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Maximum resource utilisation – Value added fish by-products

- Development and evaluation of ingredients from rest raw materials in the processing industry
- Ingredients with specific functional properties based on the demands from the market and the industry
- Improved competitiveness of the fish industry



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Abstract: The aim of the project was to improve the competitiveness of the fish industry by industry driven research. Both existing and improved ingredients from rest raw materials in the fish processing industry was evaluated for utilization in processing lines of whitefish fillets and emulsion based foods. In addition to general raw material properties and application, special emphasis was put on properties and production of fish protein isolates (FPI), fish protein hydrolysate (FPH), homogenized fish protein (HFP) and gelatin. Rest raw materials from the processing industry have different properties and are basis for different ingredients and applications. The project has demonstrated the potential of increasing the value of processing water, rest raw material and under-utilized species. It has also shown how the quality and value of fish mince as an ingredient can be improved, and demonstrated the effects of protein ingredients on whitefish fillets and emulsion based products. Using fish proteins as ingredients in processing lines for whitefish fillets generally improved the final products, resulting in lower drip loss and higher total yield. Addition of fish proteins in emulsion based products affected different functional properties. Indications were found of antioxidative and some specific bioactive properties of FPH. Extraction of gelatin from cold water fish species can take place at room temperature, giving a gel strength thigh enough to expand the application area of cold water fish gelatin. Further specification and documentation of raw material and processes is needed for production and commercialisation of fish protein ingredients. Without those, it can be difficult to claim addition benefits achieved compared to traditional products. Selection of raw materials and documentation of beneficial health effects to support new health claims is important. Standardized protein products for specific food products, should be developed.		
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Executive summary

Main objectives

The purpose of the project was:

- To evaluate both existing and improved ingredients from rest raw materials in the processing industry for utilization in (1) processing lines for whitefish fillets (fresh, frozen and salted fillets of cod and saithe) as well as production of (2) emulsion based foods.
- To develop ingredients with specific functional properties based on the demands from the market and the industry.
- To improve the competitiveness of the fish industry by industry driven research

The study has achieved this aim by:

- Establishing a knowledge base from literature studies and interviews with the industry.
- Identifying the demands from the industry for specific functional, antioxidative and bioactive properties of different FPH (commercially available and lab produced (small scale))
- Showing antioxidative activity of FPH against the most common prooxidant in food system: haemoglobin and iron.
- Verification of selected effects in fish food systems (lean and fatty fish food model).
- Studying the effects of raw material and process conditions concerning properties of FPH and optimization of the process.
- Evaluating the possibilities to utilize proteins and tissues in processing water from production of cod products.
- Studying the quality of FPI from rest raw materials and verification of selected effects in food systems (formed and breaded fish products).
- Studying the effects of various protein ingredients and process condition concerning yield and quality of whitefish fillets.
- Studying the effect of extraction conditions on the weight average molecular weight and the mechanical properties of cold water fish gelatin.
- Studying the relationship between the mechanical properties and the weight average molecular weight as well as the molecular weight distribution of cold water fish and

mammalian gelatins. - Quantifying the effect of the fractions of alpha- and beta-chains as well as the high and low molecular weight components

- Involving and educating several students (both national and international) resulting in student theses and recruitment to the industry.
- Good interaction between the industry and academia (meeting, workshops and conferences).

Method/implementation

The project was executed in five phases:

- The 1st phase of the project focused on defining what products and properties the industry is interested in, sharing knowledge between industry and researchers and setting the framework of the project. Based on this, experiments were planned.
- The 2nd phase included characterisation of existing products on the market by testing properties in laboratories and in industry.
- The 3rd phase involved optimisation of products from rest raw material.
- The 4th phase included verification of improved ingredients by testing properties in laboratories and in industry.
- The 5th phase focused on optimisation of processes.

Different methods were used to analyse both the properties of the raw material and the protein ingredients. Further, the effects of protein ingredients on different processed products and emulsion based foods were analysed. The following parameters were evaluated with the methods and materials used described in more details in Appendix.

Analyses of the raw materials:

- Chemical composition (Fat, water, protein, salt, free fatty acids)
- Physicochemical properties (pH, WHC, NMR, viscosity, colour, yield)
- Lipid and protein oxidation; protein solubility

Analyses of the protein ingredients:

- Chemical composition (Fat, water, protein, salt and protein)
- Molecular weight distribution
- Antioxidative properties

- Bioactive properties
- Emulsifying properties
- Physical properties (WHC, pH, viscosity, colour, protein solubility)
- Mechanical properties (Bloom value, Dynamic storage modulus, polydispersity index)
- Stability (microbial count, TVB-N)
- Degree of hydrolysis (DH)

Analyses of food products with ingredients:

- Chemical composition (Fat, water, protein, salt)
- Yield (total yield, cooking yield, frying yield, drip loss)
- Stability (microbial count, TVN, TVB, sensory)
- Physical properties (WHC, NMR, pH, texture, viscosity, MRI)

Concrete results and conclusions

- The rest raw materials from the processing industry, such as the head, backbones, trimmings (cut-offs), skin and guts, have different properties and are thus basis for different ingredients and applications. Production of quality ingredients can only be achieved by selection, documentation and correct treatment of food grade raw material.
- The project has demonstrated how value of processing water, rest raw materials and under-utilized species can be increased. Moreover the project has revealed how the quality and value of fish mince as an ingredient can be improved.
- The project has demonstrated the effects of protein ingredients on different processed whitefish fillets (fresh, frozen and salted). Using fish proteins as ingredients in processing lines for whitefish generally improved the final products. The improvements were mainly in the form of lower drip loss during storage, higher cooking yield and increased protein content.
- Using fish proteins as ingredients in emulsion based products affect different functional properties. This project has collected valuable data on to what extent different process conditions of FPH will affect important properties including potential to extend shelf life by acting as an antioxidant against haemoglobin (Hb) and iron induced oxidation. Moreover the project has revealed indications on some specific bioactive properties.
- The project has also demonstrated the importance of storage conditions and freshness of the ingredient (FPH) when applied in food and that the hydrolysate itself can add bad taste to the product by chemical degradation during storage.

- Extraction of gelatin from cold water fish species can take place at room temperature if the molecular distribution is right. As the weight average molecular weight of gelatin increases, the dynamic storage modulus and Bloom value increases. By removing low molecular weight molecules from a gelatin sample the mechanical properties, i.e. the strength, of the resulting gel increases.

Recommendations for further work

Specific model products should be selected for investigation of different aspects such as stability, health claims, convenience and other important properties. This can give a valuable platform where different partners would look at the aspects they have the most knowledge in. For the industry, this could be a good way to document health benefits which is needed to be able to label the benefits according to legislation.

Also, the results from the trials performed in this project challenged the following ideas for future work:

New project ideas:

- Feasibility study to see if it is beneficial to use protein products in specific food products, e.g. in processing lines. Make financial survey to estimate if the production companies can increase their profits.
- Develop standardized protein products for specific food products, which can be claimed to have additional desirable benefits compared to traditional food products.
- Increase documentation of beneficial health effects by using fish protein as ingredients in food products.
- Further studies with fish protein isolate (FPI) where the process is optimised with regard to stability, texture, taste and flavour of FPI. Also to study the effects of FPI on sensorial and textural properties of mince and surimi-based products (fish burgers, fish nuggets, etc.).
- Further studies with fish protein hydrolysates (FPH) where difference fish species and fractions are hydrolysed for optimisation and standardization with regard to desirable properties and available raw material. Reduce or remove bitter taste of the peptides and

demonstrate antioxidative effect in different food models. Further studies have to be done on fractionating with different separation technology and different fish proteins.

- Further studies should focus on the hydrolysis process in order to get more knowledge on how different fish species and fractions of raw materials influence the properties of the hydrolysates. The health effect of bioactive peptides from fish should be documented and different fractions of the hydrolysates should be studied and analysed for bioactive properties (or content of bioactive peptides).
- Develop and optimize methods for injection/fortification of fish protein in chilled fillets where shelf-life can be increased.
- Research on what is need to increase the properties of fish gelatin from cold water species, and how its application can be increased in the food and pharmaceutical industry.

Disseminations

Project meetings:

There were 6 meetings during the project period and 5 telephone meetings:

- Reykjavík, Iceland, January 2006
- Torshavn, Faroe islands, June 2006
- Oslo, Norway, October 2006
- Copenhagen, Denmark, September 2007
- Akureyri, Iceland, October 2008
- Trondheim, Norway, June 2009

Telephone meetings:

- December 2007
- August 2009
- September 2009
- October 2009
- November 2009

At the meetings partners have exchanged interests and information, results have been presented and discussed and next steps decided upon. An internal homepage was created for exchange of results. The project group has had a unique composition with partners from

academia, both university and research institutes, and from industries in the participating countries producing a range of different products. All partners have been actively engaged in the project. This has created a very good platform for further work on utilisation of fish and fish rest raw material.

Presentations of the *Maximum resource utilisation-Value added fish by-products* project:

Rasa Šližytė, Revilija Mozuraitytė, Oscar Martínez-Alvarez, Martine Fouchereau-Peron and Turid Rustad. Functional, antioxidative and bioactive properties of fish protein hydrolysates. *2nd international congress on food and nutrition*. October 24-26, 2007, Istanbul, Turkey.

Rasa Šližytė, Revilija Mozuraitytė, Oscar Martínez-Alvarez, Martine Fouchereau-Peron and Turid Rustad. Functional, bioactive and antioxidative properties of hydrolysates obtained from cod (*Gadus morhua*) backbones. *The 37th WEFTA Annual Meeting*. September 24-27, 2007. Lisbon, Portugal.

Rasa Šližytė, Turid Rustad, Revilija Mozuraitytė and Eva Falch. Fish protein hydrolysate as antioxidant in model and food systems. *3rd TAFT conference*, September 15-18th 2009. Copenhagen, Denmark.

Jonhard Eysturskard. *The 9th International Hydrocolloids Conference*. June 15-19th, 2008, Singapore.

Shaviklo G.R., Johansson R. and Arason S. Fish protein isolates attributes from fish by-products. *1st International Congress of Seafood Technology*. May 18-21, 2008. Izmir, Turkey.

Shaviklo G.R., Arason S. and Thorkelsson G. Effects of fish protein isolate on physical and sensorial properties of haddock mince balls. *38th WEFTA Annual Meeting*, September 17-19, 2008. Florence, Italy.

Published articles¹:

Rasa Šližytė, Revilija Mozuraitytė, Oscar Martínez-Alvarez, Eva Falch, Martine Fouchereau-Peron and Turid Rustad (2009). Functional, Bioactive and Anti-oxidative Properties of

¹ Status at the time of writing of the report.

Hydrolysates Obtained from Cod (*Gadus morhua*) Backbones. *Process Biochemistry*, 44(6), 668-677.

Eysturskarð, J., Haug, I.J., Elharfaoui, N., Djabourov, M., & Draget, K.I. (2009). Structural and mechanical properties of fish gelatin as a function of extraction conditions. *Food Hydrocolloids*, 23, 1702–1711.

Eysturskarð, J., Haug, I.J., Ulset, A.-S., & Draget, K.I. (2009). Mechanical properties of mammalian and fish gelatins based on their weight average molecular weight and molecular weight distribution. *Food Hydrocolloids*, 23, 2315-2321.

Eysturskarð, J., Haug, I.J., Ulset, A.-S., Joensen, H., & Draget, K.I. (2009). Mechanical properties of mammalian and fish gelatines as a function of the contents of α -chain, β -chain, low and high molecular weight fractions. *Food Biophysics*, “In Press”.

Articles ready for publication and planned publications (under writing):

Shaviklo, G.R., Arason S. and Thorkelsson G. Functional Properties of Haddock (*Melanogrammus aeglefinus*) Protein Isolates as Affected by Additives and Various Storage Temperatures, in prep., 2009.

Eysturskarð, J., Haug, I.J., and Draget, K.I. Effect of polyols and gelatine hydrolysate on the mechanical properties of mammalian and fish gelatine gels, in prep., 2009.

Rasa Šližytė, Turid Rustad, Revilija Mozuraitytė and Eva Falch, Fish protein hydrolysates as antioxidants in model and food system, *will be submitted by the end of 2009*

Posters:

Gholam Reza Shaviklo, Gudjon Thorkelsson, Sigurjon Arason, and Hordur G. Kristinnsson. Influence of different drying methods and additives on lipid oxidation and functional properties of saithe surimi powder. Poster at the conference Innovation in the Nordic Marine Sector, 12 May 2009 Reykjavík, Iceland.

Magnea Karlsdottir, Kristín Thorarinsdottir, Irek Klonowski, Arnljótur Bergsson, Sindri Sigurðsson and Sigurjon Arason. Homogenization – Increased value of fish mince. Poster at the conference *Nordic Values in the Food Sector*, 15-17 November 2009. Reykjavík, Iceland.

Sigurjon Arason, Magnea Karlsdottir, Thora Valsdottir, Rasa Slizyte, Turid Rustad, Eva Falch, Greta Jakobsen and Jonhard Eysturskarð. Maximum resource utilization – Value added fish by-products. Poster at the conference *Nordic Values in the Food Sector*, 15-17 November 2009. Reykjavík, Iceland.

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Abbreviations

CCK	Cholecystokinin
CGRP	Calcitonin-gene related peptide
CP	Collagen peptides (hydrolysed fish collagen)
CPS	Cod protein solution
DH	Degree of hydrolysis
DSC	Differential scanning calorimetry
EEA	European Economic Area
FDA	U.S. Food and Drug Administration
Fish glue	Mixture of cut-offs, water and salt
FPH	Fish protein hydrolysate
FPI	Fish protein isolate
FPP	Fish protein powder
FT-NIR	Fourier transform Near Infrared Spectroscopy
GRAS	Generally Recognized as Safe
HFP	Homogenized fish protein
HMWD	High molecular weight fish gelatin (hydrolysed fish collagen)
HPI	Haddock protein isolate
LF NMR	Low Field Nuclear Magnetic Resonance
MRI	Magnetic Resonance Imaging
NMR	Nuclear Magnetic Resonance
PCA	Principal components analysis
PLSR	Partial least squares regression
T ₂	Transverse relaxation time
T ₂₁	Shorter transverse relaxation time
T ₂₂	Longer transverse relaxation time
TMA	Trimethylamine
TVB-N	Total volatile basic nitrogen
WHC	Water holding capacity

1 Introduction

1.1 *Why this project*

The term *rest raw material* will be used in this report, substituting the term *by-products* which can have a negative meaning for the consumers. The definition of rest raw materials in the fish industry varies with fish species as well as with both the harvesting and processing methods used. The general understanding of rest raw materials when considering round fish such as cod is that the main body flesh constituting the fillets will be considered to be the main product, but the head, backbones, trimmings (cut-offs), skin and guts constitute what is generally thought of as rest raw materials.

Utilizing rest raw material from round fish processing is very important for the fish industry where great economic, nutritional and environmental values can be obtained by increasing the yield of raw material in fish filleting operation. Increased utilization and value of the raw material can lead to better profit of the fish processing companies. The quota system has put limits on value generation; the raw material has become more expensive and sometimes difficult to get. The fishing quotas indicate allowable quantities for harvesting from some of the most important fish species in order to control their exploitation. The quota system has influenced and changed the attitude toward utilization of all harvested fish. Both fishermen and processors have become more interested in making marketable products from raw materials previously used for fish meal or discarded as waste.

The rest raw material has mainly been utilized for production of low value products such as mince, fish meal and silage, resulting in low profit. The knowledge within this field has grown and is still growing. With increased scientific understanding of the properties of proteins and fish oil, the rest raw materials may be transformed to highly valuable commodities, in some cases even higher in value than the main flesh or fish fillets. Increased knowledge gives the potential to develop and produce products with desirable quality. There are indications that isolation and modification of muscular proteins from rest raw materials can lead to their application as functional additives in food systems.

The quality of marine products is very diverse. The diversity can be due to many factors such as species, season and location of catching and onboard handling procedures. The rest raw material could be used as ingredients in the final products in the round fish processing line.

The ingredients have shown to have desired properties for the food processing industry as well as for other industry such as the pharmaceutical and cosmetic industry. Therefore it is very important to increase the knowledge on the influence of the variability in the raw material on the properties of the final product. This includes factors such as difference within species, between species, season and location of catching, variation in processing, handling etc.

The fish industry is the main customer of these products. For fulfilling their demands, important product properties need to be defined. Full utilization of all the material from the process can also improve the public opinion and the image of the fish processing companies.

Earlier research on marine proteins (Kim & Mendis 2006; Thorkelsson & Kristinsson 2009; Thorkelsson *et al.* 2009; Underland *et al.* 2009) has shown that they have bioactive properties and beneficial health effects which make them a very interesting alternative for the food industry. Production of healthy products from rest raw material gives opportunity to enter a new high end market.

Using new biotechnology based on marine raw material we can provide new, stable and healthy ingredients for food and nutraceuticals. For this purpose we need to i) increase the yield of desirable products; ii) controlled processes accounting for variation in raw material providing stable, healthy and high quality products; iii) documentation and iv) standardisation of the process and properties of the marine ingredients.

In 2000-2004 an EU project (QLK1-CT-2000-01017/QLRT-2001-02829), *Utilisation and stabilisation of rest raw materials from cod species*, was executed where the aim was to evaluate properties of all the main rest raw materials from round fish processing (cod, haddock, saithe, ling and tusk). The work focused on the fat and protein fractions mainly from liver, viscera, heads, skins and cut-offs (v-cut and belly flap). One of the main outcomes of the project was a database of properties and chemical composition of these rest raw materials according to different seasons and locations. This database contains very important information which can be utilized for more practical purpose with regard to the specific needs of the producers. Pre-project financed by NICE was: "Bærekraftig verdiskaping fra restprodukter fra fisk og skalldyr".

The project under discussion in this report can be look up on as a continuation of the above mentioned projects.

1.2 Aim of the project

The aim of the project was to improve the competitiveness of the fish industry by industry driven research. To reach this goal both existing and improved ingredients (specialty proteins) from rest raw materials in the processing industry was evaluated for utilization in (1) processing lines for whitefish fillets (fresh, frozen, salted fillets of cod and saithe) as well as in (2) emulsion based foods.

A large market for ingredients from rest raw material is within the fish industry itself. Based on the demands from the market and the industry ingredients with specific functional properties were developed. This was obtained by selecting optimized mixtures and process using suitable technology to ensure the inclusion of desired functional properties in the final products.

2 Background

Seafood processing discards and rest raw materials account for approximately three-quarter of the total weight of the catch (Shahidi 1994; Pastoriza *et al.* 2004). Fish processing rest raw materials are usually regarded as residuals left after filleting and when viscera is included this can represent up to 2/3 of the round cod (Mackie 1974; Slizyte *et al.* 2005b; Falch *et al.* 2006a; Falch *et al.* 2006b). Valuable components such as fish oil, proteins, collagen and gelatin, enzymes and minerals can be obtained from this raw material. Recent studies have identified a number of bioactive compounds from fish rest raw materials and shellfish and crustacean shells (Kim & Mendis 2006). These compounds can be extracted and purified with technologies of varying complexity. Development of new technologies to extract new bioactive compounds from marine processing rest raw materials may bring more value from what is today considered a waste.

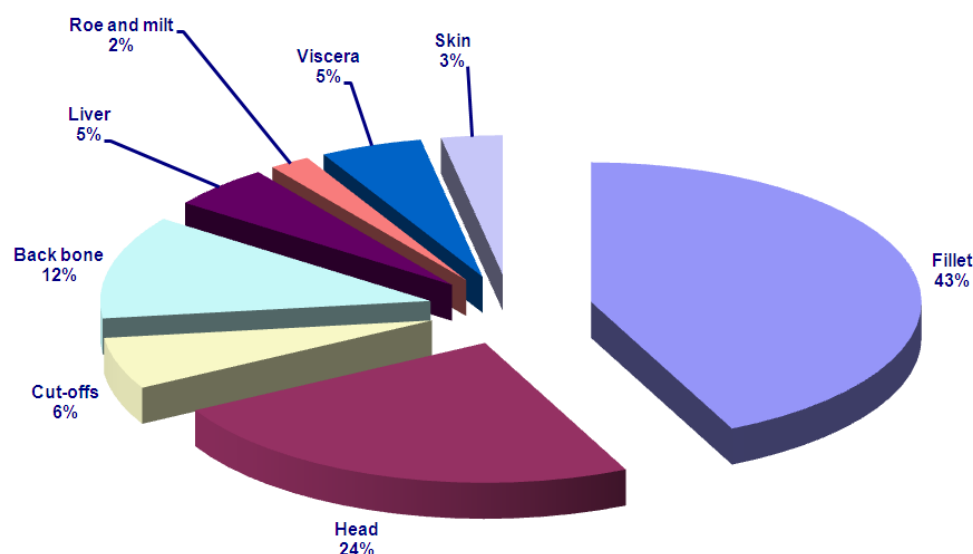


Figure 2.1. Products and rest raw materials from shore processing of cod.

Proteins or other macromolecules are often added to a food to improve their quality or functional properties. One much used definition of functional properties is: “*Those physical and chemical properties that influence the behaviour of proteins in food systems during processing, storage, cooking and consumption*” (Kinsella 1979). A description of the properties of the proteins important for functional properties was given by Damodaran 1997: “*The physico-chemical properties that influence functional behaviour of proteins in food include their size, shape, amino acid composition and sequence, net charge, distribution,*

hydrophobicity, hydrophilicity, structures (secondary, tertiary and quaternary), molecular flexibility/rigidity in response to external environment (pH, temperature, salt concentration), or interaction with other food constituents.” Nutritional, sensory and biological values are sometimes included in the functional properties.

Functional properties can be divided into several groups. Classification into 3 main groups according to mechanism of action is a common practise: i) properties related to hydration (absorption of water/oil, solubility, thickening, wettability), ii) properties related to the protein structure and rheological characteristics (viscosity, elasticity, adhesiveness, aggregation and gelification) and iii) properties related to the protein surface (emulsifying and foaming activities, formation of protein-lipid films, whippability).

There is a wide variety in methods used to determine functional properties of raw materials and food products. Most of these methods are empirical and are therefore lab dependent. As part of this project an overview of methods (including full method descriptions) used for characterisation of biochemical composition and functional properties in the different laboratories was compiled.

2.1 Mince

Minced fish is a comminute flesh produced by separation from skin and bones. Separation is a mechanical process (for producing minced fish) whereby the skin and bone is removed from the flesh (Codex 2005). Bone separators working on different principles are available commercially, but the separator most widely used for fish is of comparatively simple design.

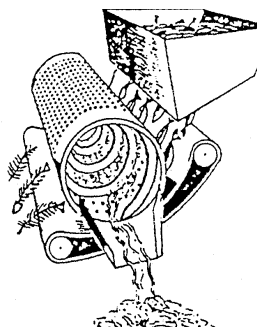


Figure 2.2. A simple bone separator.

The total yield of flesh of low bone content is higher than with filleting alone; up to twice as much can be recovered by separating flesh directly from headless gutted fish. When the fish is first filleted, an additional 8-12 per cent flesh can be separated from the filleting waste. Some people do not like fatty fish such as herring and mackerel partly because of the large numbers of small bones remaining in the fillets. Mince made from these fishes is relatively free from bones and might therefore be more widely acceptable. Flesh from underexploited species, such as blue whiting, that are difficult to fillet efficiently (small size or awkward shape) can readily be removed in a bone separator.



Figure 2.3. A frozen block of minced fish

Mincing can increase the risk of oxidation due to membrane disruption, contact with metals and air. Mince spoils faster than fillets, mainly because the structure of the flesh is destroyed during separation, and extra care has to be taken to maintain good quality. Minced fish is also more easily denatured during freezing. Thus, fish used for making mince has to be of very high initial quality, and processing has to be completed quickly, with emphasis on hygiene and low temperature.

2.1.1 Utilization of fish mince

When fish flesh is minced the texture, flavour and sometimes colour are changed; hence minced fish, and the products derived from it, have at present only limited outlets. Small amounts are used in fish cakes and in less expensive grades of fish fingers and some are used to fill voids in frozen laminated blocks of fillets from which portions and fingers are cut.

Mincing offers an opportunity to exercise greater control over flavour, appearance and keeping quality by the incorporation of additives. Rancidity in fatty fish, for example, can be controlled more easily in minced flesh by intimate mixing with permitted antioxidants, or

minces of different fat content can be mixed together to give a more desirable result but also very valuable products.

However, the mince is very difficult raw material due to high diversity, therefore it is difficult to use mince for standardized products such as ready to eat products. The present market for mince is small compared with the amount of mince that could be made available from all suitable species. Fish mince can also be successfully used directly in various food systems and in physically or chemically altered form to produce an array of nutritional and functional products (Kim & Park 2006). Recently new applications for mince has emerged, as a material in protein products for fillet injection. By solving issues related to stability of the mince and perhaps by making it available in more varied formats with further processing (i.e. isolate production, dried, freeze dried), market potential could be increased.

2.2 *Surimi*

Washing fish mince with water, mixing with sugar and/or polyphosphate followed by freezing to produce surimi increases the stability of the fish proteins. Surimi originates from Japan where it has been a traditional food source for centuries.



Figure 2.4. Two frozen blocks of surimi

The production of surimi follows several basic steps, while the degree of mechanization depends on the sophistication and scale of production. The general processing steps include treatment of raw material (chilling, heading and gutting), meat bone separation, leaching, dewatering, mixing with cryo-protective agents and freezing. The most important step of surimi processing to ensure maximum gelling, as well as colourless and odourless surimi, is

efficient washing. The leaching process involves mixing minced meat with cold water (5°C) and removing water by screening and dehydration a few times. Before the final dewatering, undesirable material particles, such as scales, and connective tissue, are removed by a refiner. The addition of cryo-protectants is important to ensure maximum functionality of frozen surimi because freezing results in protein denaturation and aggregation.

Freezing equipment and frozen storage facilities are essential to maintain the quality of surimi. Research indicates that surimi could be converted to a dried form, surimi powder (Montejano *et al.* 1994). In powdered form, surimi can be kept without frozen storage. The powdered surimi offers many advantages in commerce, such as ease of handling, lower distribution costs, more convenient storage and usefulness in dry mixes. The freeze-drying process does not damage the functionality of myofibrillar proteins (Suzuki *et al.* 1992; Montejano *et al.* 1994). Therefore freeze-dried surimi presents a more versatile structure to increase its application possibilities. Freeze drying can on the other hand lead to increased cost.

2.2.1 Utilization of surimi

Functional properties are important factors if fish proteins are to be incorporated into a food or dish as additives during preparation. The most important properties of surimi are its gelling ability as well as being a colourless and odourless stable protein mass. These features enable the application in various products i.e. crabs sticks.

Surimi is a useful ingredient for producing various kinds of processed foods. It allows a manufacturer to imitate the texture and taste of a more expensive product such as lobster tail, using a relatively low-cost material. Surimi is an inexpensive source of protein. In Asian cultures, surimi is eaten as a food in its own right and seldom used to imitate other foods. In Japan fish cakes (kamaboko) and fish sausages, as well as other extruded fish products, are commonly sold as cured surimi.

In the western countries, surimi products are usually imitation seafood products, such as crab, abalone, shrimp, calamari, and scallop. Several companies produce surimi sausages, luncheon meats, hams, and burgers. A patent was issued for the process of making even higher quality proteins from fish such as in the making of imitation steak from surimi (Hartman 1993).

Surimi is also used to make kosher imitation shrimp and crabmeat, using only kosher fish such as pollock.



Figure 2.5. Artificial crab sticks made from surimi.

Freeze-dried surimi is a commercial product in Japan. It is used as binder, dispersing agent and emulsifier in re-structured products made out of beef, pork and chicken meat. It is also used for formulation of exotic dishes.

2.3 Fish protein isolate

Fish protein isolate (FPI) is fish protein which has been purified to a protein content of at least 90% of the dry material. Up to a certain extent one can categorise surimi as FPI as the surimi process includes purification of the fish protein mass. The term FPI is however in general used for pure fish muscular proteins which have been produced by pH-shift process. This method is thought to be more efficient for complex raw material such as whole fish and rest raw materials than the surimi process (Kristinsson *et al.* 2006; Thorkelsson *et al.* 2008).

The overall process concept is simple and includes the following steps: solubilisation of the muscular proteins (pH raised with alkali or lowered with acid), separation (density difference) and precipitation. The pH shift methods involve solubilising muscle proteins by subjecting diluted, finely homogenized fish meat to either very low pH (~2.5-3) or a very high pH (~10.8-11.2) at low temperature. Solids such as bones, scales, neutral fat and disrupted cellular lipid membranes are then removed by centrifugation and the soluble protein is precipitated by adjusting the pH to the isoelectric point of the myofibrillar proteins to give a protein isolate (Kristinsson & Rasco 2000a).



Figure 2.6. A frozen block of fish protein isolate

Protein gels made from protein isolates recovered with the new process from several species have been shown to have equal and sometimes significantly better gelation properties than those produced using conventional surimi processing techniques (Choi & J.W 2002; Kristinsson & Demir 2003; Hultin *et al.* 2005). The process has also been shown to improve other functional properties. The process has given excellent results for some cold water species as well as temperate and warm water species. According to Kristinsson & Hultin (2003) the alkali treatment of cod muscle proteins improved functional properties (emulsification and gelation) of cod myosin and myofibrillar proteins.

The FPI made by pH-shift process looks like surimi when it is dewatered and packed (Figure 2.6). It may contain about 14-20% protein and 80-86% water. The quality of this product depends on several factors such as source of raw material, method of processing, pH, amount of protein and water content etc.

2.3.1 Utilization of fish protein isolate

Fortification of fish fillet by multi-needle injection of fish proteins, static soaking, or vacuum tumbling have been reported (Thorkelsson *et. al* 2008). Fish protein injection is believed to enhance the yield and improve the frozen stability of fish fillet (Kim & Park 2006). Experiments on injection of brine containing FPI has been shown to increase weight gain in cod and haddock fillets by 5-20% and increase in cooking yield has been observed. There are indications that FPI give higher cooking yield and microbiologically more stable products than products with injected fish mince (Valsdóttir *et al.* 2006). Improvements in water holding

capacity, by re-solubilisation of FPI powder and injection into fish fillets has been reported (Nolsøe & Undeland 2009).

FPI can be used as a dipping solution in battering and breading process to reduce absorption of oil in fried products. Kim *et al.* (2006) reported that when protein solutions (mixture of homogenized isolated fish protein and water (1:3)) were applied as a dipping solution for fish finger and patties before battering or breading, the quantity of oil absorbed in fried products was significantly reduced. Fish protein may form a protein film and act as fat blocker (Kelleher 2005). Thorkelsson *et al* (2008) reported however that applying FPI to reduce fat in deep-fried battered and breaded cod and saithe did not have the desired effects in the final product.

Emulsion based fish products are processed by mixing fish protein (surimi/minced fish) with different ingredients such as vegetable proteins, starches, wheat flour, spices etc. and forming fish paste into intended product shapes. Fish mince can be difficult material to work with due to varying quality (lack of standardisation and stability). FPI can be used in this case as fish protein ingredient or even replacer of whole or part of mince and surimi in the formula. It seems that a variety of emulsion based fish products can be processed by using FPI, however few studies have been published.

2.4 Fish protein hydrolysate

Use of fish protein hydrolysates (FPH) with well expressed functional and antioxidant properties in food are a subject of interest due to their ability to make products with desirable physical and sensory properties, and to produce protein enriched and oxidative stable seafood.

Table 2.1. Functional properties of fish protein hydrolysates (FPH).

Functional properties	References
High solubility	Shahidi <i>et al.</i> 1995; Pacheco-Aguilar <i>et al.</i> 2008; Wasswa <i>et al.</i> 2008
Emulsification capacity and stability	Shahidi <i>et al.</i> 1995; Pacheco-Aguilar <i>et al.</i> 2008; Wasswa <i>et al.</i> 2008
Foaming capacity	Pacheco-Aguilar <i>et al.</i> 2008; Wasswa <i>et al.</i> 2008
Reduce cooking loss	Shahidi <i>et al.</i> 1995; Onodenlore & Shahidi 1996
Water-holding capacity	Wasswa <i>et al.</i> 2007; Wasswa <i>et al.</i> 2008; Slizyte <i>et al.</i> 2009
Reduce drip loss	Kristinsson & Rasco 2000b
Studies on minimizing bitterness of FPH	Kristinsson & Rasco 2000a; Dauksas <i>et al.</i> 2004
Antioxidative properties	Shahidi <i>et al.</i> 1995; Klompong <i>et al.</i> 2007; Samaranayaka & Li-Chan 2008; Theodore <i>et al.</i> 2008; Yang <i>et al.</i> 2008; Bougatef <i>et al.</i> 2009; Slizyte <i>et al.</i> 2009
Bioactive properties (see table 2.2)	Jung <i>et al.</i> 2006; Kim & Mendis 2006; Martinez-Alvarez <i>et al.</i> 2007; Cinq-Mars <i>et al.</i> 2008; Slizyte <i>et al.</i> 2009

Enzymatic hydrolysis is one of the main methods for recovery of valuable components from fish rest raw materials (Gildberg *et al.* 2002; Dauksas *et al.* 2005; Slizyte *et al.* 2005a; Slizyte *et al.* 2005b). FPH have good solubility over a wide range of ionic strength and pH and usually tolerate strong heat without precipitating (Skanderby 1994). FPH have good functional properties and can contribute to water holding, texture, gelling, whipping and emulsification properties when added to food (Kristinsson 2006). Some studies have shown that FPH can contribute to increased water holding capacity in food formulations (Shahidi *et al.* 1995; Onodenlore & Shahidi 1996; Wasswa *et al.* 2007; Wasswa *et al.* 2008); and addition of FPH from salmon (*Salmon salar*) reduced water loss of frozen salmon patties (Kristinsson & Rasco 2000b). FPH have good foaming and emulsifying properties, thus may be used as emulsifier and emulsion stabilizing ingredients in a variety of products as well as aid in the formation and stabilisation of foam-based products. As the size of the peptides is very important for interfacial/surface activity of FPH, the degree of hydrolysis is important (Jeon *et al.* 2000). Several reports have suggested that there is an optimum molecular size or chain length for peptides to provide good foaming and emulsifying properties, and that limited hydrolysis resulting in larger peptides generally leads to improved emulsification and foaming properties, while extensive hydrolysis resulting in small peptides reduce these properties (Adler-Nissen & Olsen 1979; Lee *et al.* 1987; Quagli & Orban 1990; Jeon *et al.* 2000;

Kristinsson & Rasco 2000a). In addition, except for the deficit of a few amino acids, hydrolysates have a high nutritional value (Shahidi *et al.* 1995; Slizyte *et al.* 2005b).

Several studies have indicated that peptides derived from fish proteins have antioxidative properties in different oxidative systems (Jeon *et al.* 2000; Jung *et al.* 2003; Rajapakse *et al.* 2005; Kristinsson 2006; Klompong *et al.* 2007; Klompong *et al.* 2008; Samaranayaka & Li-Chan 2008; Yang *et al.* 2008). The antioxidant activity of proteins and peptides can be the result of specific scavenging of radicals formed during peroxidation, scavenging of oxygen containing compounds, or metal-chelating ability (Gutierrez *et al.* 2003; Kristinsson 2006).

Metal catalysed decomposition of lipid peroxides is one of the dominant oxidative pathways that occur in food (McClements & Decker 2000). Because of this, proteins can inhibit lipid oxidation by sterically hindering the interaction of metals and dispersed lipids, reducing its ability to decompose lipid peroxides. Many proteins whose specific biological function is not to store or transport metals are still capable of chelating metals. The ability of a protein to chelate metals is dependent on pH. A net anionic charge will be established on a protein at pH above the pI. That leads to electrostatic attraction between the protein and cationic metal in continuous phase, which inhibits lipid oxidation reactions (Elias *et al.* 2008).

The ability of proteins to scavenge radical has been shown in several studies (Thiansilakul *et al.* 2007; Slizyte *et al.* 2009). However, this is not conclusive evidence that they are antioxidants. To be an effective antioxidant, proteins must be more oxidatively stable than unsaturated fatty acids and the resulting protein radical must not promote lipid oxidation (Elias *et al.* 2008). Proteins are also capable of altering the development of rancidity in unsaturated fats and oils by adducting volatile aldehydes (Elias *et al.* 2008). This pathway is not truly an antioxidant mechanism, but it will inhibit rancidity by transforming lipid oxidation products into non-volatile compounds.

Production of fish protein hydrolysates with antioxidant properties will enable production of protein enriched and oxidatively stable seafood. While hydrolyzed proteins have good antioxidant activity, it is still not well-understood how the composition of peptides influences the ability to inhibit lipid oxidation.

Fish protein hydrolysates are rich in bioactive peptides, but they are less investigated than peptides from other sources such as milk (Underland *et al.* 2009). Different raw material, hydrolysis conditions, separation and isolation would influence the release and amount of these peptides. The types of enzymes and degree of hydrolysis influence the properties of bioactive peptides: short peptides (2-8 amino acids residues) have shown high ACE inhibiting (reduction of high blood pressure) activity, while longer peptides (5-14 amino acids residues) have shown good antioxidative properties in vitro (Thorkelsson *et al.* 2009). The amino acid sequence effect also the ACE-inhibition. Fish protein hydrolysates can also function as immuno-stimulants, have anti-carcinogenic effects and anti-anaemia activity (Underland *et al.* 2009).

The hydrolysis process can lead to the production of various peptides bearing a structural resemblance to hormones. These newly formed peptides can retain the biological properties of the native protein, or can show new properties. The calcitonin-gene related peptide (CGRP) is a 37-residue peptide widely distributed in the central nervous system and peripheral nerves. Different biological functions have been described for CGRP, such as vasodilatation (Gupta *et al.* 2006) and induction of satiety (Huges *et al.* 1984; Lenz *et al.* 1984). On the other hand, different authors have reported the presence of gastrin/CCK-like molecules in protein hydrolysates from fish raw materials (Cancre *et al.* 1999; Ravallec-Ple & Van Wormhoudt 2003). These molecules are the only known members of the gastrin family in humans, and could have a positive effect on food intake in humans and fish species in aquaculture. Gastrin is a gastric hormone which stimulates postprandial gastric acid secretion and epithelial cell proliferation. In humans there are two different gastrins, one with 17 and one with 34 amino acids residues. Cholecystokinin (CCK) is a group of peptides which controls the emptying of the gallbladder, as well as pancreatic enzyme secretion. It is also a growth factor, and regulates intestinal motility, satiety signalling and the inhibition of gastric acid secretion (Rehfeld *et al.* 2001). Both gastrin and CCK inhibit food intake and share a common COOH-terminal pentapeptide amide that also includes the sequences essential for biological activity.

2.4.1 Health advantages

Several beneficial health effects are linked to fish consumption in general but some of these effects are suggested to be better by intake of FPH due to the high content of easily digestible

bioactive peptides. The activity is closely related to the amino acid composition and sequence. A recent review by Underland *et al.* (2009) present health effects of different seafood products including fish proteins and FPH and a review from Kim & Mendis (2006) discussed the bioactive effects of marine rest raw materials.

Table 2.2. Health advantage of fish protein hydrolysate (FPH)

Suggested health advantages linked to FPH/peptides	References
May play a role in allergy and food intolerance (less allergenic proteins/peptides and improve glucose tolerance and insulin sensitivity) (animal model)	Lavigne <i>et al.</i> 2000
Reduce anxiety	Dorman <i>et al.</i> 1995
Enhance flow of red blood cells (in vitro)	Chuang <i>et al.</i> 2000
Obesity and diabetes II	Docmar 2006; Liaset & Espe 2008
Prevention and treatment of ulcerative conditions of the bowel	Fitzgerald <i>et al.</i> 2005
Effects on cholesterol (Zucker rats)	Wergedahl <i>et al.</i> 2004
Growth inhibitor on cancer cells	Picot <i>et al.</i> 2005
Anti-anaemia activity	Dong <i>et al.</i> 2005
Anti hypertensive (ACE-inhibition) (animal and human intervention)	Kawasaki <i>et al.</i> 2002; Je <i>et al.</i> 2009 (fractionated peptides)

2.4.2 Utilization

FPH have been tested as ingredients in different food such as cereal products, fish and meat products, desserts and crackers etc. (Kristinsson & Rasco, 2000). There are some limitations in the utilization due to e.g. unacceptable taste and smell, bitterness and also competition with other functional ingredients on the market.

Possible applications of FPH as ingredient in food systems:

- Functional food ingredients (physical properties)
- Antioxidant (Kim *et al.* 1996; Jun *et al.* 2004; Je *et al.* 2005; Khantaphant & Benjakul 2008; Je *et al.* 2009).
- Flavour enhancer - Seafood flavour (Imm & Lee 1999).
- Salt and monosodium glutamate (MSG) replacer
- Milk replacer
- Protein enrichment (i.e. for sport drinks)
- Bioactive ingredients

Looking at the international market there are some commercial fish protein products that are linked to health claims (Table 2.3). These are highly isolated products operating mainly in the health supplement market but some of them could also be potential food ingredients.

Table 2.3. Different commercial marine derived protein products with health claims Thorkelsson *et al.* 2009.

Product name	Claims	References
Hydrolysed dried bonito bowel		
Peptide ACE 3000	Lowers blood pressure	www.nippon-sapuri.com
Vasotensin	Lowers blood pressure	www.metagenics.com
PeptACE™	Lowers blood pressure	http://us.naturalfactors.com
Levenorm	Lowers blood pressure	www.onc.ca
Peptides from sardines		
Lapis Support	Lower blood pressure	www.tokiwayakuhin.jp
Collagen Peptides		
Bifidus & Collagen	Beautifies the skin	www.kagome.co.jp
Hydrolysed whitefish		
Seacure	Improves gastrointestinal health	www.propernutrition.com
Protizen	Relaxing	www.copalis.fr
AntiStress 24	Relaxing	www.fortepharma.com/fr/index.html
Fortidium	Against oxidative stress	www.biothalassol.com
Nutripeptin	Lowers glycaemic index	www.nutrimarine.com

For FPH to be used in food products the ingredient should contribute to extended shelf life and/or increased healthiness. FPH have been shown to affect specific functional and bioactive properties in food systems. Several studies have evaluated functional properties (such as water holding capacity, water binding, fat binding and texture), antioxidative properties and bioactivity (Table 2.1 and Table 2.2). All the functional properties mentioned in table 2.1 and 2.2 have great perspective for the Nordic fish industry for improving their competition and claiming advantages in their products. A very interesting property for the food industry is the possible antioxidative effect of the FPH, particularly when applied to fish products that are highly susceptible to lipid oxidation.

The main quantity of FPH produced in the Nordic countries today goes into the feed and pet-food industry, however there are companies, such as Marinova, that are producing food grade FPH for the food industry. As far as the product group knows there are no food in the Nordic countries that are enriched with FPH to increase the healthiness of the food product probably

due to the strict EU regulations. There are developments towards a higher focus on the bioactive properties of the FPH and also an increased refinement of FPH in order to increase the bioactivity. In this project both different commercial FPH and laboratory prepared FPH have been evaluated as ingredients in lean and fatty food products.

2.5 Fish gelatin

Gelatin is derived from collagen, which is the principal constituent of connective tissues and bones. Covalent cross-linking organizes collagen molecules into a three-dimensional structure while each collagen triple helix is stabilized by hydrogen bonds between three left-handed helices called α -chains. Gelatin is mainly produced from collagen sources like bovine and porcine skins, and bovine bones. Although fish gelatin has been extracted from fish skins, which is a major rest raw material of the fish filleting industry, since 1960, only small commercial volumes are available (Veis 1964; Balian & Bowes 1977; Ledward 1986; Norland 1990; Schrieber & Gareis 2007).

However, the outbreak of bovine spongiform encephalopathy (BSE) in Europe during the 1990s has generated a greater focus on gelatin from cold and warm water fish as a possible alternative to mammalian gelatins. Gelatin has been extracted from several fish species including cod (Guðmundsson & Hafsteinsson 1997), hake (Montero *et al.* 1999), megrim (Montero & Gómez-Guilén 2000), black tilapia and red tilapia (Jamilah & Harvinder 2002), brown-stripe red snapper and big-eye snapper (Jongjareonrak *et al.* 2006), Alaska pollock (Zhou & Regenstein 2005), Atlantic salmon (Arnesen & Gildberg 2007), channel catfish (Yang *et al.* 2007), horse mackerel (Badii & Howell 2006) and Nile perch (Muyonga *et al.* 2004).

Although fish gelatin in contrast to bovine gelatin, is not associated with the risk of Bovine Spongiform Encephalopathy and contrary to porcine gelatin it is acceptable for Islam, Judaism and Hinduism, the commercial interest in cold water fish gelatin has been relatively low due to its suboptimal physical properties. Gelatin from cold water fish species exhibits lower gel strength, as well as lower gelling and melting temperatures compared to mammalian gelatin and gelatin from warm water fish species. This is due to a lower content of the imino acids, proline and hydroxyproline (Piez & Gross 1960; Norland 1990; Leuenberger 1991; Guðmundsson 2002; Haug *et al.* 2004).

It is well known that the source of raw material, the degree of cross-linking and the method of manufacture, which depends on temperature, time and pH, affect the molecular weight distribution as well as the mechanical properties of the resulting gelatin.

The molecular weight of a single α -chain has been reported to be 95-100 kg/mol, but during the pre-treatment and the extraction of gelatin, peptide bonds in the primary structure are ruptured providing subunits of the α -chains. Covalent cross-links between the α -chains can survive the manufacturing treatments, providing fractions of β -chains (two covalently cross-linked α -chains), γ -chains (three covalently cross-linked α -chains) and components with even higher molecular weights (Veis 1964; Piez 1968; Finch & Jobling 1977; Hinterwaldner 1977; Ledward 1986; Norland 1990).

The mammalian gelatins with good gel forming properties are produced during the initial extractions made at low temperature while the subsequent extractions made at successively higher temperatures provide gelatins exhibiting reduced mechanical properties due to increasing hydrolysis (Finch & Jobling 1977; Hinterwaldner 1977; Ledward 1986; Schrieber & Gareis 2007).

The procedures used for preparing fish gelatins typically involve acid or alkaline pre-treatment of the fish skins prior to gelatin extraction. Although a number of different pre-treatment conditions have been reported the initial extraction temperatures for mammalian gelatins (between 45 and 60°C) have been adopted for the extraction of cold water fish gelatins (Guðmundsson & Hafsteinsson 1997; Zhou & Regenstien 2005; Arnesen & Gildberg 2007). From optical rotation measurements the denaturation temperature of cold water fish collagen was estimated to be between 15 and 20°C (Joly-Duhamel *et al.* 2002). This indicates that the temperature adopted for the extraction of cold water fish gelatin (45°C or above) is unnecessarily high.

2.5.1 Utilization of fish gelatin

The commercial interest in cold water fish gelatin has been relatively low due to its suboptimal physical properties. This is also reflected by the worldwide annual production of

gelatin (326,000 tons), with pig skin, bovine hides, ossein gelatine accounting for 46%, 29.4% and 23.1%, respectively (Karim & Bhat 2009).

It is well known that cold water fish gelatin exhibit good emulsifying and film forming properties. The main application area is therefore the embedding of oil-based vitamins. Supplier of vitamins use cold water fish gelatin for the micro-encapsulation of oil soluble substances such as vitamins A, D, E and carotenoids. Cold water fish gelatin is also used in pharmaceutical fast-dissolving tablets and as a protein additive for nutraceutical, cosmetic and food applications. It can be very difficult for the fish gelatin to substitute the mammalian gelatin for use in food products, mainly due to the difference in melting temperature. The mammalian gelatins are more stable at room temperature than the fish gelatins, which complicates the utilization. The main potentials for fish gelatins are to utilize them in the pharmaceutical industry as material in soft capsules.

2.6 Regulations

Comparison between several countries (Canada, Iceland, UK, USA) made in 2006 showed (Valsdóttir 2006) that there are in general no specific regulations regarding fish products or fish ingredients. They thus fall under general labelling requirements. Despite variations in regulations and industry guides between the countries, in which fish-based ingredients fall under, the main conclusion is the same: Added ingredients (i.e. proteins) which are part of the final product need to be labelled except when it is a part of the name of the product (i.e. salted cod). The same applies whether the source of the fish protein is of the same specie as the fillet or not. As soon as the general processing procedures are altered, resulting in product changes such as composition, chemical- and physical properties, the alteration should be labelled. How the ingredient is produced has no influence on the basic labelling requirements (i.e. fish mince or highly processed isolates or peptides). A product that before was “unprocessed” is now “processed” and can’t be sold as fresh or fresh frozen as the term “fresh” indicates no processing beyond general processing procedures. As an example, injection is not categorised as “general processing procedures.”

In USA and Canada licence is required for application of protein in fish products and new products need to go through a certification process. As an example, FDA² has commented on the GRAS³ status of certain extracted fish proteins for injection into fish fillets of the same species.⁴ Regulations in EEA⁵ are not as explicit in this regard; however one can expect the same to apply there.

Regulations on fish based ingredients follow in main lines general application and labelling requirements for ingredients, whether they are used as basic ingredients or functional ingredients.

² FDA = U.S. Food and Drug Administration

³ GRAS = Generally Recognized as Safe

⁴ There are two fish protein isolates regulated under part 172 (172.340 and 172.385) in U.S. food law (the Federal Food, Drug, and Cosmetic Act).

⁵ EEA = European Economic Area

3 Development and evaluation of ingredients from rest raw materials in the processing industry

In the beginning of the project the industrial partners defined the most important quality parameters of ingredients for application in fillet and emulsion based products (see Table 3.1). For those companies that put emphasis on fillet processing, water holding capacity along with shelf life, cooking yield, taste and colour were the most important. For processing of emulsion based products (Mills) the emphasis was somewhat different; improvement of nutritional profile and stability without negative effects on the sensory properties. Based on those parameters, experiments were planned and executed with the companies on the ingredients under investigation.

Table 3.1. Defined important quality parameters from the industrial partners.

	Faroe Seafood	Mills	Brim	Samherji	Síldarvinnslan
Water holding capacity	X	(X)	X	X	X
Emulsification properties (capacity and stability)		X			
Fat absorption		(X)			
Solubility (water)		(X)			
Gelling		(X)			
Texture		X			
Antioxidative properties		X			
Cooking yield	X		X	X	X
Taste and colour	X	X			
Shelf life/stability	X	X	X	X	X
Nutrition		X			

In this project both laboratories made and selected commercially available protein products were investigated (Table 3.2). The rest raw material from processing lines of whitefish fillets and the protein products were both investigated in vitro and in food systems (in whitefish fillets, processed fish products and in emulsion based foods).

Table 3.2. Selected commercially and lab made protein products under investigation in food systems and in vitro.

Ingredient	Producers	Investigation in food systems
Surimi	Faroe Seafood	Ingredient in processing lines of whitefish fillets (injection).
Washed mince	Faroe Seafood	Raw material for homogenized fish protein.
Unwashed mince	Faroe Seafood/Síldarvinnslan	Raw material for homogenized fish protein, fish balls and formed fish products.
Homogenized fish protein	Síldarvinnslan/Lab made	Ingredient in processing lines of fillets (injection).
Fish protein isolate	Iceprotein and lab made	Raw material for fish balls and as ingredient in processing lines of whitefish fillets (injection).
Fish protein hydrolysates	Højmarklaboriet ⁶ and lab made	Ingredient in emulsion based foods (fish cakes and paté) and in processing lines of whitefish fillets (injection).
Hydrolysed fish collagen	Faroe Marine Biotech ⁷	Ingredient in processing lines of whitefish fillets (injection).
Hydrolysed fish collagen	Norland ⁸ and lab made	(Only in vitro)
Fish protein powder	Aroma ⁹	(Only in vitro)

3.1 The layout of the results chapter

The results chapter is divided according to the raw material and ingredients used in the project. At the beginning of each paragraph is a blue box where the aims, essential findings and challenges for further investigations are listed. Below the boxes, the trials and their results are explained in more detail. The organization of the boxes can be viewed in Table 3.3.

⁶ MariPep P, C and CK

⁷ Dried low molecular weight fish gelatin – specie not known

⁸ Dried high molecular weight gelatin from fish skin (cod, haddock, and pollock).

⁹ Type not known (bigger peptides).

Table 3.3. Organization of the results chapter with regard to rest raw material and ingredients.

Box	Trials
1	Properties of raw material from processing lines
2	Properties of ingredients from fillet production
3	Application of ingredients from fillet production - injection
4	Application of ingredients from fillet production in consumer products
5	Properties of fish protein hydrolysates (FPH)
6	Fish protein hydrolysates (FPH) in food
7	Fish gelatin
8	Comparison between protein ingredients for injection in fillets

3.2 Ingredients from rest raw material of processing lines

The research on ingredients from rest raw materials of fillet production focused on four main aims: Properties of the raw material from processing lines; properties of ingredients produced from fillet production; application of ingredients produced from fillet production (injection studies); and application of raw material and ingredients from fillet productions in consumer products.

3.2.1 Properties of raw material from processing lines

Box 1

Aim:

Analyse the effects of bleeding, processing, storage time, temperature and packaging on the properties of backbones and cut offs produced during processing.

Increase the yield and value of catch processed on land, by finding ways to recover protein and tissues from the processing water used during fish processing and evaluate their properties.

Outcome:

- Effect of bleeding, storage time and processing on properties of backbones and cut-offs (saithe).
 - ✓ Protein denaturation of the frozen fractions showed fast loss in salt soluble protein in all fractions.
 - ✓ Lipid oxidation was most pronounced in samples with blood/dark muscle/scraped backbones.
 - ✓ No difference was found between backbones and cut-offs with regard to degree of hydrolysis for FPH production.
- Effects of storage condition on lipid degradation in cut-offs and lipids from cod (*Gadus morhua*).
 - ✓ Cut-offs should be kept at -24°C rather than -18°C as it reduces the influence of storage on their water holding capacity.
 - ✓ Cut-offs from saithe are more susceptible to lipid oxidation than cut-offs from cod and should be kept in oxygen tight bags, i.e. vacuum bags, during storage.
- Recovery of material from processing water from skinning and filleting (cod)
 - ✓ If the marine products contribute 60.000 ton pr. year, 1.200 tons will be lost in the processing water.
 - ✓ The ratio of fish flesh lost during filleting of cod was 0.4% of the weight of gutted fish, 1.0% of the weight of the fillets was lost during skinning.
 - ✓ 25% of the total dried materials in the processing water from filleting were collected.
 - ✓ By using vibrating sieve separator it was possible to collect fish flesh of good quality, in the filtration range from 250 to 710 µm.
 - ✓ The recovery cost of the material in the processing water does not overcome the benefit.

Challenges:

- Collection and preservation of raw material before transformation into protein ingredients
- In order to collect protein particles below 250 µm from the processing water different equipment should be considered.

Effects of bleeding, storage time, and processing on backbones and cut-offs

A study was done on the stability of the raw material, saithe in particular, in order to find the effect of bleeding, storage time, and processing on the ingredients produced. The aim was to create standards for raw material quality and handling to produce food grade ingredients.

Three batches of saithe were analysed (dry matter, lipid (content, oxidation), protein (content, solubility and oxidation) and water holding capacity). Different fractions were made from the saithe (minced saithe fillet, minced saithe fillet with kidney tissue, minced flesh of scraped backbones of saithe, minced saithe fillet with blood, minced dark muscle of saithe). In addition backbones and cut-offs were used for production of FPH.

All the frozen fractions showed a rapid loss of salt soluble proteins with the lowest content in the scraped backbones. Proteins were oxidised during storage and lipid oxidation was most pronounced in samples containing blood/dark muscle/scraped backbones. No difference was found between backbones and cut-offs on degree of hydrolysis for production of FPH.

Effects of storage condition on lipid degradation in cut-offs and lipids from cod (*Gadus morhua*)

A study was performed on minced cut-offs from cod and saithe, and cod liver that were stored at -18/-24°C for 2 and 4 months. Effects of packing (cardboard+plastic/vacuum) were also studied. Water and fat content, amount of free fatty acids, pH and water holding capacity were evaluated in the raw material, and after freezing and thawing.

Higher contents of free fatty acids in liver were observed after storage at -18°C than at -24°C. Oxidation occurred at a faster rate in the surface layers of whole liver than in the middle part. Storage temperature and time had significant effect on water holding capacity (WHC) in cut-offs. WHC decreased with time, at higher rate at -18°C than at -24°C. Lipid degradation occurred faster in saithe than in cod, vacuum packing the cut-offs decreased the degradation. Based on these results, it is recommended to store rest raw materials at -24°C rather than at -18°C, to minimize negative changes in cut-offs and liver. The storage time before further

processing should be as short as possible and packing methods that limit access of oxygen selected.

Recovery of material from processing water from skinning and filleting

The aim of the test was to evaluate the possibilities for utilisation of proteins and tissues in processing water from production of cod products. Little is known about the properties of this protein source which is not used today but might have some valuable application. This material is flushed away, and is therefore an environmental issue (Figure 3.1).



Figure 3.1. Loss of fish mass from fish processing lines.

Experiments were carried out in three fish processing plants, where different equipments were used to collect the processing water. Processing water from skinning and filleting machines was collected. The processing water was then filtered and analysed by mass distribution analysis. Different filtration methods were tested (vibration sieve, conveyor band, screw press) and sizes of sieves (4, 2, 1, 0.5, 0.25 mm). The collected material was evaluated on chemical content, yield, particle size, colour and viscosity.

It is estimated that about 1% of the weight of the processed raw material is fish lost in the processing water. Measurements indicated that the ratio of fish flesh lost during filleting of cod was 0.4% of the weight of gutted fish and 1.0% of the weight of the fillets was lost during skinning. Over half of the recovered proteins from the processing water were recovered by the coarsest filter (4 mm) and in the 1-4 mm filters 85-90% of the total recovered proteins, thus

protein loss can be reduced with rather coarse filters. With the equipment used in this project, about 25% of all the dry matter from processing water from filleting was collected.

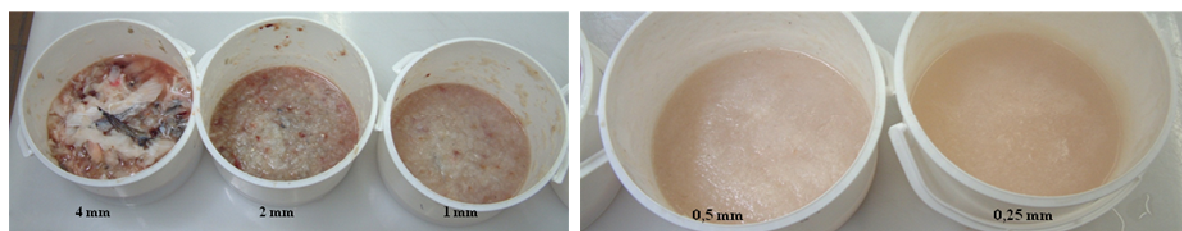


Figure 3.2. The quality of the collected fish mass with sieves (4-0.25 mm) from processing water from filleting.

The filtered mass had significantly lower dry material content than fish fillets processed at the same time and as the filters became finer the dry material content was reduced (Table 3.4). Collection of dry matter was the highest (61%) for the largest sieve (4 mm), relatively, but in general it was between 10.2-16.2%. The filtered mass was not as white as the fillets, but as the filter became finer the colour of the mass became more similar to the fillets as well as more homogenous (Figure 3.2).

Table 3.4. Proportion and dry matter content of collected fish mass with regard to sieve size, along with relative division of dry matter content in the collected fish mass with regard to sieve size. The fish mass was collected from processing water from filleting.

Sieves size (mm)	Proportion of collected fish mass (%)	Dry matter (%) of collected fish mass	Relative division (%) ¹⁰ of dry matter
0.5	21.7	6.3	12.8
1.0	14.0	12.3	16.2
2.0	6.4	17.0	10.2
4.0	57.9	11.2	60.8
Total	100.0%	48.8%	100.0%

Protein composition of the fillets versus the filtered mass was not significantly different. However, measurements on the time dependent flow behaviour viscosity of fish mass from filleting and skinning (Brabender®) show a difference between the different fish masses. This difference might be due to difference in collagens and phospholipids content. This could be a subject for further investigation, i.e. using NMR technology to analyse the phospholipids from the peptides.

¹⁰ Relative division (%) of dry matter content in the collected fish mass with regard to sieve size.

Generally, the time dependent flow behaviour viscosity of the collected fish masses decreased with temperature up to 70°C (Figure 3.3). The viscosity of the fillets was rather stable with increasing temperature, up to approximately 50°C, but between 50-85°C the viscosity increased. If these results are viewed with regard to known temperatures linked to protein denaturation, than it can be seen that viscosity decreased around 45°C. Thorarinsdottir *et al.* (2002) found that cod muscle proteins denatured in the temperature range 39-76°C according to measurements with differential scanning calorimetry (DSC). Other studies have also shown reduction in solubility at temperature below 30°C.

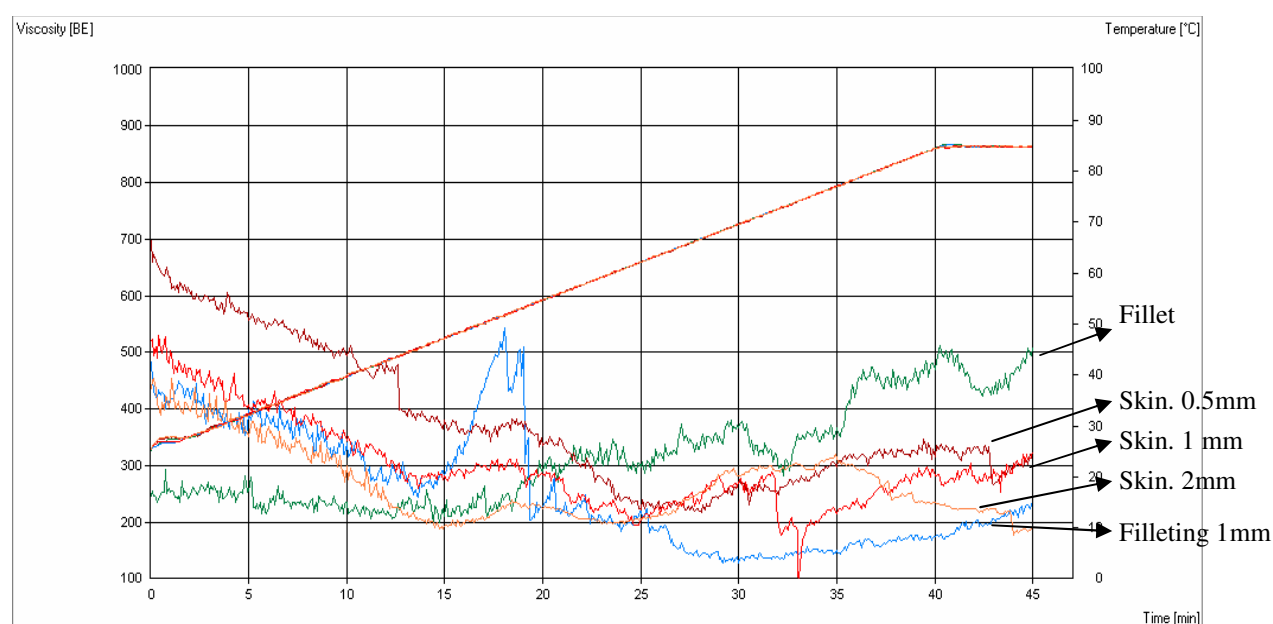


Figure 3.3. Time-dependent flow behaviour viscosity measured with Brabender® Viscograph E where samples were heated from 30°C to 85°C. Measurements were performed on collected material from skinning machine (0.5-2 mm) and from filleting machine (1mm). Minced fillet was also measured

The amount of collected material is highly variable, depending on the type of fish, quality, time of year etc. The properties of the collected fish mass are also variable depending on which part of the fish it comes from (e.g. from loin or tail). Mass balance on dry matter collected from fresh versus thawed material showed that coarser sieve is needed for the thawed material and more material is collected. The freezing of material before processing had no negative effects on the physicochemical properties of the protein from the processing water. This occurred in spite of lower solubility of the protein in processing water from thawed material compared with processing water from fresh material. The fish mass collected from processing water of thawed material had higher water holding capacity compared to processing water of fresh material.

The results shows that from a production of 60.000 tons a year, 1.200 tons will be lost with the processing water. The process developed in this study can increase the yield of fish muscle for human consumption of 0.2% with regard to gutted fish, but 0.4% with regard to product quantity. However, tests on different collection/filtering methods (vibration sieve, convey band, and screw press) indicate that the recovery cost of the material in the processing water does not overcome the benefit. Furthermore, the process requires high usage of water (about 1 kg per 1 kg fillet), thus it might only be interesting in countries were water is relatively cheap.

3.2.2 Properties of ingredients produced from fillet production

Box 2

Aim:

Evaluate influence of raw material, process conditions and/or additives on physiochemical and functional properties of mince and fish protein isolate (FPI).

Outcome:

- Fresh vs. frozen mince from cut-offs and frames
 - ✓ Fresh mince had higher water holding capacity and lower water mobility than frozen mince.
- Quality of FPI made from cut-offs from cod, saithe and arctic char
 - ✓ Good source of protein for manufacturing products that do not need high level of gel strength such as fish burgers, fish nuggets and other ready to eat fish products.
 - ✓ Texture, taste and flavour could be improved
- Influence of salt concentration and cryoprotectants on physical properties of cod protein solutions (CPS) and haddock protein isolate (HPI).
 - ✓ Stability of CPS improved by using cryoprotectants and HPI by mixture of salt and sucrose.
 - ✓ The most stable frozen cod protein solution contained 5% salt and cryoprotectants.
 - ✓ Stability of CPS during frozen storage improved adding cryoprotectants to the products at the end of the pH-shift process.
 - ✓ The cryoprotectants increased the water holding capacity and viscosity, decreased the weight loss and improved whiteness in CPS containing 1.2, 3, 5 and 15% salt.

Challenges:

- Optimise the FPI process with regard to stability, texture, taste and flavour of FPI
- Extend shelf-life of fresh protein solutions containing 1-5% salt.

Evaluation of chemical and functional properties of fish mince

Fish mince, made from cut-offs and frames from filleting processing, is often used as raw material for fish protein ingredients. The chemical and physicochemical properties of the material are therefore important. These properties can vary between species and are influenced by the treatment the mince has been through i.e. fresh or frozen.

Fresh and frozen saithe mince made from cut-offs and frames were studied. Evaluations were made on chemical composition, water holding capacity and water mobility (T_2 transversal relaxation times) of the fish minces. The fresh mince had slightly higher water content than the frozen mince. The water holding capacity was significantly higher ($p < 0.05$) in the fresh mince (Figure 3.4) which indicates the negative effects freezing can have on the fish muscle.

The T_2 transversal relaxation times were measured at room temperature. The T_{21} expresses the behaviour of tightly bound water, i.e. intra-cellular/intra-myofibrillar water or water bound to protein, while T_{22} expresses the behaviour of the less bound water, extra-cellular/inter-myofibrillar water. The water molecules are therefore more tightly bound when the T_2 is shorter. The T_{21} relaxation time of the fresh mince were significantly longer and the T_{22} relaxation time were significantly shorter than in the frozen mince. These results indicate that the water mobility was lower in the fresh mince and the water therefore more tightly bound. The normalised distribution (T_{21} and T_{22} population) of water in the mince samples was on the other hand not significantly different.

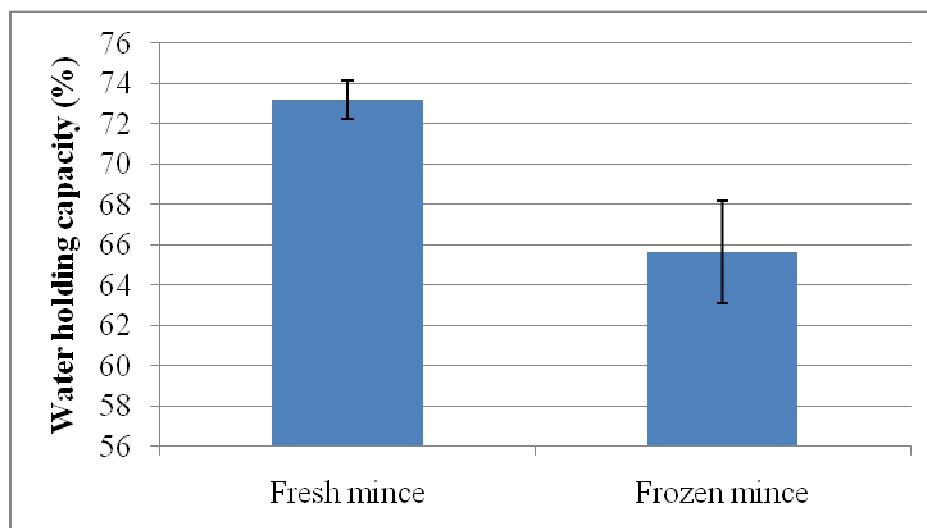


Figure 3.4 Water holding capacity of the fresh and frozen saithe mince produced from cut-offs and frames from filleting processing lines.

Evaluation on the quality of fish protein isolate from rest raw materials of cod, saithe and Arctic char filleting process

The quality attributes of fish protein isolates (FPI) made from rest raw materials (cut-offs) of filleting processes of cod (*Gadus morhua*), saithe (*Pollachius virens*), and Arctic char (*Salvelinus alpinus*) were determined based on the Codex Code of Practice for frozen surimi (FAO/WHO 2005). The results were compared to the attributes of conventional surimi and other fish protein isolates made from fish fillets.

The results indicated that although quality attributes of these products, such as: gel strength, gel forming ability and whiteness were considerably different from conventional surimi, or FPI made from fresh fillets, it is still a good source of protein for manufacturing products which do not need a high level of gel strength, such as fish burgers, fish nuggets and other ready to eat fish products. The texture, taste and flavour of FPI products, which were produced in this study, were acceptable but they could be improved by adjusting different ingredients and spices according to the target market. Based on these results, the second study was performed.



Figure 3.5. Sensorial attributes of fish protein isolates made from Arctic charr, saithe and cod, respectively.

Evaluation of the effects of salt concentration, cryoprotectants and chilled and frozen storage on physical properties of cod protein solutions and haddock protein isolate.

In the second test influence of variation in salt concentration, cryoprotectants and chilled and frozen storage of cod protein solutions (CPS) and haddock protein isolate (HPI) was measured with regard to viscosity, colour, water holding capacity and stability (microbial count, TVB-N). The fish protein solutions and fish protein isolate were extracted from cut-offs of cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*), respectively, using the pH-shift process.

The results indicate that fish protein solutions and fish protein isolates are affected by different amounts of additives (salt and cryoprotectants). The physicochemical and rheological properties of these products depend on the additives and time and temperature of storage. Using cryoprotectants for CPS and a mixture of salt and sucrose for HPI were recommended to stabilise these products. The most stable frozen cod protein solution was with 5% salt and cryoprotectants followed by the solution with 3% salt and cryoprotectants. To make fish protein solutions that would be stable during frozen storage it is recommended to add cryoprotectants to the products (preferably containing 3-5% salt) at the end of the pH-shift process. The cryoprotectants increased the water holding capacity and viscosity (Brabender and Pascal), decreased the weight loss and improved whiteness in CPS containing 1.2, 3, 5 and 15% salt. Shelf life of fish protein solution containing 3-5% protein can be extended by thermal processing (pasteurization) but risking the loss of desirable functional properties of the protein solution.

3.2.3 Application of ingredients from fillet production in processing lines – injection studies

Box 3

Aim:

Evaluate and compare protein ingredients for injection in fillet processing. Evaluate two methods for preparation of ingredients before injection.

Outcome:

- Protein ingredients (isolate, mince, Surimi) injected by Suspentec® process in fresh cod fillets.
 - ✓ Incorporation of isolate, mince or surimi in salt brine for injection increased total yield of fillets.
 - ✓ The injected groups lost their freshness earlier than the unprocessed control group.
 - ✓ Super-chilled storage increased shelf life.
 - ✓ Shelf life of the injected fillets not sufficient for exporting fresh fish products to the market
- Fish mince homogenised before injection in fillets
 - ✓ By homogenising the mince, reduced particle sizes, more homogeneous mix and decreased number of microbes can be achieved
 - ✓ Incorporation of homogenised mince in salt brine for injection increased total yield of fillets.
 - ✓ Using fresh cut-offs in HFP gave less drip in lightly salted fillets than HFP from frozen cut offs.
- Distribution of fish protein hydrolysate (FPH) injected into fillets
 - ✓ Pressure induced pockets were detected in injected fillets by magnetic resonance imaging (MRI)
 - ✓ Pre-treatment is needed to distinguish the injected proteins from the original proteins in the fillet by MRI

Challenges:

- Develop and optimize methods for injection of fish proteins in chilled fillets.
- Optimize methods for addition of ingredients in fillets with regard to species and material condition.
- Documentation of appropriate ingredient with regard to species and type of processed product

Several experiments were executed on application of fish-based ingredients in whitefish fillets. Two methods were evaluated for preparation of ingredients before injection, the SUSPENTEC® and the homogenisation process. Comparison was then made between protein ingredients for application in fillet products (fresh, frozen, salted and/or lightly salted; cod and saithe). Magnetic resonance imaging (MRI) was evaluated as a method for detecting and analysing distribution of FPH in injected fillets.

SUSPENTEC®/Cozzini process

The SUSPENTEC® process is a method for reducing meat, poultry or fish trimmings into micro-sized particles and incorporating them into traditional brines or marinades. This "suspension" is then injected into the muscle product. The process is conducted under controlled temperature to ensure efficient protein binding and complete dispersion of suspension into the product. The trim/brine suspension is automatically processed in a continuous system.

The aim of the experiments was to evaluate the application of injecting proteins by SUSPENTEC® process into cod fillets for fresh storage. Comparison was done between FPI, mince and surimi as protein ingredients.

Fresh cod fillets were injected with different brines produced through the SUSPENTEC® process (with FPI, mince, surimi or no protein ingredient). Super-chilling during storage was applied in one test to evaluate its effect on the quality and physiochemical properties of the fillets. Products were stored fresh and analysed for yield (total yield, cooking yield), stability (microorganisms, TVN, sensory) and functional properties (WHC).

The total yield¹¹ of fillets increased with injection of salt brine containing FPI, mince or surimi compared to injection with salt brine without proteins or no injection (untreated fillets). No significant difference was found in yield of fillets injected with different brines. Injection of mince and FPI improved cooking yield compared to fillets injected only with salt. Surimi did however not improve cooking yield. Injection increased number of microorganisms and formation of volatile nitrogen bases (TVN), thus reducing shelf life. Significant difference was found in sensory parameters between the characteristics of injected and untreated fillets. The injected groups lost their freshness earlier than the unprocessed control group.

Application of super-chilling during storage (comparison between FPI and mince as ingredients in the brine) resulted in reduced growth of microorganisms and formation of volatile nitrogen bases (TVN), thus increasing shelf life. Fresh fillets were stored up to 9 days at -2 and -4°C. The fillets did not freeze at -2°C, but started to freeze at -4°C. Dipping the

¹¹ Total yield is the difference between fillet weight before injection and after storage.

fillets into brine after injection made the product more stable (reduced TVN). 4 days old fish was used in the experiment. It is expected that the super-chilling would have more impact if the fish had been fresher at the beginning of the experiment.

Despite controlled cooling during processing, storage and transport, the shelf life of the injected fillets was not sufficient for export of fresh fish products to the market.

Homogenization process

Trials were executed where homogenisation was used to improve condition and properties of fish mince for injection into fish fillets. Homogenized fish proteins (HFP) were produced according to a specific continuous process. Approximately 4 parts of cold water (0-1°C) was added to 1 part of fresh or frozen mince from saithe or cod cut-offs and backbones. After infusion of water and mince, the solutions were sieved (1000 µm) to remove insoluble and undesired material. It was then homogenized at about 3000 psi by a special homogenizer and directly injected into fillets using a multi-needle injector.

Minced cut-offs (fresh and frozen) and frozen mince from backbones (washed and unwashed) were homogenized and injected into fillets. Trials with different species (pollock, cod), freshness (fresh vs. frozen) and pre-treatment (fresh vs. lightly salted) were conducted. Analyses were made on chemical and physical properties of the HFP (separation, microbes, protein solubility, colour, pH, viscosity and chemical analysis) and the fillets (yield, drip loss, water holding capacity, pH, chemical analysis, microbes, TMA and TVN).

Use of homogenisation to improve the properties of fish mince for injection into fish fillets gave good results. Trials indicated that by homogenising the mince, reduced particle size, more homogeneous mix and a decreased number of microbes can be achieved. If the pressure is increased enough, the number of microorganisms will be reduced significantly. In this experiment, reduction from ca. 2.700.000 cfu/g to 2.000 cfu/g was observed. Improvements in colour were also observed, the final product had much lighter appearance than the raw material.

The injection brine contained 20-30% mince. The total yield of the fillets was increased by injecting them with fish mince compared to untreated fillets and fillets injected only with salt brine. 10-20% weight increase was achieved, thus about 4% increase in protein content in the final product. Trials using higher mince content in the brine were not successful as the collagen in the mince clotted the sieve in the injector, resulting in less pressure and thus less brine being injected. The highest yield (Figure 3.6) was observed in fillets injected with mince less than 1mm in size and washed fish mince.

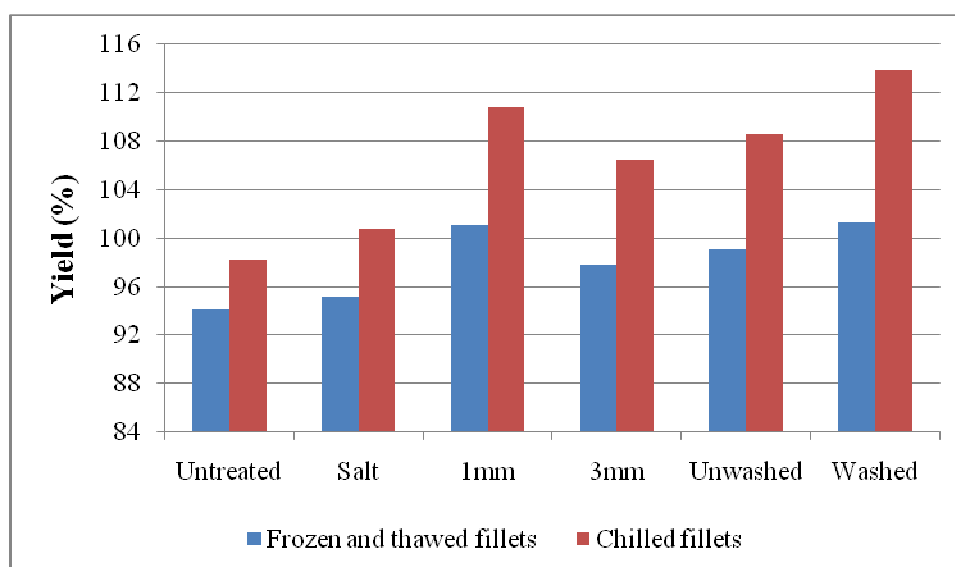


Figure 3.6. The storage yield¹² (%) of fillets injected with homogenized fish proteins (HFP) made of different processed fish mince (1mm particle size, 3mm particle size, unwashed and washed minces) after frozen and chilled storage.

Addition of HFP into fresh cod fillets decreased the drip loss and increased the storage yield significantly during chilled storage compared with control fillets and salt injected fillets. Chilled and frozen cod fillets resulted in higher total yield¹³ compared with control fillets and salt injected fillets. Addition of HFP resulted in the smallest reduction in weight through the process (closest to the original weight of the fillets).

¹² The yield of the chilled and thawed fillets after storage was determined by the observed changes in weight with respect to the weight of the raw fillets.

¹³ Evaluation of total yield was determined by multiplying the yield after storage (chilling, freezing (thawing)) and the cooking yield.

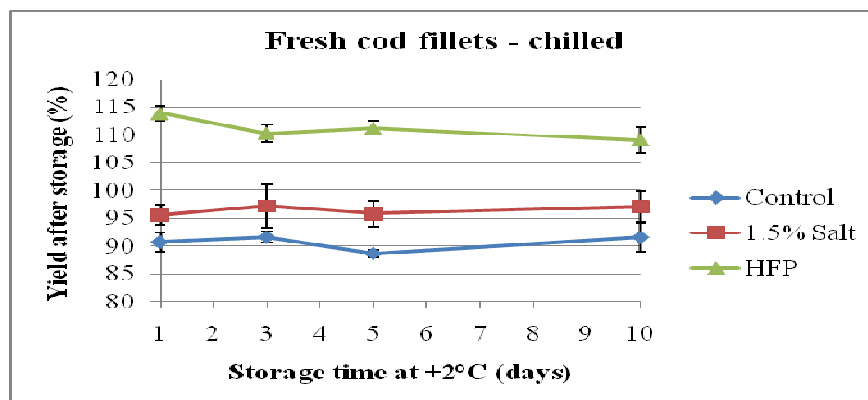


Figure 3.7. Yield (%) of fresh cod fillets as function of storage time at +2°C. (Control=untreated fillets; 1.5% Salt=fillets injected with 1.5% salt brine; HFP=homogenized fish protein).

Total yield of the lightly salted fillets (Figure 3.8) was increased by injecting them with fish mince after brining compared to non-injected fillets. The improvements were in the form of higher weight gain after injection, lower drip loss during storage and higher cooking yield. HFP had more positive effects on the lightly salted cod fillets than on the fresh fillets after frozen storage. Using cod mince from fresh cut-offs resulted in less drip from the fillet compared to using mince from frozen cut-offs.

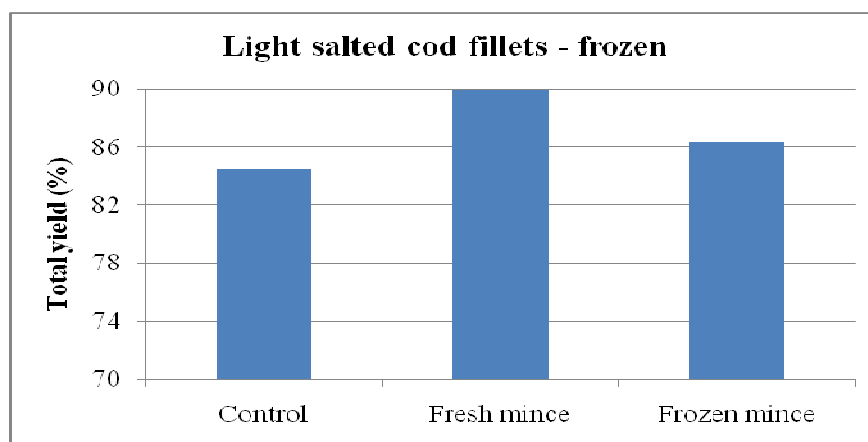


Figure 3.8. Total yield¹⁴ (%) of lightly salted cod fillets after 1 month of frozen storage. Control=untreated fillets; Fresh mince=Fillets injected with HFP made from fresh mince; Frozen mince=Fillets injected with HFP made from frozen mince.

Magnetic resonance imaging (MRI) of fillets injected with FPH

Advanced analytical (and non-destructive) methods (Magnetic Resonance Imaging) were used to study how the ingredients were distributed in the fish fillet after injection of FPH (Figure

¹⁴ Evaluation of total yield was determined by multiplying the yield after storage (chilling, freezing (thawing)) and the cooking yield.

3.9). It was possible to see the pressure induced pockets in the fillets, however the injected proteins were difficult to distinguish from the proteins in the fillets without pre-treatment (e.g. labelling) of the injected FPH. This means that pre-treatment of the proteins is needed to document the distribution of them. Distinction can perhaps been made between injected fillets and non-injected, however to detect if proteins have been injected can be more difficult.

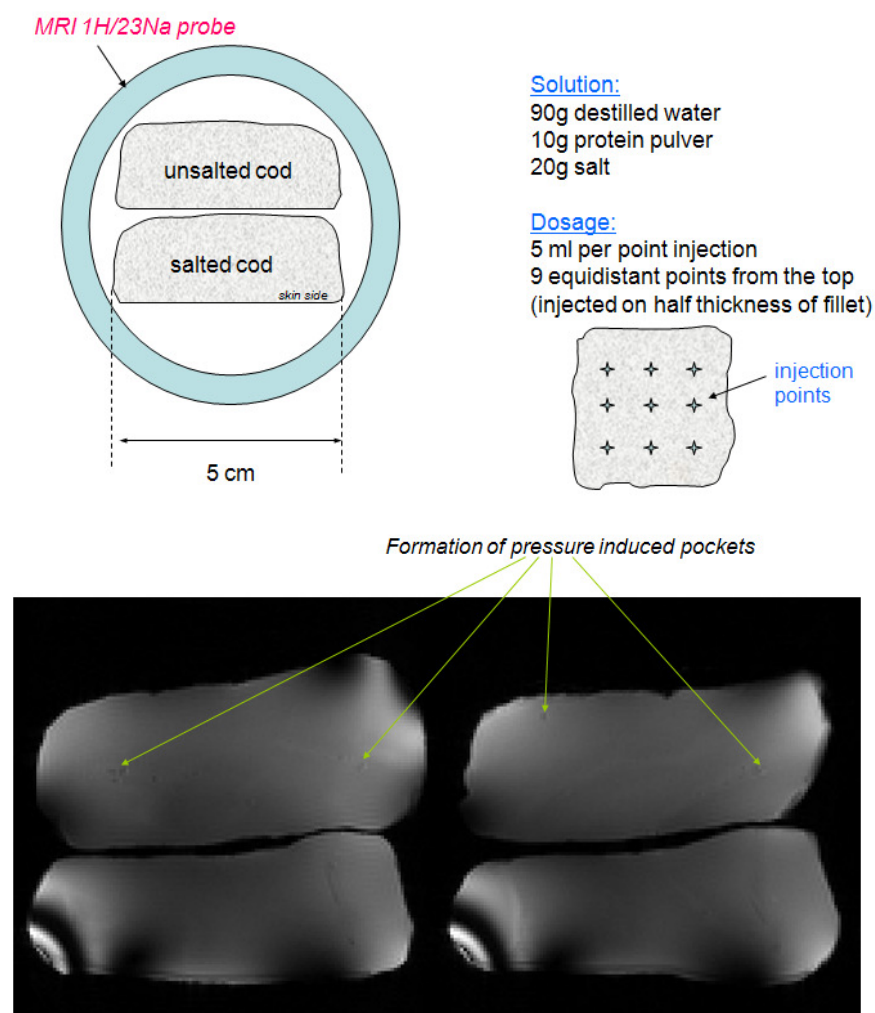


Figure 3.9. Experimental set up for injection studies of FPH in fish fillets by MRI and images from injected fillets

Application of raw material and ingredients from fillet productions in consumer products

Box 4

Aim:

Evaluate the application of raw material and ingredients from fillet production in couple of consumer products. The investigated products were breaded (formed) products and fish balls.

Outcome:

- “Fish glue” made from cut-offs and mince in formed products
 - ✓ By using “fish-glue” pressure at forming could be reduced, keeping more of the natural muscle structure intact in the final product
 - ✓ Products became more uniform, coherence improved, drip and cooking loss reduced.
- Comparison between fried fish balls containing FPI and/or mince.
 - ✓ Adding fish protein isolate to mince improved shaping and setting.
 - ✓ FPI was found to have influence on the texture of the fish balls.
 - ✓ Texture, taste and flavour of the FPI products produced were acceptable.

Challenges:

- Study effects of FPI on sensorial and textural properties of mince and surimi-based products (fish burgers, fish nuggets, etc.).
- Introduction of FPI-based products to food industry
- Further study shelf life of FPI and FPI-based products

Formed products

The aim of the experiments was to develop a process to produce formed fillet products with “fish-glue” or binding agent by using low value rest raw materials from fish processing such as cut-offs and mince. The main aim by using “fish-glue” was to reduce the pressure on forming and thus maintain more of the natural muscle structure in the final product (Manuxe 2003).

Mix of rest raw material (cut-offs), salt and water was used as glue for forming fish-cuts into formed fillet products. Comparison was made with formed products without “fish glue”. The products were breaded. Products were stored frozen and analysed on yield (total yield, cooking yield), stability (TVN, TBA, sensory), chemical (salt, water, pH) and functional properties (texture, colour).

Application of “fish-glue” resulted in a more uniform product, improved coherence, reduced drip and cooking loss. Use of the glue did not have any influence on processing (i.e. breading). A simple machine, such as a bowl meat-cutter can be used to make the glue as long as the temperature is controlled (below 4°C).

Fish balls

FPI made from haddock (*Melanogrammus aeglefinus*) cut-offs by pH-shift process was added to haddock mince in different proportions (50:50, 25:75) with the aim to manufacture two types of fried fish balls. A minced fish ball product was also prepared as a control. The products were assessed for physical properties (viscosity and cooking loss) and sensory changes within a period of 8 weeks of frozen storage at -18°C.

Viscosity decreased and forming ability improved compared to the control. Samples containing FPI had less cooking loss after frying than the control. FPI can affect texture and sensory attributes of fish mince for product development. FPI was found to have influence ($p<0.05$) on graininess and softness in texture determined by sensory evaluation. The control sample and the fish ball containing 25% FPI had a similar sensory profile.

FPI is a good source of protein for manufacturing products which do not need a high level of gel strength, such as fish balls (not Japanese style), fish burgers, fish nuggets and other ready to eat fish products. Texture, taste and flavour of the FPI products produced were acceptable, however they could be improved by adjusting different ingredients and spices according to the request of the market.

3.3 *Fish protein hydrolysate*

The work on fish protein hydrolysates was focused on the two main aims: Properties of FPH and its application in food

3.3.1 *Properties of fish protein hydrolysates*

Box 5

Aim:

Influence of raw material properties and process conditions on the biochemical, functional, antioxidative and bioactive (gastrin/CCK- and CGRP-like peptides) properties of FPH. Comparison between lab made and commercially available fish powders.

Outcome:

- Fresh versus frozen raw material for hydrolysis:
 - ✓ Fresh gives higher yield of FPH (as dried powder), gives lighter powders with better emulsification properties.
- Time of hydrolysis:
 - ✓ Longer time of hydrolysis increase the amount of FPH, increase degree of hydrolysis (DH) and decrease water holding capacity (WHC) of the powders.
 - ✓ Moderate time of hydrolysis (15 to 45 min) yields FPH with the best emulsifying properties
- Backbones from pre-rigor versus post rigor:
 - ✓ No difference in yield, degree of hydrolysis or water holding capacity but influenced amount of gastrin/CCK like molecules
- Whole versus cut
 - ✓ Cut bones give up to 18% increase in yield and darker powders
- Bioactive molecules
 - ✓ All FPH obtained from cod backbones by protein hydrolysis obtained bioactive (gastrin/CCK- and CGRP-like peptides) molecules.
- Comparison of lab-made and commercial products:
 - ✓ Differences in molecular weight distribution and in ash content (high in some of the commercial products tested due to high concentration of NaCl).
 - ✓ All tested fish powders showed an antioxidative activity, but differences in radical scavenging ability, different kinetic behaviour for iron chelating ability, reduction of Hb and iron induced oxidation were observed among the products
 - ✓ A reduction of Hb induced oxidation was observed, however, the hydrolysates were more effective towards iron induced oxidation.

Challenges:

- Collection and preservation of raw material before hydrolysis
- Optimize hydrolysis process with regard to desired properties and available raw material
 - ✓ Taste and stability of the powders
 - ✓ Content and variety of bioactive peptides
- Standardization and documentation of the process and properties of the fish protein hydrolysates

FPH obtained from cod backbones are powders with a light yellow colour (Figure 3.10), a fishy odour and desirable functional (e.g. emulsification, water holding properties), antioxidative and bioactive properties.



Figure 3.10. Dried FPH powders.

A series of hydrolysis trials have been carried out using backbones from cod that were initially fresh or frozen. In this study it was analysed how the state (fresh vs. frozen, pre-rigor vs. post-rigor filleted, whole vs. cut) of raw material and the time of hydrolysis influence the properties of fish protein hydrolysates obtained from cod (*Gadus morhua*) backbones. Hydrolysis of fresh raw material significantly increases yield of dry FPH and gives lighter powders with better emulsification properties compared to frozen raw material. Longer time of hydrolysis increases the amount of FPH, increases DH and decrease WHC of the powders. A short time of hydrolysis: 15 and 30 min (when hydrolysis times from 15 till 130 min were compared) and 25 and 45 min (when hydrolysis times from 10 till 60 min were compared) gave FPH with the best emulsifying properties (Figure 3.11).

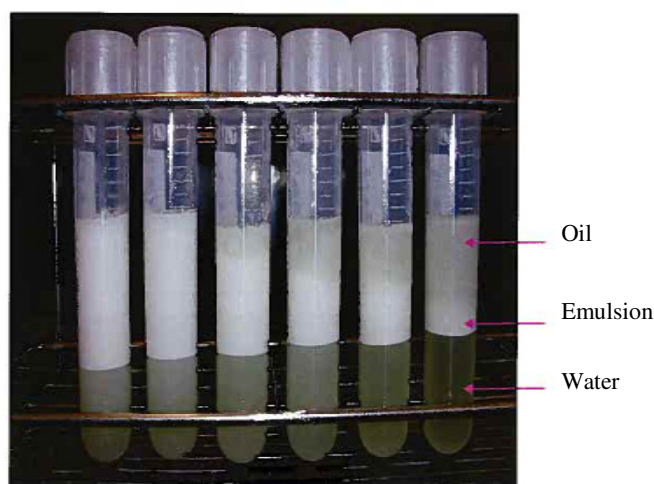


Figure 3.11. Emulsifying capacity of FPH obtained after different time of hydrolysis (15, 30, 45, 60, 120 and 130 min).

Comparison of the chemical composition of selection several commercial fish powders (MariPep P, C and CK (all from Danish Fish Protein), Norland HFC (hydrolyzed fish collagen), Aroma Powder (from New Zealand) and small scale produced FPH (fresh backbones from cod (*Gadus morhua*)) showed a relatively large variation among the samples tested. These variations affect the functional properties. The biggest difference between the powders was obtained in ash, the ash content was much higher for all MariPep (up till 20% in dw) powders compared to others (0.5-7.6% in dw) due to the high salt content in those products. The high salt content increases the stability of the powders which is desirable for the market. All the samples have rather low moisture content which might be positive for storage stability but might encourage lipid oxidation. DH of powders varied from 5 to 20% and this can also play important roles for functional and antioxidative properties. FPH made in the lab with a hydrolysis time of 50 minutes gave the highest DH of all tested powders, followed by MariPep powders and FPH with 20 minutes hydrolysis time. Norland had by far the lowest DH of the tested protein powders, but determination of molecular weight profiles by gel-filtration indicates that Aroma FPP consists of a larger amount of long peptides and fewer short ones. The molecular weight size distribution of the FPHs made in the laboratory showed some similarity to the MariPep powders, with one broad peak of quite long peptides, and some narrower peaks containing intermediate and shorter peptides. The Norland and Aroma powders also showed some similarities to each other, with one narrow peak containing a large proportion of long peptides, and one or more peaks containing some shorter peptides. Analysis of protein and peptides profiles indicated that powders consist of very different peptides and can be grouped into to three groups (based on the similarity on amount and size

of peptides): 1. Powders with relatively large peptides (Aroma and Norland powders); 2. All MariPep powders; 3. Hydrolysates produced in the lab.

Fish protein hydrolysates have the potential to enhance product stability by preventing oxidative deterioration. Generally, all fish powders tested showed an anti-oxidative activity (Table 3.5). The DPPH scavenging activity showed that antioxidative activity could be due to the ability to scavenge lipid radicals. Increased degree of DH resulted in slightly increased DPPH radical scavenging activity. Iron and haemoglobin (Hb), which are two of the most important pro-oxidants in food, were used as inducers of oxidation in the model system. The different products showed different kinetic behaviour for iron chelating ability which was related to protein and peptide size. Antioxidative activity of the fish protein hydrolysates towards iron induced oxidation was observed to be pH dependent. A reduction of Hb induced oxidation was observed, however, the peptides were more effective against iron induced oxidation. Differences in radical scavenging ability were found among the products tested.

Table 3.5. Antioxidative properties of lab made and several selected commercial fish protein powders. “+” indicates the best antioxidative properties among the tested samples, while “-“ the poorest. Fe³⁺ and Hb (haemoglobin) were used as prooxidant at different pH.

	FPH 20	FPH 50	MariPep P	MariPep C	MariPep CK	Norland
DPPH radical scavenging activity	++	+			-	--
Iron chelating ability		+	++			-
Fe ³⁺ (pH 4.5)			-	+		
Fe ³⁺ (pH 5.5)	++	+			-	
Fe ³⁺ (pH 6.5)			+		--	-
Hb (pH 4.5)	+	++		+		-
Hb (pH 5.5)		+				-
Hb (pH 6.5)	+		-			
Ranging	2	1	4	3	5	6

Our work also shows that it is possible to obtain bioactive molecules from cod backbones by protein hydrolysis. The obtained molecules (gastrin/CCK- and CGRP-like peptides) could make the cod hydrolysates useful for incorporation in functional foods.

3.3.2 Application of FPH in food

Box 6

Aim:

Examination of functional, antioxidative and sensory properties of FPH when fortified into respectively a lean food model (fish cakes made from cod muscle) and fatty food model (salmon pate)

Outcome:

- Cod pate with FPH added was judged as having the best texture and cod pate where egg was exchanged with FPH was judged to have the best taste.
- Fat uptake of fish cakes varies depended on powder added and can depend on emulsification properties of hydrolysates.
- No significant differences were found in sensory acceptance among the salmon pate fortified with lab-made and different commercial powders (1% w/w).
- Concentration test:
 - ✓ 3% (w/w) addition of FPH into the salmon pate was less appreciated by the sensory panel compared to addition of 0.5 and 1%. (The usual level of addition is 1%).
- Relatively short storage of commercial products (as powder) had impact on the taste properties of fortified salmon pate

Challenges:

- Reduce or remove bitter taste and/or by-taste of the peptides
- Bioactive and health effect needs to be documented by in vivo trials
- Demonstrate antioxidative effect in different food models

3.3.2.1 Food model studies

Lab produced and commercially available powders were tested in food models. Physical and functional properties of added powders were tested both in lean fish cakes (Figure 3.12) and cod pate (Figure 3.13) and “fatty” (salmon) pate (Figure 3.14).

Lean fish model

The lab made powders were used as an ingredient in two lean fish products. For the first part fish cakes were made together with potato flour, which is a common ingredient used for the

production of fish cakes. The reference samples were made by substituting protein powders part by potato flour. The results showed that potato flour plays important roles for colour, texture and WHC of the final products. Compared to FPH powders addition of potato flour gave lighter and firmer fish cakes with very high water holding capacity. However, too firm (hard) and dry fish cakes would not be accepted by consumers. Addition of FPH powder enriches fish cakes with proteins (FPH contains approx. 85% protein). At the same time fish cakes made with FPH had similar frying yield¹⁵ as fish cakes made with potato flour.



Figure 3.12. Fish cakes made with addition of FPH.

The different FPH tested gave variations in frying yield of fish cakes (lean model), this was however not found to be due to differences in water loss, but was due to differences in fat absorption. Fat absorption of the fish cakes was shown to be related to the emulsifying properties of the added powder. Little differences was found between FPH in respect to water holding capacity, but higher DH gave a lower WHC. The different salt content of the fish cakes, caused by different salt content of the hydrolysates, has been proven to have a crucial impact on the results for most analysis of functional properties. In order to examine how added fish powders influence adsorption of oil into the fish cakes during frying, the nuclear magnetic imaging (MRI) technique was used. The obtained images were descriptive and the adsorbed oil was clearly visualized, however, differences between the individual added powders were not large enough to define significant differences between the powders.

¹⁵ The frying yield was determined by weighing the fish patties before and after frying

Sensory evaluation of lean fish pate (Figure 3.13) indicated that with regard to texture, the judges were able to find differences between the pates with added FPH and the original one (no FPH added) where pate 2 (egg exchanged with FPH) judged as having the best texture. No difference was found between pate 2 and 3. Pate 2 (egg exchanged with FPH) was also judged to have the best taste.

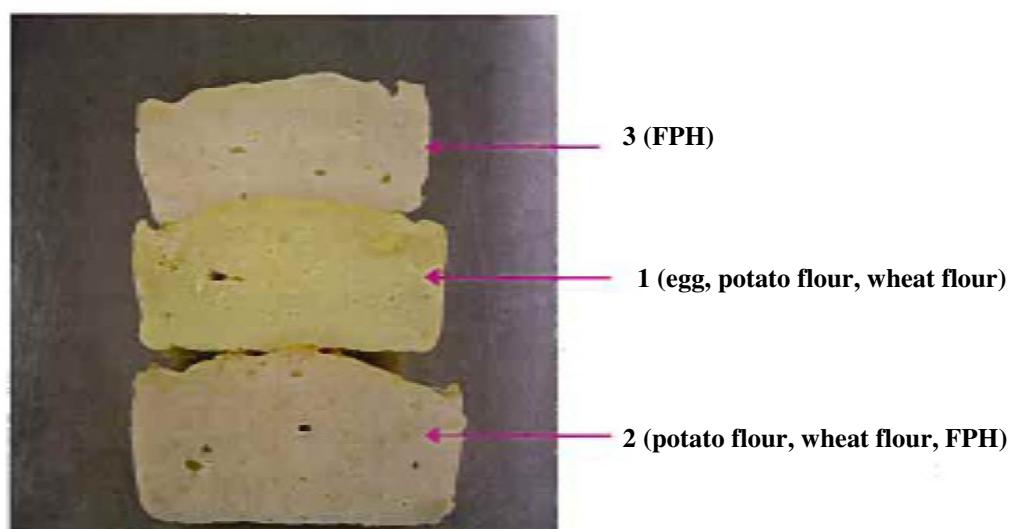


Figure 3.13. Fish pate made with different composition of ingredients.

Fat fish model

The FPH powders that were incorporated into the fatty food model (salmon pate) were evaluated by a trained sensory panel (n=7). The sensory attributes were divided into positive and negative attributes such as total score, fresh taste, fresh smell, off-taste, off-smell, bitterness, and rancidity. For the test were 1% addition of the different commercial products and the laboratory made FPH was evaluated, no significant differences were found between the groups. The products were different, however the evaluators did not agree on the scores due to different preferences and generally low acceptance for the products. Another reason for the low positive scores for the pate might be the low initial shelf-life of the powders. These powders, which were produced for these trials, are highly susceptible to lipid oxidation and maybe also other degradations that affect the sensory properties. The commercial products (MariPep P, C and CK) are usually sold as a liquid concentrate, which is a stable form.



Figure 3.14. Salmon pate made with addition of FPH.

An important test was to find sensory scores of the FPH at different concentrations in the food. The aim of this is to look at effective doses in food due to improvement in shelf-life and bioactivity. A selection of the FPH was used at 3 different concentrations (0.5, 1 and 3% w/w, Table 3.5). The same sensory attributes were used as in the 1% test. These results showed that 3% addition of the commercial product gave significantly lower positive scores and significantly higher negative scores compared to the control. Despite the normal level of addition being 1%, the aim of this experiment was to look at the sensory effect of more bioactive concentrations of the ingredients. The laboratory made FPH was comparable to the control (whey protein) and did not get as low positive scores. Overall these results showed that the difference in freshness between the powders was significant and even though one of the powders used (MariPep P) was regarded as within the shelf-life, it was degraded (see under water solution below). A sensory analysis of the same products after 5 months of storage at +4°C showed that the commercial MariPep had the least acceptable taste, all products, including the lab made FPH received higher scores for negative smell after storage. When using FPH as a powder, more investigation is needed on the stability and the shelf life.

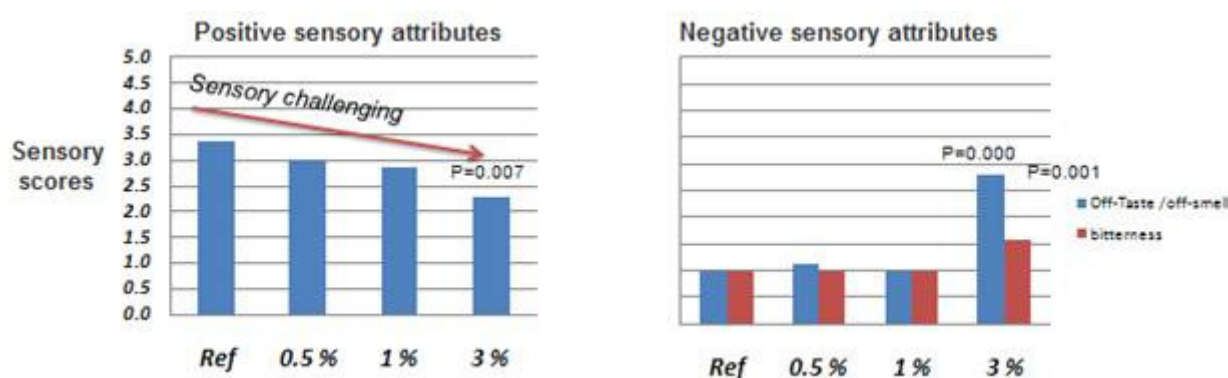


Figure 3.15. Results from the sensory evaluation of three different concentrations of MariPep P (commercial FPH) when used into salmon pate.

Oxidation of the samples with added protein powders during the first two months of storage was similar to the oxidation of control samples without added protein powders. Due to all mentioned earlier it is clear that FPH should be examined more then it comes to storage state (powders vs. liquid) and in incorporation into food systems.

Water solution: A sensory test comparing water solution (1 and 10%) of completely fresh powders and liquid concentrate (commercial form) and within shelf-life powders (approx 5 months) of MariPep-products showed that the freshly made (powders and liquid concentrate) were more appreciated by the evaluators. A colour difference was seen between the powders that were 3 months and those powders that were completely fresh. Figure 3.16 demonstrates that the water solutions of the fresh powders were lighter in colour and more transparent compared to the stored powders. A liquid concentrate is probably a better way of keeping the quality.

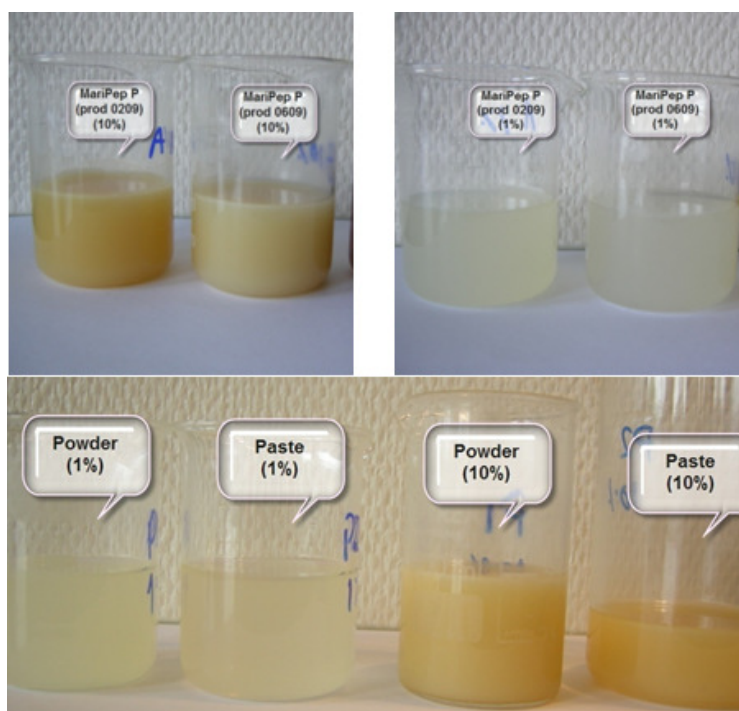


Figure 3.16. Photograph of different concentrations (1 and 10% w/w) of freshly made (a few days) and 4 months produced MariPep P in a water solution. Also water solutions of freshly made liquid concentrate and powders are shown.

3.4 Fish gelatin

Four ingredient based studies were executed on fish gelatin and one comparison study.

Box 7

Aim:

- To investigate the effect of extraction conditions on the structural and mechanical properties of cold water fish gelatin from saithe (*Pollachius virens*) skins.
- To study the relationship between the weight average molecular weight, the molecular weight distribution and the gelling properties of gelatins from different sources.
- Attempt to build a model to quantify the effect of the fractions of α - and β -chains as well as the higher and lower molecular weight components on the mechanical properties (Bloom value and dynamic storage modulus) of mammalian and cold water fish gelatins by using principal component analysis (PCA) and partial least squares regression (PLSR).
- To compare the effect of low molecular weight fish gelatin molecules and polyols (glycerol and sorbitol) on the dynamic storage modulus, gelling and melting temperatures of mammalian and cold water fish gelatins.

Outcome:

- Extraction of gelatin from cold water fish species can take place at room temperature
- The dynamic storage modulus and Bloom value for all types of gelatin increased with increasing weight average molecular weight. The Bloom values for gelatin from haddock, saithe, and cod were determined to be 200, 150 and 100 g.
- Removing low molecular weight molecules from a gelatin sample increases the mechanical properties of the resulting gel.
- Two linear relationships between the mechanical properties and the molecular weight distributions were established, one for cold water fish gelatin and one for mammalian gelatin.

Challenges:

- It would be interesting to see the results for gelation kinetics of molecular weight fractions.

3.4.1 Structural and mechanical properties of fish gelatin as a function of extraction conditions

Gelatins were extracted at different temperatures, in different acetic acid concentrations and at different extraction times. The gelatins were characterized according to their weight average molecular weight (M_w), the resulting dynamic storage modulus (G'), melting and gelling temperatures, degree of helix recovery, and compared to commercial gelatins.

The data shows that the extraction of gelatin from cold water fish species can take place at room temperature (22°C). High weight average molecular weight gelatins extracted at room temperature exhibit higher resulting dynamic storage modulus, higher gelling and melting temperatures and more helix formation compared to highly hydrolyzed gelatins extracted

under harsher conditions. The storage modulus was increased 5 times compared to commercial cold water fish gelatin.

Although the mechanical properties of gelatin from several fish species have been reported, the effect of weight average molecular weight and the molecular weight distribution on the mechanical properties of fish gelatin has only been studied to a limited extent (Gómez-Guilén *et al.* 2002; Muyonga *et al.* 2004).

3.4.2 Mechanical properties of mammalian and fish gelatins based on their weight average molecular weight and molecular weight distribution.

Acid porcine skin gelatins, lime bovine bone gelatins and gelatins from haddock (*Melanogrammus aeglefinus*), saithe (*Pollachius virens*) and cod (*Gadus morhua*) were compared according to their weight average molecular weight (M_w), polydispersity index, dynamic storage modulus (G') and Bloom value.

The dynamic storage modulus and Bloom value for all types of gelatin increased with increasing weight average molecular weight. Due to fish gelatins considerably higher weight average molecular weight and lower polydispersity, the dynamic storage moduli were comparable to the corresponding values for acid porcine skin and lime bovine bone gelatins. The Bloom values for gelatin from haddock, saithe and cod were determined to be 200, 150 and 100 g. Furthermore, the data presented in this study shows that removing low molecular weight molecules from a gelatin sample increases the mechanical properties of the resulting gel.

3.4.3 Mechanical properties of mammalian and fish gelatins as a function of the contents of α -chain, β -chain, low and high molecular weight fractions.

Principal component analysis (PCA) and partial least squares regression (PLSR) were used to relate the mechanical properties with the molecular weight distribution. The results suggest a linear relationship between the mechanical properties and the fractions of low molecular weight (LMW) molecules, alpha-chains, beta-chains and high molecular weight (HMW) molecules. The gel strength for cold water fish gelatin was positively correlated with the

fractions of beta-chains and HMW molecules and negatively correlated with the fractions of LMW molecules and alpha-chains.

It is believed that films prepared from gelatin with higher molecular weight fractions exhibit higher tensile strength and lower elongation values while films made from gelatin containing higher proportion of low molecular weight fragments exhibit lower tensile strength, but higher percentage elongation. It is therefore assumed that for a given concentration of plasticizer, a lower molecular weight gelatin can be plasticized to a higher degree (Gómez-Guilén *et al.* 2009).

3.4.4 Effect of polyols and gelatin hydrolysate on the mechanical properties of mammalian and fish gelatin gels

Glycerol and sorbitol are used in a wide variety of pharmaceutical formulations including as plasticizers of gelatin in the production of soft gelatin capsules and in film coatings. Increasing the content of plasticizers (glycerol or sorbitol) and consequently reducing the interactions between the biopolymers chains results in a less stiff, less rigid and more stretchable film.

The influence of low molecular weight gelatin molecules on the mechanical properties of mammalian and fish gelatins were investigated and compared with the effect of the plasticizers. Preliminary results suggest that fish gelatin hydrolysate could potentially be used in combination with plasticizers. The results from this study are expected to be submitted for publication in January 2010.

3.4.5 Comparison of functional properties of dried fish gelatins and their effects on fish muscle

The objective of this study was to compare functional and technological properties of two type of commercial dried fish gelatins. The gelatins ability to be injected into fish fillets were of great interest. The dried fish gelatins investigated were hydrolysed fish collagen from Norland and Faroe Marine Biotech, high molecular weight fish gelatin (HMWD) and collagen peptides (CP), respectively. Evaluation was made on viscosity (Brabender® and Bohlin), thermal properties (DSC), water activity, pH, colour, chemical composition (water, salt, fat and ash), molecular weight distribution of proteins (SDS-PAGE) and FT-NIR. To evaluate the

effects on fish muscle, the gelatin was also added dry to fish mince (*Pollachius virens*) in various concentrations (0, 0.5, 1.5 and 3.0% w/w), and frozen at -24°C for 1 week. The parameters evaluated were drip loss, WHC, T₂ transversal relaxation time and texture.

Both chemical and physical properties of the gelatins were considerable different. The melting point is one of the major physical properties of gelatin gels. This is governed by molecular weight, as well as by complex interactions determined by the amino acid composition and the ratio of α/β -chains present in the gelatin (Karim & Bhat 2009). Differential scanning calorimetry (DSC), heating (-10 to 40 °C) and cooling (40 to -10 °C) scans at 5 °C/min of 6.67% (w/v) gelatin solutions (CP and HMWD) were performed to observe the melting and the gelling temperatures. Melting temperature was observed from the maximum of the endothermic temperature peak and the gelling temperature was observed from the maximum of the exothermic temperature peak in DSC thermogram. The melting and the gelling points of these two gelatins were quite different, where CP showed significantly lower melting and gelling points. These results indicate that CP would be more suitable as ingredient in fish fillets.

The HMWD and CP showed completely different viscosity behaviour (Table 3.6). The HMWD formed very strong gel at the concentration 6.67% (w/v), which entailed that viscosity measurements were impossible at this concentration. Therefore, 3% HMWD solution were prepared and the viscosity measured. The CP solutions showed different behaviour. At the concentration of 6.67% (w/v) no viscosity was found. Increased gelatin concentration (10 and 15% w/v) had no impact on the gel forming ability. Additionally, the effects of salt on the gel forming ability was investigated by adding salt to the fish gelatin solutions (6.67% w/v) at the concentrations of 0, 1.5 and 3% salt. The salt had no effect on CP where no gel was formed with or without salt. The salt, on the other hand, increased the gel forming ability of HMWD.

Table 3.6. Viscosity of the fish gelatin solutions measured with Brabender and Bohlin equipments (CP=collagen peptide; HNWD=high molecular weight fish gelatin).

Sample	Viscosity (BU) ¹⁶	Viscosity (Pascal) ¹⁷
HMWD (6.67% w/v)	Very strong gel	Very strong gel
HMWD (3.0% w/v)	283 ± 4.24	0.041 ± 0.003
CP (6.67% w/v)	NO viscosity	NO viscosity
CP (10.0% w/v)	NO viscosity	NO viscosity
CP (15.0% w/v)	NO viscosity	NO viscosity

The CP had lower water content and higher protein (nitrogen) and salt content than HMWD. The protein patterns of HMWD and CP were, as anticipated, very different. The molecular weight of the HMWD covered the range from 212-61 kD while CP was from 66-2 kDa. The CP is therefore more degraded (smaller protein units) which can be associated with the low viscosity and low melting point.

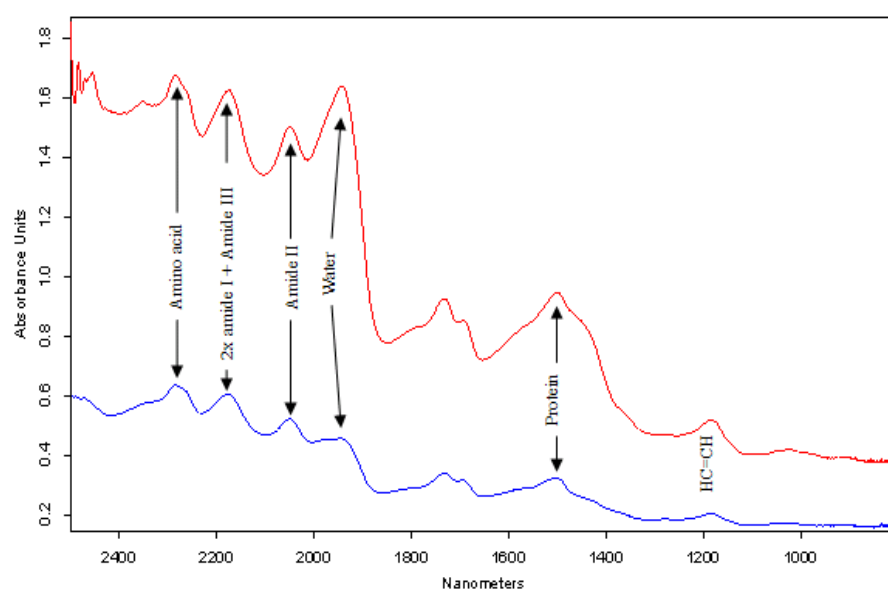


Figure 3.17. Average FT-NIR spectra of the dried gelatin (Intensity (log(1/T)) vs. Wavelength (nm)). Red=HMWD (high molecular weight fish gelatin); Blue=CP (collagen peptides).

The average FT-NIR spectra of the dried gelatins are shown in Figure 3.17. Similar spectra were observed for the dried gelatins, but the bands for the HMWD showed higher intensity compared to the CP. Assigning of peaks was done by referring to overtone reference tables (Osborne *et al.* 1993). The peak at 1945 nm is very distinct between the gelatins but it

¹⁶ At +5°C (measured with Brabender®)

¹⁷ At +5°C to +7°C (measured with Bohlin)

represents water (O-H stretching). This peak showed much higher intensity for HMWD, compared to CP, which could perhaps be correlated to higher water content in HMWD.

Because of very different physical properties, only CP was added to fish mince samples to study the effects on fish muscle, where the HMWD would not be useable for addition into fish fillets. Increased concentration of gelatin added to mince samples resulted in less drip loss and concentration above 1.5% (w/v) had the most impact. There was no significant difference in WHC, texture and T_2 transversal relaxation times between samples with various gelatin concentrations. Addition of the fish gelatin increased the water yield¹⁸ in all the treated groups compared with the control sample

¹⁸ Water yield = (water% in thawed mince samples / water% in raw material) x yield after storage

3.5 Comparison of protein ingredients for injection in fillets

Several studies were performed where different protein ingredients were injected into whitefish fillets.

Box 8

Aim:

Compare the properties of selected protein ingredients and their influence on injected fillets. The protein solutions evaluated were fish protein hydrolysate (FPH), fish protein isolate (FPI), homogenized fish protein (HFP) and gelatin.

Outcome:

- Properties of protein solutions for injection
 - ✓ FPH contained considerable higher amount of protein and salt than the other ingredients tested.
 - ✓ FPH and gelatin had lowest viscosity.
 - ✓ FPI gave less weight loss, high viscosity and relatively high pH compared to the other fish protein solutions.
- FPI and FPH injected in fresh and lightly salted cod fillets (frozen).
 - ✓ Yield of thawed lightly salted fillets increased by injection. FPH in fresh fillets gave the highest yield.
 - ✓ Protein- and water-yield in fresh and lightly salted fillets increased by injection. Water yield was highest in fresh fillets treated with FPH.
 - ✓ The FPH improved the colour (whiteness) of fresh fillets.
 - ✓ The FPH increased WHC in fillets; the water was more firmly bound (higher T_{21}).
 - ✓ Injection of fish protein solutions in lightly salted cod fillets resulted in shorter cooking time.
- ✓ HFP, FPH and gelatin injected in chilled and frozen saithe fillets.
 - ✓ Yield increased in fillets (fresh and frozen) by injection of fish protein solutions.
 - ✓ Adding gelatin, combined with other protein solutions, did not influence the yield.
 - ✓ Fresh fillets had higher total yield after cooking than the frozen fillets.
 - ✓ Addition of protein solutions increased drip in the fillets compared to control. FPH increased drip less than other protein solutions.
 - ✓ Freezing and the frozen storage reduced the quality of all fillets.
 - ✓ Least deterioration in yield and water holding capacity during frozen storage was found in fillets with added FPH.

Challenges:

- Documentation of appropriate ingredient with regard to species and type of processed product.
- Optimize methods for addition of ingredients in fillets with regard to species and material condition.

3.5.1 Comparison of properties of protein solutions for injection

The properties of selected protein solutions that are important when used for injection in fillets were compared. The protein solutions investigated were fish protein isolate

(Iceprotein), fish protein hydrolysate (MariPep C, Danish Fish Protein), homogenized fish protein and dried collagen peptide (Faroe Marine Biotech). Evaluation was made on weight loss, viscosity (Brabrand® and Bohlin), pH, chemical composition (protein, water, salt) and molecular weight distribution of proteins (SDS-PAGE).

The FPH had the lowest water content and the highest protein and salt content ($3.6\% \pm 0.1\%$). There was no significant difference found in chemical composition between the other fish protein solutions. Results obtained with SDS-PAGE showed how the fish protein solutions differ in molecular weight distribution. The FPH and the gelatin solutions contained a higher proportion of smaller protein units while the other fish protein solutions contained a higher proportion of larger protein units and even myofibrils. The FPH had a wide molecular weight distribution from 2-212 kDa, while the gelatin contained no molecules bigger than ~66 kDa.

Weight loss of the fish protein solutions indicates the ability of the soluble proteins to retain water, i.e. a high weight loss of the protein solutions can indicate that their ability to retain water is low. The FPI showed the lowest weight loss, while there was no significant difference between the other solutions. Studies have shown that increased pH, above pI, can increase water holding capacity (Wagenknecht & Tuelsner 1975; Thorkelsson 2007). The FPI was significantly more alkaline (9.28 ± 0.1) compared with the other fish protein solutions, which can explain the difference in weight loss. According to Fennema (1990), the mean isoelectric point (pI) of the myofibrillar proteins are about pH 5-6. Minimum water holding capacity, swelling and protein solubility of meat has been observed around the pI, but it increases again with either decreasing or increasing pH value.

The lowest viscosity was obtained for the FPH which may be correlated to considerable smaller protein units. Viscosity can be limiting factor with regard to injection ability of the protein solutions and may affect how well they are retained within the fish muscle.

3.5.2 Comparison of the effect of injected protein solutions on fillet properties

A couple of studies were performed where fresh and lightly salted cod fillets were injected with fish protein isolate (FPI: from Iceprotein) and fish protein hydrolysate (FPH: MariPep C from Danish Fish protein). The influence of the FPI, homogenized fish protein (HFP: from

SVN) and Gelatine (collagen peptide from Faroe Marine Biotech) on chilled and frozen saithe fillets was also evaluated.

Effects of fish protein solutions on chemical and physicochemical characteristics of fresh and lightly salted cod fillets

The objective of this research was to study the effects of added proteins on yield (total yield, cooking yield), stability (drip), functional properties (WHC, T_2 transversal relaxation time), chemical composition, colour (whiteness measured with chroma meter) and texture (hardness measured with TA-XT2 Texture Analyser) of fresh and lightly salted cod fillets. The aim was to increase yield and improve functional properties (WHC) of the fillets and thereby increase quality.

Fresh and lightly salted cod fillets were injected with FPI and FPH, stored at -24°C for 1 month and then compared to untreated control fillets. This is a short storage time at good storage conditions. Other results can be gained if the fillets are stored for longer time and/or at lower temperature e.g. -18°C.

Yield after thawing increased with addition of fish protein solutions in fresh and lightly salted fillets. The highest yield was obtained by the use of FPH in fresh fillets. The fish protein solutions had no effect on the water holding capacity of the thawed fillets.

Total yield of fillets was also evaluated (yield after storage (freezing/thawing) multiplied by cooking yield). The highest total yield was obtained in fresh fillets injected with FPI (Figure 3.18). Injection of FPI into lightly salted fillets also increased total yield (compared to the control group). Fillets injected with FPH gave similar total yield for both fresh and lightly salted fillets. Addition of fish protein solutions to the fillets increased the protein¹⁹- and water²⁰-yield for both fresh and lightly salted fillets compared to the control fillets. Water yield was highest in fresh fillets treated with FPH which is a result of a relatively low drip after thawing. The FPH had good impact on the colour (whiteness) of the fresh fillets but there was no significant difference between the lightly salted fillets. No significant change was found in texture (hardness) of the fillets by protein (FPI, FPH) addition.

¹⁹ Protein yield = (%protein in frozen fillets / %protein in raw material) x yield after storage.

²⁰ Water yield = (%water in frozen fillets / %water in raw material) x yield after storage.

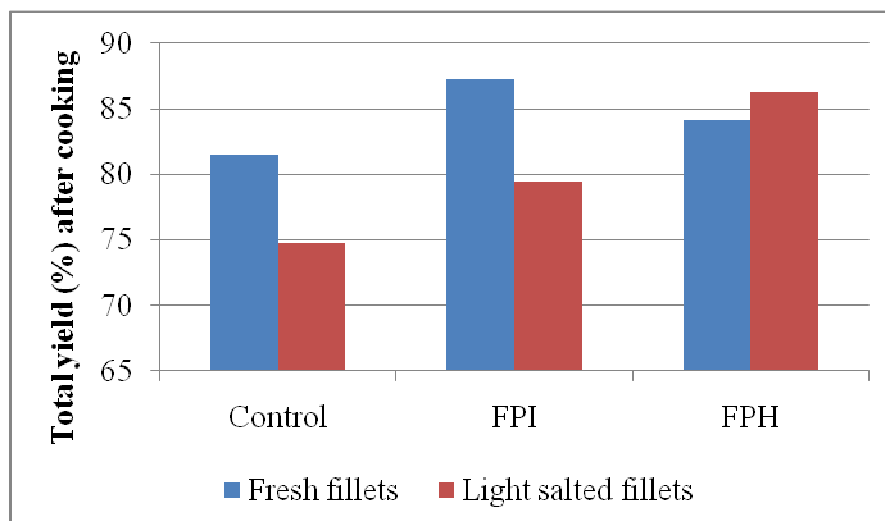


Figure 3.18. Total yield²¹ after cooking after 1 month of frozen storage. (FPI=fillets injected with fish protein isolate; FPH=fillets injected with fish protein hydrolyse (MariPep C)).

The water holding capacity (WHC) and transversal relaxation time (T_2) of fresh cod fillets, untreated (control) or injected with protein solution (hydrolysate or isolate), were also analysed. The T_2 transversal times can indicate the mobility and the location of water within the fish muscle. T_{21} indicates intracellular fluid (water bound by large molecules such as proteins); T_{22} on the other hand indicates extracellular fluid (water which is easily lost by drip).

The highest WHC was measured in fillets injected with hydrolysates, the lowest in fillets injected with isolate. Fillets injected with hydrolysate had higher T_{21} and lower T_{22} compared to untreated fillets. This indicates that the water in the hydrolysate treated fillets is more firmly bound than in untreated fillets. This is in agreement with the WHC results. Results on fillets injected with isolate showed different behaviour. WHC was lowest in these fillets. However, T_{21} was similar as in untreated fillets but T_{22} lower. This indicates that the bound water is similar, but the free water is lower in the isolate treated fillets - these were less prone to lose water which does not comply with the WHC results.

²¹ Evaluation of total yield was determined by multiplying the yield after storage (chilling, freezing (thawing)) and the cooking yield.

Effects of fish protein solutions on heat-profiles and cooking yield of fresh and lightly salted cod fillets

The objective of this study was to investigate the effects of salt and added fish proteins on heat profiles and cooking yield of cod loins. Conventional (steam cooking, boiling and baking) and microwave heating were compared. Microwave heating gave a different rate of heating.

Salt had a major influence on the heat profiles by decreasing the rate of heating compared to untreated fillets, i.e. fresh fillets took shorter time to cook (reach $>72^{\circ}\text{C}$). This difference is considered to be due to the fact that salt binds to water molecules which leads to fewer water molecules to generate heat. Adding protein to the fillets generally did not affect the heat profiles of fresh products. Addition of fish protein solutions to lightly salted cod fillets resulted in shorter cooking time (reach $>72^{\circ}\text{C}$) compared to the control group.

Boiling the loins generally gave higher cooking yield in all the groups compared to other cooking methods. On the other hand, microwave heating and baking gave considerably lower cooking yield. Addition of fish protein solutions increased the yield after cooking when boiled and baked, but had no effect on the fillets when heated in microwave.

Effects of FPH, HFP and Gelatin on chilled and frozen saithe fillets

The objective of this research was to study the effects of added proteins on yield (total yield and cooking yield), stability (drip, water yield), functional properties (WHC, T_2 transversal relaxation time) and chemical composition of fresh and frozen saithe fillets. The aim was to maintain or increase yield and improve water holding capacity (WHC) of the fillets and thereby increase quality.

The effects of homogenized fish protein (HFP), fish protein hydrolysate (FPH: MariPep C), hydrolysed fish gelatin and salt were evaluated. The fillets were stored at $+4^{\circ}\text{C}$ for 4 days and at -24°C for 1 week and 1 month, respectively.

Addition of fish protein solution increased the yield of fresh and frozen saithe fillets. Using gelatin, combined with other protein solutions, did not influence the yield. Total yield of the

fillets after cooking was higher in fresh fillets than in frozen. Addition of protein solutions increased drip in the fillets compared to control. FPH gave less increase in drip loss than the other protein solutions. Addition of protein solutions and/or salt resulted in higher water content and water yield in fresh fillets compared to control fillets. Freezing and the frozen storage significantly decreased the quality of all the fillets. Least deterioration in yield and water holding capacity during frozen storage was found in fillets with added FPH.

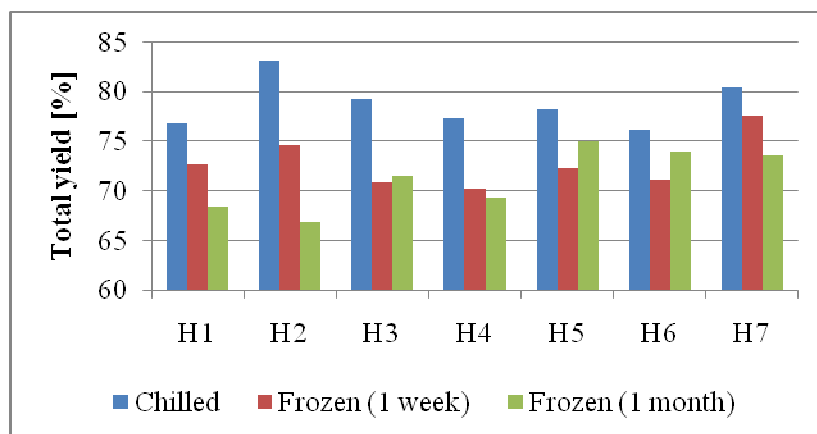


Figure 3.19. Total yield²² (%) after cooking after chilled and frozen (1 week and 1 month) storage. (H1=Control; H2=salt injection; H3=Salt and HFPI(1) injection; H4=4% salt and gelatin injection; H5=4% salt and HFP(1)+gelatin injection; H6=4% salt and HFP(2) injection; H7=FPH injection).

²² Evaluation of total yield was determined by multiplying the yield after storage (chilling, freezing (thawing)) and the cooking yield.

4 Conclusions

4.1 Main outcome of the project

In this project, analysis and improvement of protein ingredients derived from rest raw materials from the processing industry was emphasised. The rest raw materials from the processing industry, such as the head, backbones, trimmings (cut-offs), skin and guts, have different properties and are thus basis for different ingredients and applications.

A large market for ingredients from rest raw materials is within the fish industry itself. The focus was therefore on ingredient properties important for utilization in processing lines of whitefish fillets and emulsion based foods. In addition to looking at the general raw material properties and application, special emphasis was put on properties and production of fish protein isolates (FPI), fish protein hydrolysates (FPH), homogenized fish protein (HFP) and fish gelatin.

Production of high quality ingredients can only be achieved by selection and good handling, storage and processing of the raw material. Results from the project showed that frozen backbones and cut-offs of saithe were unstable, salt soluble proteins were rapidly dissoluble and lipid oxidation, especially in dark muscles, was pronounced. Cut-offs from saithe were more susceptible to lipid oxidation than cut-offs from cod which can be explained by the higher fat content. To reduce the rate of oxidation, cut-offs should be kept in oxygen tight bags, i.e. vacuum bags, during storage and should preferably be kept at -24°C or lower.

Raw material properties and storage/processing have a significant influence on the properties of FPH. Use of fresh raw material (backbones from cod) gave higher yield of FPH (as dried powder), lighter powders with better emulsification properties compared to frozen material. Only small differences in properties were however observed between pre- and post-rigor backbones. Bioactive (gastrin/CCK- and CGRP-like peptides) molecules were obtained in the hydrolysates from cod backbones and by using pre-rigor backbones, the amount of gastrin/CCK like molecules was increased.

Time of hydrolysis is an important factor for the properties of FPH. Longer time of hydrolysis increased the yield (amount of FPH), increased the degree of hydrolysis (smaller peptides)

and decreased water holding capacity (WHC) of the powders. Moderate time of hydrolysis (15 to 45 min) yielded FPH with the best emulsifying properties.

Despite growing knowledge, there are still many challenges facing production and application of FPHs. Among those are collection and preservation of raw material before hydrolysis and optimisation of the hydrolysis process with regard to desired properties (taste and stability of the powders during storage, content of bioactive peptides etc.). Before FPH can be marketed on a wider scale, further standardization and documentation of the process and properties of the FPH, both functional and health beneficial properties, is needed.

Production of mince is a common first step in processing of rest raw materials of fillet production such as cut-offs and frames. Fresh mince has many applications but is in itself a rather unstable product. It is therefore common practice to freeze it as quickly as possible. However, freezing the mince reduces the water holding capacity which is an important property when the mince is applied as an ingredient in fillet processing (injection). By homogenizing the mince, its quality (including stability) as an ingredient could be improved.

Properties such as gel strength, gel forming ability and colour of FPI made from cut-offs are significantly different from conventional Surimi and FPI made from fillets. Such FPI are however still a good source of protein for manufacturing products which do not need high level of gel strength, such as fish burgers, fish nuggets and other ready to eat fish products. Addition of salt and sucrose improved the stability of the FPI. Further optimisation of the FPI process is however needed for improved stability, texture, taste and flavour of FPI.

Extraction of gelatin from cold water fish species can take place at room temperature. As the weight average molecular weight of gelatin increases, the dynamic storage modulus and Bloom value increases. The Bloom values for gelatin from haddock, saithe, and cod were determined to be 200, 150 and 100 g. By removing low molecular weight molecules from a gelatin sample, the mechanical properties, i.e. the strength, of the resulting gel increased. Two linear relationships between the mechanical properties and the molecular weight distributions were established, one for cold water fish gelatin and one for mammalian gelatin.

The protein ingredients studied in this project (FPH, FPI, HFP and gelatin) have different properties and thus are best suited to specific applications. Viscosity was higher in FPI than in

FPH and gelatin. All tested FPH showed an antioxidative activity, but differences in radical scavenging ability, different kinetic behaviour for iron chelating ability, reduction of Hb and iron induced oxidation were observed among the products. Generally, addition of FPH would extend shelf life of products by acting as an antioxidant against haemoglobin (Hb) and iron induced oxidation.

Fish protein injection is believed to enhance the yield and improve the frozen stability of fish fillet. Injection of HFP, FPI, FPH and gelatin increased the yield of fillets. Among those, the FPH was found to have the most positive influence on the fillets (colour, WHC). Two methods were evaluated for preparation of several ingredients before injection, the Suspentec process and the homogenization process. Incorporation of FPI, mince or surimi in salt brine for injection by the Suspentec process increased the total yield of fillets. Shelf life of the injected fillets was however not sufficient for exporting fresh fish products to the market, despite application of super-chilling during storage. By homogenization of mince before injection a more homogenous mix and a decreased number of microbes was achieved. The total yield of injected fillets was also increased. Thus, homogenization resulted in decreased number of microbes in the fillets and longer storage life.

Rest raw materials can be used to improve the properties of consumer products. Mixing cut-offs with brine can create “fish-glue” which can be used to improve the texture of formed products. By using “fish-glue”, pressure at forming could be reduced, keeping more of the natural muscle structure intact in the final product. Products became more uniform, coherence improved, drip and cooking loss was reduced. Addition of FPI into fish balls improved shaping and setting as well as influencing the texture.

Addition of FPH to a lean food model (cod pate) had a positive effect on texture and taste. Concentration of the added FPH was important for sensory properties, with 3% (w/w) being less appreciated compared to addition of 0.5 and 1%. No significant differences were found in sensory acceptance among the salmon pate fortified with laboratory-made and different commercial powders (1% w/w). Relatively short storage of commercial products (as powder) had impact on the taste properties of enriched salmon pate. The bitter taste of the peptides had a negative influence on the acceptance of the product, a crucial factor to overcome in food applications. However, adding FPH (1% w/w) improved the freezing stability and juiciness of food. The effect was not as strong as by using phosphate but was still significant.

Fish is a highly perishable raw material (i.e. microbial growth, lipid oxidation, enzymatic degradation of proteins and lipids). Retaining the quality of fish and its derived products as raw material and ingredients is one of the main challenges for the whole fish industry. There are indications for stability improvement through addition of specific ingredients such as FPH.

Further search for bioactive peptides from different fish species and parts, documentation of their health effects (bioactive and health effects by in vivo trials) in various food models is an important factor in increasing the value and application possibilities of low value rest raw materials from the fish processing industry.

4.2 Market potential

In 2006, more than 110 million tonnes (77%) of the world fish productions was used for human consumption (FAO 2008). From this about 57 million tonnes were used for manufacturing products for direct human consumption. Up to 50-70% of the fish may end up as rest raw materials as the yield in filleting operation is from 30-50% (Kristbergsson & Arason 2006). About 6 million tonnes of trimmings and rest raw materials from fish processing are processed into fish meal and the rest is used in fish silage or discarded. Significant additional nutritional, economic and environmental value can be obtained by increasing the yield of raw material in fish filleting operation.

In recent years, the fish industry has placed emphasis on utilizing the whole catch and to do this with the highest possible profit. Most of the trimmings from filleting processing are utilized for mince production, e.g. backbones, belly flaps etc. It is a common practise in the meat, poultry and fish industry to add up to 12% brine to modify both fresh and processed products. This is done to improve quality, firmness and juiciness and to increase yield. The use of functional proteins as additives in food products has increased over the last years. It is well established that addition of functional proteins can increase water- and fat binding properties of the products and improve texture and stability. Results obtained from this project are valuable with regard to this. It is of great interest to utilize fish protein as additives to increase quality and value of fish products but further development and optimization is

needed with regard to desired properties. There is an increasing interest in the fish processing industry to use fish proteins to improve yield, quality and other beneficial effects of the products.

Fish fillet and minced fish in Europe and surimi in Japan and South East Asian countries have been used for manufacturing value added fish products for many years. Ingredients which are used for manufacturing these products are very important from a health and technological point of view. FPI and FPH are good sources of food ingredients that can be added to the product for value addition and improving functional properties.

In order to be used as a food ingredient, fish proteins should add a desirable property to the food. For the health food sector of the food industry, bioactive ingredients with an effect on obesity and blood pressure regulation should be highly appreciated. These effects are usually higher the more refined the products are, and high concentrations of e.g. FPH are therefore needed to gain a health effect compared to peptides that are fractionated to optimize the effect. The food industry requires considerable documentation of the health effects in order to label with health claims. Another interesting property for the food industry is the possibility to extend shelf-life. The antioxidative properties that are documented in this project are particularly interesting in fish and fatty products. When using the ingredient, in different applications, knowledge is needed on how this addition influences the functional properties. Results obtained in this project are valuable with regard to this. Finally, the food industry requires that the ingredient does not affect the sensory properties in a negative way since taste is still the major quality parameter.

Mammalian gelatin with Bloom values in the range of 150-210 g is used by the pharmaceutical industry to produce soft gelatin capsules. Soft gelatin capsule manufacturers have for some time attempted to produce capsules from cold water fish gelatin. This has now been achieved which may expand the application area of cold water fish gelatin.

Other potential use of cold water fish gelatin may be as an edible film on frozen or dry food products, in low-fat (substitutes for fat) and low-carbohydrate (substitutes for carbohydrate) food products as well as a protein source and a binding agent for cereal bars.

4.3 *Next steps*

One of the main focuses of this project was to improve the competitiveness of the fish industry by industry driven research. Raw material quality and thereby the ingredient production can be improved by specification of the raw material, keeping of a satisfactory cold chain and optimal handling procedures. These aspects need to be further specified to enable the best practice for producing high quality protein fractions (food ingredients). The industry (using the ingredients) aims to identify the product specifications for the proteins with regard to each application.

Over the years, researchers have gained more and more knowledge about the fish muscle, the protein ingredients, their applications and the beneficial effects that can be gained. It is therefore interesting to take a step back and take an overlook of the information we already have gained. We need to estimate and determine how we can use this knowledge and work further with this. There is also a need to establish what is the aim with protein production, what we can gain by this and more and foremost establish what the market and the consumers need. It is interesting and necessary to make standardized protein products where we can e.g. produce certain standardized product with certain desirable properties such as increased stability, bioactivity or health beneficial effects. In other words, be able to claim that certain protein product is more suitable for a certain food product and other protein products for totally different food product etc. Due to large variation between protein products and the raw materials, even if the producer claims it is the same products, it is very important to establish standards. The variation in product properties has a negative effect on the market and may even destroy the market. This is an urgent issue and an important subject for future projects.

It is very important to investigate the needs and demands of the part of the market where addition of protein is currently not used today, such as in fillet processing. In the fillet industry, it can be difficult to claim that protein added fillets are e.g. more stable or healthier than untreated fillets. We need to be able to show and prove that protein addition can be an advantage and therefore the market and the industry needs to be introduced to this option. Results from this project have for instance shown that protein products can be used as antioxidants. It could therefore be interesting e.g. to inject them into herring which has high fat content and examine if it has antioxidants effects and can prevent rancidity development.

The market for healthier food products is continuously growing and therefore the demand for improved and healthy food has increased. Documentation of positive health effects of protein products added to food systems is therefore important. For many producers, such as Mills, the nutritional effects of added proteins and antioxidant properties are the most important. It is a great advantage if the producers can claim that certain food product can affect e.g. the blood pressure, cholesterol or obesity. It is also important that addition of protein do not damage the final products. In that aspect it is important to develop processing methods to prevent off flavour which can be linked to protein products such as FPH.

New ideas for further studies and partnership of this unique project group is to select specific model products where different aspects are investigated such as stability, documentation to support new health claims, convenience and other important properties. This can give a platform where different partners would look at the aspects they have the most knowledge in. For the industry, this could be a valuable way to document health benefits which is needed to be able to label the benefits according to legislation.

4.3.1 Development within this field

The project group has had a unique composition with partners from academia, both university and research institutes, and from industries in the participating countries producing range of different products (Figure 4.1). All partners have been actively engaged in the project. This has created a very good platform for further work on utilisation of fish and fish rest raw material.

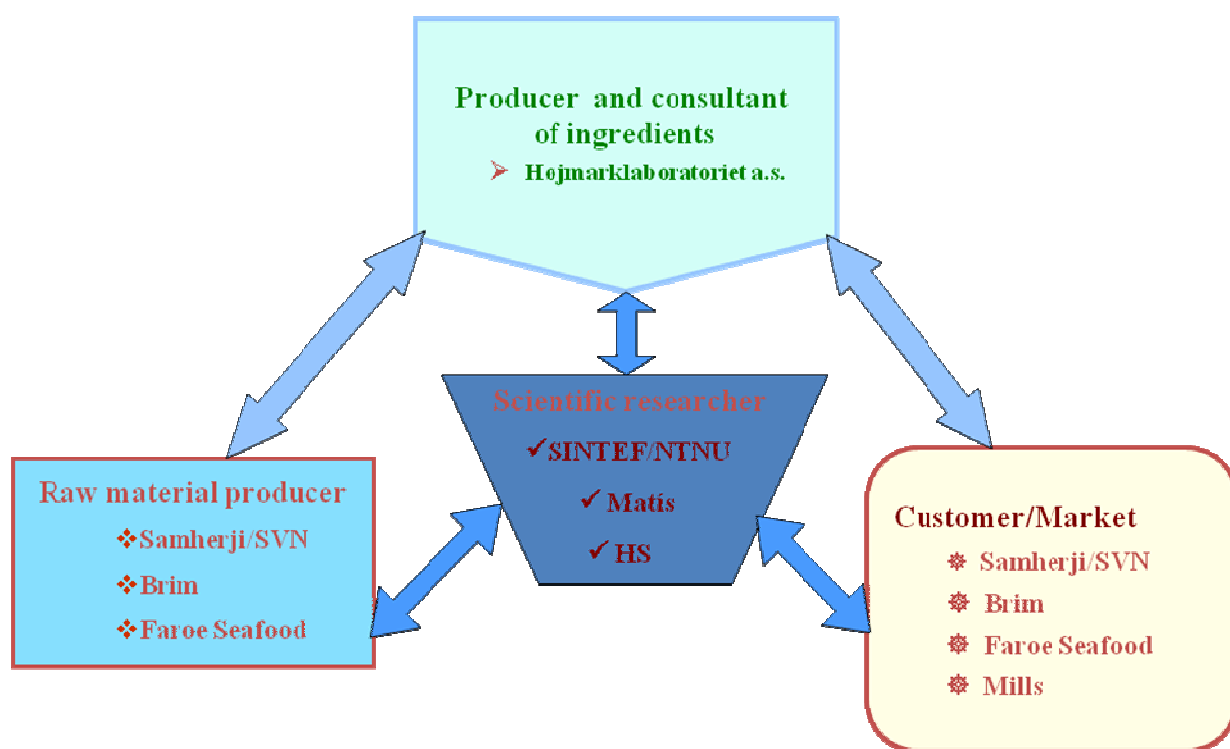


Figure 4.1. The project diagram.

This project gave also very good platform for involving and educating of students, both national and international, resulting in several student thesis and recruitment to the industry. Following is a list of involved bachelor, master and doctoral students and their project title:

- Eysturskarð, J. 2009. *Mechanical properties of gelatin gels; Effect of molecular weight and molecular weight distribution*. PhD. thesis. Dep. Biotechnology, NTNU.
- Iversen, M., Hansen, A. I. and Ertzaas, T. 2007. *Marint restråstoff*. B.Sc. thesis. Dep. Food and medical technology. Sør-Trøndelag College.
- Johansen, A. M. 2009. *Properties of fish protein hydrolysate: comparison of commercial and laboratory made hydrolysates*. M.Sc. thesis. Dep. Biotechnology, NTNU.
- Karlsdottir, M.G. 2007. *Homogenization in the fish industry*. B.Sc. thesis, Dep. Food science and nutrition, University of Iceland.
- Karlsdottir, M.G. 2009. *Application of additives in chilled and frozen whitefish fillets, effects on chemical and physicochemical properties*. M.Sc. thesis. Dep. Food science and nutrition, University of Iceland.
- Kempkes, M. 2008. *Saithe fillet by-products: Storage stability, functional properties and hydrolysis*. M.Sc. thesis. Univ. Bonn (work done at Dep. Biotechnology, NTNU).

- Shaviklo, G.R. 2008, *Evaluation of fish protein isolate products*. M.Sc. thesis. Dep. Food science and nutrition, University of Iceland.
- Úlfarsson, H. 2008. *The use of microwaves in food research*. B.Sc. thesis. Dep. Food science and nutrition, University of Iceland.

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Appendix

Materials and methods

1 Ingredients from rest raw material of processing lines

1.1 Properties of ingredients produced from fillet production

1.1.1 Evaluation of chemical and functional properties of fish mince

Physical properties of fresh and frozen saithe (*Pollachius virens*) mince made from cut-off and frames were studied. The minces were obtained from Síldarvinnslan.

1.1.1.1 Water holding capacity (WHC)

The water holding capacity (WHC) was determined by a centrifugation method (Eide and others 1982). The sample (n = 3) were coarsely minced in a mixer (Braun Electronic, Type 4262, Kronberg, Germany) for approximately 15s at speed 5. Approximately 2 g of the minced cod muscle was weighed accurately and immediately centrifuged at 210 x g for 5 min, with temperature maintained at 4°C. The weight loss after centrifugation was divided by the water content of the sample and expressed as %WHC.

1.1.1.2 T₂ transversal relaxation time measurements

The transverse relaxation time, T₂, was measured with CPMG (Carr-Purcell-Meiboom-Gill) pulse sequence. The data was processed with a bi-exponential fit, giving various relaxation times, characteristic for the different water population, i.e. water tightly bound to the muscle structure and free water (less tightly bound water). Fitting the absolute value of the CPMG is shown in following equation:

$$\text{Signal} = A_{21} \exp(-t/T_{21}) + A_{22} \exp(-t/T_{22})$$

Where T₂₁ and T₂₂ were the relaxation components, and A₂₁ and A₂₂ were the corresponding amplitudes. Since the absolute relaxation amplitudes are proportional to the amount of sample (or water) present, the relative amplitudes within the samples were used. T₂₁ population were calculated as A₂₁/(A₂₁ + A₂₂), and T₂₂ population as A₂₂/(A₂₁ + A₂₂). Four parallel samples from each group were averaged. The measurement settings for the T₂ measurement can be viewed in Table 1.1.

Table 1.1 Transverse relaxation time settings

<i>NS</i>	16
<i>RD</i> [s]	10
<i>RG</i> [dB]	70
<i>DS</i>	0
Detection mode	Magnitude
Bandwidth	Narrow
τ [ms]	0.25
<i>N</i>	8100

1.1.2 Fish protein isolate

1.1.2.1 Evaluation on the quality of FPI

The quality of FPI made from rest raw materials of filleting process of cod (*Gadus morhua*), saithe (*Pollachius virens*), and Arctic char (*Salvelinus alpinus*) were determined based on the Codex Code of Practice for frozen surimi (FAO/WHO 2005).

1.1.2.2 Influence of variation in salt concentration, cryoprotectants and chilled and frozen storage on physical properties of cod protein solutions and haddock protein isolate

Atlantic cod (*Gadus morhua*) protein solutions (CPS) were extracted from cut-offs using pH-shift process at Iceprotein ehf. in Iceland. It was stored at +2°C until used. Three samples were taken for microbial tests (total count) under hygienic conditions.

1.1.2.2.1 Preparation of test samples

36 samples of CPS were prepared as follows:

- 12 fresh samples containing 1.2, 3, 5, 10, 15 and 20% salt, stored at +2°C, 5 days.
- 12 frozen samples containing 1.2, 3, 5 and 15% salt, stored at -24°C for 14 weeks.
- 12 frozen samples with 1.2, 3, 5, and 15% salt, and cryoprotectants (sucrose, sorbitol and polyphosphate with 1.1 and 0.1% respectively), stored at -24°C for 14 weeks.

36 samples of HPI were prepared as follows:

- 9 samples without any additives
- 9 samples containing 0.8% salt and 3% sucrose

- 9 samples containing 1.3% salt and 5% sucrose
- 9 samples containing 4% sucrose and polyphosphate

4 samples were stored at +2°C for 2 days, 16 samples at -18°C for 12 weeks and 16 samples at -24°C for 12 weeks.

1.1.2.2.2 Microbiological analysis

Aerobic plate count was conducted according the procedures of Compendium of Methods for the Microbiological Examination of Foods (APHA 1992).

1.1.2.2.3 Chemical analysis

Dry matter was calculated as the loss in weight during drying at 105°C for 4 hours (ISO 1983).

TVB-N content of samples was measured using direct distillation into boric acid (based on AOAC 1990). The acid was then titrated with a diluted sodium hydroxide solution. The unbound ammonia was calculated as g/16gN.

1.1.2.2.4 Water holding capacity and weight loss

Weight loss and water holding capacity (%) was determined by centrifuging of 2 g of the FPS using Biofuge Stratos; Heraeus Instruments (GmbH&Co., Hanau, Germany). Temperature interval was set at 5°C, speed 1350 rpm and the time was 5 min. After the centrifuging had completed, the difference in weight of the samples before and after (centrifuging) was noted.

1.1.2.2.5 Viscosity

The viscosity was also analysed by using **Bohlin** BV88 viscometer (Bohlin Instruments, England). A beaker containing 200 ml of sample was put inside a 500 ml beaker containing crashed ice to control temperature. The instrument cylinder was immersed into the solution. The viscosity of the sample was recorded after 20 seconds of operating instrument at 5 to 7°C, speed setting 6, system switch 6. Measurements were done in triplicate. Viscosity was reported as Pascal.

The **Brabender** viscosity of the fish protein solutions was determined using a Brabender® Viscograph E coaxial viscometer (Brabender® OHG, Duisburg, Germany). The Brabender®

Viscograph E enables automatic analysis on samples where the material can be studied on a wide temperature scale and the effects of heating and cooling can be analyzed. It is a rotational viscometer comprised of an electronic measuring system, sample bowl (with 8 protruding pins in it), and a seven pin stirrer. A computer is connected to the device to enable visual inspection of the progress of the analysis and input of test parameters. This instrument measures a resistance of the sample against flow. It is assumed that this resistance is proportional to the viscosity of the sample. The device does this by measuring torque acting on pins that are in contact with the sample. At the same time the measuring bowl rotates and the temperature can be increased or decreased. The Brabender® Viscograph E gives the torque or viscosity results in the form of Brabender® Units.

Starting temperature was 5°C, heating rate 1.5°C/min, and maximum temperature 45°C with a holding time 3 minutes, then cooling rate of 1.5°C/min to 5°C. Measuring cartridge was 700 cmg. (0.7 Nm) and speed of the bowl 7 rpm. The measurements were done in duplicate. The temperature of the sample should be 0 to 2°C at the beginning of measurement. Samples viscosities were recorded from 5°C to 45°C and again after cooling to 15°C.

1.1.2.2.6 Colour measurement

Colour was measured with Minolta CR-400 Chroma meter (Minolta Camera Co., Ltd., Osaka, Japan) using the CIE Lab scale, with L^* (black 0 to light 100), a^* (red 60 to green -60) and b^* (yellow 60 to blue -60) to measure lightness, redness and yellowness. Whiteness was calculated by the equation: $L^* - 3^*$ as referred by Codex Alimentarius (WHO/FAO 2005).

1.2 Application of ingredients from fillet production in processing lines – injection studies

The fillets were injected using an automatic brine injection system (Dorit INJECT-O-MAT, PSM-42F-30I, Auburn NSW, Australia) with 1-2 bar pressure. The injection system was equipped with 42 needles in two rows. The needles were 4 mm in diameter and the radius around each needle was 1 cm. The needles were open in two directions. After injection, fillets were placed carefully on a grid for approx. 15 min to drain off excess solution liquid. The fillets were chilled (+2°C) and/or frozen (-24°C) and stored for various times prior analysis. The fillets were packed and stored in expanded polystyrene boxes with plastic film on the

bottom. Thawing was carried out at +2°C for approximately 36 h. Each fillet was identified with a numbered plastic tag and weighed before and after injection, frozen, after thawing and after chilling. Before analysis, fillets were skinned by hand and minced in a mixer (Braun Electronic, type 4262, Kronberg, Germany).

1.2.1 Yield after storage and cooking, drip and total yield

The fillets were weighed raw, after injection and after storage (+2°C and -24°C). The yield of the chilled or thawed fillets was calculated with respect to the weight of the raw fillets. Values less than 100% indicated that fillets had lost weight; while values over 100% indicate that fillets had gained weight.

Evaluation of yield after cooking was determined by steam cooking the chilled or thawed fillets at 95°C to 100°C for 8 min in a Convostar oven (Convotherm, Elektrogeräte GmbH, Eglfing, Germany). After the cooking period, the fillets were cooled down to room temperature (25°C) for 15 min before weighing for cooking yield determination. The yield after cooking (%) was calculated as the weight of the cooked fillets in contrast with the weight before cooking.

Evaluation of total yield after cooking was determined by multiplying the yield after storage and the cooking yield.

Thaw drip (%) was determined as the loss in weight during thawing after approx. 24 h at +2°C. Fillets were weighed frozen and again after thawing.

1.3 Application of raw material and ingredients form fillet productions in consumer products

1.3.1 Formed products

1.3.1.1 Chemical composition

The water content (g/100 g) was calculated as the loss in weight during drying at 102-104 °C for 4 h (ISO, 1983). Salt content was determined by the method of Volhard according to

AOAC 937.18 (2000). Crude protein content was estimated with the Kjeldahl method (ISO, 1979) and by multiplying the nitrogen content by 6.25.

TVB-N content of samples was measured using direct distillation into boric acid (based on AOAC 1990). The acid was then titrated with a diluted sodium hydroxide solution. The unbound ammonia was calculated as g/16g N.

1.3.1.2 Texture and colour

Prior measurements of textural properties, samples were steam cooked at 98°C for 15 min. The samples were then cooled down at room temperature and refrigerated prior analysis. Textural properties of the samples (3x3 cm and 3x5.5 cm in size) were measured by using TA-XT2 (TA-XT2 Texture Analyser, Stable Microsystems, Surrey, UK). The samples were pressed downwards twice at constant speed of 1 mm s⁻¹ into the samples until it had reached 55% of the samples height. The data processing was done in a program called Texture Expert Exceed, version 2.64.

Colour was measured with Minolta CR-400 Chroma meter (Minolta Camera Co., Ltd., Osaka, Japan) using the CIE Lab scale, with L* (black 0 to light 100), a* (red 60 to green -60) and b* (yellow 60 to blue -60) to measure lightness, redness and yellowness.

1.3.2 Fish balls

Fish protein isolate (FPI) made from haddock (*Melanogrammus aeglafinus*) cut-offs by the pH-shift process was added to haddock mince in two different proportions (Table 1.2) to manufacture two types of fried fish balls. The products were assessed for physical properties and sensory changes within the period of 8 weeks of freezer storage at -18°C.

Table 1.2. Composition (%) of the three fish balls formulas (adapted from Shaviklo 2007).

Ingredients	Formula 1	Formula 2	Formula 3
Haddock mince	70	52.5	35
Haddock protein isolate	0	17.5	35
Fresh onion	10	10	10
Bread crumbs	8.5	8.5	8.5
Wheat flour	4	4	4
Skim milk powder	3	3	3
Sunflower oil	2	2	2
Fresh garlic	1	1	1
Salt	1.5	1.5	1.5
Total	100	100	100

1.3.2.1 Thermal processing for setting

Thermal processing was a combination of boiling in hot water ($98.3 \pm 0.2^\circ\text{C}$) for 7 minutes followed by deep frying at $190 \pm 0.2^\circ\text{C}$ for 60 sec. Core temperature of each fish ball was $74 \pm 1^\circ\text{C}$ immediately after boiling. It was $59 \pm 1^\circ\text{C}$ immediately after deep frying.

1.3.2.2 Viscosity (Brabender® viscograph E)

The Brabender viscosity of mince and isolate were determined using Brabender® viscograph E (Brabender® OHG, Duisburg, Germany) as described on page 82.

1.3.2.3 Weight loss after thermal processing (cook loss)

Following draining of fish balls after boiling/frying, samples were put on 1 layer of paper towels to remove the excess moisture/oil and equilibrated to room temperature. Then 5 fish balls were selected randomly and weighed directly. The cook loss was calculated as follows: $\text{Weight loss (\%)} = [(P_1 - P_2) / P_1] \times 100$, where P_1 : fish ball initial weight (g) and P_2 : fish ball weight after boiling/frying (g).

1.3.2.4 Sensory analysis

Quantitative descriptive analysis (QDA) was used for evaluation of three fish ball samples. Two sessions were organised for training panellist at Matís ohf., Reykjavík, Iceland for

scaling procedures of sensory attributes of the fish balls under study one week before assessment of samples. Twenty sensory characteristics were evaluated by 8 trained panellists on 0-100 point hedonic scale. All fish balls samples were coded with three-digit random numbers and presented to panellists on tray in individual booths. Orders of serving were completely randomized. Water was provided between samples to cleanse the palate

2 Fish protein hydrolysates

For the first study, backbones from farmed Atlantic cod (*Gadus morhua*) obtained from a fish farm located in Central Norway were used for experiments. Weight and length of the fish was 2.7 ± 0.4 kg and 57.5 ± 2.8 cm. After hand filleting, one part of the bones after post rigor filleting were frozen (-20°C) and stored for approx. 1 month (Part A), while fresh backbones were used for the other part of the experiment (Part B). Frozen backbones were thawed overnight in a cold room, placed in plastic bags or were cut into 1-2 cm pieces with a knife and placed in plastic bags. For the second part of the experiment (Part B) fresh backbones from pre-rigor and post-rigor filleted fish were used. In order to have more uniform cutting of backbones it was decided to mince backbones in a HOBART mincer (model AE 200) using large (10mm diameter) holes.

For the second and the third study, fish protein hydrolysates were made from fresh backbone of cod. The backbones were purchased at Ravnkloa Fisk & Skaldyr AS, where the cod had been manually filleted. The fish was caught in the middle of April of the coast of Helgeland, Norway. Hydrolysis of the backbones was performed in the end of April, within four days of the cod's capture. The backbones were stored chilled on ice but not frozen, pending preparation to hydrolysis. Backbones were minced in a HOBART mincer (model AE 200) using large (10mm diameter) holes.

The fourth part on the study evaluated how time of hydrolysis influence emulsifying properties of hydrolysates from cod backbones. Sensory evaluation of lean fish (cod) pate with added FPH was performed. For this part Backbones from farmed Atlantic cod (*Gadus morhua*) obtained from Norcod fish farm located in Fosen (central Norway) were used for experiments. After hand filleting, backbones were packed in plastic bags and were frozen (-20°C) and stored for approx. 15 weeks.

Frozen backbones were thawed overnight in a cold room, minced in a HOBART mincer (model AE 200).

2.1 Enzyme and chemicals

ProtamexTM (Novozymes A/S, Bagsvaerd, Denmark) was used for the hydrolysis. This enzyme was kindly delivered by Novozymes and complied with the recommend purity specifications for food-grade enzymes given by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex (FCC) Anonymus 1998; Anonymus 2003. ProtamexTM is shown as effective proteolytic enzyme in hydrolysis of cod backbones (Gildberg *et al.* 2002) and are commonly used in industrial application.

A fish protein powder MariPep C[®], MariPep CK[®] and MariPep P[®] (all three were kindly submitted by Danish Fish Protein (Marinova aps.), Denmark), Norland hydrolysed fish collagen (HFC) (from Norland Products INC, Cranbury, USA) and fish protein powder (FPP) Aroma (from Aroma New Zealand Limited, New Zealand) were used as commercial available references.

2.2 Hydrolysis process

2.2.1 Hydrolysis process – fresh vs. frozen raw material

The hydrolysis was performed in a 4 l closed glass vessel stirred with a marine impeller (150 rpm). Thawed (or fresh in part B) backbones were mixed with warm (55°C) water at a weight ratio 1:1. When the temperature of the mixture was 55°C, the enzymatic hydrolysis was started by adding 0.1% (by weight of raw material) ProtamexTM. After different hydrolysis times: 10, 25, 45 and 60 min (A part) and 10 and 60 min (B part), enzyme inactivation was done by microwave heating for 5 min at a temperature higher than 90°C. The bones were separated from the hydrolysate mixtures by sieving and the hot mixtures were centrifuged in 1L batches at 2250*g for 15 min. Two fractions were obtained after centrifugation: the sludge (non-water-soluble part) on the bottom and fish protein hydrolysate (FPH, water-soluble compounds). The fractions were separated by decanting. Both fractions were freeze-dried. The hydrolysis was performed in duplicate.

2.2.2 Hydrolysis process – Functional and antioxidative properties

The hydrolysis was performed in a 4 l closed glass vessel stirred with a marine impeller (200 rpm). Minced backbones were mixed with warm (55°C) water at a weight ratio 1:1. When the temperature of the mixture was 55°C, the enzymatic hydrolysis was started by adding 0.1% (by weight of raw material) ProtamexTM. After different hydrolysis times: 20 and 50 min enzyme inactivation was done by microwave heating for 5 min at a temperature higher than 90°C. The bones were separated from the hydrolysate mixtures by sieving and the hot mixtures were centrifuged in 1L batches at 2250*g for 15 min. Two fractions were obtained after centrifugation: the sludge (non-water-soluble part) on the bottom and fish protein hydrolysate (FPH, water-soluble compounds). The fractions were separated by decanting. Both fractions were freeze-dried. The hydrolysis was performed in duplicate.

2.2.3 Hydrolysis process – FPH as antioxidants in model and food systems

The hydrolysis was performed in a 4 l closed glass vessel stirred with a marine impeller (200 rpm). Minced backbones were mixed with warm (55°C) water at a weight ratio 1:1. When the temperature of the mixture was 55°C, the enzymatic hydrolysis was started by adding 0.2% (by weight of raw material) ProtamexTM. After different hydrolysis times: 15,30 45,60, 120 and 130 min enzyme inactivation was done by microwave heating for 5 min at a temperature higher than 90°C. The bones were separated from the hydrolysate mixtures by sieving and the hot mixtures were centrifuged in 1L batches at 2250*g for 30 min. Two fractions were obtained after centrifugation: the sludge (non-water-soluble part) on the bottom and fish protein hydrolysate (FPH, water-soluble compounds). The fractions were separated by decanting. FPH fractions were freeze-dried. The hydrolysis was performed in duplicate.

2.3 Functional, bioactive and antioxidative properties of hydrolysates obtained from cod (*Gadus morhua*) backbones

2.3.1 Chemical and functional properties

2.3.1.1 Chemical analyses

Total nitrogen (N) was determined by CHN-S/N elemental analyser 1106 (Carlo Erba Instruments S.p.A., Milan, Italy) and crude protein was estimated by multiplying total N by 6.25. These measurements were performed in triplicate.

Ash content was estimated by charring in a crucible at 550°C until the ash had a white appearance (Aoac 1990)

2.3.1.2 Degree of hydrolysis

The degree of hydrolysis was evaluated as the proportion (%) of α -amino nitrogen with respect to the total N in the sample (Taylor 1957). Analyses were performed in duplicate.

2.3.1.3 Molecular weight distribution

Dry powder was dissolved in doubly distilled water (10 mg/ml) and centrifuged at 7840*g for 10 minutes and separated on a FPLC column (@Superdex 75 HR 10/30), the flow rate was 0.5 ml/min and the standards used were Bovine serum albumin (Mw 67000), Myoglobin (Mw 17600), Cytochrome c (MW 12270) and Vitamin B₁₂ (Mw 1355).

2.3.1.4 Colour measurements

Colour measurements were performed using a Minolta Chroma Meter CR-200/CR231. L* (lightness), a* (redness) and b* (yellowness) of the dry powders were recorded. Measurements were performed in quadruplicate.

2.3.1.5 Emulsifying properties

Emulsification capacity was measured by mixing 5 ml of rapeseed oil with 5 ml of a 1% FPH solution in water and homogenising (Ultra – Turrax TP 18/10) in 15 ml graded Nunc centrifuge tubes at 20 000 rpm for 90 s. The emulsion was centrifuged at 2400*g for 3 minutes Slizyte *et al.* 2005b. The volume of each fraction (oil, emulsion and water) was determined and emulsification capacity was expressed as millilitres of emulsified oil per 1 g of FPH (Kinsella 1976). Emulsion stability was expressed as percentage of initial emulsion remaining after a certain time (1 day at room temperature) and centrifugation at 2400*g for 3 minutes (McClements 1999). Tests were performed in duplicate.

2.3.1.6 Water Holding Capacity (WHC)

FPH powder was added to fish mince for evaluation of the ability to influence water holding capacity of fish mince. FPH powder (2% of minced muscle mass) was added to fish mince

(minced cod fillet, which were kept in the freezer and defrosted overnight at 4°C). A low speed centrifugation method was used for measuring the WHC as described by Eide *et al.* (1982) with the exception that a centrifugal force of 300*g was used instead of 1500*g. The WHC is expressed as the water retained in the mince in percentage of the original water. The test was performed in quadruplicate.

2.3.1.7 Determining antioxidant activity

The antioxidative activity of FPH was determined using an indirect spectrophotometric assay, the DPPH method as described by Thiansilakul *et al.* (2007). Liposomes have been proposed to be an appropriate model system to evaluate antioxidants for food (Frankel *et al.* 1997). Due to this, the antioxidant activity of FPH was also evaluated in a liposome model system using iron as prooxidant.

2.3.1.7.1 Determining antioxidant activity using liposomes

In some food products lipids exist in the form of small fat droplets dispersed in an aqueous matrix that may contain a variety of water-soluble components including transition metals Ghaedian *et al.* 1998. Among the transition metals, iron is one of the most important pro-oxidants for lipid oxidation (Paiva-Martins & Gordon 2002). Iron catalyse lipid oxidation due to its capacity to generate reactive oxygen species promoting breakdown of lipid hydroperoxides, which leads to an initiation of free-radical chain reaction (Minotti & Aust 1992). The antioxidant activity of hydrolysates was determined using cod roe phospholipid liposomes. The phospholipids used in these experiments were isolated from North Atlantic cod (*Gadus morhua*) roe. The extraction of total lipids was performed according to the method of Bligh & Dyer (1959). Phospholipids were isolated from total lipids using the acetone precipitation method Kates 1991, with a few modifications as described by Mozuraityte *et al.* (2006).

Liposomes were made as described by Mozuraityte *et al.* 2006. Phospholipids were sonicated in a 5mM MES buffer pH 5.5 (lipid concentration 30mg/ml) with a probe sonicator (VC501, Sonics & Material Vibra Cell, USA).

Lipid oxidation was performed in a liposome assay containing 6mg/ml phospholipids and Fe³⁺ was used to generate radicals. The consumption of dissolved oxygen by liposomes in a closed, stirred, water jacketed cell was used as a measure of lipid oxidation. The

concentration of dissolved oxygen was measured continuously by a polarographic oxygen electrode (Hansatech Instrument Ltd., Norfolk, UK). When measuring dissolved oxygen concentration, background oxygen uptake rate was observed for 4-6 min before Fe^{3+} injection. A working solution (40 μl of FeCl_3) was injected through a capillary opening in the cell to catalyse lipid oxidation. A stock solution of 15mM FeCl_3 was prepared in 0.5 N HCl. The working solution of 375 μM Fe^{3+} stock solution was prepared by diluting the stock solution 40 times in 5mM MES-buffer (pH 5.5). After injection of Fe^{3+} into the system, a linear decrease in dissolved oxygen concentration was observed. The oxidation rate was found by subtracting the background oxygen uptake rate from the rate of linear oxygen uptake observed after injection of iron.

The antioxidant behaviour of fish hydrolysates was studied by analysing the effectiveness in reducing oxygen uptake induced by iron. The hydrolysate samples were dissolved in 5mM MES buffer (pH 5.5) to obtain concentrations of 10, 5, 2 and 1%. Hydrolysate solution (40 μL) was injected in the working cell with liposomes after 6-10min of injection of Fe^{3+} and the reduction of oxygen uptake rate (%) was calculated using the following equation:

$$\% = 100 - \left(\frac{r_h}{r} \times 100 \right)$$

Where: r_h – oxygen uptake rate after hydrolysate was added, $\mu\text{M}/\text{min}$, r – oxygen uptake rate induced by Fe^{3+} , calculated as $r = r_2 - r_1$ (r_2 – oxygen uptake rate after injection of Fe^{3+} , r_1 – oxygen uptake rate before Fe^{3+} injection (background)), $\mu\text{M}/\text{min}$.

2.3.1.7.2 Determining antioxidant activity with the DPPH assay

DPPH radical scavenging was determined as described by Thiansilakul *et al.* (2007). FPH were dissolved in water at 0.25% concentration. 1.5ml of FPH solution were mixed with 1.5ml of 0.15mM DPPH in 96% ethanol and allowed to stand at room temperature in the dark for 30 min. The absorbance was measured at 517nm.

2.3.1.8 CGRP-Radioreceptor assay (RRA)

Receptor binding ability of immunoreactive molecules was developed using rat liver membranes and ^{125}I labelled human CGRP. Incubations, in a 400 μl final volume, were performed at 22°C for 1 hour (Yamaguchi *et al.* 1988). At the end of the incubation, bound and free ligands were separated by centrifugation in a solution containing 2% BSA. Each

batch was tested with at least four increasing protein concentrations, and only the straight lines presenting slopes similar to that obtained with the standard hormone (10-100 pg/tube) were considered as positive. Specific activity, as it represents the quantity of CGRP-like molecules (ng) per μg of protein, was calculated. Receptor binding ability of each purified fraction (ED_{50}) was also determined and expressed as the quantity of protein (mg) that induced a 50% inhibition of the initial binding to rat liver membranes. The experiment was performed in triplicate.

2.3.1.9 Gastrin and CGRP radioimmunoassay (RIA)

The presence of gastrin-like molecules in the crude extracts was determined by gastrin-radioimmunoassay. Rabbit antiserum, synthetic ^{125}I as tracer, and synthetic gastrin 1 as standard were used (GASK-PR, CIS Bio International). Results were expressed as pg of bioactive molecules per mg of dry weight. ED_{50} was also determined and expressed as the quantity of protein (mg) that induced a 50% inhibition of the initial binding of CCK to its specific antibody.

The quantity of immunoreactive CGRP-like molecules presented in the fractions collected after molecular sieving was measured following a previously described assay for human CGRP (Fouchereau-Peron *et al.* 1990): in brief, an anti-CGRP antiserum at a final dilution of 1/150,000 was incubated with serial dilutions of synthetic human CGRP or fractions of cod hydrolysates collected after molecular sieving (18 h at 30°C). Then, ^{125}I labelled human CGRP was added and the incubation continued during 24 hours at 4°C . Bound and free hormone were separated by charcoal-dextran precipitation. Results were expressed as pg of CGRP-like molecules per ml.

Control (specific antibody omitted) tubes were incubated in each assay. The detection limit for radioimmunoassay was 10 pg of immunoreactive peptide per tube.

2.3.1.10 Liver membrane preparation

Liver membranes were prepared using male Wistar rats according to the method of Neville until step 11 (Neville 1968). Proteins were quantified by the method of Lowry using BSA as standard (Lowry *et al.* 1951).

2.3.1.11 Partial purification of the CGRP-like molecules

The CGRP-like molecules included in the hydrolysates obtained from whole frozen and minced fresh samples in post-rigor state (ten min of hydrolysis) were pre-purified by gel exclusion chromatography on a HW 40 toyopearl column (2.5 x 33.5 cm) using ammonium acetate 0.2 M, pH 5 as eluant. The flow rate was 22 ml/hour. The column was calibrated with the following molecular weight markers: aprotinin (6000 Da), CGRP (3750 Da), and bacitracin (1411 Da). Aliquots were analyzed for CGRP-immunoreactivity. Immunoreactive fractions were then analyzed using CGRP radioreceptor assay.

2.4 Comparison of chemical and functional properties of lab made and commercial available fish powders

2.4.1 All chemical and functional analysis as described above.

2.4.2 Molecular weight distribution

Dry powder was dissolved in 50 mmol/l buffered imidazole solution at pH 7.0 (100 mg/ml). The sample was examined using a Superdex Peptide 75 10/300GL column with an Akta FPLC academic edition. Detection wavelength was set to 280 nm.

2.4.3 Amount and composition of free amino acids

Amount of free amino acids was determined by high-pressure liquid chromatography (HPLC). Dry powders were dissolved in 0.05 M phosphate buffer (pH=7.0) and centrifuged for 10 minutes at 10 000 rpm. Reversed phase HPLC by precolumn fluorescence derivatization with o-phthaldialdehyde (SIL-9A Auto Injector, LC-9A Liquid Chromatograph, RF-530 Fluorescence HPLC Monitor, all parts from Shimadzu Corporation, Japan) was performed using a NovaPak C18 cartridge (Waters, Milford, MA, USA). Glycine/arginine and methionine/tryptophane were determined together, as their peaks merged. This analysis was performed twice on each sample.

2.4.4 Amount and composition of total amino acids

The amino acid composition of powdered samples was determined by digestion in 6 M HCl at 105°C for 22 h followed by neutralisation of hydrolysates. After dilution and filtration

amount of 16 amino acids was estimated by HPLC as described earlier. These tests were performed in duplicate.

2.5 Antioxidative properties of fish powders

2.5.1 All chemical and functional properties analysis as described before.

2.5.2 Metal chelating ability

Protein ability to chelate Fe^{2+} was determined as described by Klompong *et al.* (2007) with some modifications. One ml of protein solution was mixed with 3.7 ml of MES buffer (5mM, pH 5.5). The mixture reacted with 0.1ml of mM FeCl_2 and followed by 20 min incubation with 0.2ml of 5mM 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acids)-1,2,4-triazine (ferrozine) at room temperature. The absorbance was read at 562nm. The control was prepared in the same manner except that Mes buffer was used instead of the sample. In order to eliminate the absorbance of protein itself, the absorbance of the sample, that was prepared in the same manner except that Mes buffer was used instead of the iron solution, was read. Chelating activity (%) was calculated as follows:

$$\text{Chelating activity (\%)} = \left(1 - \frac{A_{562\text{sample}} - A_{562\text{protein}}}{A_{562\text{control}}} \right) \times 100$$

2.5.3 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging was determined as described by Thiansilakul *et al.* (2007) with a slight modification. Proteins were dissolved in water at 0.25% concentration. 1.5 ml of protein solution were mixed with 1.5ml of 0.15mM DPPH in 96% ethanol and allowed to stand at room temperature in the dark for 30 min. The absorbance was measured at 517nm. The blank was prepared in the same manner except the distilled water was used instead of the sample. For control sample –ethanol was used instead DPPH ethanol solution. The scavenging effect was calculated following:

$$\text{Radical scavenging ability (\%)} = (A_{\text{blank}} - A_{\text{sample}} + A_{\text{control}}) / A_{\text{blank}} \times 100$$

2.5.4 Antioxidative activity of fish proteins in liposome system

The antioxidant activity of proteins was determined using cod roe phospholipids liposomes that were made as described by Slizyte *et al.* (2009). Lipid oxidation was performed in a liposome assay containing 6 mg/ml phospholipids. Fe^{3+} and bovine haemoglobin (hemoglobin from bovine blood (lyophilized powder), Sigma-Aldrich) (Hb), was used to catalyse lipid oxidation. The consumption of dissolved oxygen by liposomes in a closed, stirred, water jacketed cell was used as a measure of lipid oxidation. The concentration of dissolved oxygen was measured continuously by a polarographic oxygen electrode (Hansatech Instrument Ltd., Norfolk, UK).

The antioxidative activity of proteins against Fe^{3+} (15 μM) induced oxidation was studied as described by Slizyte *et al.* (2009). The antioxidant behaviour of proteins was studied by analysing the effectiveness in reducing oxygen uptake induced by iron.

Hb induced oxidation led to non-linear oxygen uptake by liposomes (Figure 2.1, A). Due to this, protein solution was injected to liposome solution before addition of Hb solution as shown in Figure 2.1, B. The antioxidative activity of proteins (4mg/ml) against Hb (0.05 mg/ml) induced oxidation was calculated following: $I(\%) = 100 - (r_{\text{protein}}/r_{\text{Hb}}) \times 100$, where: r_{protein} - oxygen uptake by liposomes after injection of protein and Hb solutions; r_{Hb} - oxygen uptake by liposomes after injection only Hb solutions (control for protein effect).

The influence of pH on protein antioxidative activity both on iron and Hb induced oxidation was performed by making protein solution with different pH. The pH of the liposome solution was adjusted by replacing some of the MES buffer that was used to dilute the 30 mg/ml liposome solution with different concentration of NaOH and HCl solution. Fe^{3+} solution used to catalyse oxidation was with pH 2 for all experiment. Hb solution used to initiate oxidation was made with the same pH as liposome solution. The pH of experiment was verified after the oxidation experiment. Oxidation experiments were performed in triplicates.

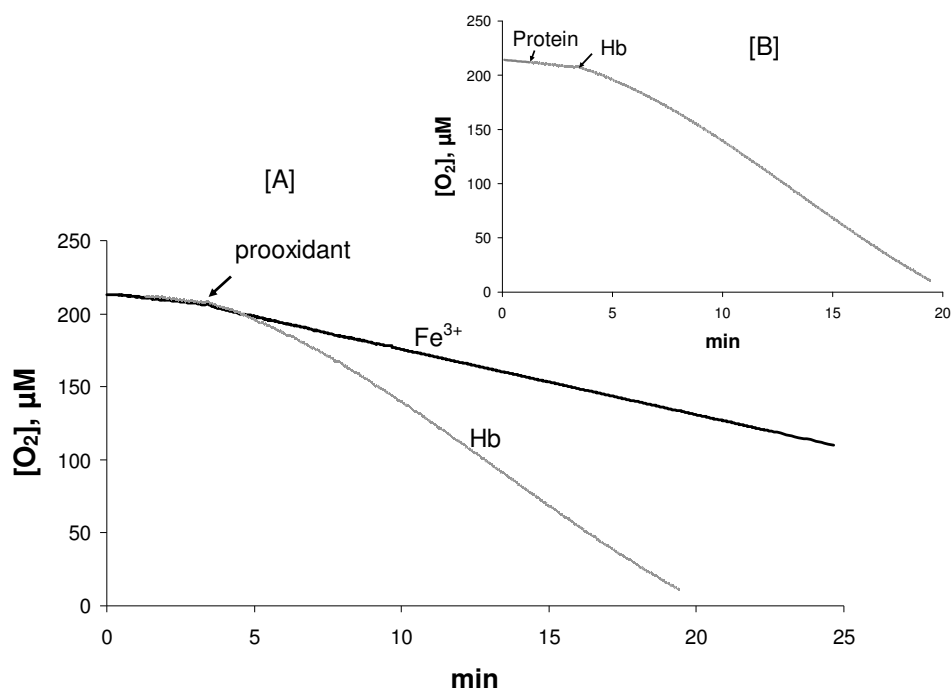


Figure 2.1. Oxygen uptake induced by iron and haemoglobin in cod roe phospholipids liposomes.

2.6 Application of FPH in food

2.6.1 Preparation of fish cakes

2.6.1.1 Preparation of fish cakes (with hydrolysates from first study hydrolysis)

- 1 kg Pollack (sei)
- 20 g FK spices
- 100 g cream powder
- 250 ml milk (H melk)
- + FPH powder
- + Potato flour

In order to evaluate how addition of fish protein hydrolysates (FPH) influences the properties of fish cakes during frying and as a final product it was decided to replace part of potato flour (which is usual ingredient in fish cakes production) with FPH. As a reference sample No 1 fish cakes were fried with addition of commercial FPH (MariPep powder). As a reference sample No 2 fish cakes were fried without addition of any powder. The following FPH powders and proportions were used:

10% (on a fish mass basis) FPH after 10 min hydrolysis of cutted backbones (10c) + 5% (on a fish mass basis) potato flour

10% FPH (25c) + 5% potato flour

10% FPH (45c) + 5% potato flour

10% FPH (60c) + 5% potato flour

10% FPH (10P: pre-rigor filleted backbones hydrolysis for 10 min) + 5% potato flour

10% FPH (60P) + 5% potato flour

10% FPH (WCP: commercial fish powder (MariPep)) + 5% potato flour

10% potato flour + 5% potato flour

Without addition of FPH or potato flour

All ingredients were mixed together in food processor and kept at room temperature for about 10-15 min. Then four fish cakes of approx 40-50 grams each were fried in 20 ml cooking oil for each of earlier mentioned group. Fish cakes were fried 1 min and 30 seconds on one side and 1 min 45 seconds on the other side. Then fish cakes were weighted, cooled down and weighed again.

2.6.1.2 Preparation of fish cakes (with hydrolysates from second study hydrolysis)

Fish cakes were made of filleted saithe (*Pollachius virens*) purchased at Ravnkloa Fisk & Skalldyr AS. The fish had been caught off the coast of Møre, Norway. A fish mince was made of the fillets. From this mince, eight batches of fish mince were made, following the recipe recounted in Table 2.1. Six batches were made with the hydrolysate powders under examination, and two reference samples were made with cream powder instead of FPH. Four patties were made from each batch, but the patties from one of the batches were left un-fried. The two batches of laboratory-made FPH with equal hydrolysis time were mixed and named FPH 20 and FPH 50. A frying pan with a non-stick surface was preheated on a common hot plate. The patties were fried four at a time. 40 ml of rapeseed oil was added to the pan 30 seconds prior to the patties. The patties were fried for 2 minutes on the first side and 3 minutes on the second side. The pan was rinsed in hot water between each batch.

Table 2.1. Ingredients for fish cake

25 g	Minced fillet of saithe
5 g	Salt
10 g	Cream powder
65 mL	Whole milk
5 g	Potato flour
12.5 g	FPH or cream powder

2.6.2 Preparation of salmon pate

The following ingredients were used for preparation of salmon pate: salmon (cooked), salmon (smoked), rainbow trout oil, whey powder, water, salt, vinegar, Na benzoate and fish proteins. Control samples instead of fish proteins contained whey powder. All ingredients were mixing in the food processor, divided in to the heat resistible dishes (approx. 80g each) and baked in the oven (190°C) for 20 min. Then samples were cold down, lead added and kept in cold room until the analysis. Different concentration of the fish powders were used for preparation of pate used for concentration test.

2.6.3 Preparation of fish pate

Cod fillets were used for making a fish pate. Three different recipes were used for these trails (Table 2.2). All ingredients for each recipe were mixed together and pates were baked in water bath at 180°C for 30 min.

Table 2.2. Recipes for cod pate (all number presented in gr.)

Recipe	1	2	3
Cod fillet	300	300	300
Egg	4	4	4
Water	-	110	110
Potato flour	110	-	-
Wheat flour	30	30	-
Full cream	20	20	-
Pepper	0,5	0,5	0,5
FPH	-	20	25

2.6.4 Properties of fish cakes with added fish powders

2.6.4.1 Frying yield

Frying yield (%) was calculated as the weight of fried fish cake over weight of mince before frying. Average value was calculated from the four replicates.

2.6.4.2 Colour measurements

Colour measurements were made using a Minolta Chroma Meter CR-200/CR231. L (lightness), a (redness) and b (yellowness) of the dry powders were recorded. Measurements were performed in quadruplets.

2.6.4.3 Texture

Textural properties were measured with a Stevens-LFRA Texture analyser equipped with flat-ended cylinder plunger (diameter 13mm) – pressure test and cone shaped plunger for cutting test. Speed of loading was set at 0.5mm/sec for flat-ended cylinder plunger and 2mm/sec for cone shaped plunger. Flat-ended cylinder plunger was pressed 0.7 cm into the sample, cone shaped plunger – 1cm. Eight measurements (4*2) were run for each batch of fish cakes.

2.6.4.4 Water Holding Capacity (WHC)

Low Speed Centrifugation method was used for measuring the WHC of fried fish cakes. Water-holding capacity (WHC) was determined as described by Eide *et al.* (1982) with the exception that a centrifugal force of 300*g was used instead of 1500*g. The WHC is expressed as the percentage of water retained in the mince. The test was performed in quadruplicate.

2.6.5 Analysis of fish cakes

2.6.5.1 Frying yield

The frying yield was determined by weighing the fish patties before and after frying.

2.6.5.2 Fat absorption

The absorption of fat into the fish cakes during frying was assayed. This was achieved by determining the total lipid content of the fish cakes during lipid extraction. The lipid content was compared to that of the unfried fish cakes, taking the differences in fat from the added cream powder into consideration.

2.6.5.3 Water holding capacity

Water holding capacity (WHC) of the fish cakes was determined by a low speed centrifugation method as described by Eide *et al.* (1982) except that the centrifugation was carried out at 210 g for 5 minutes. Water content of the fish cakes was determined in triplicate by weighing the cakes before and after drying at 105°C for 24 hours.

2.6.5.4 Texture analysis

Compression force on the fish cakes was measured using a TA.XT2 Texture Analyzer from Stable Micro Systems, England. A 1/2" flat-ended cylindrical probe was used on whole fish cakes, including its fried crust. The samples were compressed at 2 mm/s to a strain of 30% of the sample thickness.

2.6.5.5 Storage trials

A storage experiment was conducted on the fish cakes after frying. The fish cakes were stored in zip locked plastic bags in a cold storage room at 4°C. The lamp in the ceiling was kept on during the experiment to increase the rate of oxidation. The fish cakes were equally exposed to light. Samples of the fish cakes were collected after 1, 4 and 8 days of storage. Lipids from these samples were extracted following the method of Bligh & Dyer (1959) and used for further analysis.

2.6.5.6 Analysis of lipids

2.6.5.6.1 Peroxide Value

Peroxide value (PV) was analyzed by the ferric thiocyanate method as described by the International Dairy Federation 1991, and modified by Ueda *et al.* 1986 and Underland *et al.* 1998.

2.6.5.6.2 Analysis of thiobarbituric acid reactive substances.

TBARS values were determined by the spectrophotometric method as described by Ke et al. Ke & Woyewoda 1979. The absorbance values of samples were compared to a standard curve prepared with 1,1,3,3-tetraethoxypropane for the calculation of TBARS concentrations ($\mu\text{M/g}$ fat).

2.6.6 Emulsification properties and sensory evaluation of lean fish (cod) pate with added FPH

2.6.6.1 Emulsifying properties

Emulsification capacity was measured by mixing 4 ml of soya oil with 4 ml of a 5% FPH solution in water and homogenising (Ultra – Turrax TP18/10) in 15 ml graded Nunc centrifuge tubes at 21 500 rpm for 60 s. The emulsion was centrifuged at 2500*g for 3 minutes (Slizyte *et al.* 2005a). The volume of each fraction (oil, emulsion and water) was determined and emulsification capacity was expressed as millilitres of emulsified oil per 1 g of FPH (Kinsella 1976). Emulsion stability was expressed as percentage of initial emulsion remaining after a certain time (1 day at room temperature) and centrifugation at 2500*g for 3 minutes (McClements 1999). Tests were performed in duplicate.

2.6.6.2 Sensory evaluation

A semi-trained panel with 24 judges was used to rank the cod pates after how well they liked the pates. Taste and texture were the parameters evaluated.

3 Fish gelatin

3.1 Comparison of functional properties of dried fish gelatins and their effects on fish muscle

The dried fish gelatins used in this study were obtained from Kenney & Ross (Norland High molecular weight fish gelatin, Kenney & Ross limited, Nova Scotia, Canada) and Faroe

Islands (collagen peptides). Fish mince made from saithe (*Pollachius virens*) cut-offs was used to study the gelatin effects on fish muscle.

3.1.1 Preparation of gelatin gels

Samples of gelatin gel were prepared by dissolving gelatin powder in distilled water at room temperature for 30 min and then heated at 60°C in water bath for 30-60 min until gelatin was completely dissolved. The gelatin solutions were then cooled down to 25°C, and then left in refrigerator at 5-7°C for 16-18 h prior to analysis. The effect of salt on the gel properties was investigated by adding the salt to the gelatin samples at the concentrations of 0, 1, 1.5 and 3% NaCl.

3.1.2 Determination of pH of the gelatin solutions

Gelatin solution (1.5–3.0%, w/v) was made by dissolving gelatin powder in distilled water for 30 min then heated to 60°C for 30–60 min and then cooled before measuring pH. The pH was measured with combined glass electrode (SE 104 – Mettler Toledo, Knick, Berlin, Germany) connected to Portamess 913 pH meter (Knick, Berlin, Germany).

3.1.3 Determination of water, salt, ash, fat and protein content

The water content was determined by drying the sample in an oven at 102-104 °C for 4 h (ISO, 1983). Salt content was determined by the method of Volhard according to AOAC 937.18 (2000). The fat content was determined by the AOCS Soxhlet method Ba 3-38 (1998), using petroleum ether (Bp, 30-40 °C) for extraction. The samples were ashed at 550 °C, and the residues were weighed (ISO, 1978). The total nitrogen (N) content of the gelatin powders was estimated by Kjeldahl method (ISO, 1979) with the aid of a Digestion System 40 (Tecator AB, Hoganas, Sweden). Protein content was determined by the Biuret method, using bovine serum albumin as standard.

3.1.4 Determination of molecular weight using SDS-PAGE

Protein patterns of the gelatin samples were analysed using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970),

using 10% separating gel and 4% stacking gel. The samples were dissolved in distilled water and diluted as needed. Gelatin solutions (10 μ L) were mixed with SDS loading buffer (3 μ L) and heated at 100°C for 5 min. The load volume was 13 μ L in all lines. Standards from New England Biolabs were used to identify the protein fractions with molecular masses ranging from 2-212 kDa. The samples were run at 20mA for approximately 2 h. Protein bands were stained with Coomassie Brilliant Blue R-250, and then destained according to the method of Fairbanks and others (1971).

3.1.5 Differential scanning calorimetry (DSC)

Thermal properties of gelatins were determined by using differential scanning calorimetry. Measurements were performed on a Perkin-Elmer DSC-7 (Perkin-Elmer, Norwalk, USA). A refrigerated cooling system (RCS) was used in the instrument to achieve temperature of -10 °C and a nitrogen DSC cell purge at 50 mL/min. Gelatin gel (~25 mg) amounts placed in an aluminium hermetically sealed pan, were heated (-10 to 40 °C) and cooled (40 to -10 °C) at 5 °C/min. The reference was an empty pan and the equipment was calibrated with 10% NaCl solution (T_m =21.1 °C) and indium (T_m =156.6 °C and enthalpy ΔH =28.5 J/g). The endothermic peak was pointed as the melting temperature of gelatin gels during the heating trail and exothermic peaks in the cooling trails were recorded as the gelling temperature.

3.1.6 Rheology measurements

A coaxial rotational viscometer Brabender® Viscograph E was used for the viscosity measurement (Brabender® OHG, Duisburg, Germany). A measuring cartridge of 1000 cmg (0.1 Nm) and rotational speed of 75 rpm was applied. The sample viscosity was measured in a time dependent manner during heating (1.5 °C/min) from 5 °C to 45°C, with the final temperature held for 3 min then cooling (1.5 °C/min) from 45 °C to 5°C.

The viscosity was also analysed by using a Bohlin BV88 viscometer (Bohlin Instruments, England) at 5 °C to 7 °C, speed setting 6, system switch 6. Viscosity is reported as Pascal.

3.1.7 Water activity (a_w)

The water activity of the gelatin powders were determined using an a_w measuring apparatus (Novasina a_w CENTER, Novasina, Pfäffikon, Switzerland). Samples were analysed in triplicate.

3.1.8 Colour

Colour was measured with Minolta CR-400 Chroma meter (Minolta Camera Co., Ltd., Osaka, Japan) using the CIE Lab scale, with L^* (black 0 to light 100), a^* (red 60 to green -60) and b^* (yellow 60 to blue -60) to measure lightness, redness and yellowness. Whiteness was calculated according to the methods of Park (1994):

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

The instrument was calibrated against a white standard at the same light conditions and temperature (20°C). The analysis was performed five times on each sample. Gelatin solutions (3% w/v) were measured.

3.1.9 FT-NIR

FT-NIR spectra of the dried gelatins were recorded on MPA™ - Multipurpose analyser (Bruker Optics, Germany). The spectra's were collected in reflectance mode in the 12500-4000 cm^{-1} region using fiber optic module. The fiber optic probe measures over an area of 0.50 cm^2 . The scanners speed was 40 kHz and each spectra was average spectrum of 16 scans. Each sample was collected four times and the mean of four spectra of the samples was used to analyse.

3.1.10 Dry addition of the gelatins to fish mince

Samples with mince from saithe (*Pollachius virens*) were prepared (Table 3.1). The mince samples were prepared with gelatin concentration of 0, 0.5, 1.5 and 3% (w/w). The samples were then frozen at -24 °C for approx. 7 days. Samples were thawed at +2 °C for 48 h prior analysing. During thawing, additional water in the mince samples were allowed to leak out and the yield after freezing/thawing determined. Thaw drip (%) was determined as the loss in weight during thawing.

Table 3.1. Mince samples with dry addition of the gelatin powders. (CP=Collagen peptide).

Group/marking	Gelatin addition	Gelatin concentration	Storage
C	None	0%	Freezing
F1	CP	0.5%	Freezing
F2	CP	1.5%	Freezing
F3	CP	3.0%	Freezing

3.1.10.1 Determination of water holding capacity and transverse relaxation time T_2

For determination of water holding capacity and measurements of transverse relaxation times were carried out as described above, paragraphs 1.1.1.1 and 1.1.1.2, respectively.

3.1.10.2 Texture

Textural properties of the thawed mince samples with added gelatin were measured in respect to hardness (kg) by using TA-XT2 (TA-XT2 Texture Analyser, Stable Microsystems, Surrey, UK). The mince samples were put into a box, 3.0 cm high and 3.5 cm in diameter before measuring. The samples were pressed downwards at constant speed of 1 mm s⁻¹ into the samples until it had reached 50% of the samples height. The data processing was done in a program called Texture Expert Exceed, version 2.64.

4 Comparison between protein ingredients for injection in fillets

4.1 Comparison of properties of protein solutions for injection

In order to examine chemical- and physicochemical characteristics, and other properties of the protein products, specific measurements were performed on the protein products.

Weight loss, viscosity, colour (whiteness) and chemical content of the protein solutions were evaluated according to methods described above, paragraphs 1.1.2.2.4, 1.1.2.2.5, 1.1.2.2.6 and 1.1.2.2.3 respectively. Protein patterns of the protein products were analysed using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970), using 10% separating gel and 4% stacking gel (Laemmli 1970), as described above in paragraph 3.1.4 on page 103.

4.2 Comparison of the effect of injected protein solutions on fillet properties

The process of injection and storing conditions were the same as described above in paragraph 1.2 and 1.2.1 on page 83 and 84 respectively.

4.2.1 Effects of fish protein solutions on chemical and physicochemical characteristics of fresh and lightly salted cod fillets

4.2.1.1 Physical properties

Water holding capacity and T₂ transversal relaxation times were evaluated for the fillets. See description of methods above, paragraphs 1.1.1.1 and 1.1.1.2, respectively.

4.2.1.2 Chemical analysis

Salt and water content was determined by standard methods, AOAC no. 976.18 (2000) and ISO 6496 (1999), respectively. The total protein contents of the fish muscle were estimated by Kjeldahl method (ISO, 1979) with the aid of a Digestion System 40 (Tecator AB, Hoganas, Sweden) and calculated using total nitrogen (N) x 6.25. Salt, water and protein content were determined for the fillets and the fish protein solutions.

TVB-N content of samples was measured using direct distillation into boric acid (based on AOAC 1990). The acid was then titrated with a diluted sodium hydroxide solution. The unbound ammonia was calculated as g/16gN.

4.2.1.3 Microbiological analysis

A sample was taken from a muscle of each fillet and analysed for psychotropic bacteria (colony forming units) and H₂S-producing bacteria on Iron agar with overlay (IA). The plates were incubated at 15°C for five days. Bacteria forming black colonies on this agar produce H₂S from sodium thiosulphate and / or cysteine. One of the main spoilage bacteria in chilled fish, *Shewanella putrefaciens*, forms black colonies on this agar. This bacteria forms trimethylamine (TMA) from trimethylamine oxide (TMA-O), but TMA has often been used as a parameter on fish freshness.

4.2.2 Effects of fish protein solutions on heat-profiles and cooking yield of fresh and lightly salted cod fillets

4.2.2.1 Heating methods

Prior cooking, the fillets were cut in similar pieces, which weighed approximately 100g $\pm$ 20g. Heat sensors were placed in few samples of cod during baking and boiling to monitor their heat profiles.

4.2.2.1.1 Microwave heating

The microwave cooking processes were carried out in á microwave oven (Panasonic NN-T251W, Panansonic CS UK, Berks UK) at 600 W. Fibre optic sensors (03R4.2004, FISO Technologies Inc., Saint-Jean-Baptiste.av, Quebec, Canada) were connected to the microwave and the measurements were processed in computer programme (FISO Commander Microwave Workstation, version 1.10.6). Four fibre optic heat sensors were placed in predetermined places in the cod samples. The sensors were numbered 1, 2, 3 and 6. Figure 4.1 shows the sensors location: no. 1 was placed in the middle of the sample; no. 2 lateral from the middle (in the thinner part of the sample); no. 3 was placed just below the surface (in the middle of the sample); and no. 6 was placed near the skin (in the middle of the sample). The fish was cooked until all sensor showed temperature above 72°C. The samples were placed on a plastic sieve to allow excess water to drain easily.

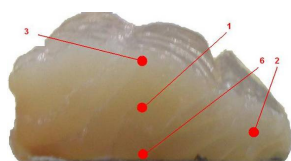


Figure 4.1. Location of the fibre optic heat sensors in the cod samples during microwave cooking.

4.2.2.1.2 Baking

The cod samples were baked at 170°C for 16 min in a Convostar oven (Convotherm, Elektrogeräte GmbH, Eglfing, Germany) on a baking sheet. After the cooking period, the samples were cooled down to room temperature (25°C) for 15 min before weighing for cooking yield determination.

4.2.2.1.3 Boiling

For the boiling process, the cod samples were dipped into boiling water for 8 min in thick plastic bags. After the cooking, any excess water was removed and the samples were cooled down for 15 min.

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