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Bioactive Peptides from Marine Sources. State of Art. Report to the NORA fund

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Ágrip á íslensku:	Skýrslan lýsir stöðu mála varðandi vinnslu og markað fyrir fiskprótein og peptíð sem fæðubótarefni og markfæði. Hún greinir frá hráefnum, kröfum til gæða og öryggis og fyrirliggjandi vísindalegum niðurstöðum um heilsusamleg áhrif. Einnig er gerð grein fyrir markaðstöðu. Markaður fyrir íblöndunarefni úr fiskpróteinum og peptíðum er enn mjög lítill en möguleikarnir mjög miklir. NORA löndin hafa tækifæri til að ná þar forustu. Aðalverkefni þeirra sem ætla sér stóra hluti á þessum markaði eru tvíþætt. Annars vegar að leysa vandamál varðandi bragðgæði og stöðugleika afurða og hins vegar að staðfesta þær niðurstöður um heilsusamleg áhrif sem fengist hafa með rannsóknastofuaðferðum með miklu meira af dýratilraunum og með því að kanna áhrif aðfurðanna á fólk. Það er hins vegar mjög dýrt og til að það sé hægt þurfa hagsmunaaðilar að snúa bökum saman og leggja bæði mannskap, aðstöðu og fé til að því markmiði að auka markfalt nýtingu og verðmæti sjávarfangs í NORA löndunum.		
Lykilorð á íslensku:	*Sjávarprótein/peptíð, vinnsla, gæði, heilsufullyrðingar, markaður*		
Summary in English:	The report describes the *state of art* in turning marine resources of the West Nordic countries into valuable protein and peptide ingredients for the food supplement and functional foods market. It covers the sources and safety and quality criteria of raw materials, processing methods of fish protein hydrolysates and peptides, documented bioactive and functional properties, products on the the market and ends by describing the challenges facing biotechnology companies producing fish protein and peptide ingredients need to overcome if they are to become successful on the market. The main conclusions and recommendations are: The soy and dairy industry dominate the ingredient market for proteins and peptides, but marine protein and peptides market is still very small but growing and with a good potential. The process of adding value to low value raw materials has just begun in the seafood industry, and the NORA countries have an opportunity of establishing themselves as leaders in this field. The two biggest challenges are documenting and verifying health claims and ensuring good sensory quality, stability and uniformity. There is tremendous competiton in the market. Producers of fish protein and peptide based ingredients need to do more to carve out a niche in the bioactive arena. There is a lot of *in-vitro* experimental evidence that fish based peptides have very high bioactivity, higher than many other proteins. There is some *in-vivo* evidence as well, but not nearly enough. The focus must be on more *in-vivo* animal and eventually human studies. The fact that most companies in this area are small makes this a difficult task as the costs can be very high for those type of trials. Therefore, it is critical that all, industry, academia and institutions join forces and help move this industry to where it needs to be and divert much of their resources into *in-vivo* testing so they can document and validate health claims for these products.		
English keywords:	*Marine protein/peptides, products, quality, health claims, market*		

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Introduction

The NORA countries rely heavily upon the fishing and aquaculture industries for exports, but traditional seafood processing faces increasing competition from low-income areas such as SE-Asia and S-America. The impact of this trend is greatest on the rural fishing communities around the North-Atlantic, where rising unemployment and a shrinking economy drives people away and into the cities. The NORA countries have implemented various programs to combat this trend, with varying success. One possible solution to this problem is to increase efforts to produce high-value products from marine resources as can be seen in figure 1.

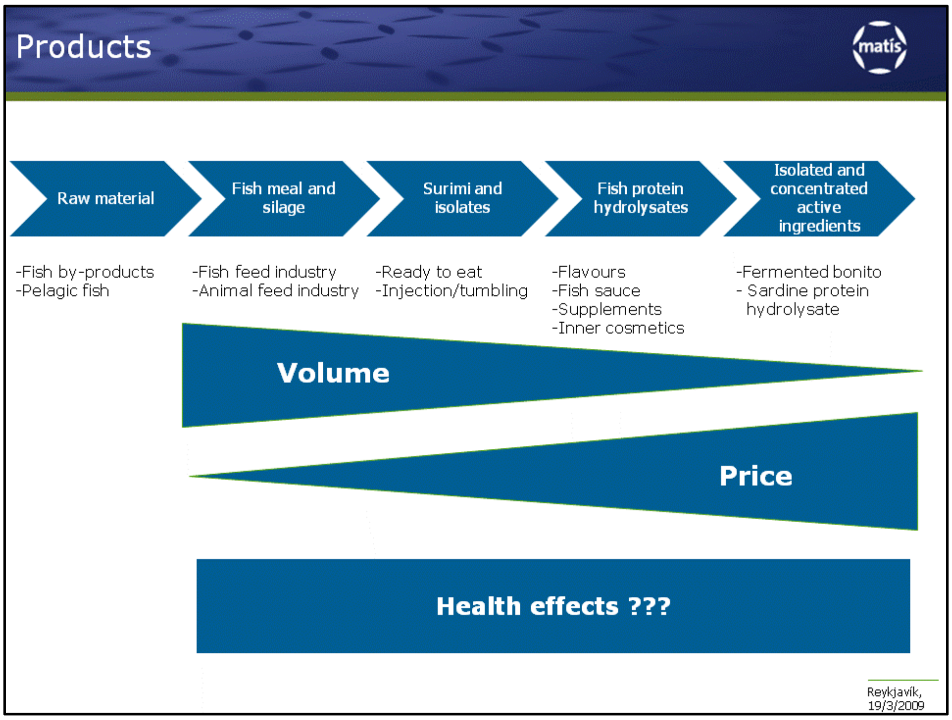

Figure 1. Added value in the processing of marine resources

It gives an overview of the added value that can be gained by up-grading fisheries by-products and pelagic fish by converting them into tailor made value added products with potential health effects instead of rendering them into fish meal for animal and fish feed. The chain goes from from fish meal to surimi and fish protein isolates to fish protein hydrolysates and finally to active ingredients for functional foods. Fish meal and surimi are commodity products with large volumes and relatively low prices. Fish protein isolates are an extension of surimi processing, but provide more value addition when incorporated into seafood products. They are already commercially produced in significant quantities and are expected to grow rapidly over the next few years. Directing by-products more to fish protein hydrolysates and isolated active ingredients is the "big dream" of the marine biotechnology industry and it is getting ever so closer to this goal, already with companies producing commercial products. Seafood flavours and ingredients for food supplements and functional foods are in this category. These products are low volume but high value and there is a tremendous future potential for these products. The two

biggest challenges are documenting and verifying health claims and ensuring good sensory quality, stability and uniformity. This is an area that efforts really needs to be focussed on in the next decades.
The process of adding value to low value raw materials has begun in the seafood industry, and the NORA countries have an opportunity of establishing themselves as leaders in the field. The transformation of fisheries and fish processing into a diversified high-tech industry can also revitalise the fragile and often monotonous economy of rural fishing communities in the area. Marine bioprospecting is also a fast growing field, where scientists search for valuable compounds in the marine environment. These compounds can serve as active ingredients in functional foods and other health related products and as a natural replacement for antibiotics in aquaculture and farming to name but a few possible uses.
The evidence that marine proteins peptides provide health beneficial effects is growing and therefore marine proteins also have a great potential as ingredients in health foods (Thorkelsson et.al. 2008). This report is about potential sources of raw materials, processing methods and documented health effects. But it also deals with safety and quality criteria for the products. Finally it mentions the products available on the market today and concludes on the future challenges in research and development.

Sources of raw materials

The raw material for proteins and peptides is underutilised fish and co-products like viscera, backbone frames, skins and cut-offs. The sources of fish proteins are very diverse and may come from many species of marine organisms. 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. Total catches in commercial fishing in the Nordic countries were around 6 million tons and fish farming supplied 0,75 million tons of fish in 2005. (Nordic Statistical Yearbook 2006). The main species can be seen in figure 2.

Raw materials

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Resources in the Nordic countries

	Million tons	Traditional products	Surimi	Fish meal	Silage
Salmon	0,6	X			X
Trout	0,1	X			
Codfish	0,5	X		x	
Saithe	0,4	X			
Flatfish	0,1	X		x	
Crustaceans/Mollusks	0,24				
Herring	1,4	X		x	
Mackerel	0,16	X		X	
Blue Whiting	1,3	x	X	X	
Capelin	0,7	X		X	
Sand eel	0,18			X	
Sprat	0,46			X	

Yearbook of Nordic Statisitics 2005

Reykjavík, 19/3/2009

Figure 2. Main resources of caught and farmed fish, crustaseans and mollucs supplied by the Nordic countries in 2005 also showing main processing categories.

The Nordic countries are also importers of raw materials from fisheries and aquaculture in other counties. The Nordic supply can be compared with a global scale of 95 m.tons of caught and 45 m.tons of farmed fish and crustaceans and molluscs (FAO Yearbooks of Fishery Statistics 2004). Annual discard from the world fisheries were estimated to be approximately 20 million tonnes.
The utilisation of protein sources from fisheries and aquaculture can be divided into three categories.

- Improved yield and utilisation of proteins in traditional fish processing
- Surimi like products from industrial fish and by-products
- Specific protein/pepide products from industrial fish and fish by-products

There is a great diversity in utilized species in the fish processing industry compared to other protein processing industries. The diversity is an opportunity, but can also create problems. There is also diversity in fishing vessels, production and processing sites and operations. This can cause problems in collecting by-products and lead to variable quality of the raw materials, sometimes making them unfit for high value products. One of the main criteria for the production of fish proteins is that the underutilized species should be processed as fresh as possible. The sustainability of marine resources and seasonal catch must be considered when production of bioactive peptides and proteins is planned. Some fishing stocks are declining while others are stable or increasing. Capelin catches in Iceland have been going down, while blue whiting,macherel and herring catches have increased. The capelin is mostly caught during the first months of the year, blue whiting in early spring and summer and herring and macherel in the summer and autumn. Demersal species, cod, haddock and saithe are caught throughout the year and fluctuation in the size of the stocks is much less than for the pelagic fish species.

Safety and stability of raw materials

The growth of spoilage bacteria can best be controlled by good chilling, storage at low temperature, hygiene and a short time from catch to processing. Storage temperature influences the type and growth rate of microorganisms (Huss, 1995). Gutting improves quality and the storage life of demersal fish species but herring and mackerel are not eviscerated immediately after catch and problems can occur during periods of heavy feeding due to belly bursting. Self-digestion can also be a problem. Good handling is essential as rough treatment will result in easier access for spoilage bacteria and enzymes that will increase spoilage rate.

Histamine formation in fish like mackerel and herring can lead to poisoning if it is not controlled. Chilling prevents histamine formation by mesophilic bacteria but cold tolerant bacteria can produce histamine in seafood at 2-5°C. In order to control histamine formation in chilled seafood, storage conditions, product characteristics and shelf-life must therefore be carefully selected (Emborg et al 2006).

Producers of fish proteins and peptides must not place unsafe products on the market. Official rules and guidelines with chemical and microbial limits in food also apply to marine proteins and peptides (Recommendation 2004/705/EC; Regulation (EC) No 178/2002; Regulation (EC No 2073/2005). The microbial guidelines include harmonised safety criteria on the acceptability of food, and the presence of pathogens. They also give guidance on the acceptability of foodstuffs and on the hygiene during processing and distribution. The microbiological criteria are a part of HACCP-based procedures and other hygiene control measures.

Quality of raw materials

Marine proteins and peptides must be of high quality to be able to make acceptable products for consumers. Changes caused by oxidation, heating, enzymes, pH changes and freezing can lead to changes in amino acids and digestibility that reduce nutritional value but also to colour changes and changes in functional properties. Chilling and freezing reduce the rate of these changes. The best way to slow or prevent them is to use fresh raw material and processing it as soon as possible in hygienic manner.

Fish protein hydrolysate and peptide processing methods

Fish protein hydrolysates (FPH) can be produced with acid or by hydrolysis with proteolytic enzymes. They can be used in fish and other food formulations, seafood flavours or ingredients for functional foods, food supplements and cosmetics (Guerard 2006; Kristinsson and Rasco 2002).

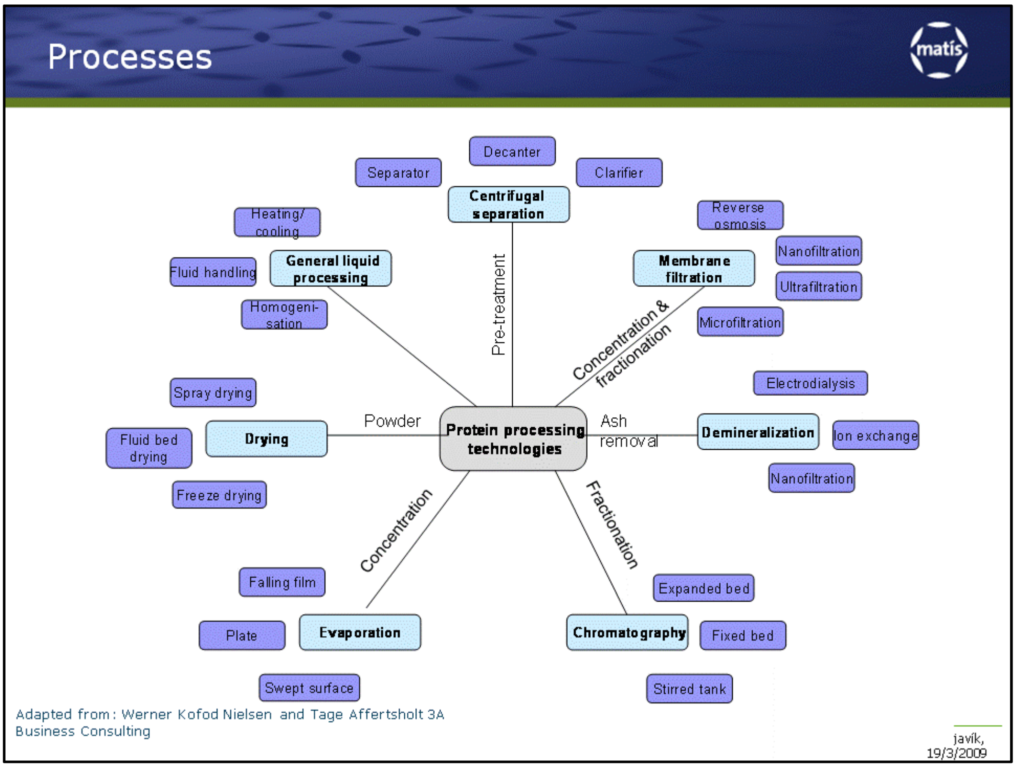

Figure 3. Processes and technologies for protein hydrolysate and peptide industries

If the fish processing industry is serious in entering the functional and bioactives market with fish proteins and peptides then attention must be paid to other well established protein industries. Figure 3 gives a good summary of protein processing technologies and is adapted from a survey on dairy or whey proteins made by 3A Consulting in Denmark. The processes include basic operations already used for fish protein ingredients like:

- General liquid processing including heating/cooling, fluid handling and homogenisation
- Centrifugal separations including separators, decanters and clarifiers
- Membrane filtration including ultrafiltration, nanofiltration, microfiltration and reverse osmosis
- Evaporations including falling film, swept surface and plate evaporations
- Drying like spray, drum drying, fluid bed drying and freeze drying

There are also more sophisticated processing technologies we still need to master with fish proteins like:

- Chromatography including expanded bed, fixed bed and stirrer tank
- Demineralisation including electrodialysis, nanofiltration and ion exchange

Ultrafiltration with molecular cut off (MWCO) of 20 kDa or higher is used to separate peptide fractions from hydrolyzed and non-hydrolyzed proteins. Membranes with 4-8 kDa can then be used to separate the fraction further. Peptide solutions can also be concentrated with Nanofiltration (NF) membranes of 0,2-0,3 kDa (Vandanjon *et al* 2007). Process chromatography is claimed to be near industrialisation in protein and peptide processing. Gel filtration is used to remove low molecular weight solutes. Ion exchange chromatography can be used to fractionate protein/peptide solutions and affinity chromatography to isolate and purify certain components (Curling 2007).

Figure 4 describes and basic step in the production of fish protein hydrolysates.

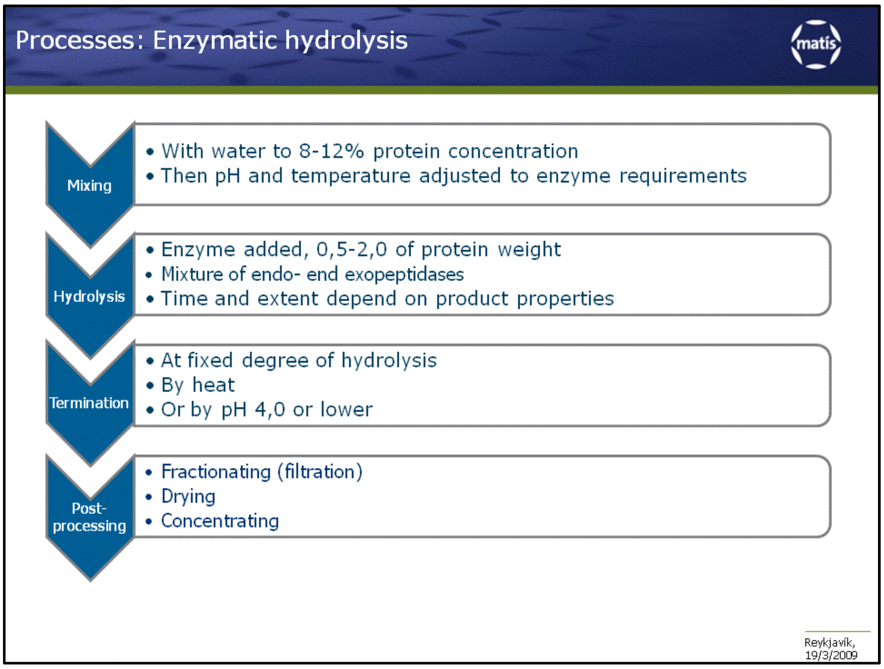

Figure 4. Basic steps in the production of fish protein hydrolysates

Production of fish protein hydrolysates starts with mincing and mixing the raw material with water to a 8-12% protein concentration before adjusting pH, adding enzymes (0,5-2,0% of protein weight) and setting the hydrolysis temperature. Cost and the properties of the end product influence the type of enzymes used. Commercial blends of enzymes are most commonly used. They are usually a combination of endo- end exopeptidases (Kristinsson and Rasco 2002). Food grade proteases from Novozymes have been used in many of the documented studies. The company has the **e**ndo-proteases: Alcalase, Food Grade®; Neutrase®; Protamex®; Novo-Pro™ D and the exopeptidase /endoprotease complex: Flavourzyme®. Endoproteases work by cleaving peptide bonds in the interior of polypeptide chains, whereas exopeptidases cleave off amino acids one at a time from the end of polypeptide chains.
In order to control the bioactive properties of the hydrolysate it is important to stop the enzyme reaction at a closely defined %DH (degree of hydrolysis) value. All the proteases can be irreversibly inactivated by heat treatment. Information on the characteristics of the proteases and suggested treatment times for inactivation at a given pH and temperature are in tables 1 and 2. Flavourzyme can be used to degrade a variety of food proteins extensively, i.e. to degrees of hydrolysis of up to 70% . Extensive hydrolysis of proteins with Flavourzyme produces protein hydrolysates, without the bitterness that often occurs with moderate enzymatic hydrolysis. Also, the potentially harmful substances formed by acid hydrolysis are avoided and the protein hydrolysate is low in salt (www.novozymes.dk).
The Flavourzyme complex can be inactivated by heating at 90°C for 10 minutes or at 120°C for 5 seconds after the hydrolysis has been completed. The proteases Alcalase, Neutrase and Protamex are all inactive at pH 4 or below. The reaction can, therefore, be stopped instantaneously by the addition of a convenient acid, e.g. hydrochloric, phosphoric, malic, lactic or acetic acid.

Table1. Characteristics of Novozymes proteases and protein hydrolysates produced by these

	Optimum pH	Optimun T°C	Max. DH%
Alcalase	8	50-60	15-25
Alcalase AF 2.4 L	8	50-60	15-25
Novo-Pro D	7-10	55-65	15-25
Neutrase	7	40-50	10-15
Protamex	7-8	50	10-20
Flavourzyme	5.5-7.5	50-55	Ca.60

Table2. Treatment times for inactivation at given pH and temperature

	pH	T°C	Time, min.
Alcalase	4	50	30
	8	85	10
Alcalase AF 2.4 L	4	50	30
	8	85	10
Novo-Pro D	4	55	30
	6-8	35	10
Neutrase	4	50	30
	7	80	4
Protamex	4	50	30
	8	85	10
Flavourzyme	6-8	90	10

Bioactive properties

Bioactive properties like antihypertensive, antioxidative, immunomodulatory, anticancer and antithrombotic activities have been documented in fish protein hydrolysates and peptides. (Kim and Mendis, 2006). The activities are most often due to 2-14 amino acids long peptides (Kitt and Weiler, 2003; Seki *et al.,* 1996; Yamamoto *et al.,* 2003).

Blood pressure reducing effect

Blood pressure reducing effect through angiotensin-converting enzyme inhibition (Anti-ACE) is the most documented bioactive property. Inhibitory peptides isolated from enzyme hydrolysates of many food proteins show *in vitro* ACE inhibitory activity and *in vivo* activity in spontaneously hypertensive rats (SHR) but only few of them have been tested in humans (Vercruysse *et al.,* 2005).
The most documented product with ACE- inhibitory effects is dried bonito bowel ('Katsuobusi') a seasoning used in Japan, made from thin slices of boiled, dried bonito. (Yokoyama *et al.,* 1992; Fujii *et al*., 1993;Fujita *et al.,* 1995;Karaki *et al.,* 1993). The active peptide is LKPNM peptide whose activities are increased 8-fold by ACE and shows prolonged effect after oral administration (Fujita *et al.,* 1999; Yoshikawa *et al*., 2000).
Sardine protein hydrolysate is second most documented ACE inhibitor from fish proteins. It has shown inhibitory effects in both *in vitro* and in *vivo* tests with SHR before and after *in vitro* digestion (Sugiyama *et al.,* 1991; Matsui *et al.,* 1993). The most active sardine peptide is a dipeptide VY that has a significant antihypertensive effect on mildly hypertensive people as well as on SHR (Kawasaki *et al.,* 2000).
Other species/raw materials with protein hydrolysates with documented ACE-inhibition are cod frames (Jeon *et al.,* 1999) and heads (Bordenave *et al.,* 2002); shrimp (Bordenave *et.al., 2002* and Hai-Lu *et al.,* 2006); salmon and chum salmon (Bordenave *et al.,* 2002 and Ono et al., 2003); yellowfin sole (Jung *et al.,*2006); Alaska pollack frames (Je *et al.,* 2004) and skin (Byun and Kim, 2001); skipjack tuna (Astwan et al., 1995) and tuna broth (Hwang and Ko, 2004) and also fermented sauce of pearl oysters (Katano *et al.,* 2003); fermented mackerel (Itou and Akahane, 2004); sea bream scales hydrolysate (Fahmi *et al.,* 2004),; and finally fermented surimi (Shan *et al.,* 2007) and hydrolysed kamaboko (Nagai *et al.,* 2006).

Antioxidative effects

Natural antioxidants are claimed to strengthen the body's defences through dietary supplementation, and inhibit lipid oxidation in foods. Normally reactive oxidative species (ROS) are effectively eliminated by the antioxidant defence system. But under pathological conditions, the balance between the generation and the elimination of ROS is broken and biomacromolecules, are damaged by oxidative stress. Lipid peroxidation in foods affects the nutritive value and may cause disease following the consumption of products that could potentially cause a toxic reaction (Je *et al.,* 2007; Jung *et al,* 2007).

A rather large fraction (10-kDa) of cod frames hydrolysates had high antioxidant activity (Jeon *et al.,* 1999) and antioxidative peptides have been isolated from hydrolyzed tuna cooking juices(Jao and Ko, 2002), hydrolysed yellowfin sole frame proteins (Jun *et al.,* 2004), Alaska pollack frames hydrolysed with a mackerel intestine crude enzyme (Je *et al.,* 2005), tuna backbones (Je *et al.,* 2007), giant squid muscle and skin (Mendis *et al.,* 2005b; Rajapake *et al.,* 2005) and hydrolysed hoki skin gelatine (Mendis *et al.,* 2005a). Antioxidative properties of round scad and yellow stripe trevally were influenced by the degree of hydrolysis and the enzyme type used (Klompong *et al.,* 2007; Thiansilakul *et al.,* 2007a and b). Processed seafood has also been documented to have antioxidative peptides for example blue mussel sauce (Jung *et al.,* 2005) and Kamaboko, a surimi-based ready-to-eat product,(Nagai *et al.,* 2006). It was concluded that Kamaboko products, with their high amounts of essential amino acids, are of benefit not only for health food diets but also to patients with various diseases such as cancer, cardiovascular diseases and diabetes (Nagai *et al.,* 2007). Finally reacting fish hydrolysate with glucose improved antioxidant activity 20-30% (Guerard and Sumaya-Martinez, 2003).

Immunostimulation

Immunomodulators can strengthen non-specific host defence mechanisms and improve stress-induced immunosupression and general well-being. They can also be used to reduce treatment costs. Immunostimulants are used in aquaculture, enhancing the resistance of cultured fish to disease and stress.(Gildberg *et al.,*1996;Pedersen *et al.,* 2004). Seacure® is a protein supplement derived from the fermentation of white fish protein. Its effects have been evaluated in an *in vivo* model and the conclusion was that the product is an immunomodulating food that can enhance non-specific host defence mechanisms (Duarte *et al*., 2006).

Other properties

The presence of a biologically related CGRP molecule in sardine hydrolysates has been reported (Fouchereau-Peron *et al.,*1999). Cholecystokinin (CCK)-like peptides and a calcitonin gene-related peptide (CGRP) respectively were detected in Alcalase(R) hydrolysates of cooked sardine wastes (Ravallec-Plé *et al.,* 2001, Rousseau *et al.*, 2001) and measured in cod muscle and shrimp head extracts and alcalase hydrolysates (Fouchereau-Peron *et al.*, 1999, Ravallec-Plé and Wormhoudt, 2003). Cholecystokinin is a peptide hormone that reduces gastric acid secretion and stimulates the intestinal digestion and absorption of fat and protein, and controls satiety/appetite. The calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide with widespread effects such as in the heart, blood vessels, pituitary, thyroid, lung and gastrointestinal tract, where it decreases food intake by expressing gastric acid secretion.

Calming or Diazepam-like effects of cod protein hydrolysate on stress responsiveness in rats have been reported (Bernet *et al.,* 2002). It has also been demonstrated that feeding cod protein hydrolysate prevents obesity-induced muscle insulin resistance in high-fat-fed obese rats (Lavigne *et al.,* 2001).

Fermentation

Traditional fermentation methods can also produce peptides from food proteins for example fish sauce and dry-cured fermented meat products are good examples. Fermentation is also used to produce functional dairy products with ACE inhibitory peptides (Chen *et al.,* 2007; Gobbetti *et al.,* 2000; Nakamura *et al.,* 1995; Yakamoto *et al.,*1999). ACE inhibitory effects have been demonstrated in surimi fermented with lactic acid bacteria (Shan *et al.,* 2007). Hydrolysed and fermented minced mackerel has been shown to have antioxidative activity (Yin *et al.,* 2005). Recent results from research on dry-cured ham indicate that dipeptides with a strong ACE inhibitory activity can be produced during meat ageing (Sentandreu and Toldra, 2007).

Fermented fish sauce is popular in South-East Asia. It is developed microbiologically 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). 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).

Functional properties

Functional properties of proteins and peptide blends include solubility, water-binding capacity, gelation and emulsifying capacity. They are important if the products are to be used in food formulations (Figure 5).

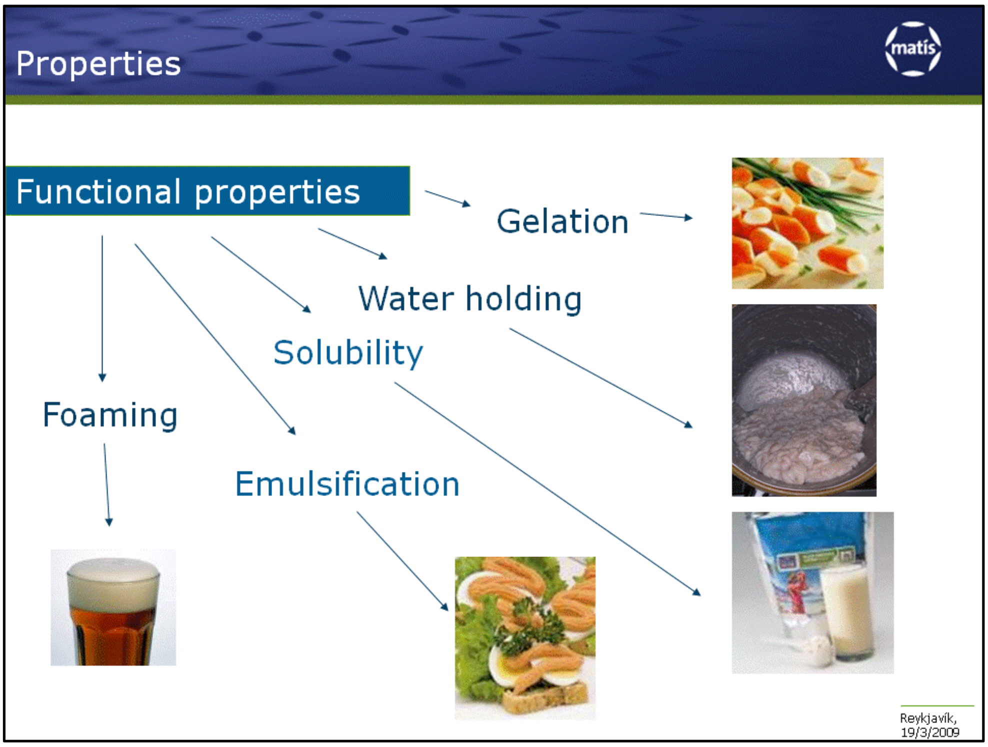

Figure 5. Functional properties of fish proteins and peptides

The source, environmental, production and processing factors influence functional properties of proteins and peptides. The source can be any of the species and stocks of fish and other marine organisms utilized for human consumption. Production and processing parameters are isolation, precipitation, drying or dehydration, concentration, enzymatic or chemical modification and environmental parameters include temperature, pH and ionic strength (Kinsella,1976).
Enzymatic hydrolysis improves solubility but reduces emulsifying properties, water holding is still rather good (Kristinsson and Rasco,2002; Slyzyte et al.,2005). Heating that is used in stopping enzyme reaction and in drying may reduce functional properties and nutritional properties and sensory quality factors. Spray and freeze dried products have better functional properties than drum dried products. High

solubility over a wide range of pH is important for many food applications as it also influences emulsifying and foaming properties. Intact fish myofibrillar proteins are quite insoluble in water over a wide pH range. Smaller peptides produced by hydrolysis can bind more easily to water than the intact protein can (Kristinsson and Rasco, 2000). Good solubility of fish protein hydrolysates over a wide range of pH that increases with degree of hydrolysis has been reported many times (Gbogouri *et al*., 2004; Geirsdottir *et al*., 2007; Klompong *et al.,* 2007; Sathivel and Bechtel, 2006; Sathvivel *et al.,* 2005; Shahidi *etal.,*1995; Thiansilakul *et al.,* 2007).

Sensory properties

Taste and odour of fish protein hydrolysates must be acceptable to the targeted consumers if the products with the bioactive peptides are to be successful on the market.

The sensory properties depend both on the raw material, the kind of protease applied and the hydrolytic conditions. High quality fish sauce has a delicious flavour (Thongthai and Gildberg,2005), but bitter taste is a problem with many fish protein hydrolysates (Kristinsson and Rasco,2002). The problem may be solved either by mild hydrolysis to reduce production of medium size peptides or by extensive hydrolysis to digest the troublesome peptides to free amino acids. But mild hydrolysis normally reduces the yield significantly. Extensive digestion is probably more practical. Figure 7 summarizes the quality challenges the marine protein/peptide companies are facing.

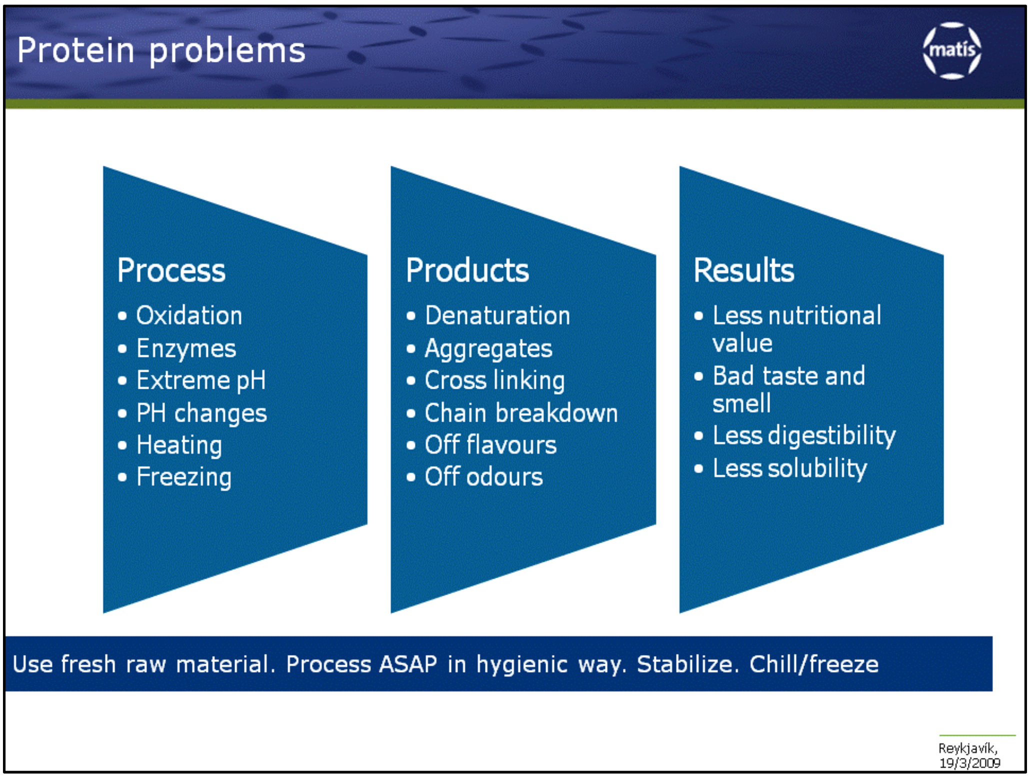

Figure 7. Quality challenges in the production of marine proteins and peptides

Although the future is exciting and bright for specialty ingredients based on fish proteins, there are still some significant issues we need to overcome to truly produce superior bioactive ingredients which can be competitive on the market. Oxidation, enzymatic processes, heating, reactions with lipid oxidation products, changes in pH and freezing can lead to adverse protein changes such as denaturation, formation of aggregates, cross linking, protein radicals and further uncontrolled hydrolysis. These changes can lead to reduced nutritional value due to changes in amino acids or reduced digestibility. They can also lead to negative changes in colour, solubility and other functional properties. The rates of most of these changes are highly temperature dependent and chilling and freezing reduce the reaction rate. Operating at slightly alkaline pH also helps to minimize these problems. The best way by far to slow or prevent problems to use fresh raw material and process them as soon as possible in the best hygienic manner. The task is to find suitable processing techniques and methods to stabilize the residual lipids associated with the products (Bragadottir et al.,2007; Geirsdottir, 2006). Mild processing conditions during hydrolysis and drying and lean raw material have been suggested to minimize lipid oxidation (Kristinsson and Rasco,2002). Oxidation is influenced by storage time and temperature. Other means include adding antioxidant before hydrolysis and by selecting enzymes that do not operate at low pH and high temperatures (Kristinsson,2006).

Fish protein and peptide products on the market

The soy and dairy industry dominate the ingredient market for proteins and peptides, but marine protein and peptides market is still very small. Seafood flavours are the traditional products. Annual production of fish collagen/gelatine accounts for only 1-2% of the global collagen/gelatine production. The market for functional foods and food supplements is growing very fast. The number of collagen based products is growing very fast. The number of collagen health foods and supplement products in Japan is remarkable (Functional Foods. Japan 2006). In Europe and the United States they are also sold as food supplements and to the cosmetic industry. Fish protein and peptide products with approved health claims don't exist in Europe and North America. There they are sold as food supplements.

Figure 6. Examples of fish protein hydrolysates on the food supplement marke

Figure 6 shows examples of commercial products containing fish protein hydrolysates. Two products have been approved by Japanese authorities. One is the Katsuobushi oligopeptide made by hydrolysis of dried bonito. It is marketed as PEPIDE ACE 3000 in Japan. 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. 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 Norway for reducing blood sugar.

Conclusions and recommendations

From a scientific point of view it has been concluded that despite difficulties in excluding the influence of LC n-3 PUFA and uncertainty whether effects from ingested peptides (i.e. hydrolysates) differ from those that arise from peptides formed in the gastrointestinal (GI)-tract after ingesting intact proteins, that seafood diet and seafood derived proteins, peptides and amino acids are all strong candidates as carriers of health effects. From an industrial point fish protein hydrolysates will continue to be produced and sold as flavours. The market based on special bioactive properties of fish protein hydrolysates is small and in the emerging phase of development. It has been forecasted to grow annually about 8-12% until the year 2012. There are also opportunities in adapting traditional food processes like fermentation to increase the bioactive properties of fish protein hydrolysates and to employ them into product that consumers already know. Low salt fish sauce and fish flavours with tailor-made bioactive properties are also likely products in the future. Figure 8 demonstrates possible future use of fish ingredients in traditional "functional" foods.

Figure 8. Examples of traditional food with "bioactive" ingredients from marine sources

These are classical food products with added fish calcium, fish collagen and fish protein hydrolysates to deliver bioactivity to the products. This is well known for other protein sources, but new for fish proteins. In these products the functional properties of the products are not derived from these fish peptide ingredients but traditional ingredients and processes used in making thes products.
This photo is from the company Copalis in France and are of products that have been tested in the pilot plant with their ingredients.
The success of marketing of fish protein and peptide ingredients as functional foods or health food supplements depends, besides supplying substantial scientific evedence, on the taste and odour of the products. Good sensory quality of the products depends on simple and easily understandable critera like fresh raw material, good handling and short processing time as well as on gaining more knowledge through research on the complicated ineractions between process and environmental parameters in order to control oxidation and formation of bitter taste.

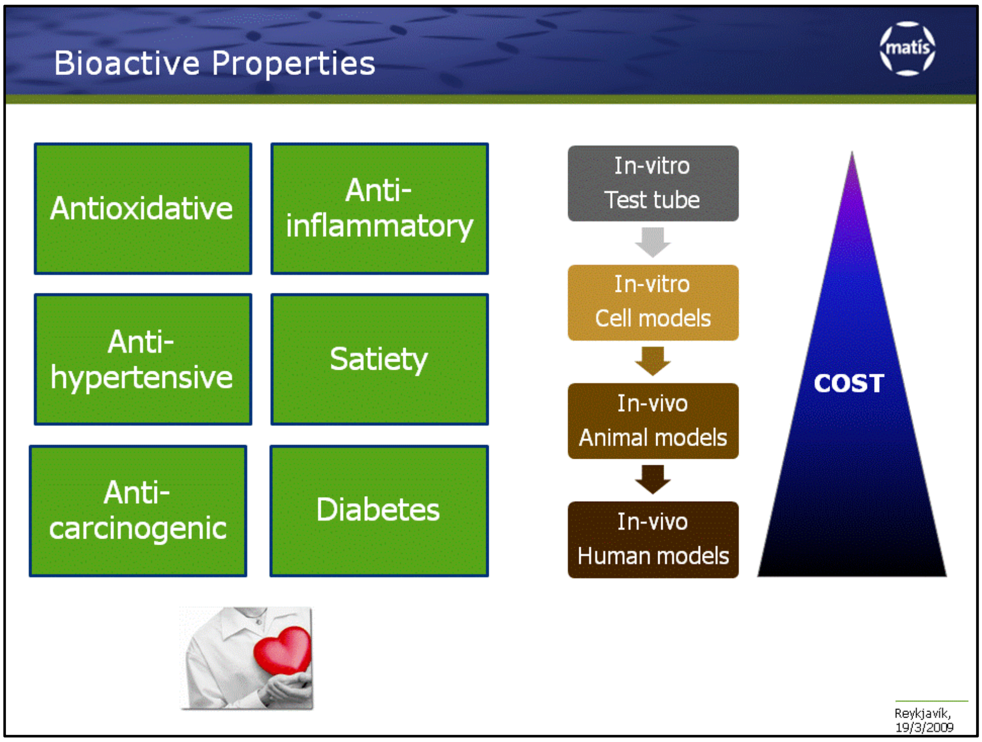

Figure 9. Ways of testing health effects of marine proteins and peptides

Recommendations for future scientific activities are demonstrated in figure 9 and include:

- To establish the positive effects of fish protein consumption in future research in both animal models of disease and in human clinical trials. It will permit identification of the bioactive components of fish protein. This, in turn, will make the benefits of fish consumption available to large numbers of people who have limited opportunities to (or do not chose to) include fish as part of their normal diet by using fish derived ingredients as functional food components.

- Further investigation of new marine protein/peptide sources

- Human intervention studies to verify if interesting results obtained by animal experiments or by *in vitro* experiments that may provide useful medical achievements.

- Search for other valuable bioactivities from peptides, still undetected, that will also be an important part of future research within this field.

Producing an ingredient with good functional properties is good, but there is tremendous competiton out there in that market. Producers of fish protein and peptide based ingredients need to do more to carve out a niche in the bioactive arena. We already have lots of in-vitro experimental evidence that fish based peptides have very high bioactivity, higher than many other proteins. We have some *in-vivo* evidence as well, but not nearly enough. Now we have to move more and more towards *in-vivo* animal and eventually human studies. The fact that most companies in this area are small makes this is not an easy task as the costs can be very high for those type of trials. Therefore, it is critical we all, industry, academia and institutions join forces and help move this industry to where it needs to be and divert much of our resources into in-vivo testing so we can document and validate health claims for these products.

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