Characteristics of freeze-dried fish protein isolated from saithe (*Pollachius virens*)

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Abstract Fish proteins isolated from by-products or low commercial/underutilized species using pH-shift process is a new source of proteins that may be used as wet or dried ingredients to develop value-added convenience foods. This paper reports the effects of freeze-drying on characteristics of fish protein isolates (FPI) from saithe (*Pollachius virens*) with or without lyoprotectants. Freeze-dried saithe mince from the same lot and without additives was used as a control. The resulting fish protein powders contained 71–93% protein, 1.5–3% moisture, 0–21% carbohydrate and 0.5–2% fat. Lipid oxidation (assessed by TBARS) of FPI powder groups was higher than that of fish mince powder. The results revealed that oxidation started during the pH-shift process and was increased by freeze-drying. Functional properties and sensory attributes were influenced by the advanced oxidation. However, the mince powder was less oxidized and had higher functional properties such as water binding capacity, gel forming ability, emulsification, foaming properties and colour and lower sensory scores for rancid odour and flavour than the FPI powders. Further studies on how to prevent oxidation of fish flesh during pH-shift and drying processes are recommended.

Keywords Fish protein isolates (FPI) · Saithe mince · Freeze-drying · Lipid oxidation · Functional properties · Sensory attributes

Introduction

Fish protein isolate (FPI) is manufactured from different kinds of raw materials by the pH-shift technology and it mostly contains myofibrillar proteins extracted from the fish muscle, in addition to membrane lipids, depending on how they are processed (Hultin et al. 2005). FPI can be used as an ingredient for production of value added and ready-to-eat products based on minced fish or surimi (Shaviklo et al. 2010a).

In the pH-shift process, proteins of the muscle tissue are first solubilised. The solubilisation can be accomplished by adding 5–10 volumes of water with alkali then added to obtain a pH of approximately 10.5 or higher or by adding acid to bring the pH below 3 (Hultin et al. 2005). The mixture is then centrifuged. This allows the light oil fraction to rise to the top of the suspension. At the same time the lipids of the membrane are removed due to density differences compared to the main protein solution. Other insoluble impurities, such as bones or skins are also sedimented at this stage. The muscle proteins are then precipitated by adjusting the pH to a value near the isoelectric point and collected by a process such as centrifugation (Kristinsson et al. 2005). Fish protein isolate (FPI) obtained from this process can be frozen like surimi or minced fish or might be dried for further utilization (Thorkelsson et al. 2008).

Dried fish protein or fish protein powder (FPP) is an excellent dietary supplement and can be added to a diverse range of products to provide a healthy source of easily

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digested proteins (Venugopal et al. 1996). FPP is utilised as a binder, dispersing agent and emulsifier in producing restructured products, formulated seafood and other food products and due to its strong interactions with other proteins and its high gel forming ability (Ramirez et al. 1999, Chung et al. 2000, Carvajal et al. 2005).

Functionality, stability and sensory properties of FPP play important roles when incorporating proteins in food formulation (Damodaran 2008) and have a great importance on the general sensory acceptance of formulated products (Schenz and Morr 1996). These characteristics are influenced by drying methods, additives (lyoprotectants) and process conditions (Venugopal et al. 1996, Chung et al. 2000, Huda et al. 2001, Eikevik et al. 2005, Bragadottir et al. 2007, Shaviklo et al. 2010b).

There are very few published scientific papers on how to preserve FPI during processing. Adding additives (cryoprotectants) to FPI to preserve proteins against denaturation during freezing and frozen storage have been recommended (Thawornchinsombut and Park 2006, Campo-Deano et al. 2010, Shaviklo et al. 2010c) but there is no published documents regarding how to protect FPI during drying process. However, it has been reported that drying of fish protein isolated from herring (*Clupea harengus*) can increase lipid oxidation and the resulting FPI powders had poor functionality with an intense rancid odour with a dark yellowish or grey colour (Geirsdottir 2005). Therefore, using FPP as an ingredient in food systems depends on whether it is possible to stabilize the residual lipids by suitable processing methods (Thorkelsson et al. 2008).

Saithe is a ganoid fish found only in the North Atlantic areas including Iceland (Olsen et al. 2010). In 2009, landing of saithe in Iceland was 61,000 t, a decrease of approximately 13% compared to 2008 (HAFRO 2010). Saithe is very similar to cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) but it has relatively low commercial value and receives less attention in markets due to its appearance and sensory attributes (Smith and Hardy 2001). Therefore, adding value to saithe through drying may provide an opportunity for better utilisation of this low commercial value fish. The objectives of this work were to investigate the possibility of drying saithe protein isolate to increase its potential commercial value by developing a functional fish protein powder, and to study the effects of using additives in FPI before freeze-drying on its stability, functional properties and sensory attributes.

Materials and methods

Raw materials (fish mince and FPI) Saithe was caught in south west waters of Iceland in February. The chilled (<+4°C) fresh skinless fillets were 3 days old when they

brought to laboratory. The length and weight of fillets were 40–45 cm and 800–1,000 g, respectively. They were provided by a local fish processing company in Reykjavik, Iceland and packed in 4 polystyrene boxes (net wt. of each 10 kg). All fillets were from the same lot. Ten kg of the fillets were kept at 4°C for 24 h and then minced and freeze-dried and 30 kg were used for isolating proteins immediately after receiving the raw material. The alkali-aided process was applied to isolate fish proteins at Iceprotein Company in Saudarkrokur, Iceland (Hultin et al. 2005). Temperature was monitored throughout the process and isolated fish proteins were stored overnight at 1±1°C. The next day, FPI was transferred to Matis processing laboratory in Reykjavik, Iceland, in 7 polyethylene (PE) buckets (each bucket 10 kg) under chilled conditions (<+4°C).

Preparation of test samples All test samples were prepared within 6 h after receiving FPI and mince. Since FPI was too watery (7.4% dry matter), it was squeezed manually using cheese cloth to remove as much water as possible. Fish fillets were also minced using an industrial meat mincer (Sirman-TC12E, Curtarolo, Italy). Five types of samples were prepared from FPI (13.3% dry matter) and mince as follows:

- Fish mince without additives as a control group (Group A);
- FPI without additives (Group B);
- FPI containing 5% sucrose (Group C);
- FPI containing 5% sucrose and 0.2% phosphate (Group D);
- FPI containing 5% sucrose, 0.2% phosphate and 0.1% erythorbate (Group E);

FPI and additives were weighed separately and mixed for 3 min using a vertical cutter (VCB-62, HILDE® Sweden) at high speed. Temperature of the mixture was less than 7°C during mixing. Fish mince and FPI samples (40 kg) were packed separately in aluminium trays. Each tray contained a 500 g sample of 3 cm thickness; the trays were blast frozen at -24°C for 12 h. Freeze-drying was done based on a previous study (Shaviklo et al. 2010b). The samples were loaded into a pilot lyophilizer (VirTis Co., Genesis Series, USA) at a pressure of 250 mm Hg in the chamber and condensing plate temperature was -80°C. The freeze-drier was activated and run for 120 h. Freeze-dried saithe (FDS) samples were milled and sieved using a laboratory test sieve with 500 microns screen mesh (BS410/1986-Endecotte LTD, London, England). About 1,000 g of freeze-dried fish protein powder was obtained for each treatment. The powders were vacuum-packed and stored at -80°C until further analysis.

Physico-chemical analysis Proximate composition of wet and dried mince and FPIs was determined according to AOAC

(1990) methods. Crude protein content was determined using the Kjeldahl method (Kjeltec System, FOSS Tectator, Hoganas, Sweden). Crude lipid content was measured by the Soxhlet method (Soxtec System-Textator, Sweden). Ash content was determined by ashing samples over-night at 550°C. Moisture content was determined by drying samples 4 h at 105°C until constant weight was achieved. Carbohydrate content was calculated by difference.

The pH of the FDS samples was measured by the method of Bragadottir et al. (2007). Five g of FDS was mixed with 20 mL of deionized water and stirred for 5 min prior to measurement with an Ag/AgCl combination electrode connected to a pH meter (model PHM80 Radiometer, Copenhagen, Denmark).

A water activity meter (model RTD 500, Novasina, Lachen, Switzerland) was used to measure water activity (a_w) of FDS samples. About 2 g of samples were put into the instrument and a_w was measured automatically after starting the program.

Density was measured by placing each sample in a pre-weighed 10 mL graduated cylinder up to 10 mL mark with gentle tapping. The graduated cylinder weighed again and the density was calculated as g powder per mL volume (Venugopal et al. 1996).

Thiobarbituric acid reactive substances (TBARS) were measured by a slightly modified steam distillation method (Tarladgis et al. 1960), where the sample size was reduced to 5 g and antioxidants (5 mL of 0.5% propyl gallate and 0.5% ethylene diamine tetra acetic acid (EDTA) in water) were added to the sample during blending. Malondialdehyd-bis-(diethyl acetate) was used as a standard.

Colour characteristics of FDS samples were measured by placing them in a test tube (25 mm in diameter) which was read in a Minolta CR-400 Chroma Meter (Minolta Camera Co. Ltd. Osaka, Japan) in Lab* measuring mode with CIE Illuminant C. The colour was measured 3 times per sample, turning the test tube 120° among measurements. Results were given as lightness (L^*), redness (a^*) and yellowness (b^*). Whiteness was calculated by the following equation according to Codex Alimentarius (Park 2005).

$$\text{Whiteness} = L^* - 3b^* \quad (1)$$

Functional properties Gel forming ability was determined for each FDS sample by the method used by Huda et al. (2001) and modified by Shaviklo et al. (2010b). FDS was added to 10 mL of distilled water in a test tube to make solutions containing 1–10% protein, and mixed in a high speed mixer (IKA Disperser, T25 Digital Ultra-Turrax®, Boutersem, Belgium) for 1 min. The tubes were heated at 90°C for 30 min in a water bath (JULABO open heating bath circulator, ED-13, Seelbach, Germany), then placed in a chilled water bath (0–2°C) for 30 min. Gel forming

ability was reported as the lowest concentration at which samples did not fall down or slip from an inverted test tube.

A Bohlin BV88 viscometer (Bohlin Instruments, Malvern, UK) was used to determine viscosity of FDS samples. A sample solution containing 10% protein was prepared from each FPP. A kitchen immersion hand blender was used at high speed for 1 min to make a homogenized solution. The solution was kept refrigerated overnight. It was homogenized the day after, before running viscosity measurement. The instrument cylinder was immersed into a beaker containing 200 mL of the sample solution for viscosity measurement. The viscosity of the sample was recorded after 10 s of operating the instrument at 5–7°C, speed setting 6 and system switch 6 (Shaviklo et al. 2010b).

To measure water binding capacity (WBC) the FDS (25 g) was weighed into a centrifuge bottle and 100 g of distilled water added. The mixture was left to stand for 30 min at room temperature with intermittent stirring and then the sample was centrifuged using a high speed centrifuge (Sorvall® RC-5B refrigerated super speed centrifuge, du Pont Instruments, Wilmington, DE, USA) for 30 min at 1,675×g. Afterwards the supernatant was weighed and used for protein solubility measurement, but the remaining powder plus liquid retained by the powder was determined gravimetrically. Results were calculated as follows (Beuchat 1997):

$$\text{WBC}(\%) = \frac{A - B}{C} \times 100 \quad (2)$$

Where A = Amount of water added to FPP before centrifugation (g), B = Amount of water removed from wet protein after centrifugation (g), and C = Sample (g).

The supernatant from the WBC measurement was used to determine protein solubility of FDS samples. The Kjeldahl method was used to determine the amount of protein ($N \times 6.25$) in both the supernatant and the original protein powder (Bragadottir et al. 2007). The protein solubility (PS) in water (%) was determined in duplicate per sample according to the following equation (Vojdani 1996):

$$\text{PS} = \frac{\text{Amount of soluble protein}}{\text{Total amount of protein}} \times 100 \quad (3)$$

Emulsion capacity (EC) and emulsion stability (ES) were measured using the method described by Yasumatsu et al. (1972). An amount of FDS was added to 25 mL distilled water and 25 mL corn oil (Mazola, Summit, IL, USA) to give a final protein concentration of 1%. The mixture was then blended in a high speed mixer (IKA disperser, T25 Digital Ultra-Turrax®, Boutersem, Belgium) for 1 min and transferred to a 50 mL calibrated tube and

centrifuged at $7,500\times g$ for 5 min (Sorvall® RC-5B). The EC was calculated using the same formula as below:

$$EC \text{ and } ES(\%) = \frac{\text{Emulsion volume after centrifugation (mL)}}{\text{Emulsion volume before centrifugation (mL)}} \times 100 \quad (4)$$

The same procedure and equation was carried out to determine ES except that before centrifugation the emulsion was heated at 90°C for 30 min, followed by cooling under tap water for 10 min.

Foaming capacity (FC) and foaming stability (FS) were determined by the method applied by Miller and Groniger (1976). An amount of powder was added to distilled water to make a solution with 10% protein. It was blended in a high speed mixer (IKA disperser, T25 Digital Ultra-Turrax®, Boutersem, Belgium) for 1 min at highest speed. The mixture was transferred carefully into a 250 mL calibrated beaker for volume measurement. FC was calculated as the volume of mixture after blending compared to the original volume. FS was the ratio of the foam capacity after 8 h observation divided by the original FC.

Microbial analysis Total plate count, total coliforms, *E. coli*, *salmonella spp.* moulds and yeasts were measured in 5 sample groups according the Methods for the Microbiological Examination of Foods (APHA 1992).

Sensory analysis Generic descriptive analysis (GDA) was applied to evaluate odours and flavours of FDS sample. Eight panellists were selected and trained based on an international standards (ISO, 1993). Panellists were trained within two sessions and during training they used a scale which was applied by Shaviklo et al. (2010b) to describe sensory attributes of the fish protein solutions. As the FPI solutions had very strong odour and flavours, 1% solution of each sample group was prepared 1 h before evaluation. An unstructured scale (0–100%) was used by the trained panellists to evaluate the intensity of each sensory attribute (Shaviklo et al. 2010b). All sample observations were done based on an international standard (ISO 1988). All sample groups were coded with 3-digit random numbers and presented to panellists on a tray in individual booths. Serving orders were completely randomized. Panellists evaluated the 5 sample solutions in duplicates in 2 sessions. Water was provided between samples to cleanse the palate.

Statistical analysis Analysis of variance (ANOVA) was carried out on all other results in the statistical program NCSS 2000 (NCSS, UT, USA). The program calculates multiple comparisons using Duncan's test to determine if sample groups are different. Significance of difference was

defined at the 5% level. PanelCheck software (version V1.3.2, MATFORSK, Norway) was applied to monitor panelist's performance.

Results and discussion

Physico-chemical analysis Significant differences were observed for moisture, protein, ash, carbohydrate and pH between the fish mince and wet FPI samples (Table 1). Freeze-dried FPIs had lower pH than freeze-dried mince powder because of using relatively low pH during the precipitation step in pH-shift process for isolating fish protein (Table 1). Among FPI powder groups pH of FPI samples containing phosphate (groups D & E) was higher than in FPI without phosphate (groups B & C) due to effect of phosphate (Knipe 1992). The lower content of protein and the higher content of carbohydrate in FPI groups with additives were due to the inclusion of 2.5% sucrose to isolate to protect proteins during freeze-drying (Chung et al. 2000, Huda et al. 2001, Musa et al. 2002, 2005 Shaviklo et al. 2010b).

Water activity values of freeze-dried fish mince and FPIs varied significantly because of different levels of moisture in the samples and possibly the influence of additives (Table 2). Freeze-dried FPI containing sucrose and phosphate (groups D & E) had higher value of a_w than other groups followed by the freeze-dried mince. Water activity of all FDS samples was less than 0.3. Food/food ingredients in this range of a_w have a long shelf life (Fontana 1998).

Significant differences were found for density among FDS samples. Density of freeze-dried mince and FPI without additives was lower than in other samples. However the density of the freeze-dried mince and FPI samples was higher than the freeze-dried and spray-dried saithe surimi reported by Shaviklo et al. (2010b) and freeze dried surimi from lizard fish (*Saurida tumbil*), threadfin bream (*Nemipterus japonicus*) and purple-spotted big-eye (*Priacanthus tayenus*) prepared by Huda et al. (2001) and than freeze-dried surimi prepared from threadfin bream by Musa et al. (2005). Factors such as amount of additives, drying method, fish powder preparation (Huda et al. 2001, Musa et al. 2005, Shaviklo et al. 2010b) and a_w (Fontana 1998) may influence the density of fish protein powders.

TBARS levels in freeze-dried mince without additives were the lowest among all treatments. TBARS levels were more than $80 \mu\text{mol/kg}$ in all FPI powders suggesting that FPIs were oxidized before freeze-drying and that additives could not prevent oxidation (Fig. 1). Although, the pH-shift process has been reported to reduce lipids and active pro-oxidants from FPI (Hultin et al. 2005) there were still a small amount of lipids in the product enough to lead to

Table 1 Proximate composition of fish mince and FPI

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Salt (%)	Carbohydrate (%)	pH
Wet							
A	77.6±0.41 ^b	17.7±0.16 ^a	0.24±0.01 ^a	1.2±0.09 ^a	0.14±0.02 ^a	0 ^b	6.7±0.07 ^a
B	86.6±0.50 ^a	12.7±0.14 ^b	0.15±0.01 ^a	0.43±0.03 ^b	0.22±0.01 ^a	0 ^b	5.7±0.06 ^b
C	82.4±0.63 ^a	12.4±0.12 ^b	0.21±0.01 ^a	0.51±0.06 ^b	0.21±0.02 ^a	5.0±0.15 ^a	5.7±0.07 ^b
D	83.2±0.57 ^a	12.2±0.14 ^b	0.14±0.01 ^a	0.63±0.02 ^b	0.24±0.03 ^a	5.2±0.14 ^a	5.8±0.08 ^b
E	82.7±0.38 ^a	12.2±0.10 ^b	0.13±0.02 ^a	0.62±0.05 ^b	0.23±0.01 ^a	5.3±0.17 ^a	5.8±0.06 ^b
	*	*	*	*	*	*	*
Dried							
A	1.9±0.04 ^b	93.5±0.42 ^a	1.9±0.05 ^a	6.1±0.38 ^a	1.0±0.12 ^c	0.0 ^b	6.7±0.03 ^a
B	1.4±0.07 ^c	94.0±0.35 ^a	0.62±0.04 ^b	2.7±0.16 ^c	1.9±0.09 ^a	0.0 ^b	5.5±0.06 ^c
C	1.6±0.08 ^c	73.6±0.35 ^b	0.54±0.05 ^b	2.2±0.29 ^c	1.4±0.06 ^b	20.7±0.15 ^a	5.7±0.07 ^c
D	2.8±0.05 ^a	71.5±0.28 ^b	0.42±0.03 ^b	3.9±0.25 ^b	1.4±0.06 ^b	20.3±0.14 ^a	6.1±0.09 ^b
E	1.9±0.07 ^b	71.6±0.14 ^b	0.44±0.03 ^b	4.1±0.20 ^b	1.4±0.08 ^b	20.5±0.12 ^a	6.3±0.08 ^b
	*	*	*	*	*	*	*

Values are means of 2 analyses

Different small letters show significant different within a column (* $p<0.05$)

(A) Mince without additives, (B) FPI without additives, (C) FPI with 5% sucrose, (D) FPI with 5% sucrose and 0.2% phosphate, (E) FPI with 5% sucrose, 0.2% phosphate and 0.1% erythorbate

significant development of oxidation products. The increase in TBARS indicates the formation of secondary oxidation products (Frankel 1998).

The acid-aided pH-shift process can lead to more rapid oxidation of the lipid fraction of fish flesh than the alkaline-aided process because of activating of haem proteins as pro-oxidants at low pH (Hultin et al. 2005). In the alkaline-aided process there is a subjection to relatively low pH (around 5.5) during the precipitation step. Below pH 6.3 or so FPI as well as minced fish are very unstable to lipid oxidation (Kristinsson and Liang 2006). FPI samples were all between pH 5.5–6.1 explaining the high levels of TBARS.

Studies on lipid oxidation of catfish (*Ictalurus punctatus*), croakers (*Johnius dussumieriri*), mullet (*Mugil cephalus*), and Spanish mackerel (*Scomberomorus maculatus*) during production of acid- and alkali-aided protein isolates as well as during conventional surimi revealed that the conventional

surimi had lower levels of lipid oxidation products than acid- and alkaline-processed isolates. The acid protein isolate was most prone to oxidation while the alkali made isolates fell in between the other 2 methods (Kristinsson and Demir 2003). Other studies have also reported lipid oxidation in fish protein isolates during pH-shift process (Kristinsson and Hultin 2004, Kristinsson et al. 2005, Kristinsson and Liang 2006).

Freeze-dried mince showed the highest lightness and whiteness compared to freeze-dried FPIs (Table 2). However, L^* values of all FDS samples used in this study were lower than that found for freeze-dried saithe surimi reported by Shaviklo et al. (2010b), freeze dried threadfin bream surimi reported by Musa et al. (2005) and Huda et al. 2001 or air-dried Alaska pollock (*Theragra chalcogramma*) surimi (Park 1995).

Significant differences were found in redness values of FDS samples. Redness values of FPI powders lower than

Table 2 Water activities, density and colour characteristics of freeze-dried saithe proteins

Sample	A	B	C	D	E
Water activity	0.05±0.002 ^b	0.03±0.001 ^c	0.04±0.004 ^c	0.07±0.003 ^a	0.05±0.005 ^b
Density (g/mL)	3.7±0.07 ^b	3.9±0.03 ^b	4.6±0.05 ^a	4.7±0.06 ^a	4.7±0.04 ^a
Lightness (L^*)	41.2±0.16 ^a	36.6±0.28 ^b	39.2±0.27 ^b	39.3±0.23 ^b	38.6±0.25 ^b
Redness (a^*)	-1.12±0.07 ^a	-2.50±0.01 ^b	-2.56±0.06 ^b	-2.71±0.12 ^b	-2.67±0.03 ^b
Yellowness (b^*)	5.2±0.09 ^c	6.9±0.08 ^a	6.2±0.02 ^b	6.1±0.05 ^b	6.4±0.07 ^b
Whiteness ($L-3b^*$)	25.4±0.37 ^a	15.8±0.54 ^c	20.7±0.17 ^b	21.1±0.43 ^b	19.5±0.14 ^b

Values are means of 3 analyses

Different small letters show significant different within a row ($p<0.05$)

Fig. 1 Lipid oxidation of fish mince and FPI powders as assessed by formation of thio-barbituric acid reactive substances. Different small letters show significant difference ($p<0.05$)

redness values of freeze-dried surimi were reported by Shaviklo et al. (2010b). The yellowness values of freeze-dried mince were lower than freeze-dried FPI significantly. Similar results were reported by Shaviklo et al. (2010b) for yellowness of freeze-dried saithe surimi. Yellowness values of all fish protein powders studied in this work were lower than the values reported by Huda et al. (2001) for freeze dried lizardfish surimi powder and by Musa et al. (2005) for freeze-dried threadfin bream surimi.

Myoglobin and haemoglobin which are responsible for colour characteristics of fish flesh (Park 1995, Venugopal and Shahidi 1996) remain in the FPI and oxidation of these proteins can cause a yellow-brownish colour in FPI (Cortes-Ruis et al. 2001, Choi and Park 2002, Yongsawatdigul and Park 2004, Perez-Mateos and Lanier 2006, Kristinsson and Liang 2006). In addition lipid oxidation causes poor visual appearance and a yellowish discoloration (Gill 1995, Belitz et al. 2009). Based on data from this study factors such as pre-drying treatment, temperature used and lipid oxidation may affect the colour of the fish protein powder.

Functional characteristics The gel forming ability varied significantly among FPI with additives and free additive fish mince (Table 3). Only mince powder and FPI with sucrose and FPI with sucrose, phosphate and erythorbate could make gel at a concentration of 6–8% protein. Shaviklo et al. (2010b) reported that freeze-dried and spray-dried saithe surimi gelled at 1% and 2% protein concentration respectively. Gel forming ability is affected by the extent of denaturation of myofibrillar proteins, relative concentration of myofibrillar versus sarcoplasmic and/stroma protein, and additives used for protecting proteins during frozen storage and drying (Lanier and Carvajal 2005). However, gel forming ability of FPPs depends on factors such as drying method, amount of additives, protein concentration and pH (Musa et al. 2005, Shaviklo et al. 2010b).

Viscosity of all FDS samples was low (Table 3). Freeze-dried mince had the highest level of viscosity followed by the freeze-dried FPI containing sucrose, phosphate and erythorbate. This may be due to denaturation and modification of protein conformation in FPI powders.

Table 3 Functional properties of freeze-dried saithe protein samples

Properties	A	B	C	D	E
Water binding capacity (%)	315.0±7.1 ^a	287.4±4.2 ^b	236.3±6.4 ^d	253.1±5.6 ^c	259.0±5.6 ^c
Protein solubility in water (%)	37.4±1.1 ^a	20.8±0.9 ^c	14.5±0.8 ^d	26.8±0.9 ^b	20.1±0.8 ^c
Viscosity (Pa)	0.90±0.00 ^a	0.13±0.00 ^d	0.06±0.00 ^c	0.27±0.00 ^c	0.29±0.00 ^b
Gel forming ability (%)	6.0±0.5 ^b	>10	8.0±0.5 ^a	>10	6.0±0.5 ^b
Emulsification capacity (%)	74.2±1.4 ^b	78.5±2.1 ^a	82.5±1.9 ^a	82.5±1.8 ^a	82.5±1.9 ^a
Emulsification stability (%)	71.3±1.3 ^b	76.5±2.1 ^a	80.5±1.8 ^a	76.5±1.6 ^a	78.5±1.6 ^a
Foaming capacity (%)	145.0±6.8 ^b	155.4±6.9 ^b	190.4±13.7 ^a	189.7±13.8 ^a	190.3±14.1 ^a
Foaming stability (%)	125.2±5.9 ^{bc}	100.3±3.1 ^c	100.5±4.9 ^c	145.5±5.7 ^a	145.2±6.7 ^a

Values are means of 2 analyses

Different small letters show significant difference within a row ($p<0.05$)

Fig. 2 Principal Component Analysis Bi-Plot describing sensory quality scores of 1% freeze-dried mince and FPI solutions as evaluated by a trained sensory panel. O= odour, F= flavour

The protein solubility in water is greatly dependent on pH and on salt concentration (Belitz et al. 2009) and the results presented in Table 3, indicate significant differences in protein solubility due to pH differences. The highest value for protein solubility was for freeze-dried mince (pH, 6.7) followed by the freeze-dried FPI containing sucrose and phosphate (pH, 6.1). Freeze-dried FPI with sucrose had the lowest solubility of the isolate powders (pH, 5.7). Solubility is influenced by protein denaturation (Cordova-Murueta et al. 2007). Protein solubilities of all FDS samples were higher than reported for surimi powders dried by freeze-drying (Huda et al. 2001), oven-drying (Huda et al. 2000), and air-drying (Venugopal et al. 1996) but lower than that reported by Shaviklo et al. (2010b) for freeze-dried surimi indicating that the method of pre-treatment and processing affects the solubility of proteins (Kilara and Harwalkar 1996).

There was a significant difference in WBC between treatments as illustrated in Table 3. The highest value of WBC was for freeze-dried mince followed by freeze-dried FPI without additives. WBC values of FPI powders were lower than freeze-dried surimi reported by Shaviklo et al. (2010b) due to lower pH in FPI. FPI powders with additives had the lowest WBC possibly because of increasing oxidation products during freeze-drying that could affect functional properties (Damodaran 2008).

No differences were seen for emulsion capacity and stability of freeze-dried FPI. Emulsion capacity and stability of freeze-dried mince without additives was lower than all FPI powders. All FDS samples showed higher results than the emulsion capacity reported by Huda et al. (2001) for freeze-dried lizard fish and threadfin bream surimi and that by Musa et al. (2005) for freeze-dried threadfin bream. Proteins with both hydrophilic and

hydrophobic residues act as an emulsifier and when they have a balance between hydrophilic and hydrophobic residues the emulsion capability is optimum (Belitz et al. 2009). While some proteins lose their hydrophilic residues during the drying process additives that are used in surimi preparation could increase hydrophilic residue to the optimum level and this results in improving in emulsion properties (Matsuda and Noguchi 1992).

Table 4 Average sensory scores (scale: 0–100) for 5 treatments of fish mince and FPI powders (at 1% solution)

Attribute		A	B	C	D	E
Odour						
Fish liver oil	***	11.2 ^b	17.8 ^b	21.3 ^b	20.4 ^b	44.6 ^a
Dried fish	**	13.1 ^b	29.7 ^a	30.6 ^a	32.3 ^a	24.1 ^{ab}
Rancidity	***	3.9 ^b	6.7 ^b	5.3 ^a	4.2 ^b	21.7 ^a
TMA	NS	4.2	7.2	4.8	5.1	10.2
Flavour						
Fish liver oil	***	13.3 ^b	28.9 ^b	22.2 ^b	23.6 ^b	53.1 ^a
Dried fish	**	16.7 ^c	35.2 ^{ab}	32.8 ^{ab}	35.6 ^{ab}	26.9 ^{bc}
Rancidity	***	5.1 ^b	10.4 ^b	8.9 ^b	8.2 ^b	21.5 ^a
Sweet	NS	6.3	9.7	9.2	7.8	10.4
Seaweed	NS	7.4	14.8	15.1	15.5	12.3
TMA	NS	4.1	7.8	8.9	9.3	10.0
Bitter	NS	8.2	5.6	5.3	6.4	6.7
Off-flavour	NS	1.7	1.9	2.1	2.4	4.3

Values are means of 2 evaluations

Different small letters show significant difference within a row (** $p < 0.01$, *** $p < 0.001$)

NS Not significant

Freeze-dried FPIs with additives had higher foaming capacity than freeze-dried FPI and mince without additives (Fig. 2). Freeze-dried FPI with sucrose and phosphate and freeze-dried FPI containing sucrose, phosphate and erythorbate had the same value of foaming stability, higher than other treatments. All FDS samples showed higher results than the emulsion capacity reported by Huda et al. (2001) for freeze-dried lizard fish and threadfin bream surimi and by Musa et al. (2005) for freeze-dried threadfin bream. Foaming properties are influenced by protein concentration, pH, salt, sugar, lipids, and the method used for foam formation. Foams collapse because large gas bubbles grow at the expense of smaller bubbles (disproportionate). The protein films counteract this disproportionate. That is why the stability of foam depends on the strength of the protein film and its permeability for gases (Belitz et al. 2009). A protein may have excellent foamability but it may not necessarily produce stable foam and vice versa (Wilde and Clark 1996). The highly stable foam from isolates with sucrose and phosphate and isolate containing sucrose, phosphate and erythorbate may be related to its highly stable protein films indicating positive effects of additives for preserving proteins during drying.

Microbial analysis No significant differences were found among all freeze-dried samples for microbial analysis. The total plate count was less than 3×10^3 log cfu/g. Neither *E. coli* nor *salmonella* was detected in the samples and total coliforms, mould and yeast, counts were below 10 log cfu/g indicating that the product was microbiologically safe (Jay 1992). It also confirms sanitation quality of used water, proper handling and processing of FPP based on Good Hygiene Practices (GHP) in food production (Fewtrell et al. 2007; WHO/FAO 2001).

Sensory analysis The sensory attributes of freeze-dried mince and FPIs were assessed in terms of odour and flavour (Table 4). The FDS samples were described by the following attributes: dried fish odour and flavour, fish liver oil odour and flavour, rancid odour and flavour and seaweed flavour. Freeze-dried mince without additives (group A) had lower intensities of dried fish odour and flavour, fish liver oil flavour than the freeze-dried FPI samples. On the contrary freeze-dried FPI with sucrose, phosphate and erythorbate (group E) which had the lower lipid oxidation value than freeze-dried FPI with sucrose (group C) had the highest intensities of fish liver oil odour and flavour and rancid flavour and odour among all other sample groups possibly because of combination of oxidation products with additives during drying and accelerating formation of such odours and flavours in this sample group.

A multivariate analysis of the data showed that 98% of variation between the samples was explained in the first

two components. FPI powder with sugar, phosphate and erythorbate (group E) located on the upper right side of Fig. 2 was more described by fish liver oil and rancidity odours and flavours. No attributes were described for the mince powder (group A), but dried fish odour and flavour were addressed for FPI powder without additives (group B), FPI powder with sucrose (group C), and FPI powder with sucrose and phosphate (group D) in were in the lower left side of this figure. FPI powder without additives (group B), FPI powder with sucrose (group C) and FPI powder with sugar and phosphate (group D) were not different in terms of fish liver oil, and rancidity odours and flavours.

The high rancid odour and flavour in FPI powders indicates that oxidation occurred during processing (Geirsdottir 2005). Lipid oxidation in fish muscle (Gill 1995) and in FPI (Kristinsson and Demir 2003, Undeland et al. 2005) which can produce off-odour and off-flavour can negatively affect protein functionality (Gutteridge 1988, Belitz et al. 2009). Thus precautions should be taken to protect fish mince/proteins against oxidation during and after processing.

Conclusion

Lipid oxidation is a problem in dried fish protein and it is influenced by freshness of raw material, pre-treatment and processing conditions. The sensory, functionality and stability results of the freeze-dried FPIs were found to be influenced by development of oxidation products in FPI treatments. The results revealed that mixing additives to the FPI before freeze-drying could not prevent oxidation possibly because of oxidation of FPI during pH-shift process. Freeze-dried mince without additives had lower value of oxidation and higher functionality and lower sensory scores such as fish liver oil flavour compared to freeze-dried FPIs. Further studies on how to prevent oxidation of fish flesh during pH-shift and drying processes are recommended if dried FPI are to be used as ingredients in food formulations.

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