Fiskeriforskning

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Protein Isolation from Herring

Textural and colour properties of different fish proteins isolated at high or low pH

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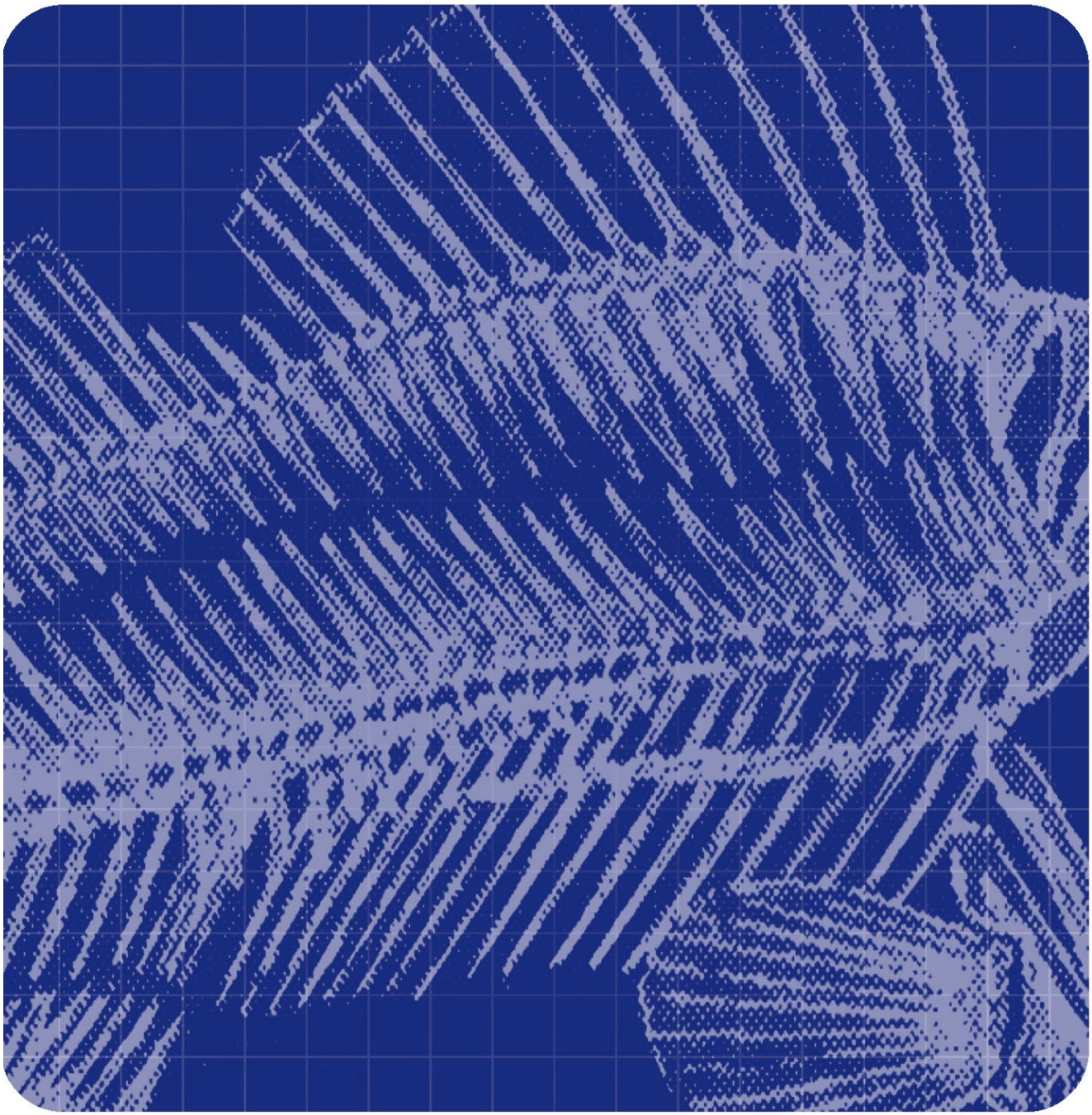

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REPORT

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Textural and colour properties of different fish proteins isolated at high or low pH.

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Summary:
Fiskeriforskning has been partner in a project financed by industry and the Nordic Innovation Centre, with the aim to scale up a laboratory method for producing functional fish protein isolates. The pilot plant was located in Iceland and four Nordic countries participated, representing four research institutes and three industry companies.
The choice of extraction method, acid or alkali, is essential for the quality of the FPI. The extraction methods give significant different results regarding all parameters assessed, except for true strain. Texture values were generally higher in alkali samples than acid samples.

The sensory analysis gave a preference to alkali treatment regarding textural properties, while the acid treatment seemed to result in better colour and less rancid odour and taste.
Addition of phosphates (TSPP) gave a significant difference in all parameters except for shear stress. The differences resulted in higher texture values and lower scores on all colour characteristics.

Both Trehalose and Raftilose®P95 seem to have at least as good capacities as sorbitol and sucrose, to be used as cryoprotectants. When comparing the two extraction methods within the same cryoprotectant group, the alkali treated samples scored higher in rancid odour with exception of sorbitol samples.

Optimum pH for good texture properties was found to be around pH 7,1. A negative effect of increased pH is a decrease in colour values. Decreasing b*-values did not outweigh the reduced L*-values.

Addition of soy protein isolate had no or rather negative effect on all parameters. Samples containing soy protein seem to be less affected to pH changes.

The dried samples had an intense rancid odour, with a dark yellowish or grey colour. The re-hydrated protein isolate samples did not produce a proper gel, and did not hold water very well. Rehydration time and homogeneity of the dried samples depended on granule size.

SUMMARY

Demand for proteins as ingredients in food production is high and increasing. Several methods have been developed to increase the commercial use of fish muscle from under-utilised fish species, by-catches, and processing by-products. Recently a process which utilises acid or alkali treatment of the muscle, followed by isoelectric precipitation of proteins (Hultin and Kelleher, 2000) has been investigated in this project with the intent to separate muscle tissue from skin and bones on an industrial scale.

The alkali treatment is preferred regarding textural properties, but the acid treatment seems to give better colour and less rancid odour and taste. Addition of phosphates (TSPP) is recommended due to favourable effects on texture and colour values.

Cryoprotection of fish minces and protein isolates, is a primary concern in maintaining quality during frozen storage. In order to improve quality, new alternative cryoprotectants have been explored. Sweetness reduction was one of our main objectives in this study.

Raftiline®P95 was the only cryostabiliser that differed significantly from the other in all tests except for gel strength and shear stress. The Raftiline®P95 samples gave higher values in gel strength and shear stress tests, but lower scores on true strain and distance. Raftilose®P85 seemed a little less effective than Trehalose and Raftilose®P95. Both Trehalose and Raftilose®P95 seemed to have at least as good capacities to protect proteins during frozen storage as sorbitol and sucrose without loosing textural or colour properties.

When comparing the two extraction methods within the same cryoprotectant group, the alkali treated samples in general scored higher in rancid odour. The addition of different cryostabilisers in the protein isolates were mainly reflected in odour and taste scores. Samples containing sorbitol (N5 and N17) gave the highest scores in rancid odour and rancid taste. Sucrose samples differed significantly compared to the rest of the samples as scoring high in sweet taste.

Relatively low rheological values were experienced on the fish protein isolate (FPI). Low cost soy protein isolate was tested for its ability to enhance functional quality. Several earlier works found positive effect by adding soy protein to low quality surimi. In this project, soy protein isolate had no or rather negative effect on all parameters. Samples containing soy protein seem to be less affected to pH changes though.

Optimum pH for good texture properties was found to be around pH 7,1. A negative effect of increasing pH is a decrease in colour values. Decreasing b*-values did not outweigh the reduced L*-values.

The dried samples of saithe protein isolate had an intense rancid odour, with a dark yellowish or grey colour. The re-hydrated protein isolate did not produce a proper gel, and did not hold water very well. Rehydration time and homogeneity of the dried samples was depended on granule size. No other FPI were dried.

In December 2004 a test production of FPI from herring was run at the Icelandic pilot plant. The conditions and results were:

- The alkaline process was used
- It was impossible to use a dish centrifuge to remove the fat phase
- It was impossible to use decanter centrifuge or squeezing trough cloth to remove water
- The samples contained ~90 % moisture on arrival in Norway
- The herring- FPI did not form a gel

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1 INTRODUCTION

Demand for proteins as ingredients in traditional and novel food products is high and increasing. Several methods have been developed to increase the commercial use of fish muscle from under-utilised fish species, by-catches, and processing by-products. Mechanical devices (e.g. Baader or Wolfking) have been used to produce fish mince of various quality from different raw material.

Recently a process which utilises acid or alkali treatment followed by iso-electric precipitation of proteins (Hultin and Kelleher, 2000) has been investigated with intent to separate herring muscle tissue from fat, skin and bones. In the laboratory the product is a highly functional fish protein isolate (FPI) with low fat content. In this Nordic project, financed by fish industry and the Nordic Innovation Centre, three industrial companies and four research institutes were involved in scaling up this process, with the aim of using herring as the raw material for the FPI. The companies were Haraldur Bøgvaldson - Iceland, D-Tech - Norway, AlfaLaval - Denmark. The research institutes were Icelandic Fisheries Laboratory - Iceland, SIK - Sweden, Sintef – Norway and Fiskeriforskning – Norway.

The main objective of the work at Fiskeriforskning was to assess the gelling ability and textural properties of the fish protein isolates, preferably from herring, produced in the Icelandic pilot plant. The work plan included the use of different non-conventional cryoprotectants to reduce sweet taste in the fish protein isolate, surimi and, fish mince, and to evaluate their protective or stabilising effects after freezing and freeze-thaw abuse. A second goal was to investigate the effects of soy protein isolate at different pH values, on the textural properties of the fish protein isolate. A third goal was to investigate the textural properties of re-hydrated dried fish protein.

The Icelandic pilot plant experienced technical challenges, which resulted in using a lean fish, fillets of saithe, rather than herring as raw material for most of the fish protein isolate production. At Fiskeriforskning FPI-samples received were from saithe, commercial surimi from Blue whiting, dried samples of saithe – FPI, and a few FPI made from herring.

1.1 Cryoprotection

Previous experience have documented that disadvantageous characteristics of frozen fish mince are texture hardening or toughening, poor functionality (e.g. gelling, formability, water holding ability), discoloration, and lipid oxidation and development of fishy odour (Lee & Lian, 2002). Therefore, cryoprotection of fish proteins is a primary concern in maintaining quality during frozen storage. In order to improve quality, new alternative cryoprotectants are continually being explored and some of these were the basis for the experiments at Fiskeriforskning.

Currently in commercial surimi production, a mixture of 4 % sucrose, 4 % sorbitol in conjunction with phosphate, has been adapted as the primary cryoprotective additives. There are several reasons for this choice: relatively low cost, good availability, good safety record, broad legal status, good solubility, and beneficial functional effects. When focusing on sweetness reduction, cryostabilisation by polymers such as maltodextrins and polydextrose (Park *et al*, 1988; Sych *et al*, 1991; Carvajal *et al*, 1999; Herrera *et al*, 1999; Auh *et al*, 1999)

could be viable alternatives. These high-MW polyols and glucose polymers are believed to stabilise proteins by raising the glass transition temperature (Tg) of a solution (Levine & Slade, 1988a, b). Maillard browning reactions from reducing sugars may limit applications in light- or white coloured products of fish (MacDonald & Lanier, 1991), and may also interact with and severely affect the activity of proteins (Hinrichs *et al*, 2001). Several workers have shown that addition of sugars like sucrose, maltose, and trehalose can stabilise proteins to the denaturing influence of drying (Matsuda, 1979, 1981; Carpenter *et al*, 1987a, b, 1988, 1990; Crowe *et al*, 1990). The same stabilising mechanisms have been believed to be valid also during frozen storage. Crowe *et al* (1990) and Carpenter *et al*, (1990) have demonstrated that specific steric requirements found in certain disaccharides such as trehalose, sucrose, and maltose are needed in order to effectively replace water molecules on the surface of the protein and thus stabilise the native protein structure.

According to literature, trehalose, which is a non-reducing disaccharide with 60% less sweetness than sucrose, shows promise as a stabiliser for various food commodities. Hunt *et al* found that texture values during frozen storage were best maintained by samples containing 0,3% $NaHCO_3$ and other sugars followed by the sample with 5% trehalose and 4% sucrose. Inulins are naturally occurring storage oligomers found in plants such as chicory and Jerusalem artichoke and have a degree of polymerisation (DE) of 2-60. These are comprised of linear β-D-(2→1) linked fructose oligomers ending with a α-D-(1→2) glucopyranose ring (Roberfroid *et al*, 1993; Spiegel *et al*, 1994). Raftilose®P95 is an oligofructose about 30% as sweet as dextrose. Hinrichs *et al*, (2001) found that the Tg and Tg' of inulins with a number/weight average degree of polymerisation (DP_n/DP_w) higher than 5.5/6.0 were higher than those of trehalose glass, thus making them interesting non-sweet substitutes for sucrose as cryoprotectants. The choice of cryoprotectants in the experiments was based on these data.

1.2 Soy protein isolate

In order to improve the gelling ability in FPI, the relatively low cost soybean protein have been used as an additive to several fish and surimi-based food products. Several studies have focused on the effect of different ingredients on the rheological properties of surimi or fish protein gel. Chang-Lee *et al* suggested that egg white and whey protein concentrate resulted in higher gel strength in whiting surimi gel formulations, while soy protein isolate (SPI) did not significantly alter gel strength. Park (1994), indicated that 1% SPI dried additive increased the shear stress and decreased the shear strain in medium-grade Alaska Pollock surimi. Lee and Lian indicated that a 2-6% addition of SPI increased penetration force and reduced fishy odour in red hake mince. Lou *et al* concluded that effects of SPI on surimi gel properties were dependent on setting conditions and surimi quality. An addition of 10 or 20% SPI and cooked at 85°C in 30 min after incubation at 40 or 50°C in 60 min had a positive effect in low grade Alaska Pollock. More SPI or different setting conditions had a negative effect on gel properties. A similar positive effect was seen on high-grade surimi only after the latter setting regime. Addition of SPI did not have the same effect on common carp surimi.

1.3 pH effects on gelling ability

The effects of pH on surimi gelling ability are well known. The PI being produced in this project, has been exposed to low or high pH before setting, which may alter the pH effect when gelling. Esturk *et al* (2002) showed that shear stress and strain values of Pacific Whiting

surimi increased as pH increased from 6.0 to 8.0. In catfish surimi, pH optimum was 6.5, while alkali treated catfish FPI made stronger gels at pH 6.0, 7.5, and 8.0 with an optimum at pH 6.0. Acid treated catfish FPI created lower quality gels all over the pH range. (Theodore *et al*, 2003). There have been no reports concerning the influence of pH or addition of SPI on the gel properties of FPI from saithe. In order to develop this product further, information concerning the effect of SPI on this products gel properties at different pH is required.

Moisture content greatly affects gel properties when using SPI. Therefore, in this study, total moisture (protein isolate + SPI) in all samples was adjusted to 72% to determine the effect of SPI on gel properties. Protein concentration of SPI was to 4% of total weight.

Protein isolates from vegetable origin are often dried and used as additives in several food applications. In this project, it was of great interest to investigate functional properties of re-hydrated fish protein isolate from this novel isolation method. Only saithe FPI were dried and rehydrated in this project.

2 MATERIALS AND METHODS

2.1 Raw material

Several fish minces, surimi samples and fish protein isolates have been used as raw material in the following trials at Fiskeriforskning.

2.1.1 Saithe mince

Fillets of fresh, chilled saithe (*Pollachius virens*) were purchased from Tromsdalen Fisk, Tromsø, minced, and cryoprotectants added. Four different components were tested for their ability to stabilise the mince from fresh saithe: Trehalose (provided by Brøste AS), Raftiline®, Raftilose®P95 (provided by MultiChem Wallinco AS), and a 50% blend of sucrose (kitchen) and sorbitol (Sigma). To all samples, 8,0% cryoprotectant and 0,3% tri-sodium-poly-phosphate (Sigma) were added before freezing at -30°C. A control group with no cryoprotectants added was also included. The combination of sucrose and sorbitol is the most used commercial blend in surimi, hence used as a reference.

2.1.2 Saithe protein isolate

Saithe protein isolate was obtained from the pilot plant at Haraldur Bøgvaldson, Iceland. The protein isolate was manufactured using either the acid or alkali treatment. In addition, the acid and alkali groups were divided into two groups, with or without 0,3% tri-sodium-poly-phosphate (TSPP) respectively. Within the four groups, five different components have been tested for their ability to stabilise saithe protein isolate. The selected cryoprotectants were Trehalose (provided by Brøste AS), Raftiline®, Raftilose® (provided by MultiChem Wallinco AS), sucrose (kitchen), or sorbitol (Sigma). A control group containing a blend of sucrose and sorbitol 1:1 and 0,3% TSPP added was also included.

2.1.3 Blue whiting surimi

Blue whiting surimi was provided by “Næraberg” factory ship, Faeroe Islands. The selected cryoprotectants: Trehalose (provided by Brøste AS), Raftiline®, Raftilose®P85, and Raftilose®P95 (provided by MultiChem Wallinco AS) and 0,3% tri-sodium-poly-phosphate (Sigma) were added on site. Samples were frozen to -30°C and shipped to Fiskeriforskning for further analysis. Samples of commercial Blue Whiting surimi from a previous batch were used as reference.

2.1.4 Protein isolate of saithe, addition of soy protein at different pH

Eight samples of exactly one kg frozen alkali treated protein isolate from saithe were taken from storage at -30°C, and kept overnight at -4°C and then chopped while adding soy-protein (Arcon®S, provided by Multichem Wallenco) to 4% and ice cold water to 72% of total weight. Chopping continued until the mince reached -1,5°C before mixing into the mince 3,0% granular NaCl of total weight and $NaHCO_3$ until desired pH were reached. Initial pH of the samples was about 6,5.

2.1.5 Dried samples

Fish protein isolate (FPI) from the pilot plant in Iceland was dried to between 5-12 % wetweight using a heat pump fluidized bed dryer at Sintef's pilot plant in Trondheim. Each dried sample was vacuum packed and sealed in 60-120 g batches and sent to Fiskeriforskning, Tromsø, for re-hydration and test of functionality. Samples were kept at -30°C before re-hydration. Cold water up to 70 or 75% was added to the samples and gently chopped until the mince became smooth and homogenous (3-5 min). The re-hydrated PI was frozen at -5°C overnight before samples for torsion analysis was made.

2.1.6 Freeze-thaw treatment

Some samples of saithe mince and Blue whiting surimi, were exposed to five freeze-thaw cycles (Kim *et al*, 1986) as follows: 18 h at -30°C, 6 h at room temperature (see figure 1). The rest of the samples were kept on -30°C for about a week before analysis.

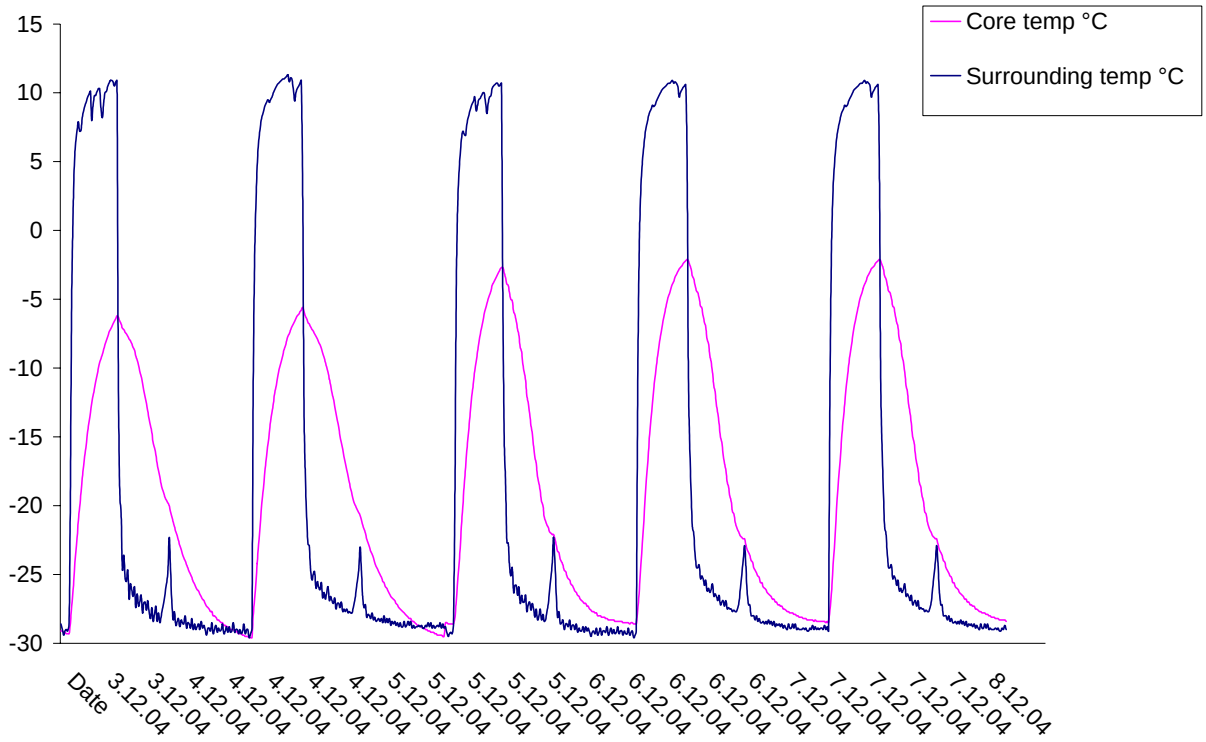

Figure 1: Example of the freeze-thaw cycle regime performed on saithe mince and commercial blue whiting samples with different cryo protectants added. Temperature is shown on y-axis and time on x-axis.

2.2 Chemical composition

All chemical analyses were performed after addition of cryoprotectants except for the control group, which did not contain cryoprotectants. Water content was determined by drying at 105° C overnight according to AOAC 950.46 (1991). PH was measured in 10 g mince suspended in 20 ml 0,15M KCl. Protein content was measured by total Kjeldahl nitrogen (TKN) according to AOAC 976.05 (1990).

2.3 Whiteness

A Chroma Meter CR-300 (Minolta) or a X-Rite was used to measure L* (lightness), a* (red-green), and b* (yellow-blue) values in the gelled samples.

Whiteness I and whiteness II was calculated according to Park, (2002) using the following formulas:

$$Whiteness\,I = 100 - \left[(100 - L^*)^2 + a^{*2} + b^{*2}\right]^{1/2}$$

$$Whiteness\,II = L^* - 3b^*$$

2.4 Gel failure properties by penetration, TPA, and torsion testing

Protein gels were made by first thawing the mince to -4,0°C and then chopping until -1,5°C , was reached in the mixture, before adding into the mince 2,0% granular NaCl. The Mince was stuffed into cellulose casings (40 mm Ø; 20-30 cm long) for analysis of gel strength by penetration. Mince used for torsion testing were moulded in 1,9 mm Ø cylinders. All samples were placed in water baths at the desired temperatures for the specified times: [Penetration: 40°C for 30 min (high temperature setting) followed by 90°C for 20 min. Torsion: 40°C for 20 min followed by 90° for 15 min. All samples were chilled in ice water for 30 min (Solberg, Fiskeriforskning)]. Ten cylindrical samples, 40 mm Ø and 30 mm in length, were prepared.

Gel strength was measured using a TAx4 Texture Analyser (Stable Micro Systems, UK). The same samples were used for texture profile analysis (TPA). Six cylindrical samples (2,87 cm length) of each preparation were reduced to a dumbbell shape with minimum diameter of 1 mm by rotating against a shaped rotating grinding wheel as described by Montejano et al (1983). A special test fixture was mounted on a Brookfield 5X HBTD viscometer (Wu et al., 1985). The viscometer torque reading and time of rotation at 2,5 rpm were read from the Wingather software (Brookfield). Shear stress, and shear strain were calculated from recorded torque and angular displacements using equations given by Hamann (1983).

2.5 Statistical analysis

Standard deviations (SD), pearson's correlation, and significant means coefficients separated by paired *t* test, were calculated using Minitab. Values were reported as significantly different when $p \leq 0{,}05$.

3 RESULTS

3.1 Saithe mince

3.1.1 Chemical composition

As shown in table 1 there is no significant difference in moisture or protein content (TKN – total Kjeldahl nitrogen) in any of the samples where cryoprotectants were added. Addition of cryoprotectants did not seem to significantly affect pH in the samples. There is a significant difference in moisture between the control and raw material. This may have been caused by drip loss during processing, or vaporisation during freezing and cooking.

Table 1: Chemical composition and pH of saithe mince before gelling (n=2).

Cryoprotectant	pH	TKN	± SD	Moisture	± SD
Control (no cryo)	6,44	17,6	± 0,0	76,3	± 0,4
Sucrose-sorbitol	6,41	16,3	± 0,1	69,8	± 0,1
Trehalose	6,42	16,1	± 0,1	70,2	± 0,3
Raftiline®HP	6,44	15,9	± 0,2	70,2	± 0,3
Raftilose®P95	6,42	16,0	± 0,2	71,2	± 0,3
Raw material	6,43	18,2	± 0,1	79,3	± 0,3

PH below about 6,5 can lead to denaturation of myofibrillar proteins, thus reducing their ability to form good gels. Hence, one should aim at adjusting pH close to neutral. In addition, gelling ability of fresh fish muscle is optimal at neutral pH and decreases with decreasing pH. The importance of having control over this factor during production of surimi and surimi products is self-evident.

3.1.2 Colour and whiteness

In table two, effects on colour when adding cryoprotectants to saithe mince which is frozen or freeze-thaw abused is shown. Raftiline® increases the values of all lightness, blueness, and redness compared to the other additives. In addition, the increased blueness will greatly reduce calculated whiteness values in the Raftiline® samples, and more so in the abused samples. Addition of trehalose seems to give the lowest b* value, and one of the lowest L* and W I values. Raftilose® seems to prevent increased b* values during freeze-thaw abuse.

Table 2: Colour measurements of saithe mince gels where lightness (L), red-green (a*), and yellow-blue (b*) in addition to calculated values of whiteness (W I & W II) are shown as affected by freezing and freeze-thaw abuse. (n=6)*

Treatment	C-protectant	L*	±SD	a*	±SD	b*	±SD	W I	±SD	W II	±SD
Frozen	Control	75,5	±0,6	-1,14	±0,09	7,11	±0,21	74,5	±0,6	54,2	±0,9
	Commercial	74,5	±0,5	-1,50	±0,08	7,39	±0,19	73,4	±0,4	52,4	±0,6
	Trehalose	74,1	±0,5	-1,43	±0,08	6,68	±0,42	73,2	±0,5	54,0	±1,4
	Raftiline	77,9	±0,4	-0,83	±0,05	8,48	±0,16	76,3	±0,4	52,4	±0,7
	Raftilose	74,3	±0,6	-1,68	±0,10	7,84	±0,32	73,1	±0,6	50,8	±1,2
Abused	Control	75,6	±0,4	-0,94	±0,05	7,95	±0,18	74,3	±0,4	51,7	±0,5
	Commercial	74,6	±0,4	-1,10	±0,08	7,63	±0,20	73,5	±0,3	51,7	±0,5
	Trehalose	74,8	±0,4	-1,12	±0,03	7,61	±0,17	73,6	±0,3	51,9	±0,5
	Raftiline	77,6	±0,2	-0,69	±0,03	9,47	±0,16	75,6	±0,2	49,2	±0,6
	Raftilose	74,3	±0,6	-1,27	±0,05	7,66	±0,26	73,2	±0,7	51,3	±1,3

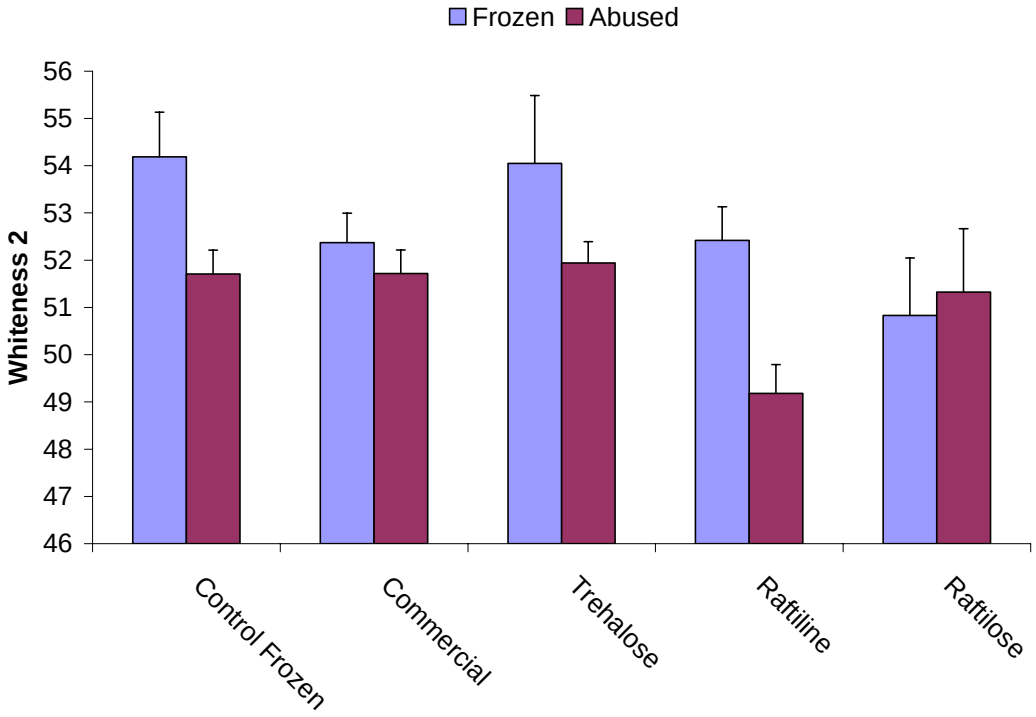

Figure 2: Whiteness 2 in gels made from frozen or freeze-thaw abused minced saithe with or without various cryoprotectants added.

3.1.3 Gel failure properties by penetration or torsion

It appears from figures two and three that freeze-thaw abuse severely reduces the functional properties of the saithe proteins. The reduction is evident in the samples containing Raftiline® and control samples without cryoprotectant. When looking at the penetration tests alone, there is no evidence of significant differences between the commercial blend, trehalose, and Raftilose® regarding protection of the functional proteins. The higher stiffness value in the

Raftiline® samples may indicate that an arrangement is formed by the Raftiline® itself, and the reduction after abuse could be due to setting of this arrangement.

The results show that the torsion test may be somewhat more sensitive than the penetration test. In the abused samples, commercial blend and Raftilose® have the highest true shear strain and shear stress values, whereas trehalose and Raftilose® score highest in the samples frozen once.

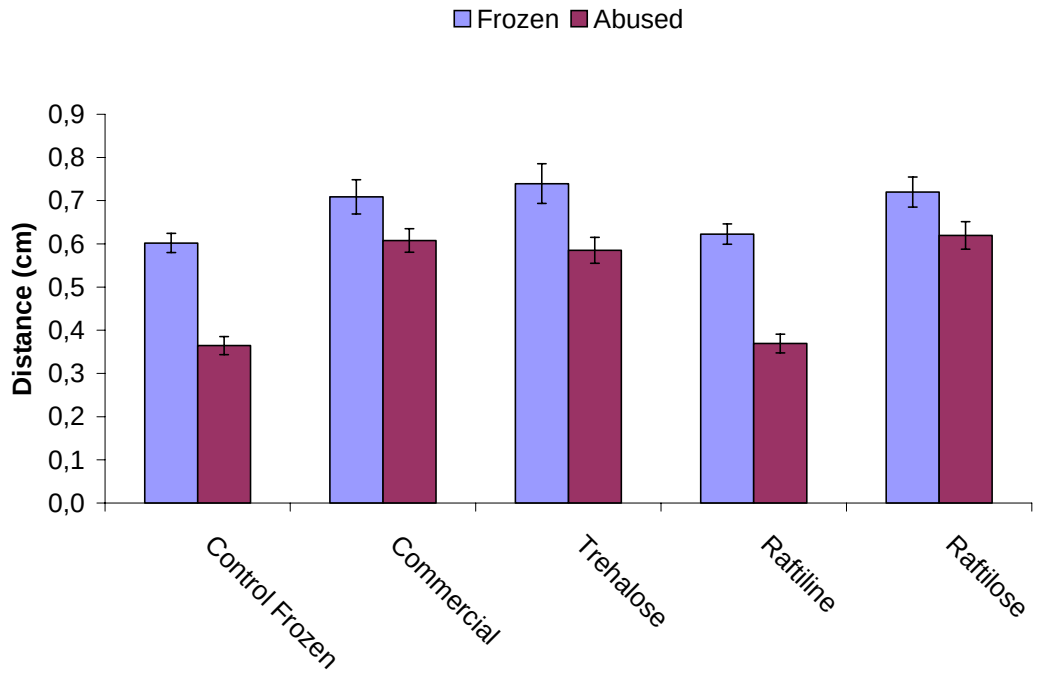

Figure 3: Distance from penetration experiments on gels made of frozen or freeze-thaw abused minced saithe with or without various cryoprotectants added.

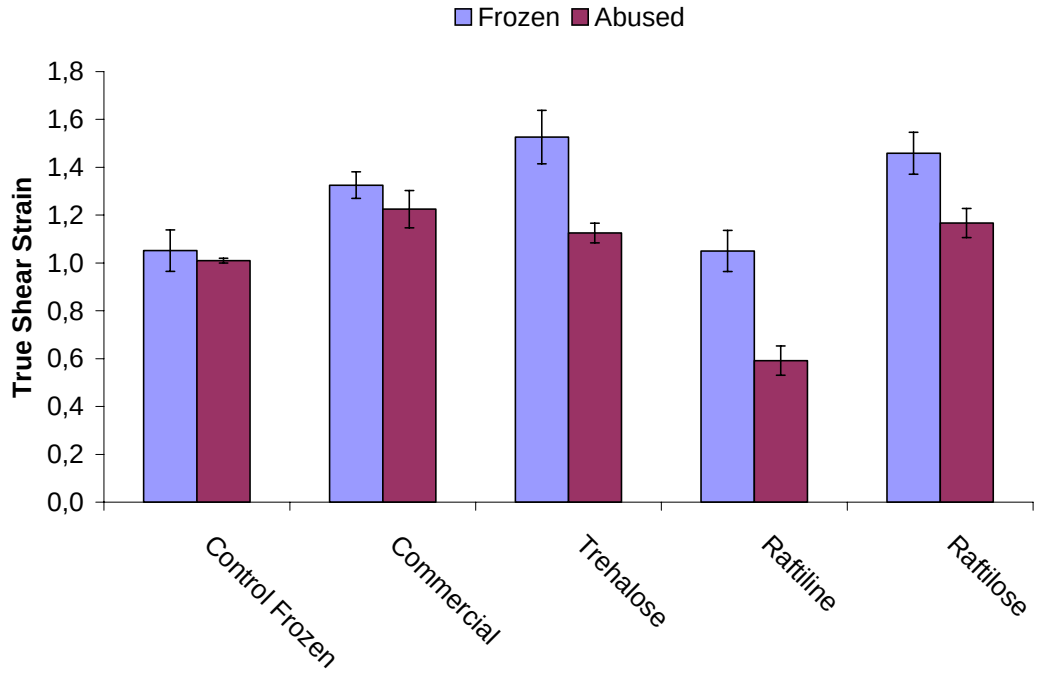

Figure 4: True shear strain from torsion tests on gels made of frozen or freeze-thaw abused minced saithe with or without various cryoprotectants added.

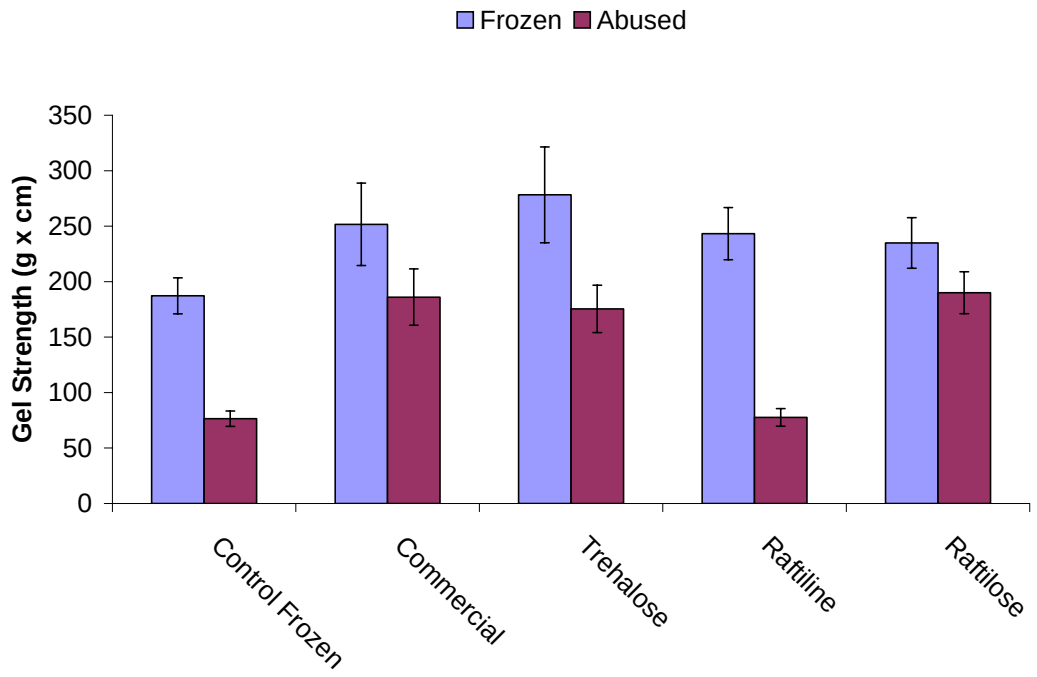

Figure 5: *Gel strength from penetration tests on gels made of frozen or freeze thaw abused minced saithe with or without various cryoprotectants added.*

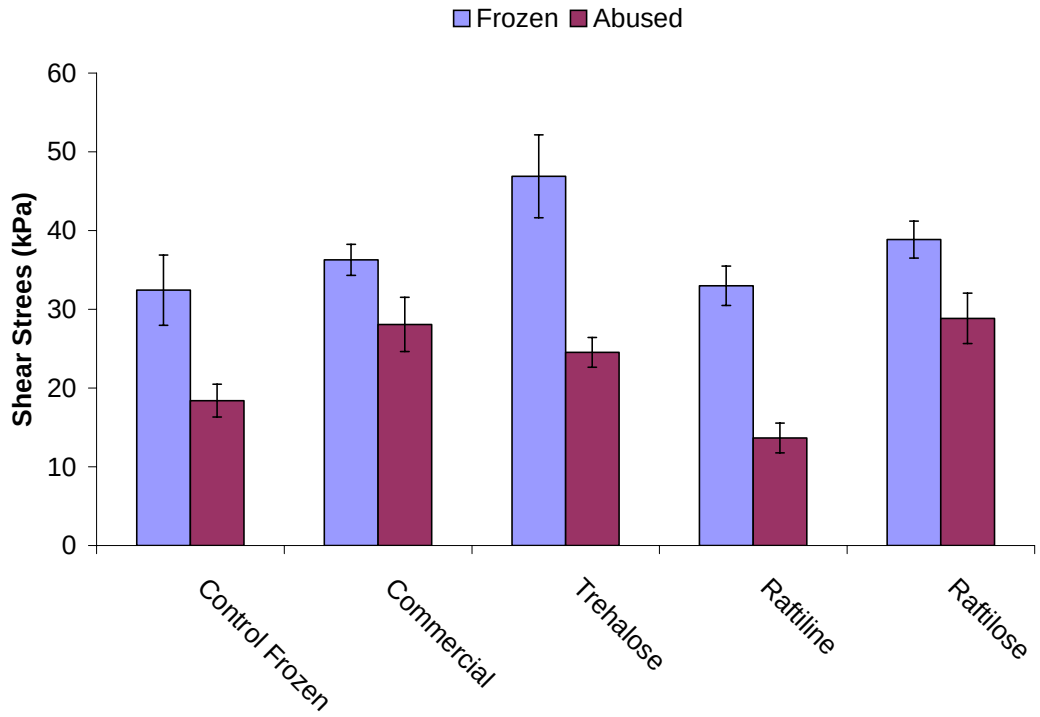

Figure 6: *Shear stress from torsion experiments on gels made of frozen or freeze-thaw abused minced saithe with or without various cryoprotectants added.*

3.2 Saithe protein isolate

3.2.1 Moisture content and pH

As shown in table 3, there is a small difference in moisture content between the alkali and acid saithe protein isolate samples. Different cryoprotectants did not seem to significantly affect pH in the samples. Addition of 0,3% TSPP significantly increased pH in the samples. The pH in alkali produced samples is slightly higher than in acid produced samples.

Table 3: Moisture and pH in protein isolate before gelling. Protein isolate was made by acid- or alkali process, with or without TSSP added.

Extraction	TSPP	Cryoprotectant	pH	Moisture
Acid	Added	Trehalose	6,27	82,1
Acid	Added	Raftilose	6,33	82,9
Acid	Added	Raftiline	6,14	79,6
Acid	Added	Sucrose	6,19	
Acid	Added	Sorbitol	6,17	
Acid	Added	Mix-Raftilose	6,17	
Acid	Absent	Trehalose	5,74	81,1
Acid	Absent	Raftilose	5,72	78,5
Acid	Absent	Raftiline	5,72	
Acid	Absent	Sucrose	5,73	
Acid	Absent	Sorbitol	5,71	79,5
Acid	Absent	Mix-Sorbitol	5,70	79,5
Alkali	Added	Trehalose	6,30	79,3
Alkali	Added	Raftilose	6,27	78,3
Alkali	Added	Raftiline	6,28	79,2
Alkali	Added	Sucrose	6,25	78,1
Alkali	Added	Sorbitol	6,26	78,5
Alkali	Added	Mix-Raftiline	6,26	78,6
Alkali	Absent	Trehalose	5,92	77,8
Alkali	Absent	Raftilose	5,89	79,3
Alkali	Absent	Raftiline	5,91	79,0
Alkali	Absent	Sucrose	5,90	77,9
Alkali	Absent	Sorbitol	5,90	77,8
Alkali	Absent	Mix-Sucrose	5,90	78,3

pH below about 6,5 can lead to denaturation of myofibrillar proteins, thus reducing their ability to form good gels. Hence, one should aim at adjusting pH close to neutral. In addition, the gelling ability of fresh fish muscle is optimal at neutral pH and decreases with decreasing pH. The importance of having control on this factor during production of surimi and surimi products is self-evident.

3.2.2 Colour and whiteness

As shown in table 4, lightness (L*) increases by addition of Raftiline®. Addition of TSPP has a significant positive effect on lightness. Alkali extraction gives lower lightness values compared to acid extraction method. Previous studies have shown that an increased moisture content in surimi gels also increases the L*-value. Acid samples contain more water. In addition to the difference in method used to isolate the proteins, water content could explain some of the difference in colour between the two groups.

Table 4: Colour measurements of gels where lightness (L), red-green (a*), and yellow-blue (b*) in addition to calculated values of whiteness (W I & W II) are shown. (n=6)*

Method	TSPP	Cryo	L*	±SD	a*	±SD	b*	±SD	W II	±SD	W I	±SD
Acid	+	Trehalose	76,8	±0,6	-2,2	±0,1	2,4	±0,2	69,6	±1,2	76,6	±0,6
Acid	+	Raftilose	76,3	±0,4	-2,1	±0,0	2,7	±0,2	68,3	±0,8	76,0	±0,4
Acid	+	Raftiline	78,8	±0,4	-1,7	±0,0	5,0	±0,2	63,8	±0,4	78,1	±0,3
Acid	+	Sucrose	76,5	±0,2	-2,2	±0,0	3,3	±0,1	66,7	±0,4	76,2	±0,2
Acid	+	Sorbitol	77,9	±0,6	-1,8	±0,3	4,5	±1,2	64,4	±3,1	77,4	±0,4
Acid	+	Mix-Raftilose	78,0	±0,4	-2,0	±0,1	3,5	±0,3	67,5	±0,8	77,6	±0,4
Acid	-	Trehalose	79,3	±0,4	-1,5	±0,1	4,9	±0,8	64,7	±2,7	78,7	±0,6
Acid	-	Raftilose	78,3	±0,6	-1,8	±0,3	4,4	±1,3	65,0	±3,4	77,7	±0,4
Acid	-	Raftiline	79,3	±0,6	-1,1	±0,1	7,0	±0,3	58,4	±1,1	78,1	±0,5
Acid	-	Sucrose	77,9	±0,7	-1,6	±0,1	6,2	±0,3	59,2	±1,7	77,0	±0,8
Acid	-	Sorbitol	78,9	±0,4	-1,4	±0,0	6,2	±0,5	60,3	±1,6	78,0	±0,5
Acid	-	Mix-Sorbitol	79,2	±0,3	-1,3	±0,1	6,6	±0,3	59,3	±1,3	78,1	±0,4
Alkali	+	Trehalose	74,4	±0,4	-2,6	±0,1	5,9	±0,2	56,7	±0,7	73,6	±0,4
Alkali	+	Raftilose	74,5	±0,7	-2,3	±0,1	5,9	±0,2	56,8	±1,0	73,7	±0,7
Alkali	+	Raftiline	77,2	±1,0	-1,9	±0,0	7,9	±0,2	53,4	±1,5	75,8	±1,0
Alkali	+	Sucrose	74,0	±0,6	-2,7	±0,1	5,6	±0,5	57,3	±1,3	73,3	±0,6
Alkali	+	Sorbitol	74,8	±0,7	-2,5	±0,1	6,0	±0,2	56,9	±0,9	74,0	±0,7
Alkali	+	Mix-Raftiline	74,4	±0,6	-2,5	±0,1	6,1	±0,3	56,0	±1,3	73,5	±0,6
Alkali	-	Trehalose	76,2	±0,5	-1,9	±0,1	7,5	±0,3	53,6	±1,3	74,9	±0,5
Alkali	-	Raftilose	75,7	±0,7	-2,0	±0,1	7,6	±0,3	53,0	±1,6	74,5	±0,8
Alkali	-	Raftiline	77,9	±1,6	-1,4	±0,2	9,0	±0,2	51,0	±2,1	76,1	±1,5
Alkali	-	Sucrose	75,4	±0,5	-2,0	±0,1	7,2	±0,3	53,9	±1,3	74,3	±0,6
Alkali	-	Sorbitol	76,5	±0,2	-2,0	±0,1	6,9	±0,2	55,8	±0,5	75,4	±0,2
Alkali	-	Mix-Sucrose	76,1	±0,7	-1,6	±0,1	7,3	±0,3	54,2	±1,4	75,0	±0,7

An increase in blueness (b*) in the alkali group and in the Raftiline® samples is observed, which reduce the calculated whiteness II value. The b*-value is also greatly reduced by addition of TSPP.

Raftiline® seem to reduce the a*- value compared to the other cryoprotectants, but less evident (p=0,022) than the other observed effects. Alkali extraction and addition of TSPP both significantly increase a*-value.

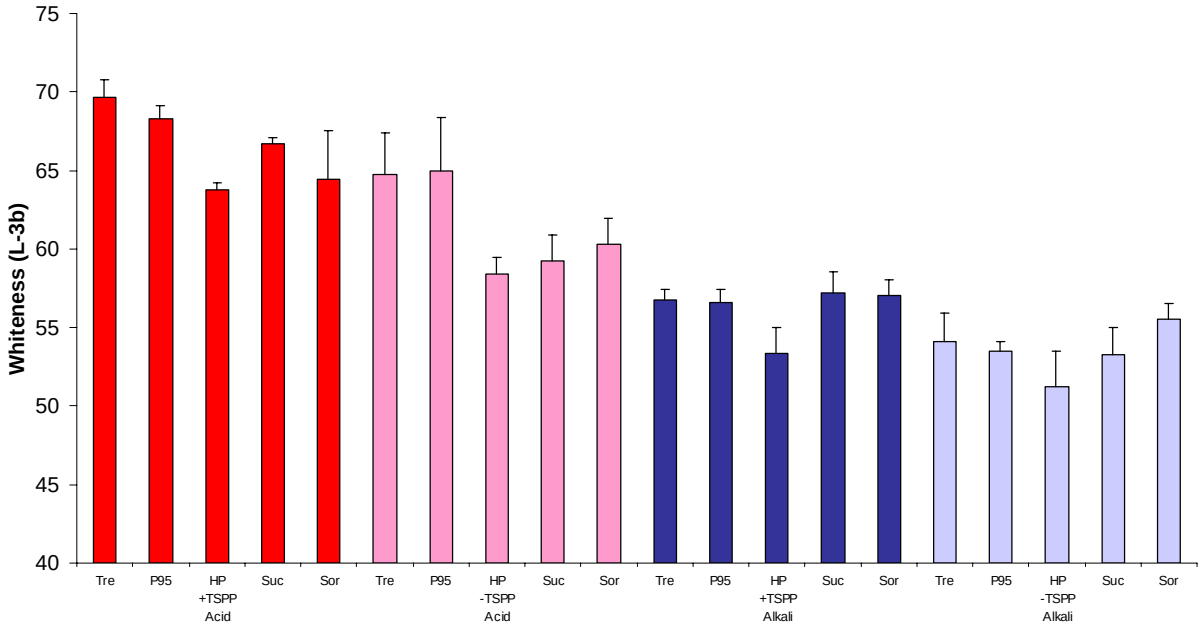

Figure 7: Whiteness (L-3b) in acid or alkali made gels of protein isolate from saithe with or without TSPP (+/-) and various cryoprotectants added. Red bars indicate acid extraction and blue bars indicate alkali extraction. Darker colour indicates addition of TSPP, lighter colour is without TSPP. The order of cryoprotectants added within the four groups is trehalose (Tre), Raftilose® (P95), Raftiline® (HP), sucrose (Suc), and sorbitol (Sor).

3.2.3 Gel failure properties by penetration or torsion

It appears from penetration and torsion tests (figures 8-11) that addition of TSPP protects the functional properties of saithe proteins measured as true strain and distance. When looking at the penetration tests alone, there are no significant differences between the commercial blend, trehalose, and Raftilose® regarding protection of the functional proteins. Raftiline samples seem to give a harder but less elastic gel.

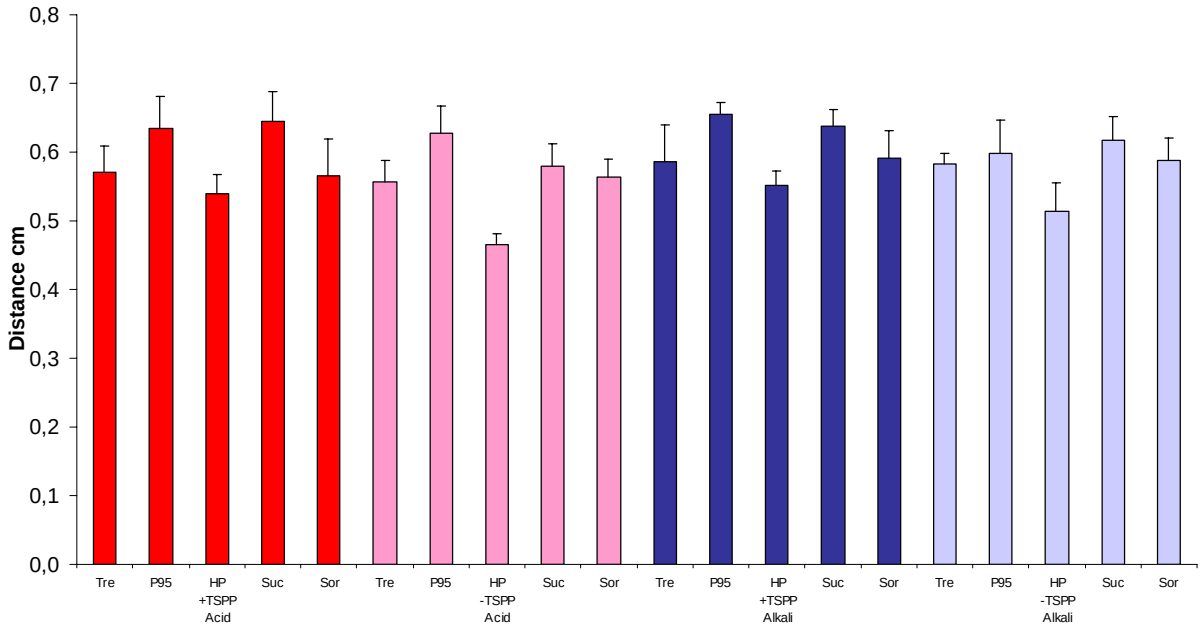

Figure 8: *Distance from penetration experiments on gels from frozen saithe protein isolate with or without various cryoprotectants and TSPP added. Explanations for colours, see fig.7.*

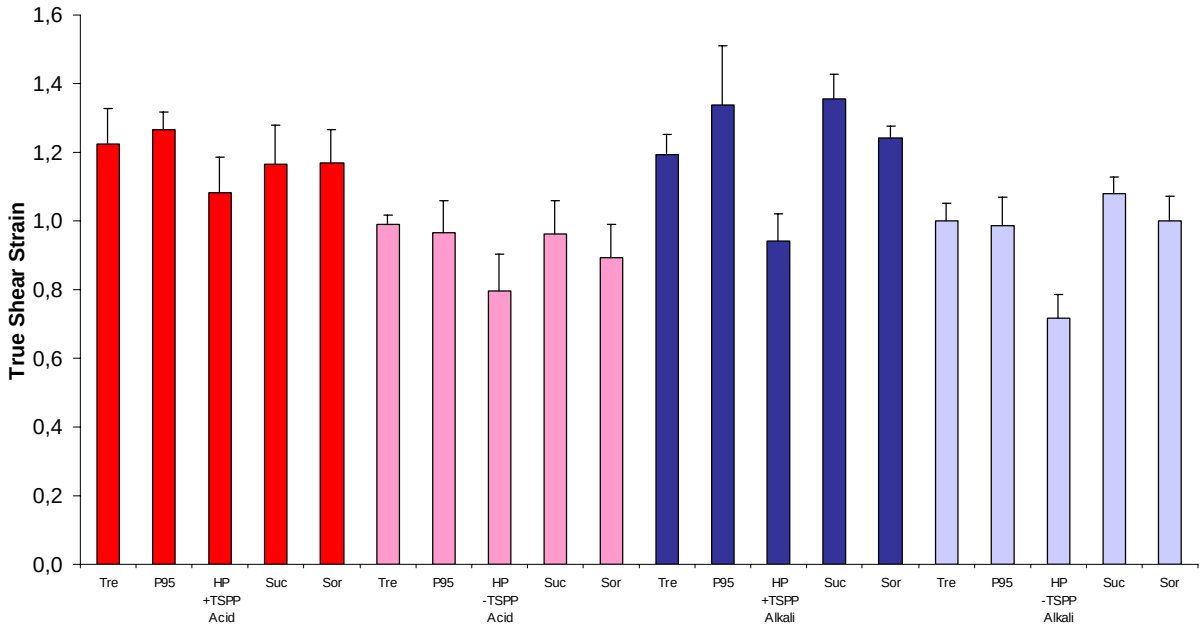

Figure 9: *True shear strain from torsion experiments on gels made from frozen protein isolate with or without various cryoprotectants and TSPP added. Explanations for colours, see fig.7.*

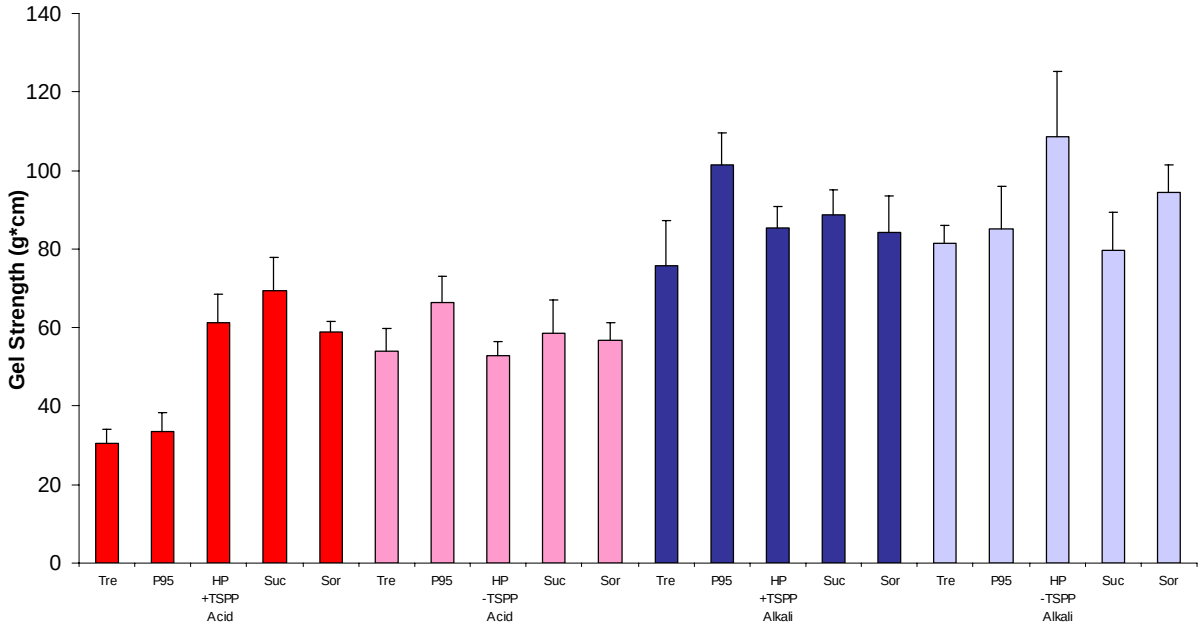

Figure 10: Gel strength from penetration experiments on gels made from frozen saithe protein isolate with or without various cryoprotectants and TSPP added. Explanations for colours, see fig.7.

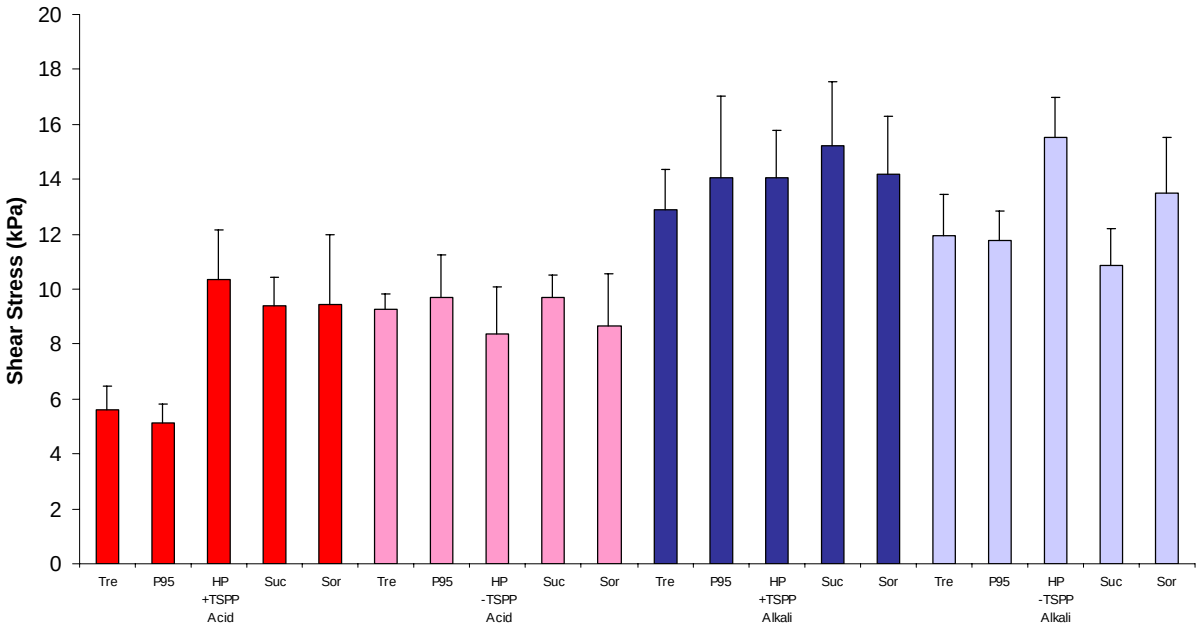

Figure 11: Shear stress from torsion experiments on gels made from frozen protein isolate with or without various cryoprotectants and TSPP added. Explanations for colours, see fig.7.

The two first samples contained more water (table 3) than the rest, and this is probably the reason for lower gel strength and shear stress values. Distance and stress values are less affected by water content and give good evaluations on the protein quality in the products.

The results show that the torsion test may be somewhat more sensitive than the penetration test. Particularly regarding the true strain versus distance, the torsion test seems to differ more between sample groups.

3.2.4 Sensory evaluation of saithe protein isolate

Eight different products made from saithe protein isolate and two reference products (commercial blue whiting surimi) were investigated according to a multivariate design where the following three factors were varied;

- Production process (acid/alkali)
- Phosphate (added/absent)
- Cryostabiliser (trehalose/raftilose®/raftilin®/sukrose/sorbitol/mix).

The samples included in the sensory evaluation, table 5, were chosen from the most promising products after texture and colour analysis.

Table 5: Samples chosen for sensory analysis.

Samples	Method	TSPP	Cryostaboliser
N-01	Acid	Added	Trehalose
N-02	Acid	Added	Raftilose®
N-04	Acid	Added	Sucrose
N-05	Acid	Added	Sorbitol
N-13	Alkali	Added	Trehalose
N-14	Alkali	Added	Raftilose®
N-16	Alkali	Added	Sucrose
N-17	Alkali	Added	Sorbitol
REFERENCE/A	Standard	Added	Commercial blend
REFERENCE/B	Standard	Added	Commercial blend

A descriptive sensory analysis was performed. The analysis seeks to provide answers regarding existing differences in the protein isolates, and if so the amount of difference. Relevant sensory characteristics were chosen in agreement with the sensory panel, panel leader and the project leader. Test products from the protein isolate were available for training the panellists before the actual analysis. This was an advantage, and was very helpful in choosing the most important characteristics before the analysis. Ten sensory characteristics were evaluated using an unstructured line scale from 0 to 10 points, from no to high intensity. A description of the chosen characteristics and how they were used is given in table 6.

Table 6: Sensory characteristics used to describe different protein isolate products from saithe.

Shellfish odour	A positive, somewhat sweet odour which reminds you of fresh shellfish, such as crabs and shrimps.
Rancid odour	An odour that reminds of fish-oil, spoiled stockfish or paint.
Grey colour	Total visible impression of the samples using white-grey, grey or dark grey.
Coherence	Pull the sample between your fingers to describe the force needed to rupture it.
Sweet taste	Intensity of sweet taste in the sample.
Salt taste	Intensity of salt taste in the sample.
Sour taste	Intensity of sour taste in the sample. A taste associated with something positive.
Watery	A watery sample contains loosely associated liquid that is easily released when chewed.
Toughness	Evaluate how much chewing is required before it feels natural to swallow.
Rancid taste	A taste that reminds of fish-oil, spoiled stockfish or paint.

After preparing the samples, they were stored at -30°C awaiting the sensory analysis. The samples were thawed overnight in a refrigerator at +4°C and cut in approximately 2 cm^2 pieces. They were then put in plastic containers with lid, individually marked with a three numbered code and served at room temperature. Each of the panellists was served the samples twice in a random order. The sensory panel consisted of 6-7 trained panellists.

3.2.5 Data analysis, sensory analysis saithe protein isolate

The eight samples and the reference samples were tested in two sessions: Tuesday four samples, and one reference, Thursday four samples, and one reference. Data from the two sessions were pooled before statistical analysis.

The sensory data were analysed by a two-way analysis of variance (ANOVA) and Tukey's test was used to test for significant differences among mean values at a 5 % level. Statistical evaluations were carried out with the software FIZZ, (BIOSYSTEMES, FRANCE).

In addition, a principal component analysis (PCA) was performed on the simple means using Unscrambler software, (Camo Trondheim).

3.2.6 Results and discussion, sensory analysis saithe protein isolate

Table 7 gives an overview of significant differences in the products' sensory characteristics. Shellfish odour was the only non-significant sensory attribute.

The reference samples differ most from the other samples. They score highest in coherence and hardness, and lowest in watery. These differences were very pronounced, thus described by the panellists as totally different compared to the test samples and as chewing rubber bands. The reference samples also score high in grey colour.

Acid versus alkali protein extraction method was also a significant factor. Products made using acid treatment (N1, 2, 4, and 5) seem less coherent when pulling and being softer and more watery when chewing. In addition, the acid treated samples were considered saltier than the alkali samples. By the exception of N4, all acid treated samples were considered significantly different in the parameters coherence, watery, and hardness compared to the alkali treated samples, table 3.

The alkali treated samples (N13, 14, 16, and 17) had a significant higher grey colour score than the acid treated samples. When comparing the two extraction methods within the same cryoprotectant group the alkali treated samples score higher in rancid odour with exception of sorbitol samples N5 versus N17. These two samples had the highest intensity in both rancid odour and taste.

The addition of different cryostabilisers in the protein isolates was mainly reflected in odour and taste scores. Samples containing sorbitol (N5 and N17) gave the highest scores in rancid odour and rancid taste. Sucrose samples differed significantly compared to the rest of the samples as scoring high in sweet taste. The panel commented this sweet taste as being somewhat artificial, excessive, and nauseating.

Table 7: Comparison of the sensory attributes of nine different protein isolate products. Acid treatment; N-01, NO-2, N-04, N-05. Alkali treatment, N-13, N-14, N-16, N-17. Simple mean, ANOVA, and Tukey's test. Samples with the same letter are not significantly different on 5 % level. N=6-7.

Characteristics	Sign.	N-01	N-13	N-02	N-14	N-04	N-16	N-05	N-17	REF/A	REF/B
Shellfish odour	Is	4,6a	3,8a	4,1a	3,9a	4,7a	4,8a	4,5a	4,6a	4,1a	3,5a
Rancid odour	***	1,4ab	2,0ab	1,1b	1,9ab	1,7ab	2,2ab	2,6a	2,5a	1,6ab	1,1b
Grey colour	***	1,2cd	4,2a	1,0d	4,8a	2,3b	4,6a	1,9bc	4,4a	4,9a	4,3a
Coherence	***	2,1ef	5,3b	2,0f	5,7b	4,2d	5,2bc	2,7e	4,5cd	9,8a	10,0a
Sweet taste	***	4,6b	4,2b	4,3b	4,2b	7,0a	6,9a	4,3b	5,0b	4,6b	5,0b
Salt taste	***	6,2ab	4,9cde	6,4a	4,8cde	4,3de	4,1ef	5,7abc	5,4bcd	4,0ef	3,1f
Acidulous taste	*	3,4ab	3,7ab	3,7ab	3,4ab	4,1a	3,8ab	3,8ab	3,7ab	2,9ab	2,7b
Watery	***	7,0a	4,4cd	7,0a	3,8d	5,3bc	4,5cd	6,1ab	4,0d	0,8e	1,1e
Toughness	***	1,3e	2,8bc	1,5de	3,0b	2,1cd	3,3b	1,9de	3,2b	6,7a	7,2a
Rancid taste	***	2,0bc	2,5abc	2,3bc	1,8bc	2,1bc	2,5abc	3,8a	3,1ab	1,8bc	1,5c

Symbols ANOVA; ***: p< 0,001 **: p< 0,01 *:p<0,05 is: non-significant p> 0,05

A principal component analysis, PCA, makes it possible to compare all information in the sensory attributes and the different products simultaneously. Bi-plot for factor 1 (x-axis) and factor 2 (y-axis) are shown in figure 12. This plot confirms the findings in the ANOVA analysis, table 7. 85% of the information in the data is explained in the first component, which is relatively high. Hardness and coherence scores in the double reference samples are the main factors contributing to this. These samples gave distinct scores and characterised as rubbery by the sensory panel. Samples N1, N2, N4, and N5 are located on the left side of the plot and are characterised as watery, white colour, and salty taste. These characteristics are located on the left side of the plot as well, hence indicates a positive correlation. The second component separates the products regarding taste attributes. Sample N4 and N16 containing sucrose was by the panel characterised as very sweet, and are close to the sweet taste variable in the plot.

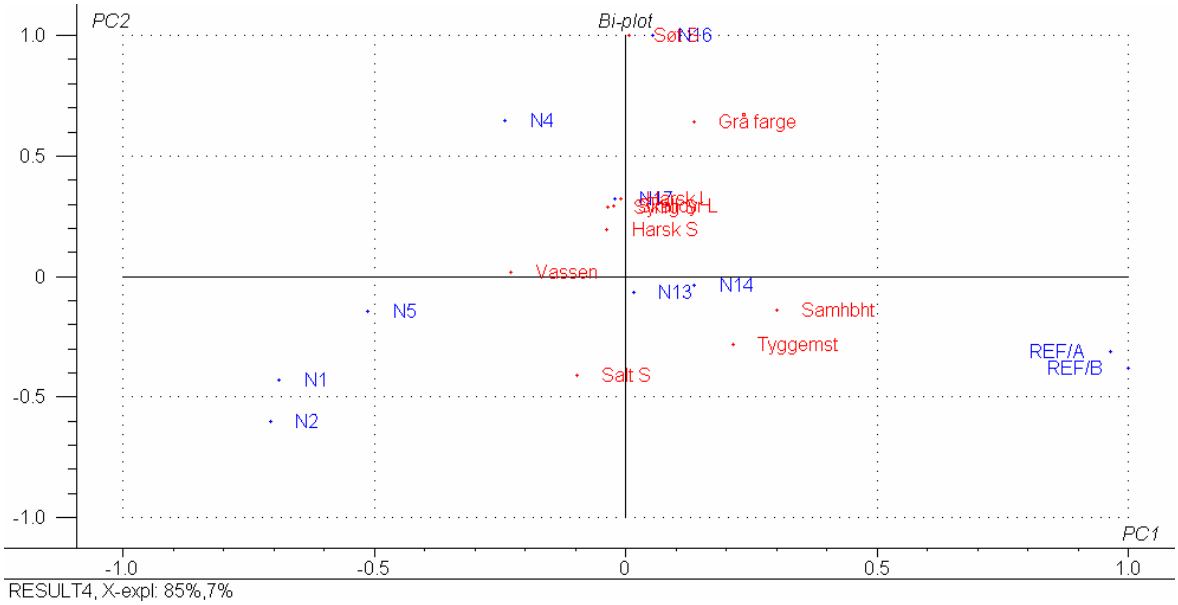

Figure 12: Principal component analysis (bi-plot) of simple means over PC1 and PC2.

3.3 Blue whiting surimi

3.3.1 Colour and whiteness

Figure 13 shows that whiteness is not affected by the freezethaw cycles. Lightness values are similar in all samples except for the two Raftiline®HP samples. They have about five units higher L*-value compared to all other samples. Thus, the observed differences in whiteness values are mainly caused by the higher b*-values. The commercial Blue Whiting samples (No#) have both the lowest L*- and b*-values. This is suspected to be caused by a higher protein content in these samples.

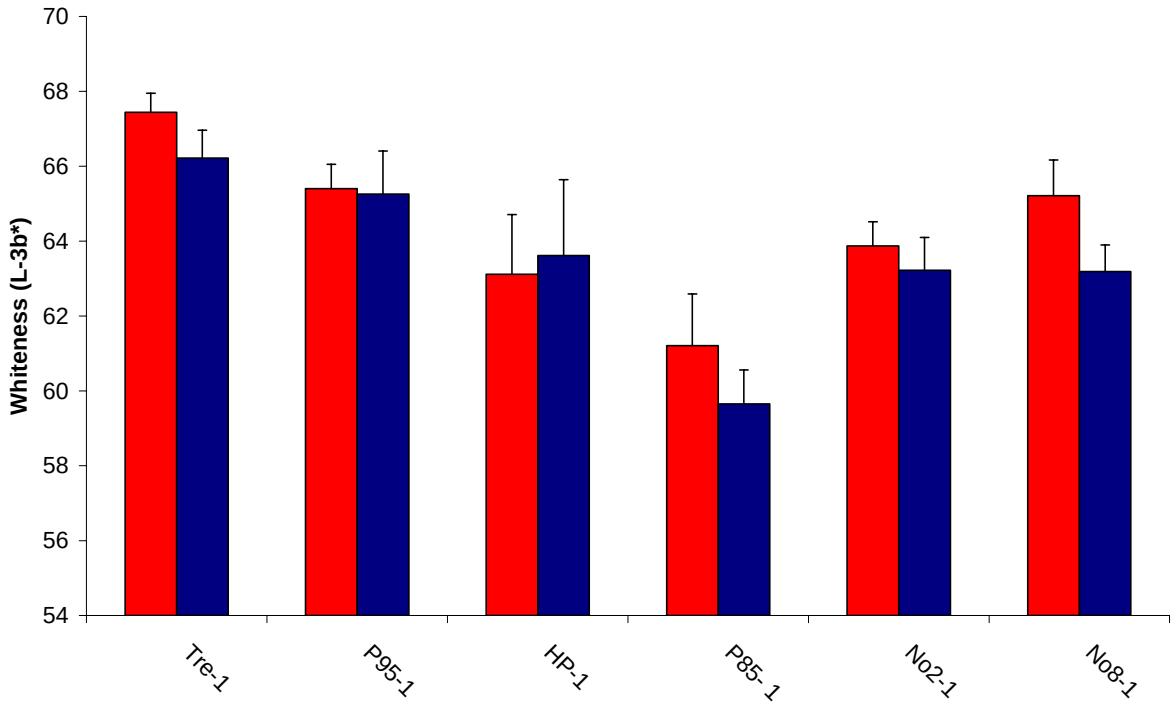

Figure 13: L-3b* values on gels from blue whiting surimi with different cryoprotectants. Red bars are treated with five freeze thaw cycles, and blue bars are frozen once and kept frozen in two weeks. The cryoprotectants are trehalose (Tre), Raftilose® (P95), Raftiline® (HP), Raftilose® (P85), No are commercial with sucrose (Suc) and sorbitol (Sor).*

3.3.2 Texture properties by penetration or torsion

The distance and true shear strain results indicate that the five freeze thaw cycles have a negative effect on the protein quality. The commercial samples are more affected than the test samples, except for the HP sample. It seems evident both from torsion and penetration tests that the Raftiline®HP are less suited as cryoprotectant than the other tested ingredients..

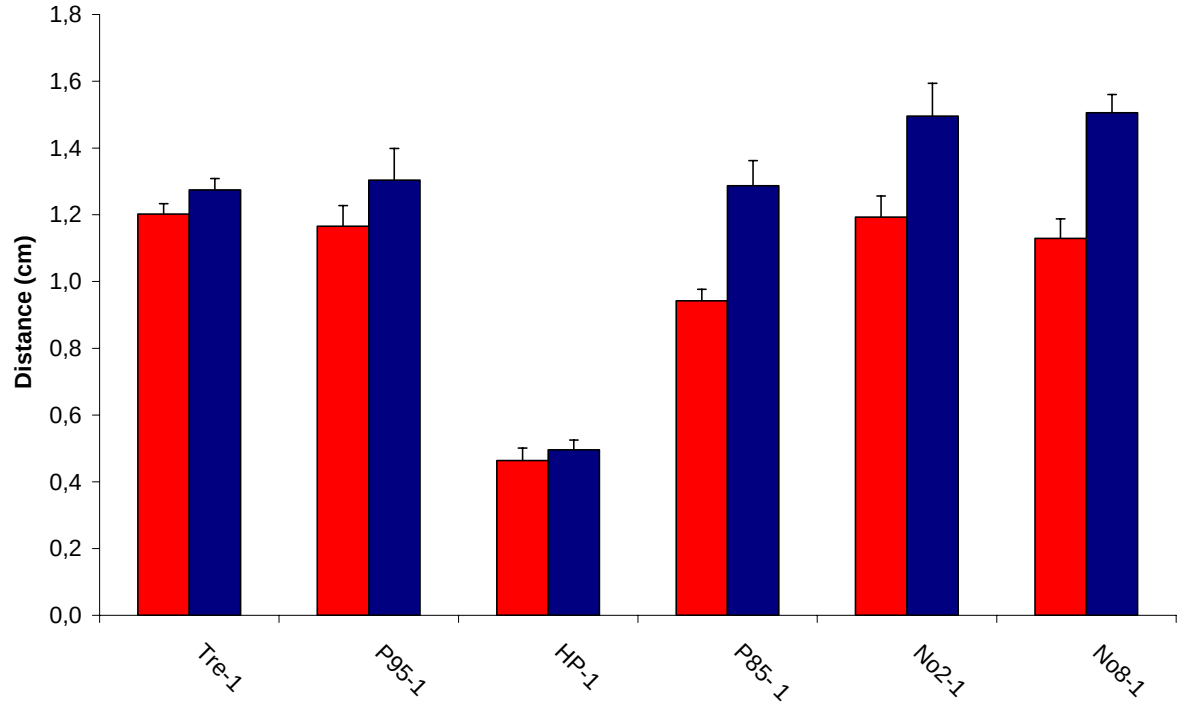

Figure 14: *Distance (cm) penetration in gels from blue whiting surimi with different cryoprotectants. Red bars are treated with five freeze thaw cycles. The cryoprotectants are trehalose (Tre), Raftilose® (P95), Raftiline® (HP), Raftilose® (P85), No are commercial with sucrose (Suc) and sorbitol (Sor).*

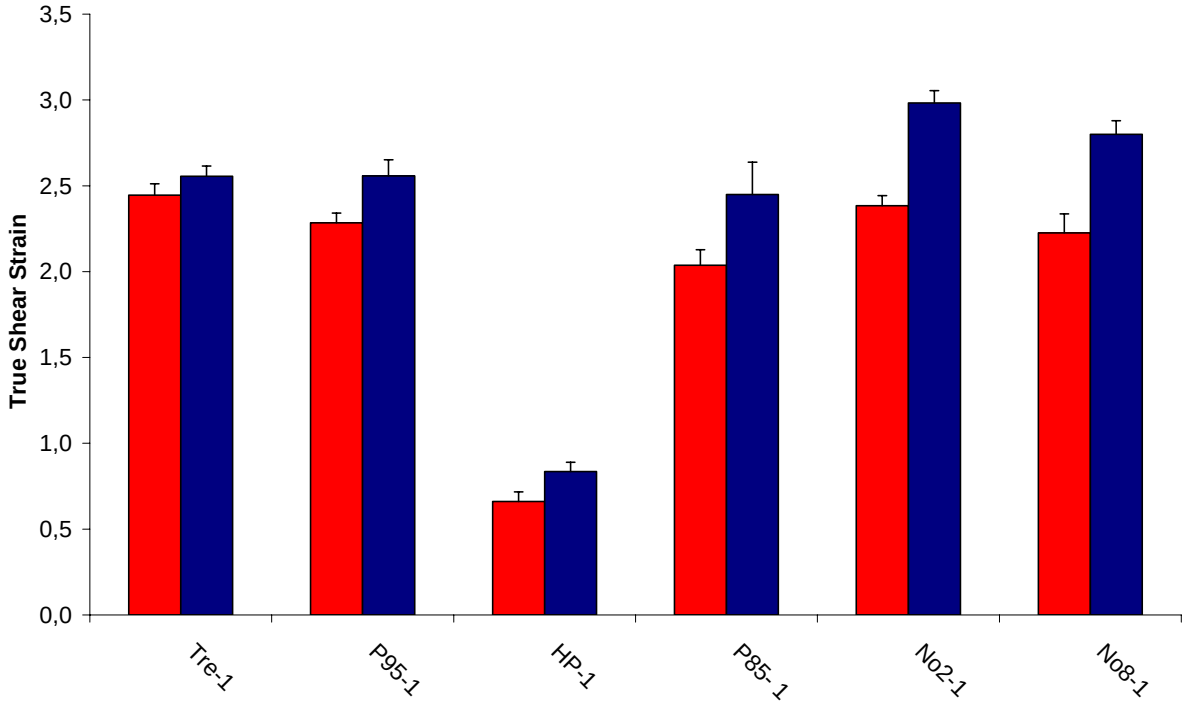

Figure 15: *True shear strain values by torsion test in gels from blue whiting surimi with different cryoprotectants. Red bars are treated with five freeze thaw cycles. The cryoprotectants are trehalose (Tre), Raftilose® (P95), Raftiline® (HP), Raftilose® (P85), No are commercial with sucrose (Suc) and sorbitol (Sor).*

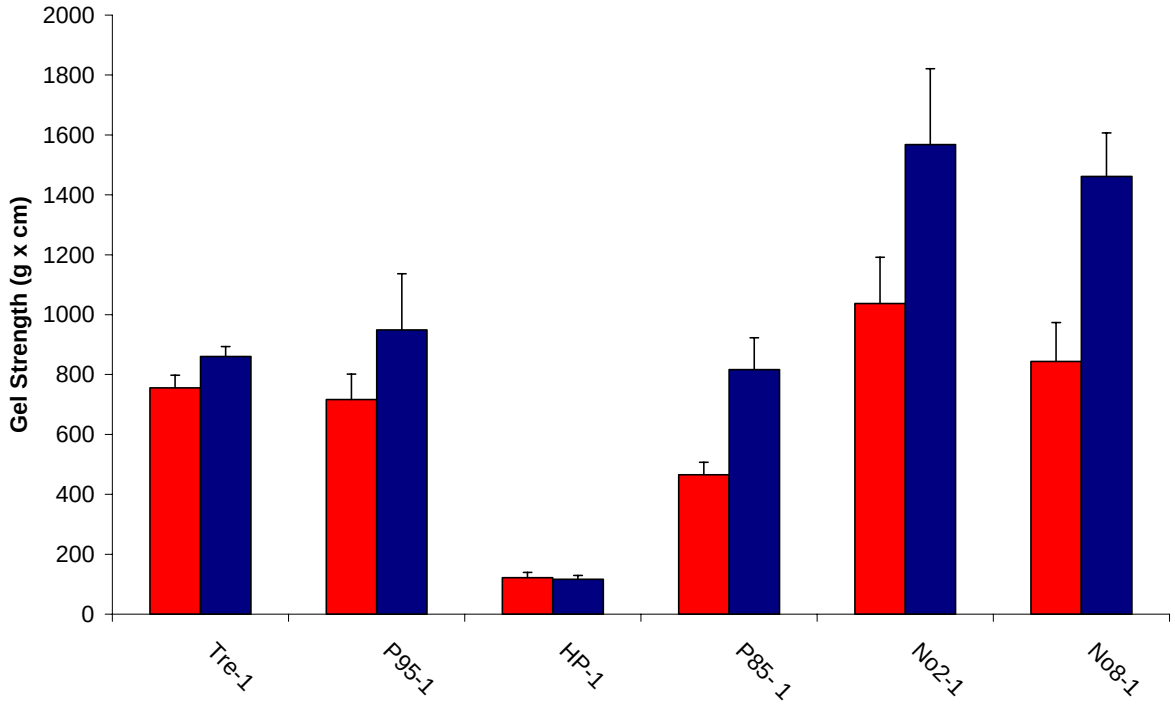

Figure 16: Gel strength (GS=g×cm) from penetration tests on blue whiting surimi with different cryoprotectants. Red bars are treated with five freeze thaw cycles. The cryoprotectants are trehalose (Tre), Raftilose® (P95), Raftiline® (HP), Raftilose® (P85), No are commercial with sucrose (Suc) and sorbitol (Sor).

Gel strength, and shear stress values support the findings from the distance and strain measurements. The higher GS and stress values in the commercial samples are suspected to be caused by a higher protein content. Of the newly introduced cryoprotectants, Raftilose®P85 seem a little less effective than Trehalose and Raftilose®P95. In contrast to the previous trials, the HP samples did not produce a hard and brittle gel before the freeze thaw abuse. One reason might be a uncontrolled thawing during transport from the factory ship to Fiskeriforskning, which we have no information of.

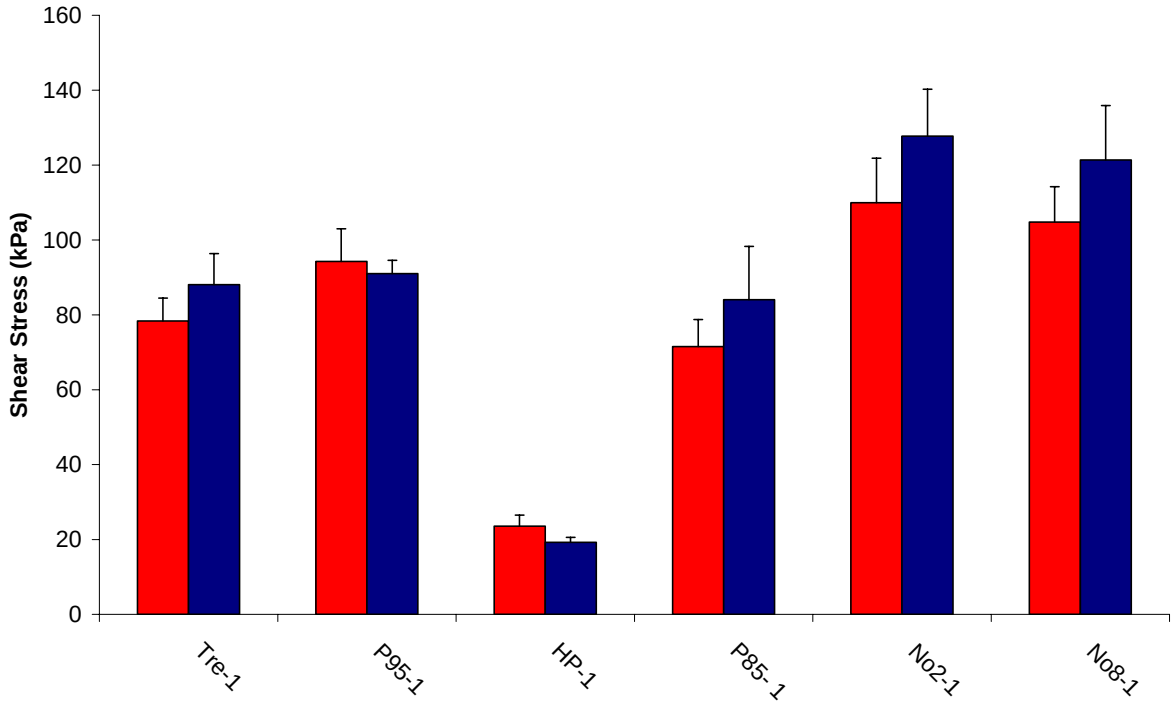

Figure 17: Shear stress values from torsion test on blue whiting surimi with different cryoprotectants. Red bars are treated with five freeze thaw cycles. The cryoprotectants are trehalose (Tre), Raftilose® (P95), Raftiline® (HP), Raftilose® (P85), No are commercial with sucrose (Suc) and sorbitol (Sor).

3.4 Soy and pH samples

3.4.1 Chemical composition

Table 8: Water content, pH and soy protein added to the saithe fish protein samples.

Sample	Soy protein	pH	Water %
1	0 %	6,5	70,77
2	0 %	6,8	71,34
3	0 %	7,1	70,13
4	0 %	7,4	71,19
5	4 %	6,5	72,46
6	4 %	6,8	72,37
7	4 %	7,1	72,11
8	4 %	7,4	71,76

3.4.2 Colour and whiteness

It is shown in figure 17 that an increase in pH from 6,5 to 7,4 decreases the L*-values in both soy and non-soy samples. Even if b*-values also decrease with increasing pH it was not sufficient to hinder a decrease in whiteness (L*-3b*). Addition of soy protein seem to cause higher a*- and b*-values, but lower L*-values regardless of pH.

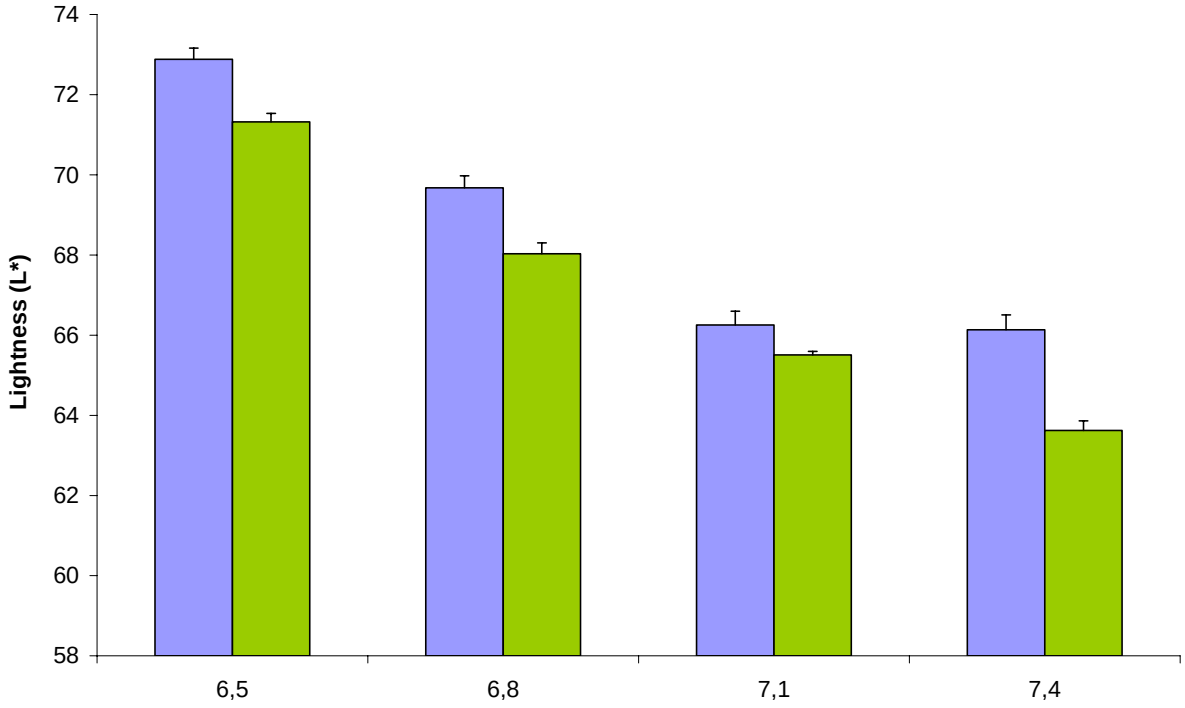

Figure 18: *Lightness (L*) on gels of protein isolate from saithe (Pollachius virens) at different pH, (x-axis). Green bars are samples with 4% arcon®s soy protein added.*

3.4.3 Gel failure properties by penetration or torsion

There is a small increase in texture values for the saithe protein isolate, with increasing pH up to 7,1. The texture values are reduced at pH 7,4 for both soy and non-soy samples, figures 19-22. This indicates an optimum pH at around 7,1. Except for pH 6,5, the soy samples seem to produce weaker gels than the non-soy samples. It also appears that soy samples are less affected by changes in pH regarding texture values.

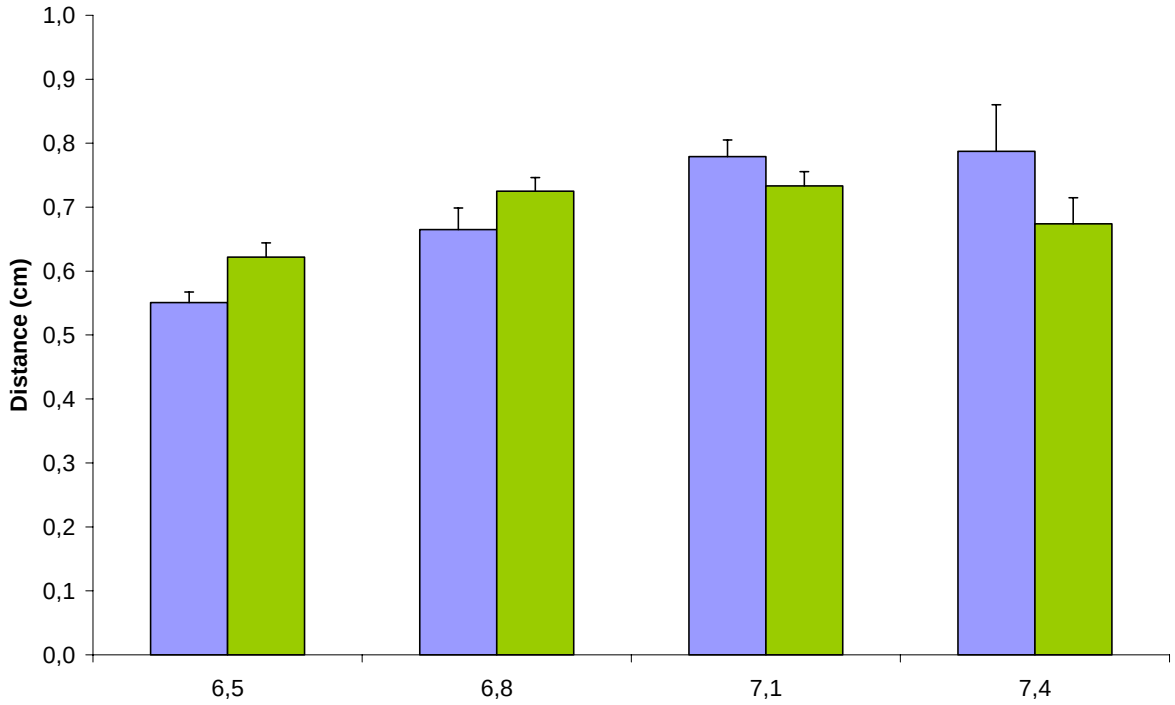

Figure 19: Distance (cm) from penetration tests on gels of protein isolate from saithe (Pollachius virens) at different pH (x-axis). Green bars are samples with 4% arcon®s soy protein added.

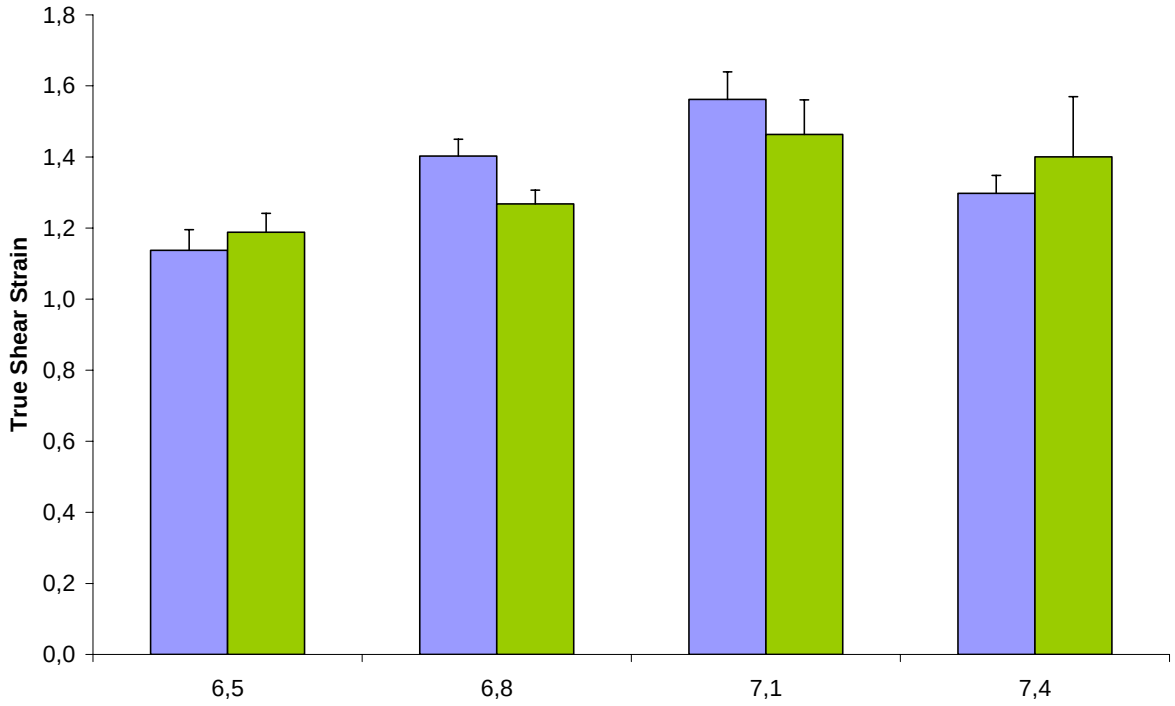

Figure 20: True shear strain from torsion tests on gels of protein isolate from saithe (Pollachius virens) at different pH (x-axis). Green bars are samples with 4% arcon®s soy protein added.

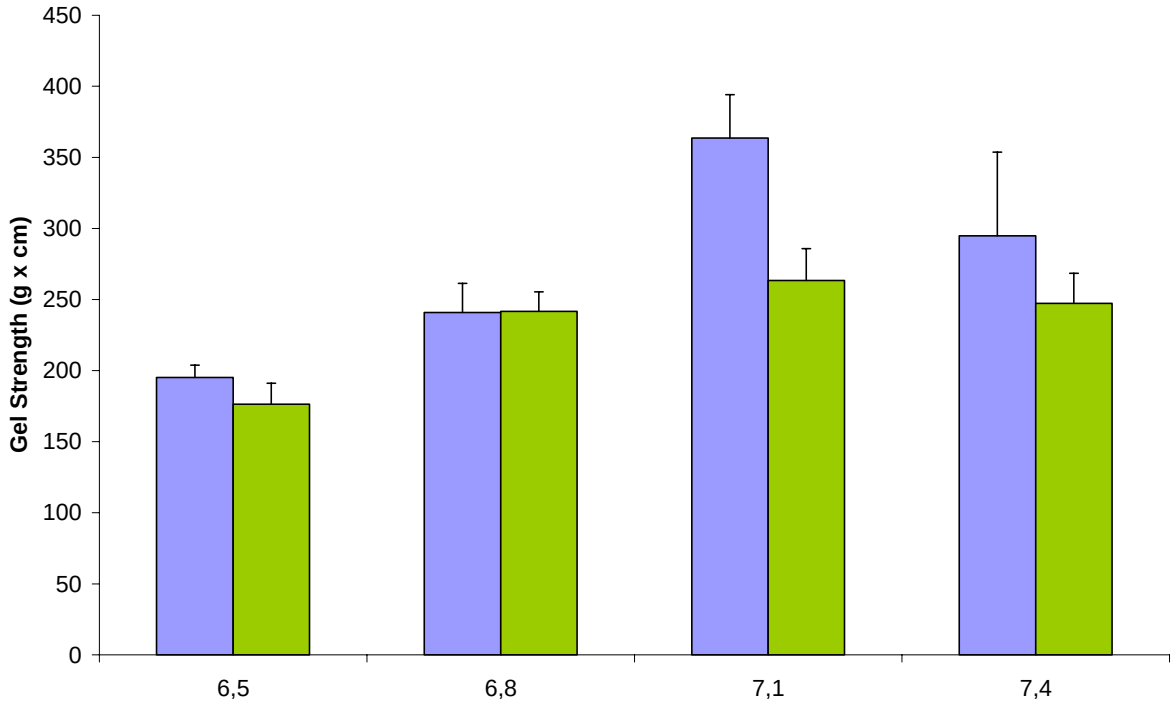

Figure 21: Gel strength (GS=g×cm) from penetration tests on gels of protein isolate from saithe (Pollachius virens) at different pH (x-axis). Green bars are samples with 4% arcon®s soy protein added.

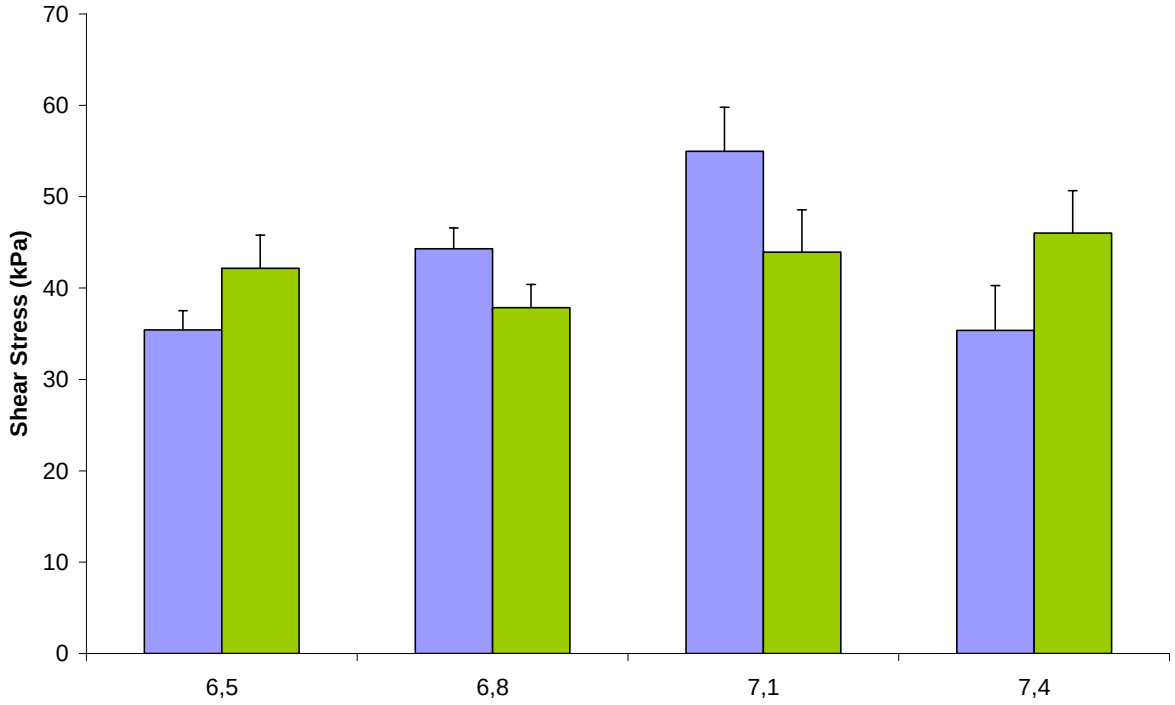

Figure 22: Shear stress values from torsion tests on protein isolate gels from saithe (Pollachius virens) at different pH (x-axis). Green bars are samples with 4% arcon®s soy protein added.

3.5 Dried samples

Dried samples were obtained from Sintef –Trondheim. The difference between the samples are the drying conditions. These are presented in an separate report from Sintef.

Immediately after removing the vacuum seal, we observed an extreme rancid odour from the dried samples. This odour seemed to increase after addition of water. Previous studies on similar protein isolate indicate extreme development of TBARS even before drying. This is believed to be caused by phospholipids being exposed to protein bound iron during the acid or alkali treatment.

Table 9: Moisture contents in raw material, dried material, and re-hydrated gelled products named Mo, Mf, and Mg respectively. Stress and true strain measurements were performed on the gelled products. For sample 2 and 10, m- indicates that value is missing, because the gels were too weak to be measured. Sample 13 do not exist.

Sample #	Mo (%)	Mf (%)	Mg (%)	Stress τ (kPa)	True strain γ_t
1	72,18	8,61	75	10,02	0,34
2	73,17	5,87	75	m	m
3	72,18	7,08	75	4,18	0,29
4	73,67	4,91	75	4,68	0,32
5	71,77	5,39	75	4,35	0,30
6	72	5,17	75	4,41	0,33
7	72,53	6,28	75	76,59	2,11
8	75,49	6,38	70	72,18	1,55
9	71,91	5,6	70	24,99	0,34
10	71,4	5,57	75	M	m
11	71,38	5,28	70	40,84	0,53
12	72	12,32	70	16,04	0,31
14	70,61	5,83	75	9,38	0,70
15	72,88	8,5	70	32,27	0,73

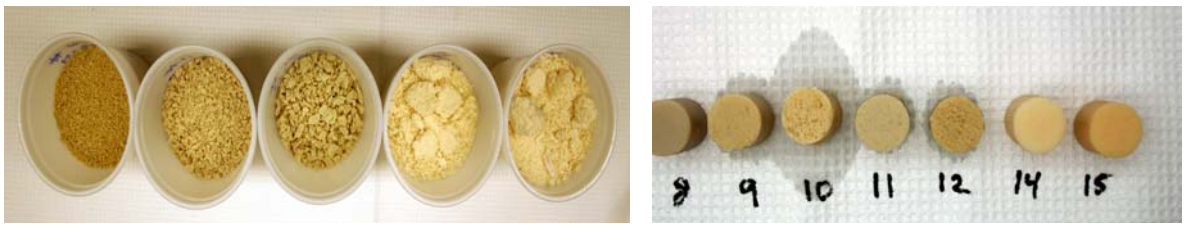

Figure 23: Left: Dried samples #10-15. Right: Gelled products of re-hydrated dried samples #8-15. Observe the wet area around sample 10.

From the pictures in figure 23, it is clear that samples 9-12 do not hold water very well. All gels in the picture contain 70% moisture, except 10 and 14 that contains 75% moisture. Samples 1-6 looked and behaved very much like sample 10. Sample 7 looked like sample 8, but less grey in colour most likely due to higher moisture content.

Textural properties on re-hydrated dried protein isolate samples measured by torsion test are shown in figures 24 and 25. Compared to dried untreated fish mince and commercial surimi samples, the PI creates very weak gels. Texture values in the dried samples are also lower relative to corresponding samples, which have not been dried.

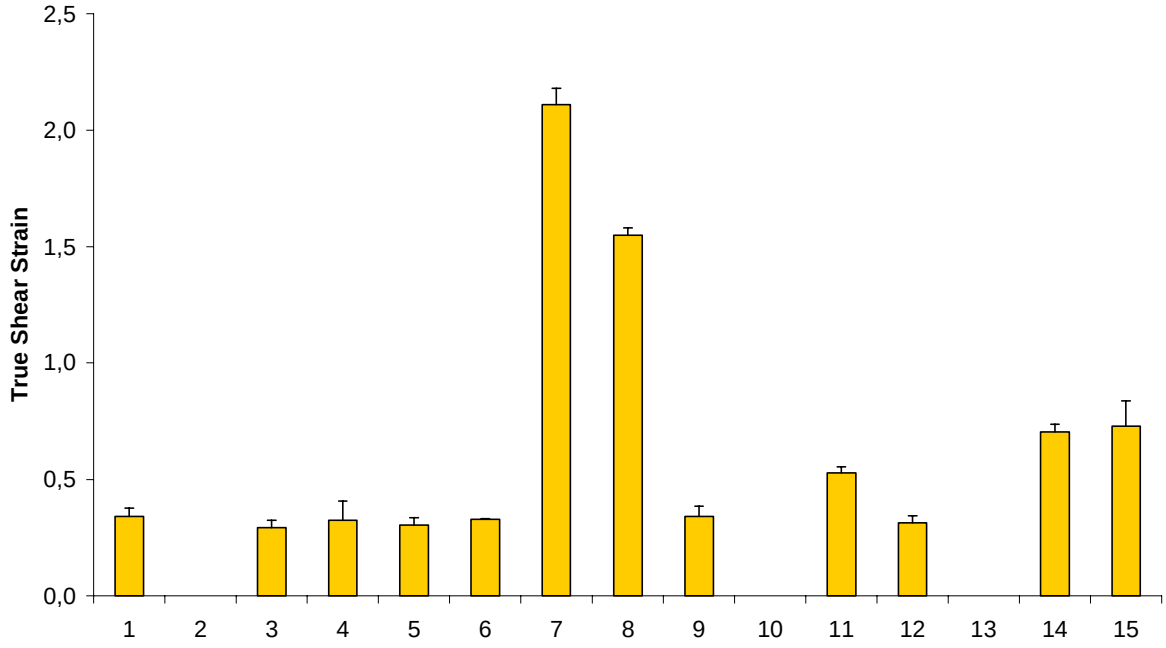

Figure 24: True shear strain from torsion tests on gels from saithe (Pollachius virens) protein isolates at different drying conditions (1-12), control samples (14 & 15). Samples 2 and 10 were too weak to be measured. Sample 13 does not exist.

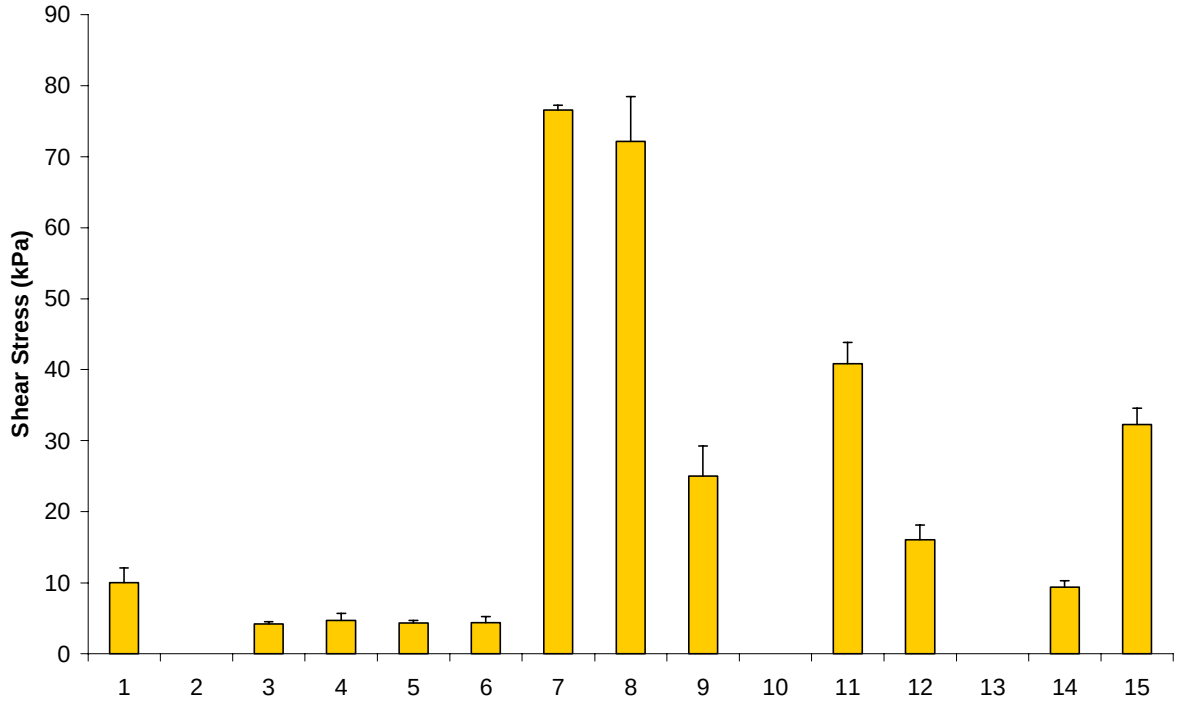

Figure 25: True shear stress from torsion tests on gels from saithe (Pollachius virens) protein isolates at different drying conditions (1-12), control samples (14 & 15). Samples 2 and 10 were too weak to measure. Sample 13 does not exist.

4 CONCLUSIONS

The choice of acid or alkali extraction method is essential for the quality of the FPI. The extraction methods give a significant ($p<0,05$) difference in results regarding all parameters assessed, except for true strain ($p=0,056$). Texture values were generally higher in alkali samples than acid samples. Alkali samples also had lower L*- and a*-values, but higher b*-values. This results in overall lower whiteness (L*-3b*) scores in the alkali treated samples. The sensory analysis gives a preference to alkali treatment regarding textural properties, while the acid treatment seems to give better colour and less rancid odour and taste. Products made using acid treatment (N1, 2, 4, and 5) seem less coherent when pulling, and being softer and more watery when chewing. In addition, the acid treated samples were considered saltier than the alkali samples. By the exception of N4, all acid treated samples were considered significantly different in the parameters coherence, watery and hardness compared to the alkali treated samples, table 3. The alkali treated samples (N13, 14, 16, and 17) had significant higher grey colour scores.

Addition of phosphates (TSPP) gave a significant difference in all parameters except for shear stress. These differences were manifested by higher texture values and lower scores on all colour characteristics. The lower b*-values compensated for the lower L*-values, thus, phosphate samples gave higher whiteness scores. Hence, addition of TSPP is recommended due to their favourable effects on texture and colour values.

Raftiline®P95 was the only cryostabiliser that differed significantly from the other in all tests except for gel strength and shear stress. The Raftiline®P95 samples gave higher values in gel strength and shear stress tests, but lower scores on true strain and distance. We also observed higher L*- and b*-values, but lower a*-values. In spite of high L*-values, they did not entirely compensate for the increase in b*-values, and the whiteness scores remained low.

Raftilose®P85 seems a little less effective than Trehalose and Raftilose®P95.

Both Trehalose and Raftilose®P95 seem to have at least as good capacities to protect proteins during frozen storage as sorbitol and sucrose without loosing textural or colour properties. When comparing the two extraction methods within the same cryoprotectant group, the alkali treated samples scored higher in rancid odour with exception of sorbitol samples N5 versus N17. These two samples are considered having highest intensity in both rancid odour and taste. The addition of different cryostabilisers in the protein isolates was mainly reflected in odour and taste scores. Samples containing sorbitol (N5 and N17) gave the highest scores in rancid odour and rancid taste. Sucrose samples differed significantly compared to the rest of the samples as scoring high in sweet taste.

Optimum pH for good texture properties was found to be around pH 7,1. A negative effect of increased pH is a decrease in colour values. Decreasing b*-values did not outweigh the reduced L*-values.

Soy protein isolates had no slightly negative effect on all parameters. Samples containing soy protein seem to be less affected to pH changes.

The dried samples had an intense rancid odour, with a dark yellowish or grey colour. The re-hydrated protein isolate samples did not produce a proper gel, and did not hold water very well. Rehydration time and homogeneity of the dried samples depended on granule size.

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