14 Mild processing techniques and development of functional marine protein and peptide ingredients

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14.1. Introduction

The raw materials that come from traditional fisheries and aquaculture can be regarded as a great and valuable source of proteins both for animal and human nutrition. Fish meal, fish sauce, surimi and fish silage are traditional protein based products. Over 6 million tonnes of fishmeal is produced worldwide each year from about 25-30 million tonnes of industrial fish (Fish Meal Information Network, 2007). The demand is increasing with the growth in aquaculture and the price has been rising (Klinkhardt, 2006). About 2-3 million tons of wild fish is processed each year worldwide into about 750 000 tonnes of surimi. It has doubled in the last 10 years (GRP, 2007). Fish sauce is produced in a quantity of about 400 000 tonnes each year (Dissaraphong *et al*., 2006). Fish silage is almost entirely used for feed. Norway is the major fish silage producer – producing about 140 000 tonnes pr year, mainly from aquaculture by-products (Rustad, 2003).

There are promising opportunities in the upgrading of marine by-products and underutilized fish by using mild processing techniques to convert them into protein and peptide ingredients both to improve yield in traditional seafood and to be used as nutraceuticals or in functional foods. Mild techniques like pH shift, fermentation, enzymatic hydrolysis, filtration, centrifugation and spray and freeze drying are used in the processing and production of valuable products.

The utilisation of protein sources from fisheries and aquaculture can be divided into two categories.

- Improved yield and utilisation of proteins in traditional fish processing
- Marine proteins and peptides with functional and bioactive properties

This chapter is an overview of the latest developments in the use of mild processing techniques in the production and use of functional marine protein and peptide ingredients. It is also written in connection with the PROPEPHEALTH project within SEAFOODplus (www.seafoodplus.org). The aims of PROPEPHEALTH are to screen, map and recover 'new' (health) beneficial compounds from seafood by-products by advanced mild refining processes and to develop 'new' bioactive (functional) seafood ingredients. And to use the ingredients for the development of new functional seafood products, accepted by the target consumers. The project is divided into three blocks. The first is on the use of pH-shift methods to produce protein isolates from by-products and pelagic fish and the application of the protein isolates into fish fillets and ready to eat seafood products. The second is on fish protein hydrolysates and bioactive peptides from an industrial point of view. Commercial products of fish protein hydrolysates (FPH) from three companies have been screened *in vitro* for different biological activities. The activities of the most promising FPH were subjected to ultra- and nanofiltration

in order to optimize the amount of the active components. Chromatographic separation is being carried out on few of the FPH in order to isolate and identify the active peptides. In the last part of the project the *in vivo* activity of two selected products will be tested.

14.2. Improved yield in traditional fish processing

About 76% of the global fish supply is used for human consumption in 2002 (FAO, 2004). About 40 million tonnes were used for manufacturing products for direct human consumption. But up to 50-70% of the fish may end up as co-products as the yield in filleting operations is from 30-50 % (Arason, 2003; Kristinsson *et al.,* 2006; Mackie, 1982). About 6 million tonnes of trimmings and by-products from food fish processing are processed into fish meal (Fish Meal Information Network, 2007) and the rest is used in fish silage or a an fertilizer or discarded. Great additional economic, nutritional and environmental values can obtained by increasing the yield of raw material in fish filleting operation and in the production of ready to eat seafood products. The meat, poultry and fish industries in USA and other parts of the world are adding up to 12% of brine to modify both fresh retail and further processed products. This is done to improve quality, firmness and juiciness and to meet increasing consumers demand for convenience food items while at the same time increasing yield. The brine is made up of water, salt, phosphates, and sometimes also other functional or flavour ingredients, like sodium lactate, polysaccharide gums, hydrolysed whey and soy proteins and modified starches (Xiong, 2005).

Innovative technologies used in improving yield in fish filleting operation are using raw material from co-products like cut offs and backbones of the same species. This applies both to the SuspenTec® system (Christensen, 2006; www.suspentec.com) and acid and alkali extraction (pH-shift) of fish proteins (Batista, 1999; Hultin and Kelleher, 1999; Hultin *et al.*

2004; Nishioka and Shimizu, 1983). Chemical processing methods for protein recovery have been extensively reviewed in two recently published books (Hultin et al., 2005 and Shahidi ed.2006).

The SuspenTec® process is an automated method of reducing fish trimmings at low temperatures (- (4-6) °C) to micron-sized particles and incorporating them into traditional brines to create homogeneous suspensions. The controlled temperature ensures efficient protein binding and dispersal of suspension into the whole-muscle product (Christensen, 2006). The pH-shift protein isolate can be added to fresh seafood of the same species by needle injection into fillets, static soaking, or vacuum tumbling. Another interesting aspect is NutraPure® protein processing a technology to reduce fat in deep-fried fish products (Kelleher and Williamson, 2007; www.proteusindustries.com). Acid and alkali extracted fish protein isolates have a GRAS (Generally Regarded as Safe) status in the United States (FDA, 2004).

The pH shift methods are an alternative to surimi production. They are more suitable than the surimi process for complex raw materials like whole fish and co-products. The process is shown in figure 1. It involves 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 temperatures. Solids such as bones, scales, neutral fat and disrupted cellular lipid membranes are then removed by centrifugation. The soluble protein is then precipitated by adjusting the pH to the isoelectric point of the myofibrillar proteins to give a protein isolate (Kristinsson *et al.,* 2006).

The method gives higher yield of proteins than surimi processing. Sarcoplasmic proteins, that are washed away in surimi processing, are mostly recovered in the pH-shift process (Choi and Park, 2002; Kristinsson and Demir, 2003; Kristinsson *et al.*2005; Undeland *et al*., 2002).

Microorganisms are also partly removed during the first centrifugation step (Hultin and Kelleher, 1999). Protein isolate had lower number of aerobic bacteria and longer bacterial shelf life than surimi from catfish (Kristinsson *et al.,* 2005).

The protein isolate from the pH processes is claimed to have a substantial absence of both neutral and membrane lipids and their removal is expected to greatly improve the oxidative stability of the product. The alkali aided process gives a more oxidative stable isolate than the acid aided process and sometimes more stable than surimi (Kristinsson and Demir, 2003; Petty and Kristinsson, 2004). Haemoglobin, a main catalyst of oxidation in fish muscle, is highly pro-oxidative at low pH but it stabilized at high pH (Kristinsson and Hultin, 2004). Heme proteins are also more actively removed than from surimi, yielding a whiter product and more stable to oxidation if processed with high pH (Kristinsson, 2002).The acid process leads to denaturation and co-precipitation of heme proteins giving a less stable and darker product (Kristinsson and Hultin, 2004; Kristinsson et al., 2005 and Choi and Park, 2002).

There are conflicting results on the influence of high and low pH on gel forming and water binding properties of the isolated fish protein. They depend both on species, condition of the raw material and processing conditions. The alkali aided process is claimed to produce gels superior over both the acid-aided process and the surimi process (Davenport and Kristinsson, 2004; Kristinsson and Ingadottir, 2006 and Yongsawatdigul and Park, 2001). The lower strength of the acid process gels was explained by proteolysis that can decrease gelation and water binding properties. This has however not been demonstrated in cod (Hultin and Kelleher, 1999; Kristinsson and Hultin, 2003) and catfish (Kristinsson *et al.,* 2005).

Sarcoplasmic proteins are removed from surimi because they are believed to interfere with gelation (Park *et al.,* 1997; Shimizu *et al*., 1992). Other studies have on the other hand shown that the presence of sarcoplasmic protein had either no or a positive effects on gel strength.

(Hultin and Kelleher, 1999; Kristinsson and Crynen, 2003; Kristinsson and Liang, 2006; Ko and Hwang, 1995; Morioka and Shimizu, 1990).

Usually separations in the pH shift process are performed by centrifugation with relatively expensive equipments that require qualified personnel. Decanter centrifuges are usually used. These require relatively large amounts of material to get steady-state running conditions. Most small- and medium-sized fish processors do not have the necessary amounts of fish material for a processing solution using decanter centrifuges. It has been shown that by replacing the first centrifugation with sieving improves the yield of protein isolate, but worsens the quality of the produced gel. Replacing the second centrifugation with filtration has no influence on the yield or the quality of the protein isolate (Nolsoe et al, 2007).

The production and application of the pH shift process has been tried on an industrial scale in Iceland in the PROPEPHEALTH project. A pilot plant with a continuous process was set up. There were difficulties in removing fat during protein isolation of herring and there was an extensive lipid oxidation during processing. Trials to reduce the oxidation were unsuccessful (Geirsdottir, 2006). But on a laboratory scale high speed centrifugation (10 000 g) reduced initial TBARS levels about 50% and process induced oxidation was prevented by adding reducing agents (0.2% erythobate) and metal chelator (0.2% EDTA or sodium tripolyphosphate) either during the prewashing step or homogenization. To gain good stability during ice storage ETDA or sodium tripolyphosphate had to be added in the homogenization step (Undeland *et al*., 2005).

Injection of brine with fish protein isolates or homogenized muscle have increased weight gain in cod and haddock fillets 5-20% and also increased cooking yield. There are indications that fish protein isolates give higher cooking yield and microbiologically more stable products

than products with injected fish mince (Thorarinsdottir *et al.,* 2006; Valsdottir *et al.,* 2006a; Valsdottir et al.., 2006b).

The use of fish protein isolates to reduce fat in deep fried fish products has also been tested on a laboratory scale. The effects of frying time, addition of cod protein isolate (5-20%) in fish block by tumbling and coating of 1x10x10 cm cut pieces with 2-6% isolate solution on fat uptake in deep fried battered and breaded cod and saithe were evaluated. The fat uptake increased more than 50% that is from 8% to 12% by increasing the frying time from 60 to 180 seconds but the addition of protein isolate in the fish block and having a pre-batter with protein isolate did not change the fat content of the finished product in the set up and conditions used in the tests. (Einarsdottir *et al.,* 2007).

The alkali aided process produced better gels than the acid-aided process (Batista *et al.*, 2006) but not as good as those obtained from the fish muscle. The proteins recovered after alkaline solubilisation of unwashed mince showed the best textural properties but the gels obtained were of medium quality according to the results of a folding test score. The protein isolates were used as ingredients in the preparation of Frankfurt-type sausages (Pires *et al.*, 2007).

14.3. Processing of marine proteins and peptides

14.3.1. Enzyme hydrolysis

Enzymes are used industrially to produce fish protein hydrolysates. The hydrolysates can be processed further by separating them into fractions using centrifugation, sieving, ultra- and nanofiltration and concentrating them by evaporation and drying. The products can be

ingredients in fish and other food formulations, seafood flavours or ingredients for functional foods, nutraceuticals and cosmetics. The most recent reviews are by Kristinsson, 2006 and Guerard, 2006b.

The enzymes are active under mild pH conditions and temperatures. The types of enzymes applied depend on the intended final product. The production of wide range of protein ingredients from by-products and underutilized fish using controlled conditions has been documented.

A preliminary step in the production of fish protein hydrolysates is the selection, storage and handling of the raw material. It depends both on the needs and demands of end users as well as needs and wishes of the raw material producers. The raw material is either by-products or underutilized fish. The by-products come both from fisheries and aquaculture. The need is to collect, dispose of and/or utilize the by-products in the most cost effective manner. Diversity, unstable and very perishable are the words that best describe the supply and quality. Spoilage and development of off-flavour must be prevented by controlling protein degradation and lipid oxidation both in the raw material and end products. Raw material with amounts of chemical contaminants like PCBs and dioxins exceeding maximum limits and action limits set by authorities must be avoided (Recommendation 2004/705/EC). Development of biogenic amines can limit the use of certain species and care must be taken to using only fresh unspoiled raw material.

Cost limits the choice of raw material to underutilised fish and co-products like viscera, backbones, skins, cut-offs, saw dust and washing waters and cooking juices. Collection of raw material to centralised production facilities is difficult due to spoilage and transportation cost. The fish protein supply is very diverse compared to other protein sources. The diversity is an opportunity, but can also create problems and make things difficult. Besides diversity in species, there is diversity in fishing vessels, production and processing sites and operations.

This can create difficulties in collecting by-products and in the quality and condition of the raw materials so it becomes unfit for processing into high value products. Co-products are not stored under the same conditions as fish fillets and spoil much more rapidly. One of the main criteria for the production of fish proteins is that the underutilized species and co-products receive the same treatment as the raw material, and that they are processed as fresh as possible. A stable and sustainable supply is necessary to start up businesses in fish proteins and peptides. The conditions of the wild fish stocks and the seasonality of the catches must be considered and can be a problem when planning a big scale production of proteins and peptides. Many of the stock are declining while other are in good condition and even increasing. In Iceland for example pelagic fish is about 70% of a total catch. Capelin has been going down, blue whiting has been increasing as well as the herring. The catch is seasonal, capelin is mostly caught during the first months of the year, blue whiting in early spring and summer and herring in the summer and autumn. Groundfish like cod, haddock and saithe are however landed throughout the year and fluctuation in the size of the stocks is much less that for the pelagic fish species.

Mincing, homogenization and mixing with enough water to ensure good access of the enzymes is the first step in the production of fish protein hydrolysates (Slizyte et al., 2005). The second step is the hydrolysis itself that includes setting the slurry to a right temperature, adjusting the pH and adding the enzyme. The choice of enzymes depends on end product but also on cost. Mixing fish viscera into the slurry is the least expensive method and the fish enzymes are active at very low temperatures which minimize microbiological and quality problems but variations in enzyme level and activity as well as lower yield can be a problem. Commercial enzymes, usually a combination of endo- end exopeptidases are used in controlled hydrolysis (Guerard, 2006), producing FPH with very different properties. Limited

hydrolysis requires specific enzymes but a mixture of enzymes is used in more extensive hydrolysis. Several studies have been documented using enzyme preparations from Novo Nordisk, Alcalase, Flavourzyme, and Protamex; Neutrase, Kojizyme (Guerard, 2006), but other like Newlase from Amana and fungal protease type II from Sigma (Guerard, 2006) and Colrolase PN-L and 7089 have also been tested with success (Kristinsson, 2006).

14.3.2. Membrane filtration

Ultrafiltration (UF) using membranes with different molecular weight cut-offs (MWCO) is used in the production of ingredients with different biological and physicochemical properties from protein hydrolysates. Peptides can be separated from non-hydrolyzed proteins and proteolytic enzymes with UF membranes with high MWCO, approximately 20 kDa or above. The peptides in the permeate can then be fractionated according to their molecular weight (MW) with UF membranes of intermediate MWCO, approximately 4–8 kDa. Peptide solutions can also be concentrated with nanofiltrtion (NF) membranes of low MWCO, approximately 200–300 Da which will retain almost all the peptides excepted the smallest ones (amino-acids, di- or tri-peptides) (Vandanjon et al 2007). Ultrafiltration and nanofiltration can also be used in a diafiltration mode for refining solutions (e.g. desalination, partial deodorization with a nanofiltration membrane) (Simon *et al*., 2002 and Vandanjon *et al.,* 2005).

High concentration of pepsin can be obtained by ultrafiltration of the aqueous phase from cod stomach silage preserved with formic acid. And a concentrate of trypsin-like enzymes can be obtained by ultrafiltration of fish sauce produced by salt fermentation of cod intestines. The

permeate from the ultrafiltration contained the major part of proteinous material (peptides and amino acids), and had a palatable taste similar to traditional fish sauce (Gildberg , 1992). Cod frame protein hydrolysate can be separated into fractions with different physicochemical and bioactive properties. Permeate from a 10 kDa MWCO showed high antioxidative activity, while a permeate from a 3 kDa membrane had excellent ACE inhibitory activity. A fraction between 10-30 kDa showed excellent emulsion properties and whippability (Jeon *et al.,* 1999). Antioxidative properties of ultrafiltrated fraction of yellowfin sole frame protein hydrolysate has been studied and the active peptide from the fraction showing the highest activity was isolated and identified using chromatographic methods to be a 13 kDa peptide molecule with 10 amino acids (Jun *et al.,* 2004). Ultrafiltration was used to fractionate Alaska pollack frame protein hydrolysate to concentrate and separate peptides with antioxidative properties. The fraction with the smallest MWCO had the highest activity. It was further purified using consecutive chromatographic methods. The sequence of the purified peptide was Leu-Pro-His-Ser-Gly-Tyr (Je *et al.,* 2005).

Enzymatic hydrolysis is usually a batch operation. It has been criticized for being costly. The enzymes cannot be reused and the process is labour intensive with low yield and inconsistent quality. An UF membrane reactor for the hydrolysis of proteins has been applied to overcome those problems. Several studies have concluded that production of protein hydrolysates in a continuous membrane reactor results in higher productivities and more uniform products than batch-type reactors(Chiang *et al.*, 2006). Figure 2 shows an outline of a continuous reactor.

14.3.3.Fermentation

There are challenges and opportunities in studying and adapting and changing traditional foods made by methods like fermentation . Fish sauce and dry cured fermented meat products are good examples. Fermentation is a traditional food processing and food preservation method. Many traditional food products are fermented. Starter cultures of lactic acid bacteria and other microorganism are used in the production of fermented beverages, dairy, meat and vegetable products. Fermented products have extended shelf life, with distinct flavour profiles and texture. The preservation is due to the production of lactic acid and other organic acids, which reduce the pH and inhibit growth of pathogenic and spoilage organisms. Fermentation is also used to produce functional dairy products with ACE inhibitory peptides that may exert an antihypertensive effect (Chen *et al.,* 2007; Gobbetti *et al.,* 2000;Nakamura *et al.,* 1995; Yakamoto *et al.,*1999).

Lactic acid bacteria have not been used commercially to any large extent for the production of seafood for human consumption. Fermentation of surimi with lactic acid bacteria to obtain a product with Angiotensin I-converting enzyme (ACE) inhibitory activity that can facilitate improvement in surimi fish sausage has been reported (Shan *et al,.* 2007). Hydrolysed and fermented minced mackerel showed an antioxidative activity (Yin *et al.,* 2005).

In Norway traditional northern European dry-cured meat sausages technology was used to develop a new kind of fermented, smoked, and dried fish product resembling a firm and sliceable dry-cured meat sausage but with a high content of polyunsaturated (PUFA) omega-3 fatty acids (Nordvi *et al.,* 2007). It is made from salmon and saithe, fish oil with whey protein-based ingredients and fermented with *Lactobacillus sakei*. The fish oil is microencapsulated simultaneously with the microparticulation of the protein. This product

can be in liquid, powder or emulsion form and is suitable for enrichment of variety of food articles and beverages or it may be consumed as such (Bakkene et al., 2007).

Resent results from research on dry cured ham indicate that dipeptides with strong ACE inhibitory activity can be produced during meat ageing.(Sentandreu and Toldra, 2007).

Processing of fermented sauce is a traditional way of preserving fish in South-East Asia. Fish sauce is mainly produced from anchovies, mackerel and herring. Heavily salted fish with two to three parts water in closed tanks is used for producing fish sauce. The fish is fermented at 30-40°C for 6-12 months or longer. The fermentation process normally takes a long time to ensure the solubilisation as well as the flavour and colour development (Dissaraphong, 2006). Biochemically, fish sauce is salt-soluble protein in the form of amino acids and peptides. It is developed micriologically with halophilic bacteria, which produce proteases that along with proteases from the fish muscle and viscera hydrolyse the fish and are principally responsible for flavour and aroma (Lopetcharat *et al.,* 2001; Gildberg and Thongthai, 2001 ; Fukami *et al.,* 2004).

There is a great interest in producing low salt fish sauce. The high salt content inhibits the action of the enzymes in the fish which makes the processing time very long and the great amount of salt in the product makes it difficult to market it as a health product. The processing time can be shortened by the addition of fish viscera or proteases (Kim *et al.,* 1997 and Morioka *et al.,* 1999) or the reduction of salt concentration under 20% (Gildberg and Thongthai, 2001; Morioka *et al.,* 1999). It has also been documented that high pressure treatment under 60 MPa at 50°C for 48 hours can be used to produce autolytate such as fish sauce without any addition of salt (Okasaki et al., 2003). Fish sauce can also contain bioactive peptides. A peptide with antioxidative properties has been isolated from fermented blue

mussel sauce (Jung *et al*., 2005) and ACE inhibitory dipeptides have been isolated from fermented anchovy sauce and it also stimulated insulin secretion by cultured insulinoma cells (Ichimura *et al*., 2003). Fish sauce is mostly produced in South East Asia but successful production from Arctic species of pelagic fish like capelin has been reported (Gildberg 2001; Hjalmarsson *et al.,* 2007).

14.4. Bioactive properties of fish protein hydrolysates and peptides

Various fish protein hydrolysates and peptides have shown different types of *in vitro* bioactivities such as antioxidative, immunomodulatory, antihypertensive, anticancer and antitrombotic activities (Kim and Mendis, 2006). The peptides are usually 2-14 amino acids long and usually have hydrophobic amino acid residues in addition to proline, lysine or arginine groups. Bioactive peptides are also resistant to the action of digestion peptidases (Kitt and Weiler, 2003; Seki *et al.,* 1996; Yamamoto *et al.,* 2003). Tables 1 and 2 show published examples of FPH with biological effects, the raw material they are produced from, the enzymes used and the amino acid sequence of the active peptides. Most of the publications are on hypotensive or inhibition of angiotensin converting enzyme (Anti-ACE) and antioxidative effects.

14.4.1. ACE inhibition

Inhibitory peptides for angiotensin I-converting enzyme having anti-hypertensive effect have been isolated from enzymatic digests of various food proteins. Many of them show *in vitro* ACE inhibitory activity but also *in vivo* activity in spontaneously hypertensive rats (SHR).

And few of them have been tested in humans (Vercruysse *et al.,* 2005). To exert an antihypertensive effect after oral ingestion, ACE inhibitory peptides have to reach the cardiovascular system in an active form. Therefore, they need to remain active during digestion by human proteases and be transported through the intestinal wall into the blood stream. The bioavailability of some ACE inhibitory peptides has been studied. For example (hydroxy)proline-containing peptides are generally resistant to degradation by digestive enzymes. Peptides can be absorbed intact through the intestine by paracellular and transcellular routes, but the potency of the bioactivity after absorption is inversely correlated to chain length (Vermeirssen *et al.,* 2004).

Peptides from chicken muscle and ovalbumin failed to show antihypertensive activity in SHR rats (Fujita *et al*, , 2000). The authors classified the peptides into 3 groups: (1) inhibitor type that is not affected after preincubation with ACE. (2) Substrate type peptides that are hydrolyzed by ACE to give peptides with weaker activity; and (3) prodrug-type peptides that are converted to true inhibitors by ACE or gastrointestinal proteases. Peptides belonging to the 1st and the 3rd groups exert antihypertensive activities even after oral administration in SHR.

Dried bonito bowel ('Katsuobusi'), is a seasoning used in Japan made from thin slices off boiled dried bonito. Thermolysin hydrolysate of bonito bowels has shown *in vitro* ACE inhibitory activities (Yokoyama *et al.,* 1992) that was increased 16 fold by treating it with ultrafiltration and chromatography (Fujii *et al*., 1993). The hydrolysate reduced systolic blood pressure in SHR rats and humans (Fujita *et al.* 1995). The active peptides were isolated and the effects confirmed in an *in vivo* test on SHR rats (Karaki *et al.,* 1993). LKPNM peptide, which was isolated from the thermolysin digest of dried bonito is a pro-drug ACE-inhibitor that was activated 8-fold by ACE itself and showed a prolonged effect after oral

administration (Fujita *et al.,* 1999; Yoshikawa *et al*., 2000). Recent results suggest that antihypertensive mechanism of the effect induced by dried bonito peptides involves direct action on vascular smooth muscle in addition to ACE-inhibitory activity (Kouna *et al.,* 2005).

Sardine protein hydrolysate produced by *Bacillus licheniformis* alkaline protease showed inhibitory effects both *in vitro* and in *vivo* tests with SHR rats before and after *in virto* digestion. When FPH was orally administered at a dosage of 2.0 g protein/kg to SHR rats, blood pressure was reduced and the reduction continued for 6 hours after administration. Diet containing FPH as the sole protein source had a stronger hypotensive effect and a higher survival ratio in stroke-prone SHR rats than commercial diet (Sugiyama *et al.,*1991; Matsui *et al.,*1993). The most active peptide is a dipeptide,VY that has a significant antihypertensive effect on mild hypertensive people via angiotensin- I-converting enzyme inhibition, as well as SHR rats (Kawasaki *et al.,* 2000).

Other raw materials that have been converted into hydrolysates and active peptides with ACE inhibitory effects include Alaska pollack frames (Je *et al.,* 2004) and skin (Byun and Kim, 2001); cod frames (Jeon *et al.,* 1999) and heads (Bordenave *et al.,* 2002); skipjack tuna (Astwan et al., 1995 and tuna broth (Hwang and Ko, 2004); shrimp (Bordenave *et.al., 2002* and Hai-Lu *et al.,* 2006); salmon and chum salmon (Bordenave *et al.,* 2002 and Ono et al., 2003); fermented sauce of pearl oysters (Katano *et al.,* 2003); fermented mackerel (Itou and Akahane, 2004); sea bream scales (Fahmi *et al.,* 2004), yellowfin sole (Jung *et al.,*2006) and finally fermented surimi (Shan *et al.,* 2007) and hydrolysed kamaboko (Nagai *et al.,* 2006).

Commercial samples of FPH from the companies Copalis (France), Marinova (Denmark) and Primex (Iceland) partners in the PROPEPHEALTH project were screened for ACE inhibititory effects.*In vitro* ACE inhibitory activities of some of the fish hydrolysates in

PROPEPHEALTH is summarized in Table 3. Most them exhibited a moderate ACE inhibitory activity with IC_{50} ranging from 4 to 1350 µg.mL^{-1} of hydrolysate. The highest activity was measured for plaice hydrolysates (IC_{50} = 4 µg.mL^{-1}) was still 836 fold less active than captopril, the most potent synthetic inhibitor of ACE. These results are in agreement with previous data indicating that fish protein hydrolysates constitute a good source of peptides exerting a moderate activity on ACE. LKPNM the prodrug peptide isolated from hydrolysed dried bonito had IC_{50} = 2,4 µg.mL^{-1} that increased 8-fold in activity whwn hydrolysed by ACE.(Fujita and Yoshikawa, 1999).

14.4.2. Antioxidant properties

There is a growing interest in finding safe and natural antioxidants that enhance the body's antioxidant defences through dietary supplementation, and inhibit lipid oxidation in foods. Under normal conditions, reactive oxidative species (ROS) are effectively eliminated by the antioxidant defence system, such as antioxidant enzymes and nonenzymatic factors. However, under pathological conditions, the balance between the generation and the elimination of ROS is broken and biomacromolecules, including DNA are damaged by ROS-mediated oxidative stress. Lipid peroxidation in foods affects the nutritive value and may cause disease conditions following consumption of potentially toxic reaction products (Je *et al.,* 2007; Jung *et al,* 2007).

Ultrafiltrated fraction of 10-30 kDa with a high antioxidant activity was isolated from cod frames hydrolysed with crude protease extracted from tuna pyloric caeca (Jeon *et al.,* 1999).. Antioxidative peptide was isolate from tuna cooking juice hydrolysed by Protease XXIII, from Aspergillus oryzae (Jao and Ko, 2002). The antioxidant activity of fish enzyme

hydrolysates could by improved 20-30% by reacting them with glucose (Guerard and Sumaya-Martinez, 2003). An antioxidative peptide of 10 amino acids was isolated from yellowfin sole frame proteins (Jun *et al.,* 2004). The same group of scientists also tested Alaska pollack frames hydrolysed with mackerel intestine crude enzyme. They isolated a six amino acid peptide after fractionation with ultrafiltration and further purification using chromatographic methods (Je *et al.,* 2005). The most active peptide from tuna backbones had 14 amino acid residues. It significantly inhibited lipid peroxidation in linoleic acid emulsion system and also quenched free radicals (DPPH, hydroxyl and superoxide) in a dose-dependent manner (Je *et al.,* 2007). Antioxidant peptides were also isolates from giant squid muscle and skin (Mendis *et al.,* 2005b; Rajapake *et al.,* 2005). A seven amino acid peptide was isolated from hoki skin gelatine after hydrolysis with trypsin (Mendis *et al.,* 2005a).

Another group has recently published result on the antioxidative properties of round scad and yellow stripe trevally.. The results revealed that antioxidative activity of protein hydrolysates from yellow stripe trevally meat were determined by degree of hydrolysis and the enzyme type used (Klompong *et al.,* 2007; Thiansilakul *et al.,* 2007a and b).

Antioxidative peptides can also be found in processed seafood. Antioxidative peptide with five amino acids was isolated from blue mussel sauce after 6 and 12 months fermentation (Jung *et al.,* 2005). Kamaboko, a surimi based ready to eat product, also showed higher oxidative activities after hydrolysis using three gastrointestinal proteases and protein proteases (Nagai *et al.,* 2006). These results were confirmed on walleye pollack hydrolysates. The authors conclude that Kamaboko products are of benefit not only for health food diets with high amount of essential amino acids, but also to patients undergoing various diseases, such as cancer, cardiovascular diseases and diabetes (Nagai *et al.,* 2007).

Antioxidant and free radical scavenging activities were demonstrated in many FPHs in PROPEPHEALTH project of the SEAFOODplus (Chabeaud *et al*., 2006a). More active fractions of saithe and shrimp processing waste hydrolysates could be isolated using ultra- and nanofiltration (Chabeaud *et al.,* 2006b; Guerard *et al*., 2007). Table 4 summarizes the results of the antioxidant assays in the PROPEPHEALTH project(Guerard, 2006a).

14.4.3. Immunostimulation

There is an increasing interest to identify new immunomodulators to enhance non-specific host defence mechanisms for improvement of stress-induced immunosupression, general well-being and as a way to reduce treatment costs. Immunostimulants are used in aquaculture, enhancing the resistance of cultured fish to disease and stress. Acid peptide fractions from Atlantic cod stomach hydrolysate stimulated superoxide production in Atlantic salmon macrophages (Gildberg *et al.,*1996) and supplementation of cod milt cationic proteins to the feed of juvenile fish improves their resistance to this bacterial infection (Pedersen *et al.,* 2004).

Seacure® is a protein supplement derived from the fermentation of fish protein by a proprietary yeast strain. Its effects on the mucosal immune response in an *in vivo* model were evaluated and the conclusion was that the product is an immunomodulating food with a demonstrated capacity to enhance non-specific host defence mechanisms (Duarte *et al*., 2006).

14.4.4.. Secretagogue and calciotropic activities

Gastrin and cholecystokinin (CCK) are small intestinal hormones belonging to the secretagogue family. Cholecystokinin is a peptide hormone that reduces gastic acid secretion and stimulates the intestinal digestion and absorption of fat and protein and controls satiety/appetite. CCK mediates satiety by acting on the CCK receptors (CCK1 and CCK2) distributed widely throughout the central nervous system. In humans, CCK administration causes nausea and anxiety and weakly decreases the desire to eat.(Fink *et.al.*1998).Calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide derived from the calcitonin gene with widespread expression such as in the heart, blood vessels, pituitary, thyroid, lung, gastrointestinal tract where it decreases food intake by expressing gastric acid secretion and a wide array of biological effects including neuromodulation, vasodilatation, cardiac contractility and bone growth(Wimalawansa, 1996).The presence of a biologically related CGRP molecule in sardine hydrolysates has been reported (Fouchereau-Peron *etal.,*1999). CCK-like peptides and calcitonin gene-related peptide (CGRP) respectively were detected in Alcalase(R) hydrolysates of cooked wastes of sardine (Ravallec-Ple *et al.,* 2001, Rousseau *et al.*, 2001) and measured in cod muscle and shrimp heads extracts and alcalase hydrolysates Fouchereau-Peron *et al.*, 1999, Ravallec-Ple and Wormhoudt, 2003.

The FPH in the PROPEPHEALTH project were screened for Gastrin/Cholecystokinin(CCK) and calcitonin gene related peptide like molecules. The highest activities were detected in Portuguese dogfish or siki. Ultrafiltration had no effects on yield of gastrin/CCK like molecules but improved yield of CGRP in a siki protein hydrolysate. Molecular exclusion chromatography was used to purify and isolate a 1500 Da fraction that could stimulate adenylate cyclase activity. (Martinez-Alvarez *et al*, 2007).

14.4.5. Other properties

Diazepam-like effects of cod protein hydrolysate on stress responsiveness in rats has been reported (Bernet *et al.,* 2002) and the authors concluded that these effects of the hydrolysate (Gabolysat PC60) agree with anxiolytic properties of this nutritional supplement, previously reported in both rats and humans.

High-fat feeding led to severe whole body and skeletal muscle insulin resistance in casein or soy protein fed rats, but feeding cod protein fully prevented the development of insulin resistance. It was demonstrated that feeding cod protein prevents obesity-induced muscle insulin resistance in high fat-fed obese rats at least in part through a direct action of amino acids on insulin-stimulated glucose uptake in skeletal muscle cells (Lavigne *et al.,* 2001; Tremblay *et al.,* 2003).

Antiproliferative activity of FPH from the companies Copalis (France), Marinova (Denmark) and Primex (Iceland) was measured in the PROPEPHEALTH project of SEAFOODplus on two human breast cancer cell lines grown *in vitro*. Samples of blue whiting, cod, plaice and salmon hydrolysates were identified as significant growth inhibitors on the two cancer cell lines (Picot et al., 2006).

14.5. Functional properties of dried marine proteins and peptides

Food proteins especially soy and dairy proteins are used in many food applications because of their good functional properties, that is water-binding capacity, oil holding capacity, viscosity, foaming properties, emulsifying properties, gelation, and solubility. Functional properties are influenced by protein source, production and environmental parameters. Production parameters are isolation, precipitation, drying or dehydration, concentration, modification

(enzymatic, alkaline, acid hydrolysis, chemical) and environmental parameters include temperature, pH and ionic strength. Each protein has a different isoelectric point based on its source and thus the functionality of proteins differs when measured at different pH values (Kinsella, 1976).

Enzymatic hydrolysis of changes the functional properties of fish proteins. Heating is used to stop enzyme reaction and in drying of FPH. It may have a deleterious effect on the functional properties and on nutritional and quality factors because of denaturation of proteins (Kinsella, 1976). Spray drying is considered a gentle form of drying and gives a product where some retention of functional properties is observed. Drum drying can on the other hand cause losses in solubility.

14.5.1. Water holding capacity

Good water-holding capacity of fish is important as it affects both economic and sensory attributes of the products. There is about 80% water in lean fish muscle. Most of it is held within the myofibrils mainly by capillary forces. The water in fish muscle is divided into bound water, immobilized water and free water. Bound water is less than 10% of the total water. It forms layers of water molecules around the myofibrillar proteins. This water has reduced mobility and is very resistant to freezing and being evaporated by heat. Immobilised water is held within the structure of the muscle but is not bound to proteins. Factors that can influence the retention of immobilized water include manipulation of the net charge on the myofibrillar proteins and the structure of the muscle cell and its components, as well as the amount of extracellular space within the muscle itself. Free water flows from fish during pressing or centrifugation (Kolczak *et al,* 2007). Free water can be lost from fish muscle during handling, transport, storage and processing. Maintaining as much water as possible is the goal of many fish processors. Shrinkage during cooking causes water to be expelled by

pressure out of the fish. This shrinkage causes the great water loss that occurs on heating. The water losses depend on the method of heating. Too much shrinkage influences the quality and sensory properties of the cooked fish. Maintaining low cooking loss is therefore both the goal of the processors as well as the buyers and users of the fish. The latest trend is to add water to processed fish by adding brine to modify both fresh retail and further processed products. This is done to improve quality, firmness and juiciness and to meet increasing demand for convenience food items while at the same time increasing yield (Xiong, 2005). Homogenized fish mince, protein isolates and hydrolysed fish proteins have been tested in the brines. Factors that affect water-binding capacity of proteins are for example, protein concentration, pH, ionic strength, temperature, other food components like polysaccharides, lipids and salts, rate and length of heat treatment (Zayas, 1997).

Very little has been documented about the use of dried hydrolysed fish protein in injected or tumbled products. It has been reported that dried cod protein hydrolysates had better water holding capacity than soy proteins but that the soy protein resulted in lower drip and higher cooking yield (Thorarinsdottir *et al.,* 2004).

14.5.2. Solubility

Intact fish myofibrillar proteins are quite insoluble in water over a wide pH range. Smaller peptides produced by hydrolysis have more of the hydrophilic polar amino acid side groups exposed and can bind more readily to water than the intact protein can (Kristinsson and Rasco, 2000). High solubility over a wide range of pH is important for many food applications as it influences other functional properties, such as emulsifying and foaming properties. Good solubility of fish protein hydrolysates over a wide range of pH that increased with degree of hydrolysis has been reported several times (Gbogouri *et al*., 2004; Geirsdottir

et al., 2007; Klompong *et al.,* 2007; Sathivel and Bechtel, 2006; Sathvivel *et al.,* 2005; Shahidi *e.al.,*1995; Thiansilakul *et al.,* 2007).

14.5.3. Emulsifying properties

There is a growing trend within the food industry to replace synthetic emulsifiers with more natural ones. Proteins can be used as emulsifiers in foods because of their ability to facilitate the formation, improve the stability, and produce desirable physicochemical properties in oil-in-water emulsions. The protein emulsifiers have the advantage to protect polyunsaturated lipids from iron-catalyzed oxidation (Hu *et al.,* 2003). At pH values below their isoelectric point proteins form positively charged interfacial membranes around oil droplets that electrostatically repel any Fe^{2+} and Fe^{3+} ions present in the aqueous phase. Thus, iron is prevented from catalyzing oxidation of the polyunsaturated lipids contained within the droplets.

Emulsifying properties of many fish proteins and fish protein hydrolysates have been reported. Interfacial activities (emulsion activity index, emulsion stability index, foaming capacity, foam stability) increase with limited hydrolysis but increased hydrolysis decreases them (Thiansilakul *et al.,* 2007; Klompong *et al.,* 2007; Sathivel and Bechtel, 2006; Sathvivel *et al.,* 2005; Gbogouri *et al.,* 2004; Shahidi *et al.,* 1995). Good solubility, emulsifying activity, and cooking stability of purified fish collagen has been reported (Kim and Park 2005). Initial heating of raw material before hydrolysis decreased emulsifying properties of FPH (Slizyte *et al.,* 2005).

FPH often show poorer emulsifying properties than dairy and soy proteins. Emulsifying capacity and stability was lower in hydrolysed herring and herring by-products than in egg and soy proteins (Sathivel *et al.,* 2003). Fish gelatin had much lower oil-in-water emulsification capacity than beta-lactoglobulin (Suhr *et al.,* 2006). Superior emulsifying properties of sodium caseinate, the susceptibility of whey protein emulsions to increasing flocculation on storage, and the coalescence of fish gelatin emulsions following centrifugation has been demonstrated (Dickinson and Lopez, 2001). Freeze dried salmon protein hydrolysates had comparable solubility to that of egg albumin and water holding capacity was better than for egg albumin and soy protein but emulsifying properties similar or lower (Kristinsson and Rasco, 2002).

Soluble fraction of ca.12kDa of sardine sarcoplasmic proteins heated over 60°**C** showed however excellent emulsifying activity (Kawai *et al.,* 1995).

There are surprisingly few reports on emulsifying properties of purified fraction or component of FPH. Two protein fractions extracted from cod were able to form and stabilize oil-in-water emulsions (Pétursson *et al.*2004). Fraction between 10-30 kDa of cod frame protein hydrolysates showed excellent emulsion properties and whippability (Jeon *et al.,* 1999).

14.5.4. Sensory challenges

The flavour of a protein hydrolysate depends both on the raw material, the kind of protease applied and the hydrolytic conditions. Bitter flavour is a major problem with most fish protein hydrolysates (Kristinsson and Rasco, 2002). The bitterness is normally caused by a number of medium size peptides with hydrophobic amino acid residues. Principally this problem may be solved either by performing mild hydrolysis to reduce production of medium size peptides or by running extensive hydrolysis to digest the troublesome peptides to free amino acids.

Although mild hydrolysis may both improve flavour and nutritional properties, it will normally reduce the yield significantly. The latter is a big problem if maximal utilisation of the raw material is a major option. Extensive digestion is probably more convenient although it may reduce nutritional quality. Producers of commercial enzymes claim that the problem of bitterness may be solved by applying certain enzyme products with specific "non-bitter" properties. However, this does not always hold true (Gildberg *et al.*, 2002).

Lipid oxidation is a problem in dried fish proteins and fish protein hydrolysates. Kristinsson and Rasco, 2002 suggested producing powder form lean fish by enzymatic hydrolysis and spray drying since the problems of lipid oxidation can be reduced by mild processing conditions. Lipid oxidation is influenced by storage time and temperature. The prospects of fish powder as a food ingredient depends on the possibilities of stabilizing the residual lipid by suitable processing techniques. All means must be ensured to prevent oxidation during all processing steps. Oxidation problems could be controlled by washing the raw material of lipids and heme proteins, by adding antioxidant before hydrolysis and by selecting enzymes that do not operate at low pH and high temperatures (Kristinsson, 2006). There are very few publications on the oxidative stability of dried fish powders. Bragadottir *et al.,* 2007 reported advanced lipid oxidation in fresh spray dried enzyme hydrolysed saithe powder. They recommend optimisation of processing parameters that would include reducing access to prooxidants and oxygen, preservation on endogenous antioxidants in the raw material by mild processing techniques and using added antioxidants. Drying of pH-shift processed protein can even increase the problem (Geirsdottir, 2006).

14.6. Market for functional marine proteins and peptide products

The market for extracted, hydrolysed, isolated and dried functional protein and peptide products is not very great compared with dairy and plant proteins and peptides. The traditional and most significant market is in seafood flavours and extracts with several small companies with products in liquid form, as pastes, dried flakes or powders. Markets for them are primarily in Europe and Asia. The annual production of collagen/gelatine is about 300 thousand tonnes. Fish gelatines account for only 1-2% of the production. It is both sold for its physical and bioactive properties.

The market for functional foods and food supplements is the fastest growing part of the food market. In Japan it grew from 3.8 in 1990 to17 billion dollars in 2006. FOSHU (Food for Specified Health Use) accounts for 30% of that market. There are many products with proteins and peptides mainly soy and dairy based. The number of collagen based products is growing very fast. The number of collagen health foods and supplement products is remarkable (Functional Foods Japan, 2006). In Europe and the United States they are mostly sold as food supplements and to the cosmetic industry. Fish collagen peptides have a variety of functions, the most representative ones being improving skin quality and preventing increases in blood pressure. In particular, it has been found to improve skin dryness and roughness, and is therefore already being used widely in health and beauty applications.

Fish protein and peptide products with approved health claims are much fewer than similar soy and dairy products. They don't exist in Europe and North America. They are mostly sold as food supplements. In table 5 there are examples of some of them. Only two products have been approved by authorities. They are both from Japan and have reached FOSHU status. Both are claimed to reduce blood pressure. One is the Katsuobushi oligopeptide made by hydrolysis of dried bonito with the enzyme thermolysin. It is marketed as PEPIDE ACE 3000

in Japan and was the first dietary supplement that was approved as FOSHU. Sales of it in 2005 were 3.5 million dollars (Functional Foods Japan 2006). It is also marketed in the United States as Vasotensin® and PeptACE™ and Levenorm™ in Canada. The other is the Sardine peptide SP100N a hydrolysed extract from sardine muscle and is among other products sold as a drink, LAPIS SUPPORT in Japan for 1.5 million dollars in 2005. SECURE® a white fish protein hydrolysate concentrate has been on the market since 1994 in the United States. It is claimed to support the cells in your gastrointestinal tract and regulate bowel functions. Nutripeptin® is a peptide powder from codfish from France/Norway for reducing blood sugar. It can be added to several different types of food, such as bread, chocolate, ice cream, hamburger, and beverages.

14.7. Future trends

This chapter is an overview of the latest developments in the use of mild processing techniques in the production and use of functional marine protein and peptide ingredients. The pH shift methods hold a great promise in better utilisation and upgrading of by-products and underutilised species. (Kristinsson et al., 2006). Applying protein isolates as water binders in injected and tumbled products will result in great additional economic, nutritional and environmental values by increasing the yield of raw material in fish filleting operation and the production of ready to eat seafood products. An even greater economic advantage would be if pH-shift methods could be used to produce high quality isolates from raw material that today is unfit for traditional processing. There are oxidation and technological problems that must be solved and much more research and development is needed into applying the isolates as commercial food ingredients or food products.

Fish protein hydrolysates cannot compete in price, size and quality with plant and dairy proteins on the functionality ingredient market. Plant and dairy ingredients will continue to be a part of formulating ready to eat convenience seafood product and the marine ingredients will be used for culinary and nutritional reasons and for their special bioactive properties. But we still have a long way to go. More research is needed into process optimization and how to scale up the hydrolysis, separation and concentration processes and how to solve problems with lipid oxidation. There is a long way from molecules to megatonnes. There are no big scale factories in specific marine proteins and peptides like we have for the manufacturing of fish meal and for protein ingredients from other sources.

Ten years ago it was concluded in a review article that biotechnology within the fish industry was still in its extreme infancy compared with other areas of food production and processing. The main reason was said to be the small size and number and diversity of utilized species and processing methods of the industry compared with agricultural production. And it was forecasted that the gestation period for a biotechnological fish processing industry would be long (Vilhelmsson 1997). Since then the production and utilisation of FPH has come a long way as has been described in this chapter. Future priorities of research for healthy, safe and nutritious seafood based on results from SEAFOODplus were presented at the 2nd Joint Trans-Atlantic Fisheries Technology Conference in Québec City 2006 (Børresen, 2006). Among the priorities was to develop lean, nutrious, tasty and convenient seafood products to control weight and reduce obesity in Western populations. But there are other needs that must also be addressed. Some of them have been mentioned in this chapter and in some cases there are already products on the market to meet those needs.

Many seafood products are deep-fried. One solution to a healthy diet would be to reduce the fat uptake during deep-frying by coating them with a barrier made of isolated fish proteins.

More supplements from FPH can be developed to reduce high blood pressure but they will face heavy competition from other protein sources. Antioxidant properties of FPH can be employed in supplements and food products to enhance the antioxidant defences of the body against oxidative stress. They can also be used as immunomodulators to enhance non-specific host defence mechanism. Specific protein products can even be made to control food intake in the fight against obesity. And there are also products on the market and future possibilities in developing FPH to lower glychemic index. The market for such products from fish proteins is not big but it will grow and there are also opportunities in adapting traditional food processes like fermentation to enhance the bioactive properties of FPH and to employ them into product that consumers already know. Low salt fish sauce and fish flavours with tailor made bioactive properties are likely the future.

Bioactive properties and functional seafood bring us straight to the complicated situation around the scientific documentation and official acceptance of health claims. Sufficient scientific evidence must be produced if companies are to produce and sell products with health claims. It means providing evidence that the active ingredient is present in a quantity and in a form needed to exert a specific function and also providing evidence from human studies, having a scientific valid design that the effect of the food or food component and finally evaluating and excluding the risk that the consumption of the products could pose to public health, including allergic potential.

Much research and legal work needed to get a health claim accepted. Private companies, universities and other research organisations will work together on special hydrolysates or peptides but the cost may be too high for small companies so a global collaboration may be need for the interests of fisheries, fish processing industries and consumers worldwide.

14.8. Sources of further information and advice (300 words)

The following books are recommended for further information as they are of special relevance to fish proteins and peptides and upgrading of co-products and underutilized species

2004. M.Sakagushi (ed.). More Efficient Utilization of Fish and Fisheries Products. Elsevier Science Publishing Company, London, England, 464 pp.

2005. Park J.W. (ed.).Surimi and Surimi Seafood.2nd ed.CRC Press.Boca Raton, Florida, USA, 960 pp.

2006. Y.Le Gal and R.Ulber(eds.),Marine Biotechnology I. Advances in Biochemical Engineering/Biotechnology.Vol.96.Springer Verlag, Berlin, Germany, 288 pp

2006 Shahidi. (Ed.) Maximizing the Value of Marine By-Products. Woodhead Publishing Limited, Cambridge, England.560 pp.

The following websites are suggested:

http://www.functionalfoodnet.eu/, is a network and an information centre for companies and contains many outputs and contains many outputs from functional foods research funded by the EU Commission under its framework programs

http://www.nordicinnovation.net/. The homepage of the Nordic Innovation center. It supports five Nordic projects on functional foods with a common goal to support the Nordic food industry in becoming more innovative and competitive in the functional food market, targeting health claims for marine functional foods ,consumer acceptance of marine functional

foods, innovative consumer driven marine functional foods product and ingredients development .Networking, cooperation and international positioning

http://www.marifunc.org/ is one of the Nordic projects and it focuses on the use of fish, nutrients and other bioactive substances isolated from fish, as ingredients in functional foods.

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Figure 14.1.

Schematic representation of pH-shift processes used in the production of fish protein isolates. The process involves solubilizing muscle proteins at low or high pH, using centifugation to separate undesireable muscle components and recovering the proteins of interest by isoelectirc preciptation. The final protein isolate can either be used directly or stabilized with cryoprotectants and frozen until used. (From Kristinsson et al.,2006. By permission from Woodhead)

Figure 14.2.

Schematic diagram of continuous spiral-wound membrane system.

Protein solution is prepared by stirring and heating is passed through a 100-mesh sieve to remove large particles. The reaction vessel is filled with the desired volume of the protein filtrate and the desired temperature maintained in the tank. Then the enzyme is added to the protein solution. Inlet pressure and flow rate can be controlled and adjusted. The reaction mixture is pumped to a spiral-wound membrane where the large particles, such as intact proteins or enzymes that cannot penetrate the pores of ultrafiltration membrane, are recycled to the reaction vessel. The permeate, containing particles small enough to penetrate the membrane are collected and processed further by further filtration, evaporation or drying.(From Chiang et.al.2006. By permission from Elsevier)

Table 14.1.

Examples of bioactive protein hydrolysates/peptides with ACE inhibitory/hypotensive effects derived from fish and crustaceans.

Table 14.2.

Examples of bioactive protein hydrolysates/peptides with biological effects derived from fish and crustaceans.

Table 14.3.

Examples of ACE inhibitory/hypotensive effects commercial fish protein hydrolysates from the PROPEPHEALTH project compared with reference sample

Table 14.4.

Antioxidative properties of commercial fish protein hydrolysates from the PROPEPHEALTH project compared with reference samples

Table 14.5.

Examples of commercially available functional foods or food ingredients carrying bioactive peptides

Table 14.1. Examples of bioactive protein hydrolysates/peptides with ACE inhibitory/hypotensive effects derived from fish and crustaceans.

Raw material	Process	Peptide sequence (Single letter code)*	References
Katsuobushi	Thermolysin	LKPNM;GYPHK;	Yokojama et al.,1992;Fujii et al.,1993;Matshumura et al.,1993
Dried bonito bowels		IRPVQ	Karaki et al.,1993;Fujita et al.,1995; Fujita et al.,1999
			Yoshikawa et al.,2000;Kouna et al.,2005
Sardine	*Bachchillus licheniformis*	VY	Sugiyama et al.,1991;
	Alkaline protese		Matsui et al.,1993;Matsufuji et al.,1994;
			Kawasaki et al, 2000;Matsui et al.,2002;
			Matsumoto et al.,2004
Skipjack tuna	Pepsin	VAWKL;WSKVVL	Astwan et al.,1995
		SSKVPP;CWLPVY	
Cod frames	Crude proteinase from tuna		Jeon et al.,1999
	Pyloric caeca		
Alaska pollack skin	Alcalase,Pronase E,collagenase	GPL	Byun and Kim,2001
Sardine			Bordenave et al.,2002
Cod heads			
Shrimp			
Chum salmon	Thermolysin		Ono et al.,2003
Pearl oyster	Alkaline protease	FY;AW;VW;GW	Katano et al.,2003
Alaska pollack frame	Pepsin	FGASTRGA	Je et al.,2004
Heshiko	Fermentation		Itou and Akahane,2004
fermented mackerel			
Sea bream scales	Alkaline protease	GY;VY;GF;VIY	Fahmi et al.,2004
Tuna broth	Orientase		Hwang and Ko,2004
Shrimp	Protease from *Bacillus* sp.98011	FCVLRP;IFVPAF;	Hai-Lu et al.,2006
		KPPETV	
Kamaboko	Gastrointestinal proteases		Nagai et al., 2006
	Protein proteases		
Yellowfih sole	Chymotrypsin	MIFPGAGGPEL	Jung et al.,2006
		IAW;YNR	
Arabesque greenling	*Lactobacillus delbrueckii*		Shan et al.,2007
surimi			

* A – Alanine, C – Cysteine, D - Aspartic Acid, E - Glutamic Acid, F - Phenylalanine (Phe), G – Glycine, H – Histidine, I – Isoleucine, K – Lysine, L – Leucine, M – Methionine, N – Asparagine, P – Proline, Q – Glutamine, R – Arginine, S – Serine, T – Threonine, V – Valine, W – Tryptophan, Y - Tyrosine

Table 14.2. Examples of bioactive protein hydrolysates/peptides with biological effects derived from fish and crustaceans.

Biological activity	Raw material	Process	Peptide sequence (single letter code)*	References
Antioxidative	Cod frames	Crude proteinase from tuna pPyloric caeca		Jeon et .al.,1999
	Tuna cooking juice	ProteinaseXXIII *Asp.oryzea*	Seven antiox.peptides	Jao et .al.,2002
	Yellowfin sole frame	Pepsin and mackerel crude enzyme	RPDFDLEPPY	Ju et .al.,2004
	Fish			Guerard et .al.,2003
	Fermented sauce of Blue mussels	Fermentation	FGHPY	Jung et .al.,2005
	Giant squid muscle	Pepsin, trypsin and α-chymotrypsin	NADFGLNGLEGLA NGLEGLK	Rajapakse et .al.,2005
	Giant squid skin	Trypsin	FDSGPAGVL NGPLQAGQPGER	Mendis et .al.,2005b
	Hoki skin gelatin	Trypsin	GPLGPL	Mendis et .al.,2005a
	Alaska pollack frame	Mackerel crude enzyme	LPHSGY	Je .et .al.,2005
	Shrimp (Acetes chinensis)	Protease from *Bachillus* sp.98011		Hai-Lu et .al.,2006
	KKamaboko	Gastrointestinal proteases		Nagai et .al.,2006 Nagai et .al.,2007
	Mussels (Mytilus coruscus)	in vitro gastrointestinal digestion system	LVGDEQAVYAVCVY	Jung et .al.,2007
	Tuna backbone	Protein proteases Pepsin	VKAGFAWTANQQLS	Je .et al.,2007
	Round scad	Flavourzyme		Thiansilakul et .al.,2007a Thiansilakul et .al.,2007b
	Yellow stribe trevally	Alcalase,Flavourzyme		Klompong et .al.,2007
Immunomodulation/	Cod stomach			Gildberg et al.,1996
immunostimulation	White fish	Fermentation		Duarte et al.,2006
	Cod stomach			Gildberg et al,1996
	White fish	Fermentation		Duarte et.al.2006
Satety/Growth/	Sardine	Alcalase		Ravallec-Ple et al.,2001
Secretion of digestive	Cod muscle	Alcalase		Ravallec-Ple and Wormhoudt,2003
eEnzymes	Shrimp heads			

	Sardine	Alcalase	Ravallec-Ple et.al.2001
	Cod muscle	Alcalase	Ravallec-Ple and Wormhoudt,2003
	Shrimp heads		
Calcitoton			
Ccalcitonin gene	Cod muscle	Alcalase	Fouchereau-Peron et .al.,1999
related peptide	Shrimp heads	Alcalase	
	sardine	Alcalase	Rousseau et al.,1999
	Portugese dogfishsiki	Copalis	Martinez-Alvarez et al., 2007

** A – Alanine, C – Cysteine, D - Aspartic Acid, E - Glutamic Acid, F - Phenylalanine (Phe), G – Glycine, H – Histidine, I – Isoleucine, K – Lysine, L – Leucine, M – Methionine, N – Asparagine, P – Proline, Q – Glutamine, R – Arginine, S – Serine, T – Threonine, V – Valine, W – Tryptophan, Y - Tyrosine

Table 14.3.

Examples of ACE inhibitory/hypotensive effects commercial fish protein hydrolysates from the PROPEPHEALTH project compared with reference sample

Sample	**ACE IC_{50} (µg. mL^{-1})***
Captopril	$4.78.10^{-3}$
Blue whiting 1	50
Blue whiting 2	1350
Cod	75
Plaice	4
Saithe	200
Salmon	220
Portugese dogfish	260

*IC_{50} corresponds to the hydrolysate concentration (µg. mL^{-1}) inhibiting 50% of ACE activity.

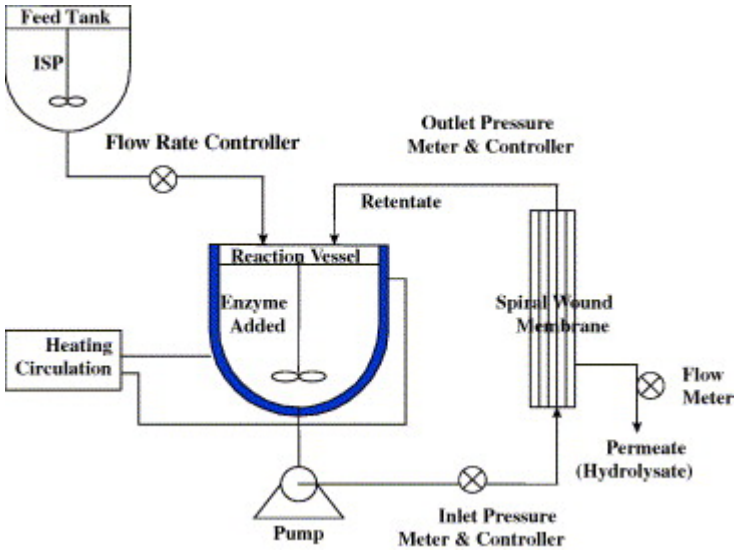
Table 14.4. Antioxidative properties of commercial fish protein hydrolysates from the PROPEPHEALTH project compared with reference samples

Samples	β-carotene	DPPH scavenging assay	Chelating activity	Reducing power
	IC5O	IC5O	IC5O	at OD=0,5 (mg/mL)
FPH from Propephealth	0,17 – 1,8	10-36	0,3 – 7,7	4,1-18,5
Ascorbic acid				0,043
BHA	0,0049			0,057
Trolox		0.056		0,084
EDTA			0.06	

- From Guerard 2006

Table 14.5. Examples of commercially available functional foods or food ingredients carrying bioactive peptides

Product name	Manufacturer	Type of food	Health claims	References
Hydrolysed dried bonito bowels				
Peptide ACE 3000	Nippon Supplements,Japan	Nutraceutical	Lowers blood pressure	www.nippon-sapuri.com/english/
		Soup mix		
Vasotensin®.	Meatgenics,USA	Nutraceutical	Lowers blood pressure	www.metagenics.com
PeptACE™	Natural factors,USA	Nutraceutical	Lowers blodd pressure	http://us.naturalfactors.com/
Levenorm™	Ocean Nutrition,Canada	Nutraceutical	Lowrs blood pressure	http://www.onc.ca/
Peptides from Sardines				
Lapis Support	Tokiwa Yakuhin, Japan	Functional drink	Lowers blood pressure	http://www.tokiwayakuhin.jp/
Peptidea	Abyss Ingredients		Relaxing	
Collagen peptides				
Bifidus & Collagen	Kagome Japan	Yogurt drink	Beautifies the skin	http://www.kagome.co.jp/
Enoceride®.	Laboratories LeStum	Nutraceutical		http://www.labo-lestum.com/
Hydrolysed whitefish				
Seacure	Proper Nutrition,USA	Nutraceutical	Improves gastrointestinal health	http://www.propernutrition.com/
Protizen	Copalis,France	Nutraceutical	Relaxing	http://www.copalis.fr/
AntiSress 24	Forte Parma, France	Nutraceutical	Relaxing	http://www.fortepharma.com/fr/index.html
Fortidium		Nutraceutical	Agains oxidative stress	http://www.biothalassol.com/
Nutripeptin		Food ingredient	Lowers glychemic index	www.nutrimarine.com


Feed Tank
ISP
Flow Rate Controller
Outlet Pressure
Meter & Controller
Retentate
Reaction Vessel
Enzyme
Added
Spiral Wound
Membrane
Heating
Circulation
Flow
Meter
Permeate
(Hydrolysate)
Pump
Inlet Pressure
Meter & Controller