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Protein Isolation from Herring

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<i>Ágríp á íslensku:</i>	<p>Notkun próteina sem hjálparefna í matvælaframleiðslu er mikil þar sem þau gegna margvíslegum hlutverkum, s.s. sem bindiefni, ýrugjafar, o.fl. Algengustu próteinin eru sojaprótein og ýmis mjólkurprótein. Fiskiðnaður hefur ekki stundað nýsköpun á þessu sviði og fiskprótein til notkunar í matvælaíðnað hafa vart verið fáanleg. Á það sérstaklega við um prótein úr dökkum, fitumiklum fisktegundum eins og síld og loðnu.</p> <p>Nýleg aðferð til að einangra fiskprótein, sem gerir það mögulegt að fjarlægja óæskilega fitu og þætti sem hafa áhrif á lit og lykt, getur breytt þessari stöðu og þar með gera mögulegt að vinna prótein úr uppsjávarfiskum.</p> <p>Aðalmarkmið þessa verkefnis var að skala upp þessa vinnsluáðferð frá tilraunastofu yfir á tilraunaverksmiðjuskala með síld sem hráefni.</p>		
<i>Lykilorð á íslensku:</i>	<i>Síld, próteineinangrun, vinnsla.</i>		
<i>Summary in English:</i>	<p>The use of proteins as ingredients in food production as binders, emulsifiers, gelling agents, etc. is widespread and increasing. The most widely used proteins in the food industry are from soybeans and milk. Fish proteins have not gained the same position on the market since suitable extraction techniques, which can preserve the functionality and remove unwanted quality decreasing components from the proteins, have been missing. The latter is particularly true concerning dark-fleshed species and has led to serious over-use of certain white fish species.</p> <p>However, the recent invention of a new extraction technique may drastically change this by making food production from the large, low value, fatty pelagic fish species in the North Atlantic technically possible. The main objective of this project was to scale up this process from lab scale to a pilot plant scale with herring as raw material.</p>		
<i>English keywords:</i>	<i>Herring, protein isolation, processing.</i>		

Summary

The use of proteins as ingredients in food production as binders, emulsifiers, gelling agents, etc. is widespread and increasing. The most widely used proteins in the food industry are from soybeans and milk. Fish proteins have not gained the same position on the market since suitable extraction techniques, which can preserve the functionality and remove unwanted quality decreasing components from the proteins, have been missing. The latter is particularly true concerning dark fleshed species and has led to serious over-use of certain white fish.

The recent invention of a new extraction technique may drastically change this situation by making food production from the large, low value, fatty pelagic fish species in the North Atlantic technically possible. The main objective of this project was to scale up this process from lab scale to a pilot plant scale with herring as raw material.

Aims

1. **Optimise the process on a lab scale using** industrially produced herring (fresh or frozen; whole, headless or filleted fish).
2. Develop a **continuous protein isolation process** from herring (*Clupea harengus*) on a **pilot scale**.
3. Develop methodologies for further processing of the protein isolate: (i) **Dehydration** and (ii) **Surimi processing**

The long term vision of the project is that the protein isolate will increase the percentage of the Nordic herring catch being used for food production.

Results

Lab work showed that herring as raw material for the process is affected by storage. This is seen both for freezing and storage on ice. The protein solubility decreased and viscosity increased resulting in lower yield of the process. Methods were developed to reduce the increased viscosity but they all affected the solubility and therefore also the yield of the process. Further storage of the frozen raw material up to around six months does not greatly affect the viscosity and protein solubility properties.

The project was successful in developing a continuous protein isolation process and the pilot plant works very well in adjusting pH and other parameters. There are still problems with fat removal when working with fatty species.

Drying of the protein isolate (lean species) was successful but the quality parameters might have been better. It was not possible to make a gel (surimi) from protein isolate of herring made in the plant. It was possible to make a gel from lean species protein isolate but the gelation parameters were not satisfactory. A market survey showed that the future for fish proteins could be promising, both as protein isolates to be used in marination and injection and also as ingredients in functional foods. However further trials, research and development than were made in this project are necessary to reach that goal.

Conclusion

The project was delayed since the scale up took more time than originally planned. That resulted in less time for optimisation of the different products, surimi, marinade and dried powder. The results, which were obtained in the project, give hopes that further optimisation of the process will result in a high quality protein product that will increase the value of the Scandinavian fish industry.

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A. Background

The use of proteins as ingredients in food production is considerable and increasing. Proteins act as binders, emulsifiers, gelling agents, etc. Currently the most widely used proteins in the food industry are derived from soybeans or milk. The soybean and milk industries have placed high emphasis on extraction, production and utilisation of proteins from their by-products with good success. The same cannot be said about the fishing industry where many underutilised fish species and protein rich by-products are still basically used for production of *animal feed/low value products* (fish meal and mince). High value protein such as surimi is however produced from white fish, mainly from Alaska Pollock and not from underutilised fish species such as pelagic species, mainly due to a lack of suitable extraction technique.

In 1998 a new isolation technology emerged which may improve the functionality, storability, odour and taste of proteins extracted from fish (Hultin & Kelleher, 1999). The technology opens up possibilities to produce functional and food grade proteins from fatty fish such as herring. The method is based on acid extraction of the proteins, high-speed centrifugation to remove fat and impurities and, finally, precipitation of the proteins under alkaline conditions. Two possible end-uses are envisioned for the resulting protein isolate: a protein mass (e.g. surimi) and, assuming the development of an appropriate dehydration technique, a nutritious functional food ingredient powder. Furthermore, the cold process may lead to oil of excellent quality.

Herring (*Clupea harengus*) is today underutilised for human consumption, although it is still a traditional backbone for the Nordic fishing industry. The world catch in 1997 was ~2.5 million tonnes (FAO, 1997) whereof Norwegians and Icelanders caught approximately 50%. The fish is increasingly being used for other purposes than for human food, e.g. as fish meal and oil. If Iceland is taken as an example, the part used for food production has decreased from traditionally 40-50% down to ~10% from 1988 to 1998 (Útvegur, 1998). The reason for this is that markets for traditional food products from herring (e.g. salted and frozen) have been declining. For many reasons, there is therefore a strong urge today to find new ways of incorporating herring into food. This would not only be a more responsible way of using a highly nutritious marine resource, but is also strongly suggested as a strategy for maintaining the marine bio-diversity as well as the sustainability and competitiveness of the Nordic fishing/fish processing industry.

In this project the aim is to develop **high value** products on a continuous **industrial scale** from **herring** using this new technology.

1. State of the Art

The difficulties in processing herring arise from its small size, seasonality and short storage life due to its unfavourable chemical composition. The **seasonality** causes uneven raw material supply and unpredictable product quality. The **unfavourable composition**, linked to the abundance of dark muscle, pollutes waste-water and lowers quality e.g. via: (i) rancidity development, (ii) poor protein functionality (Hultin & Kelleher, 2000a; Shimizu *et al.*, 1992; Tanikawa, 1985), (iii) dark colour and (iv) histidine content. Overcoming these problems has therefore been a major goal when developing novel processing techniques for herring and other pelagic species.

1.1. Previous processing approaches

Previous processing approaches of herring include mincing, washing and enzymatic hydrolysis.

Mincing allows for incorporation of compounds changing the stability, taste and functionality of the muscle which largely improves possibilities for further processing of herring as compared with fillets. However, as the surface area is increased and normally separated reactants are brought in close contact, mincing also favours degradation reactions. In the early 1990's, development, stability and consumer acceptance of herring mince products was evaluated in a large project involving most Swedish fish industries. Although the products were considered tasty and oxidative stability was obtained, factors such as dark colour and a negative consumer attitude towards herring mince as a replacement for minced meat lowered the acceptance and commercialisation of these products.

Washing minced fish with water has positive effects on functionality, colour and stability (Nishioka *et al.*, 1990; Tanikawa, 1985). *Surimi*, i.e. washed fish mince with added cryoprotectants, was developed in Japan to preserve the gelling properties of washed fish mince during frozen storage. Gels derived from surimi by salt and heat are incorporated into various traditional products found in Asia and in shellfish analogues, successfully marketed in Europe, America and Australia. Although white fish such as Alaska Pollock is the most common raw material for surimi, pelagic species such as herring (Tanikawa, 1985), menhaden (Hurley, 1989), mackerel (Hotton *et al.*, 1990; Tanikawa, 1985), sardines (Nishioka *et al.*, 1990) and capelin (Broch-Due *et al.*, 1986; Chandra, 1988; Eide *et al.*, 1980; Eide *et al.*, 1982; Langmyhr *et al.*, 1988; Spencer *et al.*, 1988; Wray, 1989) have also been tested as an alternative raw material. In Japan, small-scale commercial production using pelagic species has existed for some time (Nishioka *et al.*, 1990; Okada, 1990). A broad commercial exploitation and acceptance of surimi from pelagic fish has been limited because of the very high quality requirements of the Asian market. Many technical difficulties hinder the development of large scale commercial process. One problem is the exceedingly low protein yield (as surimi mass) from pelagic fish in comparison with white fish, e.g. 10-12% and 12-15% of initial fish weight for menhaden and mackerel, respectively (Hurley, 1989), compared with 22-24% for Alaska Pollock (Anon, 1987). Moreover surimi from pelagic fish has little stability, limited by rancidity (Gandemer, 1999; Hultin *et al.*, 1990; Katoh *et al.*, 1989; Machii *et al.*, 1988; Richards *et al.*, 1998; Tanikawa, 1985).

Due to all the difficulties of processing surimi from fatty species like herring, there is no large scale commercial process available today.

Enzymatic hydrolysis has been used to produce protein isolates e.g. from mackerel fillets (Gartzia & Pérez-Villarreal, 2000) and trimmings from cod (Stoknes & Hellevik, 2000). The isolate is mostly used for production of dried nutritional, flavouring and emulsifying ingredients. In the hydrolysis process, only a minimum amount of water is added and temperature is raised to activate (55-60°C) the enzymes. By using different enzymes and by controlling temperature, time and pH, different end-products can be produced. Great emphasis has been placed on developing enzymatic hydrolysis methods for production of fish protein-based food ingredients. However, as the process does not remove pro-oxidants and pigments, the isolates are often **fishy/rancid** and dark coloured. Also,

peptide formation yields bitterness and the high temperature used in the enzyme inactivation step denatures the sensitive fish proteins which destroy their functionality (Lanier, 1994). Today, the few commercially available enzymatically produced fish protein concentrates have found limited use due to poor product quality, lack of functionality and a fishy odour/taste.

1.2. New possibilities

In 1998 a new method for fish protein extraction emerged (Hultin & Kelleher, 1999). The end product of this method is an isolate that may be used as surimi or, provided the development of a suitable dehydration technique, a nutritious functional food ingredient powder.

The process involves the following steps (Hultin & Kelleher, 1999) (**Figure A.1**): (i) homogenisation of ground fish in ~6-9 volumes of water, (ii) protein solubilization by acidification (~pH 3) or alkalisation (~pH 11), (iii) removal of lipids and impurities by centrifugation; neutral lipids rise to the top whereas membrane lipids, insoluble proteins, scales, bones and other impurities form a sediment, (iv) removal of the protein containing supernatant followed by iso-electric protein precipitation and (v) protein recovery and removal of non-protein soluble material by centrifugation. The purified proteins may be used as surimi or as functional food ingredients. By *drying* the washed protein isolate/surimi, transport costs can be decreased and possibilities are opened for new ways of incorporating them into food. However, drying fish proteins is not straightforward. The conditions provided by conventional dryers have negative effect on the final dried protein, presenting low quality and functional properties. This is generally due to the long exposure at low pressure or long exposure to high temperatures. The use of other techniques such as freeze-drying is also limited by high costs and adverse quality changes of the proteins. The latter concerns particularly lipid oxidation. Milder and more cost efficient way is heat pump drying, but this technique has to date not been applied to surimi. Its strength is replacement of vacuum by a recyclable inert gas significantly lowering processing time (days → hours).

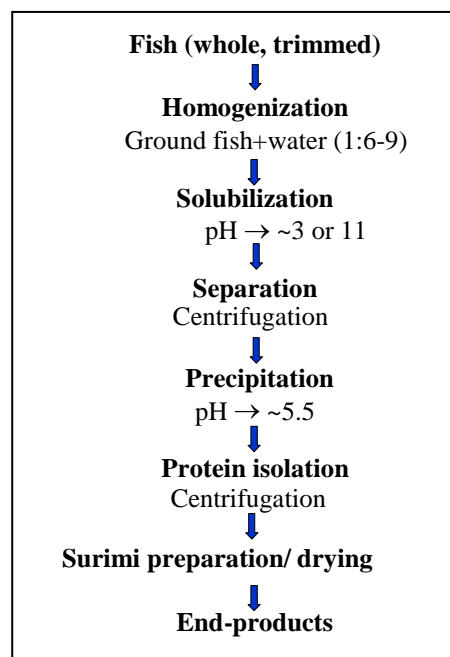


Figure A.1. Illustration of how the suggested tasks tie into the new protein isolation process (Hultin & Kelleher, 1999).

The completely new **protein isolation technique** (Hultin & Kelleher, 1999) is basically a “flip-flop” of standard surimi techniques. The novelty lies in the protein solubilization step which, by allowing density based removal of membrane lipids, bones and other impurities, reduces the risk for rancidity and the required degree of trimming the fish. In lab-scale comparison between this method and traditional surimi production from cod, the former gave better protein yields (95% compared with 55-70%), gelling capacity and colour as well as a less polluted waste-water (Hultin & Kelleher, 2000a). However, the process not only improves the efficiency of white fish processing, but obviously also **has the potential to produce good quality protein isolate from pelagic fish**. Preliminary trials with herring (Undeland *et al.*, 2000) have proven very promising. They gave protein yields of ~75%, removed most lipids and produced an isolate with good gelation capacity. Further improvements are to be expected if some viscosity related problems in the separation step can be solved. The latter would allow for successful up-scaling and commercialisation of the process, a substantial technological breakthrough that would doubtlessly open up new opportunities for the Nordic fish processing industry.

B. Results

The work packages (WP's) in this project reflected the cornerstones **protein isolation**, **protein processing** and **product** as well as **administrative tasks** (Figure B.1). The central point in the project was **WP I-Task 2** pilot plant. The Tasks in WP 1, (raw material optimisation, pilot plant, waste water and oil/by-products) were selected to ensure an environmentally friendly and efficient production of a **high quality protein isolate**. In WP II, the isolate was converted to a **powder/surimi** via development of innovative dehydration techniques, conditions for surimi production and mapping of storage stability/protection strategies. In WP III, the market for **products** based on the protein powder or surimi was evaluated. Since success in WP I-III was built on close collaboration between partners, WP IV covered efficient logistics and internal result dissemination, but also ensured external dissemination of results.

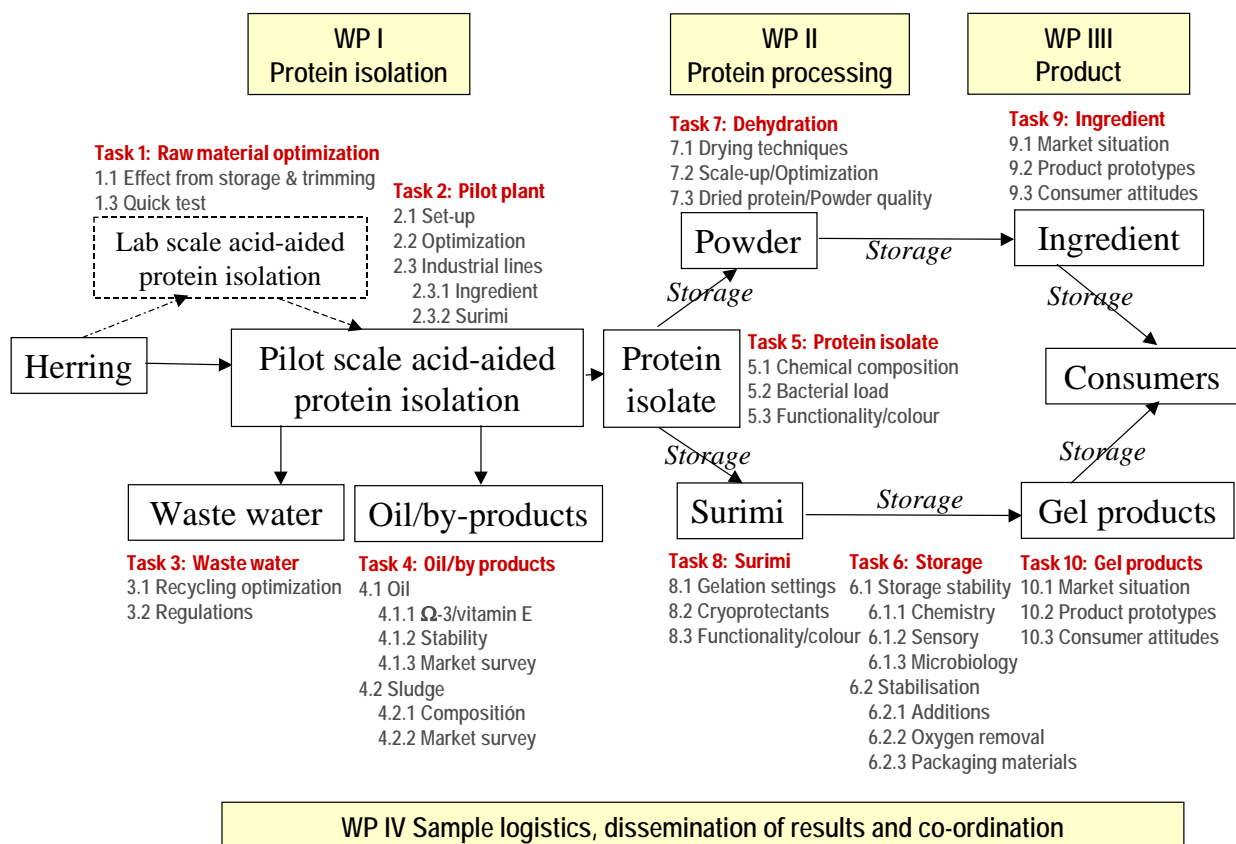


Figure B.1. Layout of the project in Work Packages (WP) and Tasks.

In the next chapters each tasks objects, materials & methods, discussion and conclusions will be presented.

TASK 1 – RAW MATERIAL OPTIMISATION ON LAB SCALE

1. Objectives

The main object in this task was to evaluate the effects of freezing and frozen storage on the stability of lipids, protein solubility and viscosity of herring proteins and to observe if it is possible to make sure that the raw material is stable from one herring season to the next for protein isolation. Furthermore, the effects of which parts of the herring are used, i.e. dark or light muscle and the effects of pre-washing to remove colour and pro oxidants. Both SIK and Icelandic Fisheries Laboratories (IFL) worked on this task. Results will be presented individually but collective conclusion presented by the end of the task.

2. SIK work

2.1. Results & Discussion

In the early work on acid/alkaline protein isolation from herring light muscle (Undeland *et al.*, 2002), it was found that unwanted high viscosity developed when the 1:9 herring muscle homogenate was acidified or alkalinized in order to solubilize the proteins. This viscosity peaked around pH 3.5 and pH 10, just before the proteins reach maximum solubility. Under favourable conditions, the viscosity then declined again below pH 3.5 and above pH 10 (**Figure 1.1**).

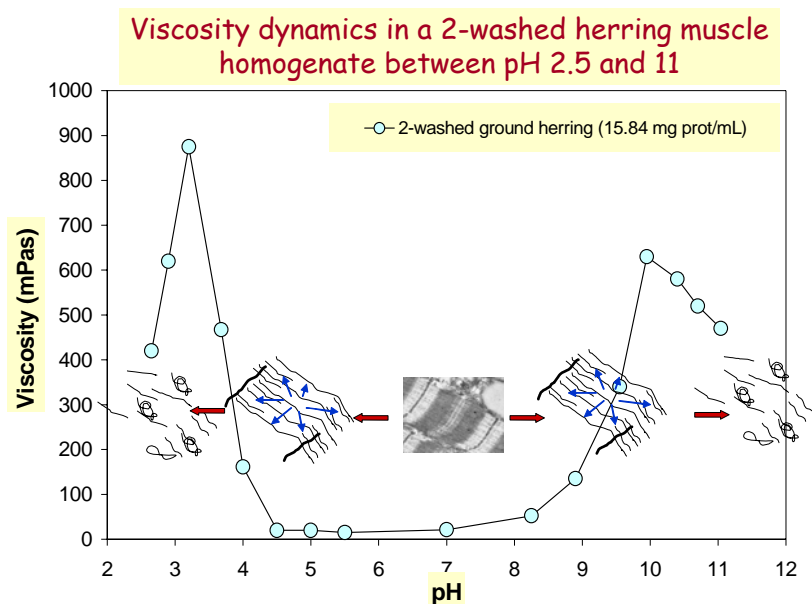


Figure 1.1. The link between viscosity of a 1:9 herring in water homogenate and pH of the homogenate. The pictures inserted illustrate the “expansion” and solubilization of the muscle proteins as a function of pH.

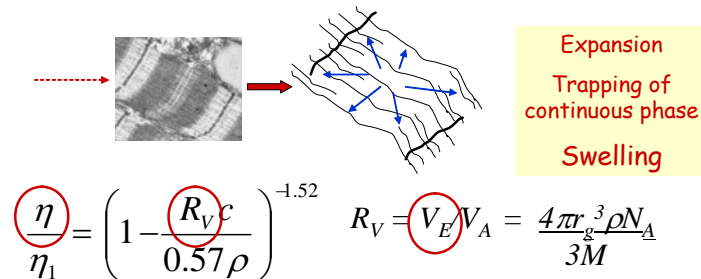
Quite often, the viscosity decline at extreme pH-values was very limited, something that rendered the density-based separation of lipids and proteins difficult and also produced large protein-entrapping sediments during high-speed centrifugation. All-in-all, this reduced the purity and yield of proteins, why it constituted a problem that needed to be addressed before scale-up. It was found that the sarcoplasmic proteins of the herring did not contribute to the illustrated raises in viscosity, instead, it appeared to be mostly linked to changes in the myofibrillar proteins (small inserts in **Figure 1.1**). In the work done by SIK, it was found that both the maximum viscosity intensity reached and the extent of the viscosity decline at extreme pH-values varied largely with the pre-processing history of

the herring used (e.g. post mortem age, freezing etc). The viscosity also varied with the specific process conditions used (pre-filtering of homogenate, presence/absence of salt, pyrophosphate etc.). Based on what the literature describes around development of viscosity in protein solutions, the following hypotheses were set up to explain the behaviour of the herring proteins during acid or alkaline solubilization (**Figures 1.2 and 1.3**).

Why does it happen?

Acidification/alkalization increase the protein net charge

- a) → repulsion → expansion of the structure
- b) → protein solubilization



η = homogenate viscosity, η_1 = viscosity of liquid surrounding the particles, c = protein concentration (in kg m^{-3}), ρ = protein density (in kg m^{-3}), R_V = effective volume ratio of the protein molecules/aggregates V_E = "effective" volume of the protein molecule/aggregate, V_A = actual volume occupied by the protein molecule/aggregate, r_g = radius of gyration of the molecule/aggregate, N_A = Avogadro number, M = molecular weight.

Figure 1.2. Explanation of how the effective volume ratio of proteins/protein aggregates is linked to the herring muscle homogenate viscosity. For deeper explanation, see Undeland et al., 2003.

Effective volume

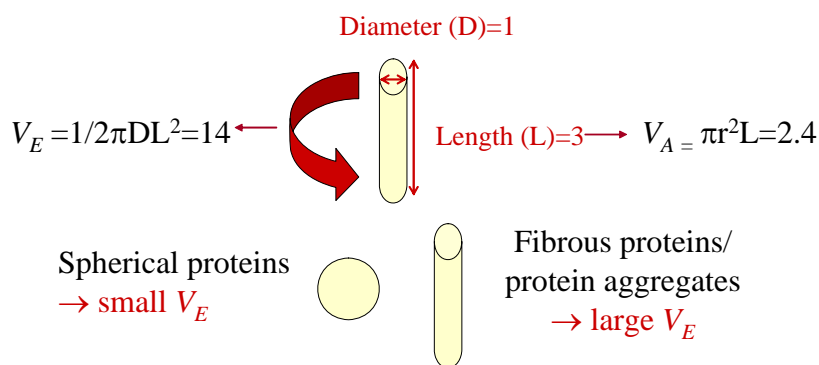


Figure 1.3. Explanation of the term "Effective volume" and why fibrous muscle proteins can give higher viscosity than globular (sarcoplasmic) ones.

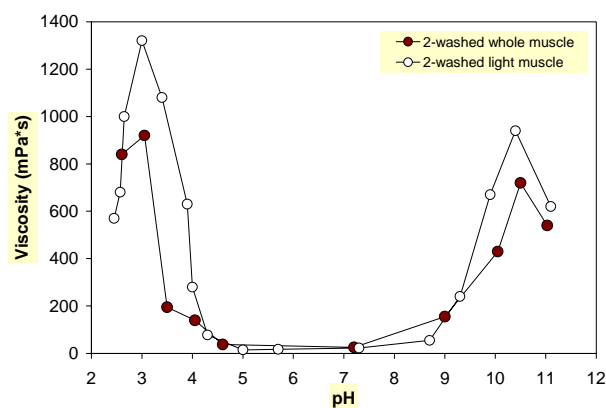
In the work done by SIK, viscosity during acidification and alkalization of a herring light muscle homogenate was studied as a function of variations in the raw material and as a function of different "thinning"-strategies:

- Presence and absence of dark muscle (**Figure 1.4a**)
- Post mortem age (**Figure 1.4b**)
- Pre-freezing of the herring (**Figure 1.4c**)
- Pre-washing (one or two 1:3 water washes) (**Figure 1.4d**)
- Filtering of non-pH adjusted homogenate (**Figure 1.4e**)
- Addition of pyrophosphate (10 mM) (**Figure 1.4f**)
- Addition of salt (NaCl) (**Figure 1.4g**)
- Holding of acidified and alkalized homogenates on ice (**Figure 1.4h-j**)

The experiments were made by preparing a 1:9 homogenate of herring light muscle in water. The pH was then gradually adjusted down to pH 2.5 using 1M HCl, or up to pH 12 using 1M NaOH. The homogenate was constantly kept on ice, and at regular pH-intervals, the viscosity was measured. Below, some major results from these trials are shown. For more deep explanation, and discussion of the data, we refer to Undeland *et al.*, 2003.

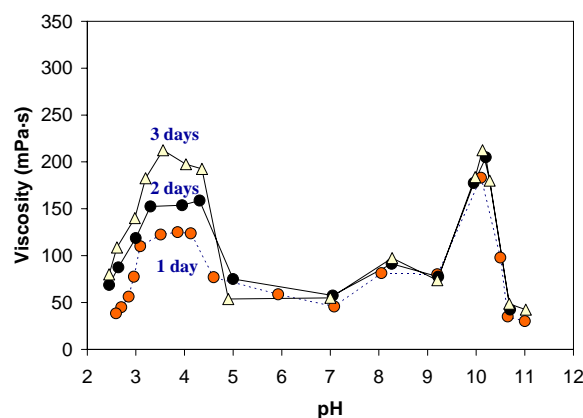
(a)

Presence or absence of dark muscle



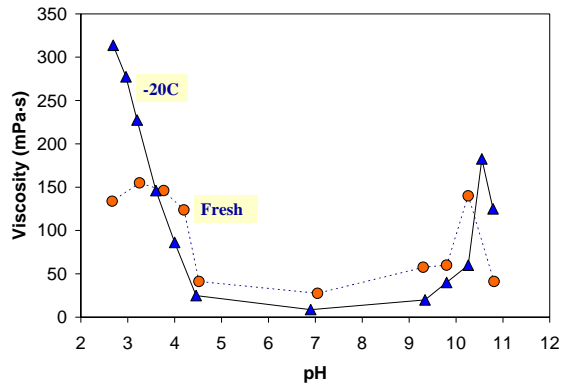
(b)

Effect of storing New England herring on ice on the viscosity of a light muscle homogenate



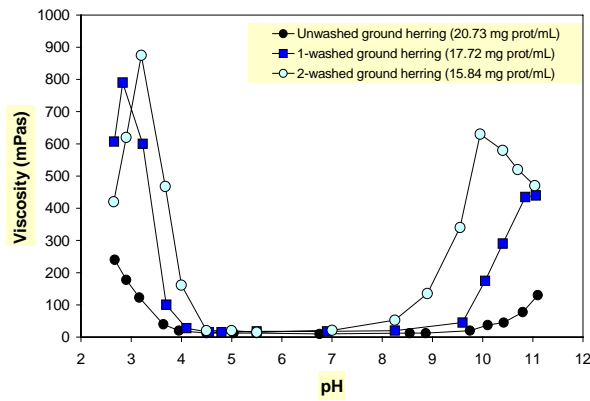
(c)

Effect of storing New England herring for 18 days at -20C on the viscosity of a light muscle homogenate



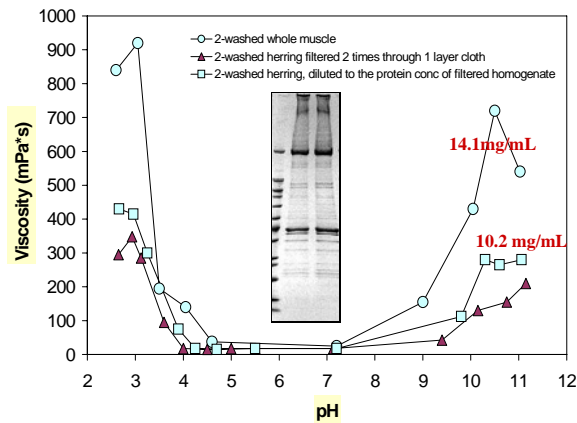
(d)

Pre-washing (one or two 1:3 water washes)

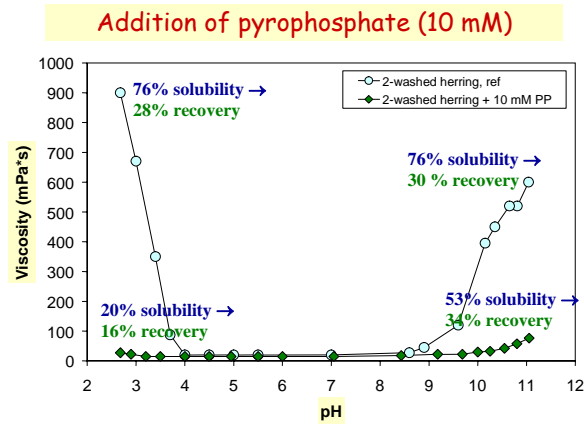


(e)

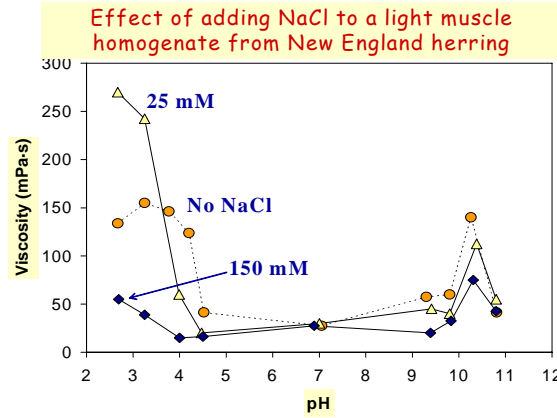
Filtering of non-pH adjusted homogenate



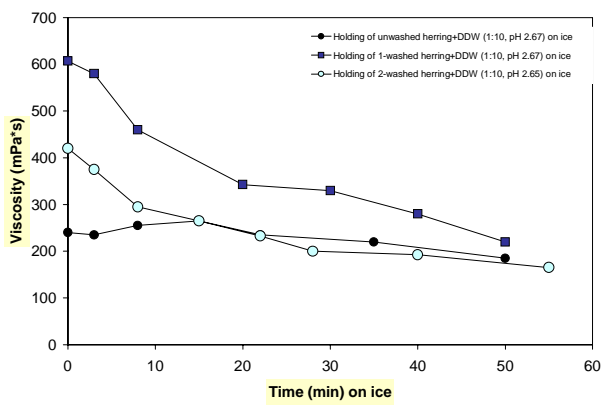
(f)



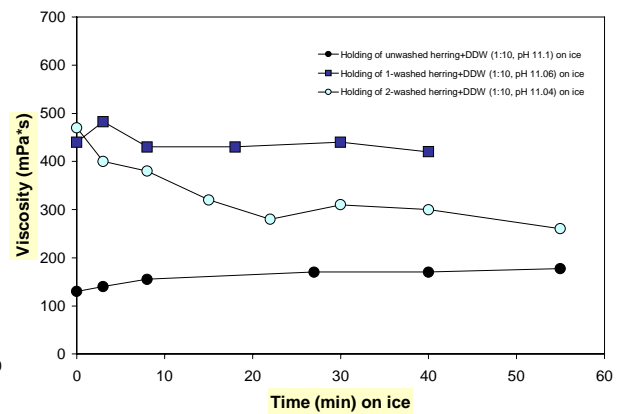
(g)



(h)



(i)



(j)

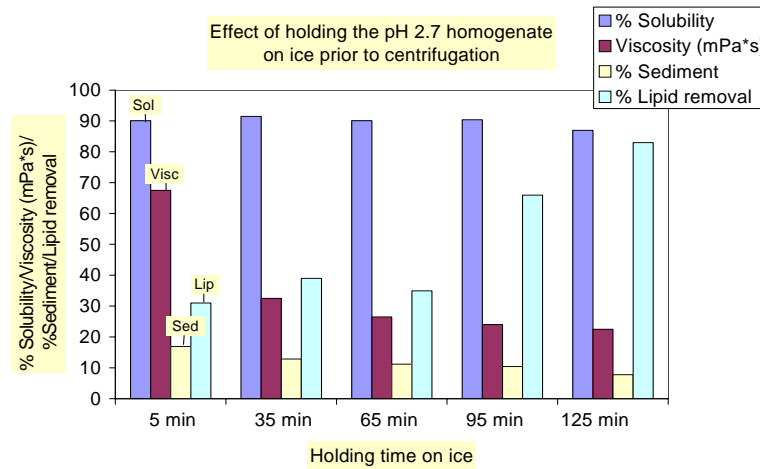


Figure 1.4. The effect of various raw-material related and process-related variables on viscosity of the herring light muscle homogenates at different pH values; 2.5-11. (a) Presence and absence of dark muscle, (b) post mortem age, (c) pre-freezing of the herring, (d) pre-washing (one or two 1:3 water washes), (e) filtering of non-pH adjusted homogenate, (f) addition of pyrophosphate (10 mM), (g) adding of salt (NaCl), (h-j) holding of acidified and alkalinized homogenates on ice.

In short, it was found that the dark muscle had less tendency than white muscle to expand, and thus, raise viscosity of the homogenate (**Figure 1.4a**). It was found that Icelandic herring had relatively more dark muscle (40% w/w) than the New England herring (20% w/w), which thus, is a factor to consider when working on viscosity related problems.

As shown in **Figures 1.4b** and **1.4c**, both extended holding of the whole herring on ice and pre-freezing of the herring prior to processing increased the viscosity after acidification and alkalization of the light muscle homogenate. A possible theory explaining this is that the storage make (certain) proteins insolubilized e.g. due to lipid oxidation. This could result in large swollen structures that do not fully “collapse”. Another possibility is that possible solubility inhibiting (PSI) peptides (see **Figure 1.5**) degrade, which could allow larger swelling/expansion during acidification/alkalization.

Role of “PSI” peptides

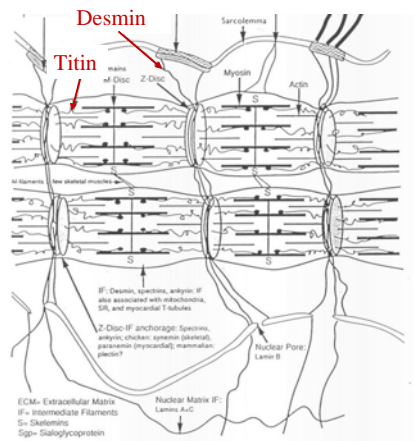


Figure 1.5. Illustration of how possible solubility inhibiting (PSI) peptides may prevent excessive, pH-driven expansion of the fish muscle by keeping the myofibrillar network together.

Pre-washing the herring mince before subjecting it to acid/alkali increased the viscosity (**Figure 1.4d**). It was hypothesized that this “swelling” was due to removal of PSI-peptides. These would be cytoskeletal proteins that hold the myofibrillar structure together, thus, preventing excessive expansion of the myofibrillar network due to pH-adjustments. Examples of such proteins are desmin, titin and alfa-actinin. It was previously found that they can be removed under the pH- and ionic strength conditions that occur in a 1:3-wash of minced muscle.

A strategy to reduce the viscosity peaks was to filter the herring light muscle homogenate through cheese cloth prior to pH-adjustments (**Figure 1.4e**). It is believed that this was due to a reduction of aggregates/polymers with large effective volume (VE), of connective tissue (collagen?), and of non-homogenized, large, muscle pieces.

Another possible strategy tested to reduce viscosity was to add 10 mM pyrophosphate to the homogenate. As seen in **Figure 1.4f**, pyrophosphate severely reduced the viscosity peaks, but it also reduced the solubility of herring light muscle proteins from 76% to 20% at pH 2.7 and from 76 to 53% at pH 10.8. It was found previously that 10 mM STPP easily undergoes hydrolysis to e.g. pyro- and monophosphate. Pyrophosphate can, just like ATP, dissociate actomyosin, which could lower the solubility of the actin. As mentioned in relation to our results from Task 6, the applicability of phosphates (STPP) as antioxidants was also limited by their tendency of reducing solubility.

In **Figure 1.4g**, it can be seen that 150 mM NaCl in the homogenate reduced viscosity while 25 mM NaCl increased it. The way proteins respond to NaCl is obviously very complex, which is known from many previous studies. Just as in the study of pyrophosphate, 150 mM NaCl not only reduced viscosity, but also solubility. This would reduce the yield of the process, and therefore the strategy of adding NaCl as a “thinning-agent” is of limited relevance. The link between salt and solubility is discussed more in the consistency-paper referred to above (Undeland *et al.*, 2003)

In **Figures 1.4h-j**, it is illustrated how a period of holding at the acid or alkaline pH prior to centrifugation made the viscosity drop quite extensively. The use of such a holding was studied under more controlled conditions, particularly on the acid side (**Figure 1.4j**). In this study, it was obvious that the drop in viscosity also helped total lipid removal. Possibly a thick consistency prevent oil from floating up to the top of the centrifuge tubes. It is also obvious that the sediment size declines with declining viscosity, which increases protein yield. We believe the effect of holding comes from that time is given for depolymerization of the protein polymers and for solubilization of partly solubilized, swollen, protein molecules. The latter makes the swollen structures “collapse”.

3. IFL work

The main emphasis of the work in this task at IFL was on the effects of frozen storage on the protein solubility in herring fillets. Two runs were made to monitor the effects of seasonality in January and September.

3.1. Materials and Methods

3.1.1. Materials

The herring used was caught off the South East coast of Iceland. In trial one in January 2001 the herring was landed about 12 hours post catch. After landing, the herring was size graded, beheaded, filleted and skinned in machines. Approximately 60 pcs. of fillets were packed in bags of 1 kg. Each pack was frozen (plate freezer) and sent two days later to the IFL laboratories in Reykjavik where upon arrival the fillets were put in frozen storage at -24°C and kept there until sampled. The herring used in trial two was also caught off the South East coast of Iceland in September. The herring was landed about 24 hours post catch. It got the same handling as the herring in January, size graded, beheaded, filleted and skinned by machines. After skinning the herring fillets were sent fresh, iced to IFL laboratories in Reykjavík. The fillets arrived at IFL 48 hours post catch. At IFL the fillets were packed in 1 kg. bags and frozen at -24°C . Part of the fillets were kept on ice until they were analysed. A longer time elapses therefore from catch until landing and freezing for the herring caught in September than in January. In trial one, samples were taken after 1, 3, 6, 9 and 12 months of frozen storage at -24°C . In trial two, samples were taken before freezing and after 7 days, 1, 3 and 6 months of frozen storage at -24°C .

3.1.2. Chemical analysis

Protein content was measured with a version of the original Kjeldahl method in a Kjeltex Auto sampler 1035/30 system. The nitrogen content is multiplied by 6.25 to get the crude protein. Water content in muscle was measured as weight loss after 4 hours at $103^{\circ}\text{C} \pm 1^{\circ}\text{C}$. Fat content was determined by the method of Soxhlet (AOAC, 1990a,b). Salt content was determined by a modified method of Volhard (AOAC, 1990c).

3.1.3. Fat stability

TBA (thiobarbituric acid) was measured by slightly modified steam distillation method (Tarladgis, 1960). Results were expressed in terms of mg malonaldehyde (MA)/kg tissue. Peroxide value (PV) was determined by titretic method and calculated as meq/kg lipid (AOCS, 1990).

3.1.4. Soluble protein

Soluble protein was measured by the Biuret method in supernatant, after centrifugation (Layne, 1957; Torten & Whitaker, 1964). Standard curve was made with bovine serum albumin. Absorbance was measured at 540 nm.

3.1.5. Viscosity

The viscosity was measured after protein solubilization at different pH from 2 to 12. The homogenates were placed into a 400 ml Pyrex beaker on ice. Viscosity was measured at $4-6^{\circ}\text{C}$ using Bohlin visco 88 BV with #C30 spindle (30 mm in diameter) at 1000 rpm. Results converted to mPa•s by a program in the equipment.

3.2. Results

3.2.1. Raw material composition and stability

Chemical compositions of the samples used in the two trials differ mainly in regard to fat composition where the fillets are higher in September than in January (**Table 1.1**).

Table 1.1. Chemical composition of herring fillets used in the different trials.

	Protein [%]	Fat [%]	Water [%]	Salt NaCl [%]	Ash [%]
Trial 1 (Jan)	17,8 ± 0,1	10,7 ± 0,2	70,2 ± 0,2	0,4 ± 0,1	1,5 ± 0,0
Trial 2 (Sept)	18,1 ± 0,2	12,4 ± 0,2	68,0 ± 0,1	0,2 ± 0,1	1,4 ± 0,0

TBA values increased during frozen storage (**Figure 1.6**) and are higher in samples in trial two than in trial one. Similar results are obtained for Peroxide value (**Figure 1.7**) although samples from raw material caught in January and stored for longer time have reached a maximum PV after 9 months, whereas TBA value is still increasing from 9 to 12 months.

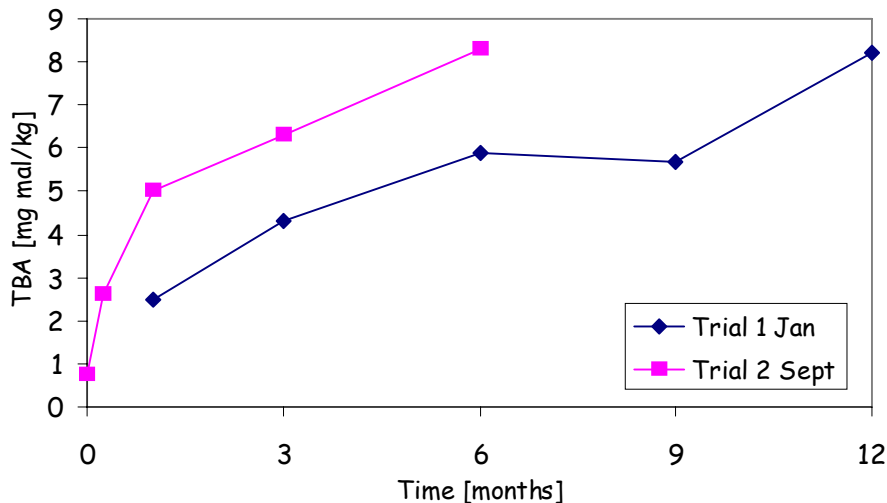


Figure 1.6. Changes in TBA-value in herring fillets stored at -24°C for up to one year. Trial 1 – herring caught in January, Trial 2 – herring caught in September.

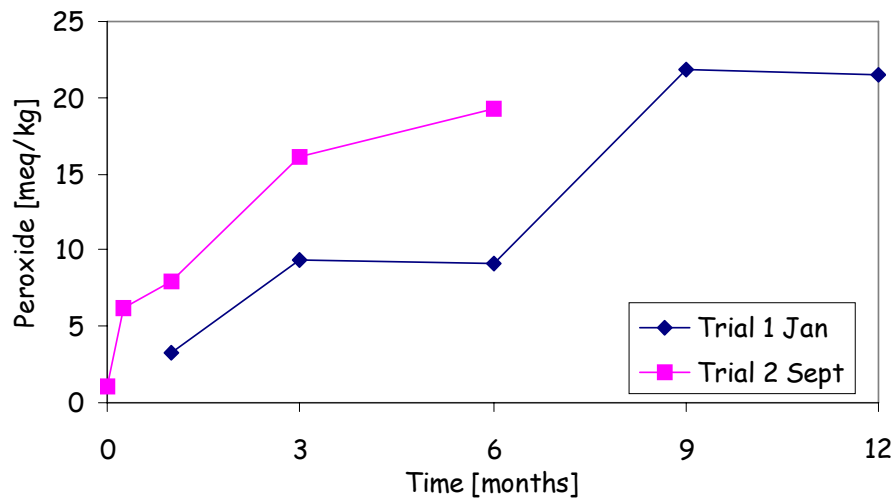


Figure 1.7. Changes in Peroxide-value in herring fillets stored at -24°C for up to one year. Trial 1 – herring caught in January, Trial 2 – herring caught in September.

Changes in protein solubility by frozen storage

A characteristic figure of changes in protein solubility with pH can be seen in **Figure 1.8** with a minimum around the isoelectric point ($\text{pI} \sim 5.0$) and maximum at highest and lowest pH values. Protein solubility decreased in both trials with frozen storage (**Figure 1.9**). After six months the solubility was around 5% lower than at the start for trial 2, measured at pH 2.7 where the protein solubility is highest. For trial 1 the solubility decreases even more. After 12 months in frozen storage the solubility increases again, indicating protein degradation. Similar results were obtained at pH 11 (**Figure 1.10**).

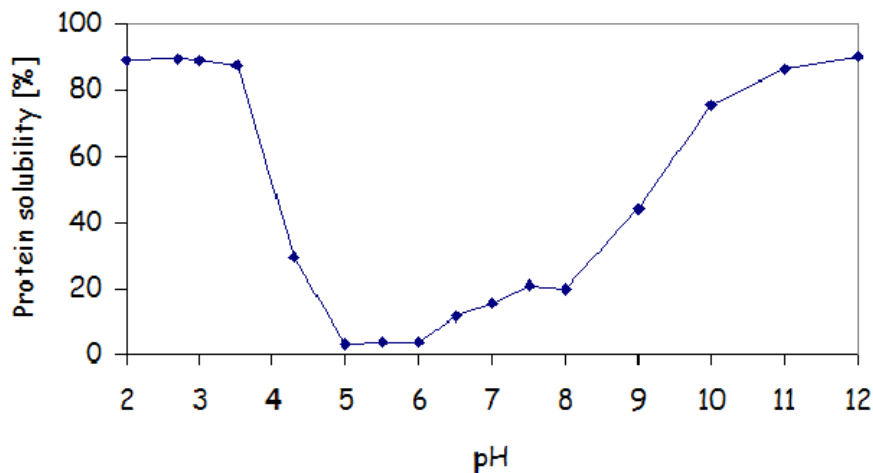


Figure 1.8. Changes in protein solubility at different pH in unfrozen herring fillets.

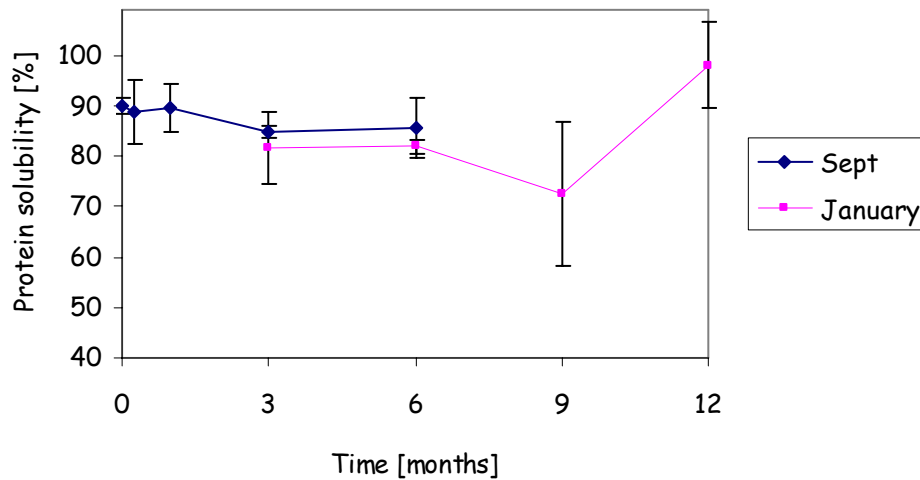


Figure 1.9. Changes in protein solubility at pH 2.7 in herring fillets stored at -24°C for up to one year. Trial 1 – herring caught in January, Trial 2 – herring caught in September.

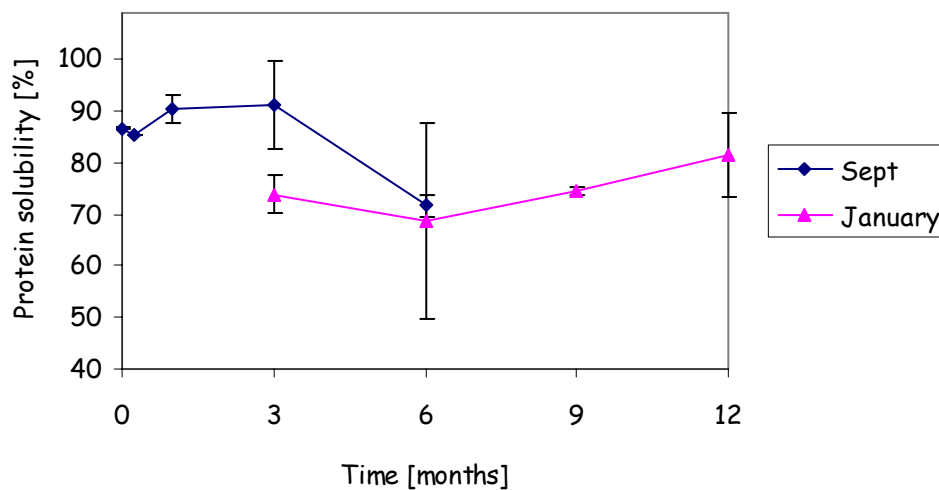


Figure 1.10. Changes in protein solubility at pH 11 in herring fillets stored at -24°C for up to one year. Trial 1 – herring caught in January, Trial 2 – herring caught in September.

3.2.2. Changes in viscosity

Viscosity was only measured in trial two and is affected by freezing and frozen storage (**Figure 1.11**) especially at pH 2.7 and 11. Freezing in itself has affect, after 7 days the viscosity has already increased and increases even more with longer frozen storage (**Figure 1.12**).

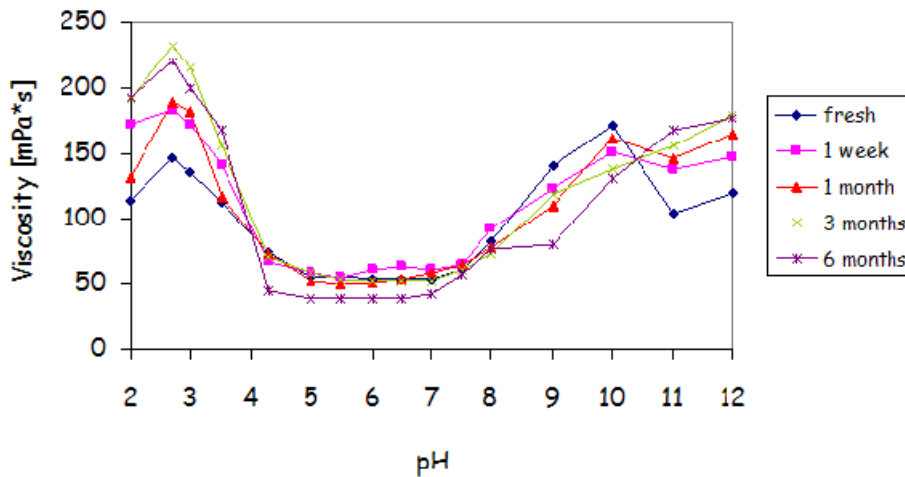


Figure 1.11. Changes in viscosity at 4-6°C of homogenate of herring fillets fresh and stored at -24°C for up to 6 months at selected pH values between pH 2 and 12. Trial 2 – herring caught in September.

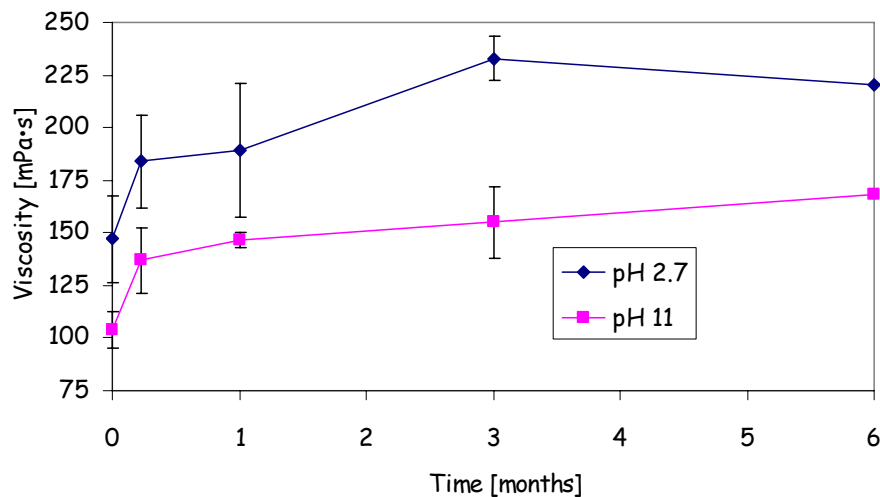


Figure 1.12. Changes in viscosity at 4-6°C of homogenate of herring fillets fresh and stored at -24°C for up to 6 months at pH 2.7 and pH 11. Trial 2 – herring caught in September.

3.3. Discussion

The difference in the chemical composition of the two samples is consistent with previously reported results (Einarsson, 1988). Two reasons might explain why the TBA values are higher in trial two than trial one. Firstly, the herring caught in September has higher fat content than the one from January. Secondly, longer time elapses from the time the herring is caught until it was processed and frozen in the samples from September than in January.

The solubility of herring proteins is higher at pH 2.7 than pH 11. At both pH values the solubility decreases during frozen storage and increases again after around 6-9 months frozen storage. This increased solubility indicates degradation of the proteins after this period of frozen storage.

The viscosity is higher at pH 2.7 than pH 11. Furthermore, viscosity increases more with freezing and frozen storage at pH 2.7 than pH 11. That might explain higher yield in the alkali process compared to the alkali process (see Task 2, Pilot plant), even though the protein solubility is higher at pH 2.7 than at pH 11 (**Figures 1.9 & 1.10**).

4. Conclusions from Task 1

It was observed that the development of high viscosity during acidification/alkalization of herring muscle homogenates clearly reduced the protein yield and also reduced the removal of lipids during high speed centrifugation. These are the reasons for trying to prevent development of viscosity. In the work done by SIK, it was discovered that both the raw material itself and variations in the process affected viscosity. Regarding the former, increased storage (on ice or at -18°C) increased the viscosity, the presence of dark muscle decreased it. When testing possible processing strategies to reduce viscosity, it was seen that the viscosity was lower when pre-washing of the mince was avoided, when the homogenate was filtered through cheese cloth, when 10 mM pyrophosphate or 150 mM NaCl was added and when the homogenate was held for an extended time at low or high pH. Among these, the additions of phosphate or salt also reduced total protein solubility, which reduced protein yield. Thus, these strategies would not be very promising to use in the pilot plant.

Similar results are obtained using Icelandic herring. The viscosity is affected by freezing, both for Icelandic herring and New England herring. For the New England herring the frozen storage is only monitored for 18 days but it's apparent that freezing affects the viscosity (**Figures 1.4c & 1.11**). The main conclusion from this task is that freezing diminished solubility and increased viscosity of herring, both decreasing yield of the process. It can however be concluded that if it is necessary to use frozen raw material, frozen storage up to around 6 months does not greatly affect the viscosity and protein solubility properties after the herring has been frozen.

TASK 2 - PILOT PLANT

1. Pilot plant

This part of the reports is divided into three parts. The first is a description of the process steps of the plant, what kind of equipment is used in each step and the original setup of the pilot plant (1.1. Process steps). In the second part (1.2 Runs in the first setup of the pilot plant), results from runs in the first setup are presented. In the third part, renovations of the pilot plant are presented, including results of yield and lipid removal from different fish species with the renovated pilot plant setup (1.3. Runs in the pilot plant – optimised layout).

1.1. Process steps

The object was to build the process in three steps:

1. Firstly, to build process using lean boneless fish proteins
2. Secondly, to build a process to handle lean fish and bone material and turn it into fish protein
3. Thirdly, to be able to process fatty fish.

The basic process steps are shown in **Figure 2.1**.

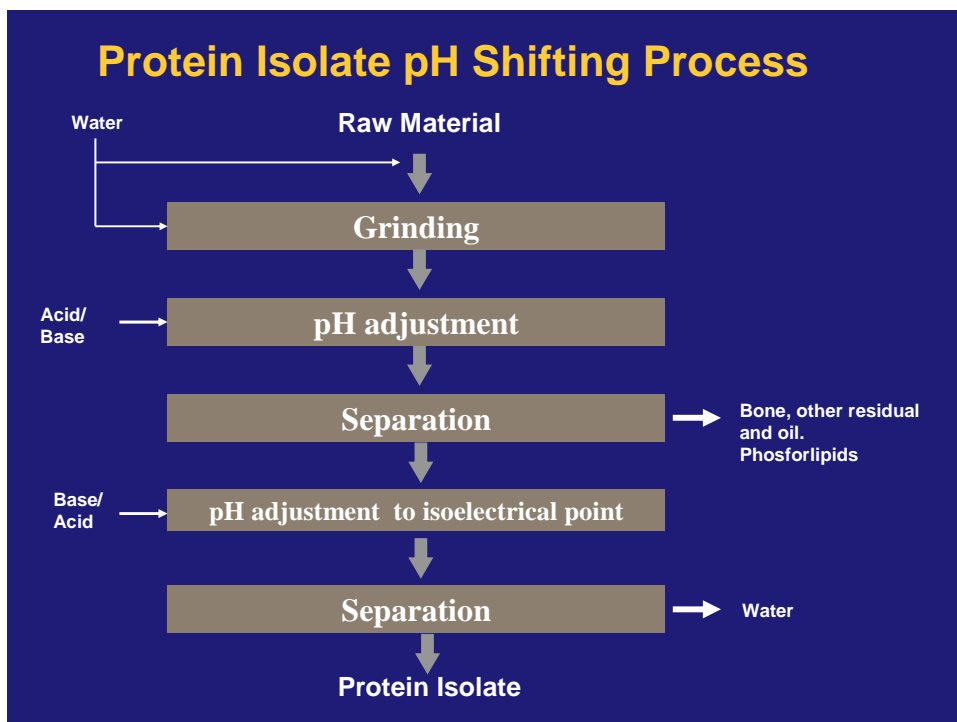


Figure 2.1. Basic process steps for the protein isolate pH shifting process.

The process is built around five processing steps:

- Grinding
- pH adjustment including the addition of water
- Separation
- pH adjustment to isoelectric point
- Dewatering

Beside these five steps, the process and the equipment must be easy to clean. A Cleaning-In-Place (CIP) module is therefore included.

The pilot plant capacity was chosen to be a system that could handle between 100-200 kg/h of mince.

Before the pilot plant was finally designed it was discussed which process parameters should be variables for testing and the following were defined:

- ✓ Particle size
- ✓ Water / mince ratio.
- ✓ Water temperature
- ✓ pH in soluble phase.
- ✓ Retention time
- ✓ Bone-, phospholipids- and oil separation
- ✓ Dewatering of protein isolate.

In the design of the pilot plant it was taken into account that those parameters should be fulfilled.

1.1.1. Grinding Process Steps

The objective of this process step is to finely grind the fish protein and add the adequate amount of water into the process.

1.1.2. pH adjustment Process Steps

The objective of this process step is to reach, in a controlled way, the desired pH that will solubilize the protein fibres (~pH 3.0 in acid process and pH~11 in alkali process).

1.1.3. Separation Process Steps.

The objective of this process step was in phase 1 to separate away all the none-soluble proteins and bone material. For this step it was decided to use a high speed separator that utilizes centrifugal force for separation of particles from a liquid phase. Furthermore, a centrifuge can under some conditions be used for separation of oil as well. To increase the part of the material that goes with the oil phase, “back pressure” can be increased.

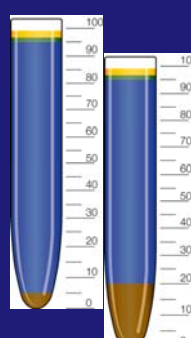
When using separation by density difference, the Stokes law applies (See Figure 2.5).

Separation by density difference

Stokes' Law

$$V_g = \frac{d^2(\rho_p - \rho_l)g}{18\eta}$$

V_g = gravitational settling velocity (m/s)
 d = particle diameter (m)
 ρ_p = particle density (kg/m³)
 ρ_l = liquid phase density (kg/m³)
 η = liquid phase viscosity (kg/ms)
 g = gravitational acceleration (m/s²)



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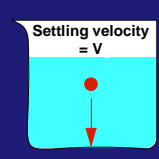
Figure 2.5. Stokes law.

As can be seen, the separation of the product depends on four factors: Particle size, density of particles and liquid plus the viscosity. In this specific process the particle size is very small and therefore density and viscosity play a significant part in the separation process. The only factor that is related to separation is the gravity acceleration. To explain the centrifugal separation principal see **Figure 2.6**.

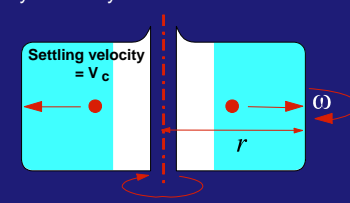
Centrifugal separation

Forces coalescence/sedimentation

Settling velocity stated by Stokes' Law



Gravity separation.
Driving force: 1g



Centrifugal separation.
Driving force: $r \cdot \omega^2$

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Figure 2.6. Optimal Centrifugal separation.

To improve the separation of solid particles, a simple tank can be modified when baffle discs are added to the tank. This way the separation can be optimized. For a centrifugal centrifuge the discs stack allows very fine particles to be separated from a liquid phase. If the liquid does hold large particles it has to be screened before it is fed into the high-speed separator. For processing a bone material the soluble liquid needs to be pre-strained in a strainer or a decanter centrifuge.

In order to remove the phospholipids the laboratory test has shown that separation at 15.000 g for 30 minutes is required. These conditions are not easy to reproduce in a pilot

plant. Even though 15.000 g is applied in the lab it is not possible to remove all traces of phospholipids. Small traces can cause negative sensory attributes. Other methods, such as the addition of antioxidants, are therefore necessary, as discussed in Task 5.

The removal of fat from fatty fish can be problematic. The Stokes law does apply for oil separation as well and here the main issues, i.e. the difference in density between oil and water/protein phase, are minimal. With increased temperature, the density difference between oil will be reduced and the water phase will stay around 1. Separation at higher temperature will be more effective but, on the other hand, temperature does also have an influence on the coagulation of the protein phase.

1.1.4. pH adjustment Process Steps to isoelectric point

The objective of this process step is to adjust the pH back to around isoelectric point (pH~5.5) to form the protein flock.

1.1.5. Dewatering and separation

The objective of this step was to separate the protein fibres from the liquid phase and to dewater the protein phase.

The separation was done in a decanter centrifuge that again utilizes Stoke law for separation of particles from a liquid phase. In this stage the size of particles is of most concern as the density of the protein flock and the water are very close. If the particles are too small when they enter into the centrifugal force they cannot be separated in time and will follow the liquid stream out of the decanter. This will be regarded as a loss in yield.

1.2. Runs in the first setup of the pilot plant

1.2.1. Cold process

Intensive trials were made in the pilot plant to get it to run properly. The main emphasis of the work was on using saithe fillets. The next step was to use herring fillets to try out lipid removal in the plant. In **Tables 2.1 to 2.5** chemical composition through the process with different raw material are presented. The main emphasis is on lipid removal. The water content in the samples was very different and to be able to compare the different samples, the ratio between lipid and protein is presented as Li/pr = g lipids / g protein. The lipid removal is calculated from these values according to:

$$\text{Lipid removal [\%]} = \frac{(\text{Li/pr}_{\text{raw material}} - \text{Li/pr}_{\text{sample}})}{\text{Li/pr}_{\text{raw material}}} \cdot 100$$

In some of the runs, only water content was measured and not the protein content. Then the lipid content is presented as g lipids / 100 g dry matter no lipids (DMNL) calculated as:

$$\text{g lipids / 100 g dry matter no lipids} = \frac{\% \text{ lipids}}{100 - \% \text{ H}_2\text{O} - \% \text{ lipids}} \cdot 100$$

Methods used for the analysis are the same as in part 3.1 in Task 1. It should be kept in mind when analysing results that the samples were very watery and the lipid- and protein content in many of the samples was very low, in fact below the sensitivity of the methods used (Kjeldahl for protein and Soxhlet for lipid). After the pilot plant had been optimised (part 1.3), lipid content was measured in samples that were not as watery as in these results presented here.

During runs in the first setup of the pilot plant dewatering after the pH had been adjusted to pH 5.5 and the protein precipitated was done in two steps. First by using a drum strainer and then in a decanter.

Saithe is a lean fish, the main lipids are phospholipids that are not easily removed in the process. As shown in **Table 2.1** the lipid content is diminished by around 30%. As stated before, the accuracy of the measurement is not very good when working with low fat content.

Table 2.1. Saithe fillets - Chemical composition through the process.

	Water [%]	Protein [%]	Lipid [%]	Salt [%]	Li/Pr *	Lipid removal [%]	Li/100 g DMNL**
Raw material saithe	79,4	19,3	0,3	0,3	0,02		2
Product – drum	93,8	4,9	0,1		0,02		2
Liquid - drum	99,4	0,4	0,1		0,34		20
Product – decanter	83,2	16,1	0,2		0,01	29 %	1
Liquid- decanter	99,2	0,47	0,13		0,28		19

* Li/Pr = g Lipids / g protein

**Li/100g DMNL = g Lipids / 100 g dry matter no lipids

Herring fillets, both with and without skin, were tried out in the runs in the original setup of the factory. Lipid content is higher in fillets with skin (**Tables 2.2 & 2.3**) since the skinning partly removes the fat layer which is under the skin of the herring. The process removes 77% of lipids in the herring fillets with skin and 60% of the lipid content in the fillets without skin. Even though the lipid removal is different, the final lipid content in the product is similar, around 0.20 g lipids /g protein.

Table 2.2. Herring fillets with skin - Chemical composition through the process.

	Water [%]	Protein [%]	Lipid [%]	Salt [%]	Li/Pr*	Lipid removal	Li/100 g DMNL**
Raw material	69,1	16,4	12,9	0,8	0,79		72
Product - drum	93,3	4,8	1,3		0,28	65 %	24
Product - decanter	86,1	11,4	2,1		0,18	77 %	18
Liquid- decanter	99,3	0,3	0,1		0,42		17

* Li/Pr = g Lipids / g protein

**Li/100g DMNL = g Lipids / 100 g dry matter no lipids

Table 2.3. Herring fillets without skin - Chemical composition through the process.

	Water [%]	Protein [%]	Lipid [%]	Salt [%]	Li/Pr*	Lipid removal	Li/100 g DMNL**
Raw material	75,8	15,3	7,7	0,5	0,50		47
Product - drum	97,6	1,4	0,7		0,49		41
Product - decanter	87,0	10,4	2,1		0,20	60%	19
Liquid- decanter	99,14	0,4	0,3		0,76		54

* Li/Pr = g Lipids / g protein

**Li/100g DMNL = g Lipids / 100 g dry matter no lipids

When the process was run in the lab, much better lipid removal was obtained. In the lab 88% of the lipids were removed and the final lipid content was 0.07 g lipids/g protein, compared to 0.20 g lipids/g protein in the plant.

Table 2.4. Herring fillets without skin - Chemical composition through the process made in the lab.

	Water [%]	Protein [%]	Lipid [%]	Salt [%]	Li/Pr*	Lipid removal	Li/100 g DMNL**
Raw material	72,3	17,0	9,4	0,5	0,56		51
After centrifuge 1	98,1	1,5	0,2		0,13	77 %	12
Product - decanter	77,6	20,9	1,4		0,07	88 %	7

* Li/Pr = g Lipids / g protein

**Li/100g DMNL = g Lipids / 100 g dry matter no lipids

1.2.2. Effect of heat and back pressure

In order to increase the lipid removal in the plant, two methods were used: 1) increase back pressure in the centrifuge, 2) heating. The effects of back pressure can be seen in **Figure 2.10** when the 45°C samples are compared. With back pressure the lipid content is around 10 g lipids / 100 g dry matter no lipids. When back pressure is not applied the value is more than double or over 20 g lipids / 100 g dry matter no lipids. The effect of heat is considerable (Figure 2.10) when linked to the back pressure. In Tables 2.1 to 2.4 back pressure was not applied and the temperature was below 10°C. The lipid content was around 20 g lipids / 100 g dry matter no lipids. Even though the heat of the mass is 45°C the lipid content is similar. It is only when back pressure is applied with heat that the lipid content is diminished. Yield was not measured in those runs but it was apparent that high amount of protein was lost when high back pressure was used and is therefore not

applicable for high yield production. It was therefore necessary to change the set-up of the pilot plant.

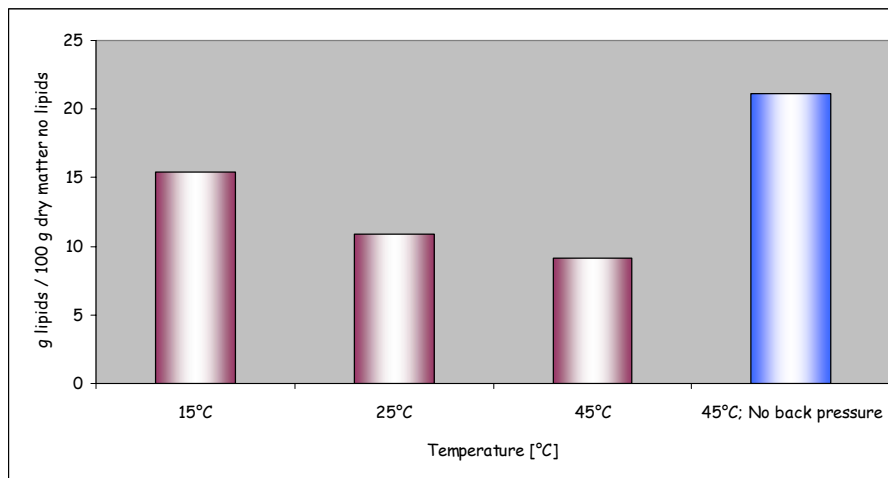


Figure 2.10. Effect of heat and back pressure on lipid content per 100 g dry matter no lipids in herring protein isolate. Sample taken after clarifier before adjustment to isoelectric point. Pilot plant set up 1, acid process.

1.3. Runs in the pilot plant – optimised layout

1.3.1. Introduction

As previously the process involves the following steps (Hultin & Kelleher, 1999; Hultin & Kelleher, 2000b; Hultin & Kelleher, 2002; Kelleher *et al.*, 2002): (i) homogenisation of ground fish in 6-9 volumes of water; (ii) protein solubilization by alkalisation ($\approx pH 11$) or acidification ($\approx pH 3.0$); (iii) removal of lipids and impurities by centrifugation - neutral lipids rise to the top whereas membrane lipids, insoluble proteins, scales, bones and other impurities form a sediment; (iv) removal of the protein containing supernatant followed by iso-electric protein precipitation and; (v) protein recovery and removal of non-protein soluble material by centrifugation.

The overall purpose of the test runs was to try out industrial scale equipment and to modify the process to maximize product volume and quality. A comparison of alkaline and acid processes was made. Some changes to the setup of the factory were made. One of the changes was that in June 2004 an extra plate heat exchanger was added in order to be able to heat/cool the flow-stream before/after fat/solid separation in dish centrifuge.

The following processes and fish types were tested:

1.3.1.1. Runs with Saithe

The purpose of the runs with saithe was to test the gelling properties of the protein concentrate from lean fish and to obtain yield of protein obtained through the process and to estimate losses through decanter liquid outlet and from centrifuge.

Other properties of interest are colour, smell and taste of products. Measurements were made on protein and water content of individual flow streams as well as the elements

calcium (Ca), magnesium (Mg), potassium (K), sodium (Na) and total phosphorus (P_{tot}). The outcome of these runs led to changes in the setup from continuous setup to a batch-wise process to facilitate the control of the process, adding 4 extra holding tanks with stirring and cooling. After modification the following trial set-up were used:

1. Saithe, using batch-wise *alkaline* process. Instead of using the dish centrifuge to remove solids at high pH the decanter was used. Measurements were made on protein- and water content of individual flow streams as well as the elements Ca, Mg, K, Na, P_{tot} .
2. Saithe, using batch-wise *acid* process. Measurements were made on protein- and water content of individual flow streams as well as Ca, Mg, K, Na, P_{tot} .
3. Saithe, *alkaline* process. Prewashed mince test of different cryoprotectors. Evaluation of gel strength as a function of cryoprotectors. Measurements were made on protein- and water content of individual flow streams.
4. Saithe, *acid* process. Prewashed mince test of different cryoprotectors. Evaluation of gel strength as a function of cryoprotectors. Measurements were made on protein- and water content of individual flow streams.

1.3.1.2. *Runs with Blue Whiting*

The purpose of the runs with headed and gutted (H&G) blue whiting was to obtain yield of protein obtained through the process and to estimate losses through decanter liquid outlet and from centrifuge. The secondary purpose of the runs was to try out more difficult raw material in the pilot plant than lean fish fillets, but lower in fat than herring.

Measurements were made on protein- and water content of individual flow streams as well as Ca, Mg, K, Na, P_{tot} . After modification, the following test runs were made:

1. Prewash with 5 parts of water prior to alkaline process. Measurements were made on protein- and water content of individual flow streams.
2. Prewashed twice prior to alkaline process.
3. Prewash with 5 parts of water prior to alkaline process.

1.3.1.3. *Runs with Herring*

The purpose here was to try to separate oil from proteins at different temperatures in centrifuge. For this purpose, hot water was mixed into the alkaline herring flow before centrifugation. The time of mixing was estimated around 15-30 seconds before it entered the centrifuge and was cooled down ≈ 15 seconds to below 10°C after centrifugation. A mixture of EDTA and erythorbate was added in the process water to minimize oxidation of lipids, at the suggestion of Ingrid Undeland at SIK in Sweden. Measurements were made on protein-, water- and fat content in outlet streams and mass balance estimated. Samples for lipid oxidation measurements were sent to SIK in Gothenburg, Sweden.

1.3.2. **Methods**

Analytical methods

The protein concentration was analyzed according to Kjeldahl method and calcium, magnesium, phosphor, potassium, sodium and total sulphur (S) with ICP-AES technique (emission atomic spectrometry with an excitation in the inductively coupled plasma). The

ICP-AES method has wide range of applications due to the possibility of the simultaneous determination of several elements in various materials. This technique allows multi-element analysis at relatively low consumption of the sample to be carried out, which is an additional advantage of the method.

The mass balance flow was estimated according to concentration ratio in different flow streams according to

$$\begin{aligned} & W_i \quad Pr_i \quad Ca_i \quad P_i \quad Mg_i \quad K_i \quad Na_i \quad S_i = \\ & aW_{o_1} \quad aPr_{o_1} \quad aCa_{o_1} \quad aP_{o_1} \quad aMg_{o_1} \quad aK_{o_1} \quad aNa_{o_1} \quad aS_{o_1} \quad (1) \\ + & bW_{o_2} \quad bPr_{o_2} \quad bCa_{o_2} \quad bP_o \quad bMg_o \quad bK_o \quad bNa_o \quad bS_o \end{aligned}$$

Where W is the water concentration in (%), P_i is protein content (%) and other letters refer to elements in mg/kg. The subscript o refers to one outflow streams and i the inflow (where $o_1 + o_2 = i$). A schematic figure of inlet and outlet parameters are shown on **Figure 2.12** with centrifugation as an example process step.

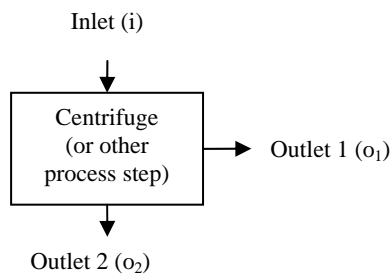


Figure 2.12. Schematic picture showing the inlet and outlet parameters.

The letter a refers to the volumetric ratio of flow stream o_1 and $b = 1 - a$ is the volumetric ratio of flow stream o_2 . The best value for a (and subsequently b) is found by Generalized Reduced Gradient (GRG2) nonlinear optimization method (Solver tool in Excel) by minimizing the sum:

$$\sum \left(\frac{aW_{o_1} - bW_{o_2}}{W_i} \right)^2 \times W_t + \dots + \left(\frac{aS_{o_1} + bS_{o_2}}{S_i} \right)^2 \times W_t \quad (2)$$

Where W_t is a weight factor adjusted according to error limits of measurements.

1.3.3. Results

1.3.3.1. Saithe

Alkaline Process

Skin- and boneless saithe fillets were used in these runs. The alkaline process was uncomplicated, dissolving the muscle proteins worked well and a reasonably clear solution was formed. A lager hold-up tank was installed to make pH adjustments easier.

The pH meters in the flow-line proved to be unreliable, especially at the beginning when chunks of matter, not fully dissolved, tend to cover the electrode. The design has to be altered for a proper functioning. After the correct pH had been reached in the holding tank the protein solutions were sent either to a disc stack centrifuge or a decanter centrifuge. There is no need for oil separation when lean fish such as saithe is used and solid materials are easy to remove.

Therefore, low back pressure was used on disc stack centrifuge when it was used and which can possibly be replaced by a decanter centrifuge. Therefore, runs using decanter for removal of connective tissue were performed as well. Trials with initial cleaning steps (water) were studied as well to observe differences in yield and quality.

Yield in alkaline process

The yield of the alkaline process was obtained by measurements of chemical composition on sub streams. The total analyses of one run of saithe are shown in **Table 2.5**. Samples were taken after mixing through the Stephan microcutter and pH adjustments to pH 10,8 (sample id. D4K21), the sub streams from centrifuge called centrifuge and shot (solid discharges) (id D4K22 and D4K23). The centrifuge steam divides into ‘decanter’ (D4K24) and ‘outlet’ (D4K25) which is the solid and liquid outlet of the decanter.

Table 2.5. Chemical analysis of saithe fillet fractions.

		Water [%]	Protein [%]	Ca [%]	P [%]	Mg [%]	K [%]	Na [%]	S-tot [%]
D4K21	Stephan	97,2	2,6	0,002	0,036	0,005	0,064	0,014	0,035
D4K22	Centrifuge	97,4	2,5	0,002	0,064	0,005	0,063	0,070	0,032
D4K23	Shot	95,8	3,9	0,004	0,036	0,005	0,061	0,068	0,045
D4K24	Decanter	79,1	20,3	0,005	0,085	0,007	0,048	0,054	0,375
D4K25	Outlet	99,0	0,7	0,002	0,029	0,004	0,062	0,069	0,064

The volume ratios were determined according to the minimization of equation 2. The results obtained from the yield estimation through a centrifuge for saithe fillets are presented in **table 2.6**.

Table 2.6. Typical results from mass balance estimations.

			Water [%]	Protein [%]	Ca [%]	P [%]	Mg [%]	K [%]	Na [%]	S-tot [%]
D4K21	Stephan		97,2	2,6	0,002	0,036	0,005	0,064	0,014	0,035
D4K22	Centrifuge	a=	0,906	88,27						
D4K23	Shot	b=	0,094	8,98						
	Target	0,7%		97,25						
				0,1%	1,2%	4,5%	3,7%	7,1%	2,4%	396,9%
	Weight			2	2	0,2	0,2	0,2	2	0
	Recovery				86	83	90	90	91	91
									91	87

Weight factors were chosen using estimated variance in measurement of components. Sodium is omitted since NaOH is added to the solution. As seen, the recovery of protein through the centrifuge is around 86% notably, the recovery of sulphur is similar or 87%, since sulphur is probably mostly found in the amino acids Methionine and Cysteine in proteins.

The overall yield of saithe fillets through the alkaline process can be viewed in **Table 2.7**. Around 64% of the proteins were recovered and around 20% of the calcium and phosphorus remained in the product.

Table 2.7. Saithe fillets - yield through process

	Protein[%]	Ca[%]	P[%]	Mg[%]	K[%]
Yield centrifuge	86,1	82,5	90,1	90	91
Yield decanter	74,5	22,2	22,9	13,4	7,2
Process yield	64,2	18,3	20,7	12,1	6,5

The use of decanter for solid removal at pH 10.8

To test the effects of different removal techniques for solid particles a decanter was employed instead of the plate centrifuge at pH 10.8.

Table 2.8. Saithe fillets - yield through process using decanter

	Protein [%]	Ca [%]	P [%]	K [%]	Na [%]
1 st decantation	86,1	82,5	90,1	90	91
2 nd decantation	74,5	22,2	22,9	13,4	7,2
Overall yield	64,2	18,3	20,7	12,1	6,5

As seen in **Table 2.8** the loss of proteins is much less in the first decantation than in centrifugation shown in **Table 2.7**. The yield through the second decantation was also somewhat higher. The overall yield of protein through the process was 74% which is close to the yield obtained on lab-scale with haddock fillets which gave 76.3% yield in our lab. The quality of removal of connective tissue with this method has to be studied in more detail.

Prewashed alkaline process

Prewash with 3 volumes of water was tried prior to the dissolution at pH 10.8 to improve colour. The resulting yield is presented in **Table 2.9**.

Table 2.9. Protein recovery.

	Protein %yield	Ca %yield
Prewashing	48,6	49,2
Centrifuge (estimate)*	(88)	86,1
Decanting	78,3	79,9
	33,5	33,9

*The yield in centrifuge was not measured and estimated from earlier results to be 88%.

It is obvious that prewashing has a great effect on yield, as expected, since large proportion of the sarcoplasmic proteins seem to be washed away. These proteins must either be adsorbed to myofibrillar proteins or coagulate after the pH 10.8 and back to pH 5.5 shift in unwashed processes.

Acid Solubilization Process

The Acid Solubilization Process, where the pH is lowered to pH 2,7 with H₂SO₄ to solubilize muscular proteins was compared to the alkaline process above (**Table 2.10**).

Table 2.10. Saithe fillets - yield through acid process

	Protein	Ca	P	Mg	K	Na
	% yield					
Recovery centrifuge	59	86	78	90	91	99
Recovery decanter	48	10	13	7	5	6
Total recovery	28	10	13	7	5	6

The yield from centrifuge, as well as the yield from decanter, was less compared to the alkaline process. The overall yield of protein was 28% through the overall acid process.

1.3.3.2. Blue Whiting

Frozen (H&G) blue whiting was used in these runs. There was some uncutted fish in the fish mass, estimated 2-3% of the total. Grinding and microcutting of the material is easy but the mince is somewhat greyish in colour due to skin and especially kidney (blood line). It is highly recommended to remove blood line prior to grinding in the next development stages.

Table 2.11 shows the protein recovery calculation H&G blue whiting through the dish centrifuge. The sample "D5K13, base before cent" shows the composition of the mixture after pH adjustment to pH 10.8 prior to separation in dish centrifuge. The D5K14 and D5K15 show the composition of the liquid after the centrifugation and in the solid discharge of the centrifuge marked as "shots".

The fraction 'a' is the mass ratio from the centrifuge and 'b' the ratio from the solid discharge. As seen in the table, around 89% of the proteins are recovered in this run whereas most of the Ca ions are removed showing removal calcium rich material such as bones.

Table 2.11. Protein recovery from H&G blue whiting through centrifuge.

	Water	Protein	Ca	P	Mg	K	Na	S-tot
	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]
D5K13 Base before cent.	97,2	2,3	0,0252	0,0388	0,0067	0,0511	0,0829	0,299
D5K14 After cent.	97,7	2,1	0,0036	0,0275	0,0062	0,0500	0,0808	0,0277
D5K15 Shot	94,8	3,9	0,3562	0,2072	0,0147	0,0547	0,0897	0,0421
a= 0,94	91,54	1,97	0,0033	0,0258	0,0058	0,0468	0,0757	0,0259
b= 0,06	5,97	0,25	0,0224	0,0131	0,0009	0,0034	0,0057	0,0027
	97,5	2,2	0,0258	0,0388	0,0067	0,0503	0,0814	0,0286
	0,3%	3,8%	2,4%	0,1%	0,2%	1,7%	1,8%	4,3%
	2	2	0,2	0,2	0,2	2	2	0
	93,9%	88,9%	13,0%	66,4%	86,2%	93,1%	93,1%	90,7%

The slurry from disk stack centrifuge is adjusted to pH 5.5. In **Table 2.12**, the composition of the slurry is marked as "D5K16". Similarly, the mass ratio is found and the yield in the solid discharge of the decanter calculated. A little more than half of the proteins were recovered through the solid discharge of the decanter. The remaining liquid was collected and the remaining proteins separated rather quickly into a lower phase that could be further concentrated using micro filtration techniques.

Table 2.12. The yield of blue whiting through decanting step.

		Water [%]	Protein [%]	Ca [%]	P [%]	Mg [%]	K [%]	Na [%]	S-tot [%]
D5K16	Tub pH 5,5	97,9	1,7	0,003	0,024	0,005	0,043	0,074	0,072
D5K17	Decanter	81,3	17,2	0,011	0,078	0,009	0,038	0,067	0,280
D5K18	Outlet	99,0	0,8	0,003	0,020	0,005	0,042	0,074	0,057
a=	0,056	4,6	0,96	0,006	0,0043	0,0005	0,0021	0,0038	0,157
b=	0,944	93,5	0,76	0,0025	0,0191	0,0047	0,0400	0,0695	0,0542
		98,01	1,72	0,003	0,023	0,0052	0,042	0,073	0,0698
		0,1%	1,1%	5,5%	2,6%	1,8%	1,3%	1,3%	2,5%
	Weight	2	2	1	1	1	2	2	0
	Yield	4,6%	56,0%	18,9%	18,5%	10,2%	5,0%	5,1%	22,5%

The overall yield through the process, i.e. from H&G blue whiting to solid discharge of the decanter, is shown in **Table 2.13**. The overall yield of the proteins is around 50%, but as noted above more proteins can be recovered from the decanter effluent using filtration techniques (see Task 3). The physical properties of those proteins need to be studied.

Table 2.13. The yield through total process

	Protein [%]	Ca [%]	P [%]	Mg [%]	K [%]	Na [%]
Total yield	49,9	2,5	12,4	8,8	4,7	4,8

1.3.3.3. Herring

The runs with herring were made with newly frozen, de-skinned butterfly fillets. The pH of minced fillets was 6,5 and the temperature was kept below 6°C. The fillets were ground to around 200 µm size and the mixture was stored in a tank for approximately 45 minutes to allow flotation of fat.

The lower phase containing the proteins was pumped into the decanter and washed mince collected. Chemical compositions of the flow streams were measured and are presented in **Table 2.14**.

Table 2.14. Flotation of herring fillets.

	Protein [%]	Fat [%]	Water [%]	Sum	Fat [% of dry weight]
Raw material + ice, after grinding	15,4	11,6	71,7	98,7	43,0
Liquid from tank	3,8	2,7	92,9	99,4	41,5
Floatable in tank	6,5	10,2	82,5	99,2	61,1
Liquid from decanter	1,5	2,0	95,9	99,4	57,1
Product washed & microcut mince	22,4	3,6	73,5	99,5	13,8

The recovery of the proteins through the process of flotation and decanting of the herring filets was around 67% and 85% of the fat was removed. The washed herring mince was dissolved again in 5 parts water and the pH adjusted to 10.8 in a holding tank. The solution was run through the centrifuge at temperatures below 8°C and sent through a heat exchanger for further cooling after the centrifuge. It proved to be very difficult to apply back pressure to separate fat from the liquid phase. The protein yield from the centrifuge was around 60% (**Table 2.15**).

Table 2.15. Mass balance trough centrifuge.

Mass balance through centrifuge		Protein [%]	Fat [%]	Water [%]
a=	D1606K202 pH 10.8 in tank	1,80	0,20	97,2
	D1606K203 From centrifuge bucket A+C	1,07	0,09	45,03
b=	D1606K205 pH 5.5 tank after centrifuge	0,86	0,16	52,49
Recovery through centrifuge		59%	46%	46%

The liquid phase was pH adjusted to pH 5.5 in a second holding tank and the slurry run through the decanter where 85% of the proteins came out through the solids outlet of the decanter and the resulting product contained 4.5% fat dry matter. The total recovery of the process was rather low or 32%, but most of the fat was removed or more than 95%. The simple floating and decanting in the beginning of the process proved to be most efficient for the removal of fat. Variations of temperature were tested to ease the fat removal within the centrifugal step. The pH 10.8 solution was heated to 25°C by adding 60°C hot water into the solution stream \approx 30 seconds before centrifugation, followed by an immediate cooling to about 8°C after the centrifugation. Back pressure was applied on centrifuge but it was not possible to control the flow in a reasonable manner. This resulted in somewhat lower fat content of product or around 5% but yield and control was not satisfactory.

1.3.4. Discussion

1.3.4.1. *Lean fish*

The process yield of lean fish through the process was satisfactory and the process is useful for cleaning protein from cut-offs, fish frames and from headed and gutted pelagic fish such as blue whiting. The process could possibly even be simplified by replacing the stack centrifuge with a simpler decanter centrifuge since fat does not have to be removed.

The trial runs showed however, that kidney (blood line) and other blood residues have to be removed prior to mincing to decrease greyish colour of the products.

Colour improvements

Methods of rinsing kidney from flesh could imply cleaning of kidney before mincing or filleting (when fish flesh from fish frames is to be used), as is commonly done in the salmon industry.

Gelation strength

As seen in results from Fiskeriforskning, the gelation strength of the products from this process is not as good as from the traditional surimi process. However our results have shown that good gelation strength is occasionally gained. Future steps will involve scrutiny of the gelation process of the muscle proteins and the interaction with pH history, ionic strength and heat profiles.

Occasional good gel strength gives us good hope to unveil the secret of gel formation. As for now, the reduced gel strength and, more importantly, the varying gel properties diminish the market potential compared to traditional surimi. However even though European surimi markets, especially the market in France, have been one of the fastest

growing markets in recent years, price has been relatively low or at 1,3 euro/kg in 2004. The price has been at same levels on the US markets. Although the price of surimi rose somewhat at the beginning of 2005, it seems evident that better value can be obtained using the protein for other purposes such as for injection into filets. For this application, cutoffs and meat from frames of same species or even the use of muscle proteins from cod related fish species such as blue whiting can be used, although blue whiting protein would rather be used in products such as fish blocks or value added products.

Trials have been made on using the muscle protein isolates as injection mass into haddock fillets with good results (Þórarinsdóttir, 2005). This method, i.e. using cutoffs and flesh from frames, seems to be a very attractive method of increasing fillet yield and adding value to blue whiting. The alkaline process has in our tests been used as an initial step in production of protein hydrolysate (not in the scope of this project).

1.3.4.2. *Herring and other fatty fish*

Most of the fat was removed in the initial cleaning process when fat was floated in initial cleaning steps, i.e. the floating of fat and decanting of herring mince prior to pH shift. Twelve percent of the fat was removed with floatation whereas 5% of the proteins were lost and when decanted 75% more fat was removed and the final fat content of the mince after washing was 13% (of dry weight) and recovery of protein 63%. The lost proteins are probably the sarcoplasmic proteins vanishing with the oil/water emulsion.

As mentioned before, it was not possible to separate the emulsion in the stack centrifuge after the pH shift to 10.8, but insoluble proteins were removed. It can be argued that a more simple kind of equipment could be utilized for this purpose, i.e. the removal of solid matter. The final product after pH dropped to 5.5 and decanting gave protein isolate with 4,6% fat (dry weight).

The main conclusions from trials with herring are that fat can not be separated from the emulsion in a stack centrifuge at 6.000g. A better solution would be an initial step where fat/oil and proteins following the emulsion are removed and treated separately for protein and fat recovery.

The remaining proteins can be cleaned from structural proteins using pH shift and decanting or milder steps of separation. Evidently, about 30% of the proteins can be recovered this way. Future research tasks will be the separation of proteins from emulsion, either by the use of higher g-force or series of microfiltration and ultrafiltration steps. The resulting proteins from pH shift treatment are of great interest. They are high in myosin and do not contain stomal proteins and could have good properties for injection into fillets.

This will be tested in our continuing work with fatty fish such as arctic charr and salmon. Approximately 5% of the muscle mass remains on frames and in cutoff material in salmon and charr processing. About 30% can be used in protein injection solutions. With increased water binding capacity around 3-5% weight gain could be expected.

2. Conclusion for Task 2

Apparently, plant works very well and proves good in adjusting pH. However, problems with fat removal remain when working with fatty species since the fat cannot be separated from the emulsion in a stack centrifuge at 6000g. A better solution would be an initial step where fat/oil and proteins, following the emulsion, are removed and treated separately for protein and fat recovery. Future research tasks will be the separation of proteins from emulsion, e.g. by use of series of microfiltration and ultrafiltration steps. Most likely the main use of the Hultin process in a plant production is to isolate proteins for uses like for injection and as raw material for enzyme hydrolysis.

TASK 3 – WASTE WATER

Preliminary trials were made to remove proteins lost in the waste water using ultra filtration. The protein content is low in the water after the decanter or less than 0,5% (**Tables 2.1 to 2.4**). Protein content this low is considered too low for it to be reasonable to recover the proteins by use of ultra filtration.

Proteins are mainly lost in the step where lipids are removed in the clarification step. There water is mixed with proteins, lipids, skin and bones. This part is discussed in Task 4.

1. Conclusion for Task 3

Waste water from the final step of the process includes very low content of chemicals. The use of ultra filtration to recover such a low protein content to use the proteins further is not feasible.

TASK 4 – OIL/BY-PRODUCTS

One of the most promising aspects of using the Hultin process to isolate proteins from herring is the use of the oil part. At the start of the project it was regarded as a new method to recover cold pressed, high quality lipid phase. The conclusion from the project is that the lipid phase is an emulsion when it is recovered from the process. The only method seen to break up this emulsion is to use heat, which is the method used commercially today.

The third phase from the centrifuge is the bottom layer, which contains bones, skin and some lipids. It is reckoned that this phase can be used for production of fishmeal in a commercial fishmeal plant.

1. Conclusion for Task 4

The conclusion of the project is that the lipid phase collected from skimming and after centrifugation is entrapped in an emulsion. With this setup of a pilot plant for the protein isolation from herring process it is not possible to obtain cold processed herring oil.

Other byproducts include skins and bones which can be used as raw material for fishmeal production in a fishmeal plant.

TASK 5 – PROTEIN ISOLATE CHARACTERISTICS & TASK 6 – STORAGE

1. Objectives

The work within tasks 5 and 6 has evolved around the development and prevention of lipid oxidation during the process itself, as well as during subsequent storage of the isolates; mostly on ice. The bulk of the work has been made using isolates made in the labs, in Iceland (IFL), in Massachusetts (UMass Maine Station) and, finally, in Sweden (SIK). The isolates made in Massachusetts were sometimes made using locally caught herring, and sometimes using herring that was shipped frozen from Iceland.

The major background for putting a lot of effort on lipid oxidation in relation to the protein isolation methods, especially the acid one, are listed below:

- ✓ Herring is rich in haemoglobin (Hb) -Hb is one of the most potent catalysts of lipid oxidation
- ✓ When herring is minced, Hb gets mixed with lipids and other proteins
- ✓ Hb is activated at low pH, i.e. reduced deoxy-Hb gets deoxygenated and oxidized into the more catalytic met-Hb form
- ✓ Lipid oxidation destroys sensory properties and also gelation properties of the isolates.

2. Materials and Methods

Figure 5.1 gives a brief description of the setup used to test storage-induced lipid oxidation. A portion (20-25 g) of isolate was flattened out in the bottom of 250 mL Erlenmeyer flasks. The flasks were then stored on ice for up to 17 days. Changes in odour intensity (mostly ocean odour, painty odour, fishiness and mineral odour) were measured by uncapping the bottles and sniffing the head space. A line scale ranging from 0-100 or 0-10 was used, with 100 or 10 being the strongest. In the initial documentation trials (**Figures 5.2-5.6**), a small internal sensory panel was used. In the two big trials, when testing the effect of process variations and antioxidants as well as when testing the stability of pilot-made isolates (**Figures 5.10 & 5.13-17**) SIK's trained external sensory panel was used. Small sample plugs (0.5-1g) were also taken out from the flasks for TBARS-analyses. Colour (L, a, b values) of isolates was measured by pressing a Minolta colorimeter probe towards the bottom of the flasks. A more extensive description of the methodology is given in Undeland *et al.* (2005).

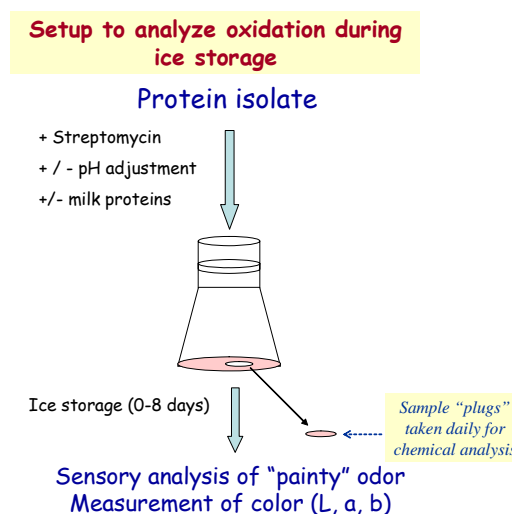


Figure 5.1. Experimental setup used to follow lipid oxidation in herring protein isolates by chemical and sensory analysis. The chemical analyses consisted of TBARS, and the sensory analyses were intensity judgement of painty, fishy, mineral and ocean odours.

3. Results

3.1. Documentation of lipid oxidation during processing and storage

In order to screen for the levels and kinetics of lipid oxidation during, and after, the acid and alkaline processes, some initial ice- and frozen storage trials were made in 2002 using lab-made isolates from pre-frozen Icelandic herring, pre-frozen Swedish herring and fresh Swedish herring. The different experiments are summarized below:

- ✓ Lipid oxidation (painty odour) during ice storage of acid- and alkali-made herring protein isolates made at UMass Marine station (Icelandic herring) (**Figure 5.2**)
- ✓ Lipid oxidation (TBARS) during frozen storage of acid- and alkali-made herring protein isolates made at UMass (Icelandic herring) (**Figure 5.3**)
- ✓ Lipid oxidation during ice storage of an acid-made herring protein isolate made at IFL (Swedish herring) (**Figure 5.4**)
 - *pH during the storage was varied*
- ✓ Lipid oxidation during ice storage of saithe protein isolates made in the pilot plant at HB (**Figure 5.5**)
 - *+/- high speed spin (10,000g, 20 min)*
- ✓ Lipid oxidation during ice storage of herring protein isolates made at SIK (**Figure 5.6**)
 - *+/- high speed spin (18,000g, 20 min)*
 - *+/- addition of 4% milk proteins*

Figure 5.2 shows the development of painty odour in acid- and alkali-made isolates from pre-frozen Icelandic herring during ice storage. Both acid and alkali made isolates started off at fairly low painty odour intensities, but increased rapidly. Somewhat higher intensities were reached for the acid made isolates. These contained 1.7% lipids (w/w) as compared to the alkali-made ones that contained 0.7%.

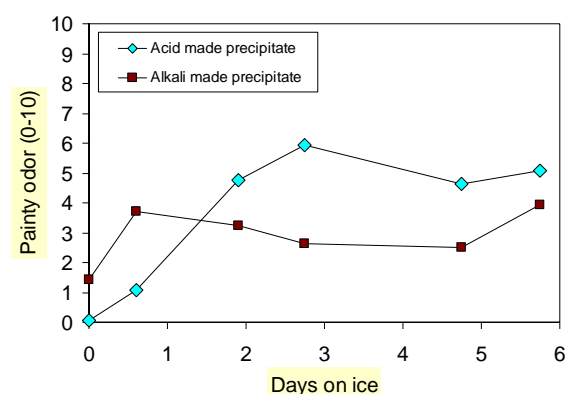


Figure 5.2. Development of painty odour during ice storage of lab-made acid and alkali processed isolates from pre-frozen Icelandic skinned d herring fillets.

In a frozen storage trial, thiobarbituric acid reactive substances (TBARS) were followed over time in isolates made with the acid and alkaline processes from pre-frozen, skinned Icelandic herring fillets. It is clearly seen in **Figure 5.3** that the raw materials used, i.e. the

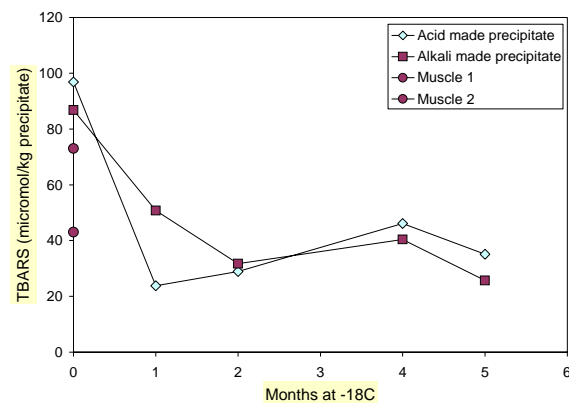


Figure 5.3. TBARS development during frozen storage of lab-made acid- and alkali-processed isolates from pre-frozen skinned Icelandic herring fillets.

muscles, were oxidized already before the processing started, and that the high level of oxidation products (TBARS), increased further during the two processes. Thus, oxidation appeared to have “peaked” already before the frozen storage started. Therefore, only a decline was detected during the actual storage. This kind of decline is commonly seen for TBARS, as the oxidation products (so called secondary products) tend to react further, e.g. with proteins.

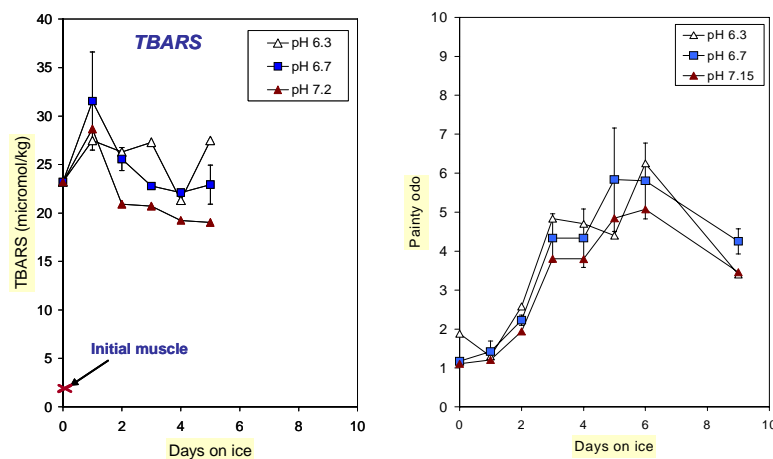


Figure 5.4. Development of TBARS and pinty odour during ice storage of acid-made isolates after adjustment to three different pH-values.

High levels of lipid oxidation products were formed during the acid aided production of herring protein isolates (Figure 5.4). TBARS values increased from about 2 $\mu\text{mol/kg}$ of mince to about 25 $\mu\text{mol/kg}$ of final protein isolate. During ice storage of these protein isolates, there were only small further increases in TBARS; rather, the levels of TBARS very soon started to decline. This indicated the limitation in using TBARS as an oxidation index during storage of acid-produced herring proteins as long as oxidation is not minimized during the actual process.

Sensory analysis of the isolates during ice storage showed that the intensity of painty odour increased rapidly between two and six days after manufacturing. There were also some changes in “old fish” odour. Furthermore, the protein isolates had a fairly sharp “boiled” odour that made the assessment of other attributes more difficult.

Small, but insignificant differences in oxidative stability were seen between isolates adjusted to different pH-values. The development of oxidation declined in the following order: pH 6.3 > 6.7 > 7.2. The likely explanation is the effect of pH on activation of Hb as a pro-oxidant. All isolates had 80% moisture.

From analyses of colour (data not shown), it was found that lightness (L) increased during ice storage while redness (a-value), decreased. The former might be due to protein denaturation and the latter to formation of met-Hb/met-myoglobin. Blueness (b-value) remained almost unchanged. The results indicate that analysis of L- and a-values might be a quick and useful means of following storage induced oxidative changes in the protein isolates, but more research is needed.

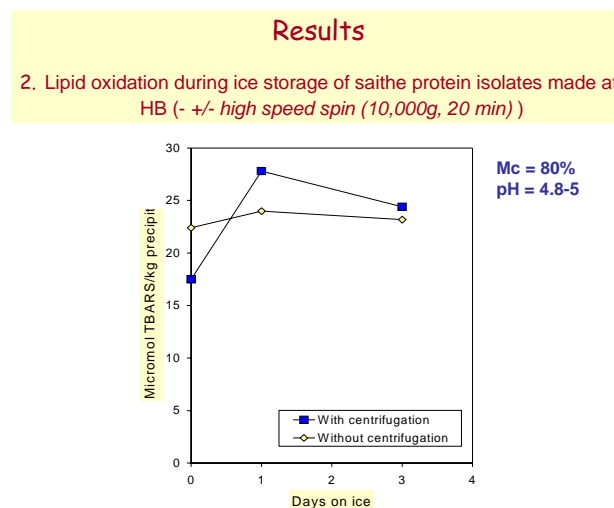


Figure 5.5. TBARS changes during ice-storage of acid Saithe isolates made in the pilot plant with and without a high-speed centrifugation at 10 000g.

Saithe protein isolates, manufactured in the pilot plant at HB, were shipped to SIK for analysis of oxidative changes during ice storage. The isolates were made with and without a “high speed centrifugation” (10, 000 g), with the intention to see how this step affected oxidation. Prior to storage, the isolates were adjusted to 80% moisture with distilled water, while the pH was left unadjusted at 4.8-5. Initial TBARS-values were 17 and 22 $\mu\text{mol/kg}$ in isolates made with and without a high speed centrifugation, respectively. As shown in **Figure 5.5**, TBARS only increased during the first day on ice, more so in the non-centrifuged sample. TBARS values then fell in both samples. Thus, in this study, there was no obvious beneficial effect from a high speed centrifugation.

In a fifth trial, herring protein isolates were prepared at SIK, with and without the inclusion of a high-speed (18, 000g) centrifugation. The isolates were then stored on ice, with and without the addition of 4% (w/w) milk proteins. The latter have been described as effective “traps” of aldehydes. Painty odour developed slightly slower in samples

subjected to a high-speed centrifugation and by the inclusion of milk proteins (**Figure 5.6**). In the centrifuged sample with added milk-proteins, no paintiness had developed after four days, but the sample fairly quickly became bacterially spoiled. In addition, L-values were higher, and a- and b-values lower in samples with milk proteins added. The inclusion of a centrifugation largely reduced a- and b-values; probably due to heme and melanin removal.

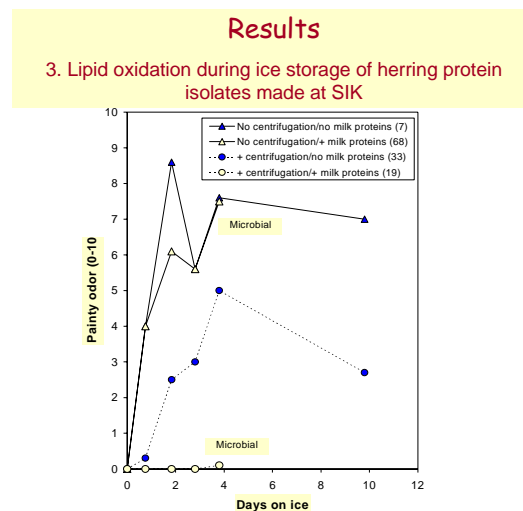


Figure 5.6. Painty odour development during ice-storage of acid produced isolates with and without a high-speed centrifugation at 18 000g, 20 min and with/without Alapro milk proteins (4%).

3.2. Strategies to prevent lipid oxidation during process and storage

Based on the results shown above in **Figures 5.2-6**, indicating that fairly extensive lipid oxidation develop during both acid and alkaline protein isolation from herring, strategies have been tested to reduce the development of lipid oxidation during the process and subsequent storage. These strategies included ¹)pre-washing to remove as much as possible of the pro-oxidative haemoglobin (Hb), ²)shorter exposure to extreme pH-values, ³)inclusion/exclusion of high-speed centrifugation and ⁴)addition of water soluble antioxidants at different steps of the process. Regarding additions of antioxidants, it is known to be difficult to simultaneously inhibit oxidation catalyzed by LMW-Fe²⁺ and Hb-Fe³⁺. One way which has been described is to reduce Hb-Fe³⁺ e.g. by ascorbic acid or erythorbate, while chelating LMW-Fe²⁺ e.g. by EDTA and STPP. For further protection, these strategies can be combined with addition of radical scavengers in the aqueous and/or lipid phase. In production of "regular surimi" it has been found (Kelleher *et al.*, 1992, Richards *et al.*, 1998) that water soluble antioxidants must be added early, i.e. in the mincing step or washing step, for having an effect on oxidation. Therefore, we tested to add antioxidants both in a pre-wash and homogenization.

3.2.1. Hb-removal via pre-washing

As one strategy to try and reduce the development of lipid oxidation during protein isolation, we tried to reduce the amount of pro-oxidative haemoglobin (Hb) during a pre-wash of the mince. In these studies, we worked after the hypothesis that Hb is better removed at physiological conditions (i.e. 150 mM, pH 7) as this would prevent red cell lysis and e.g. adhesion of heme to the unpolar membranes. In these experiments, we also wanted to maximize Hb-removal, while minimizing loss of other sarcoplasmic proteins.

We therefore tested different combinations of ionic strength and pH using factorial designs. Two trials were made, one with pre-frozen herring mince (**Table 5.1**), and one with fresh herring mince (**Table 5.2**). In both trials, the washes were made at the ratio 1:3 (herring: solution). The ionic strength was varied from 29 mM NaCl (water) to 150 mM NaCl. The pH was adjusted from the natural 6.5 or 7 up to 7.5. The results were evaluated with MLR (multiple linear regression) using the Modde® software.

Table 5.1. Factorial trial to evaluate how the removal of hemoglobin (Hb) from herring mince in a pre-wash was affected by pH and ionic strength (IS) of the pre-wash solution. The herring raw material had been frozen.

**Factorial design frozen herring mince
(herring caught in june, 2002)**

Washes: 50 g mince stirred 1 min with 150 mL washing solution
→ 15 min settling → washed mince collected in sieve

Hb (muscle): 8 µM ?? Total protein (muscle): 20.9%

NaCl	pH	Hb-removal (%)	Total protein-removal (%)
29	6.5	70	12.7
150	6.5	76	15.2
29	7.5	83.7	15.3
150	7.5	92.9	16.5
29	7	69.9	15
75	7	72.2	17.2
75	7	63.8	16.2
75	7	71.6	16.8

Table 5.2. Factorial trial to evaluate how the removal of hemoglobin (Hb) from herring mince in a pre-wash was affected by pH and ionic strength (IS) of the pre-wash solution. The herring raw material was fresh.

**Factorial design fresh herring mince
(herring caught 7th of october, 2002)**

Washes: 50 g mince stirred 1 min with 150 mL washing solution
→ 15 min settling → washed mince collected in sieve

Hb (muscle): 14.5 µM (20.8 µM) Total protein (muscle): 23%

Salt (mM)	pH	Hb-removal (%)	Total Protein removal (%)
29	7	54.6	15.14
150	7	48.4	14.05
29	7.5	51.2	13
150	7.5	52.2	16
75	7.25	52.3	15.4
75	7.25	62.3	15.5
75	7.25	59.0	15.3
75	7.25	59.7	14.6

Water! →

In short, greater effects from the studied variables were seen when using frozen herring mince. The variation in Hb-removal was here from 63-93% of the initial. The reason why frozen herring “responded” more to variations in the pre-wash could be that the Hb was harder “bound” in frozen material, and thus, that some extra help was needed to solubilize Hb. The most Hb (93%) was removed with 150 mM NaCl and pH 7.5 in the pre-wash. In the study on fresh herring, very little differences were seen between treatments and Hb-removal was only 48-62%. Following statistical evaluation of the experimental data, none

of the variables (pH or IS) were shown to be significantly affecting the Hb-removal or total protein removal. There was, however, a tendency that pH had larger effect on Hb-removal than NaCl addition. To conclude, variations in the pre-wash had only little effects on Hb-removal from herring having an initial muscle pH over 6.5. Previous studies have shown that the largest gain in adjusting the pH/salt of a pre-wash is obtained with muscle having a low initial pH (around 6).

We also investigated the effect of changing the volume of wash solution (100 instead of 150 mL/50 g mince) and the number of washes (2 instead of 1) (results not shown). Three volumes of wash solution instead of two removed about 20% more Hb. A second wash improved the Hb-removal by 35%; e.g. from 60% to 80%. With one 1:3 wash, 13-16% of the total proteins were removed.

3.2.2. Variations in the process settings and additions of antioxidants

For the purpose of making a larger systematic evaluation of the possibilities to reduce oxidation via process-variations and antioxidant-additions, we collected a large batch of herring caught in Kattegat in November 2002. The herring was filleted, skinned, minced and frozen in 120 g portions at -80°C. Such portions were then used for each of the subsequent protein isolation trials carried out on a lab-scale. To confirm that the mince remained stable at -80°C, the TBARS content of the mince was regularly checked. It was found that in the period November 2002 to June 2004, it had only increased from 8 to 13 µM/kg. **Figure 5.7** shows the basic process settings used in this large evaluation.

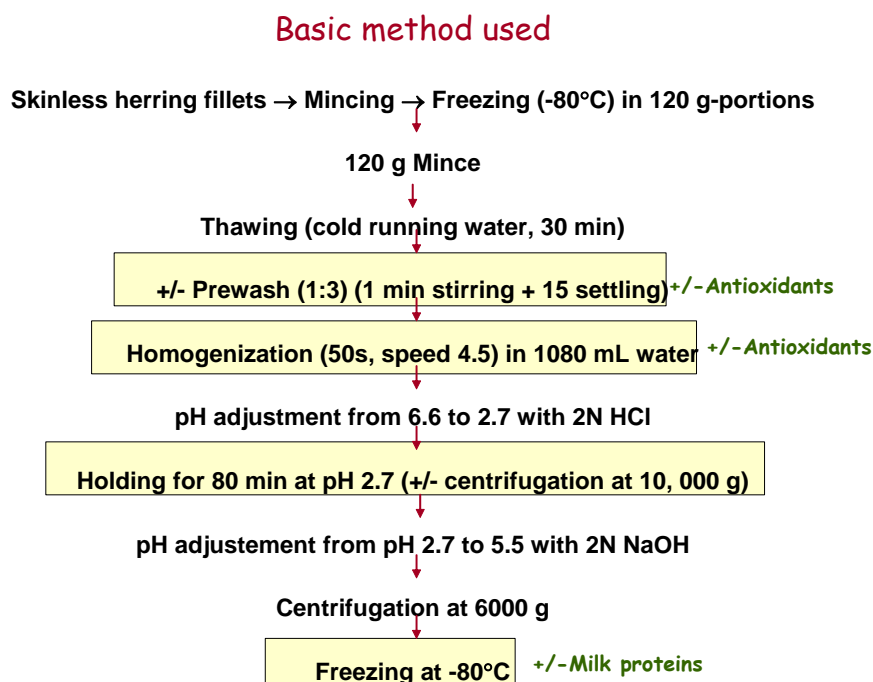


Figure 5.7. Basic process used to produce herring protein isolates on lab-scale in order to test how process-variations and antioxidants affect development of lipid oxidation.

The protein isolates that SIK produced from the raw material caught in November 2002 are summarized in **Table 5.3**. This table also show the pH, moisture and TBARS values of the isolates obtained.

Table 5.3. The 17 protein precipitates produced and ice stored in study 5. The raw material was minced skinned herring fillets with a pH of 6.6, 72% moisture and 8 μmol MDA/kg. This table shows the pH, moisture content (Mc), weight and TBARS-values of the 17 protein precipitates right after processing. Abbreviations used: Ao=antioxidants, HS=high speed centrifugation, Min.=minutes, +ao=0.2% erythorbate and STPP.

#	Pre-wash	Ao in Homogenization	HS Centr	Time at pH 2.7 [min]	Milk prot.	pH	Mc [%]	TBARS [μmol MDA/kg]
1	No	No	No	75		4.6	91	40
2	No	No	Yes	75		4.7	87	25
3	Yes	No	No	75		4.8	88	42
4	Yes	No	Yes	75		4.7	91	24
5	Yes (+ao)	Ery+STPP	No	75		4.9	76.5	5
6	Yes (+ao)	No	No	75		4.2	87	3
7	No	Ery+STPP	No	75		4.8	80	5
8	No	Ery+STPP	Yes	75		4.6	90	6
9	No	Ery	No	75		4.3	87	10
10	No	Ery+EDTA	Yes	75		4.7	90	5
11	No	Ery+EDTA	No	75		4.4	86	7
12	No	Ery+STPP*	Yes	75		5	87	9
13	No	No	No	4		4.5	84	56
14	No	No	No	30		4.7	85	62
15	No	No	No	75		4.6	86	50
16	No	No	No	75	Yes	4.6	83	56
17	No	Ery+EDTA	No	75	Yes	4.4	83	7

*STPP was added after the high-speed centrifugation.

Just as in the trials shown in **Tables 5.1** and **5.2**, the pre-wash of the mince was done by stirring/leaching the herring mince with 3 volumes of ice-cold distilled water or antioxidant solution. Both in the pre-wash and in the homogenization step, antioxidant solutions (erythorbate (Ery), STPP, EDTA) were made to give 0.2% of each antioxidant in the moisture fraction of the muscle. High speed (HS) centrifugation of solubilized proteins was done for 20 min at 10,000 \times g. In samples where the high-speed centrifugation was excluded, the homogenate was instead held for 75 min at the low/high pH (i.e., time required for the centrifugation and filtering steps to be completed). In some experiments, the exposure time to pH 2.7 was shortened to 30 and 4 min. All precipitates were frozen at -80°C until the start of the ice storage trial. Then, in order to make the 17 protein isolates comparable during the ice storage, they were all adjusted to 91% moisture and pH 6.55 after thawing. Streptomycin (200 ppm on a moisture basis) was added manually to prevent bacterial growth. Four percent milk proteins (Alapro) were added to two of the protein isolates (#16 & 17, **Table 5.3**). Ice storage and analyses were done as previously described.

Based on analyses of TBARS in muscle, washed muscle and in the different final protein isolates, it was again observed that TBARS increases drastically under the acid-aided process; from 10 to 40 $\mu\text{mol}/\text{kg}$ (**Table 5.3** and **Figure 5.8**). However, the addition of erythorbate and STPP in the 1:3 pre-wash or in the homogenization step prevented the rise in TBARS (**Figure 5.9**). No further improvement was seen after adding antioxidants in both pre-wash and homogenization. Erythorbate alone, added in the homogenization, also prevented oxidation, but not as efficiently as erythorbate plus STPP. It should be stressed that 0.2% STPP largely reduced the protein solubility at pH 2.7 and an addition is not useful together with a “high-speed” centrifugation. Most proteins will then end up in the sediment and will be lost.

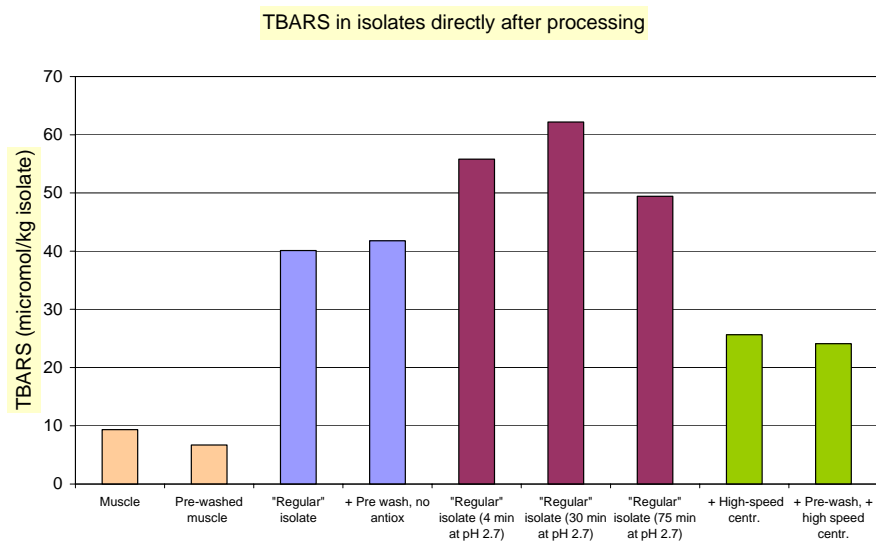


Figure 5.8. TBARS in the different isolates made without antioxidant additions.

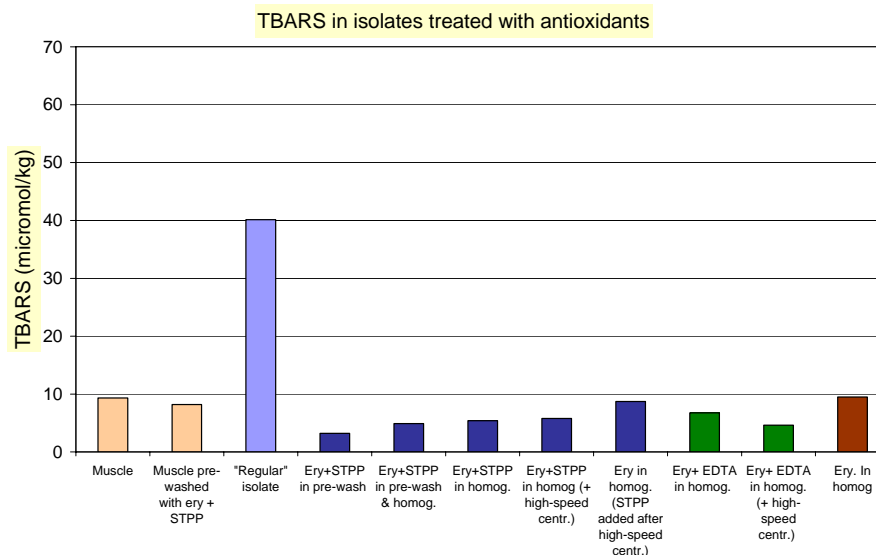


Figure 5.9. TBARS in the different isolates made with antioxidant additions.

From an oxidation perspective, inclusion of a high speed centrifugation reduced the TBARS values of the isolates by about 35%. The lipid removal in the centrifugation was 42-44%. However, when antioxidants were added, no effect from a centrifugation could be seen due to the already low TBARS values.

Here we report briefly how oxidation developed during the ice storage of the isolates. For further details, see the TAFT proceedings.

Figures 5.10-11 show how TBARS developed during ice-storage of the different isolates. **Figure 5.10** illustrates how four of the antioxidant-fortified isolates initially had low painty odour values (<20) and stayed below 20 throughout 17 days on ice. Three of these were treated with Ery and EDTA while one sample had STPP instead of EDTA. Samples with Ery and STPP added both in a pre-wash and homogenization, as well as the sample where STPP was added *after* the high-speed centrifugation, stayed below an intensity of

20 for 9 days on ice, but then rapidly increased. Samples where Ery alone had been added in the homogenization step, and where Ery and STPP had been added in the pre-wash but not in the homogenization, started at paintiness values around 30, and then immediately started to increase. Thus, EDTA seemed to be a more antioxidative chelator than STPP, and Ery alone was not as efficient as when combined with a chelator. Although antioxidant additions in the pre-wash step alone was enough to obtain good stabilization during the process, it did not yield an isolate with good storage stability.

Precipitates with no antioxidants added had initial paintiness values between 20 and 50. The higher values were for “regular” precipitates that were not subjected to a high speed centrifugation. In these samples, no further changes were seen during the storage, while the painty odour intensity increased in precipitates with lower initial values. This indicates that the non-centrifuged samples had most likely reached their “peak” in painty odour before the storage trial started. The data also show that the high speed centrifugation reduces, but does not eliminate, lipid oxidation during short term storage. The highest painty odour intensity detected, 70, was found in the precipitate made from pre-washed mince without the

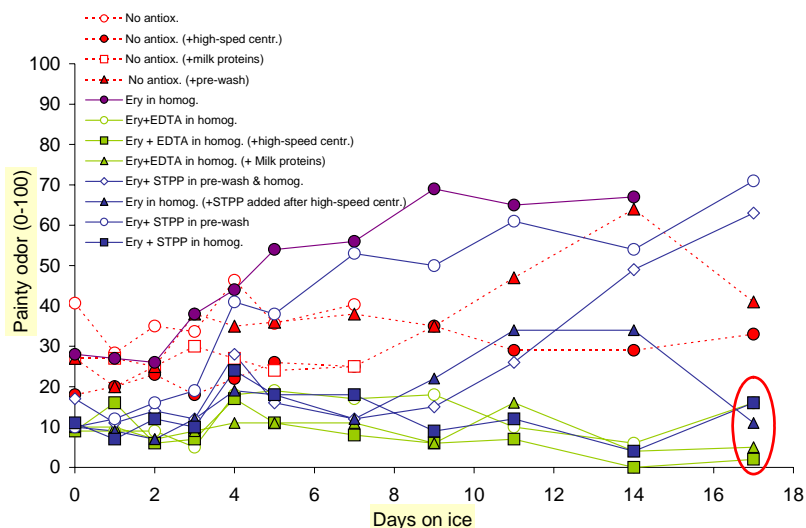


Figure 5.10. Development of painty odour (0-100) in different herring protein precipitates during storage on ice. Details on their production is shown in Table 5.3.

use of a high-speed centrifugation. This could indicate that the net-effect of a water-wash was dominated by removal of natural antioxidants rather than removal of pro-oxidative heme-proteins (Undeland *et al.*, 2003). Four percent milk proteins were added to one antioxidant-stabilized protein precipitate (0.2% Ery plus EDTA, no high-speed centrifugation), and one non-stabilized precipitate (no pre-wash, no high-speed centrifugation). There was no detectable effect from the milk proteins on painty odour in these samples. It has been reported previously that milk proteins can act both as radical scavengers and as sinks for volatile oxidation products. We will evaluate this capacity further using samples that initially are low in oxidation products, but that tend to oxidize during the storage. The samples selected here were either “already oxidized” or did not oxidize at all. There was no difference in painty odour development during storage whether the exposure time to pH 2.7 was 4, 30 or 75 min (data not shown).

The TBARS data more or less confirmed the data on painty odour (**Figure 5.11**). Samples without antioxidants had fairly high TBARS from start, and were not followed longer than seven days. Two samples (Ery added alone in homogenization and Ery+STPP added in only pre-wash) stayed stable during processing, but developed TBARS quickly during storage. Ery+STPP added in pre-wash and homogenization or in homogenization alone held back TBARS until the 7th day. The only samples not developing any TBARS at all were those with Ery + EDTA. This strengthens that EDTA was the most efficient chelator.

Regarding colour data, in short, samples subjected to high speed centrifugation initially had lower a-values (redness), most likely connected to removal of pigments (Hb and melanin) into the sediment. All samples decreased to some extent in a-value during storage. L-values (lightness) initially varied between 45-60; the higher values in pre-washed samples. There were only small changes during storage of the precipitates. B-values (yellowness) were lowest in samples subjected to high-speed centrifugation, again indicating pigment removal in this step. Yellowness only changed in one of the samples during storage; the one with erythorbate added alone.

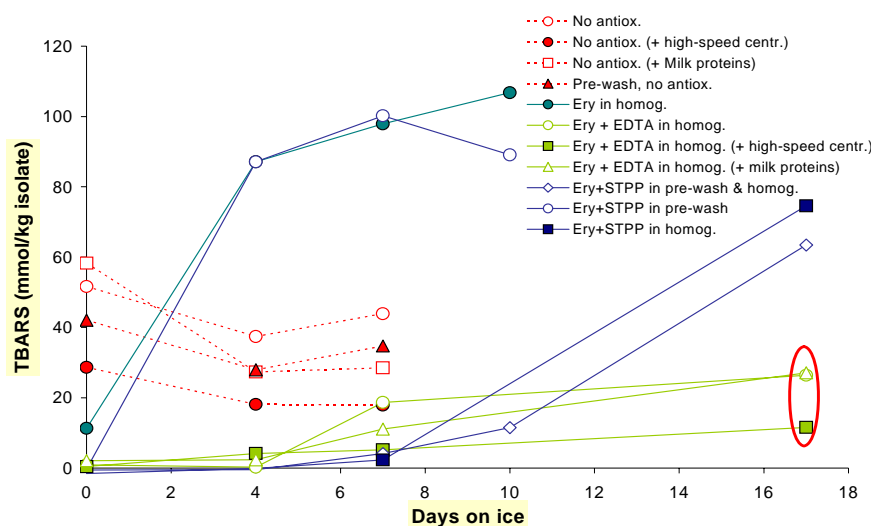


Figure 5.11. Development of TBARS in different herring protein precipitates during storage on ice. Details on their production is shown in Table 5.3.

4. Conclusions for Task 5 & 6

It was shown repeatedly, both on lab-scale and pilot-scale, that extensive lipid oxidation developed during acid and alkaline protein isolation from herring. For this reason, there were usually no further changes in lipid oxidation products during the subsequent storage on ice or at -18°C. Sometimes, the levels of the measured oxidation products even decreased during ice storage. From a long series of trials, the following was found to affect the development of lipid oxidation during the actual process:

- High-speed centrifugation at 10,000g or 18,000g reduced the level of oxidation products by 40-50%.
- Addition of a metal reducing agent (Ery) and a metal chelator (STPP or EDTA) in a 1:3 pre-wash, in the homogenization or both clearly reduced oxidation.

The following did not affect lipid oxidation:

- Pre-washing the mince (1:3) in water
- Reducing the exposure time at extreme pH-values from 75 to 4 min.
- Shifting from acid to alkaline processing

During ice storage of the final isolates, the following was found to reduce lipid oxidation:

- Addition of antioxidants (erythorbate and STPP/EDTA) in the homogenization step (+/- addition also in pre-washing), EDTA here seemed more efficient than STPP.
- Increasing the pH to >7 had a very small stabilizing effect when the lipids of the isolate were already oxidized.
- Adding four percent milk proteins in some trials seemed to reduce painty odour.

The sensory parameter that seemed to correlate most clearly with the chemical measure of oxidation (TBARS) during ice storage was painty odour. Among the other attributes analyzed, ocean odour declined in the isolates, mineral odour stayed pretty stable, and fishy odour increased. However, these last three attributes in general did not differentiate the samples. Most of these results are published in Undeland *et al.*, 2005.

From factorial trials with the use of a 1-3 pre-wash to remove Hb prior to processing it was found that variations in pH and ionic strength of the pre-washing solution did not significantly affect removal of Hb or removal of total proteins. A pre-wash without antioxidants did, according to the above, prevent oxidation.

TASK 7 – DEHYDRATION

1. Production

1.1. Introduction

Rising energy costs and concerns about the environmental impacts of fossil and other fuels used in industrial processing of fish have pushed for the need to develop new technology. This is particularly important in drying process, since it utilizes up to 50% of the total energy consumption in certain processing industries. New drying technology, such as heat pump drying (HPD) can be combined with operating strategies to produce high quality powders while reducing the net energy per unit of water removed (Alves-Filho & Mujumdar, 2002). Heat pumps are highly efficient modern technology in order to reduce energy consumption during heating and cooling, as e.g. in drying. When properly built, it is among the most thermally efficient dryers. This is associated with dryer energy utilization, which is dictated by the specific moisture extraction ratio (SMER) expressed in kg of water per kWh. The SMER depends upon the evaporating and condensing temperatures as well as in the dryer thermal efficiency and given by:

$$SMER = COP \frac{\Delta x}{\Delta h} \quad (1)$$

Alves-Filho & Mujumdar (2004) report that an adiabatic fluidized bed heat pump dryer can operate with SMER between 2 and 3kg/kWh or higher. As shown in **Tables 7.1 & 7.2**, the heat pump fluidized bed dryers perform 278 to 694% more efficiently than the conventional fluid-bed dryer and much higher than the other conventional dryers.

Fish processing is an important economical sector in the Nordic countries, but catches are restricted to specific fishing periods as well as restrictive quotas. Also, losses of valuable fish components occur in processing where large amounts of residues and by-products are discarded, causing pollution. Drying allows the recovery of fish by-products and to stabilize fish material which is often easily degradable due to lipid oxidation, even when refrigerated.

Low air drying temperatures reduce lipid oxidation in fish processing and significantly improve the quality of the final product. Furthermore, low temperature enhances other properties of the dried fish, such as colour and rehydration ability. Heat pump dryers have a wide interval of process temperature (-20 to 100°C), which is suitable for processing heat sensitive materials at atmospheric freeze conditions. Furthermore, the heat pump is the most energy efficient method to supply heating and cooling during drying. The main disadvantage of stationary atmospheric freeze drying is a low throughput. However, this process may be improved by using fluidized bed that promotes excellent contact between suspended particles and air, with subsequent higher rates of heat and mass transfer.

Protein isolate particles are highly heat-sensitive materials and quality properties considerations also place additional constraints on suitable dryers and operation schemes.

Isolated protein powders were produced by using the HPD in multiple energy input modes through changes in drying conditions, and by geometrical changes of the raw- and the drying chambers. Fresh fish material, like protein isolates, degrades relative quickly compared with a dry product.

This part of the report describes the new heat pump drying technology and the strategies established to adjust the operating conditions and preparation of the raw protein material prior to drying. The heat pump drying in multi-stage that includes drying at sub-zero temperatures under atmospheric conditions has shown to produce protein powder as an alternative to freshly isolated protein.

The granulation and sieving procedures were identified prior to drying. A heat pump dryer operating in fluidized bed mode was used after establishing the drying conditions. Fish protein powder was produced and all samples had low moisture content and low water activity that allows for a longer and safer storage at nearly atmospheric conditions. Considering that the isolated protein is a heat sensitive material, the experiments were designed and performed focusing on the following objectives:

- ✓ To identify the drying mode: stationary or agitated
- ✓ To establish the conditions for properly drying the protein: atmospheric below-above freezing temperatures
- ✓ To obtain the drying kinetics for the drying process at different sizes and conditions.
- ✓ To measure the relevant powder and gel properties: colour, enthalpy, freezing points, water activity, rehydration, density and rheological characteristics.

Twelve isolated protein powder samples were successfully produced using heat pump drying technology combined with step-up temperature, granulation and sieving processes. The protein powders were produced with controlled or pre-specified characteristics, as indicated by the results on thermal or physical properties. This provides the benefit that fish protein, which is usually seasonal, may eventually be supplied as dried powder all year round. Besides producing protein powder with adjustable characteristics there is the additional benefit that heat pump drying is an environmentally friendly technology which reduces energy utilization compared to conventional counterparts.

The overall experimental conditions and measured data are listed in detail in **Tables 7.3 & 7.5** and the results are plotted in **Figures 7.2 to 7.22**.

Table 7.1. Commercial dryers with range of energy utilization and specific moisture extraction ratio

Conventional dryer	Minimum energy [kJ/kg _w]	Maximum energy [kJ/kg _w]	Average SMER [kg _w /kWh]
Vacuum freeze	45000	115000	0.08
Spray	4500	11500	0.45
Rotary	4600	9200	0.52
Tunnel	5500	6000	0.63
Band	4000	6000	0.72
Fluid bed	4000	6000	0.72

From Alves-Filho & Mujumdar, 2002.

Table 7.2. The heat pump dryers and energy use compared to convention fluidized bed dryers

Emerging technologies	Fluid bed dryer ratio: new to conventional	SMER [kg _w /kWh]	Energy utilization [kJ/kg _w]
Adiabatic fluid bed heat pump dryer	2.78 to 4.17	2 to 3	1800 to 1200
Non-adiabatic fluid bed heat pump dryer	4.17 to 6.94	3 to 5	1200 to 720
Superheated steam dryer	3.33 to 5.00	2.4 to 3.6	1500 to 1000

From Alves-Filho & Mujumdar, 2004.

1.2. Freeze granulation and sieving

The frozen protein temperature was kept below its initial freezing point prior to granulation. Using a progressively perforated rotary grater the protein samples were granulated into shapes that were nearly spherical. Using a sieve combination of screens, a top 3 mm and a bottom 1.5 mm, allowed to select particles with size average of about 2 mm, top 5 mm and a bottom 3.5 mm for selection of particles with size about 4 mm and top 8 mm and a bottom 6.5 mm provided particles with size of 8 mm. Three large sample batches were made with particles of similar size distribution. The batches were conditioned and kept at -25°C prior to the drying tests.

Table 7.3. Heat pump fluidized bed drying conditions and experimental data for 12 protein runs.

No.	Sample label	T [$^{\circ}\text{C}$]	Size [mm]	V [L]	Ra [%]	Mo [%]	Mf [%]
1	Phnc	-10	2	2	1.26	72.18	8.61
2	Phnc	-10/+20	2	2	1.01	73.17	5.87
3	Phnc	-5	2	2	1.11	72.18	7.08
4	Phnc	-5/+20	2	2	1.36	73.67	4.91
5	Phnc	0/+20	2	2	1.29	71.77	5.39
6	Prh75%	-5/+20	2	2	1.18	72.00	5.17
7	Phnc	-5/+20	2	3.5	1.21	72.53	6.28
8	Phnc	-5/+20	2	3.5	1.12	75.49	6.38
9	Phnc	-5/+20	2	4	1.62	71.91	5.60
10	Phnc	-5/+20	2	6	1.25	71.40	5.57
11	Phnc	-5/+20	4	2	1.59	71.38	5.28
12	Phnc	-5/+20	8	2	1.28	72.00	12.32

1.3. Low temperature heat pump dryer

A heat pump dryer operates using heat exchangers to transfer energy from a low temperature source to a higher temperature source. This is done by using a throttling valve and a compressor to establish the necessary pressure or temperature differences for transfer in the main heat exchangers. The main components of the heat pump dryer are the compressor, three-way valve, condenser, receiver, expansion valve, evaporator, external condenser, blower, drying chamber and the cyclone. After loading the solids into the drying chamber the blower is activated and rotation adjusted for proper air velocity and solids fluidization. Higher quality powders can be obtained using low temperature and high drying rates attained by combining step-up temperature in a properly designed heat pump dryer. **Figure 7.1** shows a lay-out of the low and medium temperature heat pump fluidized bed dryer to produce isolated protein powder and main components in the drying circuit.

The frozen granulated protein was divided in twelve batches of similar particle size in bed volumes of 2, 3.5 and 4 and 6 litres. Each batch sample was heat pump dried by placing it into a 0.25m cylindrical drying chamber having a fine net on top and a perforated plate at the bottom. The access window was closed and air flow for fluidization at specific temperature was supplied by activation of the blower and the heat pump compressor.

The drying air inlet temperature was set to a value below the protein freezing point based on data. After reaching the critical protein moisture the air temperature was increased to a value just below the semi-solid protein fusion for finishing drying with maximum allowable water removal rate. The process variables were logged in Fluke Hydra Unit that was used for data acquisition of inlet air velocity (m/s), relative humidity (%) and drying

chamber inlet and outlet temperature ($^{\circ}\text{C}$) as well as ambient temperature and relative humidity.

The single trials were done at temperatures of -10°C and -5°C while the combined temperature tests were done by changing the air temperature at specific points at $-10/20^{\circ}\text{C}$, $-5/20^{\circ}\text{C}$, $0/20^{\circ}\text{C}$ and $-5/20^{\circ}\text{C}$. The initial moisture content of these samples ranged from about 72 to 74%wb and was dried to final moisture between 12 and 5 %wb.

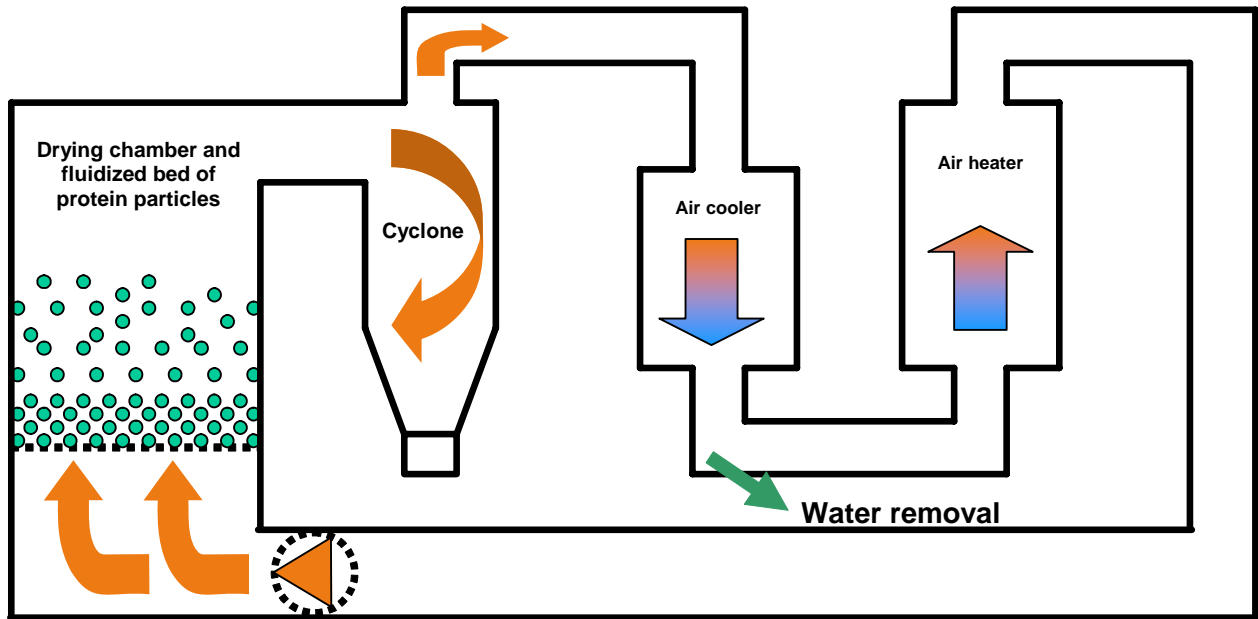


Figure 7.1. Low and medium temperature heat pump fluidized bed dryer to produce isolated protein powder and main components in the drying circuit.

1.4. Enthalpy for isolated fish protein

Avoiding protein melting or fusion by increasing the air temperature requires knowledge of the enthalpy and the protein freezing points, which depends on protein temperature and moisture content. This information is obtained from calorimetric data that is measured and plotted in **Figure 7.2**.

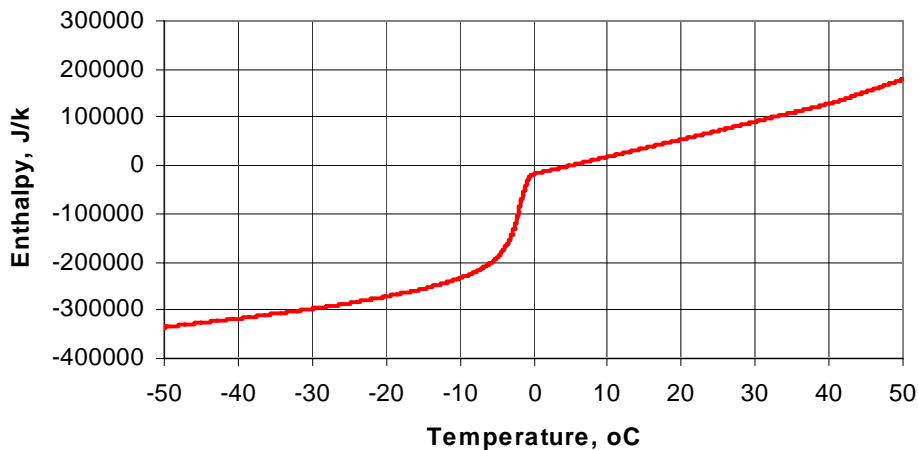


Figure 7.2. Enthalpy as function of temperature for isolated protein

1.5. Fish protein drying kinetics and water activity

Figure 7.3 shows a picture of a typical dried isolated protein sample with granules of 2 mm size produced in the heat pump dryer at combined temperatures of -5 and 20°C. The drying curves for all dried protein samples were determined by taking samples in pre-established time intervals. The moisture content measurements were made using infrared-moisture meter type Precisa HA300.

The drying kinetics presented similar trends as shown in **Figures 7.4 & 7.9**. The water removal rate varies directly with the air temperature and inversely with the protein particle size. This is shown in the plot where the drying rate is much higher for 2 mm particles than 8 mm. The other plot indicates that the drying rate is higher for 0°C than -10°C combined with the same 20°C. Thus, the highest drying rate, higher SMER or dryer throughput is reached for smaller protein particle size (2 mm) and a temperature combination of -5 and 20°C.

The water activity for all trials was measured in specific time intervals during and after drying using an Aqualab meter model CX-2 made by Decagon Devices. Usually, the protein sorption isotherm follows the “S” shape that characterizes hygroscopic materials having the solid matrix micro, meso and macro pores that provide sites for monolayer vapour sorption as well as multilayer condensation-evaporation-sublimation of gas-liquid or frozen solvents.

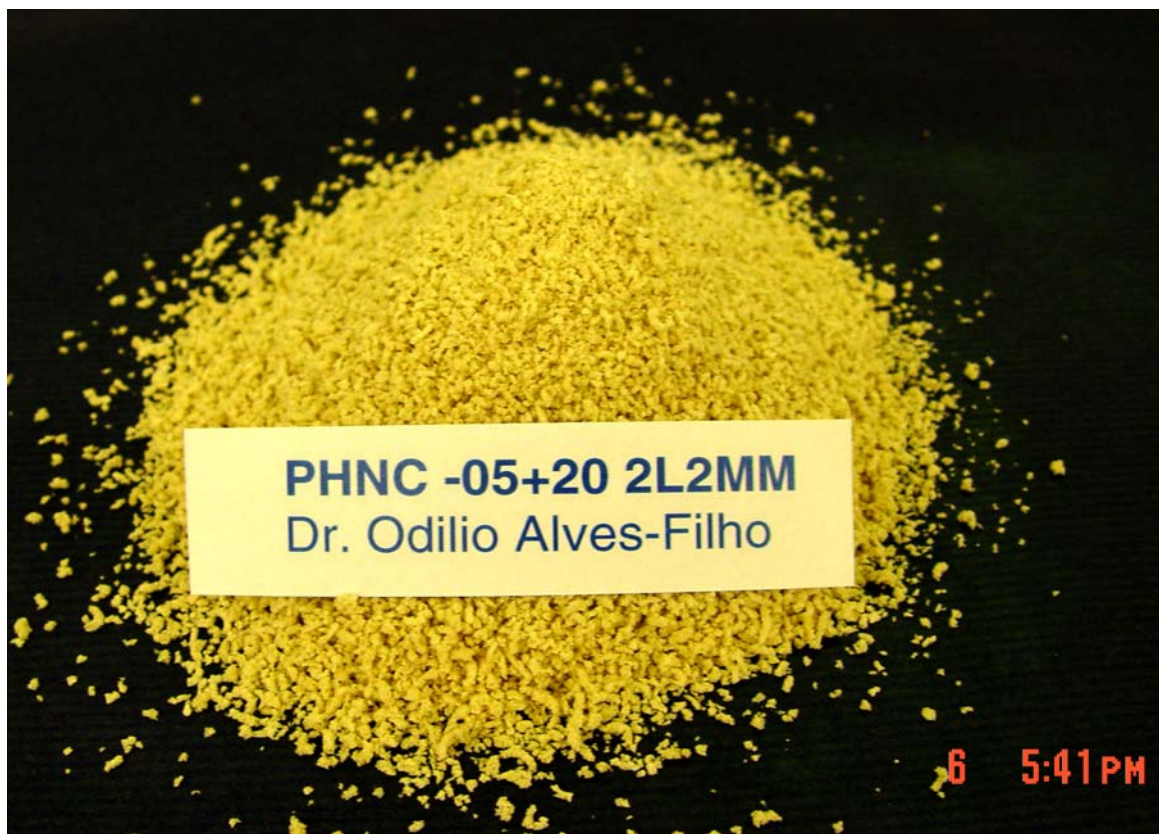
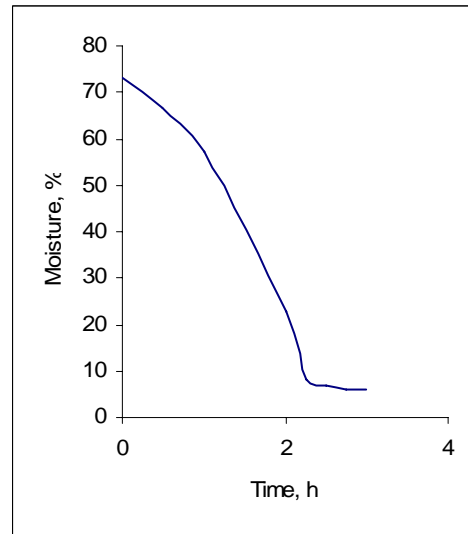
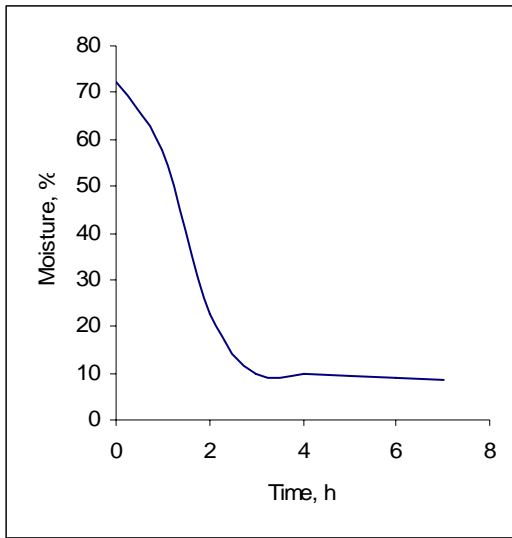
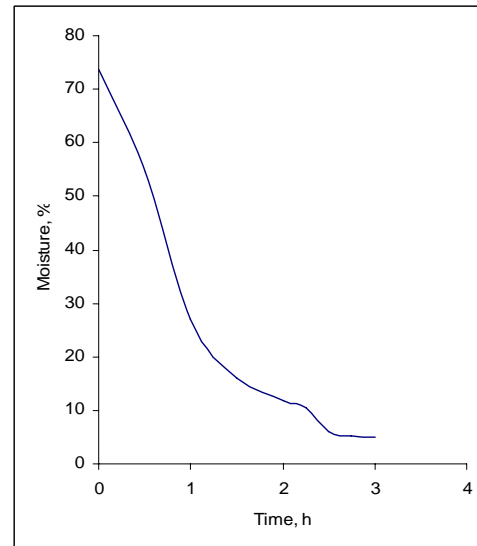
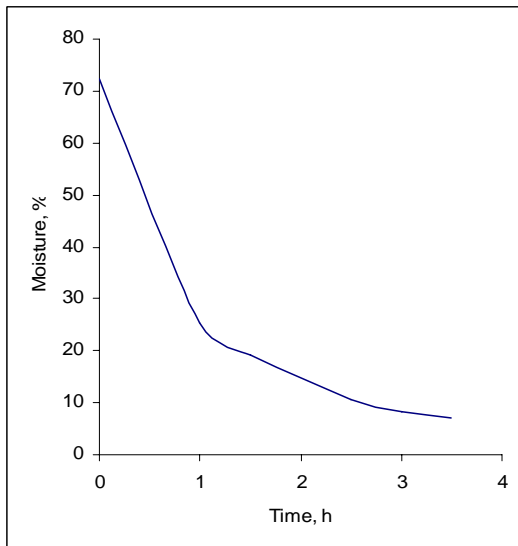


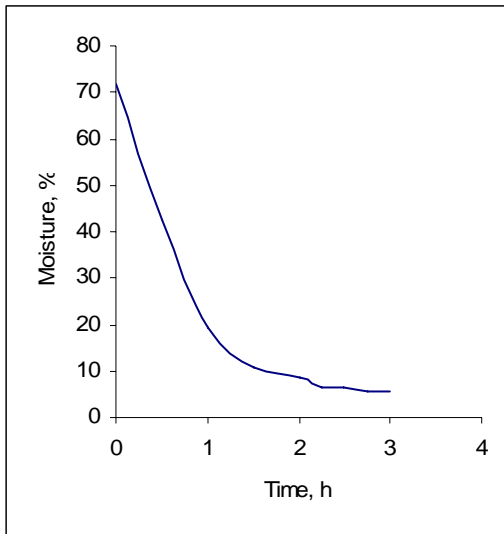
Figure 7.3. Typical dried granules of protein isolated from saithe produced in the heat pump dryer at temperatures of 5 and 20°C.



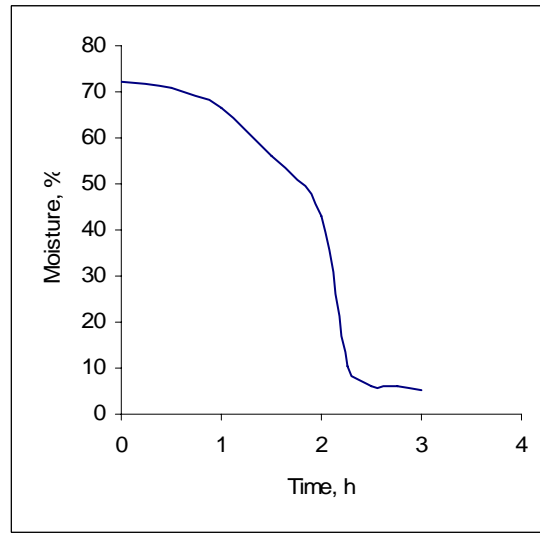
(a) (b)
 Figure 7.4. Protein drying curves in the heat pump dryer, (a) run 1 and (b) run 2



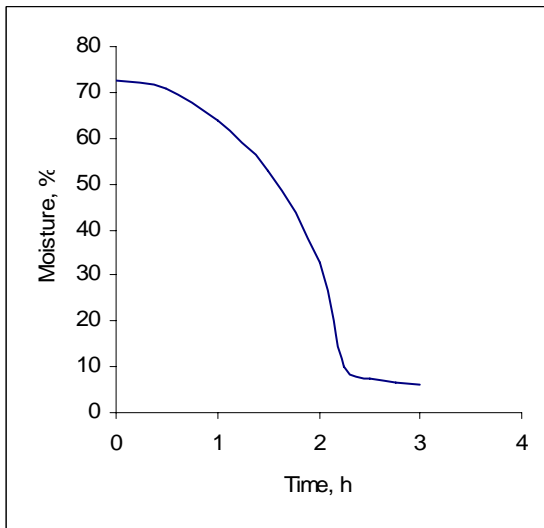
(a) (b)
 Figure 7.5. Drying curves, (a) run 3 (b) run 4



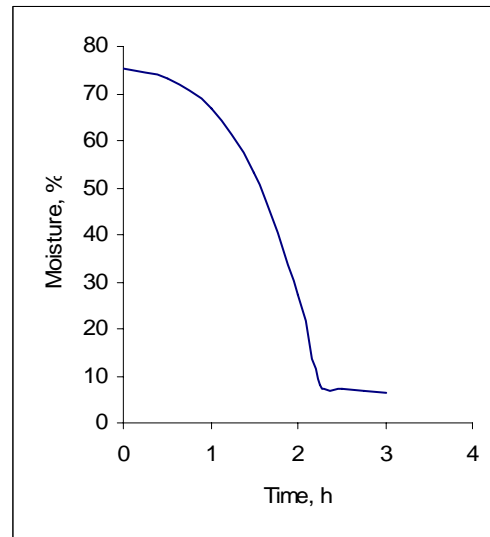
(a)
Figure 7.6. Drying curves, (a) run 5 (b) run 6



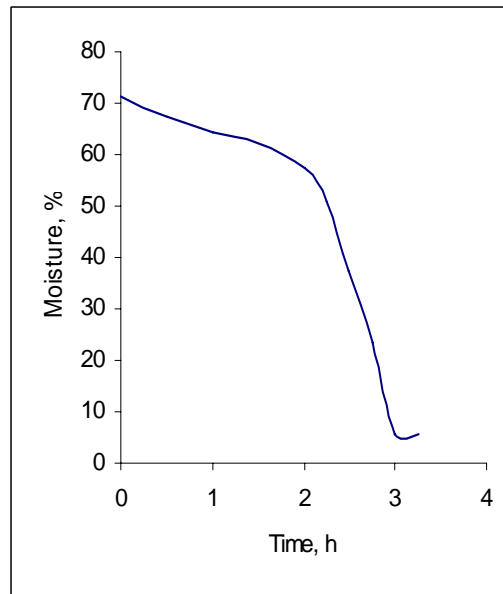
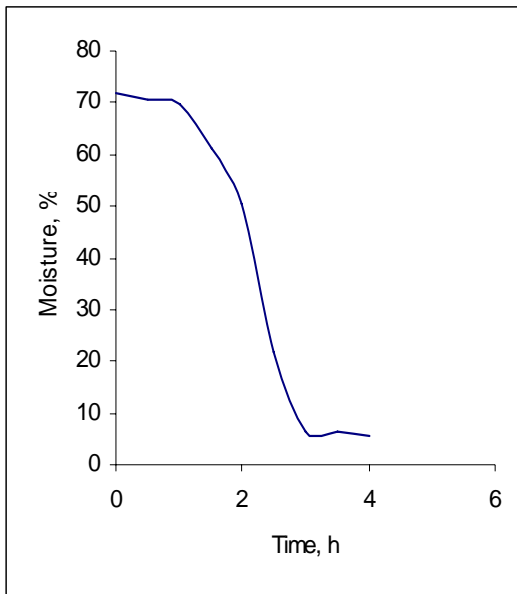
(b)



(a)
Figure 7.7. Drying curves, (a) run 7 (b) run 8

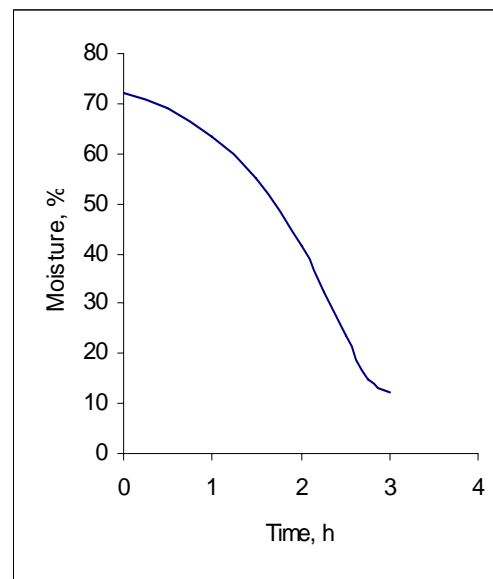
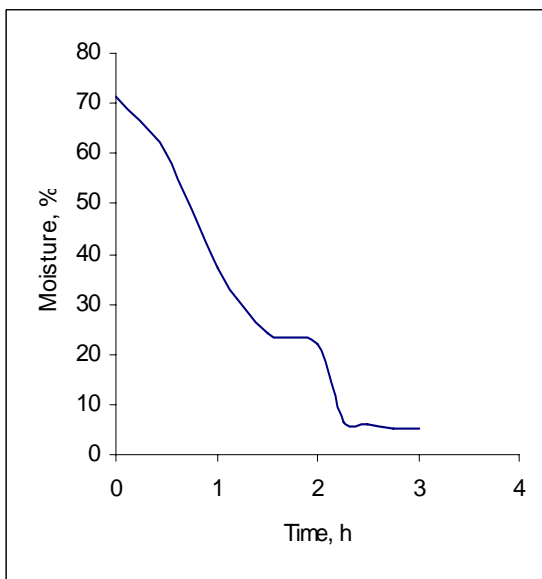


(b)



(a)
Figure 7.8. Drying curves, (a) run 9 (b) run 10

(b)



(a)
Figure 7.9. Drying curves, (a) run 11 (b) run 12

(b)

Table 7.4. Experimental data on density and colour components for 12 protein runs dried in the low heat pump fluidized bed dryer.

No.	T [°C]	ρ_o [kg/m ³]	ρ_f [kg/m ³]	L_f	a_f	b_f
1	-10	1829.4	173.87	57.83	0.05	11.93
2	-10/+20	1829.4	194.42	65.03	-0.59	13.85
3	-5	1829.4	180.47	67.15	-0.88	13.21
4	-5/+20	1829.4	206.11	67.52	0.52	13.99
5	0/+20	1829.4	233.08	60.37	0.36	13.99
6	-5/+20	1829.4	365.17	56.53	1.35	15.6
7	-5/+20	1829.4	213.60	54.68	-0.70	15.51
8	-5/+20	1829.4	205.40	53.60	0.63	13.23
9	-5/+20	1829.4	276.50	63.61	0.18	16.91
10	-5/+20	1829.4	334.60	62.30	0.36	18.37
11	-5/+20	1829.4	222.20	52.74	1.00	12.32
12	-5/+20	1829.4	236.80	53.08	1.61	11.44

2. Product properties

Properties of the dried products was measured by three participants: Sintef, IFL and Fiskeriforskning. Sintef measured the product rehydration properties along with moisture in the product. Fiskeriforskning measured the ability of the powders to make a gel. Finally IFL evaluated the use of the powders into product.

2.1. Sintef

2.1.1. Results on protein colour during drying

The effects of the processing conditions on the fish protein colour during drying was verified by measurements expressed in the CIELAB space coordinates with eight repetitions per sample. Sample colours were measured using a spectrophotometer type X-Rite 948 with a CIE 1964 10° observer and illuminant D65.

The results are plotted in **Figures 7.10 to 7.14** and in **Table 7.4** for all heat pump dried protein samples.

The yellow component is indicated by b_f and the measurements were similar for all runs with a difference of about 7. The highest yellow components was for run 10 with 2 mm and 6 L dried at -5/20°C and lowest for run 12 with 8 mm and dried at the same temperature.

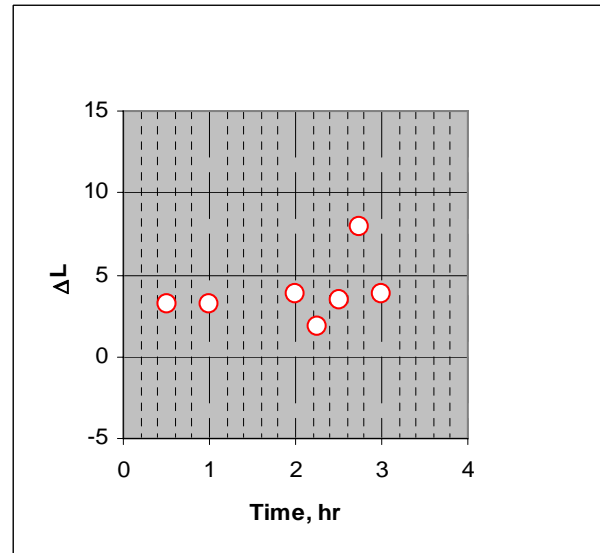
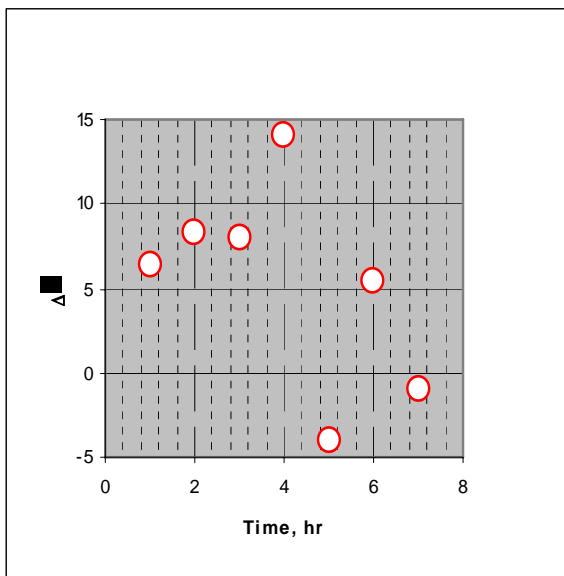
The brightness is indicated by L_f colour component. The samples with a diameter of 4 and 8 mm and volume of 2 L, dried at -5 and 20°C, presented the smallest brightness components with values of about 53 for both runs 12 and 11, this with the smallest value. The samples with diameter of 2mm and volume of 3.5 L, dried at -5 and 20°C, presented smaller brightness components with values of 55 and 53 for runs 7 and 8. However, as the volume rises the colour improves as indicated by 2 mm diameter samples in volumes 4 and 6 L, dried at -5 and 20°C, which had large brightness components with values of 63 and 62 for runs 9 and 10. The samples with diameter of 2 mm and volume of 2 L, dried at single or temperature combination of -10, -5 and 20°C, presented higher brightness components between 56 and 68. The maximum brightness is for runs 3 and 4 that were dried at -5 and -5/20°C, respectively.

Thus, improved sample brightness is attained with particle size and temperature of 2 mm and -5/20°C and the best bed volume are 2 L followed by 4 and 6 L.

The red colour component is low for all dried samples ranging about around the unit in the centre of the red-green plane coordinate axis. The red value is at maximum for the large particle size run 12. Consistently with its lightest, Run 3 with 2 mm has the lowest red and was dried at temperature of $-5/20^{\circ}\text{C}$.

Thus, colour improved by reduced red component is obtained with particle size and temperature of 2 mm and $-5/20^{\circ}\text{C}$ and bed volume of 2 L.

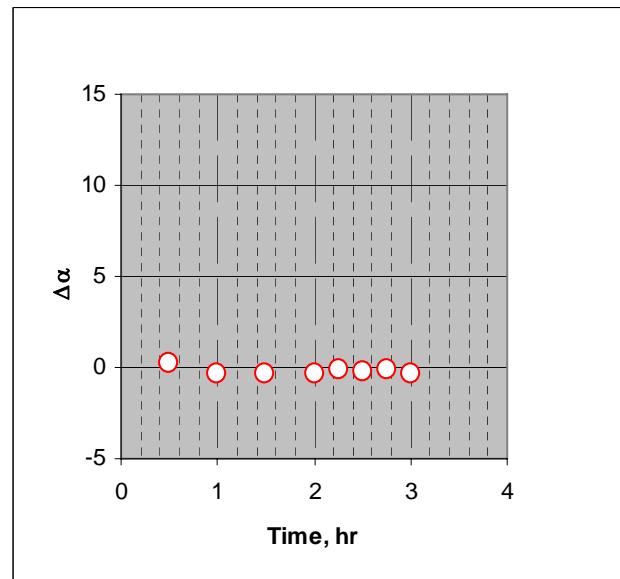
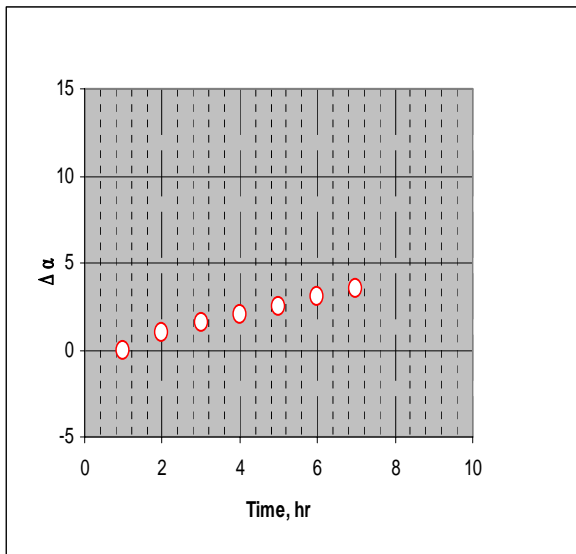
The difference in brightness, red and yellow colour components during the drying were determined in all runs as plotted in **Figures 7.10 to 7.14**. The trend is similar for all runs as indicated by the results for sample 3, which shows a small difference in the brightness with a slight increase in at about 3.5 hours and it is an indication that the original colour of the raw material was preserved during the drying.



(a)

(b)

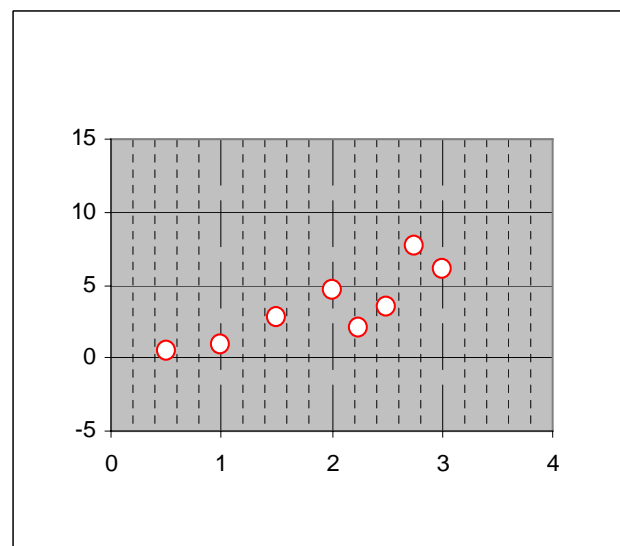
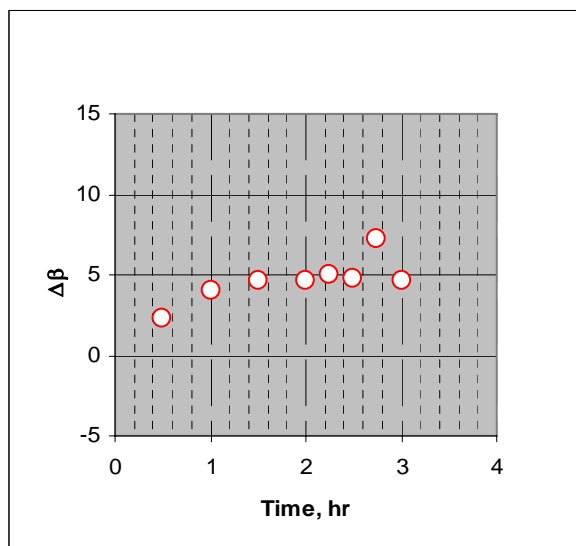
Figure 7.10. Difference in brightness for isolated protein during drying, (a) run 1 and (b) run 2.



(a)

(b)

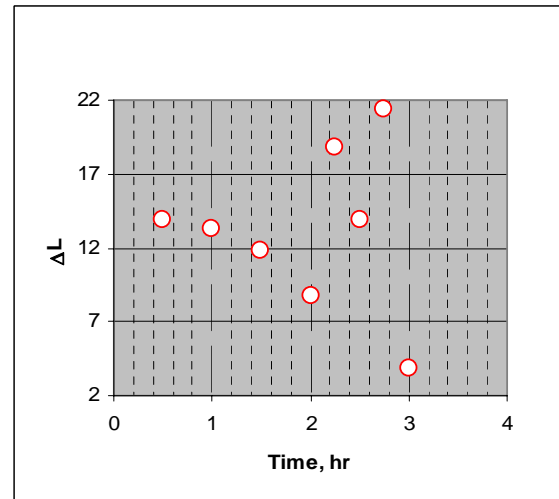
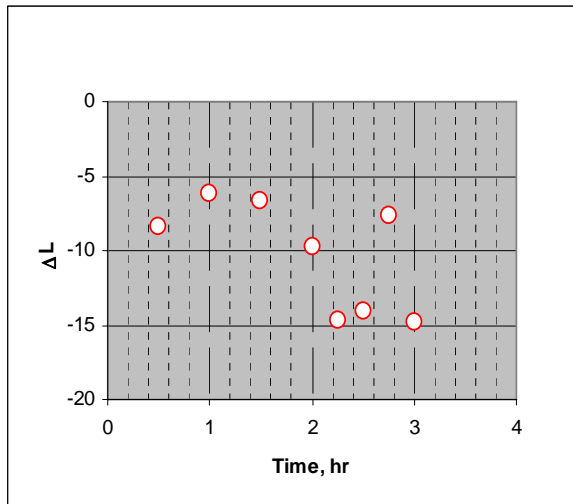
Figure 7.11. Difference in red component for isolated protein during drying, (a) run 3 and (b) run 4.



(a)

(b)

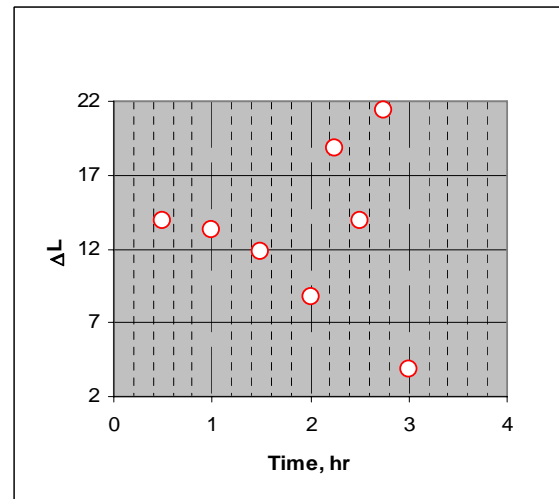
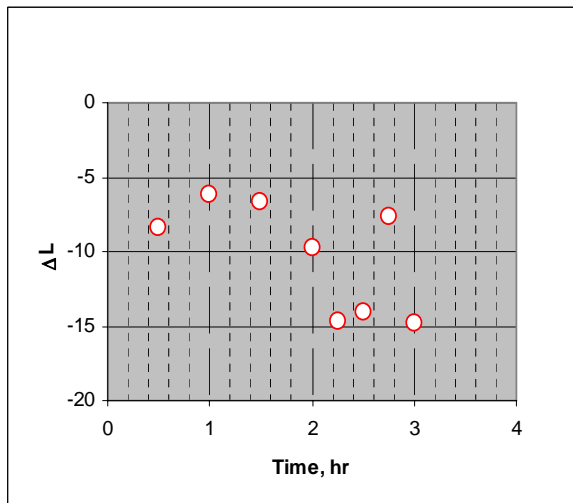
Figure 7.12. Difference in yellow colour for isolated protein during drying, (a) run 5 and (b) run 6.



(a)

(b)

Figure 7.13. Difference in brightness for isolated protein during drying, (a) run 11 and (b) run 12.



(a)

(b)

Figure 7.14. Difference in brightness for isolated protein during drying, (a) run 11 and (b) run 12.

2.1.2. Results on protein rehydration and density

The processing conditions affect the fish protein properties and can be verified by measurements rehydration and bulk density.

The rehydration in water at temperature of 20°C was measured for each sample using rehydration equilibrium apparatus, beaker water bath and an immersion device made of metal screen and shaped as a half-sphere similar to a deep spoon. The equilibrium apparatus is composed of an evacuated-sample holding chamber, perforated base, filter, hoses and drainage flask. Alves-Filho (2004 a,b) has worked extensively with fish powders rehydration and reports that satisfactory reproduction on measurements of rehydration requires a consistent procedure taken into account the solvent temperature, particle size and initial-targeted final moisture content and time for each rehydration step. The author (Filho *et al.*, Sintef) has developed and recommends a procedure for protein rehydration, which consists of 12 steps that may be summarized as follows:

- 1) Saturate a filter with pore size smaller than the smallest powder particle in water at 20°C for 2 minutes
- 2) Vacuum drain the filter using the equilibrium apparatus for 2 minutes
- 3) Remove the drained filter and record its mass (m_f)
- 4) Take 250 to 500 mg of dried protein powder sample and record its mass (m_d)
- 5) Add the mass of drained filter (m_f+m_d)
- 6) Put the dried powder sample over the drained filter
- 7) Put the filter with the sample into a immersion device
- 8) Gradually immerse the device with the filter and sample into the beaker water (20°C) bath
- 9) Keep it there for over-saturation and remove it after 6 minutes
- 10) Take the filter and over-saturated sample using a tong
- 11) Vacuum drain the filter plus sample using the equilibrium apparatus for 2 minutes
- 12) Remove the filter plus sample and record the mass (m_f+m_w)

The rehydration for a sample was calculated based on average values obtained from triplicate measurements of the mass of dried and rewetted sample according to the following equation:

$$Ra = \frac{\sum_{i=1}^n \frac{(m_{fi} + m_{di})}{n} + \sum_{i=1}^n \frac{(m_{fi} + m_{wi})}{n}}{\sum_{i=1}^n \frac{(m_{fi} + m_{di})}{n}} \quad (2)$$

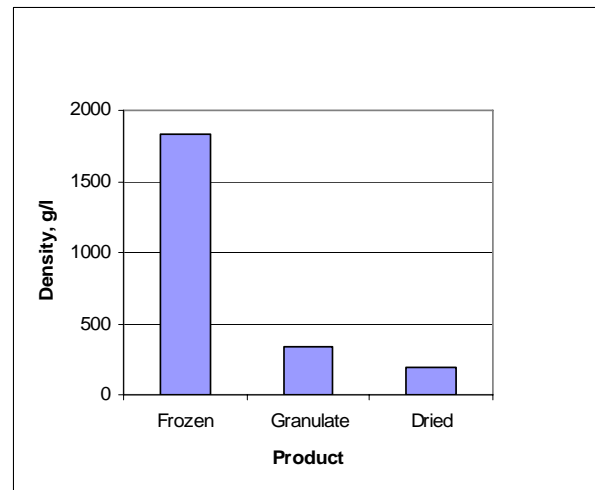
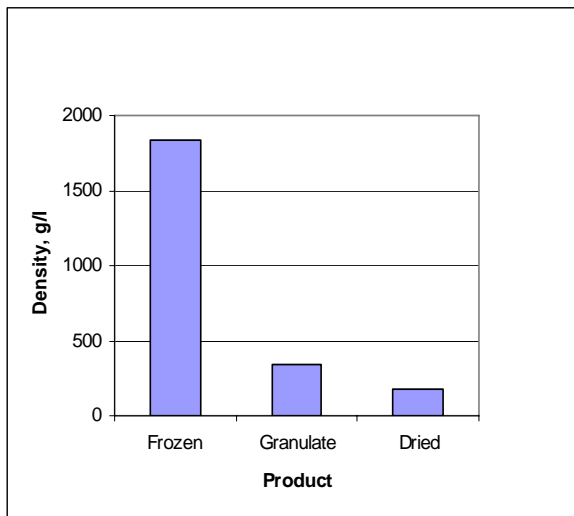
Where, i varies from 1 to n and n is equal to 3 for triplicate measurements. Also, m is the sample mass in mg and the subscripts f , d and w stands for filter, dried and wet sample.

The above procedure with timed steps and Equation (2) provided reproducible measurements of the mass of dried and reconstituted powder sample. This is clearly observed by the consistent results given in Table 3 for all runs. It indicates that for samples dried at atmospheric freezing mode only, the rehydration increases as the temperature is reduced. For similar size and volume, the sample dried at -10°C had a higher R_a value of 1.26 than that dried at -5°C with a value of 1.11.

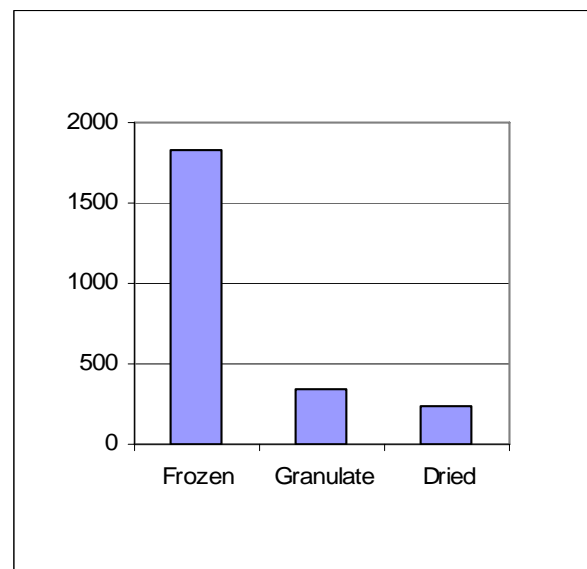
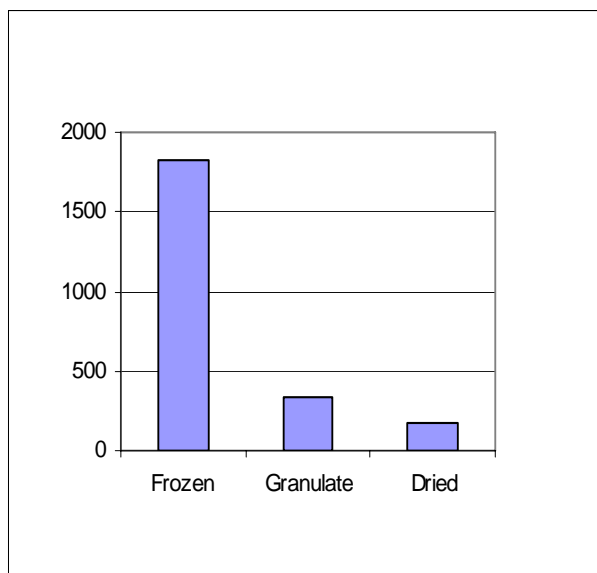
However, for samples dried at atmospheric freezing combined with medium temperature, the rehydration decreases as the first mode temperature is reduced. The sample dried at -10/+20°C had a lower R_a of 1.01 than at -5/20°C with a value of 1.36. The R_a varies inversely with the air relative humidity. Similar sample dried at same conditions but with high relative humidity of 75% had lower R_a value of 1.18.

Similar size samples had high rehydration as the particle size or bed volume increased but to a certain value. The highest R_a was 1.62 for the sample dried at -5/20°C with bed volume of 4 L, which was higher than all other runs and lower than run 10.

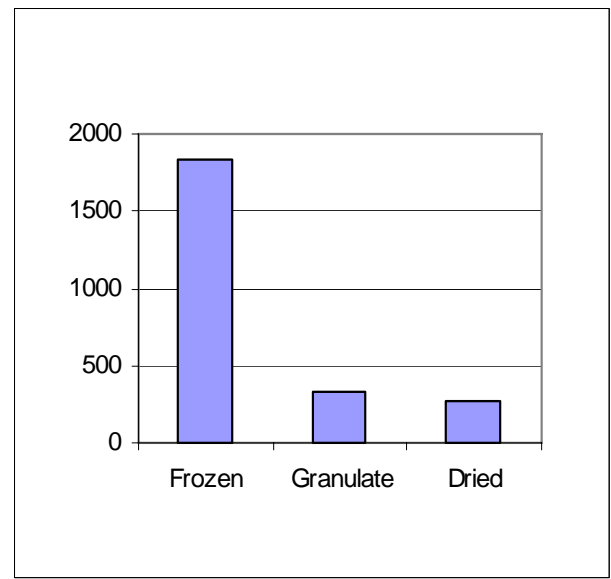
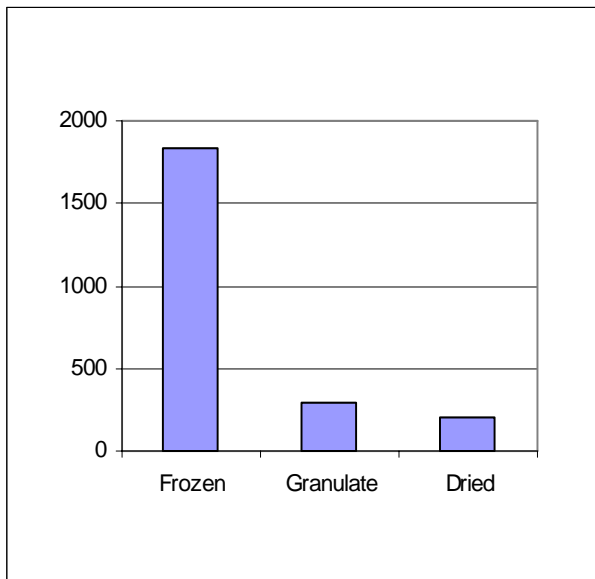
The average density was obtained from triplicate measurements of sample mass and volume. The density depends on temperature and drops continuous as the protein dried. The results are plotted in **Figures 7.15 to 7.18**. The plots show similar changes shown in **Figure 7.16** where the fresh-frozen, granulated and powder protein dried 5°C had densities changed from 1829.4 to 337.0 and finally to 180.47 kg/m³, respectively.



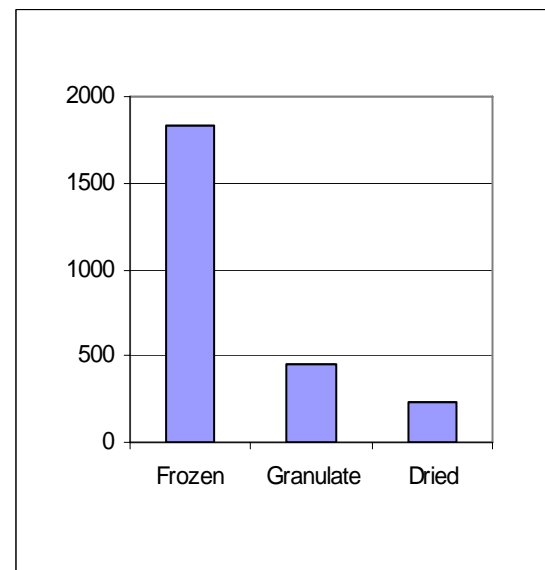
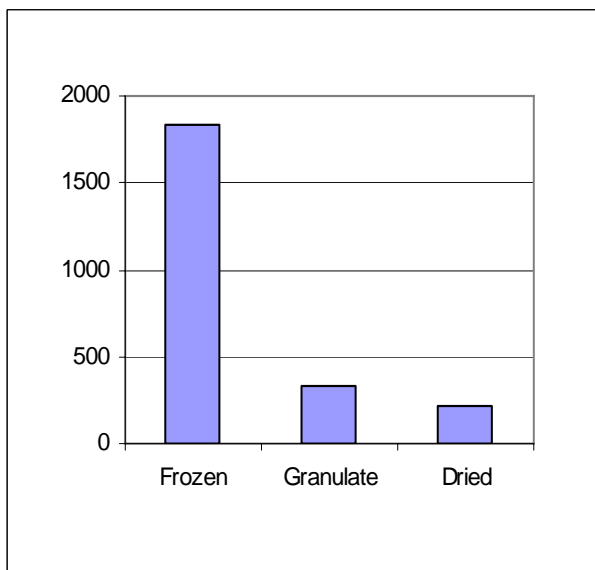
(a) (b)
 Figure 7.15. Density change for isolated protein from fresh-frozen, granulated and dried isolated protein, (a) run 1 and (b) run 2.



(a) (b)
 Figure 7.16. Density change for isolated protein from fresh-frozen, granulated and dried isolated protein, (a) run 3 and (b) run 5.



(a) (b)
 Figure 7.17. Density change for isolated protein from fresh-frozen, granulated and dried isolated protein, (a) run 7 and (b) run 9.



(a) (b)
 Figure 7.18. Density change for isolated protein from fresh-frozen, granulated and dried isolated protein, (a) run 11 and (b) run 12.

2.2. Fiskeriforskning Product properties

2.2.1. Materials and methods

Fish protein isolate (FPI) from the pilot plant in Iceland was dried using a heat pump exchange 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 were made.

Methods used for measure properties of dried proteins by Fiskeriforskning are listed with Task 8 – Surimi.

2.2.2. Results and discussion

Immediately after removing the vacuum seal, an extreme rancid odour was detected 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 7.5. Moisture contents in raw material, dried material, and re-hydrated gelled products named M_o , M_f , and M_g 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 does not exist.

Sample #	M_o [%]	M_f [%]	M_g [%]	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	72,00	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	72,00	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 7.19. 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 7.19**, 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 were measured by torsion test. Results are shown in **Table 7.5** and **Figures 7.20 & 7.21**. 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.

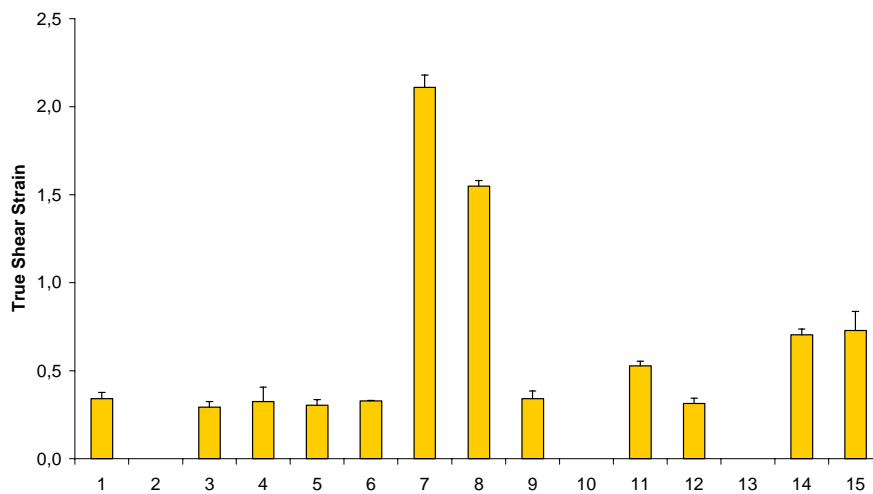


Figure 7.20. True shear strain from torsion tests on gels from *saithe* (*Pollachius virens*) protein isolates at different drying conditions (1-12), control samples (14 & 15). Samples 2 and 10 were too weak to be measured. Sample 13 does not exist.

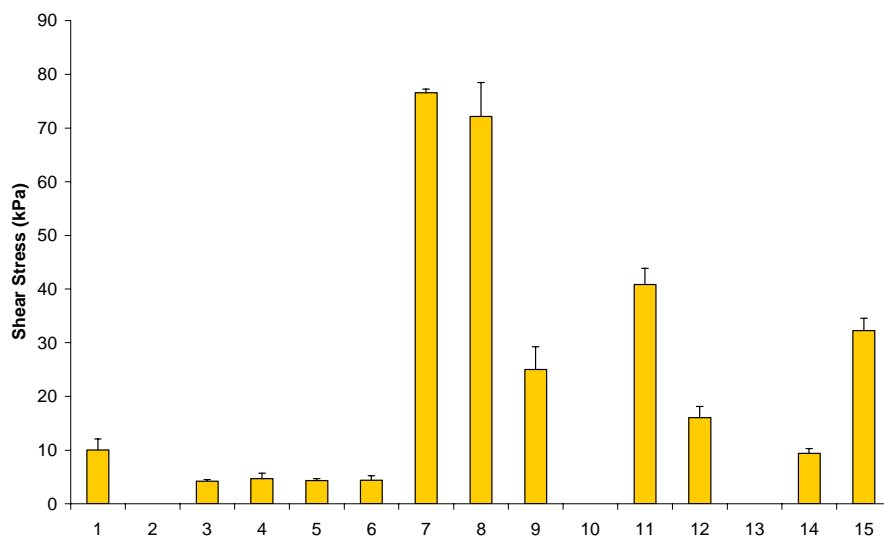


Figure 7.21. True shear stress from torsion tests on gels from *saithe* (*Pollachius virens*) protein isolates at different drying conditions (1-12), control samples (14 & 15). Samples 2 and 10 were too weak to measure. Sample 13 does not exist.

2.2.3. Discussion of stress and strain characterization

Figure 7.22 shows a picture of four dried protein runs before they were forwarded to Tromsø for measurement of rheological properties. The powder samples were rehydrated to reach analytical moisture content (M_g) of 70 to 75%wb. However, since most samples had maximum initial moisture content of 73% and higher value for analytical moisture it may disturb rehydration and stress-strain properties. Also sorption-desorption-hysteresis phenomena plays additional role in irreversibility of protein powder rehydration. The consequence is that most samples rehydrated to 75% formed weak gels and two samples extremely strong gels.

The high values for runs 7 and 8 will be not included in the present stress-strain analysis because the only difference in runs 4, 6, 7 and 8 are the bed volume. To further look at the results they are presented as relative shear stress expressed by

$$\tau_r = \frac{\tau_{\text{actual}}}{\tau_{\text{min}}} \quad (3)$$

Similarly, the relative shear strain is given by

$$\varepsilon_r = \frac{\varepsilon_{\text{actual}}}{\varepsilon_{\text{min}}} \quad (4)$$

The results are given in **Table 7.6** and plotted in **Figures 7.22 & 7.23** and indicate the same trends for relative stress and strain, which means that both are functions of similar variables, i.e., time, temperature, size, etc. The highest stress was for gels from powders dried at combined temperature of -5 and 20°C. Allied to temperature, the particle size influence the stress but not in a linear relationship. Samples with medium size samples presented higher relative stress. The highest value was for run 11, with volume of 2L and diameter of 4 mm (not linear because 2 mm and 8mm particles had lower values), followed by run 9 with diameter 2 mm and volume 2L, run 12 with diameter 8 mm and volume 2, and run 1 with 2 mm and bed volume of 2L. The highest stress was for gels made from samples dried at combined temperature of -5 and 20°C and 4 mm particle (there is an optimal size around 4 mm or between 3 and 6 mm). Also, bed volume seems to affect stress and more data are needed to confirm this aspect.

The lower stress were gels for runs 2 to 6 dried at combined temperature of -10, -5 and combination with 20°C with stress. The lowest value of 1.0 for run 3 dried at -5°C and slightly higher value of 1.12 for run 4 dried at combined mode of -5 and 10°C. The particle size and bed volume were constant for these samples and, thus, had no effect on stress-strain for these runs.

Slightly higher stress was observed for gels from samples dried at combined temperature of -5 and 20°C, 2 mm particle and volume of 2L.

In testing and analyzing dried protein gel as it is submitted to force-response, one may classify the powders in groups based on stress-strain. This is done considering an important strength parameter called elasticity modulus under compression, tension or torsion. The relative elasticity modulus of the protein gel is the derivative of the stress as

function of strain or the ratio of the relative stress to the unit relative deformation. It can also be estimated from experimental data by calculation of the tangent line crossing the extreme points for each group.

$$\xi_r = \frac{d\tau}{d\varepsilon} \cong \frac{\Delta\tau}{\Delta\varepsilon} = \frac{\tau_{ult}-\tau_0}{\varepsilon_{ult}-\varepsilon_0} \quad (5)$$

The plot in **Figure 7.24** shows a nearly linear relation between stress and strain for the two groups. It clearly indicates a higher stress group consisting of runs 9 to 12 that deforms extensively before reaching the ultimate stress or rupture. The second group deforms easily prior to rupture at medium stress values. However, calculation using the data in **Table 7.6** and plot in **Figure 7.24** leads to elasticity modulus of 7.82 for the high stress group and 8.10 for the low stress group. This means that both groups have the same elasticity behaviour and indicate that if the gel was prepared such that the protein molecular binding forces were kept, the low stress group would actually have similar strength and reach the same or higher ultimate stress as the other group.

Table 7.6. Experimental data according to Table 7.3 and relative shear stress and strain for the rehydrated protein powders (same results as in table 7.5 but here as relative values).

No	M ₀ [%]	M _f [%]	M _g [%wb]	τ _r	ε _r	Obs
1	72.18	8.61	75	2.40	1.17	
2	73.17	5.87	75			MI
3	72.18	7.08	75	1.00	1.00	
4	73.67	4.91	75	1.12	1.10	
5	71.77	5.39	75	1.04	1.03	
6	72.00	5.17	75	1.06	1.14	
7	72.53	6.28	74			OR
8	75.49	6.38	70			OR
9	71.91	5.60	70	5.98	1.17	
10	71.40	5.57	75			MI
11	71.38	5.28	70	9.77	1.83	
12	72.00	12.32	70	3.84	1.07	

MI: missing values due too low stress or value undetectable by the instrument

OR: out of range for this analysis.

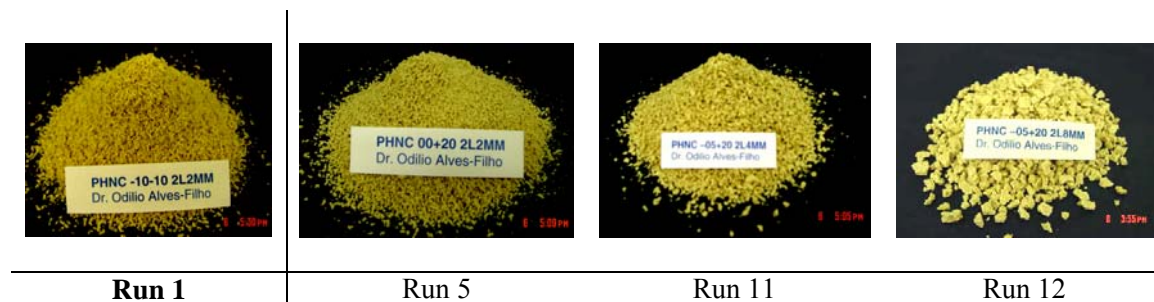


Figure 7.21. Pictures of four dried saithe isolated protein runs prior to preparation for rheological measurements.

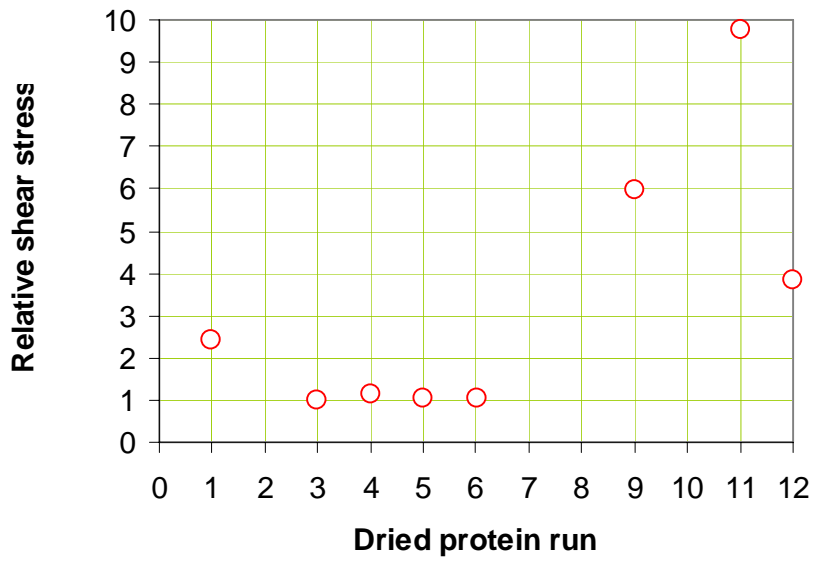


Figure 7.22. Relative shear stress in gels of dried saithe protein isolate with conditions in Tables 7.3, 7.5 and 7.6.

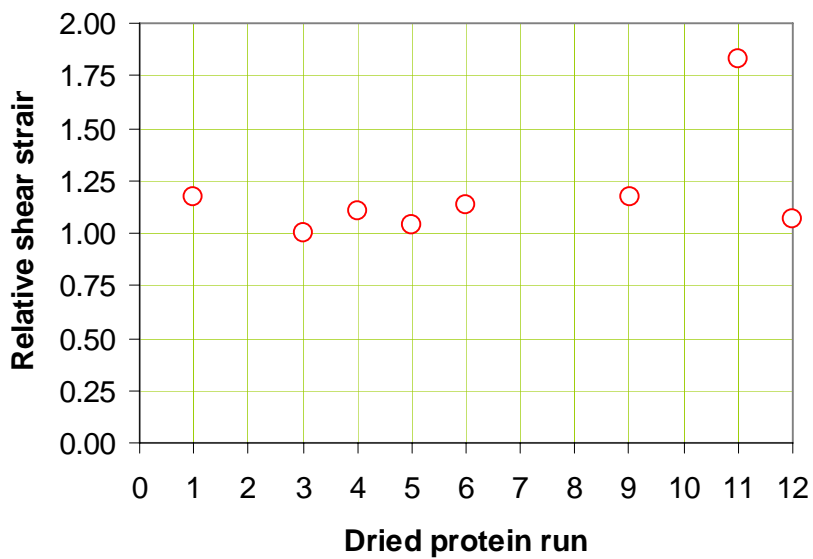


Figure 7.23. Relative shear strain in gels of dried saithe protein isolate with conditions in Tables 7.3, 7.5 and 7.6.

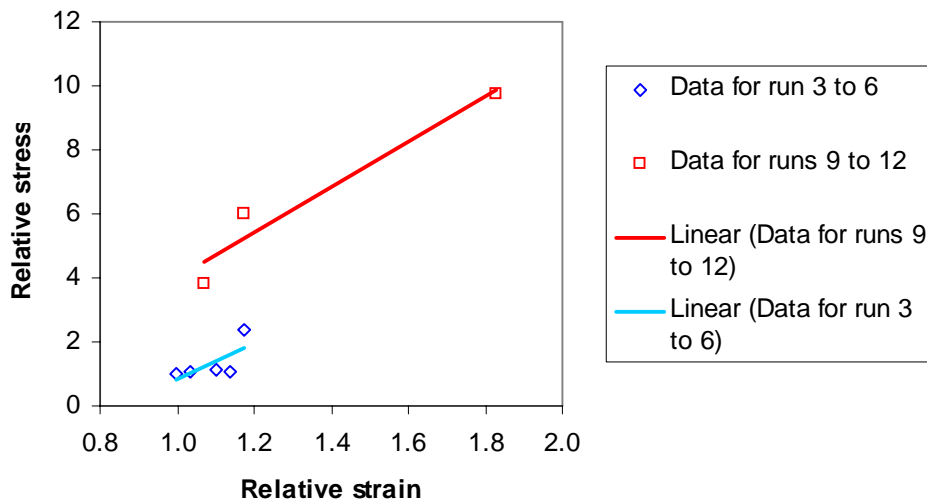


Figure 7.24. Elasticity modulus and relative shear strain versus strain for two groups of gels-dried protein isolate.

2.3. IFL – Product Properties

2.3.1. Materials and methods

IFL measured the same samples as were measured by Fiskeriforskning..

2.3.1.1. Water binding capacity

A revised method by Beuchat (1977), with modifications, was used to measure water binding capacity. About 1 g of protein powder was put into a centrifugal bottle and 15 mL of water added. pH was set at around 6,67 with 1% (w/v) HCl or 1% (w/v) NaOH. The mixture was left to stand for 30 minutes at room temperature with swirling every 10 minutes. Then the sample was centrifuged (Sorvall RC-5B Refrigerated Super Speed Centrifuge, Du Pont Instruments, USA) at 1820 x g for 30 minutes at 0-5°C. The weight of unabsorbed water was divided by the sample weight and expressed as %WBC. Analysis was done in duplicate.

2.3.1.2. pH

pH was measured in water binding capacity samples before and after centrifugation. pH was also measured in water holding capacity samples before centrifugation. Measurements were done with Knick Portames pH meter (Knick, Berlin, Germany).

2.3.1.3. Biuret protein assay

The method of Biuret (Torten & Whitaker, 1964) was used to determine protein content (soluble proteins) in supernatant, after centrifugation. Standard curve was made with bovine serum albumin. Samples were placed in experiment tubes, Biuret solution (1,5 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 6,0 g $\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ dissolved in 500 mL distilled water, 300 mL of 10% NaOH added while stirring, solution then diluted to 1L with distilled water) added and mixed on Turax for 5 seconds. Left to stand for 35 minutes. Sample put into 1 mL cuvettes and absorbance measured at 540 nm. Each sample was analysed in duplicate.

2.3.1.4. *Water holding capacity*

3,2 grams of protein powder was mixed with 100 g of minced cod muscle and 20 g of cold, distilled water in a mixer (Braun Electronic, Type 4262, Kronberg, Germany) for approximately 20 seconds on speed 5. Sample was left to stand for 30 minutes on ice. Water holding capacity was then determined by a centrifugation method of Eide and others (1982). Sample (2 grams) was accurately weighed and immediately centrifuged (Sorvall RC-5B Refrigerated Super Speed Centrifuge, Du Pont Instruments, USA) at 210 x g for 5 minutes at 0-5°C. The weight loss after centrifugation was divided by the water content of the minced cod muscle and expressed as %WHC. Each sample was measured in quadruplet.

2.3.1.5. *Colour*

Colour was measured with Minolta CR-300 chroma meter (Minolta Camera Co., Ltd., Osaka, Japan) in Lab* colour system (CIE, 1976) with CIE Illuminant C. The dried protein samples were put in a wide experiment tube, thumped on flat surface 32 times (to settle the content), put on a vortex for 10 seconds and then the colour was measured. Each sample was measured in triplicate.

2.3.1.6. *Water activity*

Water activity was measured with Novasina a_w -center (Axair Ltd., Pfäffikon, Switzerland). Temperature was set at 25°C. Each sample was measured in triplicate.

2.3.1.7. *Sensory*

Pre trial was made to evaluate the sensory properties of the protein powders. The panel consisted of four judges, all trained in sensory analysis. A score card was used and each powder was given the scores according to:

- ✓ None / just detected ~ 0
- ✓ Slide ~ 1
- ✓ Moderate ~ 2
- ✓ Strong ~ 3
- ✓ Very strong ~ 4

2.3.2. Results and discussion

2.3.2.1. *Water binding capacity*

Highest proportional binding capacity was found in sample no. 8, and the lowest in sample no. 12 (**Figure 7.25**).

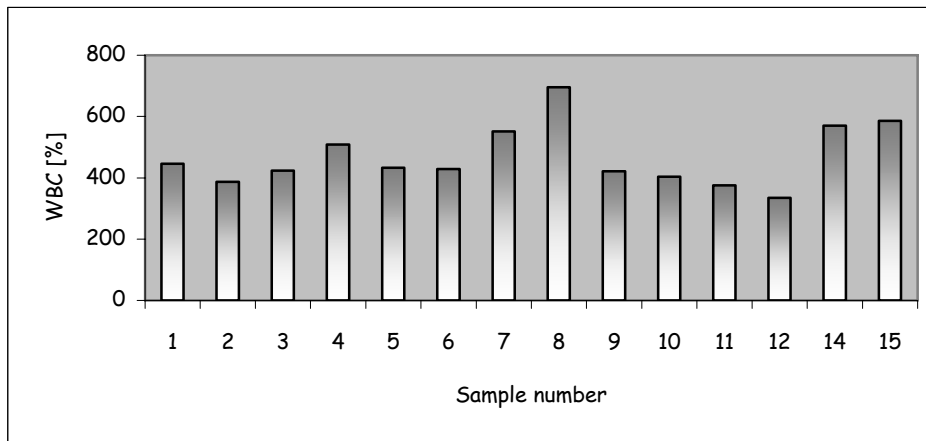


Figure 7.25. Water binding capacity in all samples.

2.3.2.2. pH

As seen on **Figure 7.26**, the pH for the supernatant and the insoluble matter showed the same tendency even though the pH in all samples was set at ca 6,67-6,68 before centrifugation.

WHC samples were more similar than the WBC samples, but sample no. 2 is different from all the other samples. Control+water has a higher pH than the control, but it is impossible to say if the difference is significant or not since only one measurement was done. It's also of interest that samples 7, 8 14 and 15 have the highest pH in the different parts after centrifugation for the WBC samples.

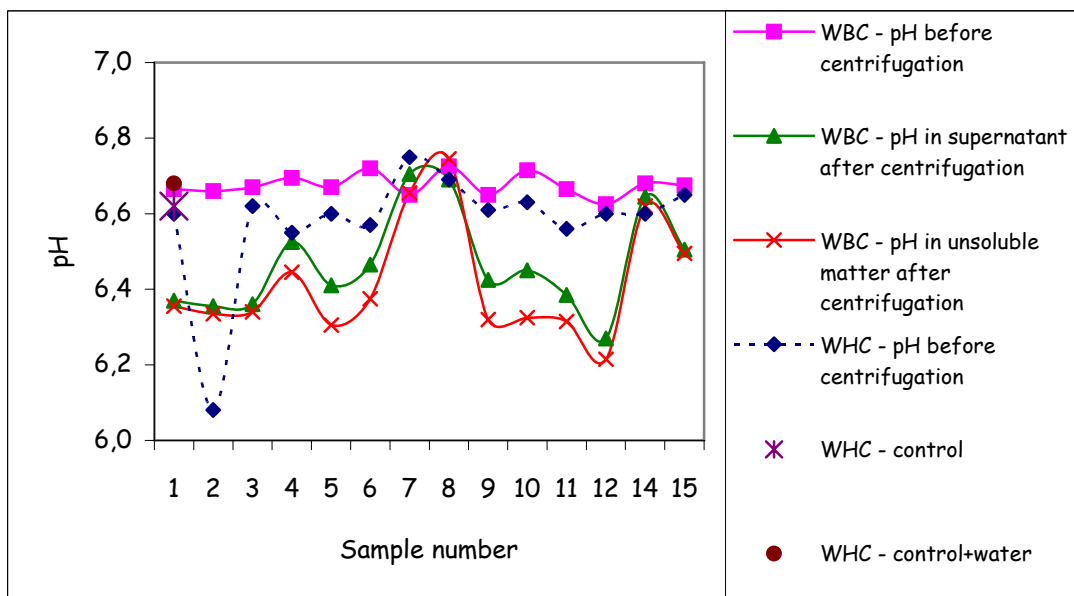


Figure 7.26. pH in WBC and WHC samples.

2.3.2.3. Biuret protein assay

As seen on **Figure 7.27**, samples no. 4, 7 and 8 had the highest protein content as measured by the method of Biuret. Other samples had a protein content in the range of 2,0-3,6 mg/mL.

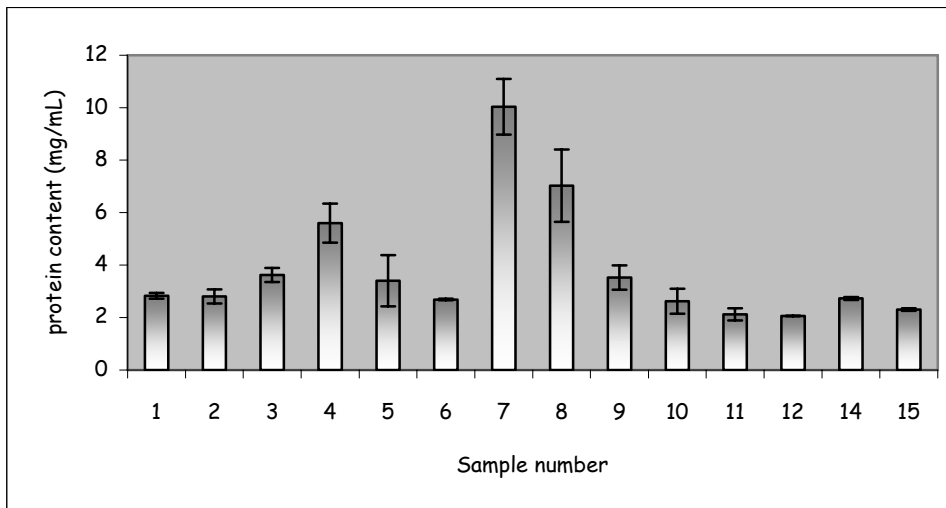


Figure 7.27. Biuret protein assay of all samples with standard deviation.

2.3.2.4. Water holding capacity

All the samples had higher water holding capacity than the control sample (protein powder and cod mince) and the control+water sample (cod mince and distilled water). Those results are as expected (**Figure 7.28**).

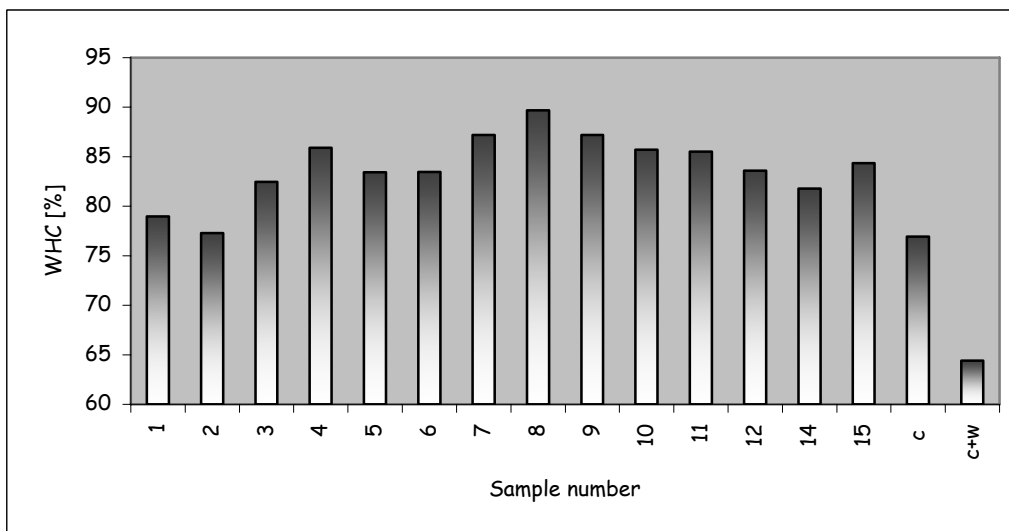


Figure 7.28. Water holding capacity (%) for all samples, control (c) and control with water (c+w).

2.3.2.5. Colour

As can be seen on **Figures 7. 29 to 7. 31**, samples no. 14 and 15 had more brightness in colour (around 85 in L* value) than other samples (L*value in the range 72,6-78,4). The samples were very similar in a* value, having a range of 1,04. The samples were quite similar in b*value, samples no. 7 and 8 had the lowest b*values.

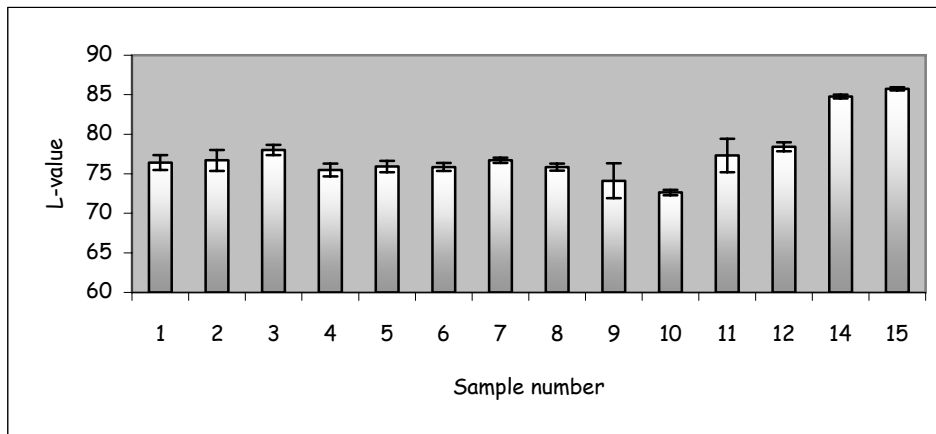


Figure 7.29. L* value of all samples.

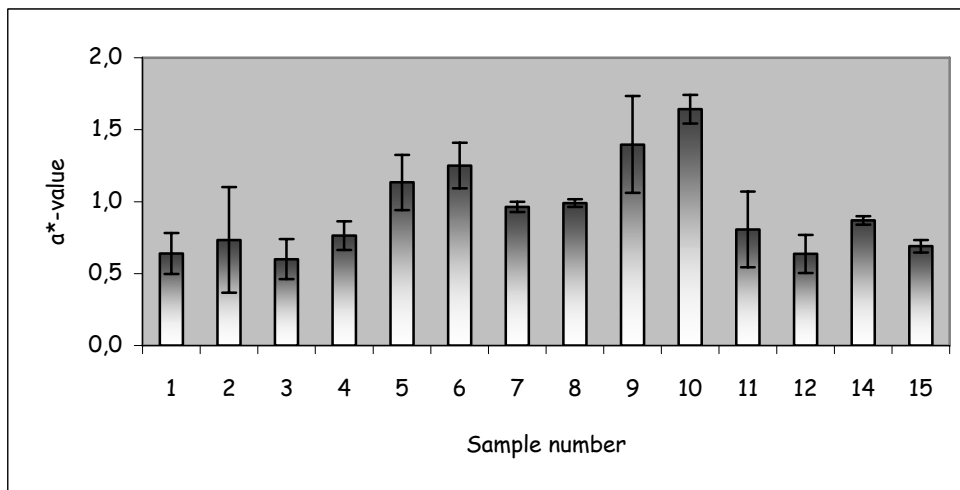


Figure 7.30. a* value of all samples.

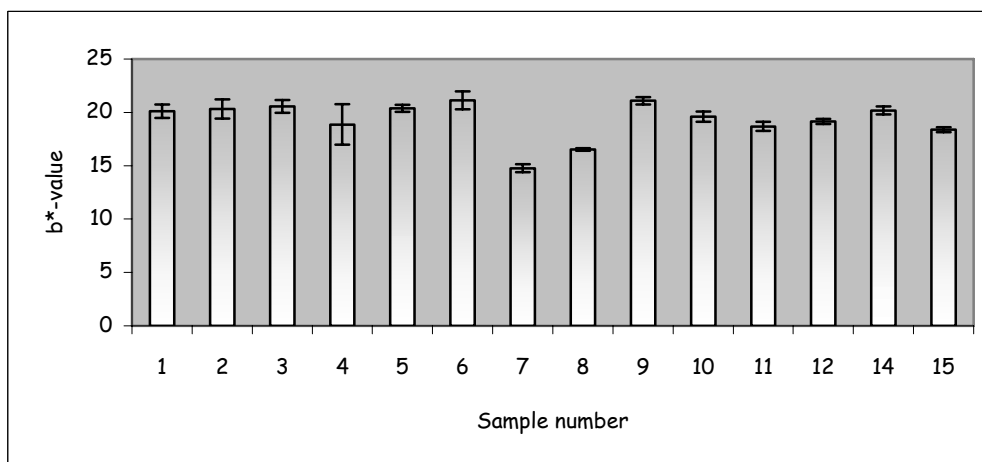


Figure 7.31. b* value of all samples.

2.3.2.6. Water activity

A large range was observed within the water activity of the samples. Sample no. 12 had the highest water activity (**Figure 7.32**).

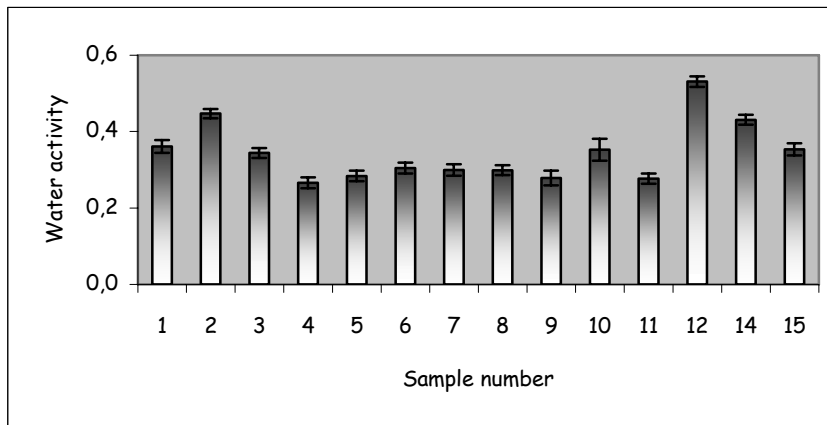


Figure 7.32. Water activity in all samples.

The results of the measurements show a high degree of similarity among all the samples, in all factors measured. In all measurements, one or two samples are very different from all the other samples.

2.3.2.7. Sensory

The sensory judges were not nearly unanimous in their evaluation of the samples (Table 7.6 and Figure 7.33). It was not possible to have a training session before the evaluation and these results should therefore only be looked up on as pre-trial. All the same, the results give us some indication of the sensory properties of the samples.

Table 7.6. Score table filled of 4 judges.

Sample	None/just detected	Slide	Moderate	Strong	Very strong	Description
1		x	x	xx		Plastic, dried fish (TMA)
2		xx	x	x		
3	xxx		x			Dried fish, butter, sour
4	x	xx	x			Plastic
5	x		x	xx		
6		xxx	x			Rancid, sour
7	x	xx	x			Dried fish, butter
8	x		x	xx		Dried fish, rancid
9	xx			xx		
10		xxx			x	Paint, dried fish, plastic, rancid
11	x	x	x	x		Plastic, dried fish, rancid
12		x	xxx			Plastic, dried fish
14		xxx	x			Grass, dried fish
15		xxx	x			Grass, dried fish

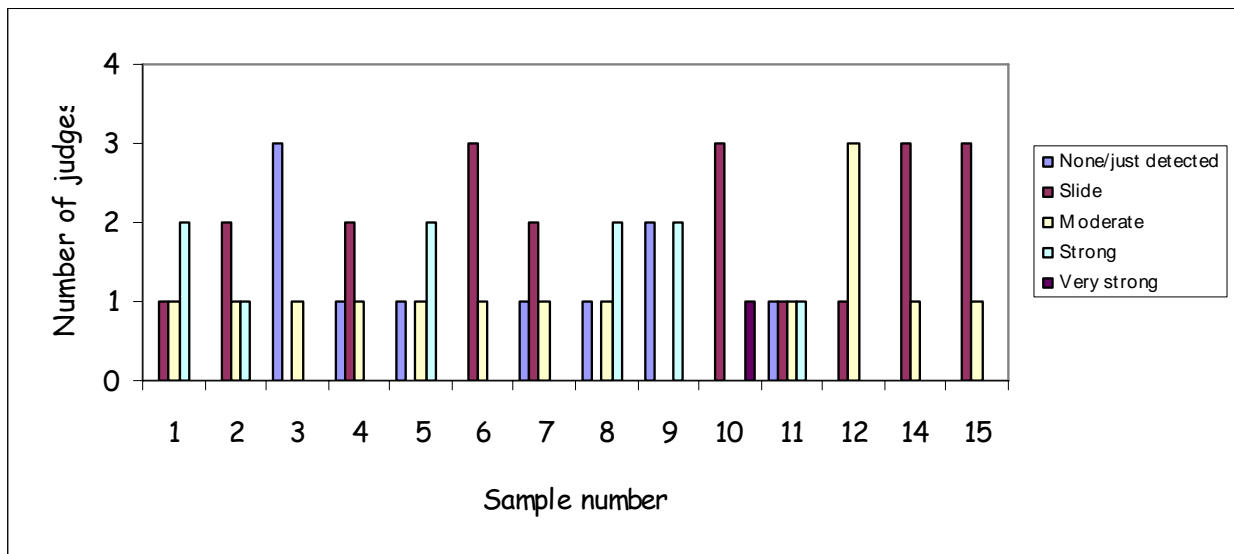


Figure 7.34. Scores given by 4 sensory judges.

Figure 7.35 shows the average score for each sample. According to those results, sample nr. 3 has the least odour and then sample number 4.

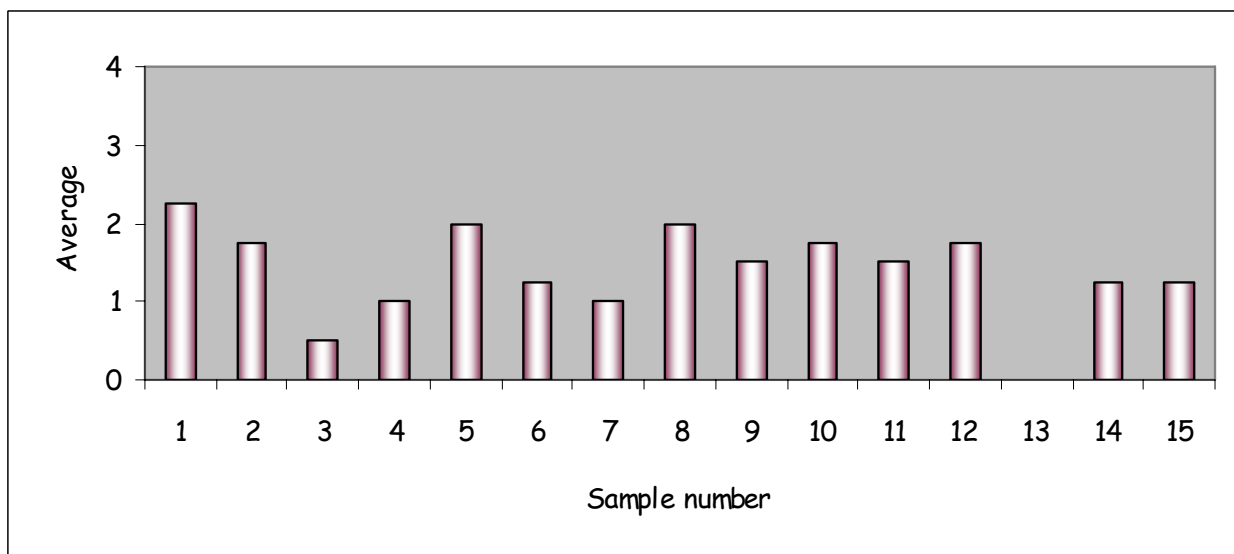


Figure 7.35. Average score from 4 judges evaluating odour intensity of dried fish protein samples. Sample nr. 13 doesn't exist.

3. Conclusions for Task 7

All dried protein samples presented excellent powder characteristics since powder runs were free flowing with high to medium rehydration ability, adjustable colour, low moisture content and low water activity as required for longer and safer storage. Generally, the relevant dried samples thermal or physical properties were improved by low drying temperature. Also, a combination of low and medium drying temperatures resulted in similar powder properties but with the advantage of higher drying rates. The results and data from this investigation are useful for further dried fish protein developments and should assist in the design and industrial scale up of equipment for drying and processing protein isolates from saithe and similar fish.

Based on the established conditions and particle characteristics, the conclusions are:

- The highest drying rate, SMER and dryer throughput is reached for smaller protein particle size (2 mm) and a temperature step-up combination (-5 and 20°C).
- Lighter dried protein powder is desirable compared to dark and improved brightness is attained for small particle size (2 mm) and step-up temperature (-5/20°C) and the best bed volume is 2 L (followed by 4 and 6 L). The red or green component is to be avoided in a protein powder. Powder colour improvement can be produced by reducing the red component using small particle size and step-up temperature (2 mm and -5/20°C) as well as small bed volume (2 L). Protein powder yellow component is desirable instead of blue (its complementary CIELAB space colour). The highest yellow component was for small diameter samples dried at step-up temperature (2 mm and -5/20°C) but with high bed volume (6 L).
- A 12 step procedure has been developed and an equation is proposed for consistent results protein rehydration measurements. This provided reproducible measurements and consistent results for all reconstituted protein powders runs. Among 2 mm and 2 L volume runs, dried the single temperature, the lowest the temperature the higher the hydration (-10°C followed by -5°C). Rehydration increased with the particle size or bed volume but there is an optimum value. The highest R_a was 1.62 for the sample dried at -5/20°C with bed volume of 4 L.
- ✓ It is advantageous to be able to adjust density of the dried protein as produced in HPD atmospheric freeze drying. If protein powder has low initial density it can be compressed to basically any desirable density. The dried protein depends on temperature and drops up to tenfold as it dries. The density was about 1830 and 180kg/m³ for the fresh-frozen protein and powder, respectively.
- The highest stress was for gels-powder dried at combined temperature (-5 and 20°C) and medium size particle (4 mm) but it seems that there is an optimal size between 3 and 6mm. Gel stress-strain behaviour was analyzed by classifying the protein powder in two groups. The higher stress group had wide range of particle size (from 2 to 8 mm) and all were dried with step-up temperature (-5 and 20°C). Even though differing in ultimate force-deformation, elasticity-analysis shows a linear relationship between stress-strain for the two groups. The elasticity modulus calculated for both indicates that they are similar stress-strain behaviour but further tests are needed to confirm this as well as the effect of bed volume.

Several isolated protein powder samples were successfully produced using heat pump drying technology combined with down-stream granulation and sieving processes. The successful drying modes are heat pump fluidized bed drying with step-up temperature.

The results from the dried samples can be summarised as having 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.

TASK 8 – SURIMI

1. Introduction

The main objective of this task 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. Another goal was to study the effects of adding soy protein isolate at different pH values, on the textural properties of the fish protein isolate.

1.1. Cryoprotection

Previous experience has revealed that disadvantageous characteristics of frozen fish mince include texture hardening or toughening, poor functionality (e.g. gelling, formability, water holding ability), discolouration and lipid oxidation and fishy odour development (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 adopted 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 (Auh *et al.*, 1999; Carvajal *et al.*, 1999; Herrera *et al.*, 1999; Park *et al.*, 1988; Synch *et al.*, 1990&1991) could be viable alternatives. These high-MW polyols and glucose polymers are believed to stabilise proteins by raising the glass transition temperature (T_g) of a solution (Levine & Slade, 1988a, b). Maillard browning reactions from reducing sugars may limit its application 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 scientists have shown that the addition of sugars like sucrose, maltose, and trehalose can stabilise proteins to the denaturing influence of drying (Carpenter *et al.*, 1987a, b, 1988, 1990; Crowe *et al.*, 1990; Matsuda, 1979, 1981). The same stabilising mechanisms are 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 the 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.* (2002) found that texture values during frozen storage were best maintained by samples containing 0,3% NaHCO₃ 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. 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 on 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 the gel properties of this product 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 up to 4% of total weight.

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.

Saithe mince

Fillets of 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 commonly used commercial blend in surimi, hence used as a reference.

2.1.1. Saithe protein isolate

Saithe protein isolate was obtained from the pilot plant at Haraldur Böðvarsson, 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.

2.1.2. Protein isolate of saithe, addition of soy protein at different pH

Eight samples of exactly one kg frozen alkali treated protein isolate from saithe was moved from -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 NaHCO₃ until the desired pH were reached. Initial pH of the samples was about 6,5.

2.1.3. 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 8.1**). The rest of the samples were kept on -30°C for about a week before analysis.

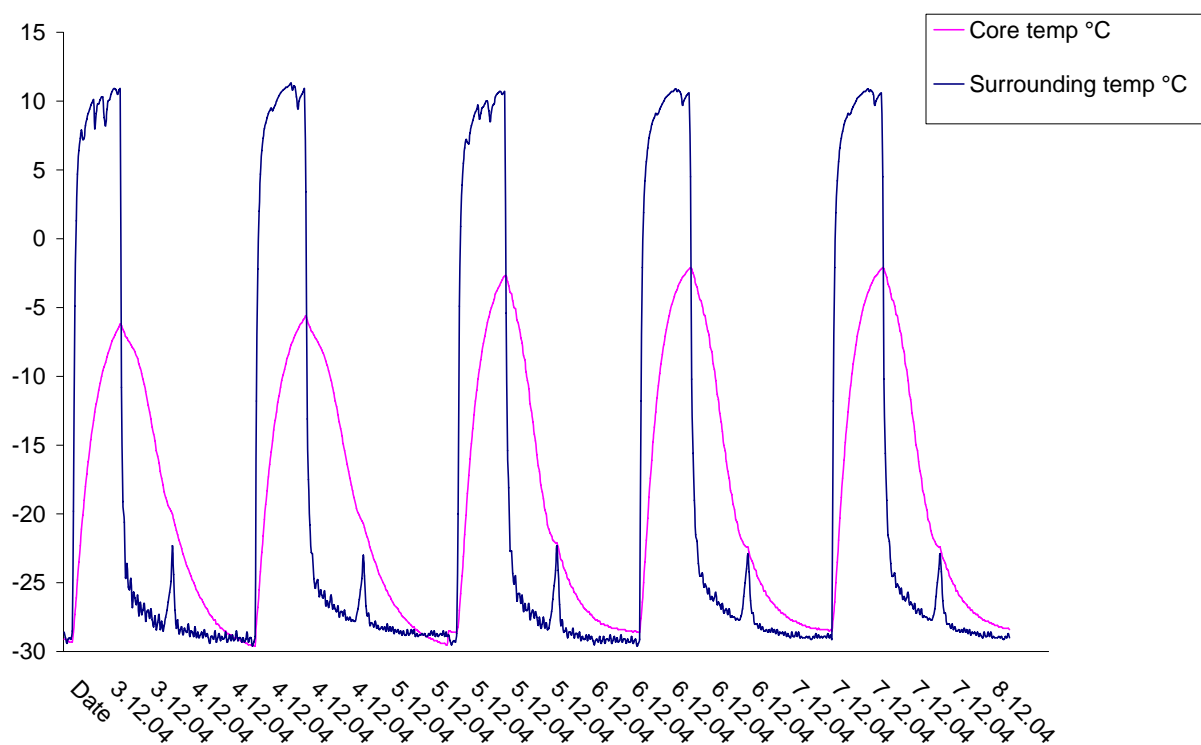


Figure 8.1. Example of the freeze-thaw cycle regime performed on saithe mince and commercial blue whiting samples with different cryoprotectants added. Temperature is shown on y-axis and time on x-axis.

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 (1990d).

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:

$$\text{Whiteness I} = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$$

$$\text{Whiteness II} = L^* - 3b^*$$

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

Protein gels were made by first thawing the mince to $-4,0^{\circ}\text{C}$ and then chopping until $-1,5^{\circ}\text{C}$, was reached in the mixture, before adding 2,0% granular NaCl into the mince. 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°C for 15 min. All samples were chilled in ice water for 30 min]. 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 8.1** 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 8.1. 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 8.2**, the 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. On the other hand trehalose gives good W II values (**Figure 8.2**). Raftilose® seems to prevent increased b* values during freeze-thaw abuse.

Table 8.2. 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

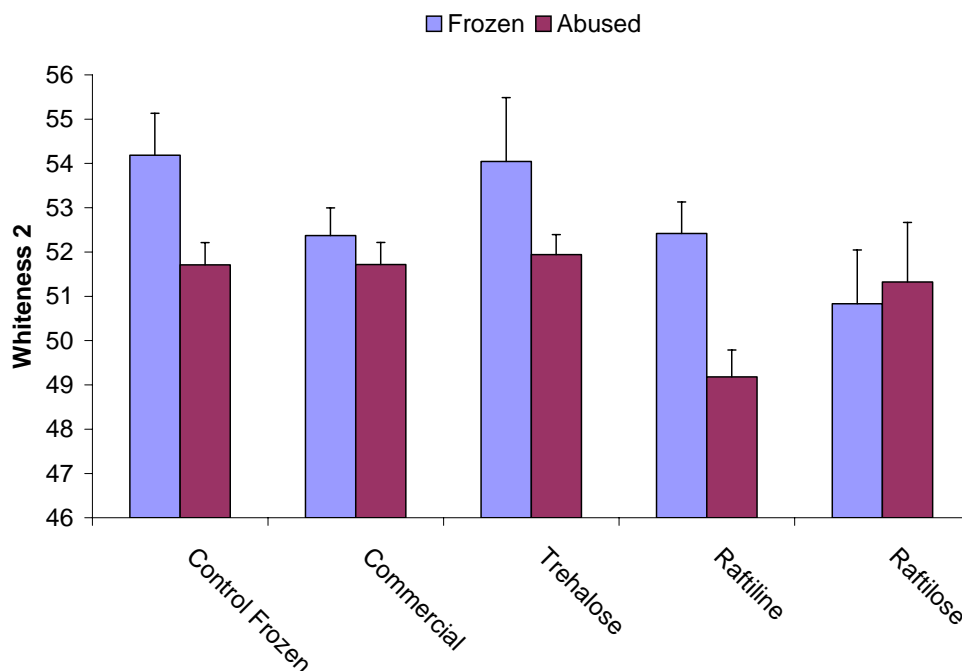


Figure 8.2. Whiteness II in gels made from frozen or freeze-thaw abused minced saithe with or without various cryoprotectants added.

3.1.3. Gel failure properties by penetration or torsion

It appears from **Figures 8.3 & 8.4** 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.

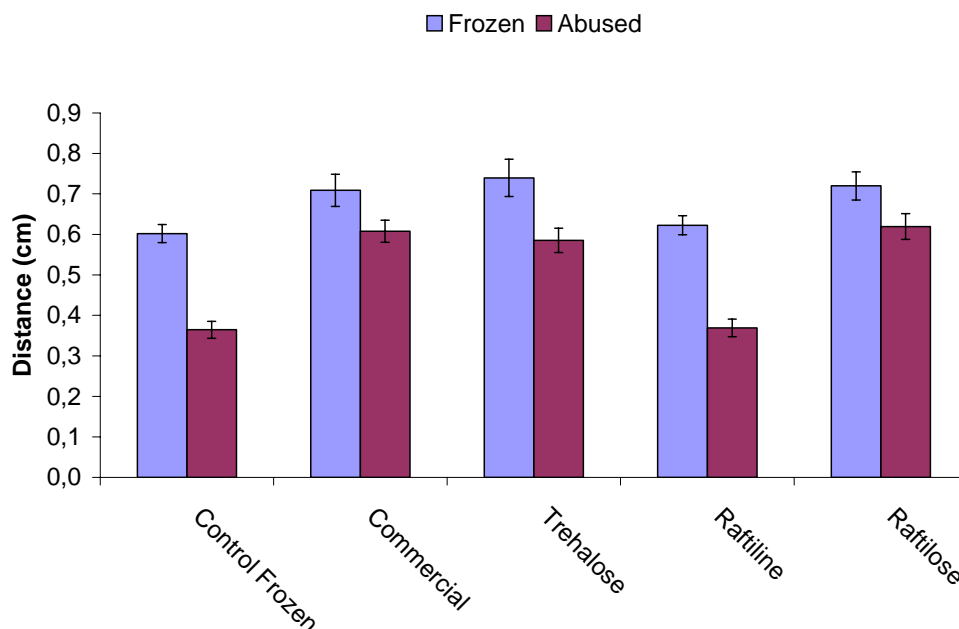


Figure 8.3. Distance from penetration experiments on gels made of frozen or freeze-thaw abused minced saithe with or without various cryoprotectants added.

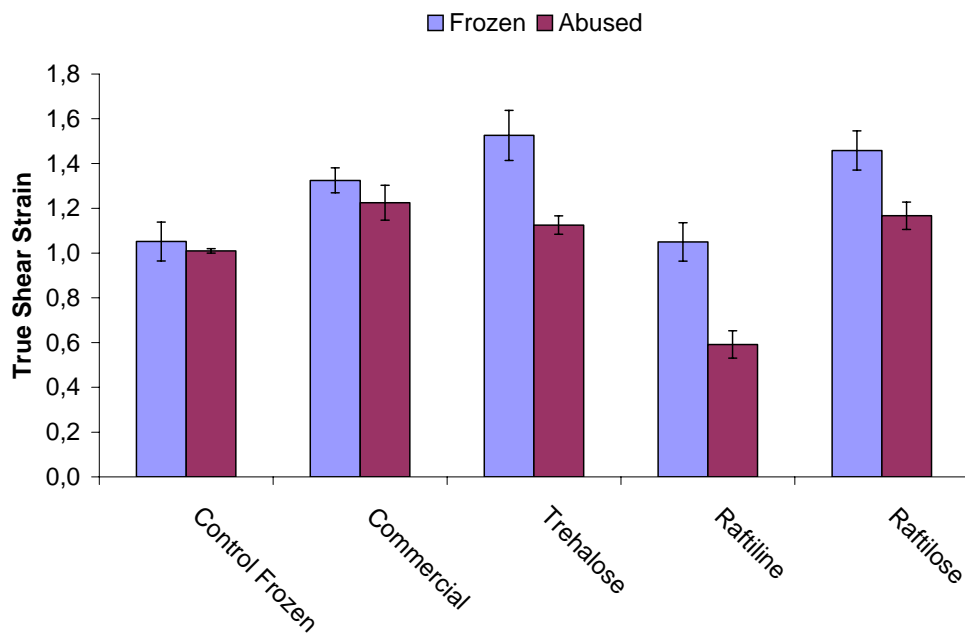


Figure 8.4. True shear strain from torsion tests on gels made of frozen or freeze-thaw abused minced saithe with or without various cryoprotectants added.

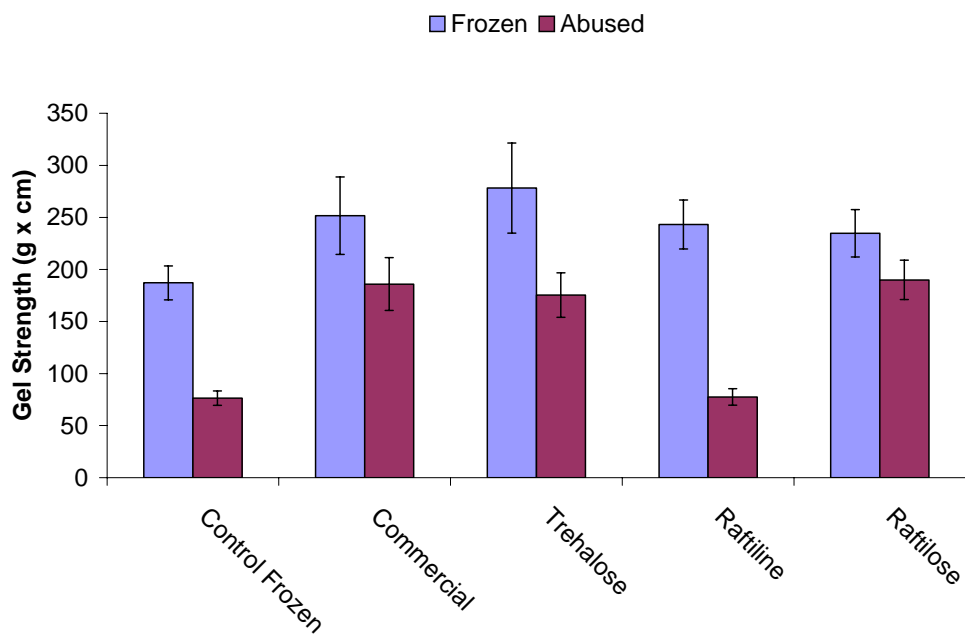


Figure 8.5. Gel strength from penetration tests on gels made of frozen or freeze thaw abused minced saithe with or without various cryoprotectants added.

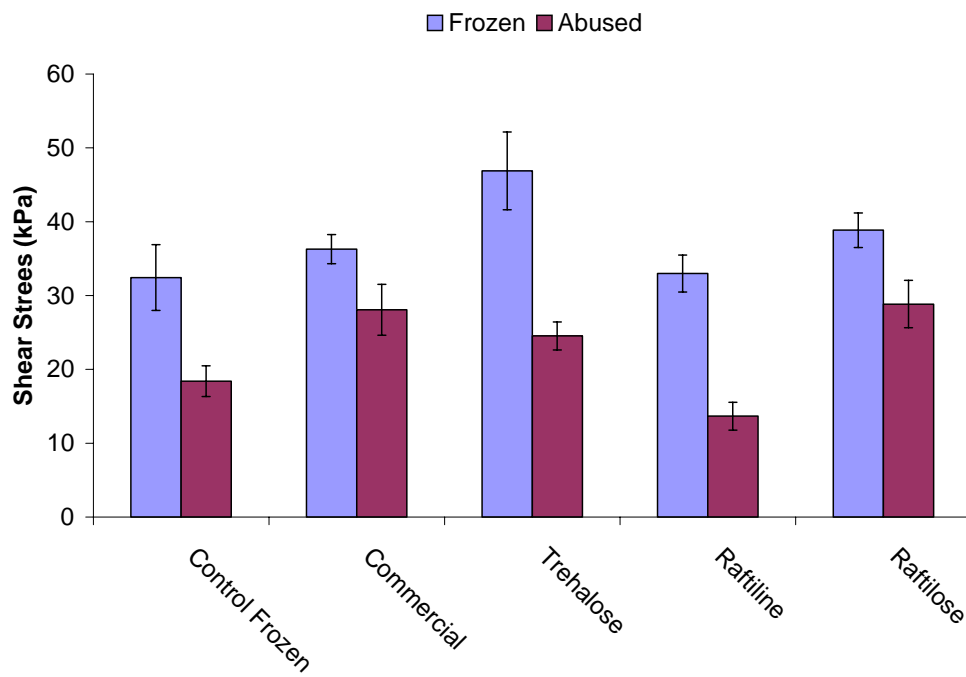


Figure 8.6. Shear stress from torsion experiments on gels made of frozen or freeze-thaw abused minced saithe with or without various cryoprotectants added.

3.2. Saithe protein isolate

3.2.1. Moisture content and pH

As shown in **Table 8.3**, there is a small difference in moisture 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 8.3. Moisture and pH in protein isolate before gelling. Protein isolate was made by acid- or alkali process, with or without TSPP 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

3.2.2. Colour and whiteness

As shown in Table 8.4, 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 1. 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)

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

There is also observed an increase in blueness (b^*) in the alkali group and in the Raftiline® samples, which greatly reduce calculated whiteness II value (**Figure 8.7**). 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.

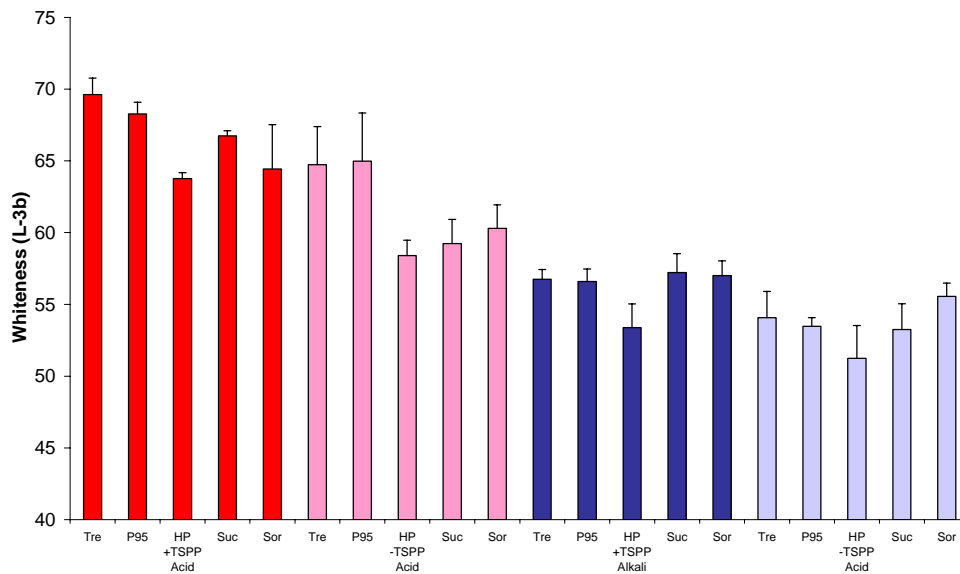


Figure 8.7. Whiteness (L-3b) in acid or alkali made gels of protein isolate from saithe with or without TSPP (+/-) and various cryoprotectants added. Red bars indicate acid extraction and blue bars indicate alkali extraction. Darker colour indicates addition of TSPP, lighter colour is without TSPP. The 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 8.8-11) that addition of TSPP protects the functional properties of the saithe proteins measured as true strain and distance. 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. Raftiline samples seem to give a harder but less elastic gel.

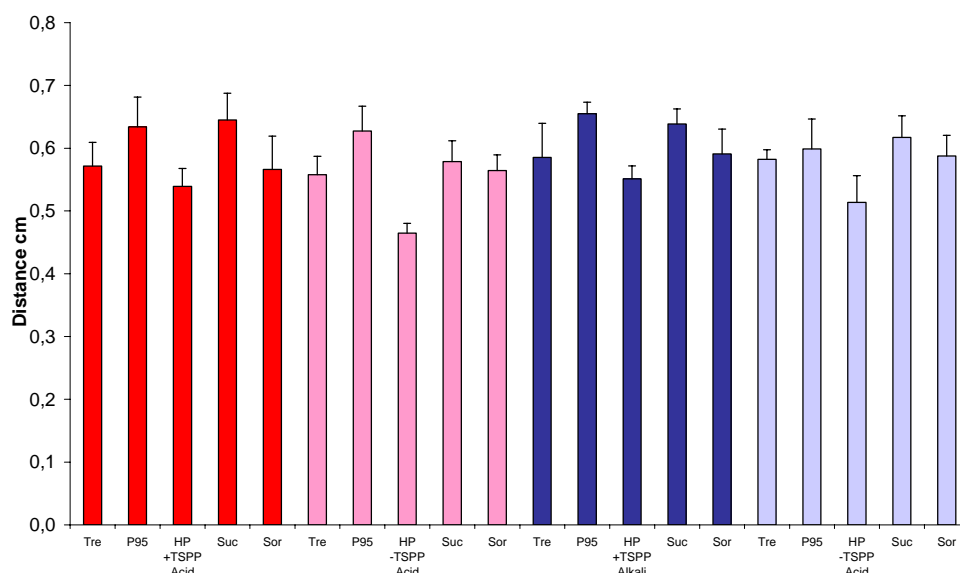


Figure 8.8. Distance from penetration experiments on gels from frozen saithe protein isolate with or without various cryoprotectants and TSPP added. Explanations for colours see figure 8.7.

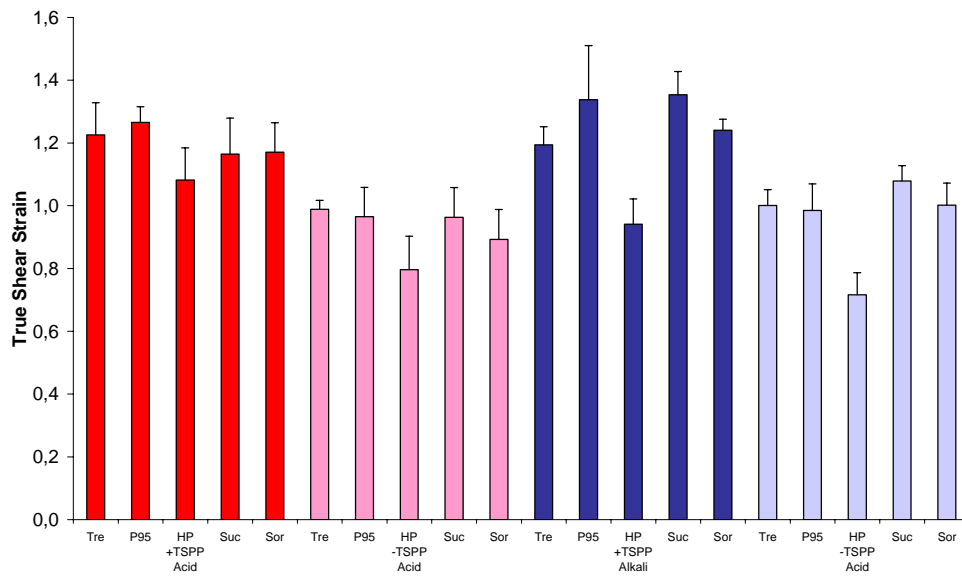


Figure 8.9. True shear strain from torsion experiments on gels made from frozen protein isolate with or without various cryoprotectants and TSPP added. Explanations for colours see figure 8.7.

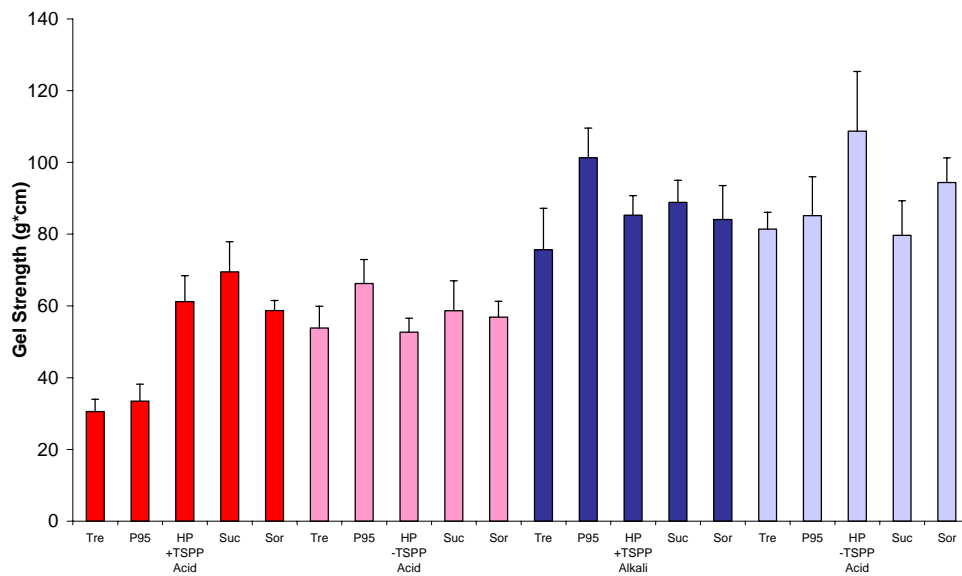


Figure 8.10. Gel strength from penetration experiments on gels made from frozen saithe protein isolate with or without various cryoprotectants and TSPP added. Explanations for colours see figure 8.7.

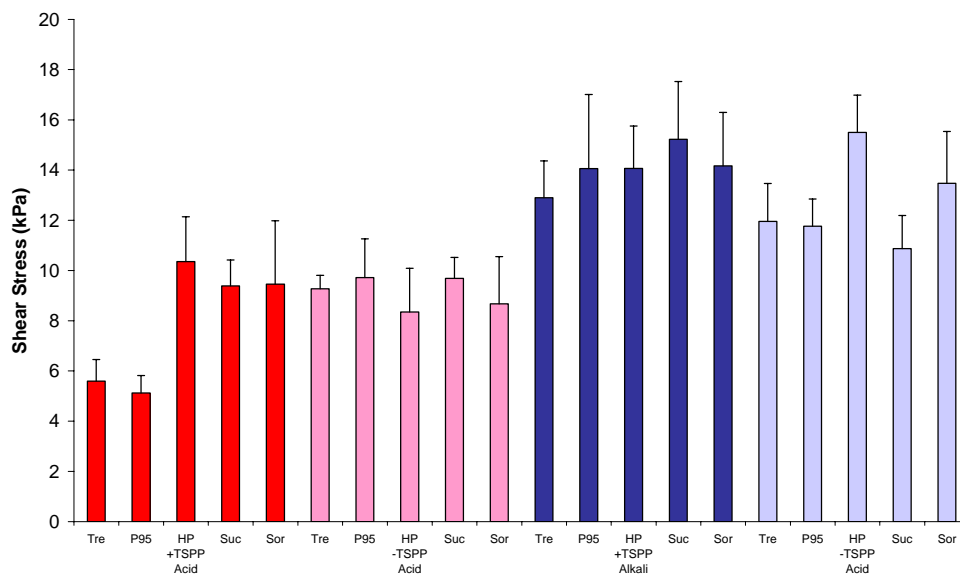


Figure 8.11. Shear stress from torsion experiments on gels made from frozen protein isolate with or without various cryoprotectants and TSPP added. Explanations for colours see figure 8.7.

The two first samples contained relatively more water (**Table 8.3**) than the rest, and this is probably the reason for lower gel strength and shear stress values. Results regarding distance and stress values, are less affected by water content and can still give good evaluations on the protein quality in the products. As previously stated 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/rafitlose®/raftilin®/sukrose/sorbitol/mix).

The samples included in the sensory evaluation, **Table 8.5**, were chosen from the most promising products after texture and colour analysis.

Table 8.5. Samples chosen for sensory analysis.

Samples	Method	TSPP	Cryostabiliser
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 zero to high intensity. A description of the chosen characteristics and how they were used is given in **Table 8.6**.

Table 8.6. 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 cm² pieces. They were then put in plastic containers with a 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 Turkey'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 8.7 gives an overview of significant differences sensory characteristics of the products. 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 8.3**).

The alkali-treated samples (N13, 14, 16, and 17) had significant higher grey colour score. 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 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. The panel commented on this sweet taste as being somewhat artificial, excessive, and nauseating.

Table 8.7. Comparison of the sensory attributes of nine different protein isolate products. 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	4,6 ^a	3,8 ^a	4,1 ^a	3,9 ^a	4,7 ^a	4,8 ^a	4,5 ^a	4,6 ^a	4,1 ^a	3,5 ^a
Rancid odour	***	1,4 ^{ab}	2,0 ^{ab}	1,1 ^b	1,9 ^{ab}	1,7 ^{ab}	2,2 ^{ab}	2,6 ^a	2,5 ^a	1,6 ^{ab}	1,1 ^b
Grey colour	***	1,2 ^{cd}	4,2 ^a	1,0 ^d	4,8 ^a	2,3 ^b	4,6 ^a	1,9 ^{bc}	4,4 ^a	4,9 ^a	4,3 ^a
Coherence	***	2,1 ^{ef}	5,3 ^b	2,0 ^f	5,7 ^b	4,2 ^d	5,2 ^{bc}	2,7 ^e	4,5 ^{cd}	9,8 ^a	10,0 ^a
Sweet taste	***	4,6 ^b	4,2 ^b	4,3 ^b	4,2 ^b	7,0 ^a	6,9 ^a	4,3 ^b	5,0 ^b	4,6 ^b	5,0 ^b
Salt taste	***	6,2 ^{ab}	4,9 ^{cde}	6,4 ^a	4,8 ^{cde}	4,3 ^{de}	4,1 ^{ef}	5,7 ^{abc}	5,4 ^{bcd}	4,0 ^{ef}	3,1 ^f
Acidulous taste	*	3,4 ^{ab}	3,7 ^{ab}	3,7 ^{ab}	3,4 ^{ab}	4,1 ^a	3,8 ^{ab}	3,8 ^{ab}	3,7 ^{ab}	2,9 ^{ab}	2,7 ^b
Watery	***	7,0 ^a	4,4 ^{cd}	7,0 ^a	3,8 ^d	5,3 ^{bc}	4,5 ^{cd}	6,1 ^{ab}	4,0 ^d	0,8 ^e	1,1 ^c
Toughness	***	1,3 ^e	2,8 ^{bc}	1,5 ^{de}	3,0 ^b	2,1 ^{cd}	3,3 ^b	1,9 ^{de}	3,2 ^b	6,7 ^a	7,2 ^a
Rancid taste	***	2,0 ^{bc}	2,5 ^{abc}	2,3 ^{bc}	1,8 ^{bc}	2,1 ^{bc}	2,5 ^{abc}	3,8 ^a	3,1 ^{ab}	1,8 ^{bc}	1,5 ^c

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 8.12. This plot confirms the findings in the ANOVA analysis, shown in **Table 8.7**. 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 were 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 coloured, with a 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. Samples N4 and N16 containing sucrose were characterised by the panel as very sweet, and are close to the sweet taste variable in the plot.

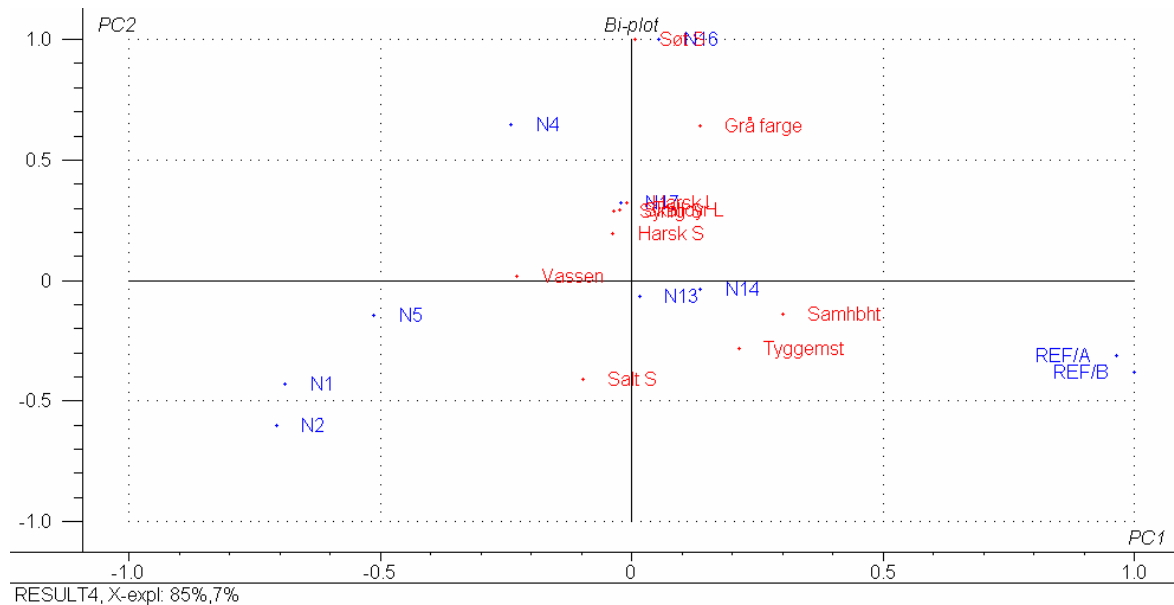


Figure 8.12. Principal component analysis (bi-plot) of simple means over PC1 and PC2.

3.3. Conclusion

The choice of acid or alkali extraction method is essential for the quality of the FPI. The extraction methods give significantly ($p < 0,05$) different results regarding all parameters assessed, except for true strain ($p = 0,056$). Texture values were generally higher in alkali samples than in 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 which are made using acid treatment (N1, 2, 4, and 5) seem less coherent when pulling and are softer and more watery when chewing. In addition, the acid-treated samples were considered saltier than the alkali samples. With 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 8.3**). The alkali-treated samples (N13, 14, 16, and 17) had significant higher grey colour score.

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 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 losing 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 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.

In December 2004 a test run for 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 dish centrifuge to remove fat phase in the Icelandic pilot plant
- It was impossible to use decanter centrifuge or squeezing trough cloth to remove water in the Icelandic pilot plant
- The samples contained ~90 % moisture on arrival in Norway
- The herring- FPI did not at all form a gel

Work package III -Product

TASK 9 – INGREDIENT

The aim of this task was a literature research and a limited market survey to evaluate the market situation regarding products with fish proteins added as nutritional/functional-raising agents. Furthermore, to develop prototypes that should be tested for consumer attitudes, stability, functionality etc.

Whereas the project was not successful in making good fish protein products, it was impossible to develop a prototype to be tested on the market. The market situation for protein ingredients was evaluated with a literature research which shows that it might be a bright future for fish proteins isolate made according to the methods developed in the project.

The protein market

The market for protein ingredients can be divided into the following categories:

- ✓ Sports nutrition
- ✓ Functional foods - also named Nutraceuticals
- ✓ Medical foods and clinical nutrition
- ✓ Protein Enrichment or marinade products

The major product categories of proteins are

- ✓ Soy based proteins
- ✓ Whey protein
- ✓ Casein
- ✓ Gelatin
- ✓ Dried egg whites
- ✓ Fish proteins

Fish proteins

The use of fish proteins has been eclipsed by the introduction of new food and nutraceutical products claiming functional properties based on soy and whey derivatives. There is some usage today for fish proteins in the dietary supplement business as a gut health enhancer and in sports nutrition as a muscle builder, with the typical ingredient being either a fish protein concentrate or isolate. Fish protein hydrolysates have mostly been limited to feed, fertilizer, and some limited use in fermentation and for media growth in the life science market.

Many in the seafood-, food science-, and fish processing industries are hopeful that fish-derived proteins, protein derivatives and extracts will soon become more appreciated in the higher value markets. Although there is great excitement regarding the recent advancements and discoveries regarding the use and value of such ingredients within this particular segment of the food industry, higher value fish proteins have not really made it to the highest echelon yet in the general markets.

The commercial markets for fish proteins, excluding fish as a “centre-of-the-plate” muscle protein for human consumption, have traditionally been driven by the use of fish meals in industrial applications, and the use of various fish by-product ingredients towards the fertilizer, feed, and more recently, the nutrition and food processing markets.

Range of Fish Protein Products

Typically, the more highly processed the fish protein, the higher the protein content of the ingredient. Fish protein ingredients in the market today include fish silage, fishmeal or flour, fish protein concentrate, fish protein isolate, fish protein hydrolysates and fish gelatin. There is a tendency in the market to use these terms interchangeably, which creates some confusion. Product specifications can help to clarify which product is being referred to. The different types will now be discussed shortly.

Fish silage. This product is considered a low-grade product, used primarily as a feed source for animals and as a fertilizer, or “liquid fish”. Fish silage is not used for human consumption. The silage may be used directly in feed or processed further by separation of the oil and evaporation to produce a protein concentrate. Norway is the biggest fish silage producer – producing about 140,000 MT per year, mainly from aquaculture by-products (salmon) (Rustad, 2003).

Fishmeal/Flour The fishmeal market is the most well defined and characterized of the fish proteins, since there is a global market for this product with well documented historical supply and price information available. The market for fishmeal is established for various livestock feeds and also in aquaculture feed (as a protein source for non same-species fish). Fishmeal is a very cheap potential Fish Protein Concentrate, but it is not intended for human consumption, because it is not normally made under sufficiently hygienic conditions to rule out occasional contamination of disease-causing bacteria. Fishmeal made under hygienic conditions is called FPC type C. Fish flour is not a precise term, but is the name sometimes given to a product such as FPC type A which, when ground to a fine powder, can be used as an unobtrusive additive to prepared foods (Rustad, 2003).

Fish protein concentrate (FPC) is defined by the FAO as any stable fish preparation, intended for human consumption, in which the protein is more concentrated than in the original fish. The Food and Agriculture Organization of the United Nations (FAO) defines three types: Type A: virtually odourless and tasteless powder having a maximum total fat content of 0.75%. Type B: powder having no specific limits as to odour or flavour, but definitely having a fishy flavour and a maximum fat content of 3%. Type C: normal fishmeal produced under satisfactorily hygienic conditions. The protein content of FPC depends on the raw material used and the extent to which water has been removed, but the products normally contain at least 65% protein and, in type A, up to 80% protein.

Fish protein hydrolysates can be defined as proteins that are chemically or enzymatically broken down to peptides, and sometimes fall under the definition of a FPC. Biological processes using added enzymes are employed more frequently to make hydrolysates.

Fish gelatin has been available on the market for many years, but to date the market for gelatin from fish still has remained a niche market. The volumes currently consumed are estimated at 1,000 to 1,500 tons on a global basis. This compares to a total gelatin market of 250,000 tons worldwide (approximately 60,000 tons total are produced in the U.S. each year). Despite concerns over BSE, consumption of gelatin has grown over the past ten years with growth for porcine and bovine gelatin estimated currently at about 2% per annum. Market applications include food, pharmaceutical, microencapsulation, photographic industry, and wound care. Hoffmann- La Roche is believed to be the largest single consumer of fish gelatin in the world, using the product primarily for

microencapsulation of vitamins and other pharmaceutical additives. The company is believed to account for some 30% of the global consumption. The rest of the market is very dispersed. The major producer in North America is Norland, believed to be producing approximately 500 tons of fish gelatin, in 5 to 6 different grades.

Fish Protein Isolates (FPI) are typically used as products and as inputs for making surimi seafood analogues. The surimi market will be discussed in Task 10. One of the more promising uses of fish protein isolates is the use of them as protein-enrichment products. They will now be discussed in more detail.

The interest and demand for new ingredients and technologies to extend shelf-life, reduce shrinkage, and increase product weight of value-added meat and seafood is strong and growing, based on the functional capabilities of certain ingredients, paired with common food processing techniques. One of the most common ways to add water and water-soluble ingredients in meat is via tumbling. Combining the process of tenderizing and marinating, tumbling mixes the meat and marinade, loosening up the muscle to allow absorption of moisture and moisture-retaining marinade/ingredients. Injection is another technique used to force curing agents into the muscle.

Typical ingredients used for moisture retention include sodium phosphate, carrageenans, alginates, and various vegetable starches. Soy protein isolates and soy protein concentrates have been promoted as allowing meat products to have optimized marination/brine levels and high succulence in high-abuse circumstances, such as freezing, precooking, microwaving from frozen, and extending hold time after cooking, according to company representatives. Other uses of vegetable and animal-based functional protein ingredients in food include increasing cooking yields, reduction of water purge, and for meat replacement and flavour enhancement. Recently, a company named Proteus Industries has applied for, and received, permission from the FDA to market fish-based protein ingredients as an enrichment product for seafood. Steve Kelleher, who originally worked with Herbert Hultin on the acid protein isolation process (Hultin & Kelleher, 1999), is one of the owners of the company. Commercialized under the NutraPure brand, Proteus has licensed several seafood companies on the East Coast, including North Atlantic, Portland, Maine, and Good Harbor Fillet, Gloucester, MA, to incorporate the fish protein enrichment into select seafood products, and test market the products in Hannaford Brothers supermarket and Sam's Club. Good Harbor has incorporated the processing technology into a line of low-fat and reduced-fat frozen seafood items, and is marketing the products to school lunch programs, the military, and healthcare facilities nationwide. According to Proteus, NutraPure is created by extracting protein from fish trimmings, removing excess water, and then the protein is injected back into seafood, which provides for less drip loss, longer product shelf life, and increased flesh moisture. At IFL, trials on injection of a protein isolate made with the alkaline process are very promising.

Fish Protein Future Outlook

Overall, value-added fish proteins seem to have a rosy future. Much work is being done to characterize the products, their bioactive properties, and the potential benefits of the protein fractions. Large research efforts such as SEAFOODplus in Europe indicate that there is a serious intent in bringing fish protein products in line with the other non-marine protein ingredients.

New research has shown that fish protein and fish protein hydrolysates (peptides) have similar or even better bioactive properties than protein and hydrolysates made from other protein sources. For example, it has been demonstrated that fish protein and fish protein hydrolysates have a positive effect on insulin intolerance in obese rats, an effect not found when the rats were fed with soya- or whey proteins (Ravallec *et al.*, 2001). Other research have also shown positive effect of fish proteins on insulin activity (González *et al.*, 2001; Hurley *et al.*, 1995).

In another research, rats suffering from high blood pressure were fed, on the one hand with 20% fish protein and, on the other, with 20% casein. Otherwise the feed had the same ingredients, including 5% isio oil. Results showed that blood pressure was significantly reduced in the rats that were fed with fish protein compared to casein (Ait-Yahia *et al.*, 2003). Furthermore, fish protein hydrolysates have been shown to have a positive effect on Calcitonin gel related peptide (CGRP) that controls digesting (Rousseau *et al.*, 2001).

To be able to market fish proteins as ingredients in functional foods and sport nutrition it is important to show those effects in humans. That is one of the goals in SEAFOODplus where several partners in this project are participating.

A summary over protein pricing is given in **Table 9.1**. Those numbers apply for the US market. As can be seen the pricing is very different. When looking at fish proteins it can be seen how big the range is, from 1,25 \$/kg fish meal to 125 \$/kg fish protein concentrate.

Table 9.1. Protein Pricing Summary*

Ingredient	Protein level [%]	Price per kg [\$]	Cost per kg protein [\$]
<i>Fish</i>			
Fish meal**	70	0,7	1,25
Fish protein concentrate	65-80	3,5 – 100	5 - 125
Fish protein isolate	95	19	20
Fish protein hydrolysates	90	3,5 - 30	4 to 33
Fish gelatin (Food grade)	Not available	15 - 20	n/a
Fish collagen hydrolysate	90-93	20 - 35	22 - 38
<i>Soy</i>			
Soy flour	47	0,4	0,8
Soy protein concentrate	70	2,5	3,6
Soy protein isolate	90	3,9	4,3
<i>Whey</i>			
Whey, dry sweet	14	0,4	2,8
Whey prot conc 34%	34	1,0	2,9
Whey prot conc 80%	80	2,8	3,4
Whey prot conc 80%, instant	80	3,3	4,0
Whey prot isolate	90	5,7	6,4
Whey prot isolate, instant	90	7,7	8,6
<i>Casein</i>			
Casein, rennet	82	5,2	6,3
Sodium caseinate	89	5,3	6,0
Casein, acid	88	5,0	5,6
<i>Egg</i>			
Egg white, dried	81	13,4	16,6
Eggs whole	47	6,8	14,6
<i>Gelatin</i>			
Gelatin	90	5,0	5,5
Gelatin, hydrolyzed	90	6,6	7,3

*Strategro, 2005

**Iffo, 2005

Conclusion for Task 9

As can be seen on Table 9.1, by converting raw material, which today is used for fishmeal into higher quality products, it is possible to increase the turnover in the fish industry. The use of the protein isolate as an injection material is a very promising possibility for use of the protein isolate. The future for fish proteins can therefore be bright, both as protein isolates to be used in marinades and injection or as ingredients in functional foods.

The results obtained in this projects is a big step toward reaching that goal.

TASK 10 – GEL PRODUCTS

1. Objectives

The aim of this task was to evaluate the market situation regarding novel surimi-based products with a literature research and a limited market survey. Furthermore to develop a few product prototypes and test for consumer attitudes, stability, functionality etc.

2. Surimi market

Since the project was not successful in making good fish protein product, it was not possible to develop prototypes to be tested on market (as for Task 9). The market situation for surimi was evaluated with a literature research. The main source is a Globefish (2005) report but other sources gave similar prospects for the surimi market (Gain, 2003; FAS, 2005). The surimi market is very dynamic and prices fluctuate between years, as can be seen on **Figure 10.1** which shows changes in the price of surimi in France in 2003 and 2004. Average unit values for French surimi imports declined over the 2003 / 2004 period. Prior to the fourth quarter of 2003, average values for surimi from the USA were significantly above those for Chile, but the gap has since closed with unit values from the two countries roughly in line with each other since then (Globefish, 2005).

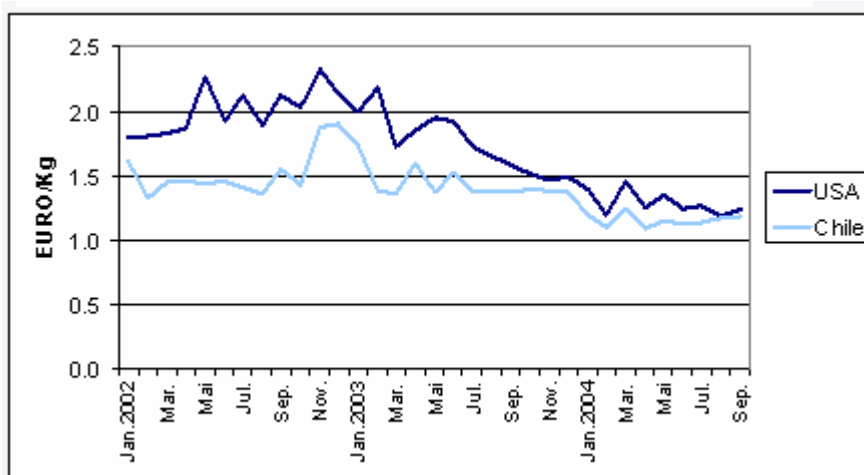


Figure 10.1. French import unit values for frozen Surimi (Globefish, 2005).

EU15 surimi imports from third countries (countries outside EU) were worth approximately €150 million per year during the last five years. The largest exporting countries to the EU15 in 2003 were Thailand (€31 million), USA (€25 million) and the Republic of Korea (€25 million). The USA has traditionally been the largest exporter of frozen surimi to the EU but Chile significantly increased its share in 2002 and 2003 (**Figure 10.2**).

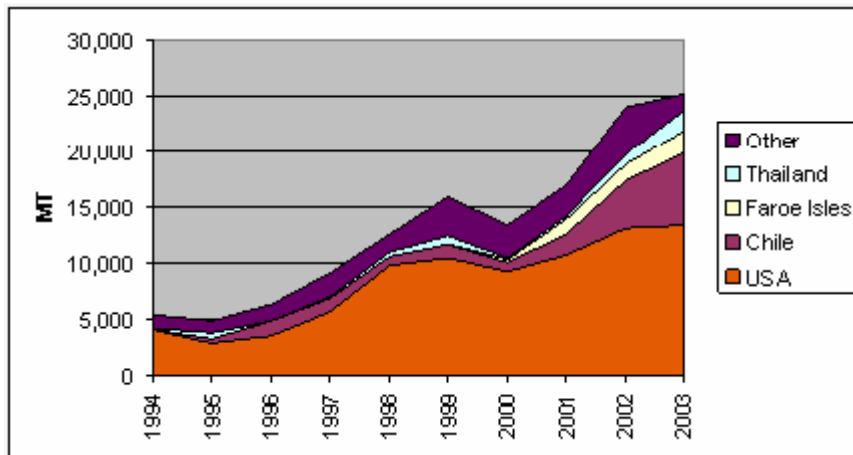


Figure 10.2. EU15 imports of frozen surimi from countries outside EU (Globefish, 2005).

The largest exporters of prepared surimi include Thailand and the Republic of Korea. China also had a large market share between 1998 and 2001, but in 2002 and 2003 Lithuania captured most of this share (**Figure 10.3**). During the first nine months of 2004, Thailand, China and USA increased volume exports to the EU while exports from all other major export countries decreased (Globefish, 2005).

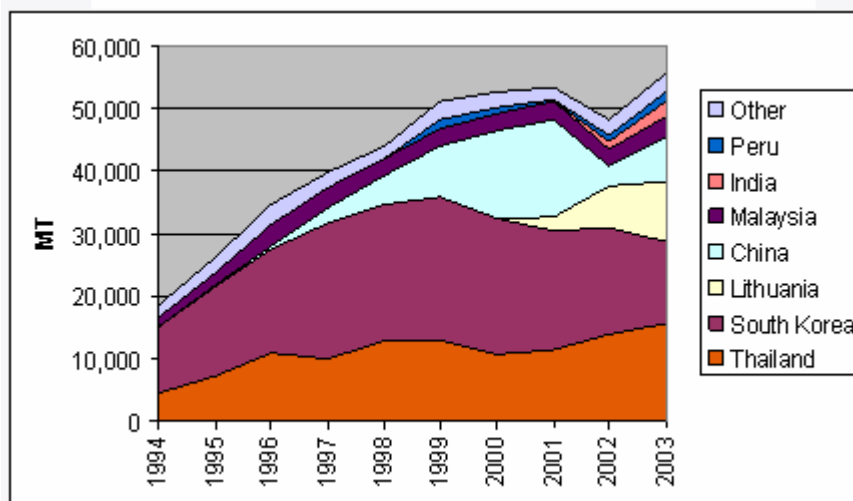


Figure 10.3. EU15 import of surimi preparations from countries outside EU (Globefish, 2005).

Export of U.S. surimi to the European Union (EU) continued to increase in 2004, driven by growing consumption of analogue seafood products and increased reprocessing of surimi in Europe. Over the past ten years, the U.S. surimi sales volume skyrocketed from 2,595 metric tons (MT) in 1995 to 28,917 MT in 2004, a more than eleven-fold increase. The export value to the EU also rose dramatically during the period, from \$6.2 million to \$44.8 million. France, Lithuania and Spain were the top EU destinations for U.S. surimi (FAS, 2005).

Exports of surimi to Lithuania in 2004 rose to 7,156 MT (up 37 percent from 2003), valued at more than \$11.1 million. According to seafood industry sources, Lithuania is primarily a re-processing market, which imports surimi and exports consumer-ready products to West European countries, such as France and Germany (as discussed in Figure 10.3). At the same time as export to Europe of US surimi has increased, the sale to Asian

countries like Japan and Korea have declined. As Europe is learning to appreciate surimi better and better, the original country for surimi production and consumption, Japan, is losing interest in their traditional products from surimi like kamaboko. Therefore the use is going down in Japan (FAS, 2005).

There are indications that the downward trend in surimi prices evident during 2004 may have come to an end. Reports of limited supplies of surimi in Asia, following the tsunami disaster, point to an increased focus on Alaska Pollock as a surimi source and a certain stabilization of prices, at least in dollar terms, in this segment during the first quarter (Globefish, 2005).

When the numbers presented here in relation to price for exported surimi from USA to Europe it shows that surimi costs around 1,6 \$/kg in 2004 (FAS, 2005). Similar number is given by Globefish (2005) in **Figure 10.1**. Commercial surimi on market includes 8.3% cryoprotectants and 75 to 80% water and 12 to 17% protein. The price for proteins is therefore 10 to 14 \$/kg in 2004. The surimi imported from USA is mainly of highest grade. It is interesting to compare this number to values given in **Table 9.1** where the prices for fish protein is from 1.25\$/kg protein for fish meal up to 125 \$/kg protein for highest value fish protein concentrate. The price for fish protein isolate is 20\$/kg protein. To aim at production of fish protein isolate might therefore give a better value than by aiming at the surimi market.

3. Conclusion from Task 10

Prices and the market for surimi is very changeable, as for other seafood products, where quotas and weather changes around the globe have a big effect. In the past few years since this project started, big changes have been in the world surimi production where new exporting countries like India, China and Russia have come into the market and affecting prices (Gain, 2003). As previously stated the main possible markets for the protein isolate is in the injection market area.

C. CONCLUSION

Raw material optimisation

Lab work showed that herring as raw material for the process is affected by storage. This is observed both for freezing and storage on ice. The protein solubility decreased and viscosity increased resulting in lower yield of the process. Methods were developed to reduce the increased viscosity but they all affected the solubility and therefore also the yield of the process. Further storage of the frozen raw material up to around 6 months does not greatly affect the viscosity and protein solubility properties.

Pilot plant

The pilot plant works very well where it is possible to adjust pH and other parameters. There are still problems with fat removal when working with fatty species. Another type of centrifuge might help but most probably the emulsion is too tight. More work on oily fish might give alternative solutions. Most likely the main use of the Hultin process in a plant production is to isolate proteins from lean species for different uses, e.g. injection, raw material, for enzyme hydrolysis, drying etc. The main use of the process for fatty species would be to make a protein isolate for use in injection in fatty species. The waste water from the final step of the process includes very low content of chemicals. Use of ultra filtration to recover such low protein content to use the proteins further is not feasible. The conclusion of the project is that the lipid phase, collected from skimming and after centrifugation, is entrapped in an emulsion. This pilot plant setup it is therefore unsuitable to obtain cold processed herring oil. Other byproducts include skins and bones which can be used as raw material for fishmeal production in a fishmeal plant.

Stability of product

It was repeatedly observed, both on lab-scale and pilot-scale, that extensive lipid oxidation developed during acid and alkaline protein isolation from herring. Because of this, there were usually no further changes in lipid oxidation products during the subsequent storage on ice or at -18°C . It is therefore very important to use antioxidants through all the steps of the process.

Drying

All dried protein samples presented excellent powder characteristics since powder runs were free flowing with high to medium rehydration abilities, adjustable colour, low moisture content and low water activity as required for longer and safer storage. Generally, the thermal or physical properties of the relevant dried samples were improved by low drying temperature. Also, combined low and medium drying temperature resulted in similar powder properties but with the advantage of higher drying rates. The results and data from this investigation are useful for further dried fish protein developments and should assist in the design and industrial scale up of equipment for drying and processing protein isolates from saithe and similar fish species. Several isolated protein powder samples were successfully produced using heat pump drying technology combined with down-stream granulation and sieving processes. The successful drying modes are heat pump fluidized bed drying with step-up temperature.

Although the drying went well and the powder characteristics were acceptable, the quality parameters were not. The results from the dried samples can be summarised as having an intense rancid odour, with a dark yellowish or grey colour. The rehydrated protein isolate

samples did neither produce a proper gel nor hold water very well. Rehydration time and homogeneity of the dried samples depended on granule size. To improve those quality parameters, further development is needed. The results give good indications about which drying parameters give the best results. The high rancid odour indicates that, as was shown for herring, oxidation occurs through the process, whereas lean fishes contain phospholipids which oxidise easily. Antioxidants should therefore be used in the process, both when working with lean as well as fatty species.

Gelation parameters

The choice of acid or alkali extraction method is essential for the quality of the FPI, as the alkali process gave a better quality product regarding all parameters assessed, except for true strain. The gel strength of saithe protein isolate was low, under 500 g*cm which is considered as the target for good surimi product.

Different cryoprotectants were tried out. Raftiline®P95 was the only cryostabiliser which differed significantly from the others in all tests, except for gel strength and shear stress. 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 losing textural or colour properties. 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, adding TSPP is recommended due to favourable effects on texture and colour values. 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.

Herring protein isolate produced in the pilot plant had too high moisture content to make gelation possible. In summary it can be said that saithe protein isolate gave a gel but not herring protein isolate.

Market situation

The future for fish proteins can be considered bright, both as protein isolates to be used in marination, and injection or as ingredients in functional foods. Further trials, research and development than were possible in this project are necessary to reach that goal.

The price and market for surimi is very fluctuating, as for other seafood products where quotas and weather changes around the globe have a great effects. Since the project was unsuccessful in making a product with good gelling qualities, it is not realistic to aim at the surimi market. As previously stated, the main possible markets for the protein isolate is in the injection market area.

D. NOMENCLATURE

a_f, b_f, L_f	Final chromacity-colour coordinates of the CIELAB space
ATP	Adenosine 5'-triphosphate
CIP	Cleaning-In-Place
COP	Coefficient of performance
DE	Degree of polymerisation
dh/dx	Dryer thermal efficiency
DMNL	Dry matter no lipids
dx	Air absolute humidity during drying or condensation, kg/kg
$d\tau/d\varepsilon = \Delta\tau/\Delta\varepsilon$	Tangent of the line crossing the initial and ultimate stress-strain point
FPI	Fish Protein Isolate
ICP-AES	Emission atomic spectrometry with an excitation in the inductively coupled plasma
m_d	Mass of dry protein powder, kg
m_f	Mass of drained filter, kg
m_w	Mass of adsorbed water, kg
PE	Peroxide value
PI	Protein Isolate
PSI	Possible solubility inhibiting
Q_o	Evaporator capacity, kW
SMER	Mass of water evaporated using one unit of energy input, kg/kWh
SPI	Soy Protein Isolate
STPP (TSPP)	Sodium Tri Polyphosphate
V	Bed volume, liters
VE	Effective volume
W_c	Compressor work, kW
ε_o	Initial relative shear strain, dimensionless
ε_r	Relative shear strain, dimensionless
ε_{ult}	Ultimate relative shear strain, dimensionless
ρ_f	Final bulk density, kg/m ³
ρ_o	Initial bulk density, kg/m ³
τ_o	Initial relative shear stress, dimensionless
τ_r	Relative shear stress, dimensionless
τ_{ult}	Ultimate relative shear stress, dimensionless

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