	38
t-meaty	*0.072*	41	36	34	40
t-clammy	*0.999*	25	26	26	26
t-rubbery	*0.862*	19	17	20	20

Table 28. Comparision of average QDA scores of cooked fillets by Duncans test after 49d storage

Sensory attribute	*p-value*	C	S	FePI	FoPI
odour					
o-sweet	*0.928*	24	23	24	22
o-shellfish	*0.368*	25	28	23	23
o-meat	*0.984*	18	19	19	18
o-vanilla	*0.759*	19	21	21	18
o-potatoes	*0.954*	29	30	31	31
o-frozen	*0.836*	17	17	19	16
o-table cloth	*0.834*	19	21	19	18
o-TMA	*0.283*	13	15	16	11
o-sour	*0.556*	11	12	13	10
o-sulphur	*0.345*	4	7	5	6
appearance					
a-dark	*0.007*	45 [b]	55 [a]	55 [a]	49
a-heterogeneous	*0.087*	42	50	49	45
a-precipitation	*0.064*	34	35	41	42
flavour					
f-salt	*0.494*	12	15	15	12
f-metallic	*0.549*	23	19	21	19
f-sweet	*0.369*	20	23	26	21
f-meat	*0.639*	24	21	21	21
f-frozen	*0.380*	16	17	21	18
f-pungent	*0.768*	9	7	9	8
f-sour	*0.124*	9	14	10	8
f-TMA	*0.363*	13	16	17	13
f-off	*0.378*	12	19	19	17
texture					
t-flakes	*0.106*	36	35	29	30
t-soft	*0.608*	37	40	40	40
t-juicy	*0.632*	33	35	37	38
t-tender	*0.166*	44	45	52	48
t-mushy	*0.844*	30	33	30	32
t-meaty	*0.677*	46	44	44	47
t-clammy	*0.347*	37	30	32	33
t-rubbery	*0.747*	20	21	18	19

Table 29. Comparision of average QDA scores of cooked fillets by Duncans test after 2 days of chilled storage, 14 and 49 of frozen storage (Different superscript letters showed that samples were different within a line).

Sensory attribute		**C-0.5m**	**C-1.5m**	**C-2d**	**F-0.5m**	**F-1.5m**	**F-2d**	**I-0.5m**	**I-1.5m**	**I-2d**	**J-0.5m**	**J-1.5m**	**J-2d**
odour	*p-value*	1	2	3	5	6	7	9	10	11	13	14	15
o-sweet	***0,000***	27 c	24 c	44 ab	29 c	23 c	46 a	25 c	24 c	42 ab	33 bc	22 c	46 ab
o-shellfish	*0,705*	34	25	39	30	28	37	32	23	32	34	23	36
o-meat	*0,178*	24	18	32	27	19	33	22	19	34	30	18	30
o-vanilla	***0,001***	17 b	19 b	29	21	21	33 a	17 b	21	25	28	18 b	26
o-potatoes	***0,002***	35 a	29	28	33	30	24 b	31	31	24 b	33	31	27
o-frozen	***0,000***	19 a	17 a	8 b	17 a	17 a	5 b	20 a	19 a	9 b	17 a	16 a	10 b
o-table cloth	***0,000***	20 ab	19	7 c	20	21	9 bc	28 a	19	9 bc	19	18	8 bc
o-TMA	***0,000***	18 a	13 ac	5 bcd	18 a	15 a	5 bcd	23 a	16 a	3 de	15 ab	11 ad	5 be
o-sour	***0,000***	16 a	11 ab	5 bcd	11 ab	12 ac	4 de	18 a	13 ac	3 de	11 ac	10 ad	5 ce
o-sulphur	***0,000***	9	4	2 bc	10 ab	7	2 c	12 a	5	2 c	9	6	4 bc
appearance													
a-dark	*0,134*	52	45	50	50	55	47	51	55	47	49	49	46
a-heterogeneous	*0,197*	50	42	44	45	50	44	47	49	43	45	45	44
a-precipitation	***0,003***	46	34 b	38	48	35 b	36 b	51 a	41	41	45	42	35 b

Table 30. Comparision of average QDA scores of cooked fillets by Duncans test after 2 days of chilled storage, 14 and 49 of frozen storage (Different superscript letters showed that samples were different within a line).

Sensory attribute		**C-0.5m**	**C-1.5m**	**C-2d**	**F-0.5m**	**F-1.5m**	**F-2d**	**I-0.5m**	**I-1.5m**	**I-2d**	**J-0.5m**	**J-1.5m**	**J-2d**
flavour	*p-value*	1	2	3	5	4	6	7	9	11	13	14	15
f-salt	***0,002***	18	12	17 b	24 a	15	27 a	21	15	28 a	19	12	20
f-metallic	***0,000***	24 b	23 b	44 a	26	19 b	43 a	22 b	21 b	38	27	19 b	44 a
f-sweet	***0,008***	24	20 c	37 ab	26	23	38 a	23	26	30	25	21 bc	35
f-meat	***0,003***	23 b	24	37	27	21 b	35	22 b	21 b	41 a	27	21 b	34
f-frozen	***0,000***	18 a	16 a	9 c	18 a	17 a	8 c	19 a	21 a	11 bc	13 ab	18 a	8 c
f-pungent	***0,035***	18	9	14	18	7	10	17	9	13	16	8	11
f-sour	***0,000***	14 ab	9 bcd	11 bcd	13 ab	14 ab	6 de	18 a	10 ac	7 ce	11 ac	8 bcd	6 de
f-TMA	***0,000***	14	13	7 bc	17 ab	16 ab	5 c	21 a	17 ab	7 bc	12	13	5 c
f-off	***0,000***	16	12	11 b	16	19	7 b	24 a	19	9 b	15	17	8 b
texture													
t-flakes	***0,002***	47 a	36	44 ab	42	35	44 ab	42	29	34 bc	42	30	32 c
t-soft	***0,000***	43	37 de	59	42 bcd	40 ce	64 ab	42 bcd	40 ce	61 ac	46	40 ce	65 a
t-juicy	***0,000***	37 ce	33 de	50 bcd	40 bcd	35 ce	54 ac	41 bcd	37 ce	56 ab	41 bcd	38 ce	61 a
t-tender	***0,000***	44 bcd	44 de	50	43 ce	45 ce	53 ac	46 bcd	52 bcd	58 ab	47 bcd	48 ce	60 a
t-mushy	*0,087*	33	30	37	40	33	44	39	30	48	38	32	40
t-meaty	***0,000***	41 ab	46 a	45	36	44 ab	39 bc	34	44 ab	43	40	47 a	37 c
t-clammy	*0,113*	25	37	27	26	30	18	26	32	24	26	33	20
t-rubbery	*0,666*	19	20	17	17	21	20	20	18	19	20	19	18