Atlantic Biotechnology

DTU Food
National Food Institute

DTU

Report from workshop on

Bioactive peptides from aquatic raw materials

Copenhagen, 2 March 2010

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Edited by

Lisa Lystbæk Andersen
Henrik Hauch Nielsen
Flemming Jessen

National Food Institute
Technical University of Denmark

Bioactive peptides from aquatic raw materials

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National Food Institute
Technical University of Denmark
Mørkhøj Bygade 19
DK - 2860 Søborg

T: +45 72 34 70 00
F: +45 72 34 70 01

Content

Preface

The open workshop "Bioactive Peptides from Aquatic Raw Materials" took place in Copenhagen on 2 March 2010 at Ingeniørforeningens (IDA) Mødecenter. The workshop was arranged by Flemming Jessen (project leader), National Food Institute, Technical University of Denmark (DTU), in cooperation with Gestur Hovgaard, director of the marine biotech company Atlantic Biotechnology S/P, Faroe Islands. Other project participants: Hordur G. Kristinsson (Matís, Reykjavik, Island), Turid Rustad (Norwegian University of Science and Technology (NTNU), Trondheim, Norway), Ingmar Høgøy (Seagarden ASA, Haugesund, Norway), Jan Stagsted (University of Aarhus, Denmark), Hóraldur Joensen (University of Faroe Islands, Tórshavn, Faroe Islands), Eddy G. Torp (DueMiljø AS, Oslo, Norway), Frank Hansen (Hansen-Øye Consult, Sortland, Norway).

This report consists of a synopsis of the presentations at the workshop with the programme and the slides accompanying each presentation presented in Appendix 1-12.

The workshop was supported by the **Working Group for Fisheries Cooperation (AG-Fisk), Nordic Council of Ministers for Fisheries and Aquaculture, Agriculture, Food and Forestry**.

March 2011

Summary

A workshop was held in Copenhagen in March 2010 addressing issues of documentation of bioactivity and the commercial utilisation of bioactive peptides and other compounds derived from aquatic raw materials. Ongoing research activities and commercial initiatives were presented by representatives from both research institutions and Nordic companies specialising in developing products containing bioactive peptides.

In conclusion of the workshop, three areas where further research is needed to fully document the bioactive effect of marine peptides were identified. These were: 1) Studies on the uptake and stability of bioactive peptides in the intestinal system, 2) the implementation of more extensive human trials to confirm positive results from *in vitro* and animal studies, and finally, 3) more research is needed to determine whether the observed effect of marine peptides on the metabolic syndrome is a general peptide effect or directly attributable to specific peptides found only in fish or other aquatic materials. In general, more studies are needed to explore the sources, bioavailabilities, and the physiological/functional properties and the mechanisms of action of bioactive peptides.

Workshop synopsis

Introduction

The aim of the workshop was to generate an overview of the current state of the art of research and development within the field of utilising bioactive peptides and other compounds of marine origin for health beneficial purposes. The ambition was furthermore to provide a knowledge platform to facilitate Nordic cooperation and research coordination between industry and research institutions through the establishment of new collaborations and joint applications for funding under national as well as EU auspices.

The aquatic ecosystem represents an as of yet not fully explored resource of biodiversity with a huge variety of organisms adapted for living conditions very different from terrestrial animals. For example, fish generally have an eminent bioactive defence system for protection against the high amounts of potentially harmful bacteria in the water. In consequence, fish and other inhabitants of the aquatic ecosystem expectedly possess a great number of biocomponents of both nutritional and pharmaceutical value, in line with what has been found in the rain forest.

During industrial processing of fish for human consumption only about half of the total fish weight is converted to edible products. The remaining part, the secondary products, is mainly used for animal feed products. Internationally, there is increasing documentation of the fact that fish contain large amounts of bioactive peptides. These bioactive peptides often have beneficial effects in respect to both human and animal health, as for example strengthened immune response, better blood pressure regulation, inhibition of cancer cell proliferation, reduction of obesity, diabetes, and antibacterial activity. Products with bioactive peptides from fish have a potential usage in healthy foods, as nutritional supplements, as ingredients, as alternatives to antibiotic growth promoters in animal feeds, and as pharmaceutical products.

Despite many years of focus on better exploitation of the secondary marine products, there is still a great need for product and market development. The access to ever more advanced technologies and research-based knowledge increasingly enables the potentials of the marine raw materials to be developed into specific innovations. New applications within the area of "bioactive peptides" are developed in a complex interplay between industries, technologies, research, and markets. This workshop has its main focus on the "niche" that bioactive peptides represent within the broad spectrum of marine ingredients.

11 presentations were given by invited speakers from both academic research institutions and Nordic companies developing health promoting products.

Health aspects

Biologically active peptides released from fish proteins, either during digestion or during food processing, may have crucial influences on the regulation and modulation of the human metabolism. Thus such peptides are potential nutraceuticals providing health benefits and prevention or treatment of diseases. Many studies have reported that fish peptides have effects on important

health aspects including blood pressure, obesity, diabetes, cancer, coronary vascular disease, inflammation, and immunological diseases (Hordur G. Kristinsson, Appendix 8, page 85).

Central to many studies on the health effects of peptides are *in vitro* investigations of the mechanisms known to be involved. Antihypertensive peptides are mainly found and studied by their inhibitory effect on angiotensin-converting enzyme (ACE). Apart from inhibiting ACE, the ACE inhibitors have the ability to influence the metabolic syndrome as they reduce fat mass in adipose tissue (Stéphanie Bordenave-Juchereau, Appendix 2, page 23). In relation to obesity the bioactive effects are registered as reduced fat depositing, probably due to a regulation of the adipocyte life cycle (Stéphanie Bordenave-Juchereau, Appendix 2, page 23), but also as reduced liver lipid and reduced adipose tissue mass (Bjørn Liaset, Appendix 3, page 31). The mechanisms of action of bioactive peptides influencing diabetes seem to be both by a reduced postprandial blood glucose response and by effects on the insulin response (Einar Lied, Appendix 4, page 43). Antimicrobial peptides from fish show direct effects on microbes and they are also potentially able to enhance the immune function (Jan Stagsted, Appendix 6, page 67). To document anticancerous effects the peptides must possess dual effects and induce programmed cell death (apoptosis) as well as inhibition of cell proliferation (Flemming Jessen, Appendix 12, page 143).

Industrial perspectives

Marine peptide hydrolysates are expected to have a great potential as supplements in a number of future products. Functional foods such as sports drinks, pet food, dietary supplements, but also cosmetics are seen as some of the most promising areas where, today already, there is a large and growing market. This is promoted by the increasing consumer awareness that a number of diseases (both concerning humans and animals) may be avoided by a healthier lifestyle, among this an intake of foods containing health promoting ingredients such as bioactive peptides (Hordur G. Kristinsson, Appendix 8, page 85).

Today already, there are a number of commercial marine peptide products with claimed bioactive properties such as activity against hypertension, intestinal diseases, stress, and insulin resistance on the market (Hordur G. Kristinsson (Appendix 8, page 85), Einar Lied (Appendix 4, page 43)).

A number of Nordic companies producing marine based hydrolysates have been established within the recent years, e.g. NutriMarine Life Science AS from Norway (Einar Lied, Appendix 4, page 43) and Atlantic Biotechnology S/P, Faroe Islands. Already established companies such as Seagarden ASA, Norway, have extended their production to also include marine hydrolysates/peptides (Bjarte Langhelle, Appendix 10, page 115).

One of the major challenges for existing and future commercial peptide products is valid documentation of the functional and health promoting properties. Presentations at the workshop showed that a considerable number of research studies are taking place in collaboration with private companies to document the bioactivity of the products. The company NutriMarine Life Science AS has tested a protein hydrolysate, NutriPeptin, in a human trial investigating blood glucose concentration and insulin sensitivity (Einar Lied, Appendix 4, page 43). The Norwegian company Seagarden ASA has collaborated with International Research Institute of Stavanger (IRIS), Norway, on identifying peptide sequences with known stimulatory effects on the gastro-

intestinal system in peptide products (Anne Hjelle, Appendix 11, page 121). Similarly, protein hydrolysates from the Danish company Marinova ApS, which produces ingredients and foods from fish protein, are tested in collaboration with the National Food Institute, DTU, and University of Copenhagen, Denmark, for anticarcinogen and antioxidative properties (Flemming Jessen, Appendix 12, page 143).

Research activities

Bioactive peptides can be found as naturally present in e.g. the mucus of fish, where they may function as an outer defence against bacteria. However, bioactive peptides are potentially present in any protein as part of the protein sequence and are only released in their functional forms upon protein degradation, e.g. during digestion. These hidden peptides or "cryptides" are usually small containing 2-20 amino acids and have functions distinct from the parent protein ("Cryptein", Stéphanie Bordenave-Juchereau, Appendix 2, page 23). It has been found that some of the bioactive peptides are multifunctional; i.e. they may possess both ACE inhibitory activity and influence the proliferation and differentiation of fat cells (Stéphanie Bordenave-Juchereau, Appendix 2, page 23). In this way, intake of marine peptides may be able to help prevent the development of the human disease known as "Metabolic syndrome", which involves a number of cardiovascular risk factors such as obesity and hypertension.

The general focus in research, as well as on industrial scale, is on producing protein hydrolysates from fish or algae using either fermentation or for the greater part enzymatic hydrolysis using food grade proteases. Elucidating the functional mechanisms behind the observed health effects of e.g. fish protein hydrolysates is not always the immediate objective as the raw hydrolysates may display health beneficial effects in themselves. The Norwegian company Nutri-Marine Life Science AS (Einar Lied, Appendix 4, page 43) has developed a very promising fish hydrolysate product from saithe fillet (NutriPeptin). The positive health effects of NutriPeptin are supported by two clinical trials. NutriPeptin is suitable for application in various foods.

Much of the health effects associated with intake of fish can be attributed to the presence of n-3 fatty acids. However, fish peptides, minerals/trace elements, vitamins, and amino acids, and not least the in seafood abundant free sulfonated organic acid taurine, which has importance for energy metabolism, may also be important contributors (Edel O. Elvevoll, Appendix 5, page 53). Various seafood items display antioxidative capacity during simulated digestion experiments. Human clinical trials with combined n-3 fatty acids and taurine supplementation resulted in reduced total cholesterol and LDL-cholesterol in the blood. Similarly, saithe fish protein hydrolysate (FPH), which contains high amounts of taurine, has been shown to reduce visceral adipose tissue in rats (Bjørn Liaset, Appendix 3, page 31). However, taurine does not seem to be solely responsible for the observed effects and, furthermore, it remains to be tested if the positive effect of FPH is specific due to its marine origin or whether protein sources from other species display the same properties.

Three fish hydrolysates produced by Seagarden ASA (Bjarte Langhelle, Appendix 10, page 115) were subjected to fractionation, liquid chromatography purification, and mass spectrometry with the aim of identifying and categorising as many bioactive peptides as possible based on known bioactive peptide sequences (Anne Hjelle, Appendix 11, page 121). Focus was put on identifying bioactive peptides with respect to gastrointestinal diseases specifically and all three

hydrolysates did to varying degrees contain bioactive peptides known to stimulate the gastrointestinal system. The PEPFISH project (Flemming Jessen, Appendix 12, page 143) has focus on identifying and characterising bioactive peptides from enzymatic hydrolysates from underutilised or low-value materials from fish processing. The hydrolysates will be tested *in vitro* for effects in relation to cancer cell proliferation and apoptosis, cancer cell migration, blood pressure regulation (ACE inhibition), immunological responses, and antibacterial activity.

Fish peptides both from laboratory scale and commercial hydrolysates display antioxidative properties in different oxidative systems (Turid Rustad, Appendix 7, page 77). This property is potentially useful for the food industry as addition of fish peptides can help avoid problems with oxidation and hence rancidity in products containing unsaturated oils. Furthermore, the fish protein hydrolysates have also been found to contain calcitonin gene-related peptide (CGRP)-like and Gastrin/Cholecystokinin (G/CCK)-like molecules. CGRP is a potent arterial and venous vasodilator involved in the control of hypertension. Gastrin and cholecystokinin are hormonal regulators of various digestive processes and feeding behaviours. Interestingly, the amount of CGRP-like molecules was highest when fresh fish material was used for hydrolysis.

A potentially enormous value addition is to be expected if peptides with documented health promoting or other effects can be derived from low-value fish material (Hordur G. Kristinsson, Appendix 8, page 85). However, most claimed bioactive effects still need to be verified. Also, a problem exists with consumer acceptance of products with added fish hydrolysates due to problems with lipid oxidation and subsequent rancid tastes and flavours. Some of these problems can be reduced by using hydrolysates prepared from protein isolates of homogenized fish materials, which contain lower amounts of lipids and pro-oxidants.

A large number of naturally occurring bioactive peptides, such as e.g. piscidines, have also been identified. The Nanofish project (Jan Stagsted, Appendix 6, page 67) has focus on isolating naturally occurring antimicrobial and immunostimulating peptides from fish. The Nanofish project has developed so-called nanoparticles containing antimicrobial fish peptides coated with a protective layer of chitosan or alginate. The nanoparticles facilitate protection of the peptides from degradation in the upper digestive tract and safe release in the lower digestive tract.

As a whole other aspect of bioactive compounds derived from fish, a variety of enzymes has been described by the Norwegian company Nofima AS (Inge W. Nilsen, Appendix 9, page 103). These include the well-known shrimp alkaline phosphatase and a heat labile shrimp double-strand specific DNase. Inhibitors of HIV-protease have been identified in marine invertebrates with very interesting pharmaceutical prospects. Currently, salmon lysozymes with antibacterial effects are being characterised.

Future challenges/research areas

The presentations and discussions at the workshop identified three main areas where further research is neccesary in order to increase the knowledge on bioactivity of marine peptides and how they can be commercialized:

1) Research on uptake of bioactive peptides from the gut and how bioactivity is influenced by variations in pH, digestive enzymes, and transport through the intestinal membrane
2) More extensive controlled human trials are needed to confirm results obtained in *in vitro* and animal experiments

3) There is a need to determine whether the positive effect of marine peptides on the metabolic syndrome is a general peptide effect or due to specific peptides, and whether the observed effect is dependent on a synergistic interplay of several peptides. In this connection, it should also be determined what role other marine components present in marine hydrolysates such as the organic acid taurine has on bioactivity

In conclusion, more research is necessary to obtain the valid and reliable documentation of bioactivity that is needed in order to introduce marine peptide products into the market and to obtain possible health claims. In general, more studies are needed exploring the sources, bioavailabilities, and possible physiological/functional properties and the mechanisms of action of bioactive peptides.

Sammenfatning

En workshop blev afholdt i København i marts 2010 omhandlende dokumentation af bioaktivitet og kommerciel udnyttelse af bioaktive peptider og andre forbindelser fra akvatiske råvarer. Igangværende forskningsaktiviteter og kommercielle initiativer blev præsenteret af repræsentanter fra både forskningsinstitutioner og nordiske virksomheder med speciale i at udvikle produkter, der indeholder bioaktive peptider.

Tre områder hvor yderligere forskning er nødvendig for fuldt ud at dokumentere den bioaktive effekt af marine peptider blev afslutningsvis identificeret på workshoppen. Disse var: 1) Undersøgelser af optagelsen og stabiliteten af bioaktive peptider i mave-tarmsystemet, 2) gennemførelse af mere omfattende humane studier der kan bekræfte de positive resultater fra *in vitro*- og dyreforsøg, og endelig 3) er der behov for mere forskning for at afgøre, hvorvidt den observerede effekt af marine peptider på det metaboliske syndrom er en generel peptid effekt, eller direkte kan henføres til specifikke peptider der kun findes i fisk og andre akvatiske råmaterialer. Generelt er flere undersøgelser nødvendige for at udforske resourcerne, biotilgængeligheden, og de fysiologiske/funktionelle egenskaber og virkningsmekanismer af bioaktive peptider.

Appendices

Appendix 1. Programme

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BIOACTIVE PEPTIDES FROM AQUATIC RAW MATERIALS

Workshop, 2 March 2010, 10:00 a.m. - 17:00 p.m.

Venue: Ingeniørforeningens Mødecenter, Kalvebod Brygge 31-33, DK-1780 København V

9:00 - 10:00	Arrival
10:00 - 10:10	Welcome by **Henrik Hauch Nielsen** (Senior scientist, National Food Institute, Technical University of Denmark)
10:10 - 10:30	**Rolf K. Berge** (Professor, Institute of Medicine, University of Bergen, Norway) "Improved health through novel peptides of marine origin"
10:30 - 10:50	**Stéphanie Bordenave-Juchereau** (Senior scientist, University of La Rochelle, France) "Marine cryptides as a tool to fight the metabolic syndrome"
10:50 - 11:10	**Bjørn Liaset** (Scientist, National Institute of Nutrition and Seafood Research (NIFES), Norway) "Fish protein hydrolysate reduces visceral adipose tissue mass in rats"
11:10 - 11:40	Coffee break
11:40 - 12:00	**Einar Lied** (Managing director/Professor, NutriMarine Life Science AS, Norway) "Marine peptides; a tool of blood glucose lowering and stabilisation"
12:00 - 12:20	**Edel Elvevoll** (Dean, Faculty of Biosciences, Fisheries and Economics, University of Tromsö, Norway) "Seafood and health - more than n-3 fatty acids"
12:20 - 12:40	**Jan Stagsted** (Senior scientist, Department of Food Science, Aarhus Universitet, Denmark) "Nanofish – utilization of natural fish antimicrobial peptides as nanoparticles?"
12:40 - 13:40	Lunch
13:40 - 14:00	**Turid Rustad** (Professor, Department of Biotechnology, Norwegian University of Science and Technology (NTNU), Norway) "Antioxidative and bioactive activities of fish protein hydrolysates"
14:00 - 14:20	**Hordur G. Kristinsson** (Head of division, Matís, Iceland) "Production, quality and bioactivity of fish derived peptides"
14:20 - 14:40	**Inge W. Nilsen** (Senior scientist, Nofima AS, Norway) "Marine enzymes and enzyme inhibitors"

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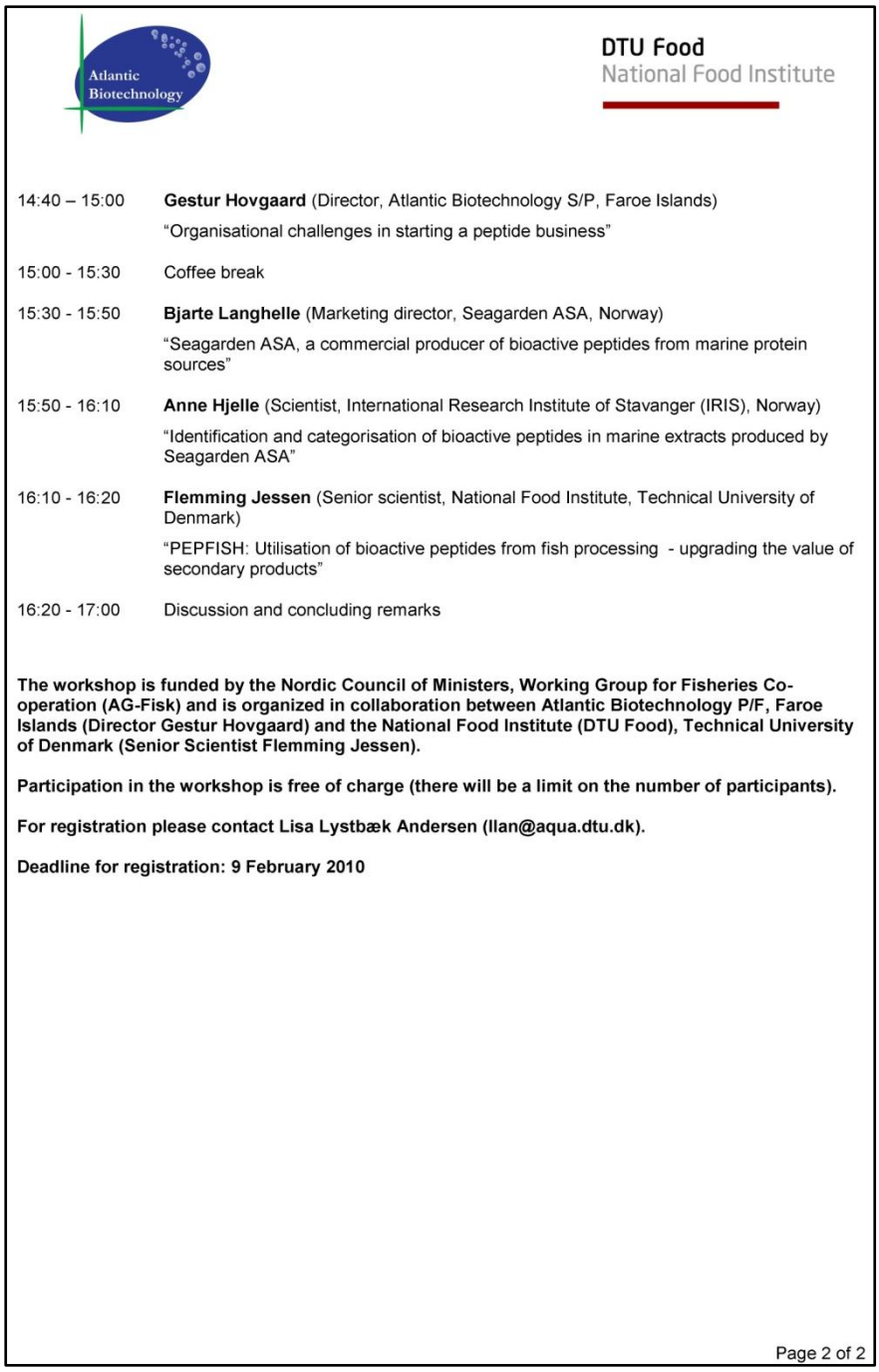

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14:40 – 15:00	**Gestur Hovgaard** (Director, Atlantic Biotechnology S/P, Faroe Islands) "Organisational challenges in starting a peptide business"
15:00 - 15:30	Coffee break
15:30 - 15:50	**Bjarte Langhelle** (Marketing director, Seagarden ASA, Norway) "Seagarden ASA, a commercial producer of bioactive peptides from marine protein sources"
15:50 - 16:10	**Anne Hjelle** (Scientist, International Research Institute of Stavanger (IRIS), Norway) "Identification and categorisation of bioactive peptides in marine extracts produced by Seagarden ASA"
16:10 - 16:20	**Flemming Jessen** (Senior scientist, National Food Institute, Technical University of Denmark) "PEPFISH: Utilisation of bioactive peptides from fish processing - upgrading the value of secondary products"
16:20 - 17:00	Discussion and concluding remarks

The workshop is funded by the Nordic Council of Ministers, Working Group for Fisheries Co-operation (AG-Fisk) and is organized in collaboration between Atlantic Biotechnology P/F, Faroe Islands (Director Gestur Hovgaard) and the National Food Institute (DTU Food), Technical University of Denmark (Senior Scientist Flemming Jessen).

Participation in the workshop is free of charge (there will be a limit on the number of participants).

For registration please contact Lisa Lystbæk Andersen (llan@aqua.dtu.dk).

Deadline for registration: 9 February 2010

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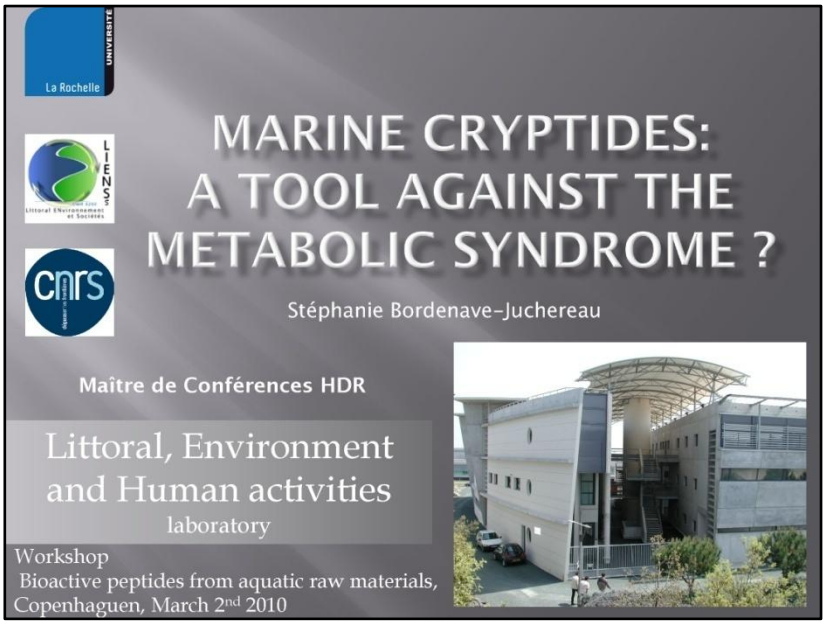
Note:
Rolf K. Berge and Gestur Hovgaard were unable to give their presentations at the workshop.

Appendix 2. ”Marine cryptides as a tool to fight the metabolic syndrome” by Stéphanie Bordenave-Juchereau

1



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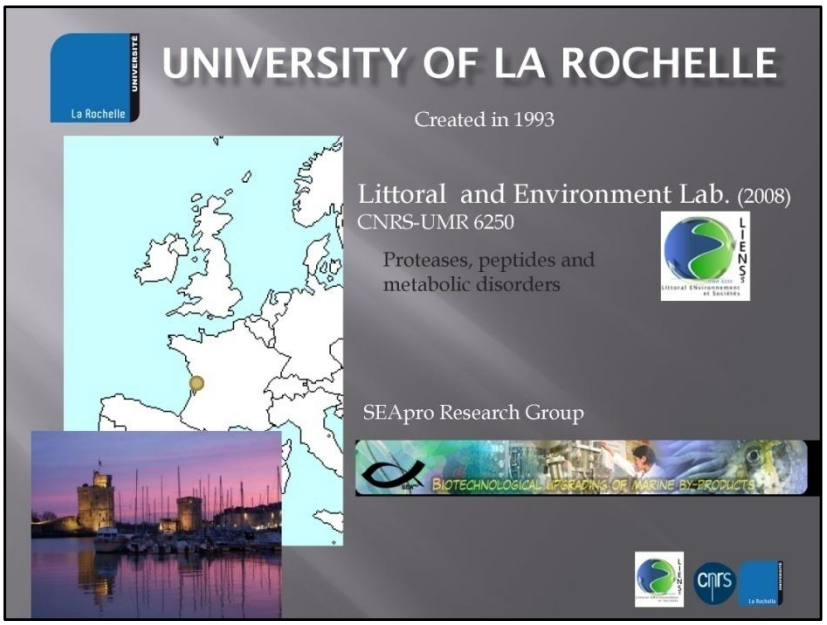

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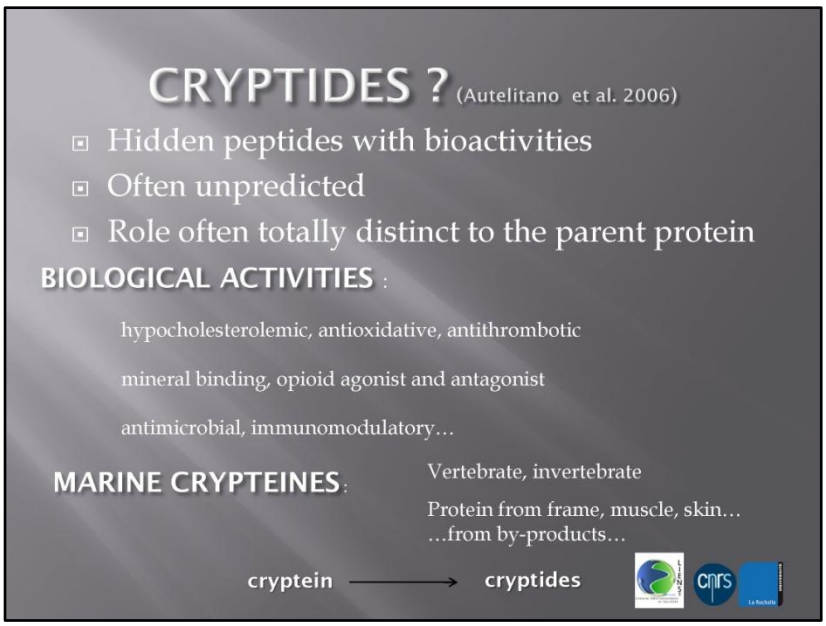
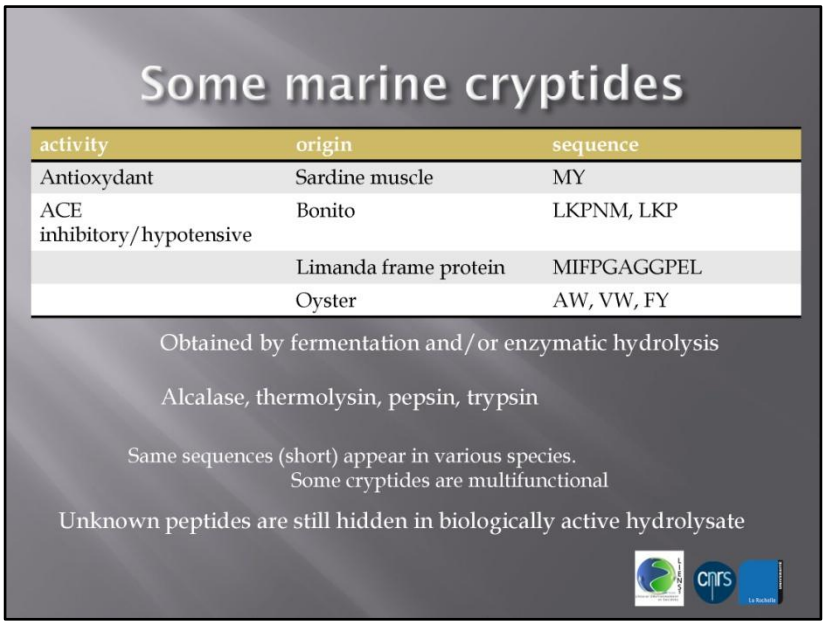
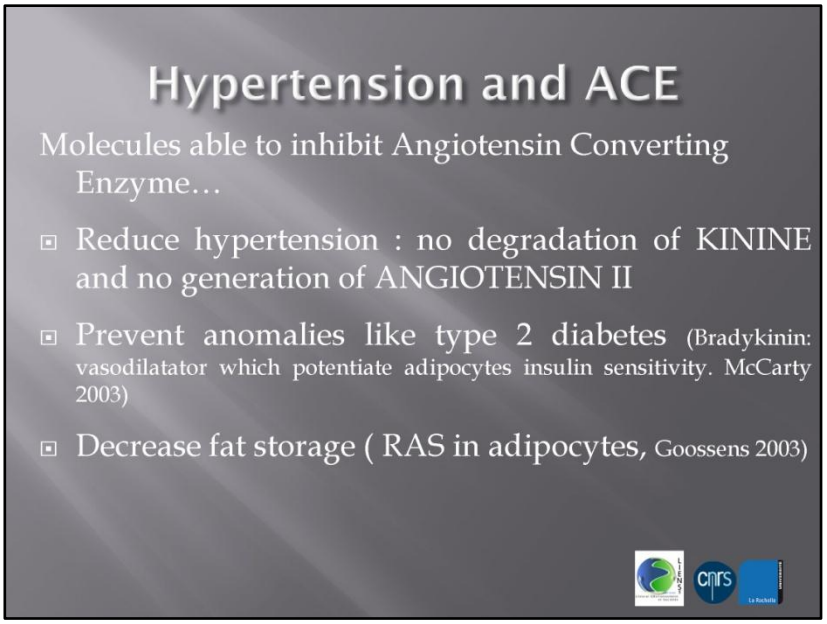

CRYPTIDES ? (Autelitano et al. 2006)
Hidden peptides with bioactivities
Often unpredicted
Role often totally distinct to the parent protein
BIOLOGICAL ACTIVITIES :
hypocholesterolemic, antioxidative, antithrombotic
mineral binding, opioid agonist and antagonist
antimicrobial, immunomodulatory…
MARINE CRYPTEINES :
Vertebrate, invertebrate
Protein from frame, muscle, skin…
…from by-products…
cryptein
cryptides

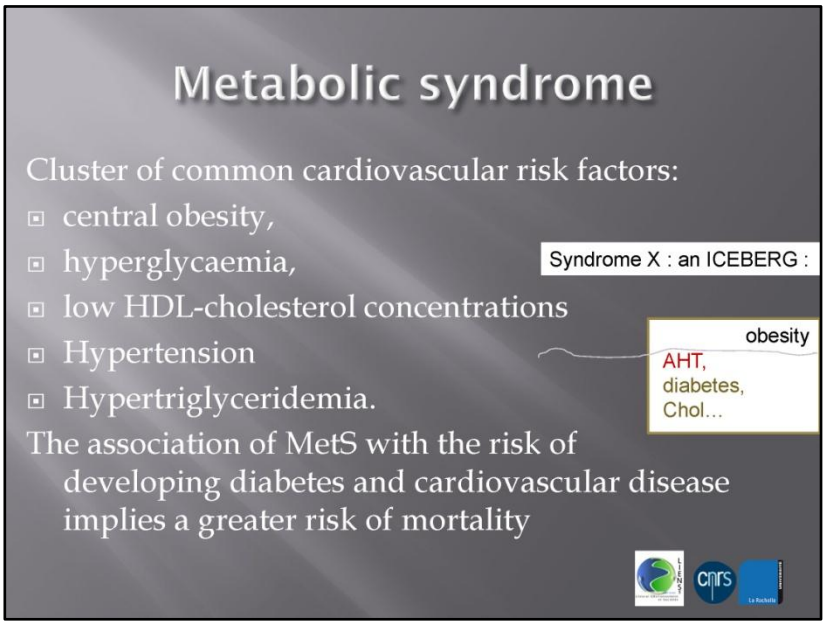
4


Some marine cryptides
activity | origin | sequence
Antioxydant | Sardine muscle | MY
ACE inhibitory/hypotensive | Bonito | LKPNM, LKP
Limanda frame protein | MIFPGAGGPEL
Oyster | AW, VW, FY
Obtained by fermentation and/or enzymatic hydrolysis
Alcalase, thermolysin, pepsin, trypsin
Same sequences (short) appear in various species.
Some cryptides are multifunctional
Unknown peptides are still hidden in biologically active hydrolysate

5


Hypertension and ACE
Molecules able to inhibit Angiotensin Converting Enzyme…
Reduce hypertension : no degradation of KININE and no generation of ANGIOTENSIN II
Prevent anomalies like type 2 diabetes (Bradykinin: vasodilatator which potentiate adipocytes insulin sensitivity. McCarty 2003)
Decrease fat storage (RAS in adipocytes, Goossens 2003)

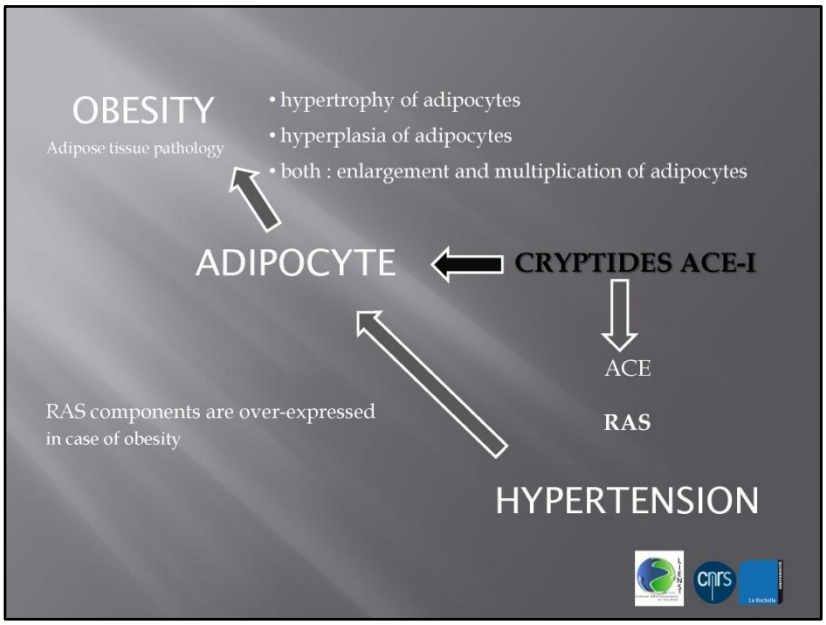
6


Metabolic syndrome
Cluster of common cardiovascular risk factors:
central obesity,
hyperglycaemia,
low HDL-cholesterol concentrations
Hypertension
Hypertriglyceridemia.
Syndrome X : an ICEBERG :
obesity
AHT,
diabetes,
Chol…
The association of MetS with the risk of developing diabetes and cardiovascular disease implies a greater risk of mortality

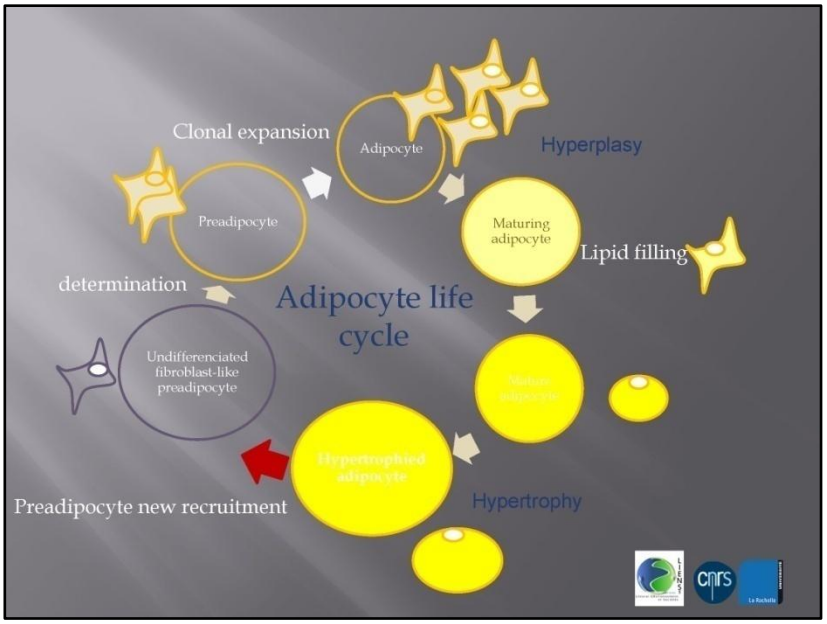
7


Obesity
Imbalance between energy intake and expenditure leading to excessive body fat deposition.
Energy expends
Energy intakes
Metabolism sport
Food
Food
OBESITY : fat tissue pathology
With physiological, psychological and social consequences

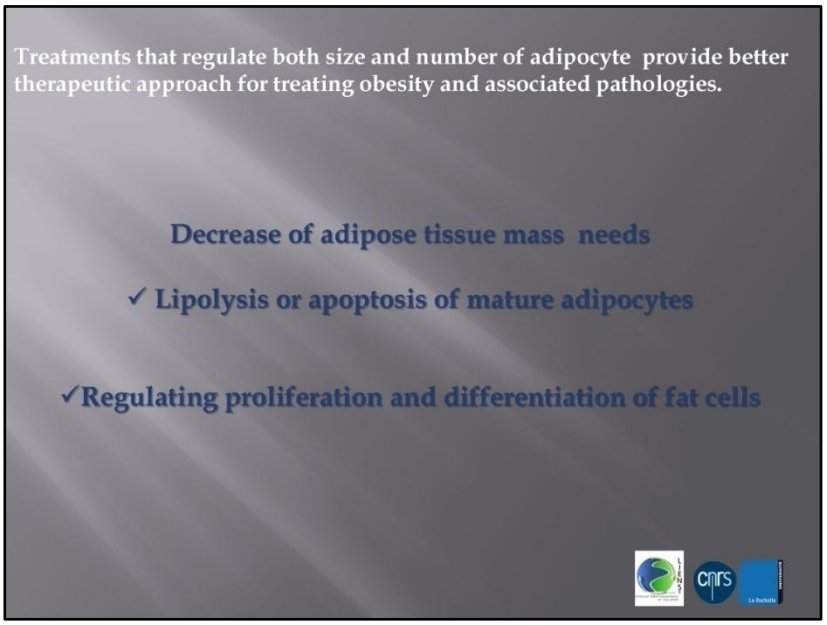
8


OBESITY
Adipose tissue pathology
• hypertrophy of adipocytes
• hyperplasia of adipocytes
• both : enlargement and multiplication of adipocytes
ADIPOCYTE
CRYPTIDES ACE-I
ACE
RAS
RAS components are over-expressed in case of obesity
HYPERTENSION

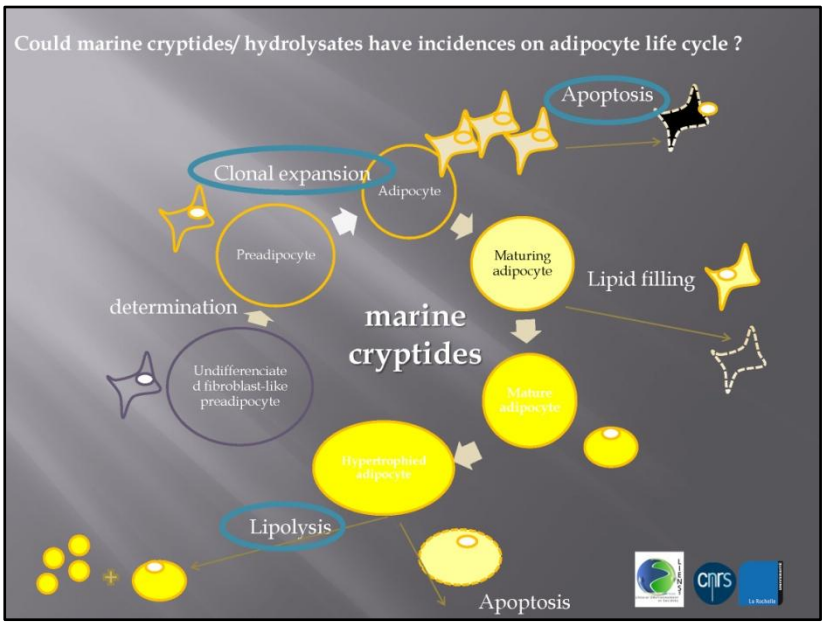
9


Clonal expansion
Adipocyte
Hyperplasy
Preadipocyte
Maturing adipocyte
Lipid filling
determination
Adipocyte life cycle
Undifferenciated fibroblast-like preadipocyte
Hypertrophied adipocyte
Preadipocyte new recruitment
Hypertrophy

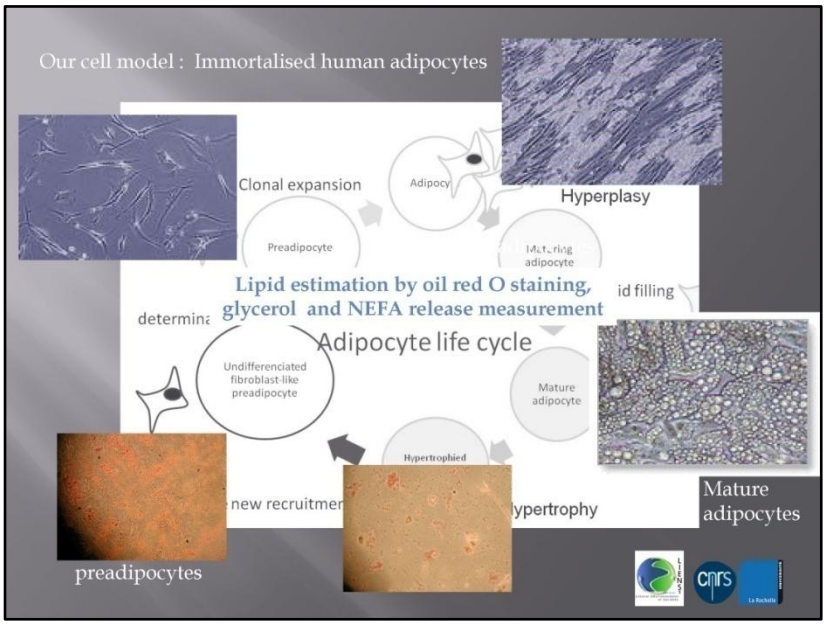
10


Treatments that regulate both size and number of adipocyte provide better therapeutic approach for treating obesity and associated pathologies.
Decrease of adipose tissue mass needs
✓ Lipolysis or apoptosis of mature adipocytes
✓Regulating proliferation and differentiation of fat cells

11


Could marine cryptides/ hydrolysates have incidences on adipocyte life cycle ?
Apoptosis
Clonal expansion
Adipocyte
Preadipocyte
Maturing adipocyte
Lipid filling
determination
marine cryptides
Undifferenciated fibroblast-like preadipocyte
Mature adipocyte
Hypertrophied adipocyte
Lipolysis
Apoptosis

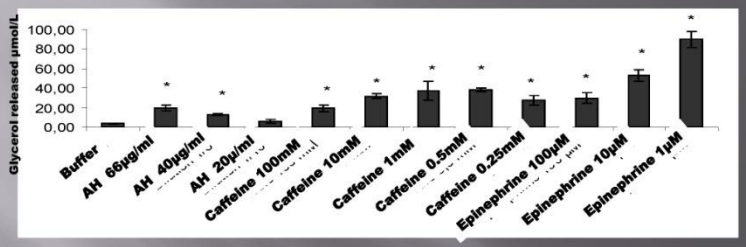
12


Our cell model : Immortalised human adipocytes
Clonal expansion
Hyperplasy
Preadipocyte
Lipid estimation by oil red O staining, glycerol and NEFA release measurement
Adipocyte life cycle
Undifferenciated fibroblast-like preadipocyte
Mature adipocyte
Hypertrophied
Mature adipocytes
preadipocytes

13

Some results obtained with immortalised human adipocytes :

Example of the lipolytic activity of an Algae Hydrolysate (AH)



Common lipolytic agents were tested on immortalised adipocytes which react as other cells lines used for lipolytic agents screening.
Algae hydrolysate (66µg/ml protein content) allowed as glycerol release as caffeine 0.25mM.

At this concentration, a reduction of 33% of fat storage was observed

Incubation : 22h

14

Marine cryptides….

- ACE inhibitory activity
- incidence on adipocyte development

-…and on the development of the metabolic syndrome …

15

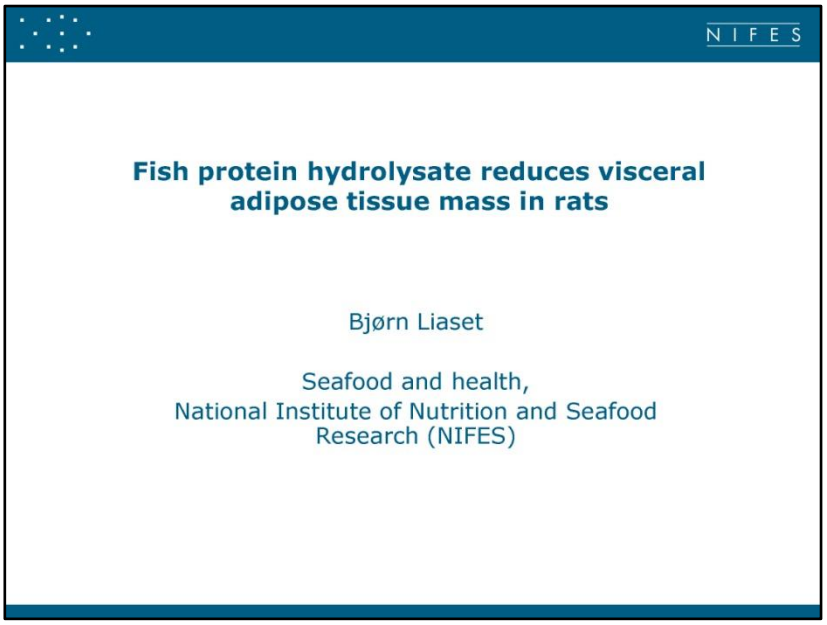

Financial support and research projects :
BIOTECMAR
BIOTECHNOLOGICAL EXPLOITATION OF MARINE PRODUCTS AND BY-PRODUCTS
Pr. Fabienne Guerard
Projet cofinancé dans le cadre du programme PSDR Grand Ouest par :
Cemagref
INRA
PAYS DE LA LOIRE
GESTION DURABLE (PSDR Grand Ouest)
Pr. Patrick Bourseau

16

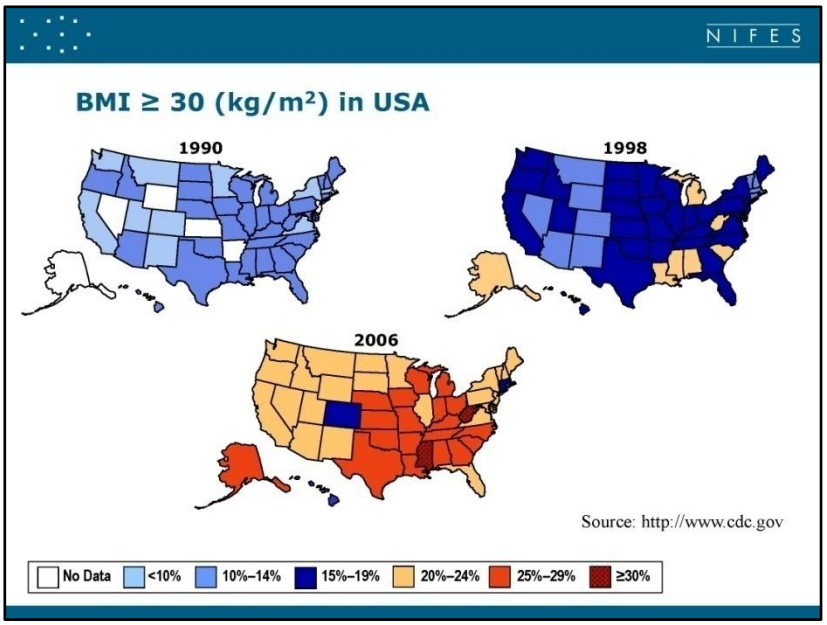

Thanks !

Appendix 3. ”Fish protein hydrolysate reduces visceral adipose tissue in rats” by Bjørn Liaset

1



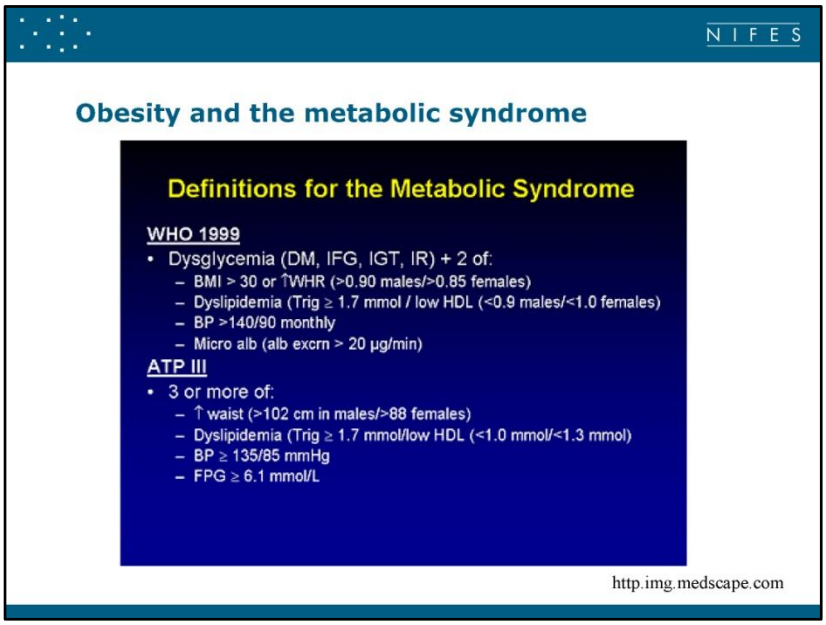
2



3


NIFES
Incidences of obesity increase– so what?
Interactions
Hepatocytes
Obesity
Adiposity
Insulin resistance
Weight control
Cardiovascular
stroke
Type 2 diabetes
High blood lipids
Hypertension
Macrophage
Interactions
Interactions
www.biolactis.com

4


NIFES
Obesity and the metabolic syndrome
Definitions for the Metabolic Syndrome
WHO 1999
• Dysglycemia (DM, IFG, IGT, IR) + 2 of:
– BMI > 30 or ↑WHR (>0.90 males/>0.85 females)
– Dyslipidemia (Trig ≥ 1.7 mmol / low HDL (<0.9 males/<1.0 females)
– BP >140/90 monthly
– Micro alb (alb excrn > 20 µg/min)
ATP III
• 3 or more of:
– ↑ waist (>102 cm in males/>88 females)
– Dyslipidemia (Trig ≥ 1.7 mmol/low HDL (<1.0 mmol/<1.3 mmol)
– BP ≥ 135/85 mmHg
– FPG ≥ 6.1 mmol/L
http.img.medscape.com

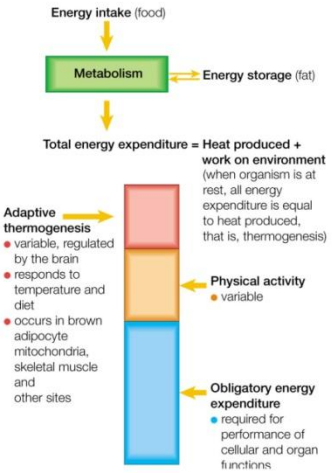
5

NIFES

Why obesity?

- **Proteins !!!**

Lowell & Spiegelman 2000
Nature 404: 652

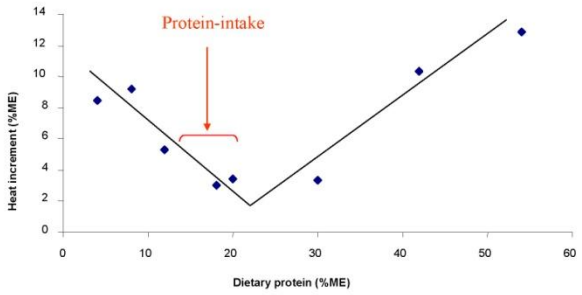


6

NIFES

Dietary protein intake and heat production

Data frå TS Hamilton 1939 J Nutr



- Recommended intake: 15 (10-35) energi%

7

NIFES

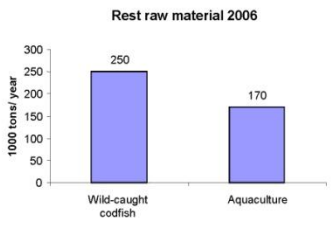
Dietary protein and energy expenditure

- Very-low dietary protein induces energy expenditure
- High dietary protein induces energy expenditure
- However, protein intake quite contstant over last decades (15-20 en%)
- Can different types of dietary proteins induce energy expenditure differently at average dietary level

8

NIFES

Fish protein hydrolysate

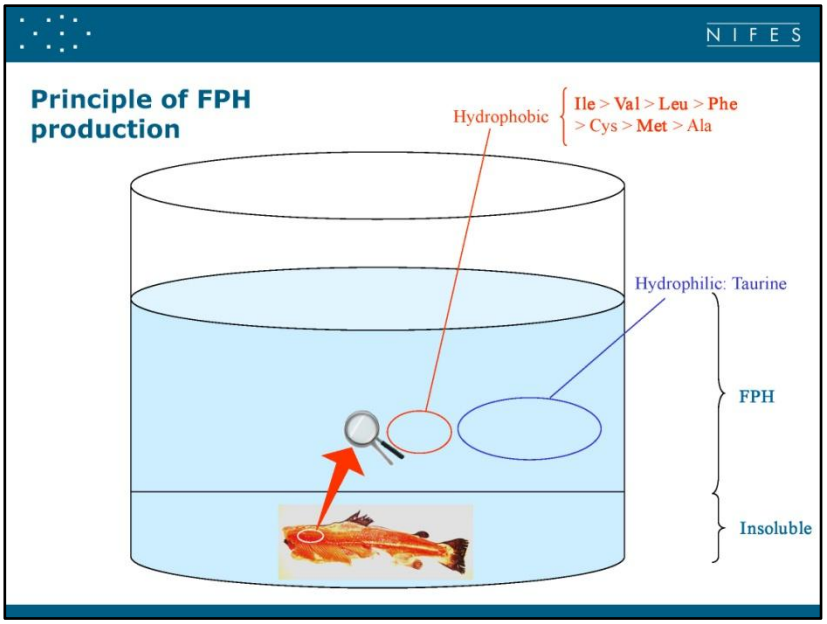


www.rubin.no

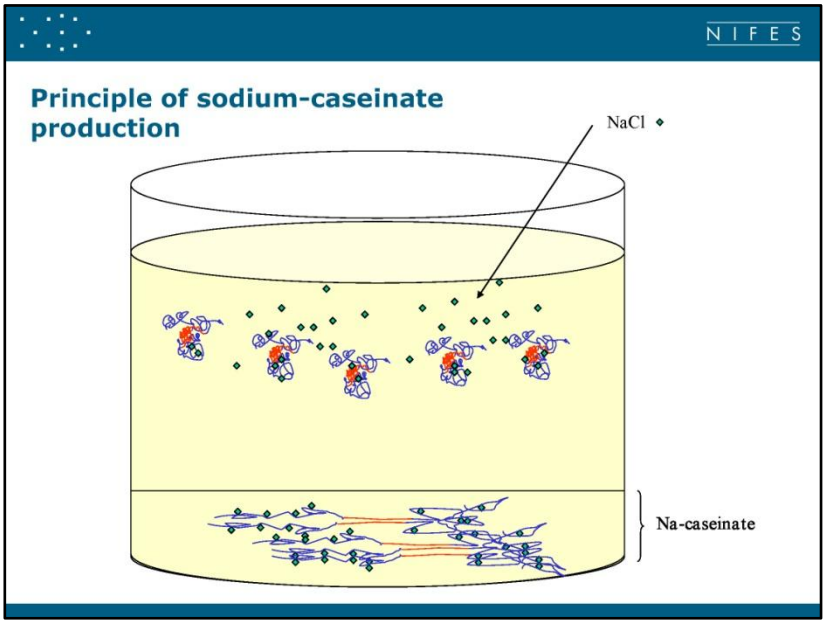
Enzymatic hydrolysis

Dried fish protein hydrolysate (FPH)

9


NIFES
Principle of FPH production
Hydrophobic
Ile > Val > Leu > Phe > Cys > Met > Ala
Hydrophilic: Taurine
FPH
Insoluble

10


NIFES
Principle of sodium-caseinate production
NaCl
Na-caseinate

11

NIFES

Rat study with saithe FPH

- Dietary saithe FPH compared to dietary soy protein and casein

Component, g/kg	Saithe FPH	Soy protein	Casein
Saithe FPH	239	-	-
Soy protein isolate	-	230	-
Casein	-	-	227
KCl	-	23	26
Salt mix AIN-93 G*	35	35	35
Vitamin mix AIN-93G*	10	10	10
Cellulose	50	50	50
Sucrose	90	90	90
Supplement AIN-95G*	10	10	10
L-Cystine	3	3	3
Choline Bitartrate	2,5	2,5	2,5
Soy bean oil	100	100	100
Potato starch, dextrinized	461	447	446

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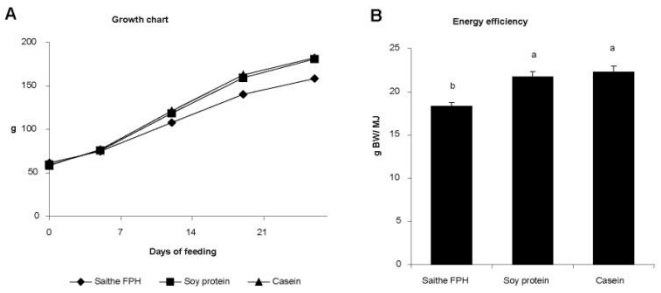
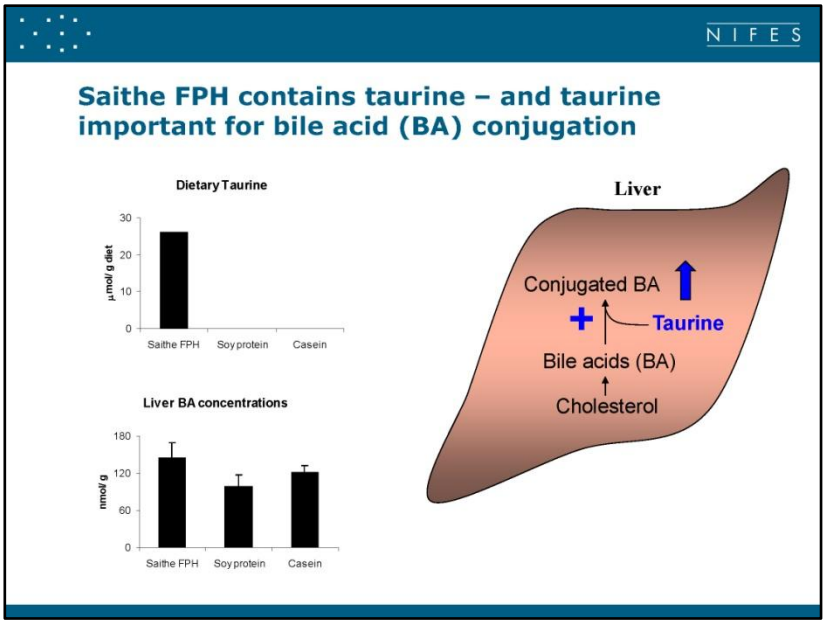
NIFES

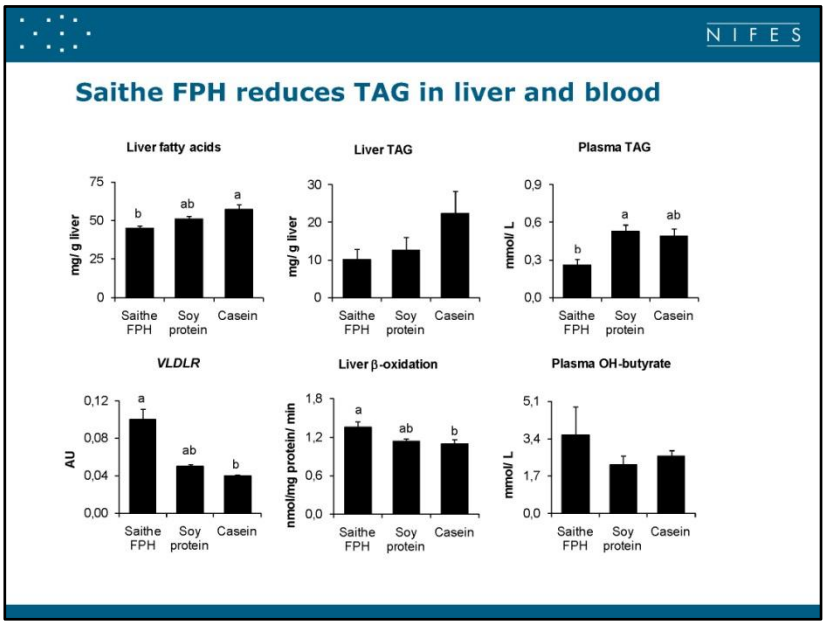
Saithe FPH reduces energy-efficiency



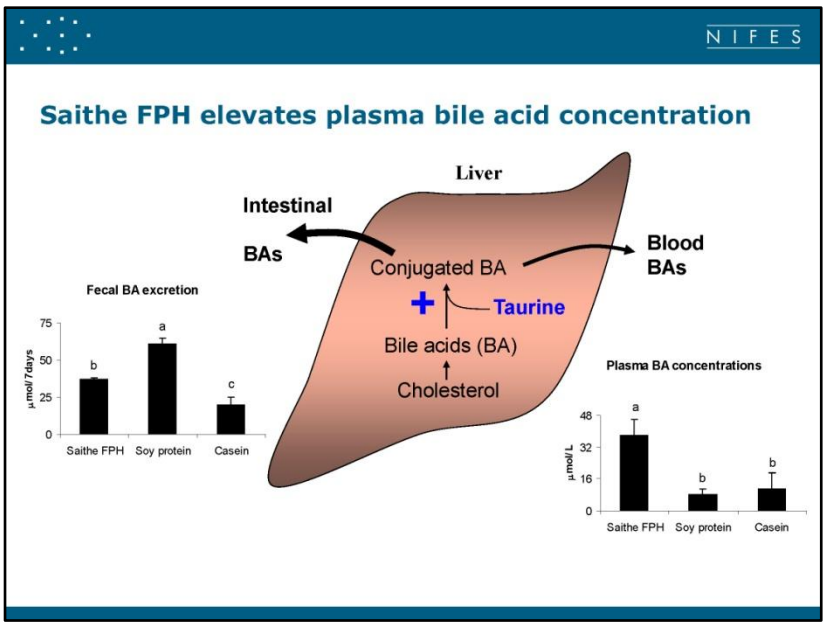
13


NIFES
Saithe FPH contains taurine – and taurine important for bile acid (BA) conjugation
Dietary Taurine
µmol/ g diet
30
20
10
0
Saithe FPH
Soy protein
Casein
Liver BA concentrations
nmol/ g
180
120
60
0
Saithe FPH
Soy protein
Casein
Liver
Conjugated BA
Taurine
Bile acids (BA)
Cholesterol

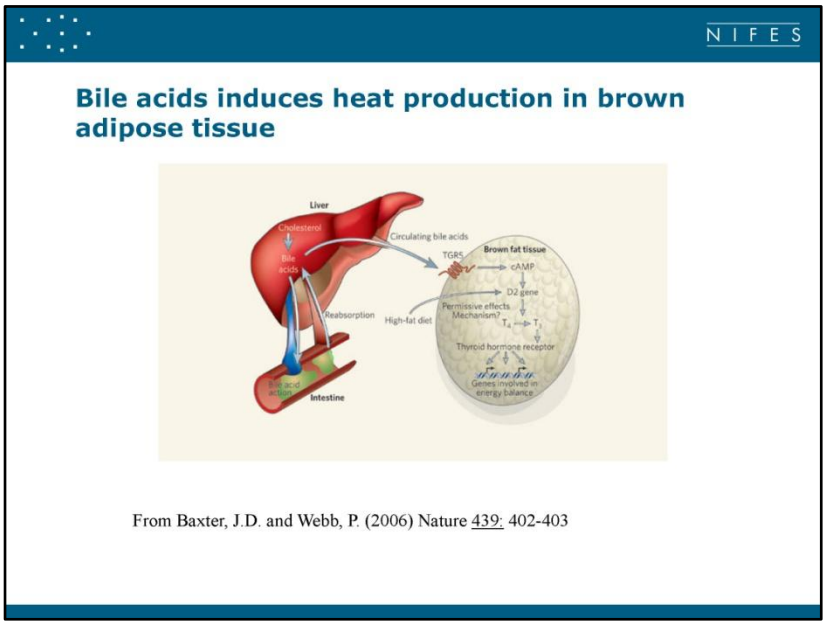
14


NIFES
Saithe FPH reduces TAG in liver and blood
Liver fatty acids
mg/ g liver
75
50
25
0
b
ab
a
Saithe FPH
Soy protein
Casein
Liver TAG
mg/ g liver
30
20
10
0
Saithe FPH
Soy protein
Casein
Plasma TAG
mmol/ L
0,9
0,6
0,3
0,0
b
a
ab
Saithe FPH
Soy protein
Casein
VLDLR
AU
0,12
0,08
0,04
0,00
a
ab
b
Saithe FPH
Soy protein
Casein
Liver β-oxidation
nmol/mg protein/ min
1,8
1,2
0,6
0,0
a
ab
b
Saithe FPH
Soy protein
Casein
Plasma OH-butyrate
mmol/ L
5,1
3,4
1,7
0,0
Saithe FPH
Soy protein
Casein

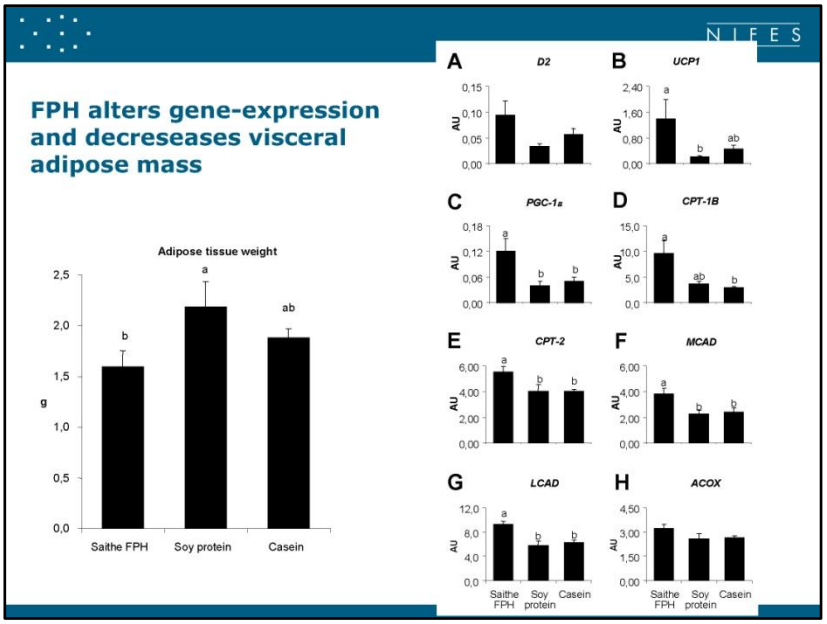
15


NIFES
Saithe FPH elevates plasma bile acid concentration
Liver
Intestinal
BAs
Blood
BAs
Conjugated BA
Taurine
Bile acids (BA)
Cholesterol
Fecal BA excretion
µmol/ 7days
75
50
25
0
a
b
c
Saithe FPH
Soy protein
Casein
Plasma BA concentrations
µmol/ L
48
32
16
0
a
b
b
Saithe FPH
Soy protein
Casein

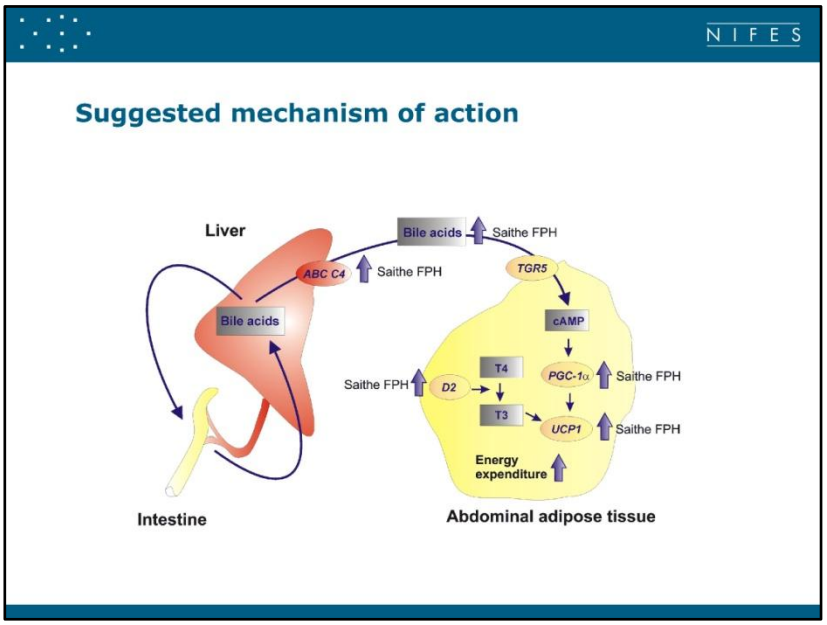
16


NIFES
Bile acids induces heat production in brown adipose tissue
Liver
Cholesterol
Bile acids
Circulating bile acids
TGR5
Brown fat tissue
cAMP
D2 gene
Permissive effects
Mechanism?
T_4
T_3
Thyroid hormone receptor
Genes involved in energy balance
Reabsorption
High-fat diet
Intestine
From Baxter, J.D. and Webb, P. (2006) Nature 439: 402-403

17


NIFES
FPH alters gene-expression and decreseases visceral adipose mass
Adipose tissue weight
g
Saithe FPH
Soy protein
Casein
A D2
B UCP1
C PGC-1α
D CPT-1B
E CPT-2
F MCAD
G LCAD
H ACOX
AU

18


NIFES
Suggested mechanism of action
Liver
Bile acids
Saithe FPH
ABC C4
TGR5
cAMP
T4
PGC-1α
D2
T3
UCP1
Energy expenditure
Intestine
Abdominal adipose tissue

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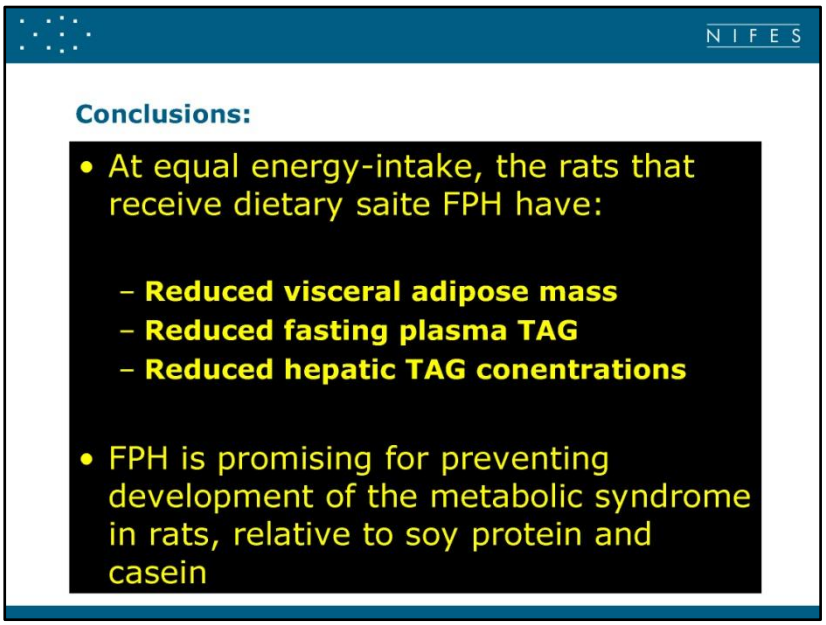
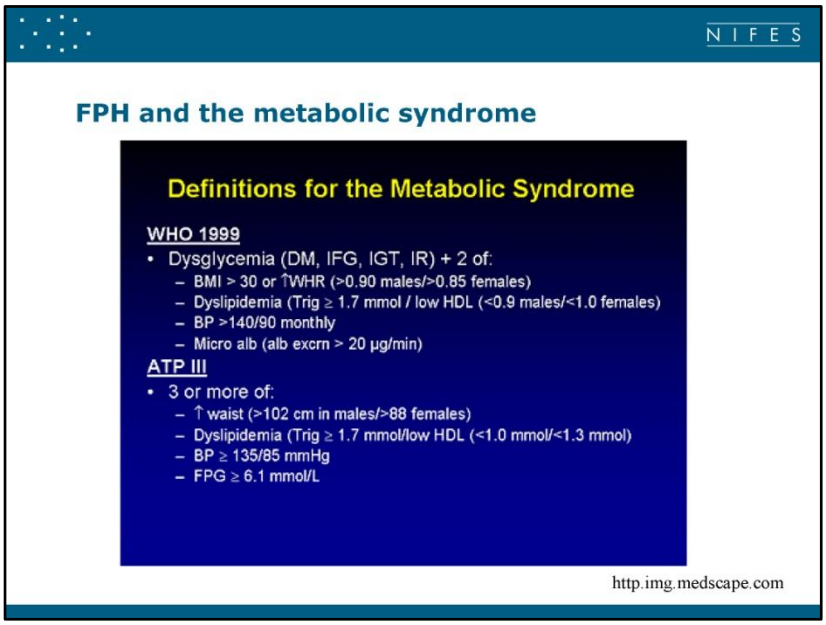

NIFES
Conclusions:
• At equal energy-intake, the rats that receive dietary saite FPH have:
– Reduced visceral adipose mass
– Reduced fasting plasma TAG
– Reduced hepatic TAG conentrations
• FPH is promising for preventing development of the metabolic syndrome in rats, relative to soy protein and casein

20


NIFES
FPH and the metabolic syndrome
Definitions for the Metabolic Syndrome
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– Micro alb (alb excrn > 20 µg/min)
ATP III
• 3 or more of:
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– Dyslipidemia (Trig ≥ 1.7 mmol/low HDL (<1.0 mmol/<1.3 mmol)
– BP ≥ 135/85 mmHg
– FPG ≥ 6.1 mmol/L
http.img.medscape.com

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NIFES
Collaborators:
University of Copenhagen
Qin Hao
Karsten Kristiansen
University of Southern Denmark
Philip Hallenborg
University of Bergen
Gunnar Mellgren
Karolinska University
Hanns-Ulrich Marschall
NIFES
Lise Madsen
Marit Espe
Gabriel Criales
Åse Heltveit
Jacob Wessels
Livar Frøyland

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NIFES
Financial support:
Rubin/ FHF
Innovation Norway
Danish Natural Science Resarch Council (Danish Obesity Research Centre)
Program Committee on Nutrition, University of Bergen
Large scale enzymatic hydrolysis:
Novozymes, Bagsvaerd, Denmark

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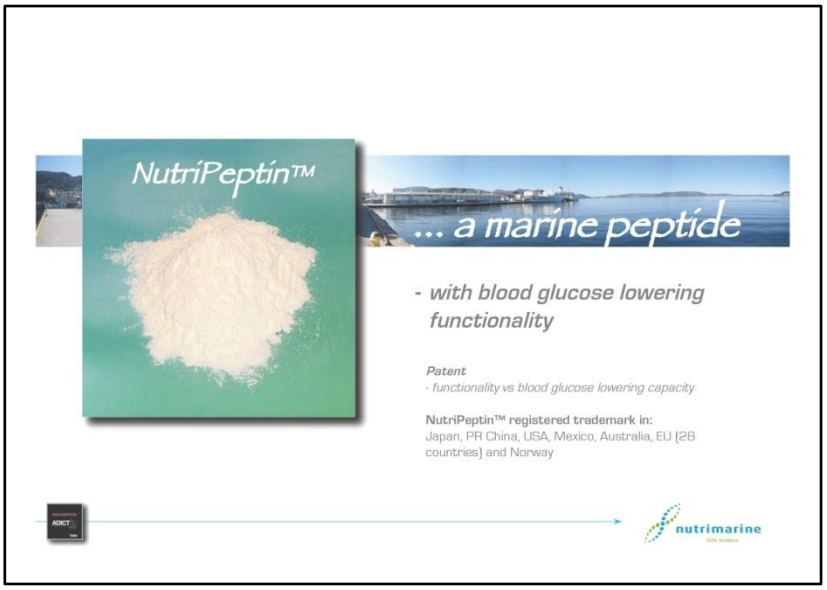
NIFES

Thank you for your attention!

1dak.com/people/biggest-beer-belly-in-the-world/

Appendix 4. ”Marine peptides; a tool of blood glucose lowering and stabilisation” by Einar Lied

1



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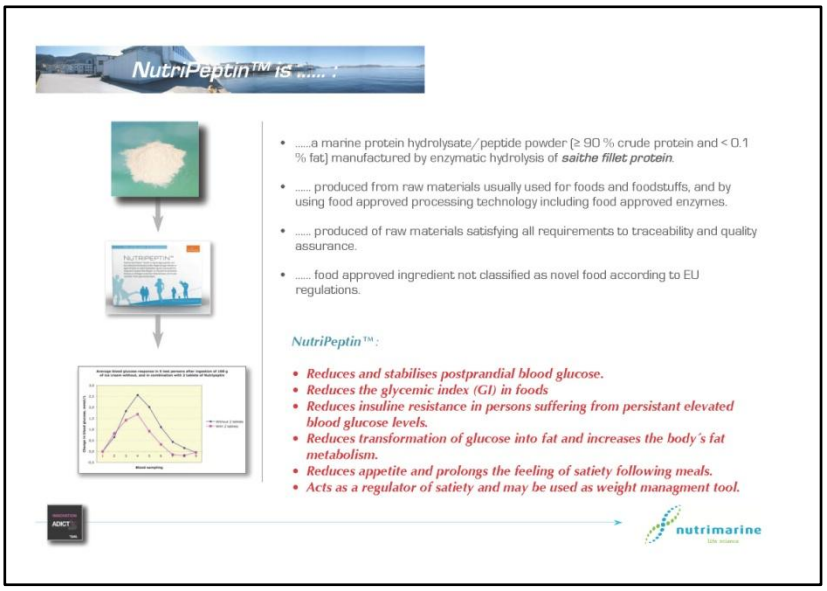

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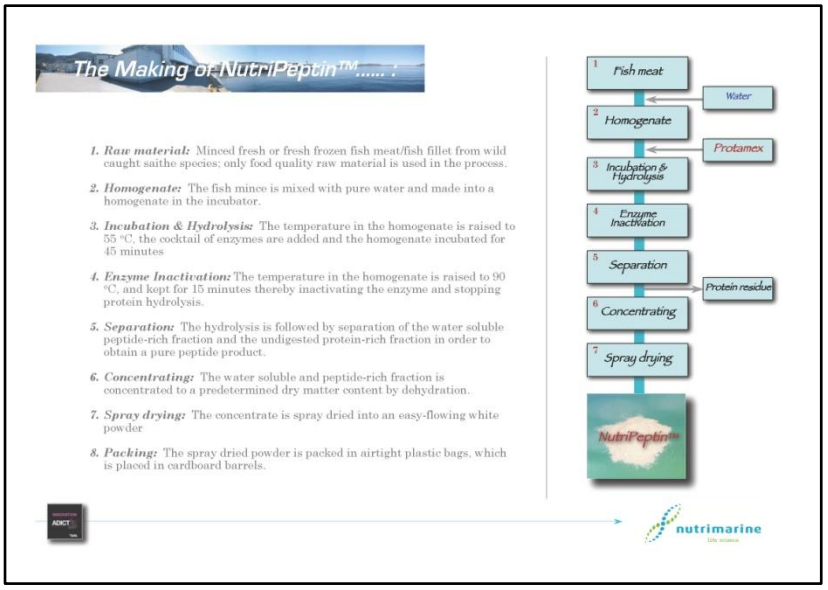
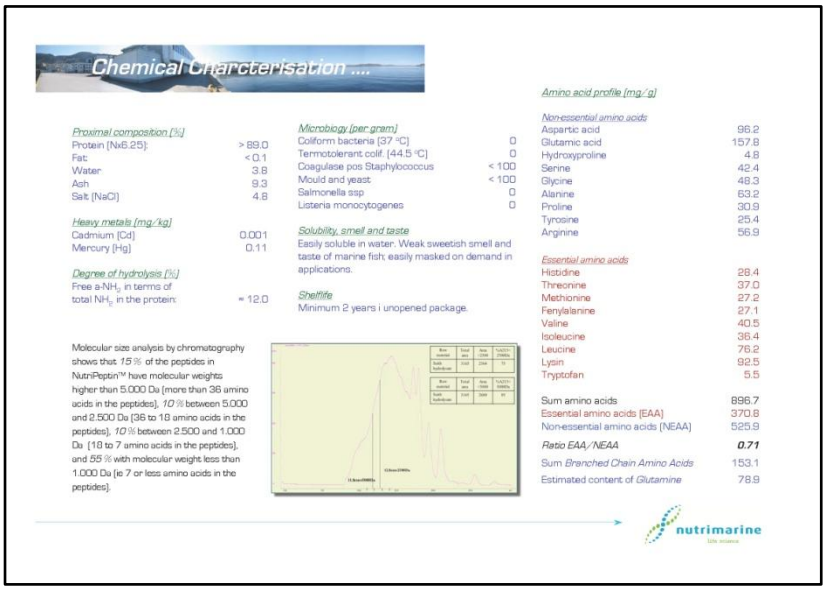

The Making of NutriPeptin™..... !
1. Raw material: Minced fresh or fresh frozen fish meat/fish fillet from wild caught saithe species; only food quality raw material is used in the process.
2. Homogenate: The fish mince is mixed with pure water and made into a homogenate in the incubator.
3. Incubation & Hydrolysis: The temperature in the homogenate is raised to 55 °C, the cocktail of enzymes are added and the homogenate incubated for 45 minutes
4. Enzyme Inactivation: The temperature in the homogenate is raised to 90 °C, and kept for 15 minutes thereby inactivating the enzyme and stopping protein hydrolysis.
5. Separation: The hydrolysis is followed by separation of the water soluble peptide-rich fraction and the undigested protein-rich fraction in order to obtain a pure peptide product.
6. Concentrating: The water soluble and peptide-rich fraction is concentrated to a predetermined dry matter content by dehydration.
7. Spray drying: The concentrate is spray dried into an easy-flowing white powder.
8. Packing: The spray dried powder is packed in airtight plastic bags, which is placed in cardboard barrels.
1 Fish meat
Water
2 Homogenate
Protamex
3 Incubation & Hydrolysis
4 Enzyme Inactivation
5 Separation
Protein residue
6 Concentrating
7 Spray drying
NutriPeptin™
nutrimarine

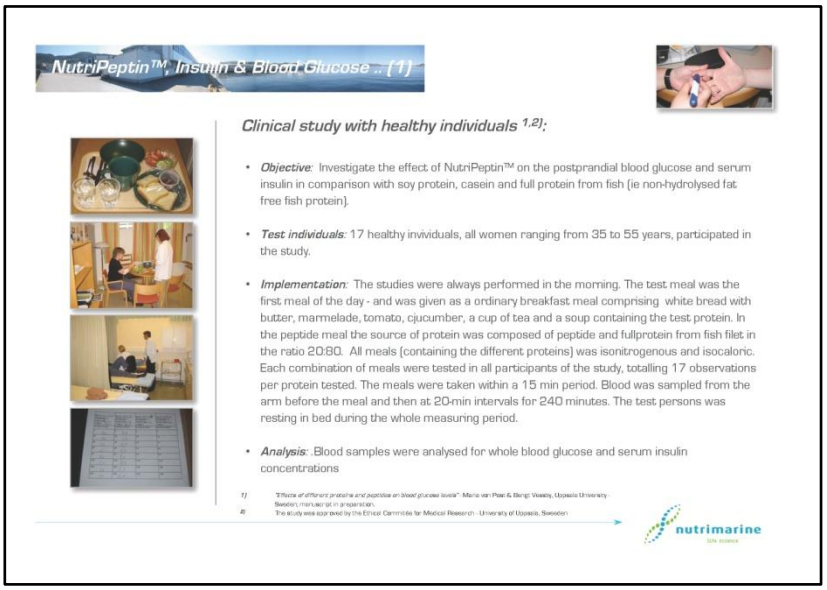
4


Chemical Charcterisation ....
Proximal composition [%]
Protein (Nx6.25): > 89.0
Fat: < 0.1
Water: 3.8
Ash 9.3
Salt (NaCl) 4.8
Heavy metals [mg/kg]
Cadmium (Cd) 0.001
Mercury (Hg) 0.11
Degree of hydrolysis [%]
Free a-NH2 in terms of total NH2 in the protein: ≈ 12.0
Microbiogy (per gram)
Coliform bacteria (37 °C) 0
Termotolerant colif. (44.5 °C) 0
Coagulase pos Staphylococcus < 100
Mould and yeast < 100
Salmonella ssp 0
Listeria monocytogenes 0
Solubility, smell and taste
Easily soluble in water. Weak sweetish smell and taste of marine fish; easily masked on demand in applications.
Shelflife
Minimum 2 years i unopened package.
Molecular size analysis by chromatography shows that 15 % of the peptides in NutriPeptin™ have molecular weights higher than 5.000 Da (more than 36 amino acids in the peptides), 10 % between 5.000 and 2.500 Da (36 to 18 amino acids in the peptides), 10 % between 2.500 and 1.000 Da (18 to 7 amino acids in the peptides), and 55 % with molecular weight less than 1.000 Da (ie 7 or less amino acids in the peptides).
Amino acid profile [mg/g]
Non-essential amino acids
Aspartic acid 96.2
Glutamic acid 157.8
Hydroxyproline 4.8
Serine 42.4
Glycine 48.3
Alanine 63.2
Proline 30.9
Tyrosine 25.4
Arginine 56.9
Essential amino acids
Histidine 28.4
Threonine 37.0
Methionine 27.2
Fenylalenine 27.1
Valine 40.5
Isoleucine 36.4
Leucine 76.2
Lysin 92.5
Tryptofan 5.5
Sum amino acids 896.7
Essential amino acids (EAA) 370.8
Non-essential amino acids (NEAA) 525.9
Ratio EAA/NEAA 0.71
Sum Branched Chain Amino Acids 153.1
Estimated content of Glutamine 78.9
nutrimarine

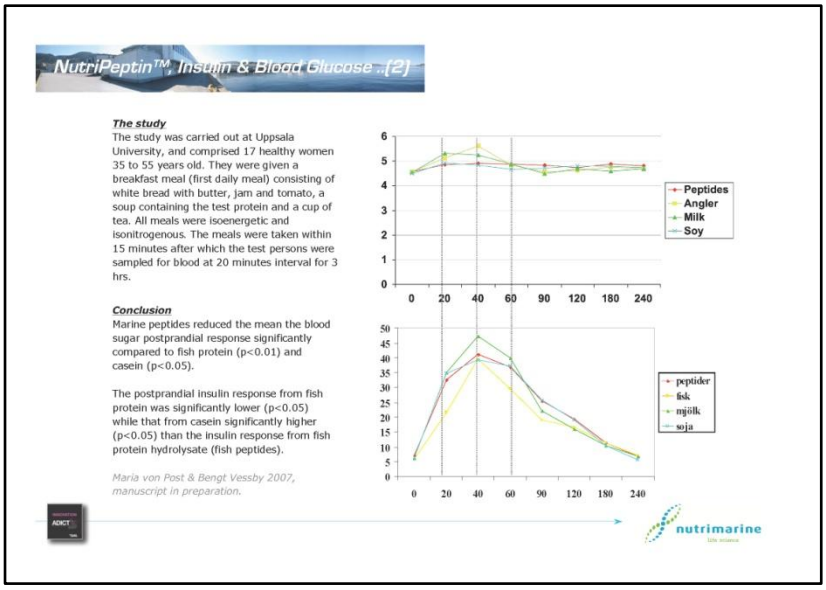
5


The Health Problem .....
About 3 % of the population in Western communities suffers from Diabetes 2; another 3 % suffers from persistant elevated blood glucose levels. It is estimated that about 7 % of the population will suffer from Diabetes type 2 by the end of 2015, and twice that number from highly elevated and unhealthy blood glucose levels.
Obesity and diabetes have turned out to be a great and fast growing health problems.
Diabetes may lead to:
Cardiac diseases: the risk of developing different cardiac diseases is increased by five-fold in people suffering from diabetes
Elevated blood pressure: 70 % of diabetic patients have blood pressure higher than 140/85 (= normal blood pressure).
Kidney disease: Diabetes increases the risk of serious kidney failure and dialysis treatment.
Blindness: Diabetes is the most frequent cause of blindness in people less than 60 years.
Amputations: Diabets affects blood circulations- the risk of amputations below the knee is increased 34-fold.
Growing health problems related to overweight and elevated blood glucose levels opens for innovative nutrition preparations and food supplements based on natural and functional ingredients to reduce health hazards.
nutrimarine

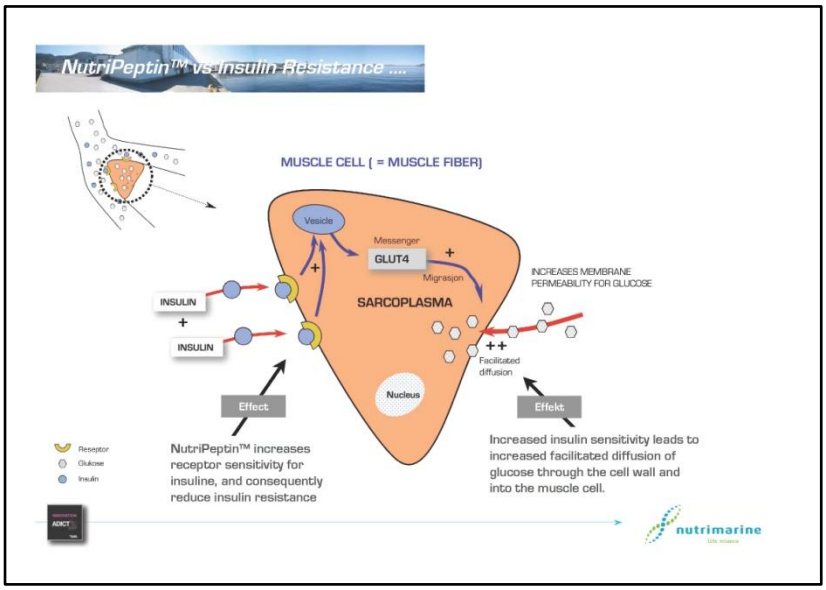
6


NutriPeptin™, Insulin & Blood Glucose .. [1]
Clinical study with healthy individuals [1,2]:
Objective: Investigate the effect of NutriPeptin™ on the postprandial blood glucose and serum insulin in comparison with soy protein, casein and full protein from fish (ie non-hydrolysed fat free fish protein).
Test individuals: 17 healthy invividuals, all women ranging from 35 to 55 years, participated in the study.
Implementation: The studies were always performed in the morning. The test meal was the first meal of the day - and was given as a ordinary breakfast meal comprising white bread with butter, marmelade, tomato, cjucumber, a cup of tea and a soup containing the test protein. In the peptide meal the source of protein was composed of peptide and fullprotein from fish filet in the ratio 20:80. All meals (containing the different proteins) was isonitrogenous and isocaloric. Each combination of meals were tested in all participants of the study, totalling 17 observations per protein tested. The meals were taken within a 15 min period. Blood was sampled from the arm before the meal and then at 20-min intervals for 240 minutes. The test persons was resting in bed during the whole measuring period.
Analysis: .Blood samples were analysed for whole blood glucose and serum insulin concentrations
1) "Effects of different proteins and peptides on blood glucose levels" - Maria van Post & Bengt Vessby, Uppsala University Sweden, manuscript in preperation.
2) The study was approved by the Ethical Commitee for Medical Research - University of Uppsala, Sweeden
nutrimarine

7


NutriPeptin™, Insulin & Blood Glucose ..(2)
The study
The study was carried out at Uppsala University, and comprised 17 healthy women 35 to 55 years old. They were given a breakfast meal (first daily meal) consisting of white bread with butter, jam and tomato, a soup containing the test protein and a cup of tea. All meals were isoenergetic and isonitrogenous. The meals were taken within 15 minutes after which the test persons were sampled for blood at 20 minutes interval for 3 hrs.
Conclusion
Marine peptides reduced the mean the blood sugar postprandial response significantly compared to fish protein (p<0.01) and casein (p<0.05).
The postprandial insulin response from fish protein was significantly lower (p<0.05) while that from casein significantly higher (p<0.05) than the insulin response from fish protein hydrolysate (fish peptides).
Maria von Post & Bengt Vessby 2007, manuscript in preparation.
Peptides
Angler
Milk
Soy
peptider
fisk
mjölk
soja
nutrimarine

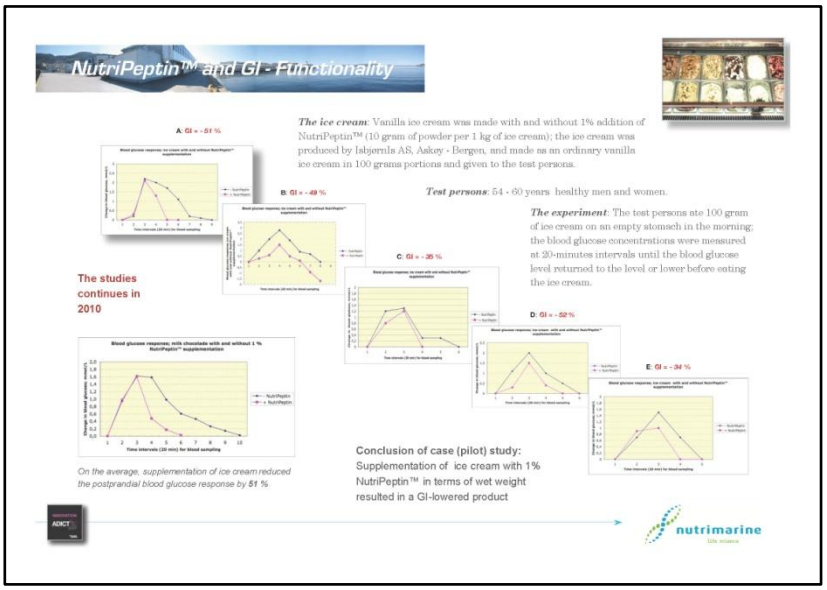
8


NutriPeptin™ vs Insulin Resistance ....
MUSCLE CELL (= MUSCLE FIBER)
Vesicle
Messenger
GLUT4
Migrasjon
INCREASES MEMBRANE PERMEABILITY FOR GLUCOSE
INSULIN
INSULIN
SARCOPLASMA
Facilitated diffusion
Nucleus
Effect
Effekt
Reseptor
Glukose
Insulin
NutriPeptin™ increases receptor sensitivity for insuline, and consequently reduce insulin resistance
Increased insulin sensitivity leads to increased facilitated diffusion of glucose through the cell wall and into the muscle cell.
nutrimarine

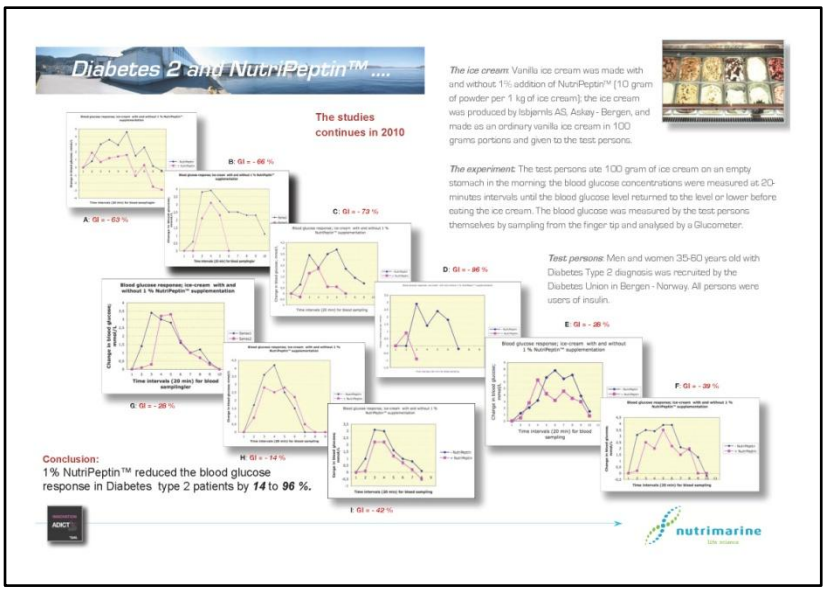
9


Potential Applications ...
NutriPeptin™'s nutritional qualities, and functional and technical properties makes it very suitable for applications in (1) formulated foods and food supplements for better performance and restitution, and (2) as a health-promoting ingredient in foods.
Marine peptides
Peptide powder manufactured from fresh or fresh frozen saithe fillets
Protein and peptide food supplements
Drinking concepts for sport and clinic
Sport nutrition
Peptides in food: Improves performance
Stimulate muscle glycogen synthesis
Senior nutrition
Peptides in food: Stimulate muscle growth
Lower age-dependent muscle degeneration
Clinical enteral nutrition
Peptides in food: Improves growth and protein utilisation
Provides easily absorbable proteins
Improved performance
Health promoting
GI-lowering drinks, foods and dishes
GI-low nutrition
Peptides in food: Lower blood glucose after a meal
Stimulate glucose uptake in cells
Cardioprotective nutrition
Peptides in food: Lower plasma cholesterol and lipids
Lower blood pressure
Fat-reducing nutrition
Peptides in food: Lower fat deposition and risk of obesity
Lower the risk of diabetes II
nutrimarine

10


NutriPeptin™ and GI - Functionality
The ice cream: Vanilla ice cream was made with and without 1% addition of NutriPeptin™ (10 gram of powder per 1 kg of ice cream); the ice cream was produced by Isbjørnla AS, Askøy - Bergen, and made as an ordinary vanilla ice cream in 100 grams portions and given to the test persons.
Test persons: 54 - 60 years healthy men and women.
The experiment: The test persons ate 100 gram of ice cream on an empty stomach in the morning; the blood glucose concentrations were measured at 20-minutes intervals until the blood glucose level returned to the level or lower before eating the ice cream.
A: GI = - 51 %
B: GI = - 49 %
C: GI = - 35 %
D: GI = - 52 %
E: GI = - 34 %
The studies continues in 2010
On the average, supplementation of ice cream reduced the postprandial blood glucose response by 51 %
Conclusion of case (pilot) study:
Supplementation of ice cream with 1% NutriPeptin™ in terms of wet weight resulted in a GI-lowered product
nutrimarine

11


Diabetes 2 and NutriPeptin™ ....
The studies continues in 2010
The ice cream: Vanilla ice cream was made with and without 1% addition of NutriPeptin™ (10 gram of powder per 1 kg of ice cream); the ice cream was produced by Isbjørnlis AS, Askøy - Bergen, and made as an ordinary vanilla ice cream in 100 grams portions and given to the test persons.
The experiment: The test persons ate 100 gram of ice cream on an empty stomach in the morning; the blood glucose concentrations were measured at 20-minutes intervals until the blood glucose level returned to the level or lower before eating the ice cream. The blood glucose was measured by the test persons themselves by sampling from the finger tip and analysed by a Glucometer.
Test persons: Men and women 35-60 years old with Diabetes Type 2 diagnosis was recruited by the Diabetes Union in Bergen - Norway. All persons were users of insulin.
A: GI = -53 %
B: GI = - 66 %
C: GI = - 73 %
D: GI = - 96 %
E: GI = - 26 %
F: GI = - 29 %
G: GI = - 28 %
H: GI = - 14 %
I: GI = - 42 %
Conclusion:
1% NutriPeptin™ reduced the blood glucose response in Diabetes type 2 patients by 14 to 96 %.
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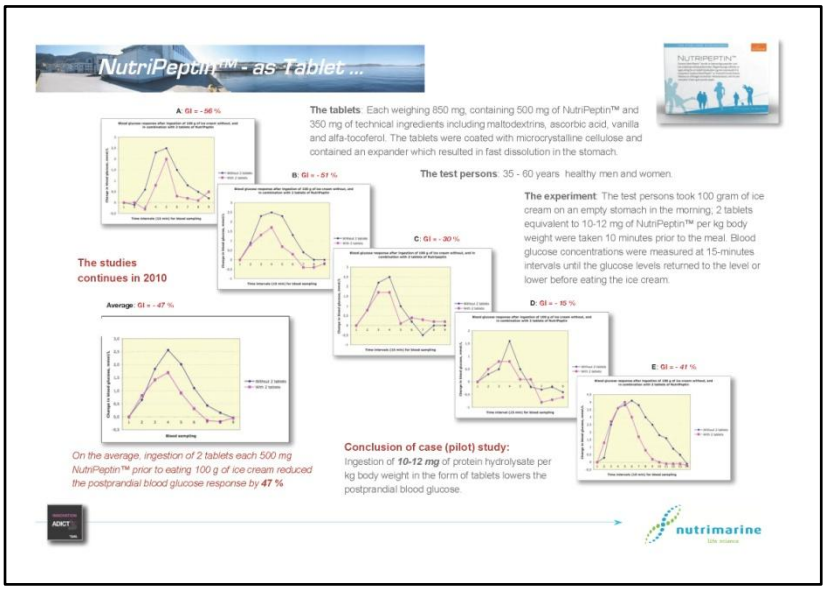
12


NutriPeptin™ - as Tablet ...
The tablets: Each weighing 850 mg, containing 500 mg of NutriPeptin™ and 350 mg of technical ingredients including maltodextrins, ascorbic acid, vanilla and alfa-tocoferol. The tablets were coated with microcrystalline cellulose and contained an expander which resulted in fast dissolution in the stomach.
The test persons: 35 - 60 years healthy men and women.
The experiment: The test persons took 100 gram of ice cream on an empty stomach in the morning; 2 tablets equivalent to 10-12 mg of NutriPeptin™ per kg body weight were taken 10 minutes prior to the meal. Blood glucose concentrations were measured at 15-minutes intervals until the glucose levels returned to the level or lower before eating the ice cream.
A: GI = -56 %
B: GI = - 51 %
C: GI = - 30 %
D: GI = - 15 %
E: GI = - 41 %
The studies continues in 2010
Average: GI = - 47 %
On the average, ingestion of 2 tablets each 500 mg NutriPeptin™ prior to eating 100 g of ice cream reduced the postprandial blood glucose response by 47 %
Conclusion of case (pilot) study:
Ingestion of 10-12 mg of protein hydrolysate per kg body weight in the form of tablets lowers the postprandial blood glucose.
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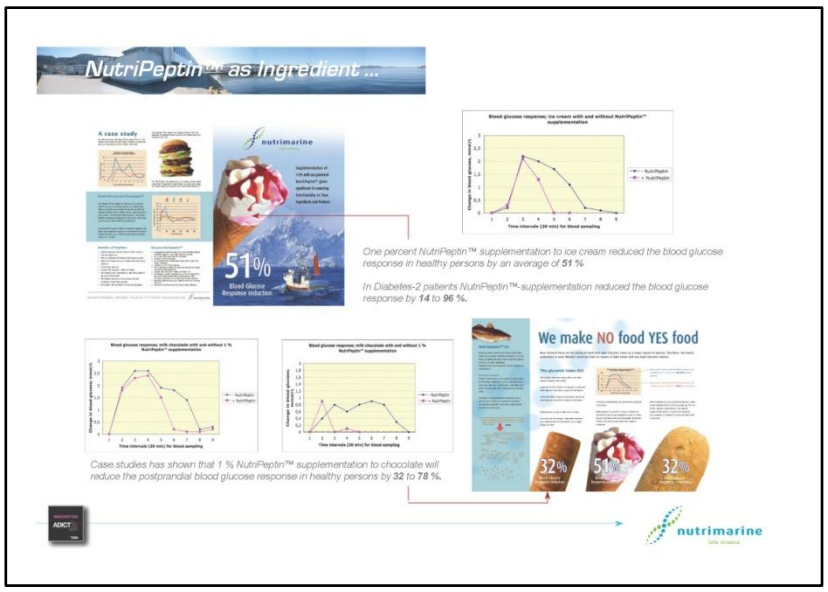
13


NutriPeptin™ as Ingredient ...
51%
Blood Glucose Response Induction
One percent NutriPeptin™ supplementation to ice cream reduced the blood glucose response in healthy persons by an average of 51 %
In Diabetes-2 patients NutriPeptin™-supplementation reduced the blood glucose response by 14 to 96 %.
We make NO food YES food
32%
51%
32%
Case studies has shown that 1 % NutriPeptin™ supplementation to chocolate will reduce the postprandial blood glucose response in healthy persons by 32 to 78 %.
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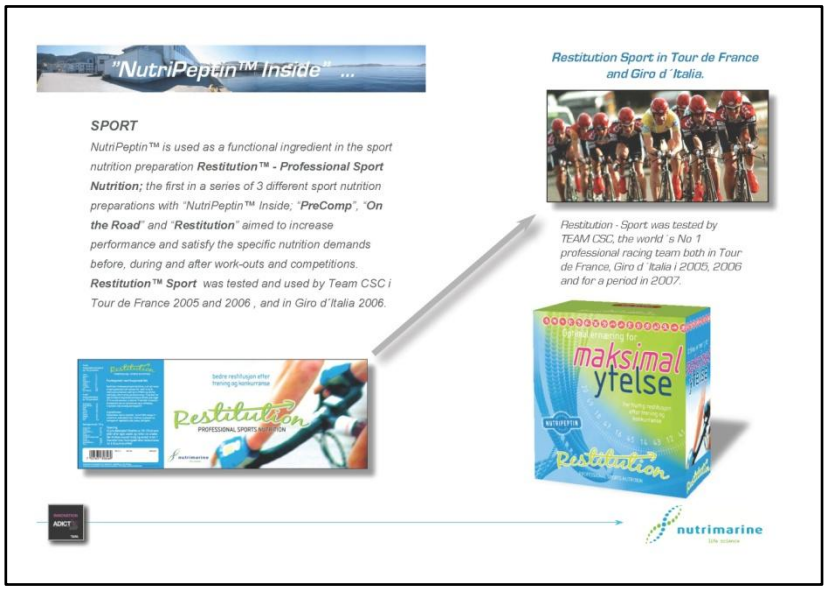
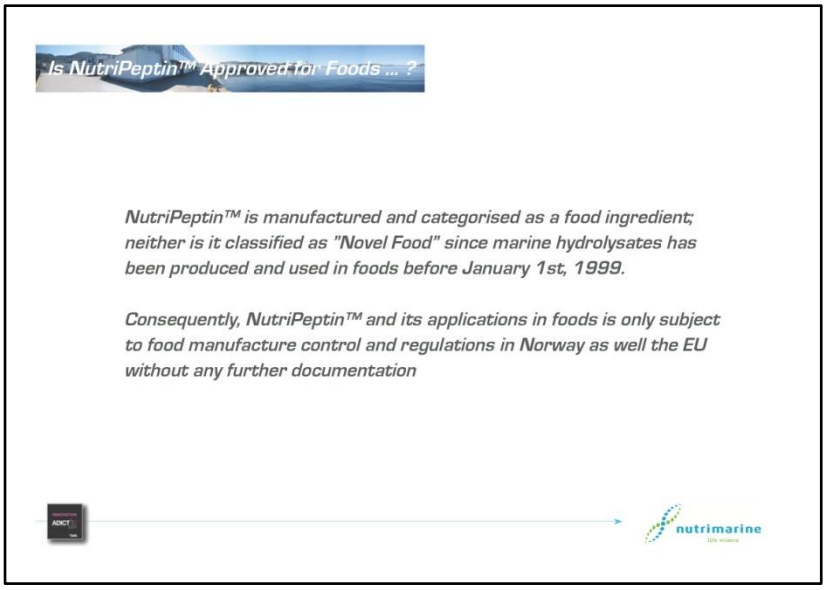
14


"NutriPeptin™ Inside" ...
SPORT
NutriPeptin™ is used as a functional ingredient in the sport nutrition preparation Restitution™ - Professional Sport Nutrition; the first in a series of 3 different sport nutrition preparations with "NutriPeptin™ Inside; "PreComp", "On the Road" and "Restitution" aimed to increase performance and satisfy the specific nutrition demands before, during and after work-outs and competitions. Restitution™ Sport was tested and used by Team CSC i Tour de France 2005 and 2006 , and in Giro d´Italia 2006.
Restitution Sport in Tour de France and Giro d´Italia.
Restitution - Sport was tested by TEAM CSC, the world´s No 1 professional racing team both in Tour de France, Giro d´Italia i 2005, 2006 and for a period in 2007.
Restitution
PROFESSIONAL SPORTS NUTRITION
maksimal yfelse
nutrimarine

15


Is NutriPeptin™ Approved for Foods ... ?
NutriPeptin™ is manufactured and categorised as a food ingredient; neither is it classified as "Novel Food" since marine hydrolysates has been produced and used in foods before January 1st, 1999.
Consequently, NutriPeptin™ and its applications in foods is only subject to food manufacture control and regulations in Norway as well the EU without any further documentation
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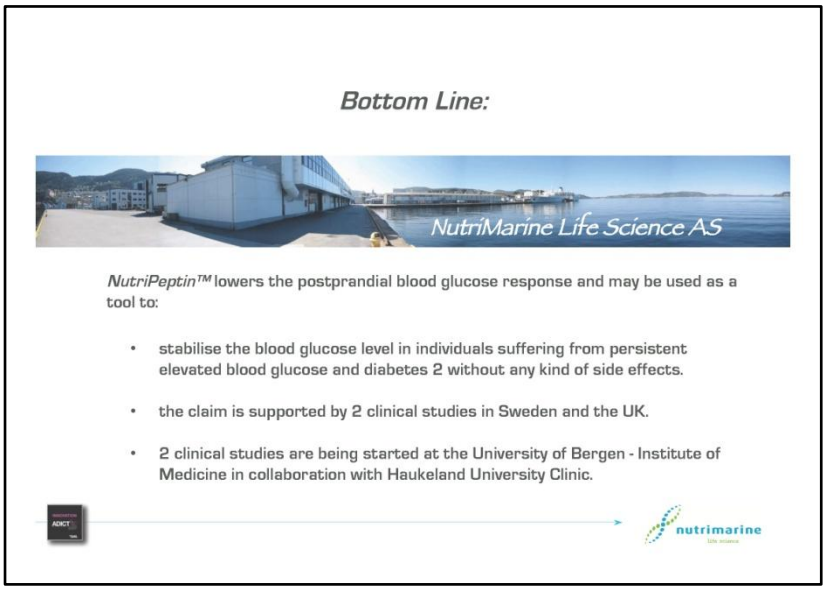
Bottom Line:
NutriMarine Life Science AS
NutriPeptin™ lowers the postprandial blood glucose response and may be used as a tool to:
• stabilise the blood glucose level in individuals suffering from persistent elevated blood glucose and diabetes 2 without any kind of side effects.
• the claim is supported by 2 clinical studies in Sweden and the UK.
• 2 clinical studies are being started at the University of Bergen - Institute of Medicine in collaboration with Haukeland University Clinic.
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Contact information:
Einar Lied, Dr Philos.
Managing Director / Professor (Clinical nutrition)
Cell phone: (+47) 95 83 34 73
E-mail: einar.lied@nutrimarine.com
Olav Hestmælingen, MD
Clinical Manager / Senior surgeon
Cell phone.: (+47) 95 83 34 73
E-mail: olav.hoestmaelingen@nutrimarine.com
NutriMarine Life Science AS
Bontelabo 2
N5003 Bergen
Norway
Tel: (+47) 55 59 65 90
Fax: (+47) 55 59 65 91
www.nutrimarine.com
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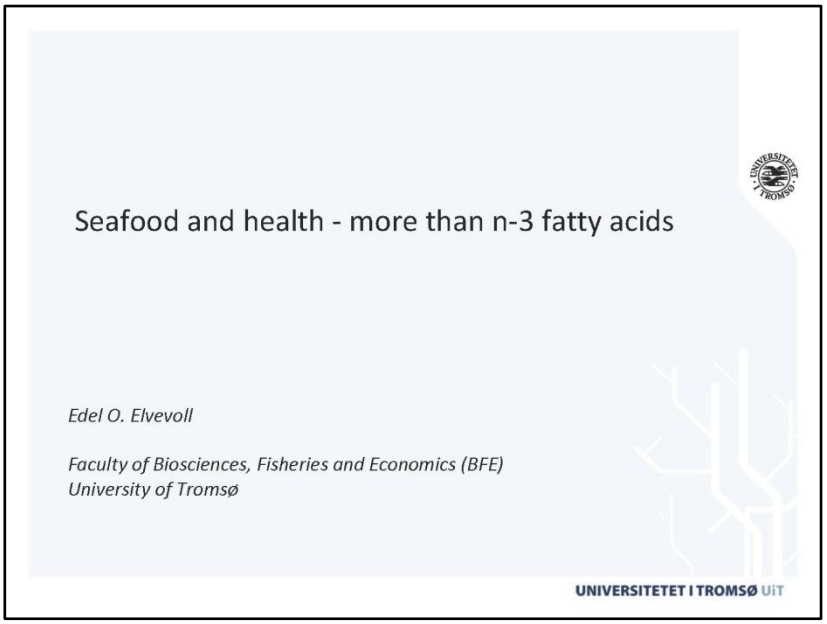
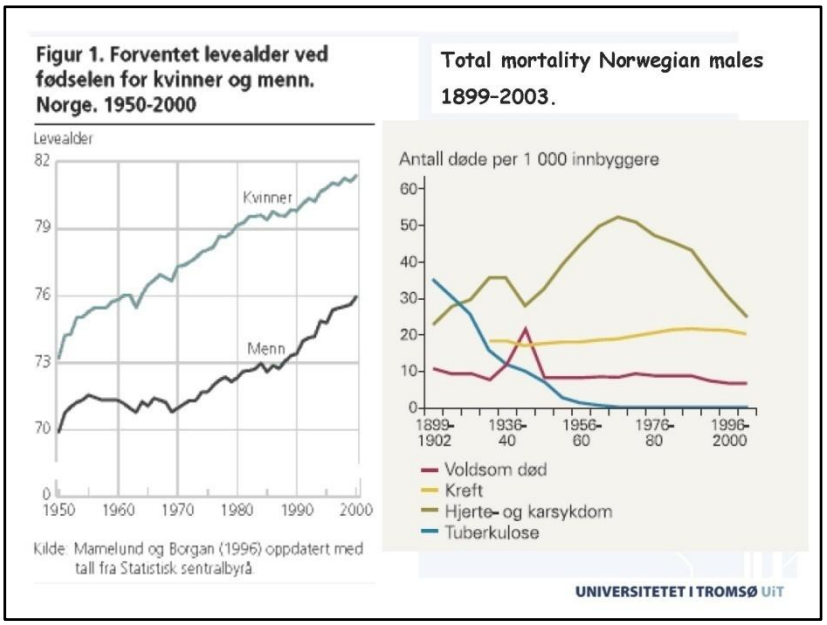
Appendix 5. ”Seafood and health – more than n-3 fatty acids” by Edel O. Elvevoll

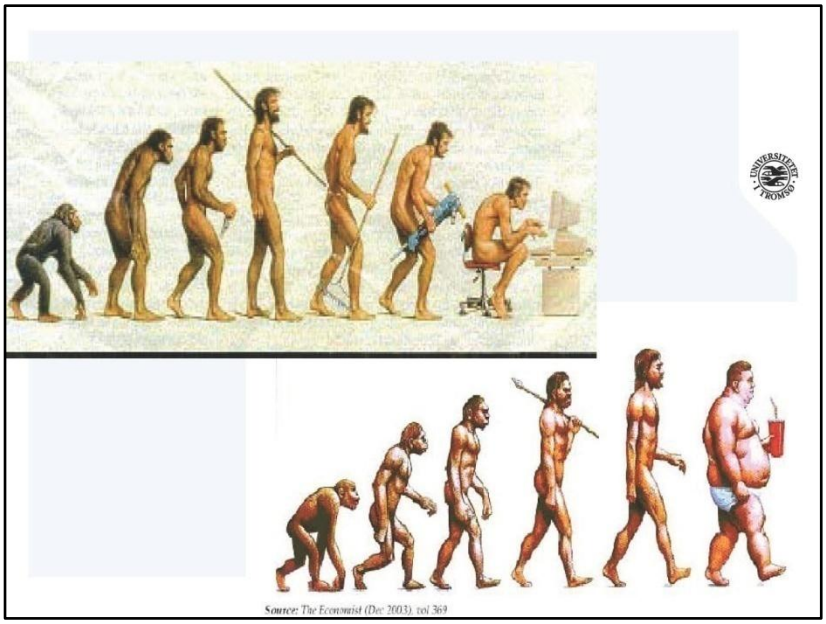
1



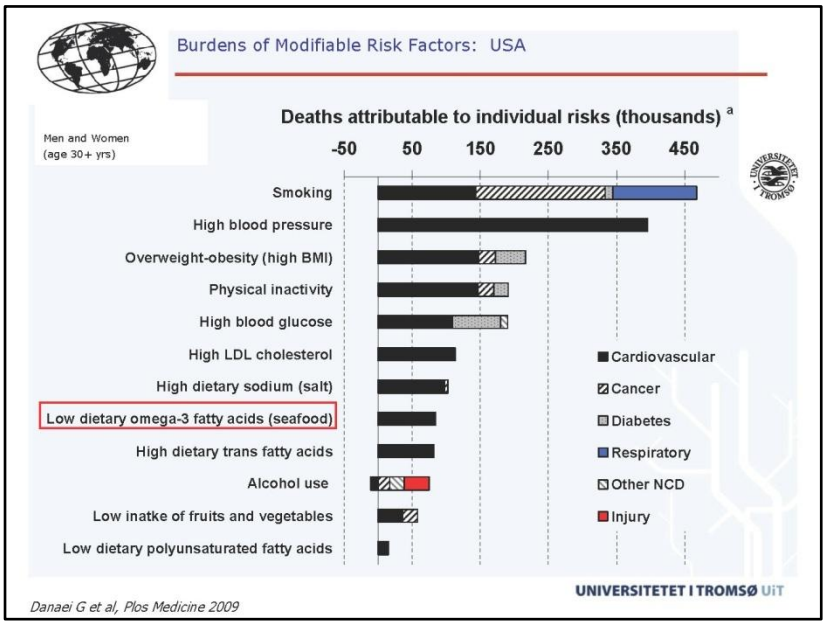
2



3


Source: The Economist (Dec 2003), vol 369

4


Burdens of Modifiable Risk Factors: USA
Men and Women
(age 30+ yrs)
Deaths attributable to individual risks (thousands) a
-50
50
150
250
350
450
Smoking
High blood pressure
Overweight-obesity (high BMI)
Physical inactivity
High blood glucose
High LDL cholesterol
High dietary sodium (salt)
Low dietary omega-3 fatty acids (seafood)
High dietary trans fatty acids
Alcohol use
Low inatke of fruits and vegetables
Low dietary polyunsaturated fatty acids
Cardiovascular
Cancer
Diabetes
Respiratory
Other NCD
Injury
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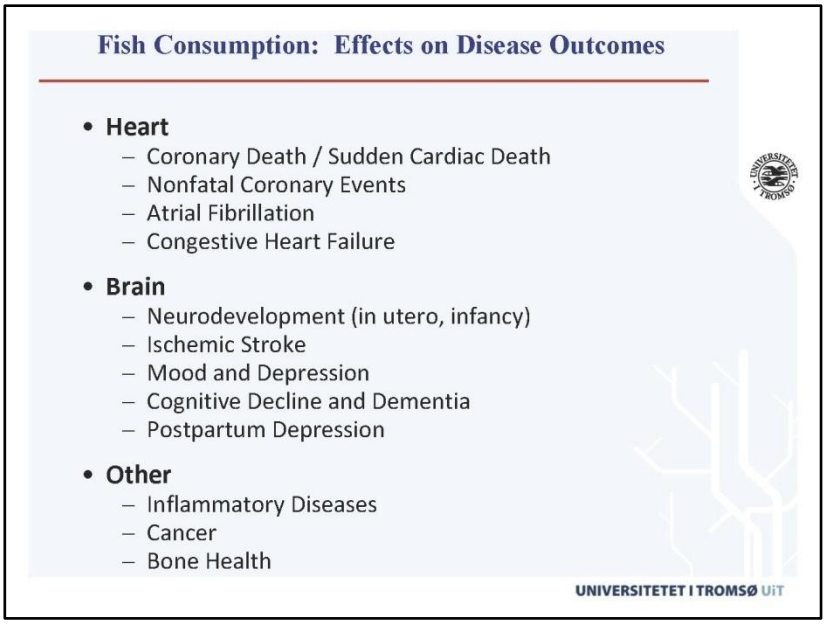
Danaei G et al, Plos Medicine 2009

5


Fish Consumption: Effects on Disease Outcomes
• Heart
– Coronary Death / Sudden Cardiac Death
– Nonfatal Coronary Events
– Atrial Fibrillation
– Congestive Heart Failure
• Brain
– Neurodevelopment (in utero, infancy)
– Ischemic Stroke
– Mood and Depression
– Cognitive Decline and Dementia
– Postpartum Depression
• Other
– Inflammatory Diseases
– Cancer
– Bone Health
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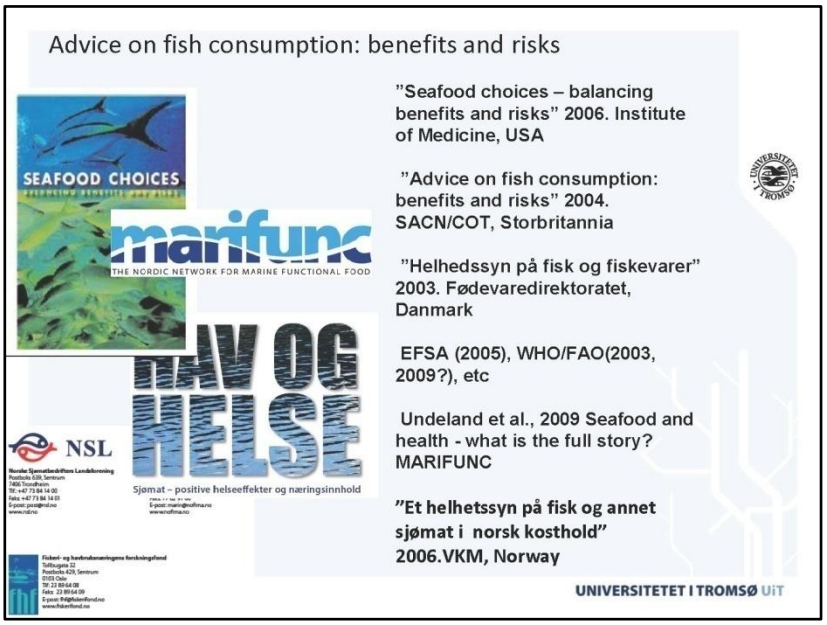
6


Advice on fish consumption: benefits and risks
SEAFOOD CHOICES
marifunc
THE NORDIC NETWORK FOR MARINE FUNCTIONAL FOOD
HAV OG HELSE
Sjømat – positive helseeffekter og næringsinnhold
NSL
"Seafood choices – balancing benefits and risks" 2006. Institute of Medicine, USA
"Advice on fish consumption: benefits and risks" 2004. SACN/COT, Storbritannia
"Helhedssyn på fisk og fiskevarer" 2003. Fødevaredirektoratet, Danmark
EFSA (2005), WHO/FAO(2003, 2009?), etc
Undeland et al., 2009 Seafood and health - what is the full story? MARIFUNC
"Et helhetssyn på fisk og annet sjømat i norsk kosthold" 2006.VKM, Norway
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7

Getting information on health effects

Human studies, clinical trials

Highest level of evidence

-Firm control of diet, study length, type of subjects, etc.
-Expensive
-Impossible to conduct on some diseases

Observational (Epidemiological) studies

Segment of population observed

-Fish intake associated with diseases
-Indication of correlation
-Many confounding factors

Animal Studies

Extrapolation to humans is limited

-Can excert tight control over experimental conditions
-Insights into designing human studies

***In vitro* (test tube) studies**

Hardest to extrapolate to humans

-Important in early phases

-Can give directions

marifunc THE NORDIC NETWORK FOR MARINE FUNCTIONAL FOOD

I. Undeland Sept 2008

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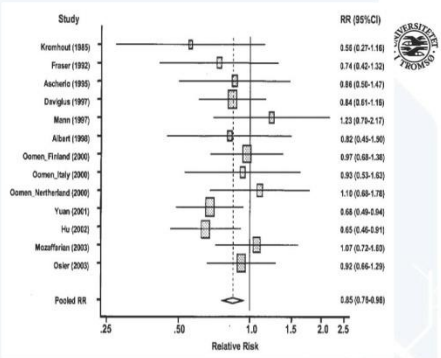
Fish consumption and CHD mortality

- Meta-analysis, observational, 222.364 individuals
- Inverse association between fish consumption and CHD mortality rates
- Fish meal once a week, reduced risk of CHD death by 15 % compared to
- May be further reduced with additional consumption
  - 7% decrease with per additional serving per week



He et al., Circulation, 2004

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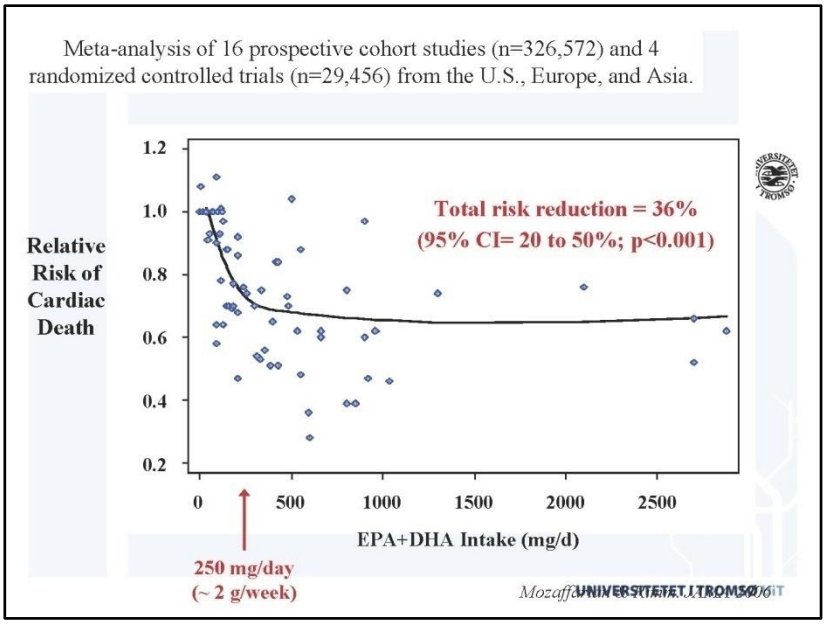
Meta-analysis of 16 prospective cohort studies (n=326,572) and 4 randomized controlled trials (n=29,456) from the U.S., Europe, and Asia.
Relative Risk of Cardiac Death
1.2
1.0
0.8
0.6
0.4
0.2
Total risk reduction = 36%
(95% CI= 20 to 50%; p<0.001)
0
500
1000
1500
2000
2500
EPA+DHA Intake (mg/d)
250 mg/day
(~ 2 g/week)
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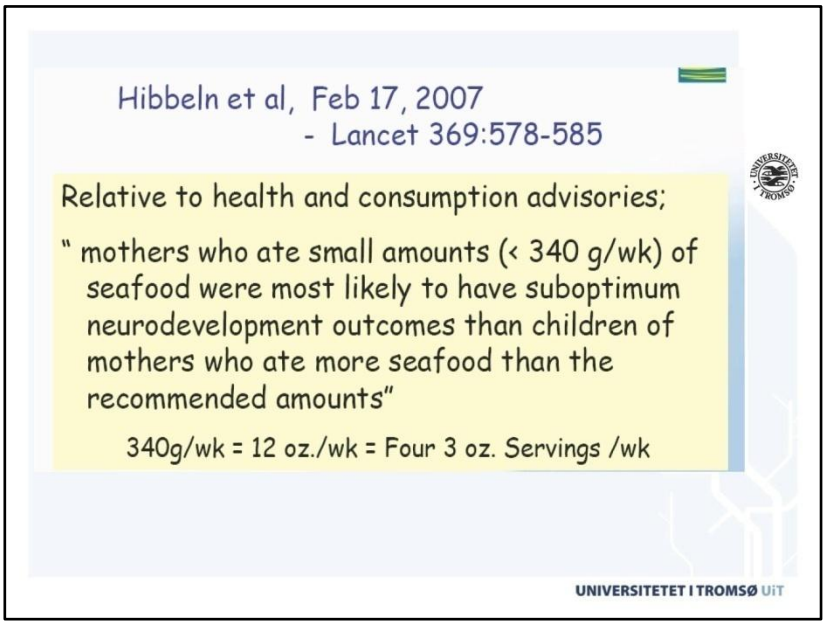
10


Hibbeln et al, Feb 17, 2007
- Lancet 369:578-585
Relative to health and consumption advisories;
" mothers who ate small amounts (< 340 g/wk) of seafood were most likely to have suboptimum neurodevelopment outcomes than children of mothers who ate more seafood than the recommended amounts"
340g/wk = 12 oz./wk = Four 3 oz. Servings /wk
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11

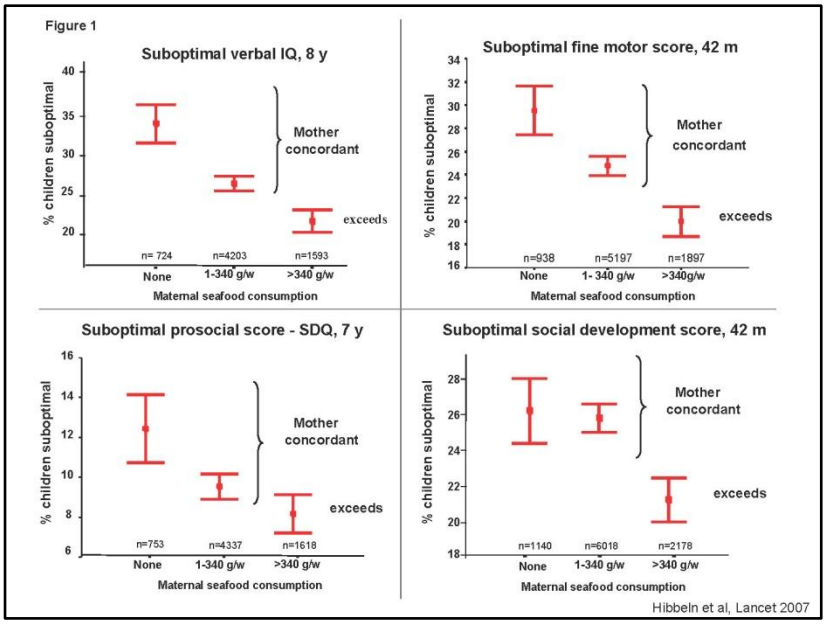
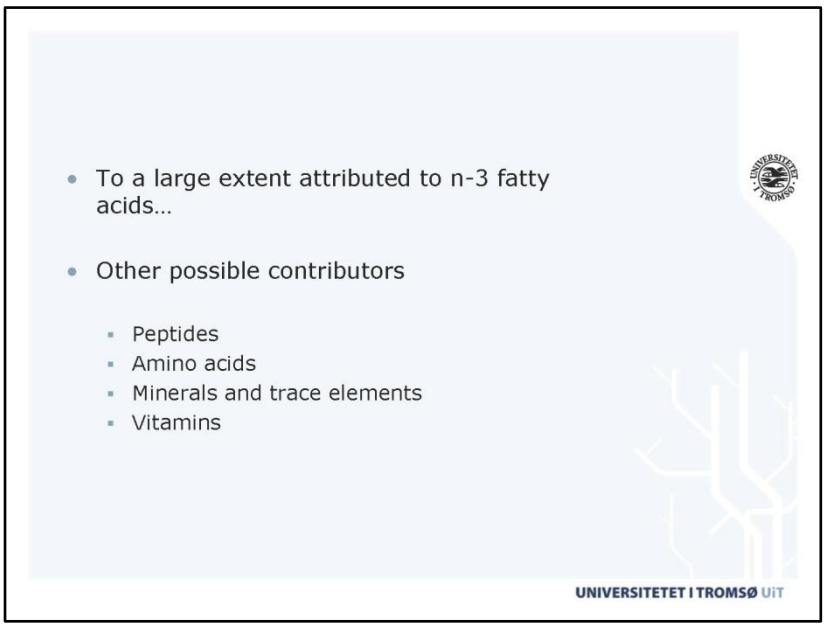


Figure 1
Suboptimal verbal IQ, 8 y
% children suboptimal
Mother concordant
exceeds
n= 724
n=4203
n=1593
None
1-340 g/w
>340 g/w
Maternal seafood consumption
Suboptimal fine motor score, 42 m
% children suboptimal
Mother concordant
exceeds
n=938
n=5197
n=1897
None
1- 340 g/w
>340g/w
Maternal seafood consumption
Suboptimal prosocial score - SDQ, 7 y
% children suboptimal
Mother concordant
exceeds
n=753
n=4337
n=1618
None
1-340 g/w
>340 g/w
Maternal seafood consumption
Suboptimal social development score, 42 m
% children suboptimal
Mother concordant
exceeds
n=1140
n=6018
n=2178
None
1-340 g/w
>340 g/w
Maternal seafood consumption
Hibbeln et al, Lancet 2007

12


To a large extent attributed to n-3 fatty acids…
Other possible contributors
Peptides
Amino acids
Minerals and trace elements
Vitamins
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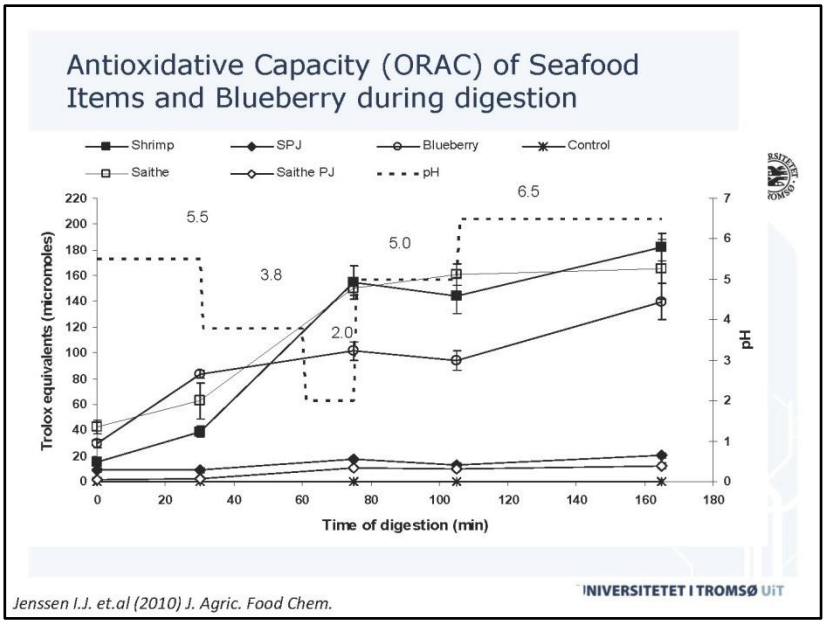
13


Antioxidative Capacity (ORAC) of Seafood Items and Blueberry during digestion
Shrimp
SPJ
Blueberry
Control
Saithe
Saithe PJ
pH
5.5
3.8
2.0
5.0
6.5
Trolox equivalents (micromoles)
pH
Time of digestion (min)
Jenssen I.J. et.al (2010) J. Agric. Food Chem.
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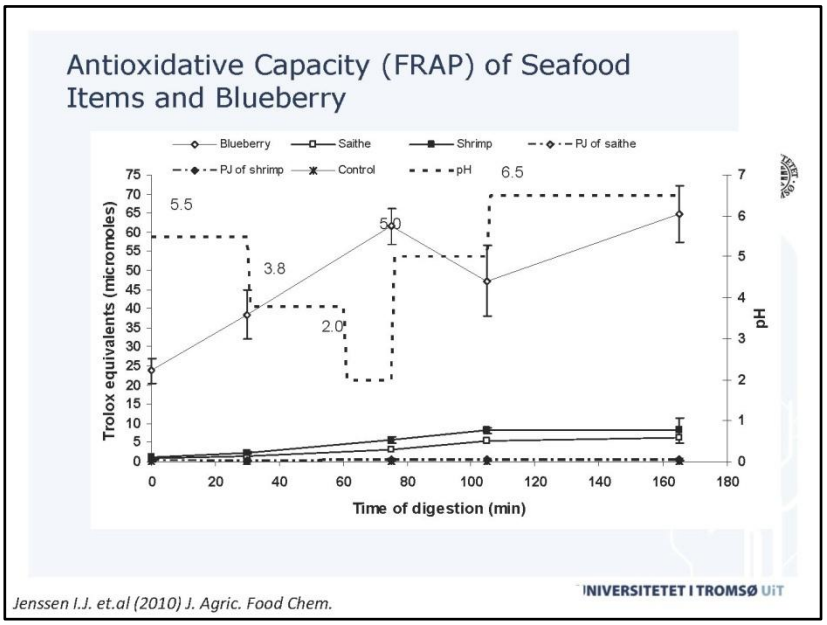
Antioxidative Capacity (FRAP) of Seafood Items and Blueberry
Blueberry
Saithe
Shrimp
PJ of saithe
PJ of shrimp
Control
pH
5.5
3.8
2.0
5.0
6.5
Trolox equivalents (micromoles)
pH
Time of digestion (min)
Jenssen I.J. et.al (2010) J. Agric. Food Chem.
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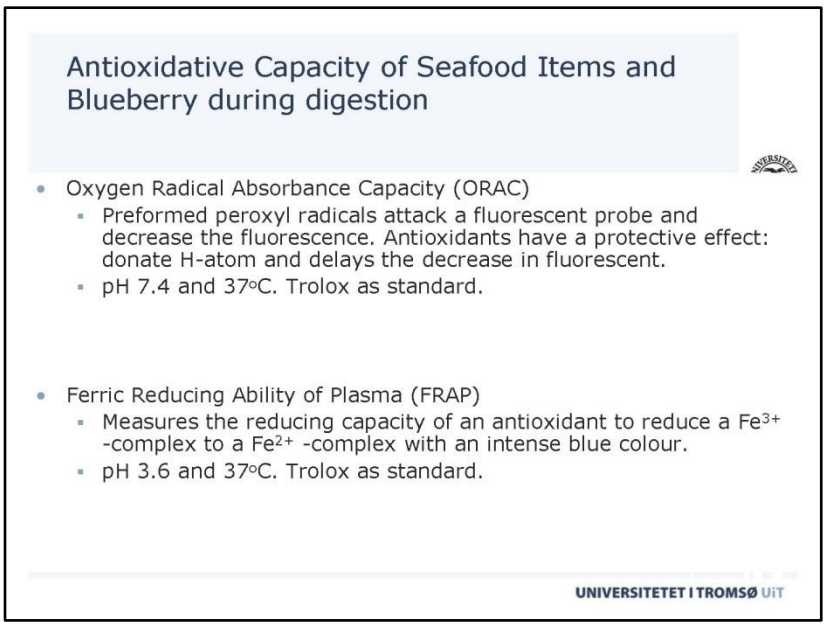
15


Antioxidative Capacity of Seafood Items and Blueberry during digestion
Oxygen Radical Absorbance Capacity (ORAC)
Preformed peroxyl radicals attack a fluorescent probe and decrease the fluorescence. Antioxidants have a protective effect: donate H-atom and delays the decrease in fluorescent.
pH 7.4 and 37°C. Trolox as standard.
Ferric Reducing Ability of Plasma (FRAP)
Measures the reducing capacity of an antioxidant to reduce a Fe3+ -complex to a Fe2+ -complex with an intense blue colour.
pH 3.6 and 37°C. Trolox as standard.
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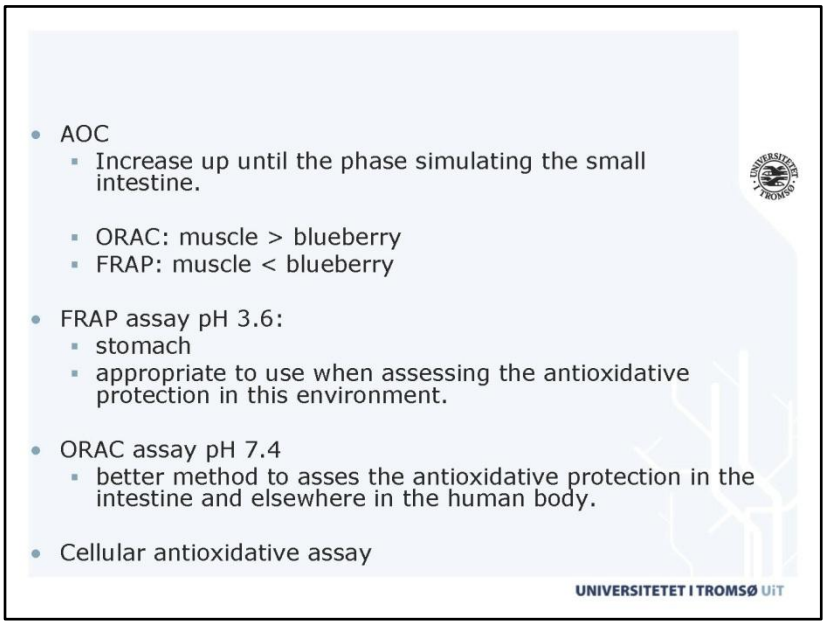
AOC
Increase up until the phase simulating the small intestine.
ORAC: muscle > blueberry
FRAP: muscle < blueberry
FRAP assay pH 3.6:
stomach
appropriate to use when assessing the antioxidative protection in this environment.
ORAC assay pH 7.4
better method to asses the antioxidative protection in the intestine and elsewhere in the human body.
Cellular antioxidative assay
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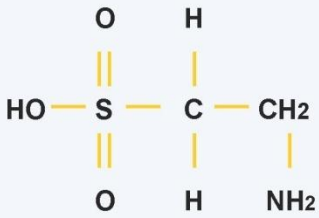
Taurine



- Free sulfonated amino acid.
- Shown to be a marker of seafood consumption.

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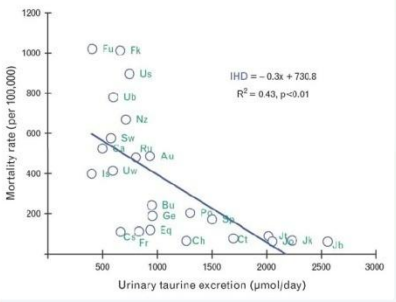
Dietary markers on IHD mortality



- Associations between various dietary markers and mortality from IHD
- Males, n=2462
- "In our present study, taurine excretion, in fact, appears as the most significant factor (inversely) in IHD mortality"

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Yamori et al., J. Hypertension, 2006

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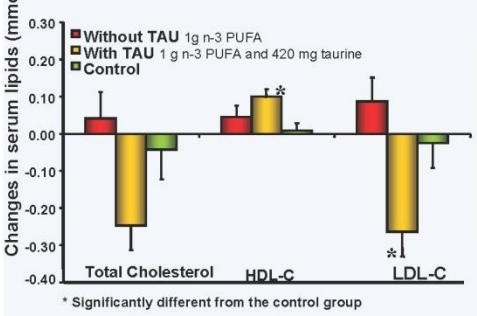
Taurine supplementation



- Effects of taurine supplementation on blood lipids in levels comparable to a diet high in seafood
- Randomized, double-blind, parallel intervention trial
  - 110 healthy individuals
  - 7 weeks, fish pâté
- Reduced total cholesterol (4 %) and LDL (8%)
- HDL increased (6%)
- More clinical trials needed

Elvevoll et al., Atherosclerosis 2008

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Taurine is abundant in seafood

- Intervention studies in animals and humans have suggested that protective effect may act through several mechanisms
  - Antioxidative properties
  - Reduce pro - inflammatory products
  - Suppress atherosclerosis
  - Platelet aggregation, reduce blood cholesterol, improved cardiac performance
- Study
  - The mechanism (s) needs to be studied
  - The relevance and the effect of taurine in **amounts** normally acquired trough **the diet** should be studied
  - Possible interactions with other components abundant in seafood

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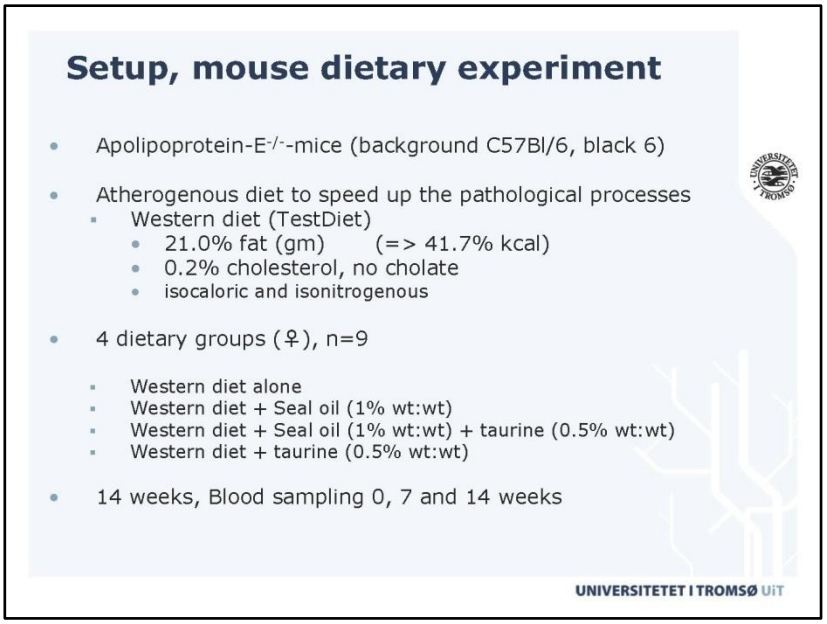
Setup, mouse dietary experiment
Apolipoprotein-E-/--mice (background C57Bl/6, black 6)
Atherogenous diet to speed up the pathological processes
Western diet (TestDiet)
21.0% fat (gm) (=> 41.7% kcal)
0.2% cholesterol, no cholate
isocaloric and isonitrogenous
4 dietary groups (♀), n=9
Western diet alone
Western diet + Seal oil (1% wt:wt)
Western diet + Seal oil (1% wt:wt) + taurine (0.5% wt:wt)
Western diet + taurine (0.5% wt:wt)
14 weeks, Blood sampling 0, 7 and 14 weeks
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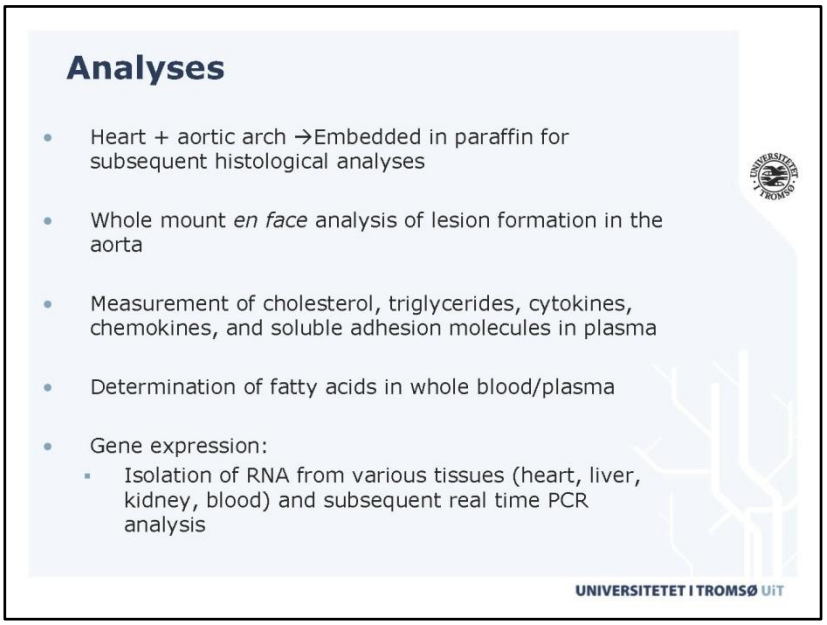
22


Analyses
Heart + aortic arch →Embedded in paraffin for subsequent histological analyses
Whole mount en face analysis of lesion formation in the aorta
Measurement of cholesterol, triglycerides, cytokines, chemokines, and soluble adhesion molecules in plasma
Determination of fatty acids in whole blood/plasma
Gene expression:
Isolation of RNA from various tissues (heart, liver, kidney, blood) and subsequent real time PCR analysis
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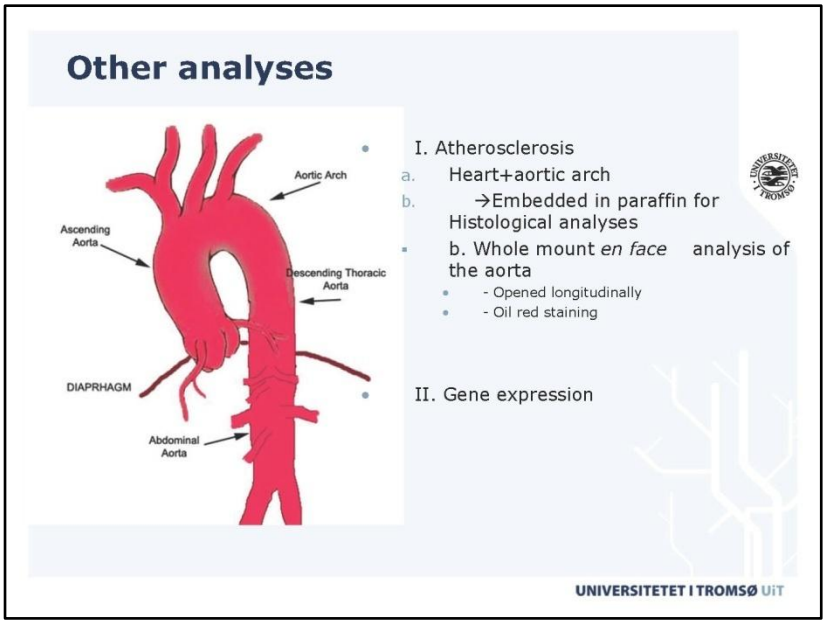
23


Other analyses
Aortic Arch
Ascending Aorta
Descending Thoracic Aorta
DIAPRHAGM
Abdominal Aorta
I. Atherosclerosis
a. Heart+aortic arch
b. →Embedded in paraffin for Histological analyses
b. Whole mount *en face* analysis of the aorta
- Opened longitudinally
- Oil red staining
II. Gene expression
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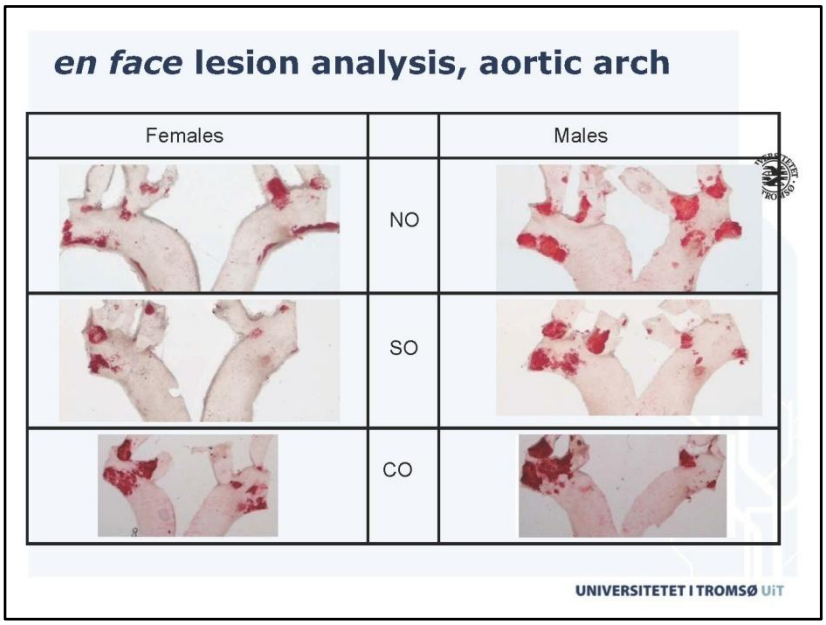
en face lesion analysis, aortic arch
Females
Males
NO
SO
CO
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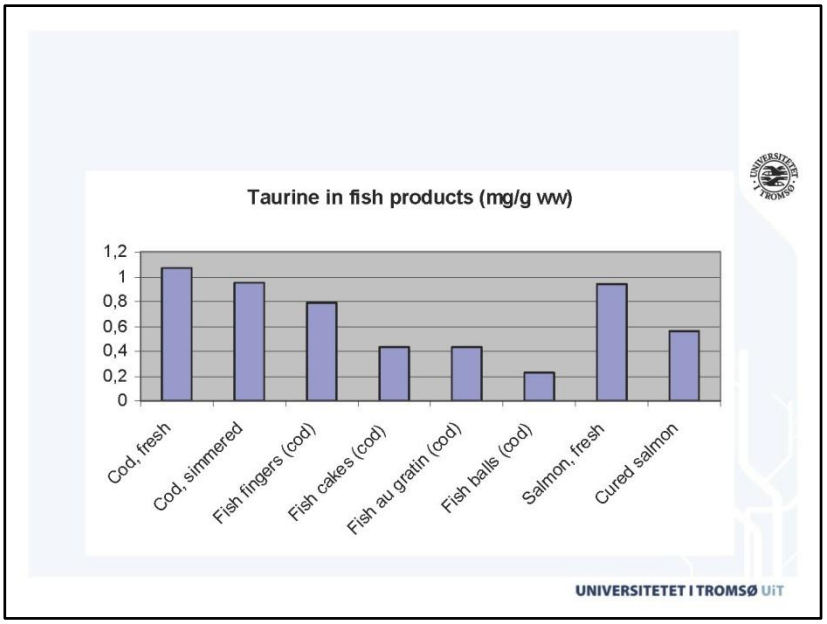
Taurine in fish products (mg/g ww)
1,2
1
0,8
0,6
0,4
0,2
0
Cod, fresh
Cod, simmered
Fish fingers (cod)
Fish cakes (cod)
Fish au gratin (cod)
Fish balls (cod)
Salmon, fresh
Cured salmon
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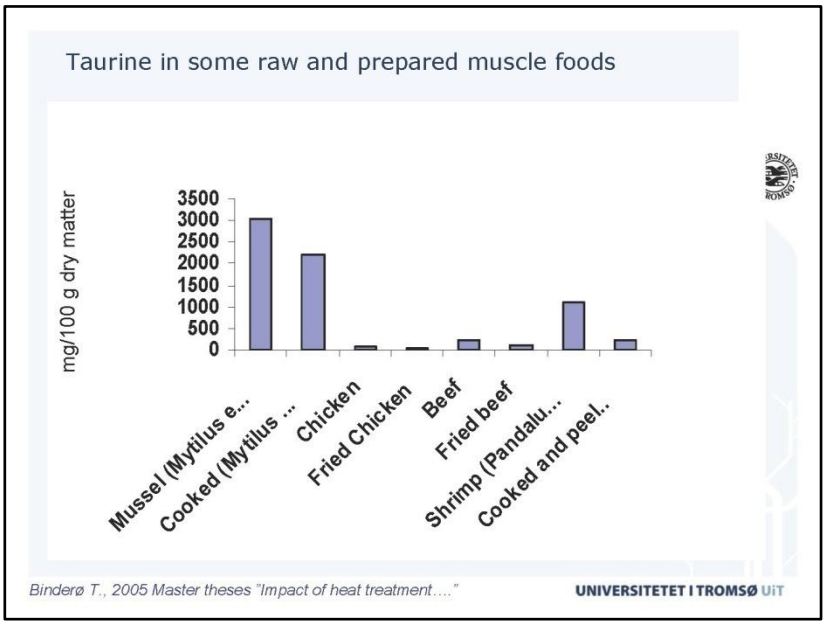
26


Taurine in some raw and prepared muscle foods
mg/100 g dry matter
3500
3000
2500
2000
1500
1000
500
0
Mussel (Mytilus e...
Cooked (Mytilus ...
Chicken
Fried Chicken
Beef
Fried beef
Shrimp (Pandalu...
Cooked and peel..
Binderø T., 2005 Master theses "Impact of heat treatment...."
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27

The end – thank you

Karl-Erik Eilertsen,
Hege Devold,
Hanne K. Mæhre,
Ida J. Jensen,
Bjørn Tore Dragnes,
Rune Larsen,
Svein Kristian Stormo,
Jan Ole Olsen,
Jan Brox,
Bjarne Østerud

Foto: Ole Torrissen, HI

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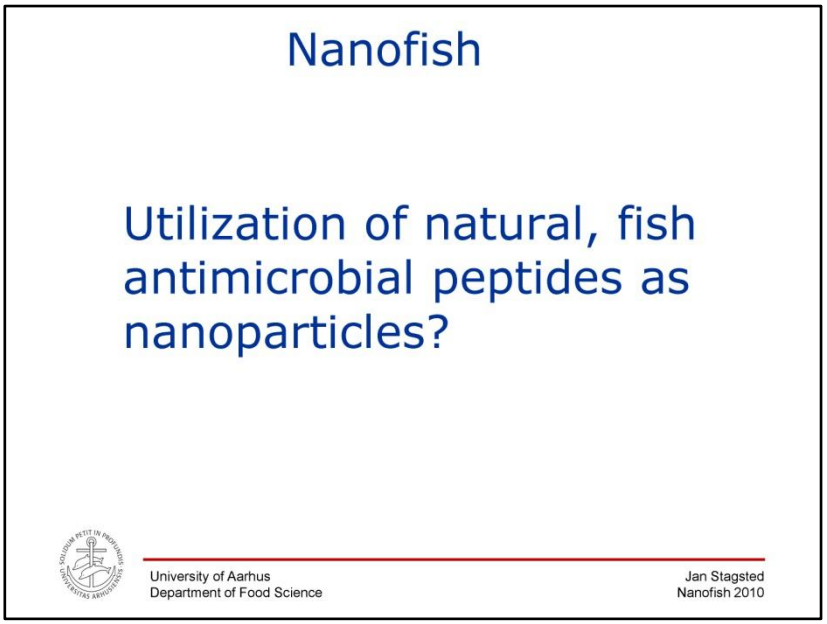
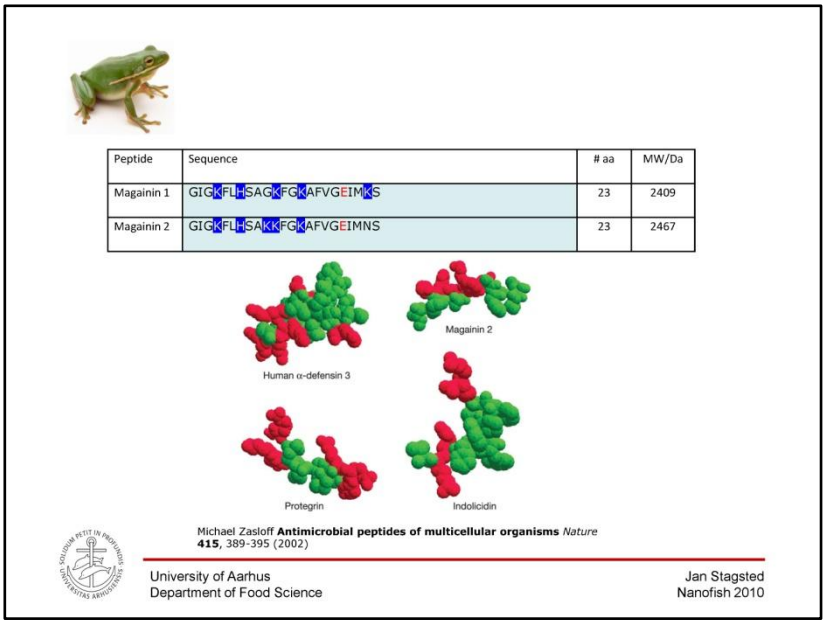
Appendix 6. ”Nanofish – utilization of natural fish antimicrobial peptides as nanoparticles?” by Jan Stagsted

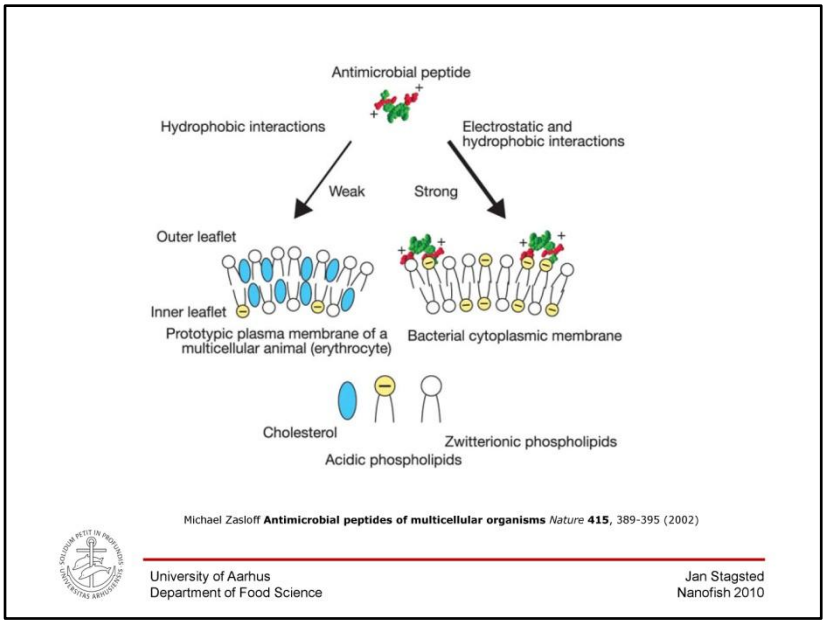
1



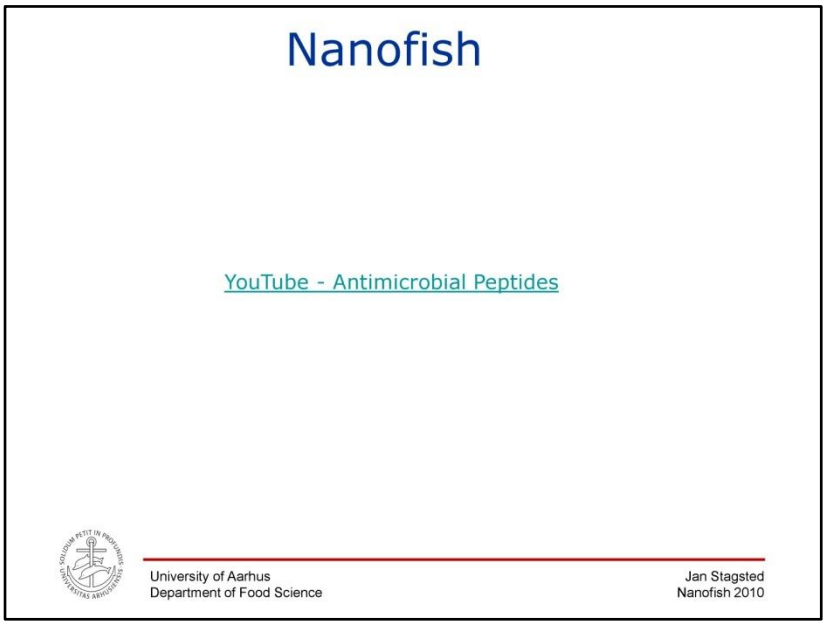
2



3


Antimicrobial peptide
Hydrophobic interactions
Electrostatic and hydrophobic interactions
Weak
Strong
Outer leaflet
Inner leaflet
Prototypic plasma membrane of a multicellular animal (erythrocyte)
Bacterial cytoplasmic membrane
Cholesterol
Zwitterionic phospholipids
Acidic phospholipids
Michael Zasloff **Antimicrobial peptides of multicellular organisms** *Nature* **415**, 389-395 (2002)
University of Aarhus
Department of Food Science
Jan Stagsted
Nanofish 2010

4


Nanofish
YouTube - Antimicrobial Peptides
University of Aarhus
Department of Food Science
Jan Stagsted
Nanofish 2010

5

Welcome to the Antimicrobial Peptide Database and Analysis System

Wang G, Li X, Wang Z. **APD2: the updated antimicrobial peptide database and its application in peptide design.** Nucleic Acids Res. 2009 Jan;37(Database issue):D933-7

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Department of Food Science

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6

Number of species:	~6,300	~30,000
		
Number of AMP:	548	50

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Department of Food Science

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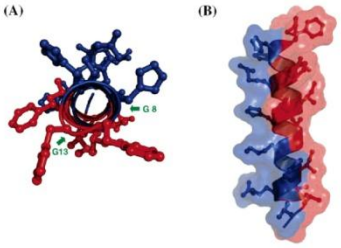
Nanofish 2010

7

Peptide	Sequence	# aa	MW/Da
Piscidin 1	FFHHIFRGIVHVGKTIHRLVTG	22	2571
Piscidin 2	FFHHIFRGIVHVGKTIHRLVTG	22	2543
Piscidin 3	FIHHIFRGIVHAGRSIGRFLTG	22	2491
Piscidin 4	FFRHLFRGAKAIFRGARQGKRAHKVVSRVRNRDVPETDNNQEEP	44	5329



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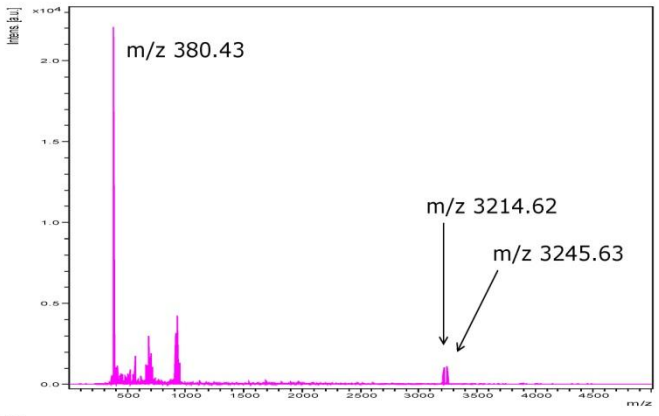
8

Monkfish peptides in acidic extract (0.1 N HCl) by MALDI-MS



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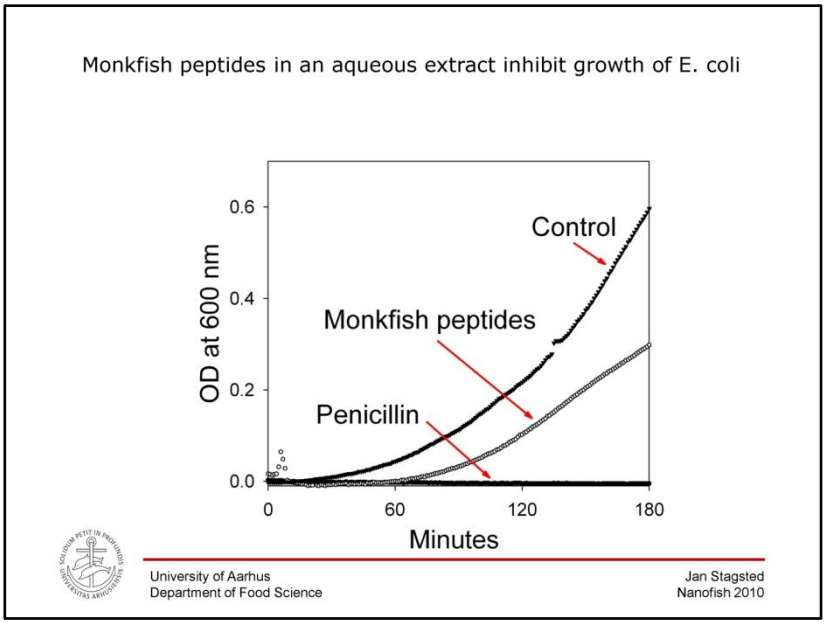
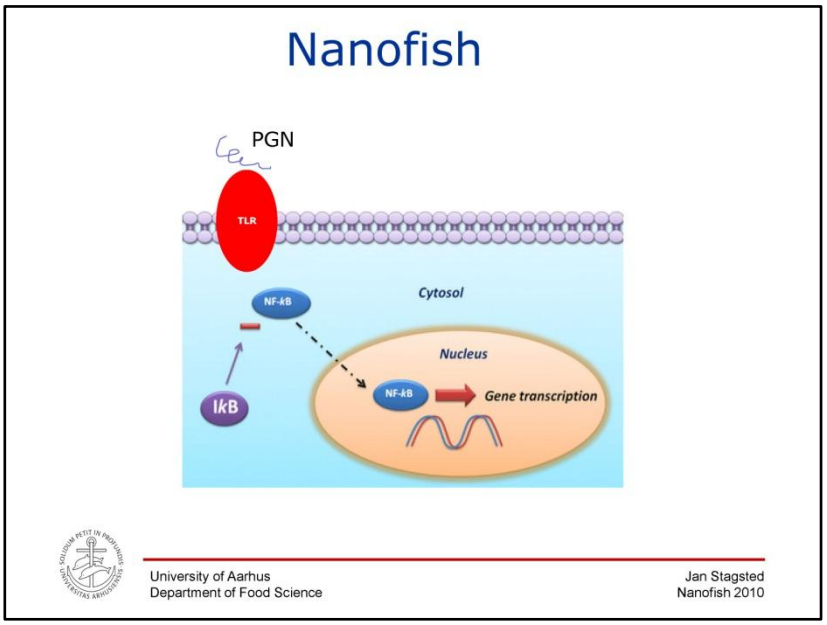
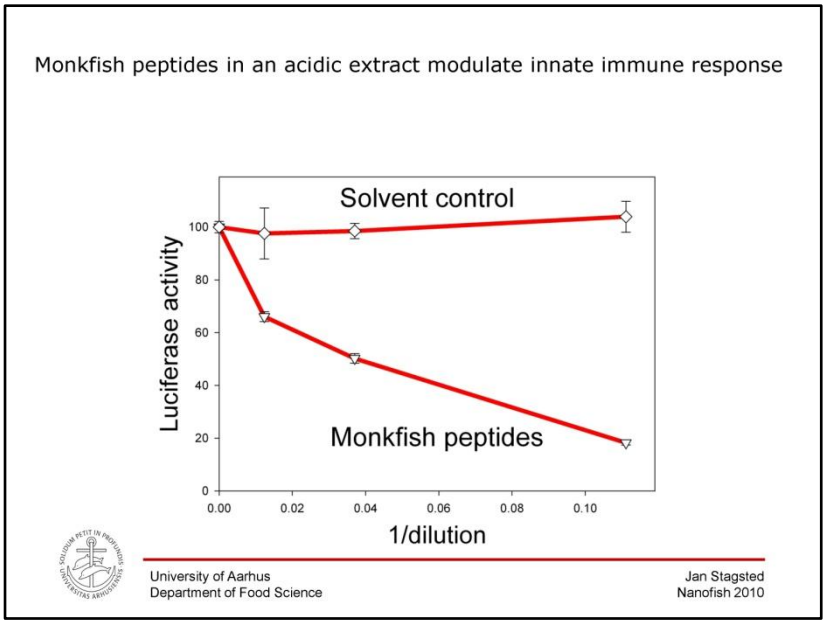
9


Monkfish peptides in an aqueous extract inhibit growth of E. coli
OD at 600 nm
0.0
0.2
0.4
0.6
0
60
120
180
Minutes
Control
Monkfish peptides
Penicillin
University of Aarhus
Department of Food Science
Jan Stagsted
Nanofish 2010

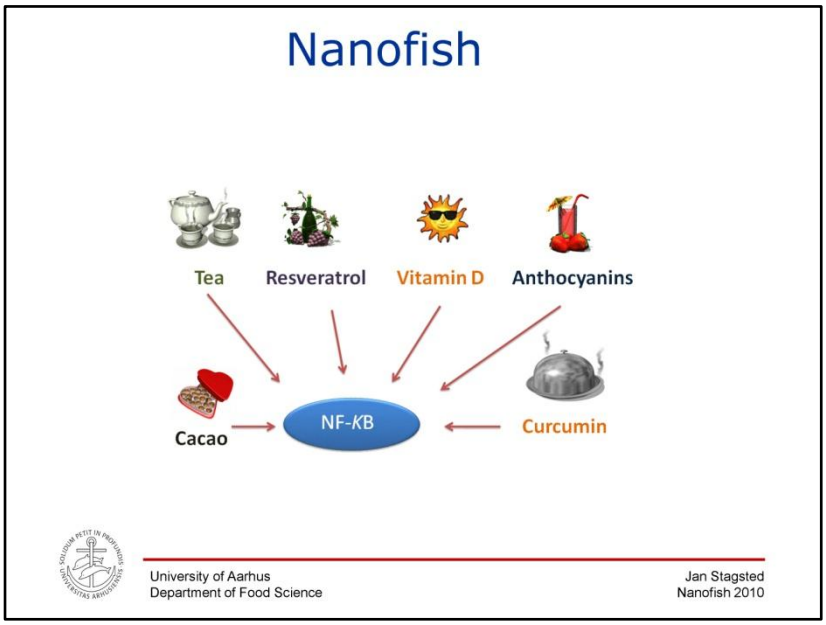
10


Nanofish
PGN
TLR
Cytosol
NF-kB
IkB
Nucleus
NF-kB
Gene transcription
University of Aarhus
Department of Food Science
Jan Stagsted
Nanofish 2010

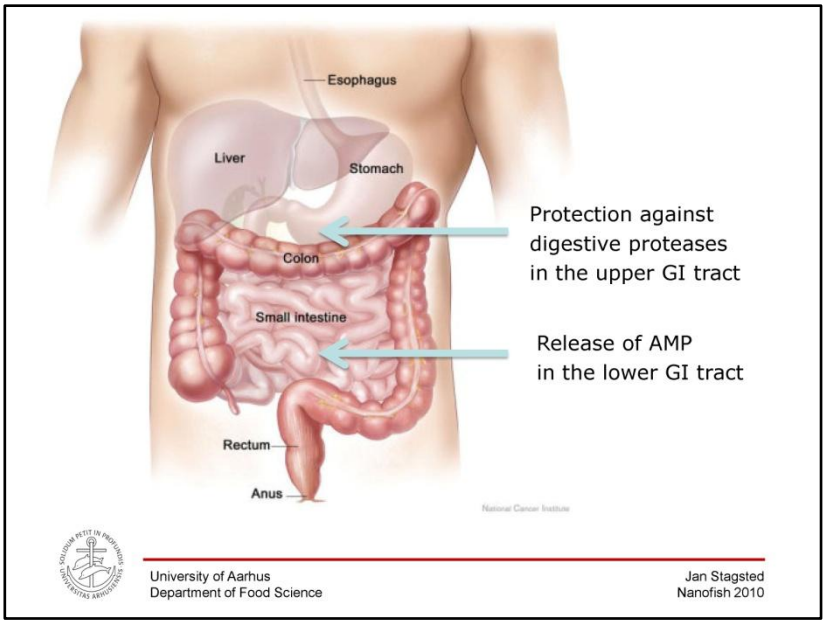
11


Monkfish peptides in an acidic extract modulate innate immune response
Solvent control
Monkfish peptides
Luciferase activity
1/dilution
0
20
40
60
80
100
0.00
0.02
0.04
0.06
0.08
0.10
University of Aarhus
Department of Food Science
Jan Stagsted
Nanofish 2010

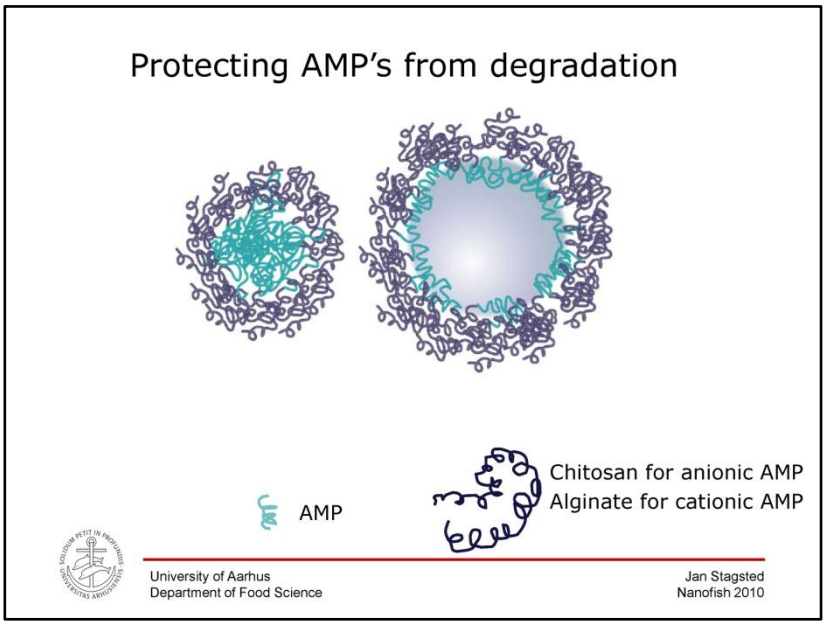
12


Nanofish
Tea
Resveratrol
Vitamin D
Anthocyanins
Cacao
NF-KB
Curcumin
University of Aarhus
Department of Food Science
Jan Stagsted
Nanofish 2010

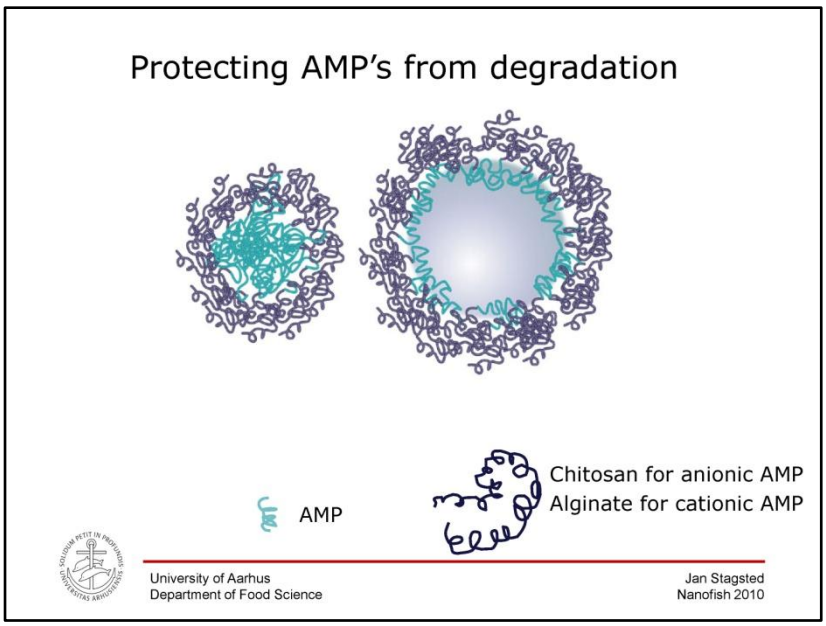
13


Esophagus
Liver
Stomach
Colon
Small intestine
Rectum
Anus
Protection against
digestive proteases
in the upper GI tract
Release of AMP
in the lower GI tract
University of Aarhus
Department of Food Science
Jan Stagsted
Nanofish 2010

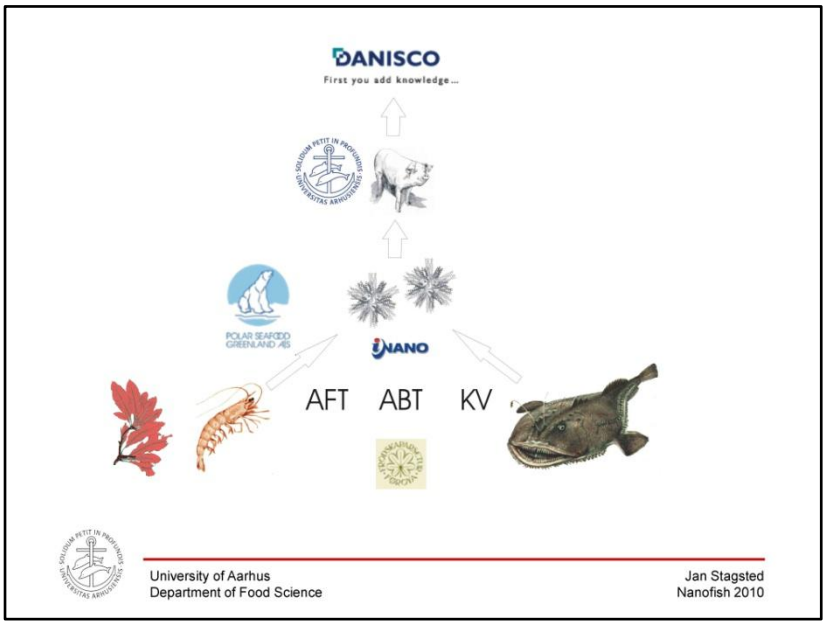
14


Protecting AMP's from degradation
AMP
Chitosan for anionic AMP
Alginate for cationic AMP
University of Aarhus
Department of Food Science
Jan Stagsted
Nanofish 2010

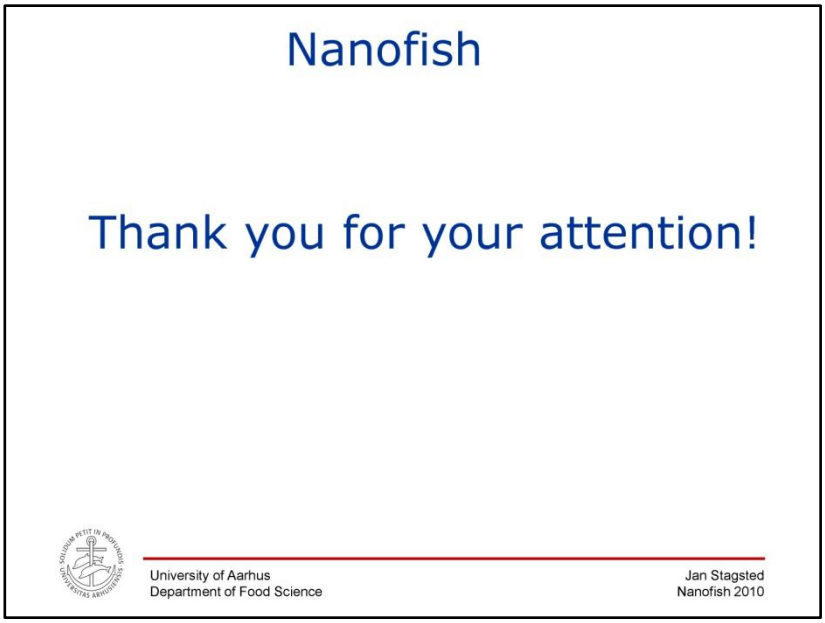
15


Protecting AMP's from degradation
AMP
Chitosan for anionic AMP
Alginate for cationic AMP
University of Aarhus
Department of Food Science
Jan Stagsted
Nanofish 2010

16

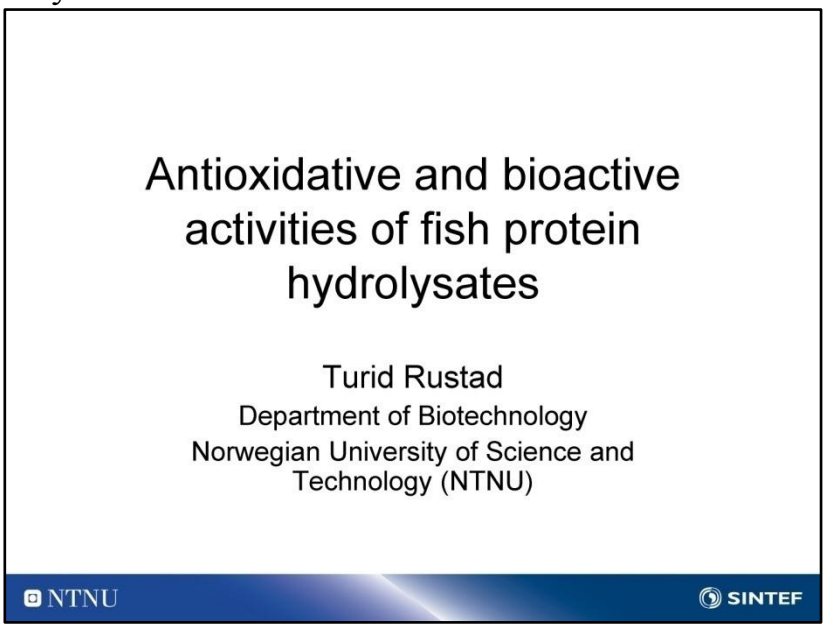

DANISCO
First you add knowledge...
POLAR SEAFOOD GREENLAND A/S
iNANO
AFT
ABT
KV
University of Aarhus
Department of Food Science
Jan Stagsted
Nanofish 2010

17

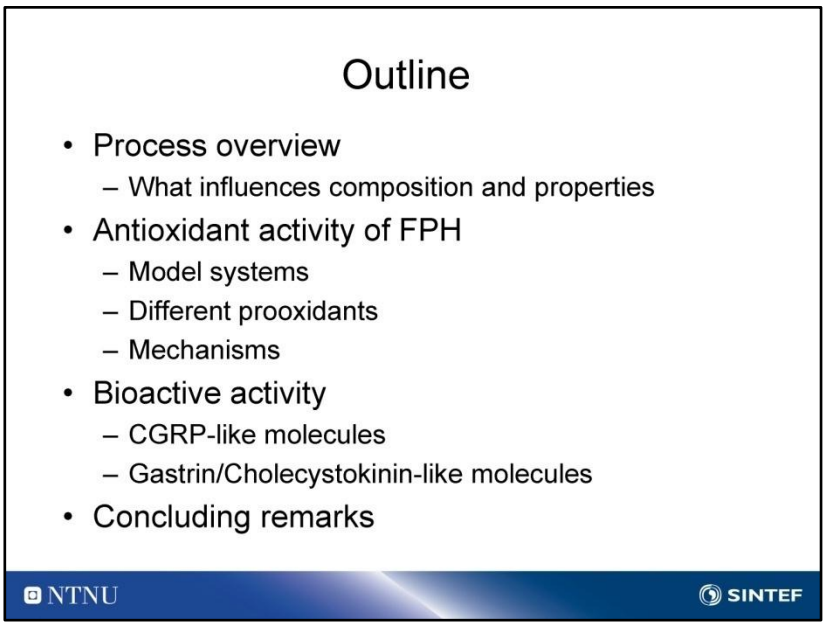

Nanofish
Thank you for your attention!
University of Aarhus
Department of Food Science
Jan Stagsted
Nanofish 2010

Appendix 7. ”Antioxidative and bioactive activities of fish protein hydrolysates” by Turid Rustad

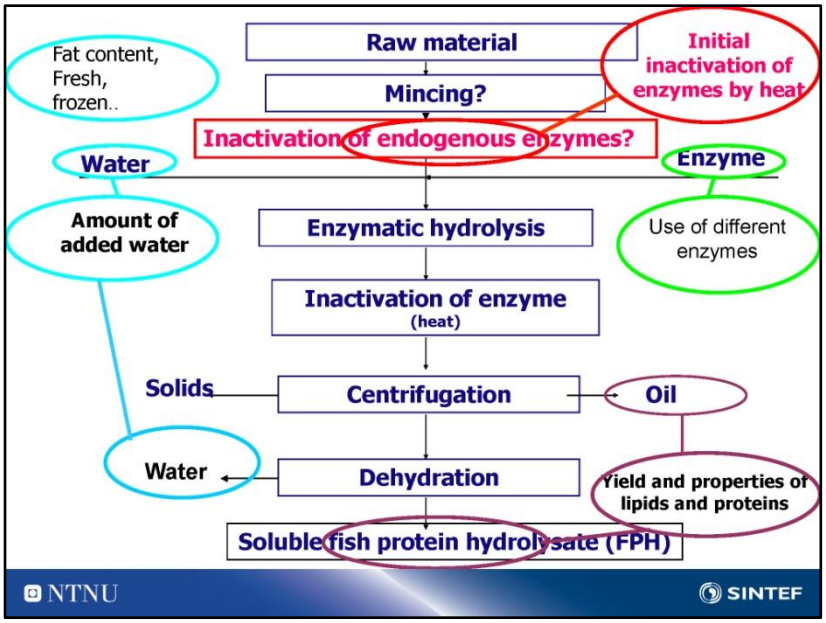
1



2



3


Raw material
Fat content, Fresh, frozen..
Initial inactivation of enzymes by heat
Mincing?
Inactivation of endogenous enzymes?
Water
Enzyme
Amount of added water
Enzymatic hydrolysis
Use of different enzymes
Inactivation of enzyme (heat)
Solids
Centrifugation
Oil
Water
Dehydration
Yield and properties of lipids and proteins
Soluble fish protein hydrolysate (FPH)
NTNU
SINTEF

4


Raw material
• Cod backbones after removal of flesh
– Frozen or fresh backbones
• Different time of hydrolysis
• Commercial fish powders
– Norland HFC
– Aroma (New Zealand)
– Powders from Danish fish protein
• MariPep C
• MariPep P
• MariPep CK
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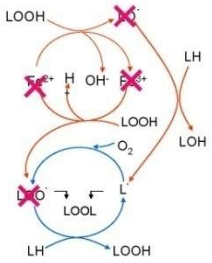
SINTEF

5

Antioxidative properties

- Peptides from fish proteins have antioxidative properties in different oxidative systems
- Antioxidative mechanisms related to the ability of peptides and hydrolysates to:
  - ***scavenge radicals***
  - ***chelate transition metals***
  - alter the development of rancidity in unsaturated oils by ***adducting volatile aldehydes***
- Antioxidative properties depends on
  - ***amino acid*** sequence
  - ***molecular weight***
  - ***pH***



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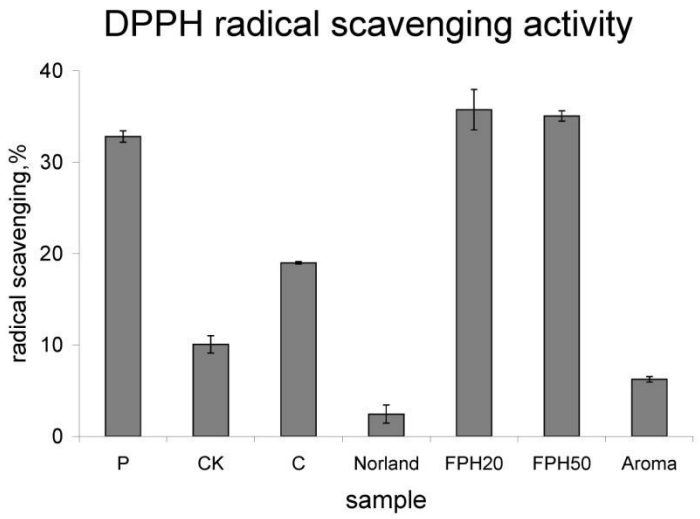
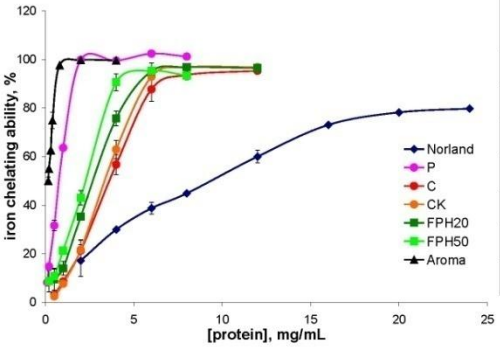
6



NTNU SINTEF

7

Iron chelating ability



	g pr/uM Fe
FPH20	1.5
FPH50	1.0
MariPep P	0.5
MariPep C	1.5
MariPep CK	1.5
Norland HFC	5.0
Aroma powder	0.2

Fe^{2+} concentration - 4nMol/mL

interaction time between protein and iron - 60 min

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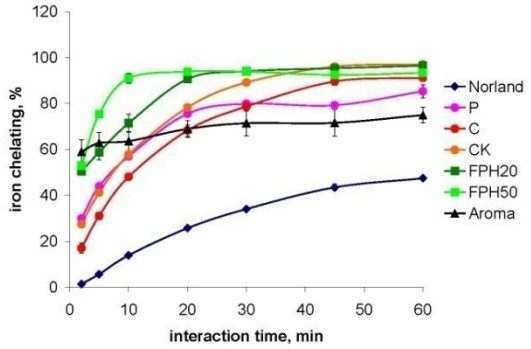
8

Kinetics of iron chelation



Protein concentration: C, Norland, CK, FPH20, FPH50 - 8 mg/mL;
P sample – 1.6mg/mL Aroma – 0.08mg/mL
The concentration of added Fe^{2+} in the final reaction mixture – 4nMol/mL

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9

Fe and haemoglobin mediated oxidation

	Two different types of lipid oxidations	
Prooxidants	**Fe**	**Haemoglobin**
Effect of prooxidant	Linear	Michaelis-Menten
Dissolved oxygen	Not dependent	Dependent (1.order kinetics)
pH	Optimum at pH 4 - 5	Increasing with pH
Temperature	Ea = 60-86kJ/mole K	Ea= 48 kJ/ mole K
Phosphate	Antioxidant	No effect
EDTA	Antioxidant	No effect

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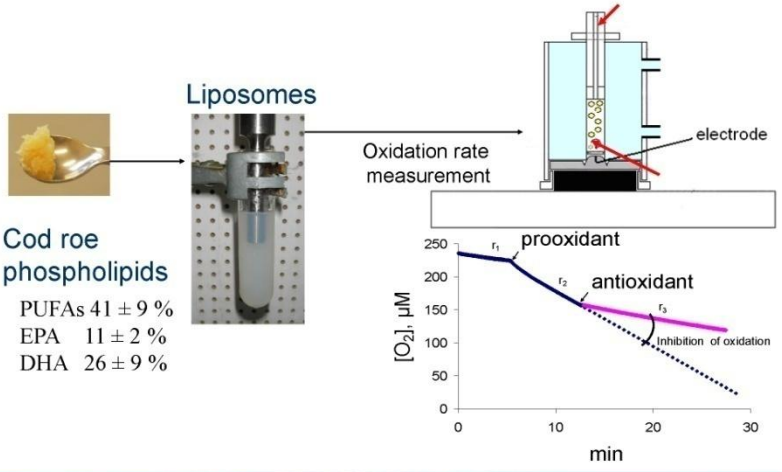
10

A system to measure the oxidation kinetics



Cod roe phospholipids

PUFAs 41 ± 9 %
EPA 11 ± 2 %
DHA 26 ± 9 %

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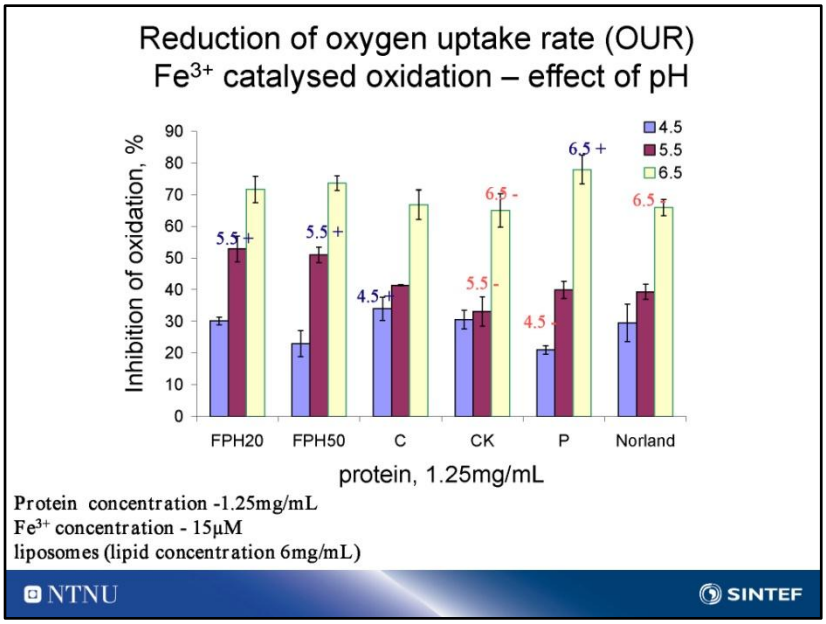
11


Reduction of oxygen uptake rate (OUR)
Fe3+ catalysed oxidation – effect of pH
Inhibition of oxidation, %
4.5
5.5
6.5
FPH20
FPH50
C
CK
P
Norland
protein, 1.25mg/mL
Protein concentration -1.25mg/mL
Fe3+ concentration - 15µM
liposomes (lipid concentration 6mg/mL)
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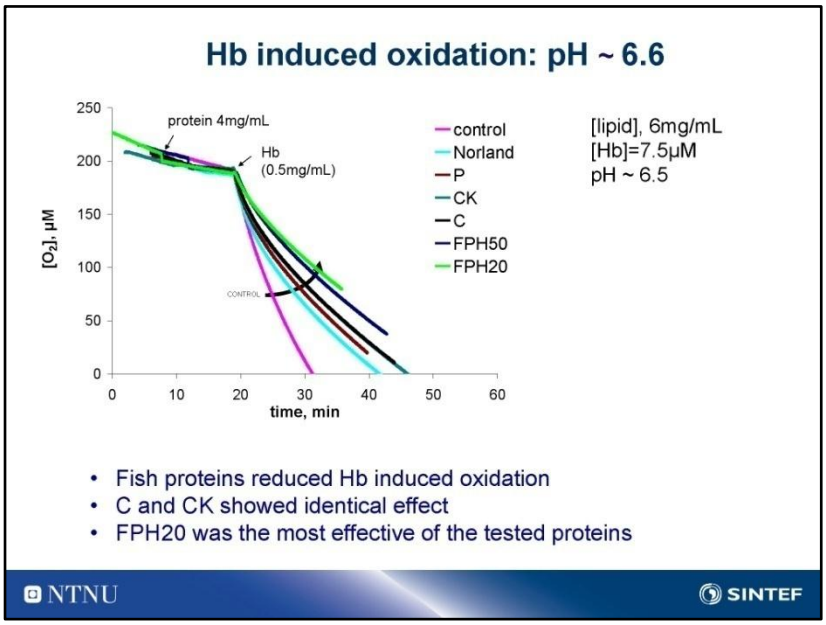
12


Hb induced oxidation: pH ~ 6.6
protein 4mg/mL
Hb
(0.5mg/mL)
[O2], µM
time, min
control
Norland
P
CK
C
FPH50
FPH20
[lipid], 6mg/mL
[Hb]=7.5µM
pH ~ 6.5
• Fish proteins reduced Hb induced oxidation
• C and CK showed identical effect
• FPH20 was the most effective of the tested proteins
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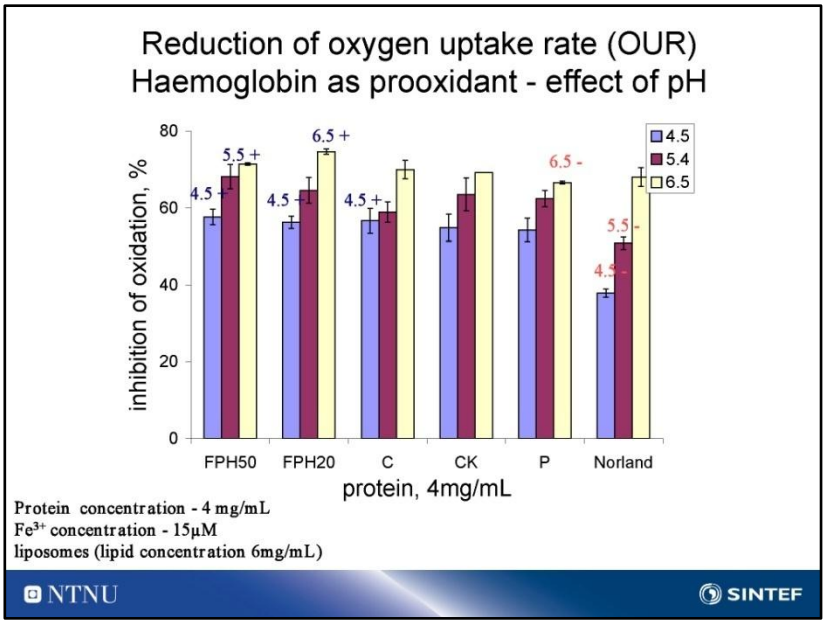
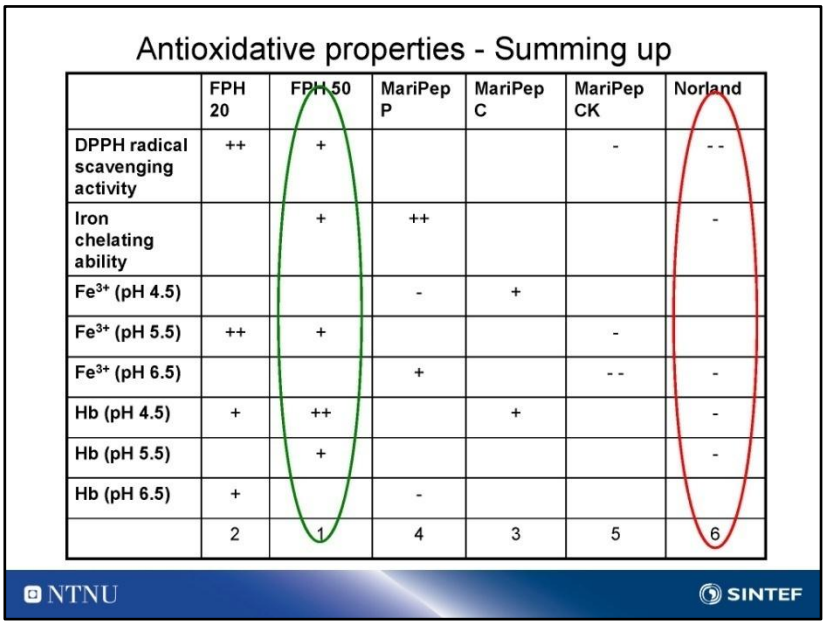
13

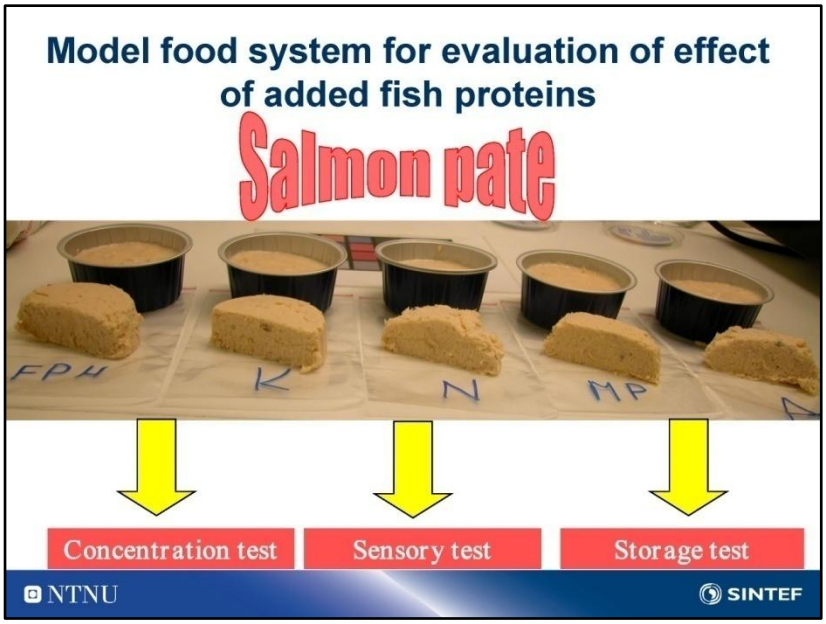


14

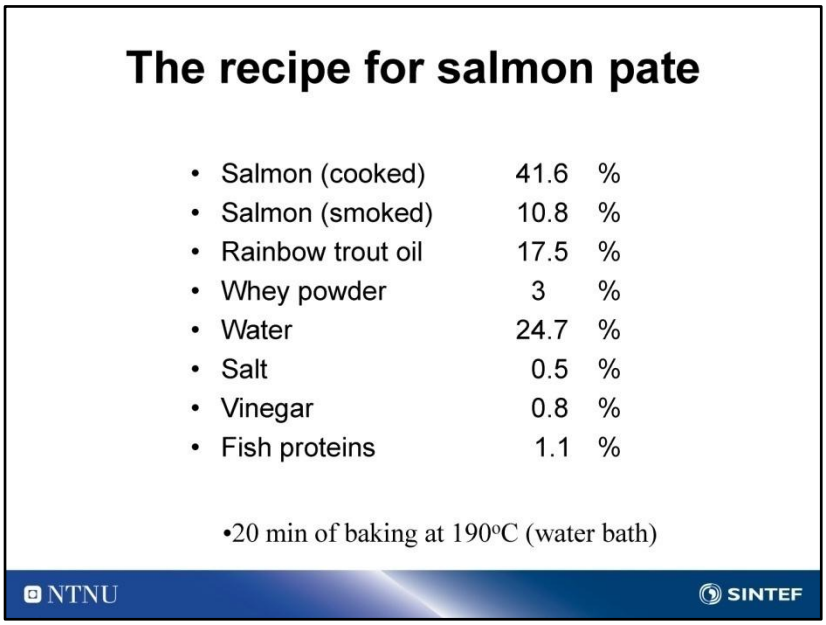


	FPH 20	FPH 50	MariPep P	MariPep C	MariPep CK	Norland
DPPH radical scavenging activity	++	+			-	- -
Iron chelating ability		+	++			-
Fe^{3+} (pH 4.5)			-	+		
Fe^{3+} (pH 5.5)	++	+			-	
Fe^{3+} (pH 6.5)			+		- -	-
Hb (pH 4.5)	+	++		+		-
Hb (pH 5.5)		+				-
Hb (pH 6.5)	+		-			
	2	1	4	3	5	6

15

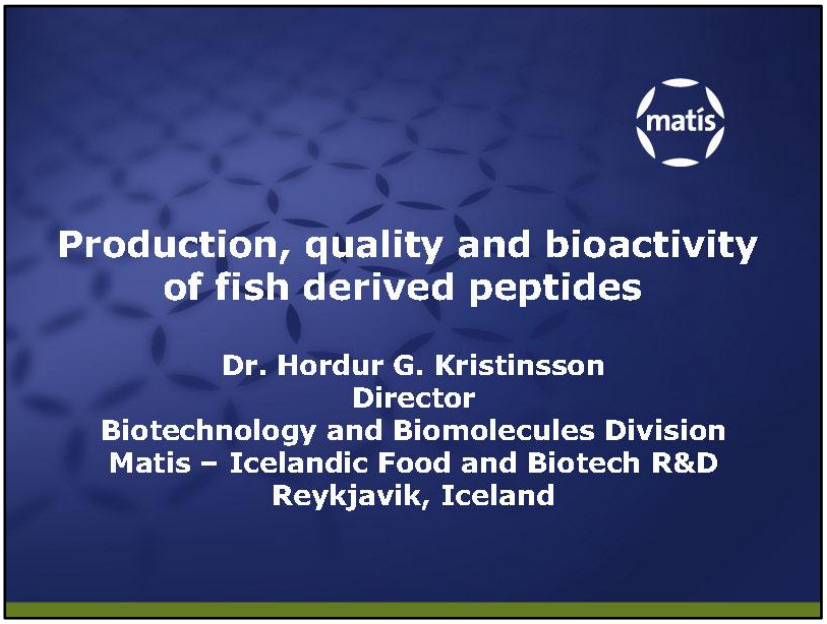

Model food system for evaluation of effect of added fish proteins
Salmon pate
FPH
K
N
MP
Concentration test
Sensory test
Storage test
NTNU
SINTEF

16

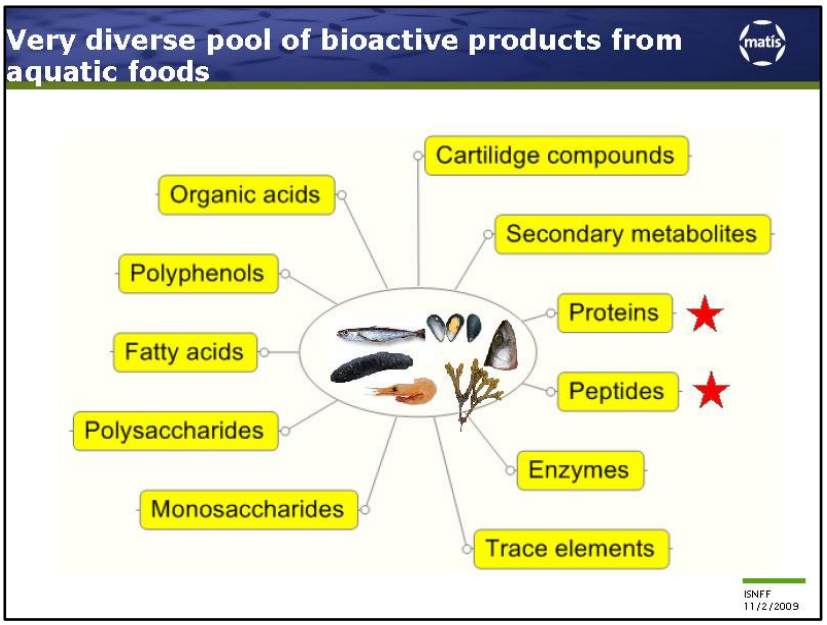

The recipe for salmon pate
• Salmon (cooked) 41.6 %
• Salmon (smoked) 10.8 %
• Rainbow trout oil 17.5 %
• Whey powder 3 %
• Water 24.7 %
• Salt 0.5 %
• Vinegar 0.8 %
• Fish proteins 1.1 %
•20 min of baking at 190ºC (water bath)
NTNU
SINTEF

Appendix 8. ”Production, quality and bioactivity of fish derived peptides” by Hordur G. Kristinsson

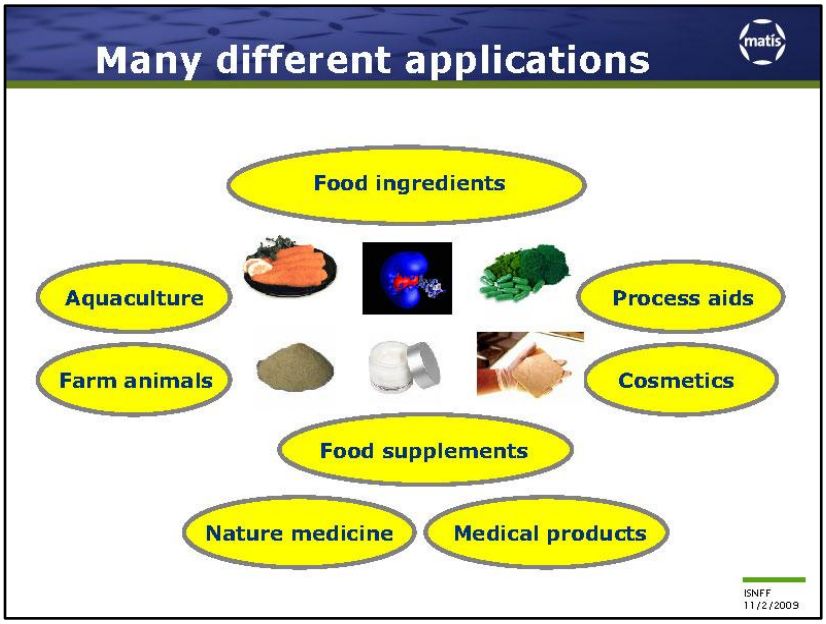
1



2



3


Many different applications
matis
Food ingredients
Aquaculture
Process aids
Farm animals
Cosmetics
Food supplements
Nature medicine
Medical products
ISNFF
11/2/2009

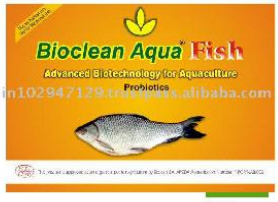
4


USA – functional foods
matis
$30-80 billion in 2009
2011
25% omega-3 products
ISNFF
11/2/2009

5

Animal food market – opportunities

USA - petfood
$50 billion in 2009



ISNFF
11/2/2009

6

Health effects

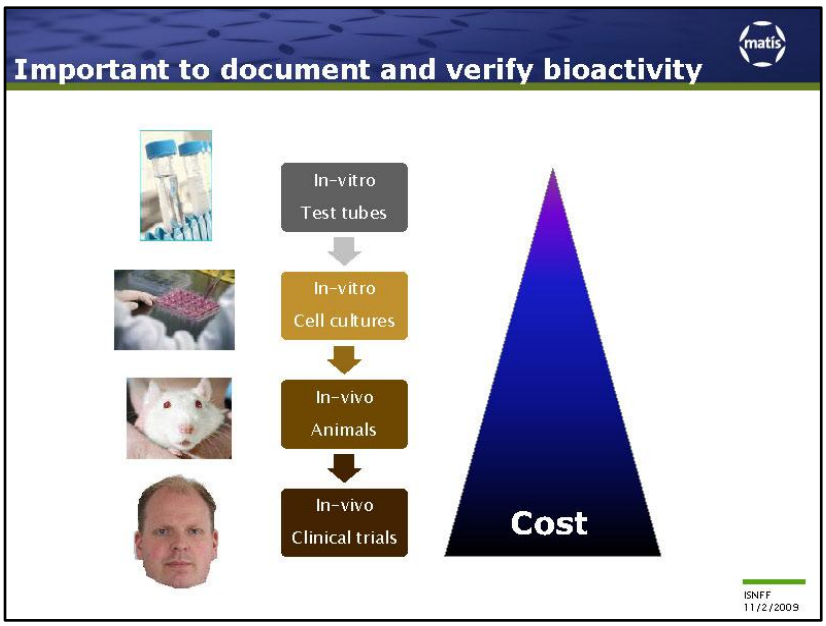
Increased consumer awareness about natural bioactive compounds

- Might work against:
  - Oxidative stress
  - Increased blood pressure
  - High cholesterol
  - Inflammation
  - Cancer
  - Diabetes
  - Obesity

Could play a role in a more healthy living

ISNFF
11/2/2009

7


Important to document and verify bioactivity
matis
In-vitro
Test tubes
In-vitro
Cell cultures
In-vivo
Animals
In-vivo
Clinical trials
Cost
ISNFF
11/2/2009

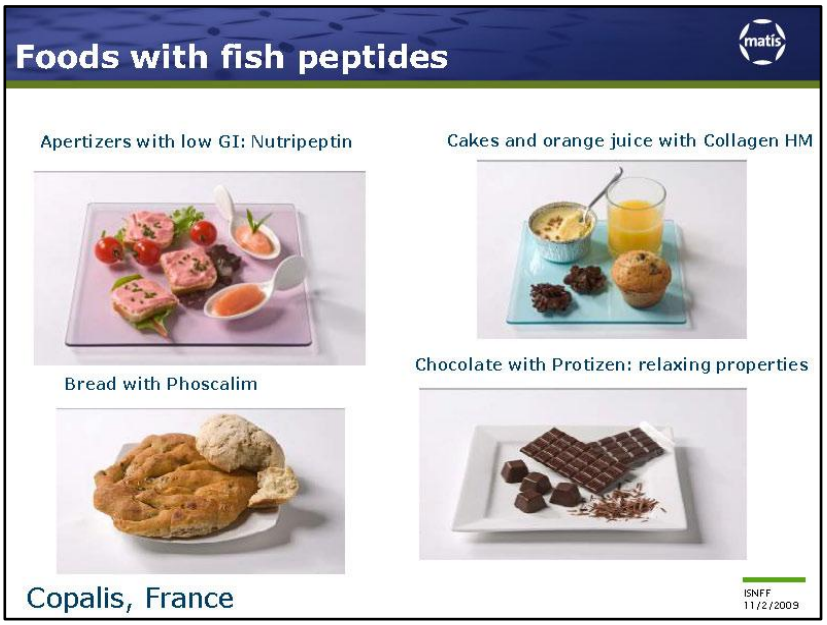
8


Substantial value addition
matis
Fishmeal
SeaCure
PeptACE
PeptACE
FISH PEPTIDES
0,13 ISK/g protein
81 ISK/g protein
132 ISK/g protein
623-1015 fold difference!
ISNFF
11/2/2009

9


Peptide products
matis
Katsuobushi oligopeptide (Vasotensin®)
Nippon Supplements
Sardine peptide SP100N
Senmiekisu
Seacure®
Proper Nutrition
PeptACE
Natural Factors
Peptides de Poisson
Grand Ocean
PeptiStress
DJFusion
AntiStress
Forté Pharma
ISNFF
11/2/2009

10


Foods with fish peptides
matis
Apertizers with low GI: Nutripeptin
Cakes and orange juice with Collagen HM
Bread with Phoscalim
Chocolate with Protizen: relaxing properties
Copalis, France
ISNFF
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Cosmeceuticals with fish peptides
matis
NATURAL COLLAGEN
moisturising cream
NATURAL COLLAGEN
regenerating cream
Vselena, Poland
ISNFF
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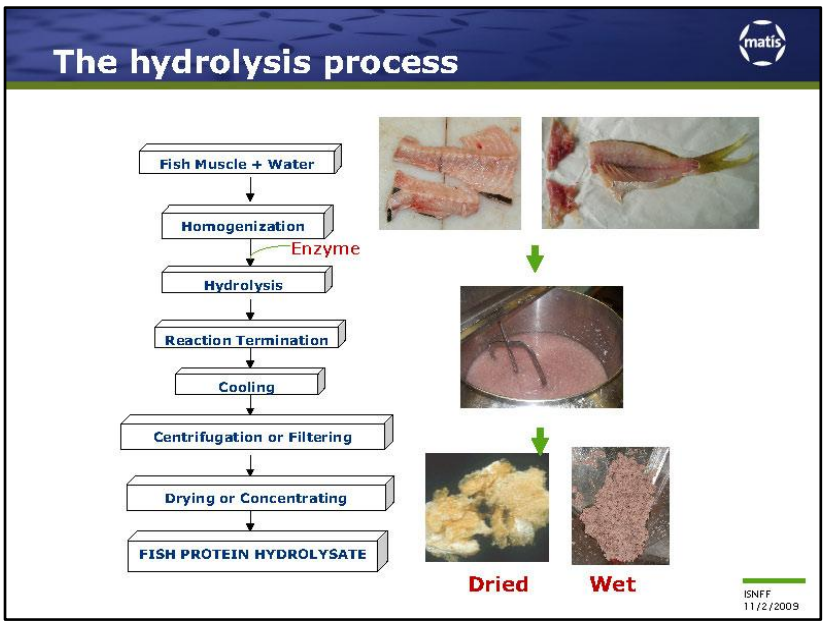
The hydrolysis process
matis
Fish Muscle + Water
Homogenization
Enzyme
Hydrolysis
Reaction Termination
Cooling
Centrifugation or Filtering
Drying or Concentrating
FISH PROTEIN HYDROLYSATE
Dried
Wet
ISNFF
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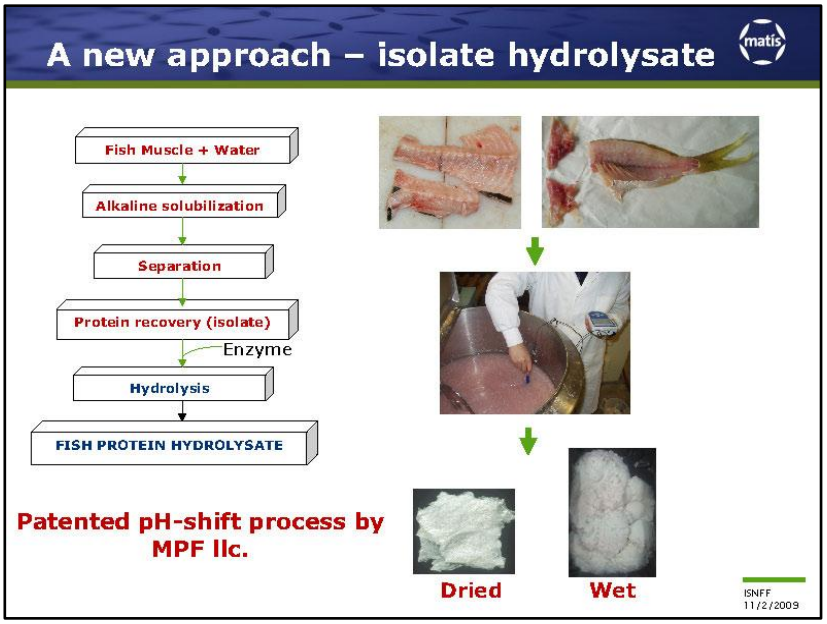
A new approach – isolate hydrolysate
Fish Muscle + Water
Alkaline solubilization
Separation
Protein recovery (isolate)
Enzyme
Hydrolysis
FISH PROTEIN HYDROLYSATE
Patented pH-shift process by MPF llc.
Dried
Wet
ISNFF
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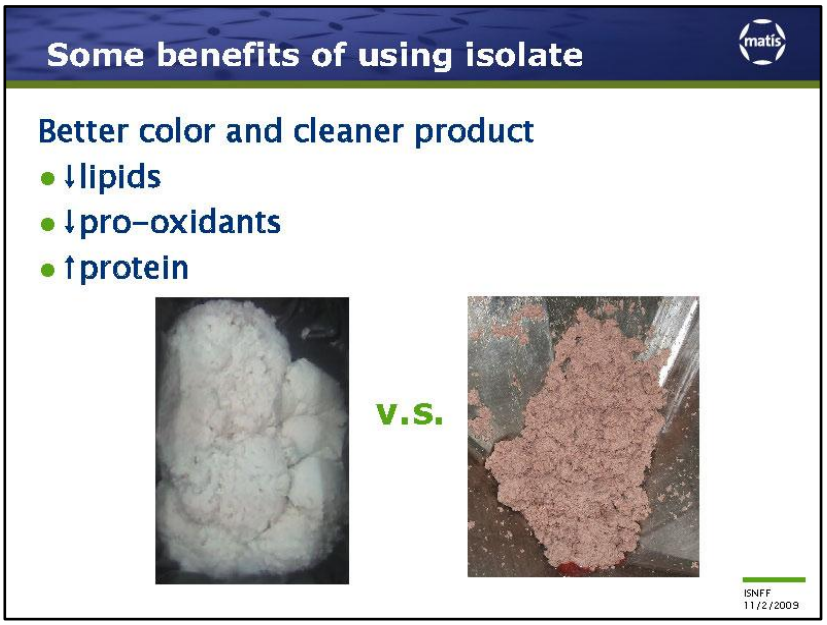
Some benefits of using isolate
Better color and cleaner product
• ↓lipids
• ↓pro-oxidants
• ↑protein
V.S.
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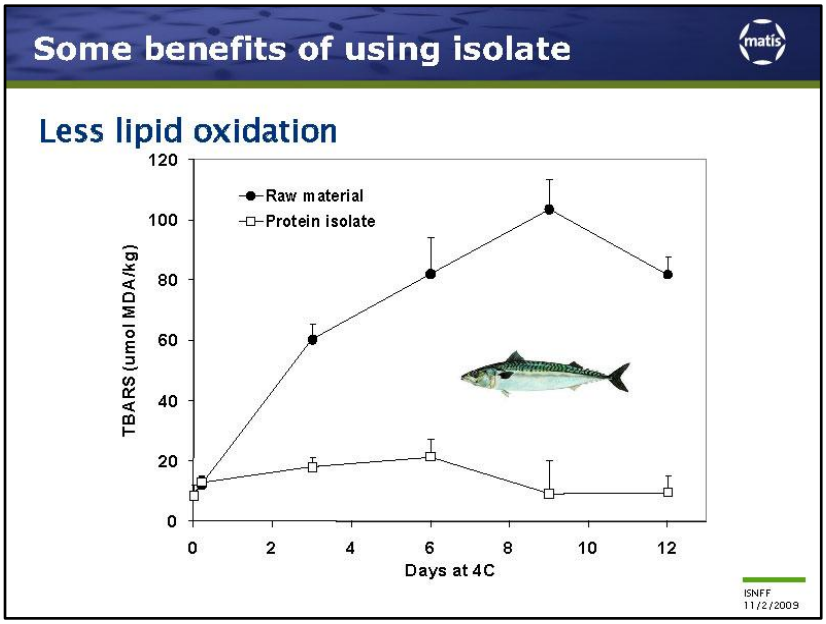
Some benefits of using isolate
matis
Less lipid oxidation
Raw material
Protein isolate
TBARS (umol MDA/kg)
Days at 4C
0
20
40
60
80
100
120
0
2
4
6
8
10
12
ISNFF
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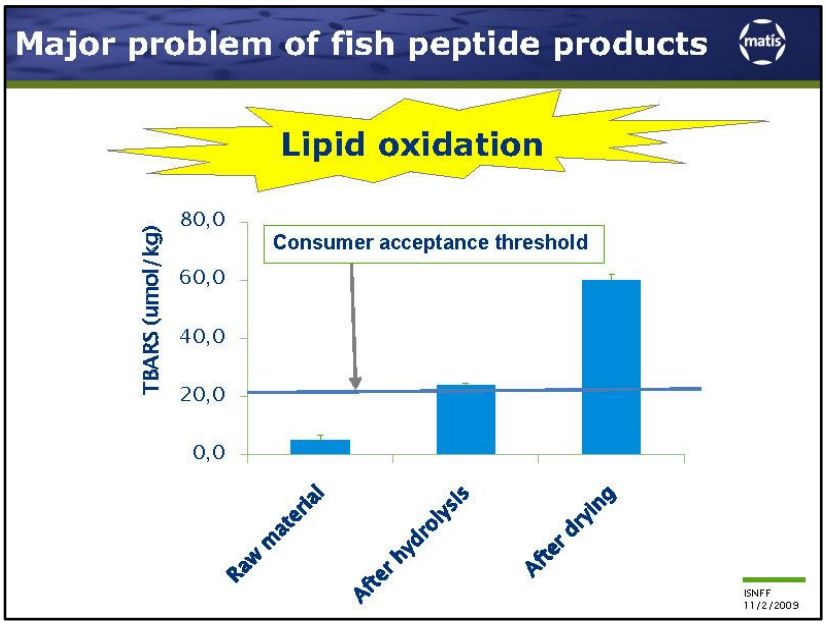
16


Major problem of fish peptide products
matis
Lipid oxidation
Consumer acceptance threshold
TBARS (umol/kg)
80,0
60,0
40,0
20,0
0,0
Raw material
After hydrolysis
After drying
ISNFF
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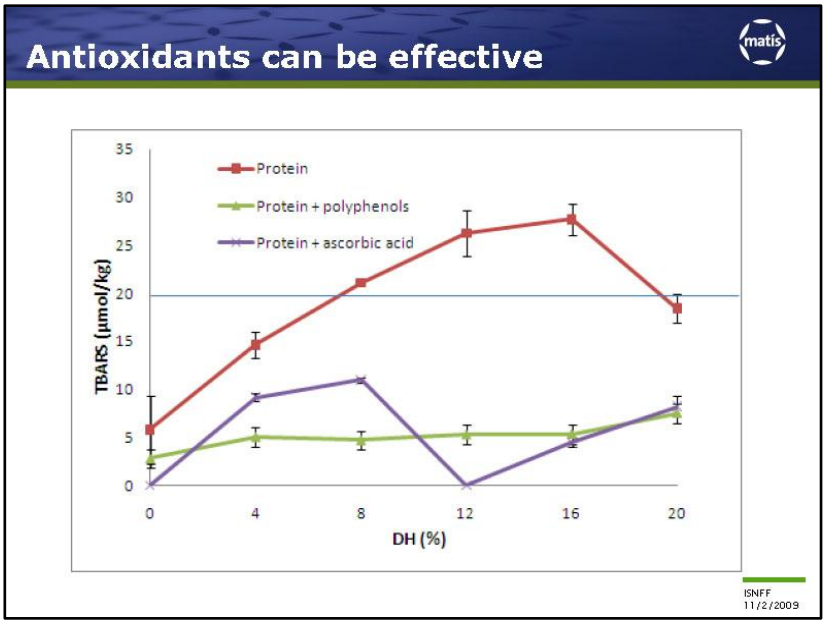
17


Antioxidants can be effective
matis
Protein
Protein + polyphenols
Protein + ascorbic acid
TBARS (µmol/kg)
DH (%)
0
5
10
15
20
25
30
35
0
4
8
12
16
20
ISNFF
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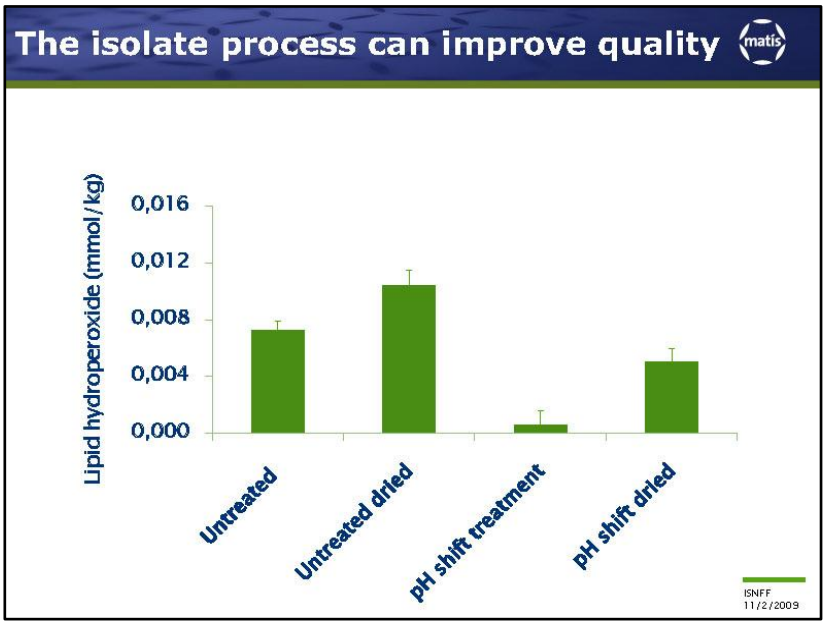
18


The isolate process can improve quality
matis
Lipid hydroperoxide (mmol/kg)
0,000
0,004
0,008
0,012
0,016
Untreated
Untreated dried
pH shift treatment
pH shift dried
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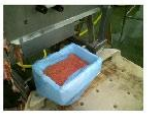
Many different species/materials studied

- Cod
- Haddock
- Saithe (pollock)
- Blue whiting
- Capelin
- Herring
- Salmon
- Clams
- Shrimp
- Lobster
- Sea cucumber
- Seaweed
- Tilapia
- Channel catfish

FOCUS: Byproducts





Hydrolysis Ultrafiltration

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Investigations

Antioxidative properties
- ORAC
- DPPH
- Metal chelation
- Radical scavening
- Monocytes
- Protein carbonyls
- Washed fish model (food model)
- Fish protein isolates (food model)
- Clinical trials

Anti-hypertensive properties
- ACE
- Clinial trials

Anti-carcinogenic properties
- Alamar blue
- Protein expression

Anti-inflammatory properties
- Dentric cell model

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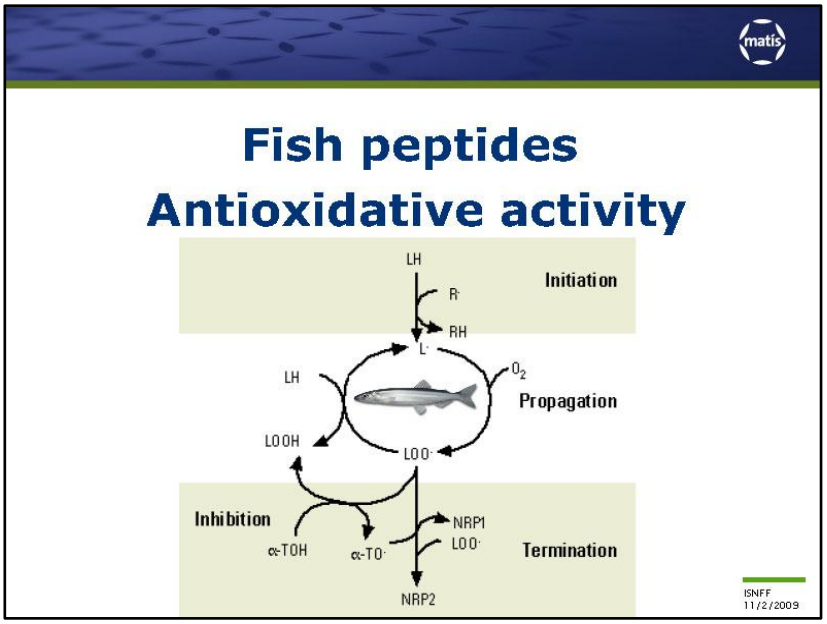
Fish peptides
Antioxidative activity
LH
Initiation
R·
RH
L·
LH
O_2
Propagation
LOOH
LOO·
Inhibition
NRP1
LOO·
α-TOH
α-TO·
Termination
NRP2
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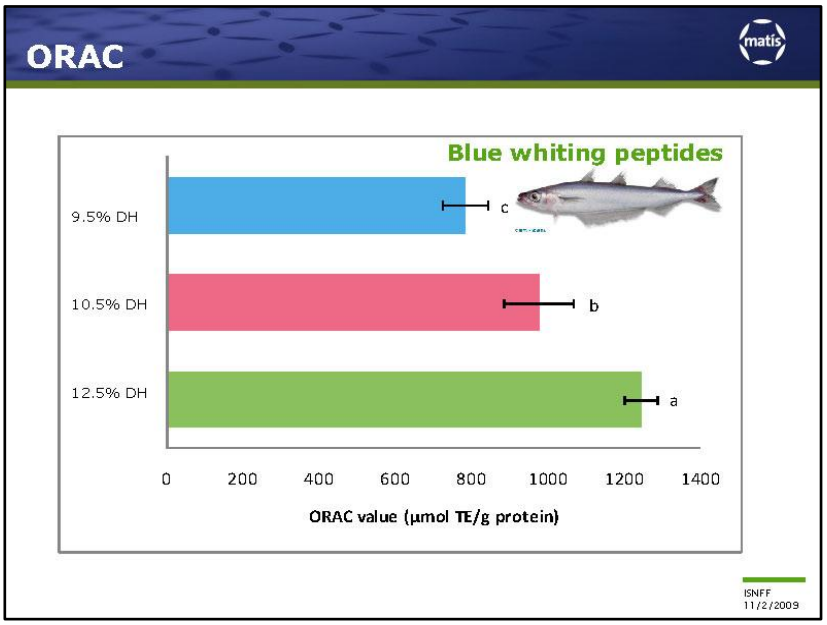
22


ORAC
Blue whiting peptides
9.5% DH
10.5% DH
12.5% DH
c
b
a
0
200
400
600
800
1000
1200
1400
ORAC value (µmol TE/g protein)
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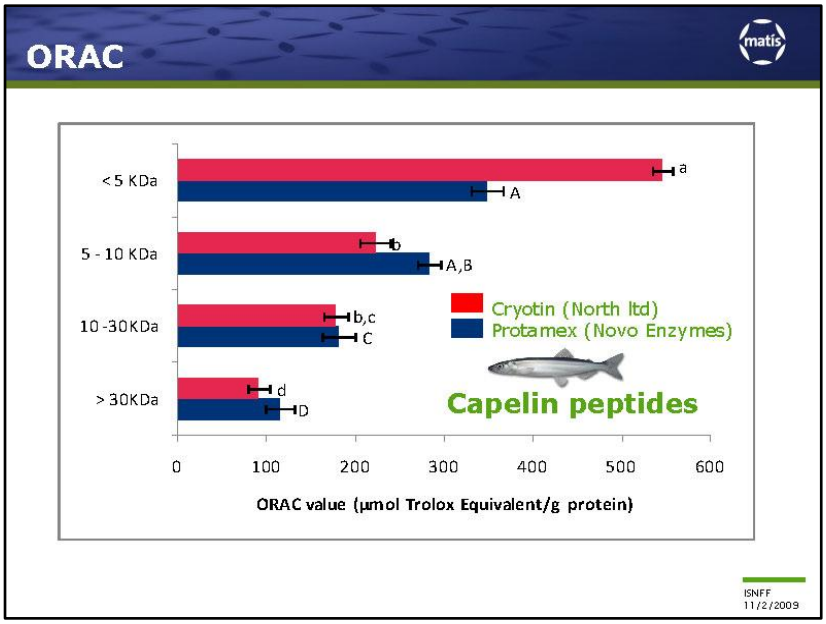
ORAC
matis
< 5 KDa
5 - 10 KDa
10 -30KDa
> 30KDa
a
A
b
A,B
b,c
C
d
D
Cryotin (North ltd)
Protamex (Novo Enzymes)
Capelin peptides
0
100
200
300
400
500
600
ORAC value (µmol Trolox Equivalent/g protein)
ISNFF
11/2/2009

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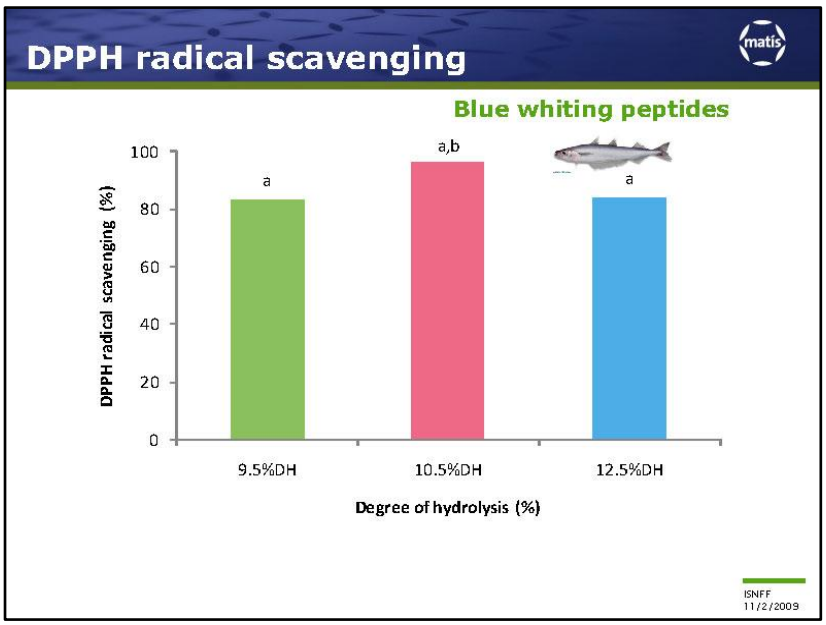
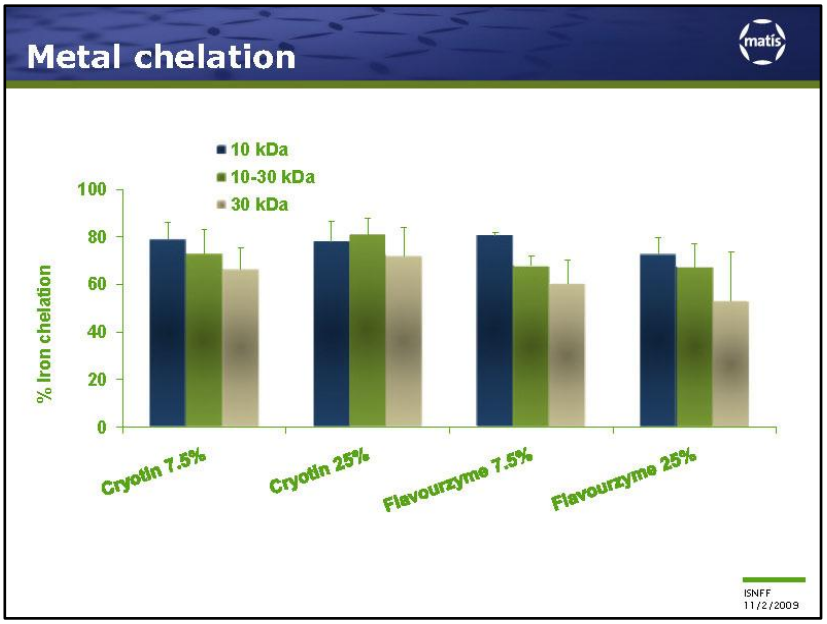

DPPH radical scavenging
matis
Blue whiting peptides
DPPH radical scavenging (%)
100
80
60
40
20
0
a
a,b
a
9.5%DH
10.5%DH
12.5%DH
Degree of hydrolysis (%)
ISNFF
11/2/2009

25


Metal chelation
matis
10 kDa
10-30 kDa
30 kDa
% Iron chelation
100
80
60
40
20
0
Cryotin 7.5%
Cryotin 25%
Flavourzyme 7.5%
Flavourzyme 25%
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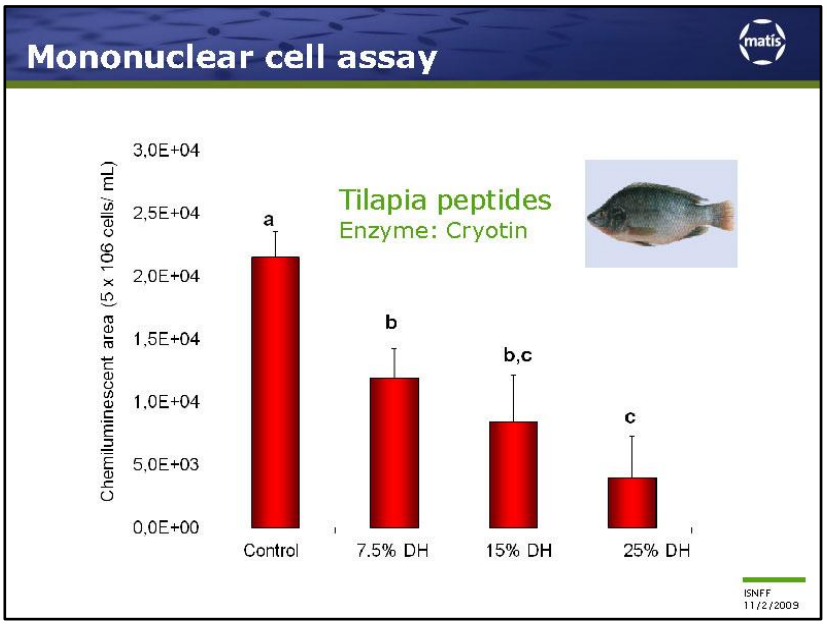
Mononuclear cell assay
matis
Chemiluminescent area (5 x 106 cells/ mL)
3,0E+04
2,5E+04
2,0E+04
1,5E+04
1,0E+04
5,0E+03
0,0E+00
a
b
b,c
c
Tilapia peptides
Enzyme: Cryotin
Control
7.5% DH
15% DH
25% DH
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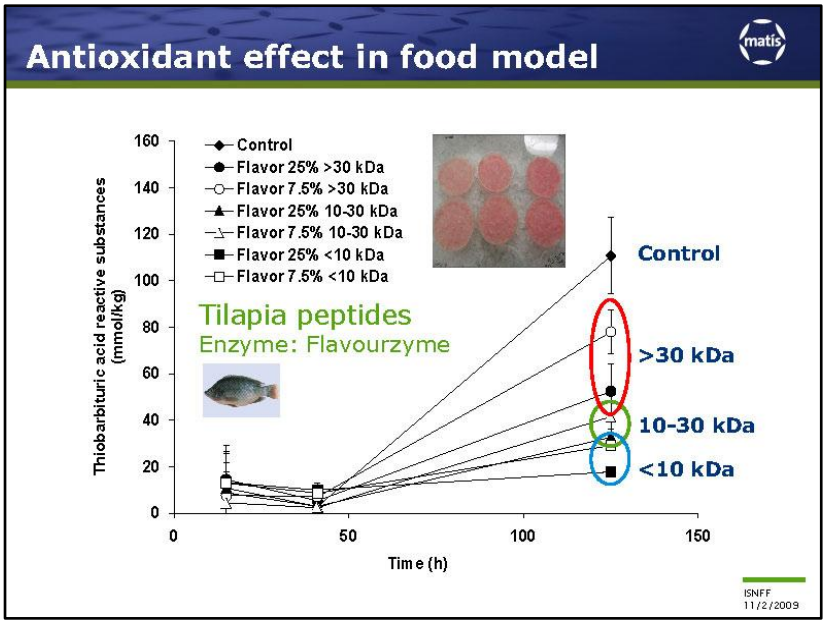
Antioxidant effect in food model
Control
Flavor 25% >30 kDa
Flavor 7.5% >30 kDa
Flavor 25% 10-30 kDa
Flavor 7.5% 10-30 kDa
Flavor 25% <10 kDa
Flavor 7.5% <10 kDa
Tilapia peptides
Enzyme: Flavourzyme
Thiobarbituric acid reactive substances (mmol/kg)
Time (h)
Control
>30 kDa
10-30 kDa
<10 kDa
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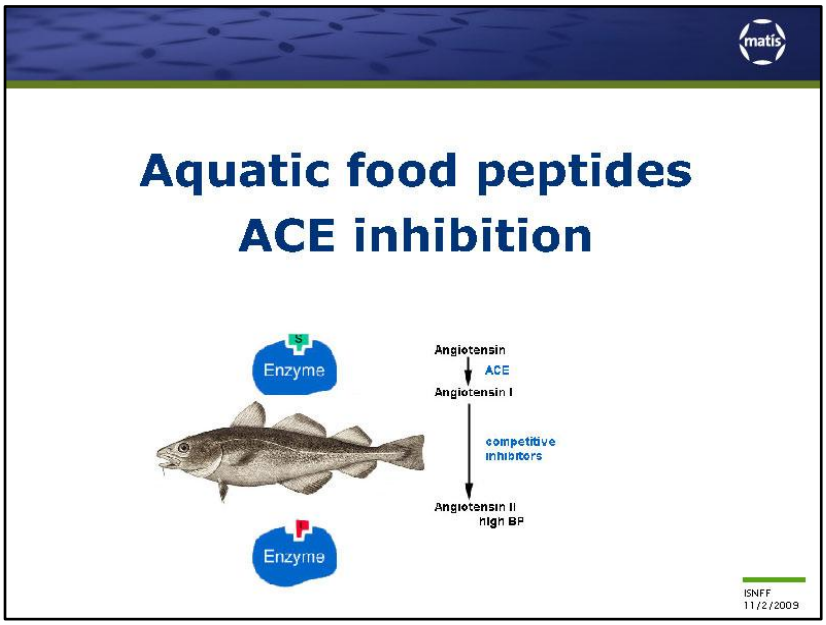
Aquatic food peptides
ACE inhibition
Enzyme
Angiotensin
ACE
Angiotensin I
competitive inhibitors
Angiotensin II
High BP
Enzyme
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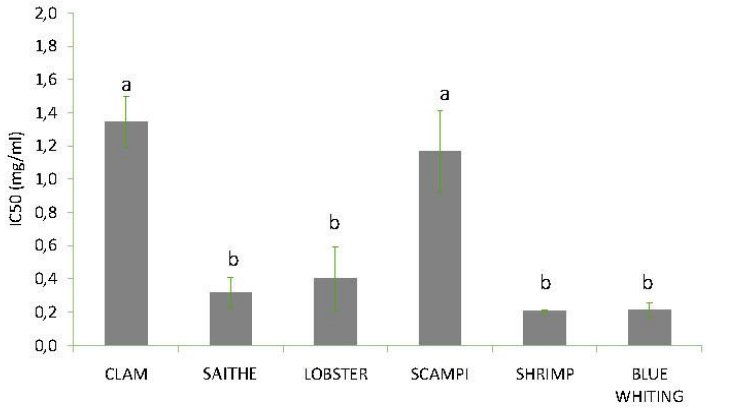
ACE inhibition of various marine peptides



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Cod peptides – 15% DH & fractionated

Fraction	Protein [mg/mL]	ACE inhibition [%]	IC_{50} [mg/mL]
>30 kDa	20,6	94,9	1,4
< 30 kDa	19,1	85,9	0,8
< 10 kDa	16,3	70,5	0,2
< 5 kDa	9,8	78,2	0,1

Lower IC_{50} value => higher activity

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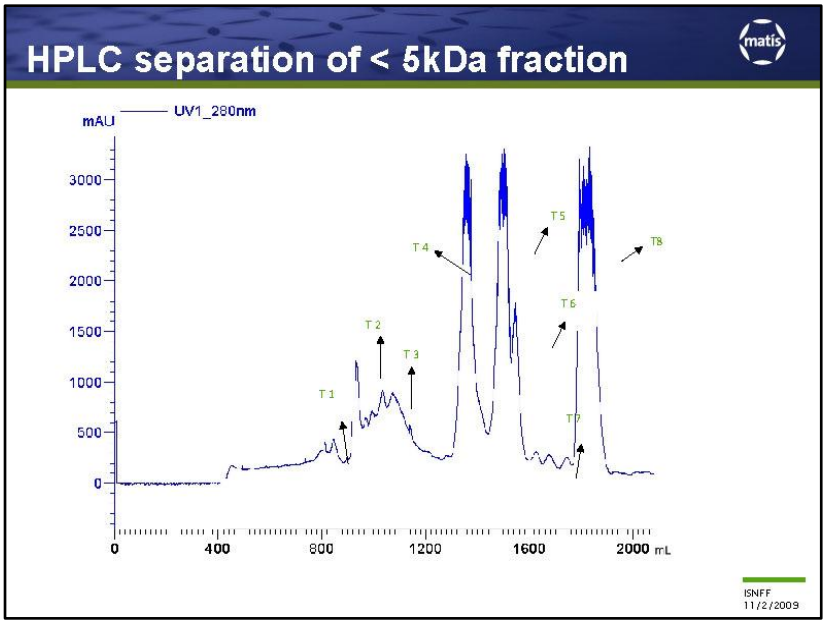
HPLC separation of < 5kDa fraction
matis
mAU
UV1_280nm
3000
2500
2000
1500
1000
500
0
T1
T2
T3
T4
T5
T6
T7
T8
0
400
800
1200
1600
2000 mL
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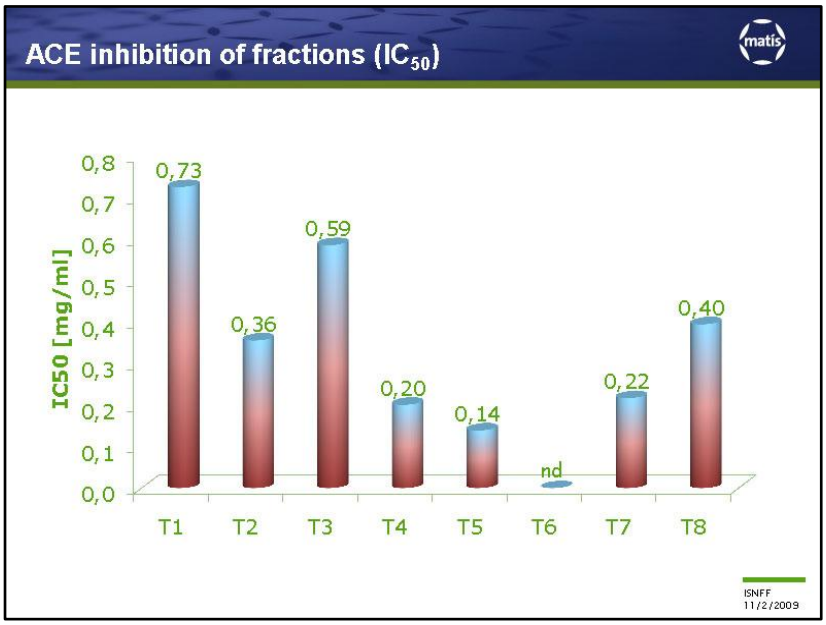
ACE inhibition of fractions (IC50)
matis
IC50 [mg/ml]
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
0,73
0,36
0,59
0,20
0,14
nd
0,22
0,40
T1
T2
T3
T4
T5
T6
T7
T8
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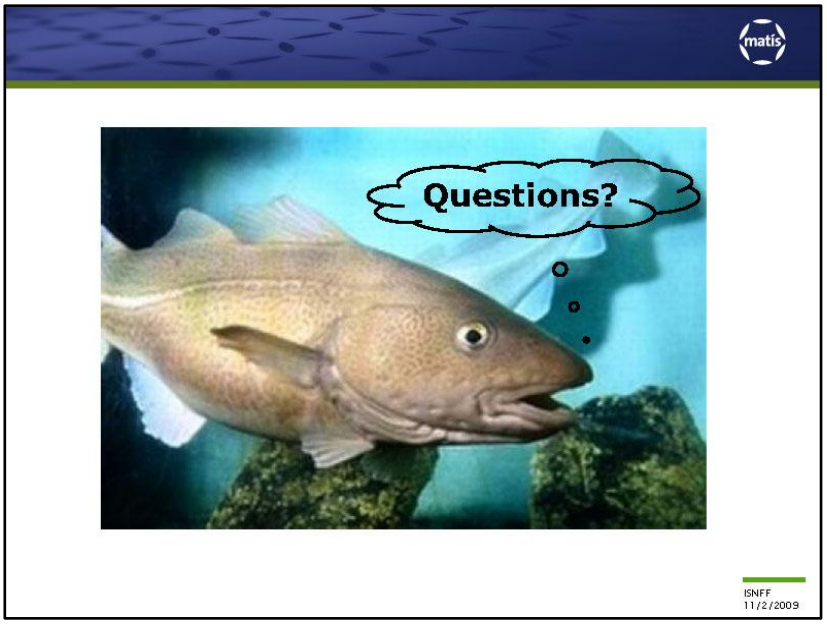
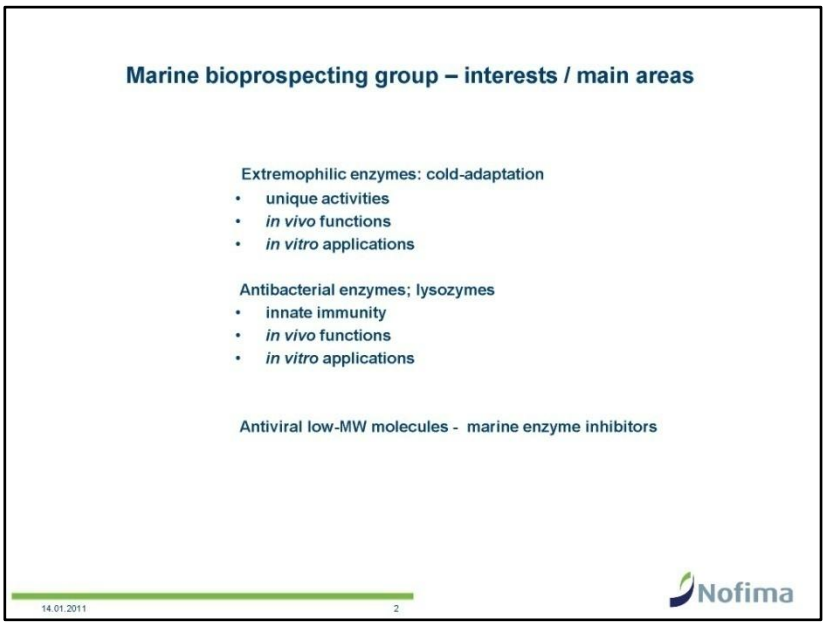


Appendix 9. ”Marine enzymes and enzyme inhibitors” by Inge W. Nilsen

1

2



3

In focus: enzymes with unique features

- Cold-adapted
- ± Low-temperature activities
- ± Heat lability
- High catalytic rates
- Fish & marine invertebrates
- Basic / molecular and applied research

Past – recent - present studies →

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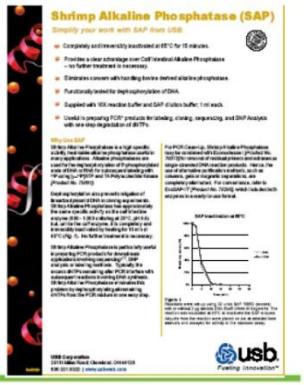
Stories from "the past" - 1

1. **Shrimp alkaline phosphatase (SAP); successful commercial product**
- **Sold as native & recombinant product**
- **Heat labile (→ efficient & irreversible heat inactivation), not cold-active**
- **Dephosphorylates DNA and nucleotides**
- **Used prior to DNA sequencing and in cloning**



Nilsen I.W., Øverbø K., and Lanes O. (2007) Shrimp alkaline phosphatase. European Patent EP1326890.

Nilsen I.W., Øverbø K., and Olsen R. (2001) Thermolabile alkaline phosphatase from Northern shrimp (*Pandalus borealis*): Protein and cDNA sequence analyses. *Comp Biochem Physiol* 129:853-861.

Olsen R., Øverbø K., and Myrnes B. (1991) Alkaline phosphatase from the hepatopancreas of shrimp (*Pandalus borealis*): A dimeric enzyme with catalytically active subunits. *Comp Biochem Physiol* 99: 755-761.

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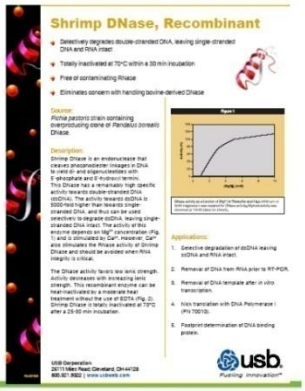
Stories from "the past" - 2

2. Shrimp double-strand specific DNase (dsDNase); commercial product
- Sold as recombinant product
- Heat labile (→ efficient & irreversible heat inactivation), not cold-active
- Degrades dsDNA
- Used to prevent false positives of carry-over products in PCR amplification reactions



Nilsen I.W., Sandsdalen E. and Stenberg E. (2003) A method of removing nucleic acid contamination in amplification reactions. US patent 6,541,204.

Nilsen I.W., Øverbø K., Elde M., Gjellesvik D.R. and Lanes O. (2010) The enzyme and the cDNA sequence of a thermolabile and double-strand specific DNase from Northern shrimps (*Pandalus borealis*). PLoS *ONE (in press).*

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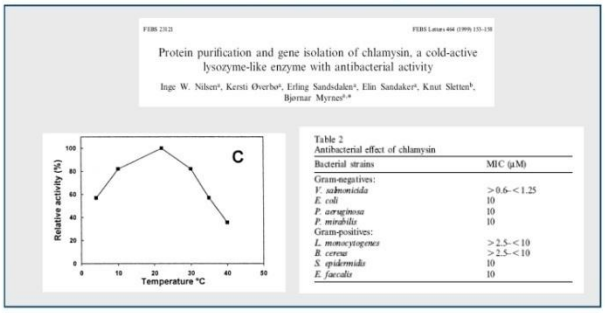
6

Stories from "the past" - 3

A lysozyme from Icelandic scallops



Protein purification and gene isolation of chlamysin, a cold-active lysozyme-like enzyme with antibacterial activity

Inge W. Nilsen[a], Kersti Øverbø[a], Erling Sandsdalen[a], Elin Sandaker[a], Knut Sletten[b], Bjørnar Myrnes[a,*]

Table 2
Antibacterial effect of chlamysin

Bacterial strains	MIC (μM)
Gram-negatives:	
V. salmonicida	> 0.6- < 1.25
E. coli	10
P. aeruginosa	10
P. mirabilis	10
Gram-positives:	
L. monocytogenes	> 2.5- < 10
B. cereus	> 2.5- < 10
S. epidermidis	10
E. faecalis	10

Not commercialized due to problems in recombinant production

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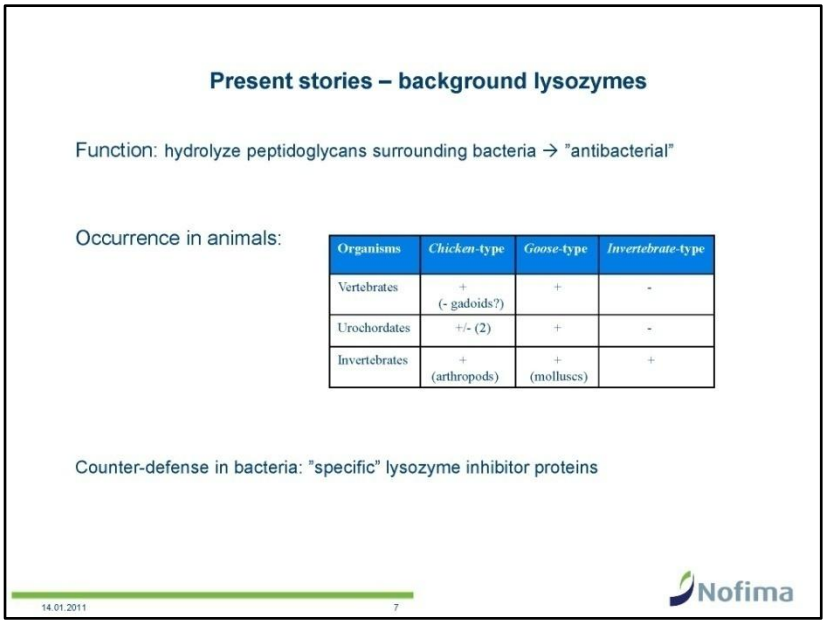
Present stories – background lysozymes
Function: hydrolyze peptidoglycans surrounding bacteria → "antibacterial"
Occurrence in animals:
Organisms | Chicken-type | Goose-type | Invertebrate-type
Vertebrates | + (- gadoids?) | + | -
Urochordates | +/- (2) | + | -
Invertebrates | + (arthropods) | + (molluscs) | +
Counter-defense in bacteria: "specific" lysozyme inhibitor proteins
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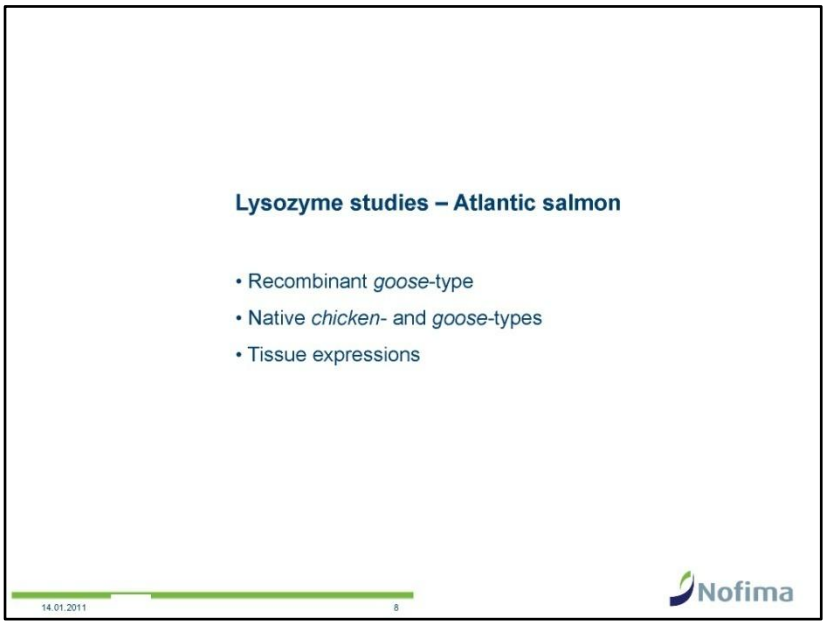
Lysozyme studies – Atlantic salmon
• Recombinant goose-type
• Native chicken- and goose-types
• Tissue expressions
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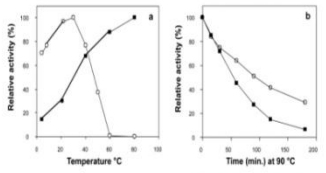
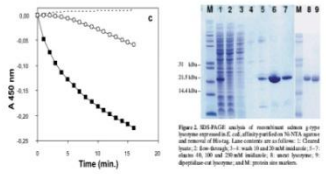
Recombinant SaLG – cold-active, heat-labile but reactivates





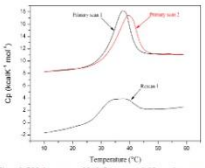


Figure 1. DSC thermograms of SaIG. Primary scan 1- Measured at a scan rate of 1 °C min^{-1} for 1.6 mg/ml protein; Primary scan 2- 2 °C min^{-1} for 1.6 mg/ml protein; Rescan 1- Following the initial scan at 1 °C min^{-1} for 1.6 mg/ml and overnight incubation at 4 °C, the sample was rescanned.

Table 2. Effect of temperature on SaIG activity at high and low protein concentrations

SaIG (mg/ml)	Heating	Cooling 12 h on ice	Relative activity (%)
1.60	-	-	100
1.60	DSC*, 20→60 °C	+	33
1.60	60 °C, 15 min	+	2
1.60	90 °C, 15 min	+	30
0.05	-	-	3
0.05	90 °C, 15 min	+	8
			100
			91

NB!

* scan rate 2 °C/min ; **30 fold dilution of heated SaIG sample before cooling

Kyomuhendo P, Myrnes B, and Nilsen IW (2007) A cold-active salmon goose-type lysozyme with high heat tolerance. *Cell. Mol. Life Sci.* 64:2841-2847.

Kyomuhendo P, Myrnes B, Brandsdal BO, Smalås AO, Nilsen IW and Helland R (2010) Thermodynamics and structure of a salmon cold-active goose-type lysozyme. (*submitted*)

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Recombinant SaLG and bacterial lysozyme inhibitors

SaLG – not inhibited by bacterial Ivy, unlike terrestrial *g*-type lysozymes and all *c*-type lysozymes

→ Used to isolate novel specific *g*-type inhibitor from bacteria

↓

PliG – to be published

Collaboration with Chris Michiels, Catholic University of Leuven, Belgium

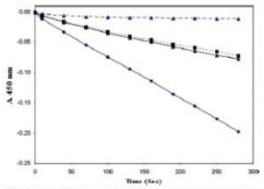

Fig. 1 Lysis of *Micrococcus lysodeikticus* cell suspension by hen-egg white lysozyme (HEWL; *diamonds*) and recombinant salmon g-type lysozyme (*triangles*) in the absence (*filled symbols*) or presence (*open symbols*) of the bacterial inhibitor of vertebrate lysozyme (Ivy).

Kyomuhendo P, Nilsen IW, Brandsdal BO and Smalås AO (2008) Structural evidence for lack of inhibition of fish goose-type lysozymes by a bacterial inhibitor of lysozyme. J Mol Model 14:777–788

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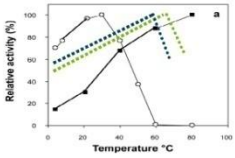
Native salmon lysozymes: isolation and characterization

Myrnes B, Øverbø K and Nilsen IW (*unpublished*)

- SaLC and SaLG isolated from adult individuals
- Confirmed by bacterial inhibitors PliG/Ivy and MS analyses

- Both native enzymes are cold-active
- Both native enzymes have temperature optimum at 60 ºC (!!)

→ Temp-optimum discrepancy for recombinant versus native SaLG



- SaLC is bifunctional !!
  - minimum 50-200 fold higher (endo- and exo-) chitinase activity than SaLG and HEWL

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12

Salmon lysozymes – antibacterial studies

- Expressed interest for activities against enterobacteria *E. coli* / *Salmonella*
- Lysozyme enzyme activitiy of SaLG tested on cells after removal of outer cell wall
- Antibacterial effects tested in different liquid media under various growth conditions
- Lysozymes tested alone or in combination with low-MW material from salmon

Nofima

13

Salmon lysozymes – antibacterial studies

- SaLG displays very high enzymatic activity on E. coli (and other) cell walls

  - but:

- No antibacterial effect of SaLG (40 nM - 40 µM → ~1 µg – 1 mg/ml)
- No antibacterial effect of SaLC (max 5 µM)
- No antibacterial effect of SaLG+SaLC
- No synergy in antibacterial effect of SaLG+SaLC+low-MW fraction

  - however:

- Some antibacterial effect of salmon low-MW fractions
- Potential synergistic effect of two different low-MW fractions

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14

Lysozyme gene-expression and enzyme activity studies reveal that goose-type dominates over chicken-type in tissues of juvenile Atlantic salmon (*Salmo salar*).

Myrnes B, Seppola M, Callewaert M, Vanderkelen L, Michiels C and Nilsen IW (*unpublished*)

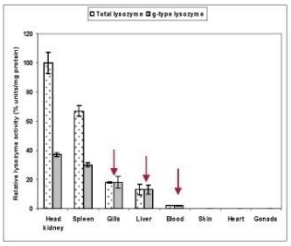

Figure 1. Relative distribution of lysozyme activities (mean values ± SD) in juvenile Atlantic salmon organs. G-type lysozyme activity is determined from the activity suppressed by the PliG inhibitor as well as the activity remaining in the presence of the Ivy inhibitor.

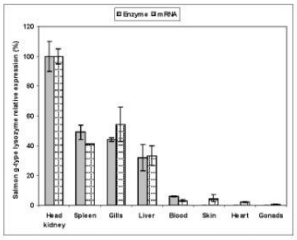

Figure 2. Relative amounts (mean values ± SD) of enzyme activities and mRNA transcripts of the g-type lysozyme in juvenile Atlantic salmons.

- Discrimination of lysozyme enzyme activities by specific inhibitors (Ivy and PliG)
- Gene expression studies in agreement with enzyme activities
- Enzyme activities in tissues of adult salmon show the same distribution pattern

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15

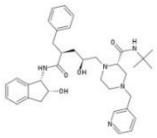
Inhibitors of HIV-1 protease

Frequent HIV "therapy" - a trippel coctail of inhibitors:

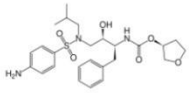
1 inhibitor of reverse transcriptase + 2 inhibitors of protease

Life-time treatment and few available drugs → resistance

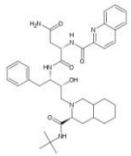
Examples of HIV protease inihibitors:



INDINAVIR (IDV)



AMPRENAVIR (APV)



SAQUINAVIR (SQV)

14.01.2011 test 15

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16

Marine inhibitors of HIV-1 protease

Øverbø K, Arnesen JA, Christopeit T, Danielson H, Nilsen IW (unpublished)

- Extracts of marine invertebrates
- Sequential steps of low-MW components purification
- Fractions assayed for inhibition of HIV-protease enzymatic activity
- Compared to enzyme activity inhibition from two anti-HIV drugs (APV, SQV)
- No inhibiton of pepsin activity (another aspartic protease)

- when criteria are met:

- Analysed for its binding kinetics to HIV-protease (Biacore biosensor)
- Compared to its binding to human serum albumin (HSA)
- Compared to binding of clinical drug (IDV) to HIV protease

14.01.2011 test 16

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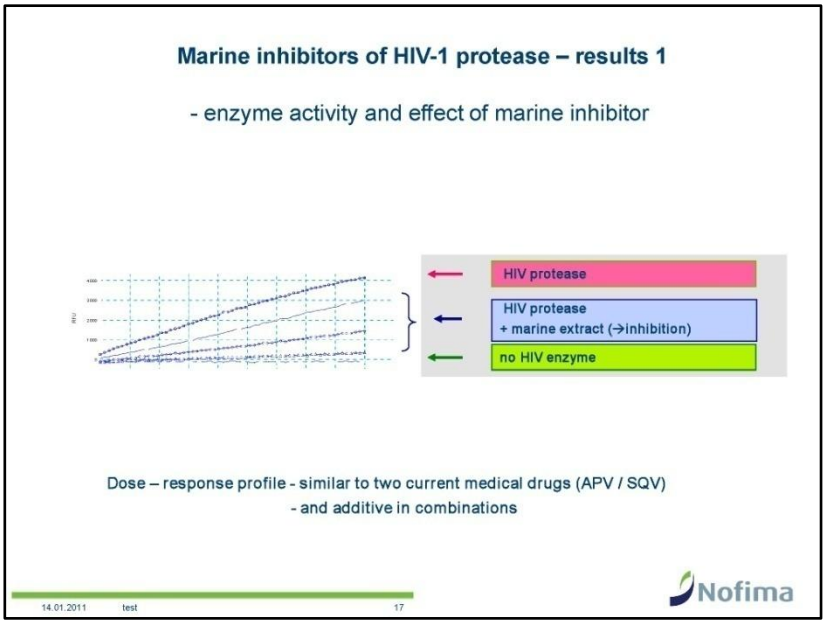
17


Marine inhibitors of HIV-1 protease – results 1
- enzyme activity and effect of marine inhibitor
HIV protease
HIV protease
+ marine extract (→inhibition)
no HIV enzyme
Dose – response profile - similar to two current medical drugs (APV / SQV)
- and additive in combinations
14.01.2011 test 17
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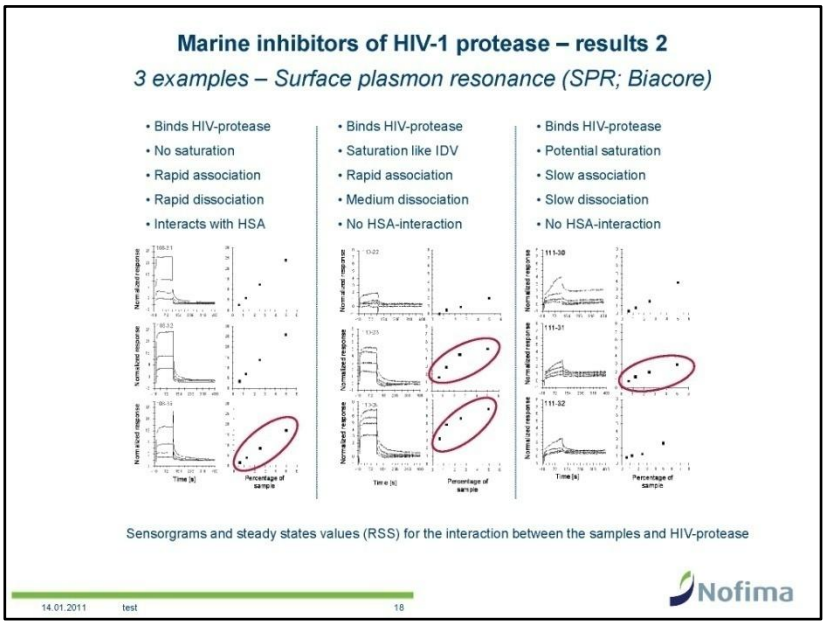
18


Marine inhibitors of HIV-1 protease – results 2
3 examples – Surface plasmon resonance (SPR; Biacore)
• Binds HIV-protease
• No saturation
• Rapid association
• Rapid dissociation
• Interacts with HSA
• Binds HIV-protease
• Saturation like IDV
• Rapid association
• Medium dissociation
• No HSA-interaction
• Binds HIV-protease
• Potential saturation
• Slow association
• Slow dissociation
• No HSA-interaction
Sensorgrams and steady states values (RSS) for the interaction between the samples and HIV-protease
14.01.2011 test 18
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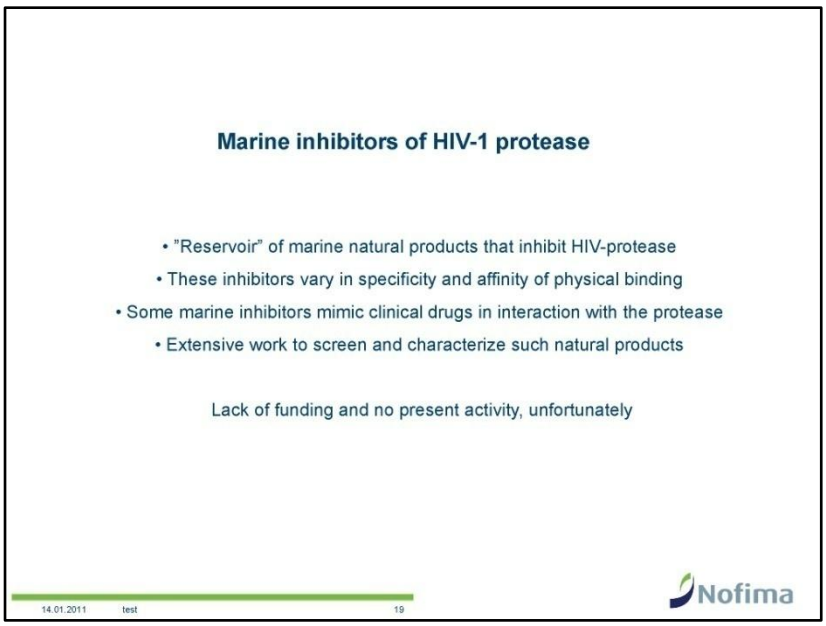
19


Marine inhibitors of HIV-1 protease
• "Reservoir" of marine natural products that inhibit HIV-protease
• These inhibitors vary in specificity and affinity of physical binding
• Some marine inhibitors mimic clinical drugs in interaction with the protease
• Extensive work to screen and characterize such natural products
Lack of funding and no present activity, unfortunately
14.01.2011 test 19
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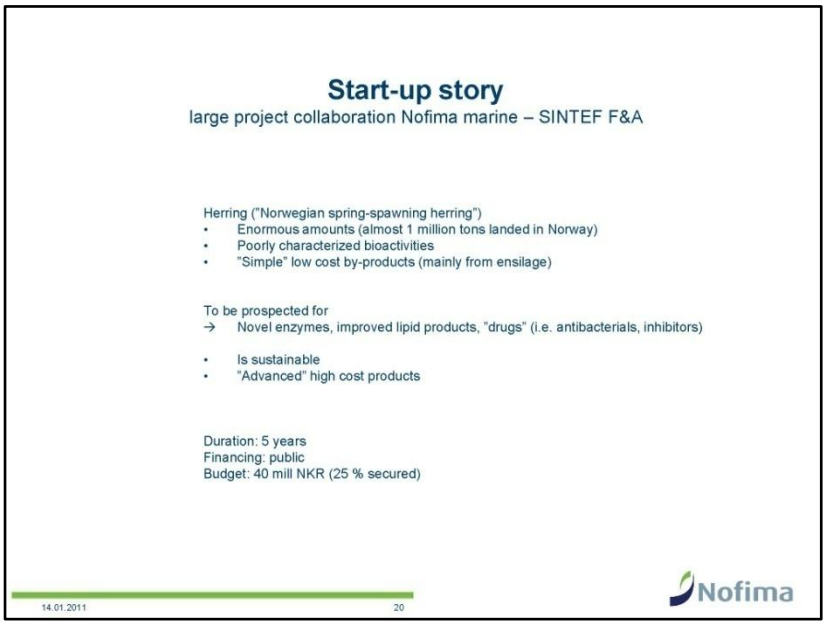
20


Start-up story
large project collaboration Nofima marine – SINTEF F&A
Herring ("Norwegian spring-spawning herring")
• Enormous amounts (almost 1 million tons landed in Norway)
• Poorly characterized bioactivities
• "Simple" low cost by-products (mainly from ensilage)
To be prospected for
→ Novel enzymes, improved lipid products, "drugs" (i.e. antibacterials, inhibitors)
• Is sustainable
• "Advanced" high cost products
Duration: 5 years
Financing: public
Budget: 40 mill NKR (25 % secured)
14.01.2011 20
Nofima

21


Sommarøy, January 2010
Photo: Rama Bangera
Thank you for listening!
14.01.2011
21
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Appendix 10. ”Seagarden ASA, a commercial producer of bioactive peptides from marine protein sources” by Bjarte Langhelle

1

2

3


seagarden
Arctic seafood ingredients
ChitiNor
seagarden
mariproTM
Atlantic

4


seagarden
Arctic seafood ingredients
MARINE SAVOURY INGREDIENTS
WHITE FISH PRODUCTS
Fish Powders
Codfish Powders
Saithe Powders
White Fish Powders
Arctic Fish Powder
Fish Protein Extract Powder
SALMON PRODUCTS
Smoked Salmon Powder
Salmon Powder
Smoked Salmon Granulate
Salmon Granulate
SHELLFISH PRODUCTS
Shrimp Powders
Shrimpshell Powders
Crab Powder
Squid Powder
Scampi Powder
Mussel Powder
Shellfish Powder
Lobster Extract Powder
Seaweed Powder
SEAFOOD PASTES
Fish Protein Extract
Atlantic Salmon Extract
Lobster Extract

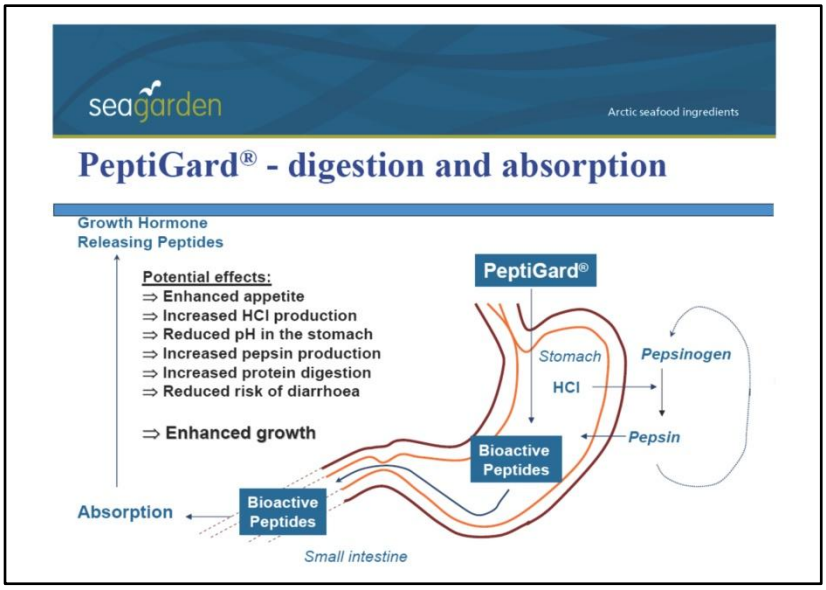
5


seagarden
Arctic seafood ingredients
MARINE BIO-ACTIVES
COMMERCIAL PRODUCTS
Minipro™ (Eur. Pat Appl. 02751374.6)
Microencapsulated starter feed for shrimp and fish larvae
API Chitin
Approved API (active pharmaceutical ingredient)
Intermediate for GMP grade glucosamines and chitosans
PRODUCT PIPELINE
Peptigard™ (Eur. Pat 0951837A1)
Piglet and poultry growth-enhancer. Bio-active peptides derived from fish, used as an alternative to porcine plasma for weaning pigs and poultry.
Collagen
Fish Collagen Hydrolysates

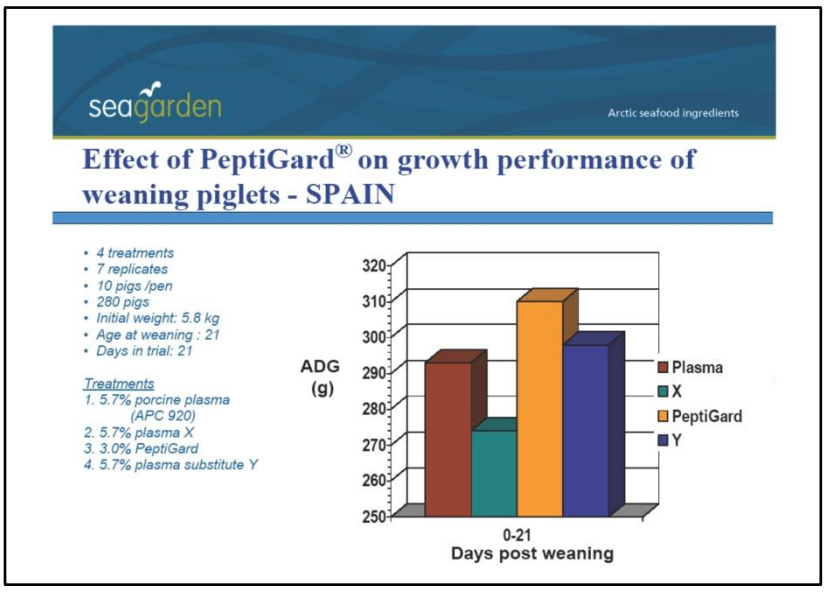
6


seagarden
Arctic seafood ingredients
CURRENT DEVELOPMENT PROJECTS
MARINE SAVOURY INGREDIENTS
Development of new marine savoury ingredients
Client-specific formulations
GME-products from process-water, marine powders Avaldsnes.
MARINE BIOACTIVES
Peptigard™
MiniPro™
Collagen hydrolysates
Bioactive peptides – IRIS
Fermentation growth media
Chitin-derivatives

7


seagarden
Arctic seafood ingredients
PeptiGard® - digestion and absorption
Growth Hormone
Releasing Peptides
Potential effects:
⇒ Enhanced appetite
⇒ Increased HCl production
⇒ Reduced pH in the stomach
⇒ Increased pepsin production
⇒ Increased protein digestion
⇒ Reduced risk of diarrhoea
⇒ Enhanced growth
PeptiGard®
Stomach
Pepsinogen
HCl
Pepsin
Bioactive Peptides
Absorption
Bioactive Peptides
Small intestine

8


seagarden
Arctic seafood ingredients
Effect of PeptiGard® on growth performance of weaning piglets - SPAIN
• 4 treatments
• 7 replicates
• 10 pigs /pen
• 280 pigs
• Initial weight: 5.8 kg
• Age at weaning : 21
• Days in trial: 21
Treatments
1. 5.7% porcine plasma (APC 920)
2. 5.7% plasma X
3. 3.0% PeptiGard
4. 5.7% plasma substitute Y
ADG (g)
320
310
300
290
280
270
260
250
Plasma
X
PeptiGard
Y
0-21
Days post weaning

9

seagarden

Arctic seafood ingredients

EVALUATION FACTORS IN PRODUCT DEVELOPMENT FROM BIOPROSPECT TO MARKET

COMMERCIAL CONSIDERATIONS

- Cost-Efficacy / Cost-Benefit
- Reproducibility
- IPR
  - Patents
  - Disclose or not disclose?
- Regulatory Requirements
- Development Time and Costs

➢ **Return on Investments**

CLINICAL AND REGULATORY CONSIDERATIONS

- Structure-Function Claims
- Clinical studies
  - Efficacy Studies
  - Dose-Response
  - Mode of Action
  - Food Vehicle Studies
  - Safety
- Novel Food Registration, GRAS, Pharma/Medical, etc.

➢ **Clinical and Regulatory Strategy**

10

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Arctic seafood ingredients

EVALUATION FACTORS IN PRODUCT DEVELOPMENT FROM BIOPROSPECT TO MARKET

COMMERCIAL AND ACADEMIC CO-OPERATION

- Review Scientific and Market Status – What is State of the Art?
- Scientific and Commercial Planning

➢ **Commercial and Academic Interdependency and Co-operation**

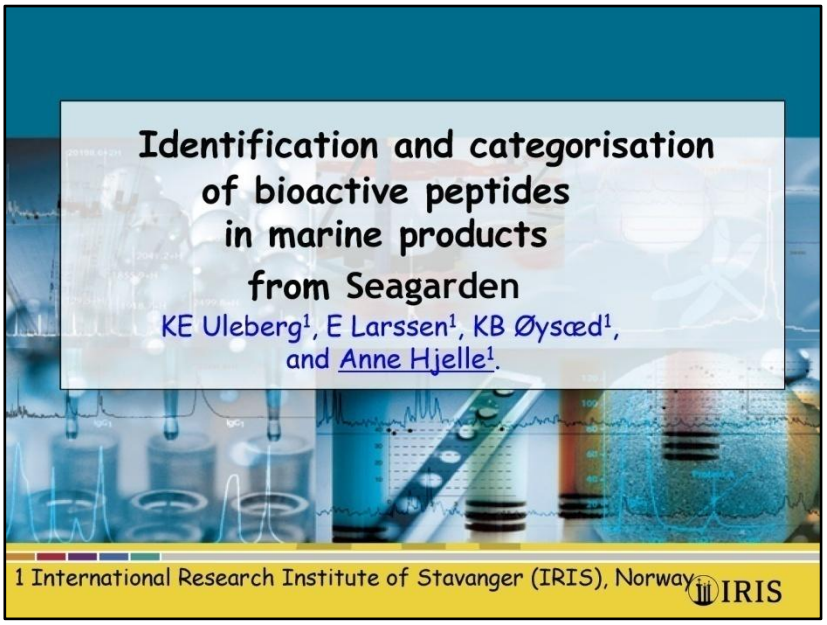
BIOPROSPECTING

- Bioprospecting at Seagarden:
  - What *Do* we have?
  - What *Can* we have?

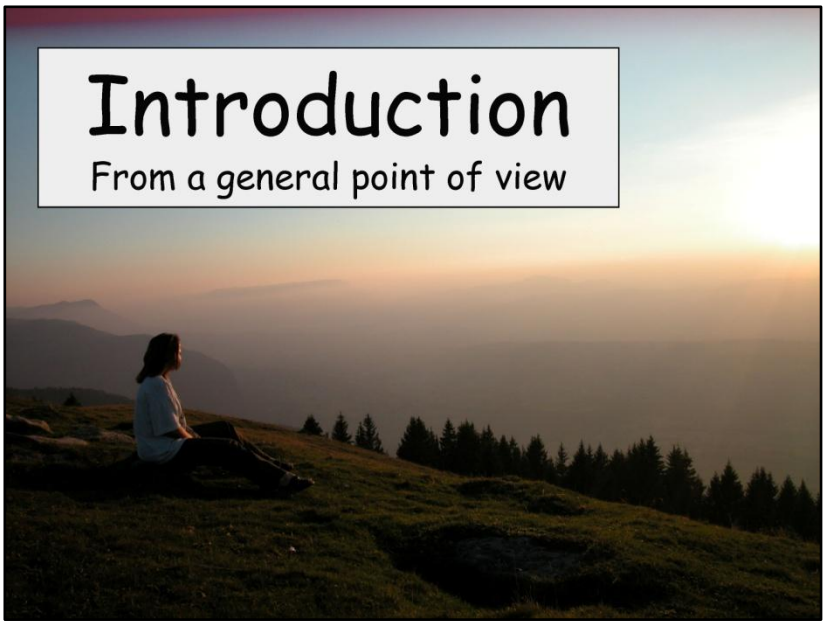
➢ **Bioprospecting into Existing Product Portfolio**
-> SG-IRIS Co-operation

Appendix 11. ”Identification and categorisation of bioactive peptides in marine extracts produced by Seagarden ASA” by Anne Hjelle

1



2



3

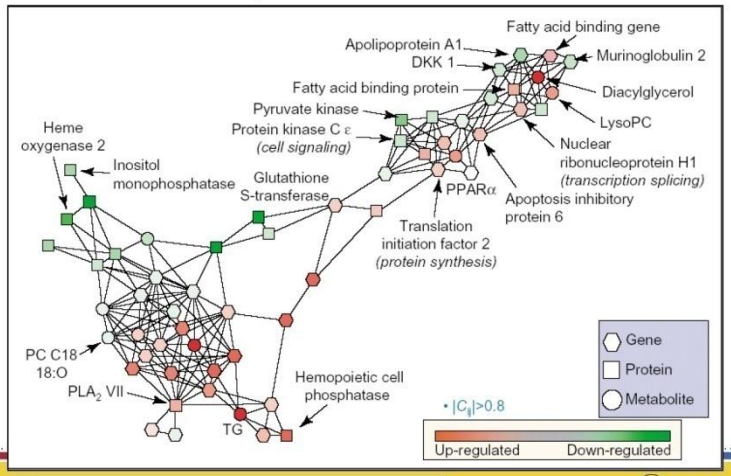
International trend: (e.g. EU) Nutrition & Health, 7th Framework)

- Understanding beneficial and harmful dietary factors
- Interaction between nutrition and physiological/psychological functions
- **Reformulation of processed food, development of novel foods and ingredients, dietetic foods and foods with nutritional- and health claims ("Functional food").**
- Dietary strategy: development and application of nutrigenomics and systems biology

IRIS

4

Example systems biology: Biomolecules (de)activated by onset of atherosclerosis:



C.B. Clish et al. 2004; J. Van der Greef et al. 2004

IRIS

5

Marine ingredients as " functional food"

Seagarden (SG) is a supplier of high quality marine ingredients to customers worldwide, including a wide range of powders and extracts as regards both marine savoury ingredients and marine bioactives.

However, this project is limited to evaluation of bioactive PEPTIDES in SG products (fish hydrolysates).

IRIS

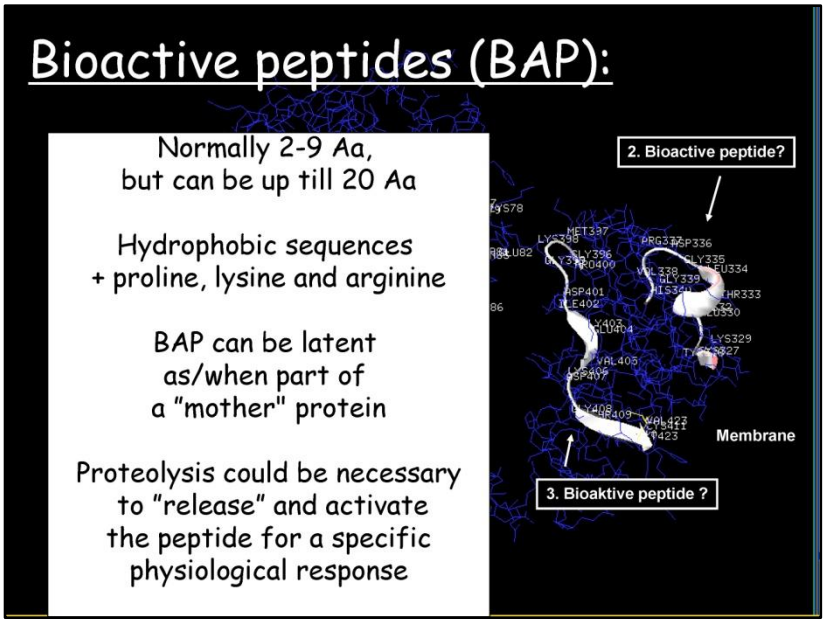
6

Proteins & peptides: "functional building blocks in all organisms"

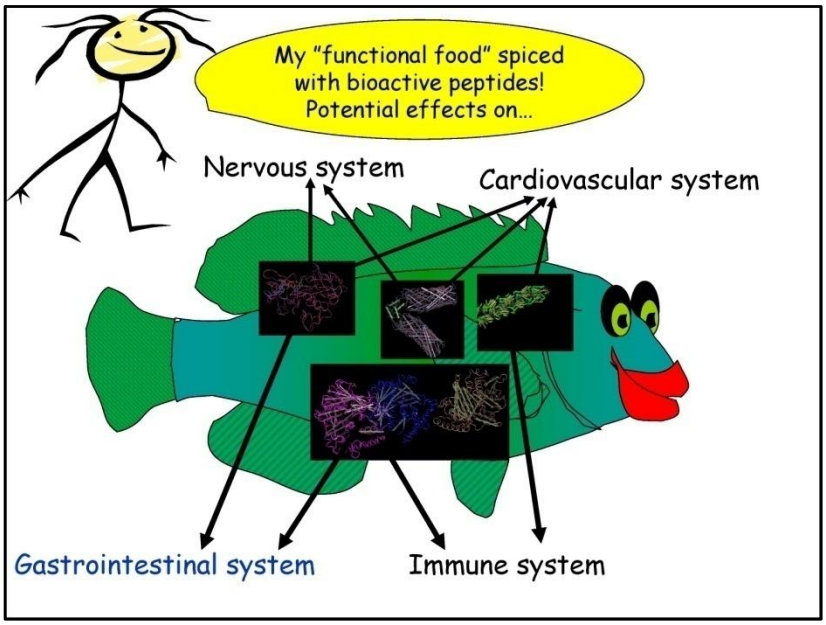
= unique sequences of Aminoacids:

IRIS

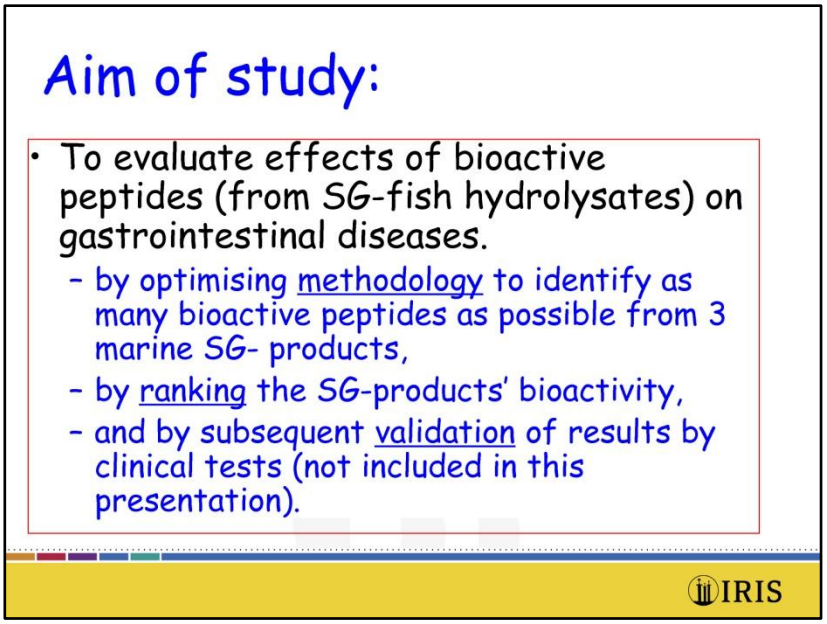
7


Bioactive peptides (BAP):
Normally 2-9 Aa,
but can be up till 20 Aa
Hydrophobic sequences
+ proline, lysine and arginine
BAP can be latent
as/when part of
a "mother" protein
Proteolysis could be necessary
to "release" and activate
the peptide for a specific
physiological response
2. Bioactive peptide?
Membrane
3. Bioaktive peptide ?

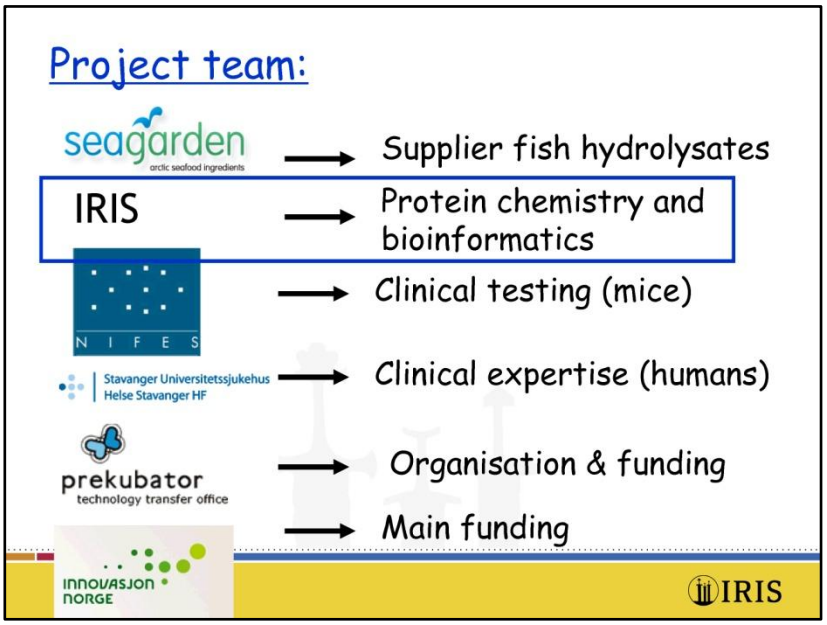
8


My "functional food" spiced
with bioactive peptides!
Potential effects on...
Nervous system
Cardiovascular system
Gastrointestinal system
Immune system

9


Aim of study:
• To evaluate effects of bioactive peptides (from SG-fish hydrolysates) on gastrointestinal diseases.
– by optimising methodology to identify as many bioactive peptides as possible from 3 marine SG- products,
– by ranking the SG-products' bioactivity,
– and by subsequent validation of results by clinical tests (not included in this presentation).
IRIS

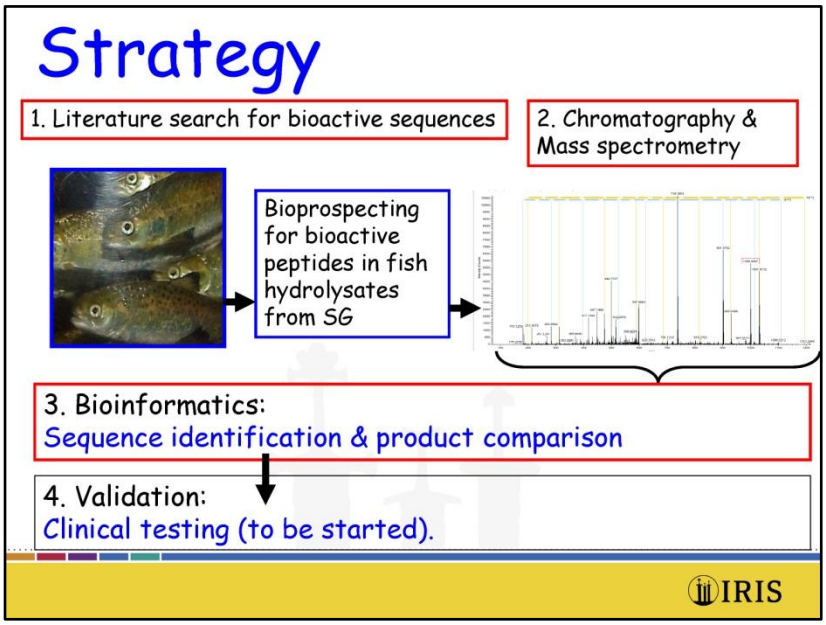
10


Project team:
seagarden
arctic seafood ingredients
Supplier fish hydrolysates
IRIS
Protein chemistry and bioinformatics
N I F E S
Clinical testing (mice)
Stavanger Universitetssjukehus
Helse Stavanger HF
Clinical expertise (humans)
prekubator
technology transfer office
Organisation & funding
INNOVASJON NORGE
Main funding
IRIS

11


Methods

12


Strategy
1. Literature search for bioactive sequences
2. Chromatography & Mass spectrometry
Bioprospecting for bioactive peptides in fish hydrolysates from SG
3. Bioinformatics:
Sequence identification & product comparison
4. Validation:
Clinical testing (to be started).
IRIS

13

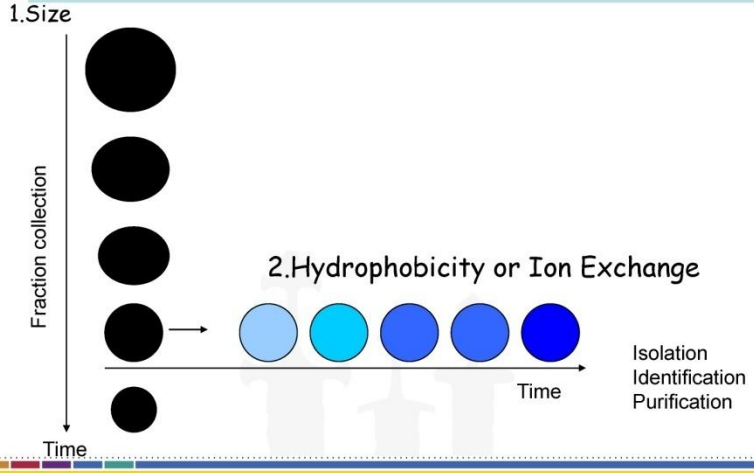
1. Which peptides are bioactive?

- Product evaluation in the present study is based on information from published literature:
- Literature search at 'ISI Web of knowledge' (http://apps.isiknowledge.com)

IRIS

14

2A. Sample preparation by chromatography

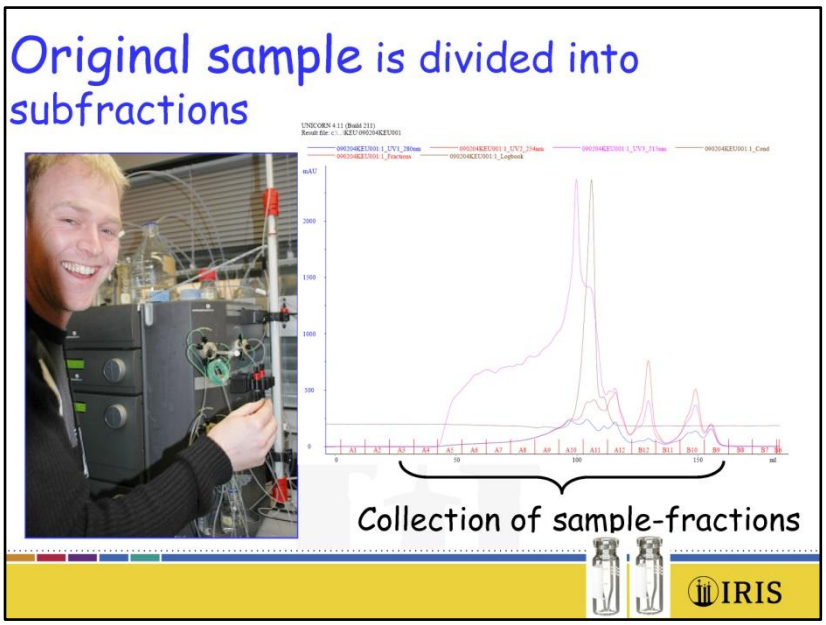


IRIS

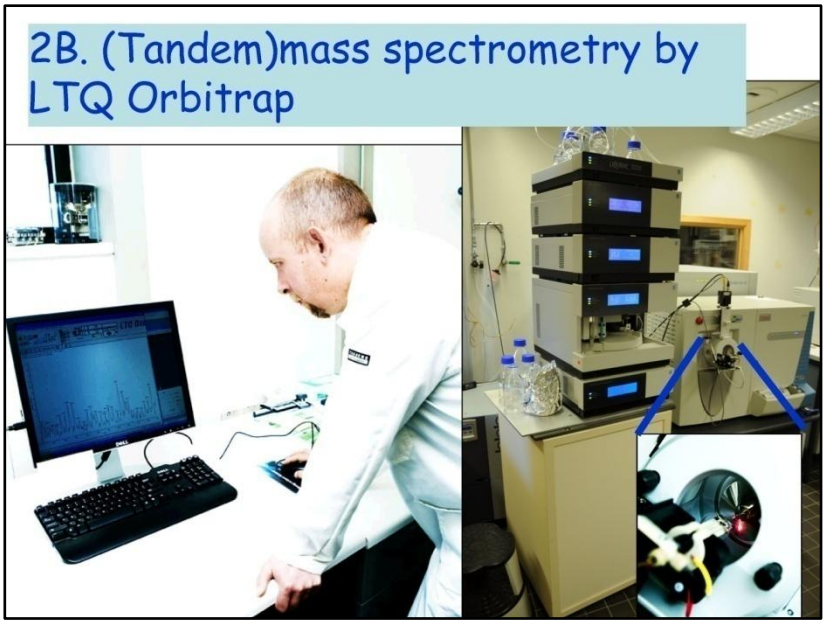
15


Challenge:
Sample-COMPLEXITY
IRIS

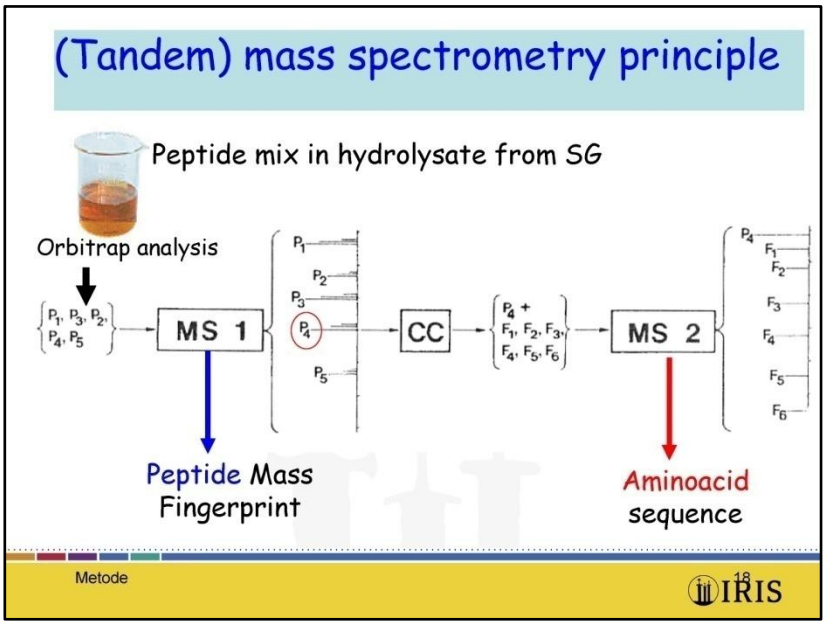
16


Original sample is divided into subfractions
Collection of sample-fractions
IRIS

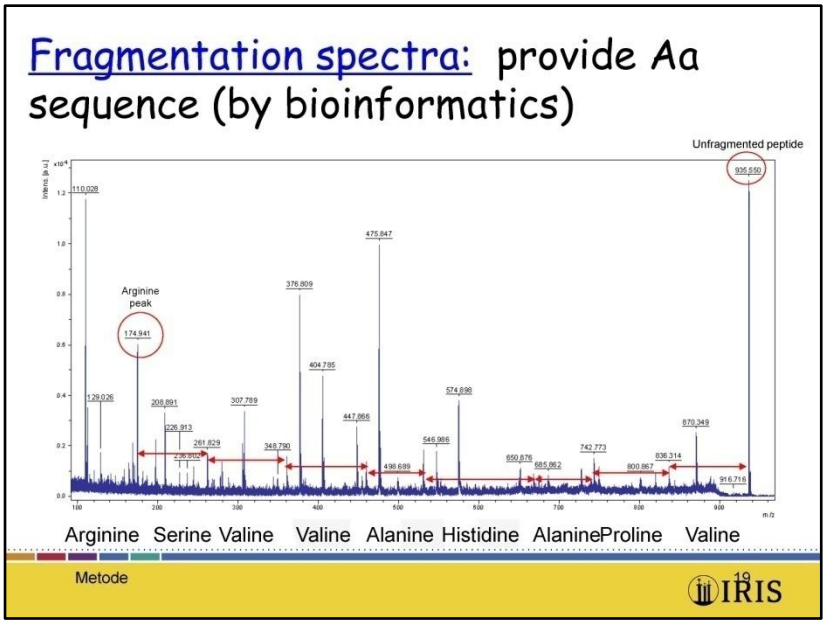
17


2B. (Tandem)mass spectrometry by LTQ Orbitrap

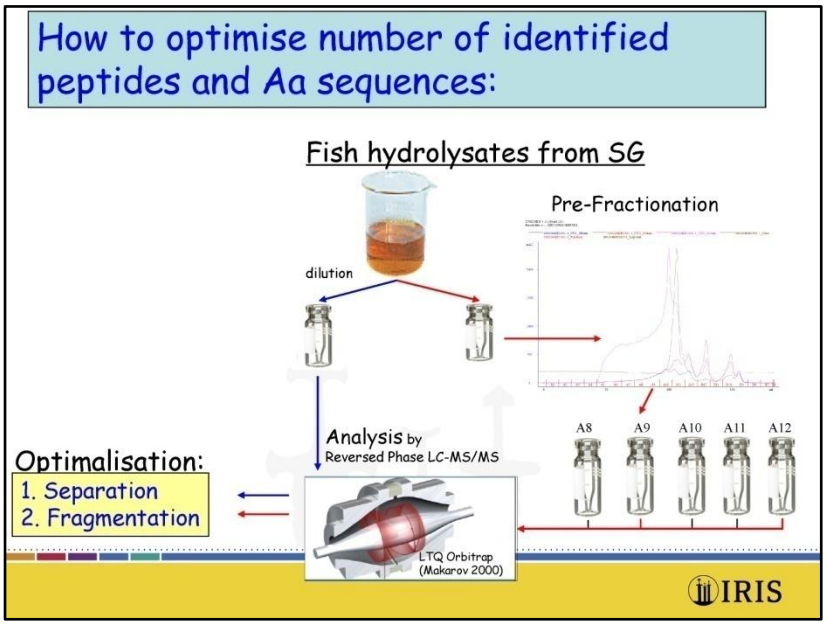
18


(Tandem) mass spectrometry principle
Peptide mix in hydrolysate from SG
Orbitrap analysis
MS 1
CC
MS 2
Peptide Mass Fingerprint
Aminoacid sequence
Metode
18
IRIS

19


Fragmentation spectra: provide Aa sequence (by bioinformatics)
Unfragmented peptide
Arginine peak
Arginine Serine Valine Valine Alanine Histidine AlanineProline Valine
Metode
19
IRIS

20


How to optimise number of identified peptides and Aa sequences:
Fish hydrolysates from SG
dilution
Pre-Fractionation
A8
A9
A10
A11
A12
Analysis by Reversed Phase LC-MS/MS
Optimalisation:
1. Separation
2. Fragmentation
LTQ Orbitrap (Makarov 2000)
IRIS

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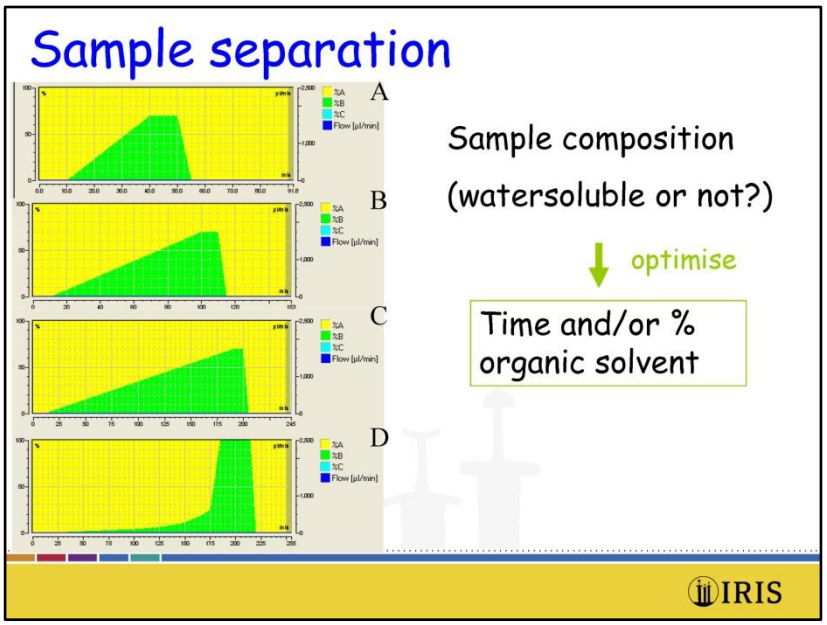
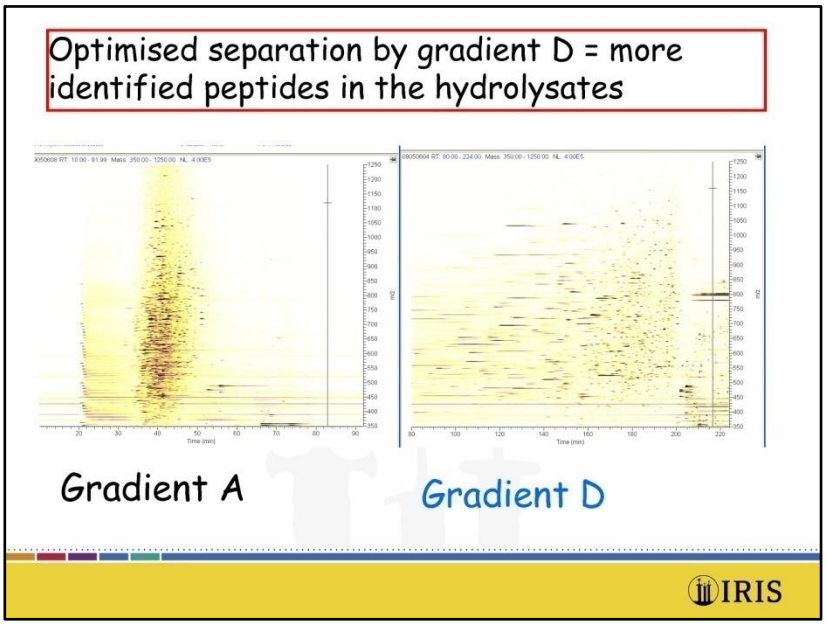
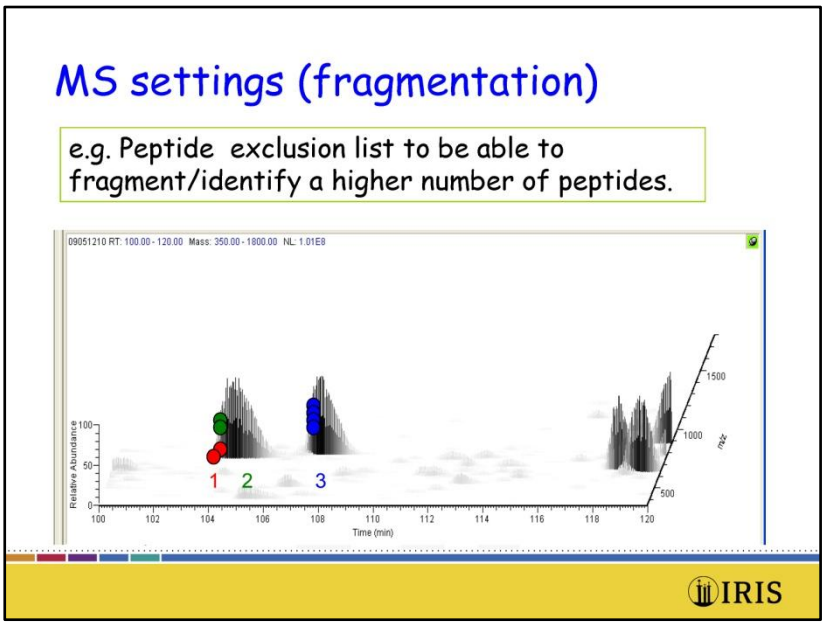

Sample separation
A
B
C
D
Sample composition
(watersoluble or not?)
optimise
Time and/or %
organic solvent
IRIS

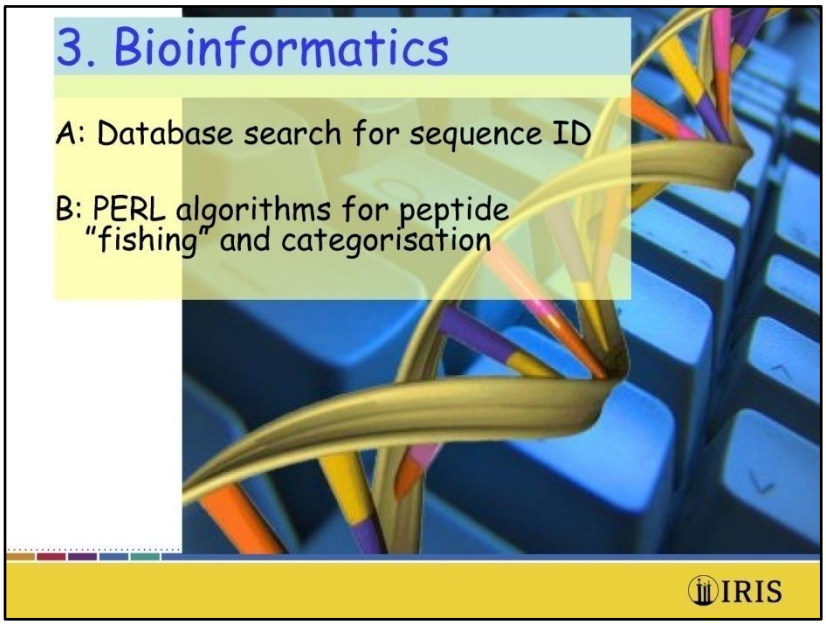
22


Optimised separation by gradient D = more identified peptides in the hydrolysates
Gradient A
Gradient D
IRIS

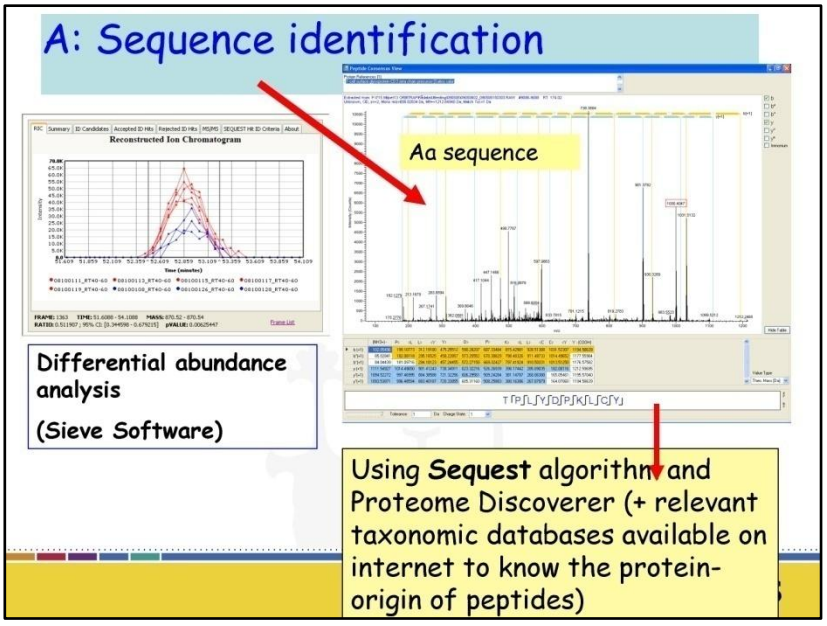
23


MS settings (fragmentation)
e.g. Peptide exclusion list to be able to fragment/identify a higher number of peptides.
Relative Abundance
Time (min)
1
2
3
m/z
IRIS

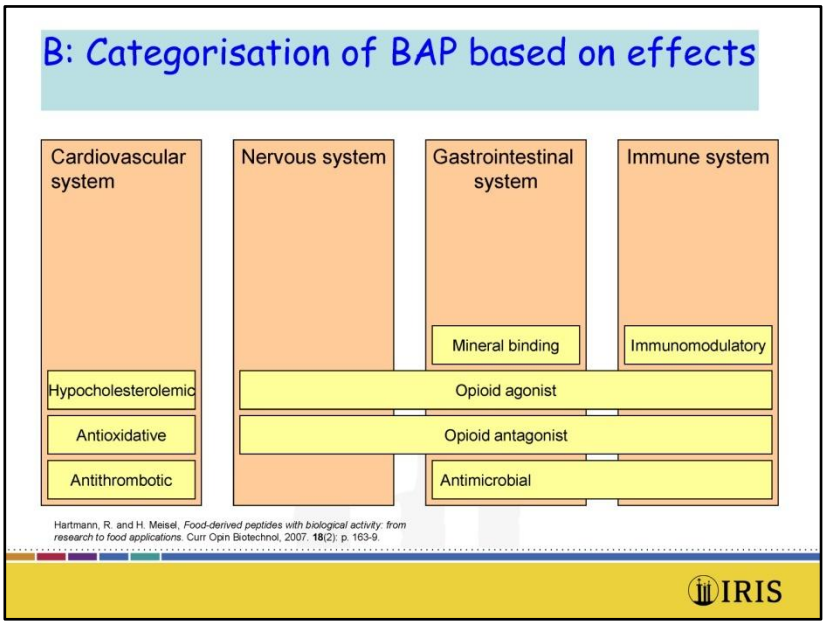
24


3. Bioinformatics
A: Database search for sequence ID
B: PERL algorithms for peptide "fishing" and categorisation
IRIS

25


A: Sequence identification
Reconstructed Ion Chromatogram
Aa sequence
Differential abundance analysis
(Sieve Software)
Using Sequest algorithm and Proteome Discoverer (+ relevant taxonomic databases available on internet to know the protein-origin of peptides)

26


B: Categorisation of BAP based on effects
Cardiovascular system
Nervous system
Gastrointestinal system
Immune system
Mineral binding
Immunomodulatory
Hypocholesterolemic
Opioid agonist
Antioxidative
Opioid antagonist
Antithrombotic
Antimicrobial
Hartmann, R. and H. Meisel, Food-derived peptides with biological activity: from research to food applications. Curr Opin Biotechnol, 2007. 18(2): p. 163-9.
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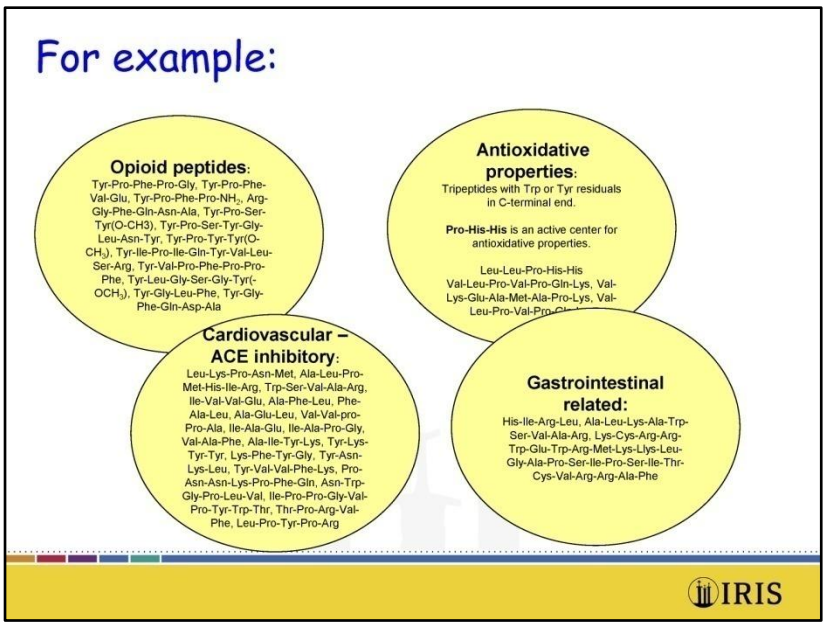
For example:
Opioid peptides:
Tyr-Pro-Phe-Pro-Gly, Tyr-Pro-Phe-Val-Glu, Tyr-Pro-Phe-Pro-NH2, Arg-Gly-Phe-Gln-Asn-Ala, Tyr-Pro-Ser-Tyr(O-CH3), Tyr-Pro-Ser-Tyr-Gly-Leu-Asn-Tyr, Tyr-Pro-Tyr-Tyr(O-CH3), Tyr-Ile-Pro-Ile-Gln-Tyr-Val-Leu-Ser-Arg, Tyr-Val-Pro-Phe-Pro-Pro-Phe, Tyr-Leu-Gly-Ser-Gly-Tyr(-OCH3), Tyr-Gly-Leu-Phe, Tyr-Gly-Phe-Gln-Asp-Ala
Antioxidative properties:
Tripeptides with Trp or Tyr residuals in C-terminal end.
Pro-His-His is an active center for antioxidative properties.
Leu-Leu-Pro-His-His
Val-Leu-Pro-Val-Pro-Gln-Lys, Val-Lys-Glu-Ala-Met-Ala-Pro-Lys, Val-Leu-Pro-Val-Pro-Gl
Cardiovascular – ACE inhibitory:
Leu-Lys-Pro-Asn-Met, Ala-Leu-Pro-Met-His-Ile-Arg, Trp-Ser-Val-Ala-Arg, Ile-Val-Val-Glu, Ala-Phe-Leu, Phe-Ala-Leu, Ala-Glu-Leu, Val-Val-pro-Pro-Ala, Ile-Ala-Glu, Ile-Ala-Pro-Gly, Val-Ala-Phe, Ala-Ile-Tyr-Lys, Tyr-Lys-Tyr-Tyr, Lys-Phe-Tyr-Gly, Tyr-Asn-Lys-Leu, Tyr-Val-Val-Phe-Lys, Pro-Asn-Asn-Lys-Pro-Phe-Gln, Asn-Trp-Gly-Pro-Leu-Val, Ile-Pro-Pro-Gly-Val-Pro-Tyr-Trp-Thr, Thr-Pro-Arg-Val-Phe, Leu-Pro-Tyr-Pro-Arg
Gastrointestinal related:
His-Ile-Arg-Leu, Ala-Leu-Lys-Ala-Trp-Ser-Val-Ala-Arg, Lys-Cys-Arg-Arg-Trp-Glu-Trp-Arg-Met-Lys-Llys-Leu-Gly-Ala-Pro-Ser-Ile-Pro-Ser-Ile-Thr-Cys-Val-Arg-Arg-Ala-Phe
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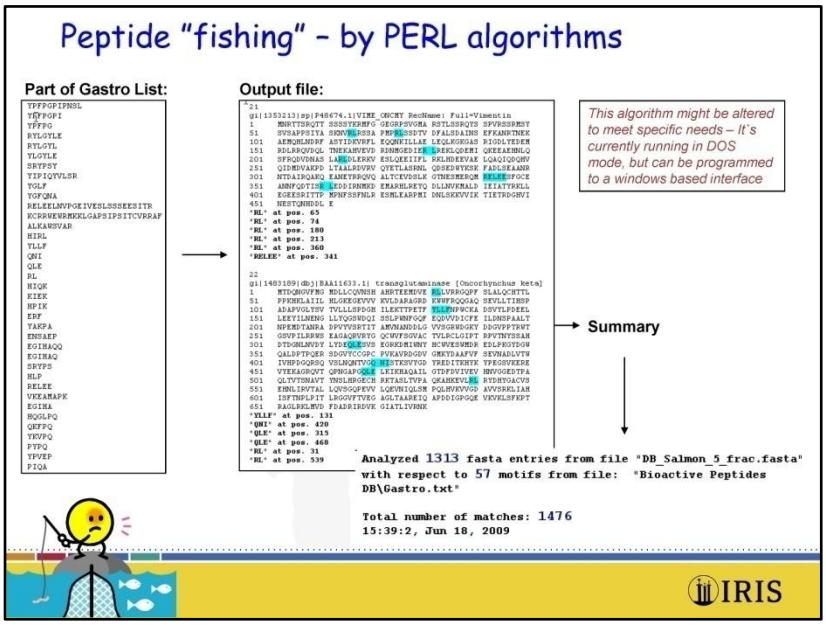
Peptide "fishing" – by PERL algorithms
Part of Gastro List:
YPFPGPIPNSL
YPYPGPI
YPFPG
RYLGYLE
RYLGYL
YLGYLE
SRYPSY
YIPIGYVLSR
YGLF
YGFGNA
RELEELNVPGEIVESLSSSEESITR
RCRRWEWRKKLGAPSIPSITCVRRAF
ALKAHSVAR
HIRL
YLLF
QNI
GLE
RL
NIQK
KIEK
HPIK
ERF
YAKPA
ENSAEP
EGIHAQQ
EGIHAQ
SRYPS
HLP
RELEE
VKEAMAPK
EGIHA
HQGLPQ
QKFPQ
YKVPQ
PYPQ
YPVEP
PIQA
Output file:
This algorithm might be altered to meet specific needs – It`s currently running in DOS mode, but can be programmed to a windows based interface
Summary
Analyzed 1313 fasta entries from file "DB_Salmon_5_frac.fasta" with respect to 57 motifs from file: "Bioactive Peptides DB\Gastro.txt"
Total number of matches: 1476
15:39:2, Jun 18, 2009
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Results

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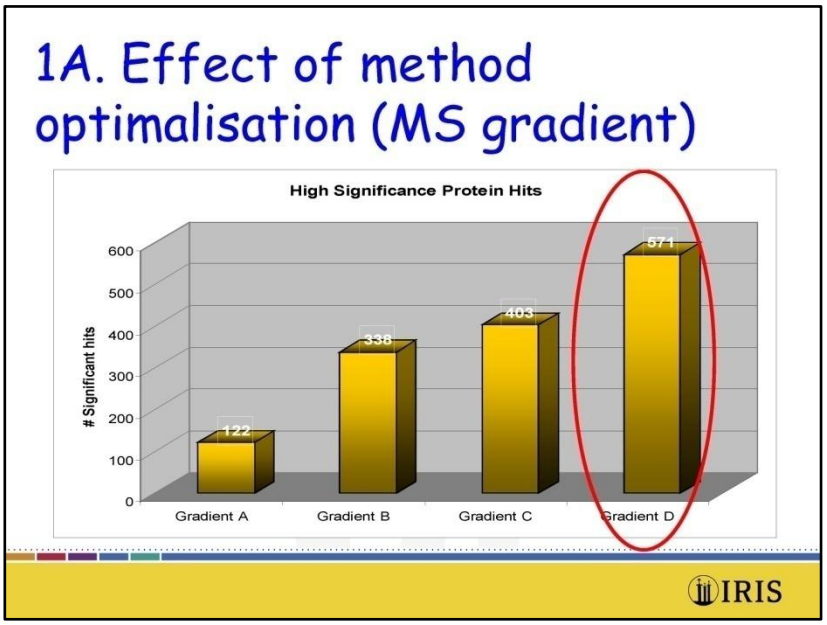
1A. Effect of method optimalisation (MS gradient)
High Significance Protein Hits
Significant hits
0
100
200
300
400
500
600
122
338
403
571
Gradient A
Gradient B
Gradient C
Gradient D
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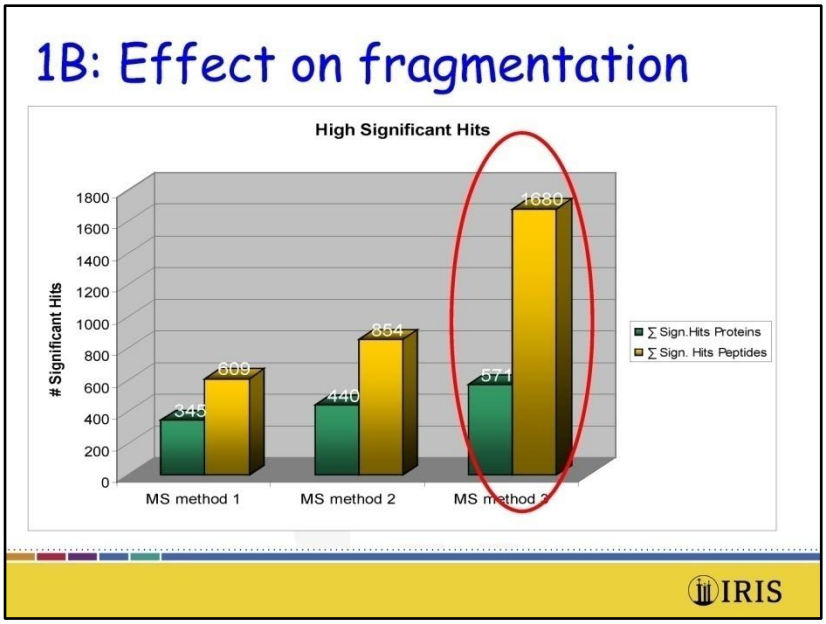
1B: Effect on fragmentation
High Significant Hits
Significant Hits
1800
1600
1400
1200
1000
800
600
400
200
0
609
440
854
1680
MS method 1
MS method 2
∑ Sign.Hits Proteins
∑ Sign. Hits Peptides
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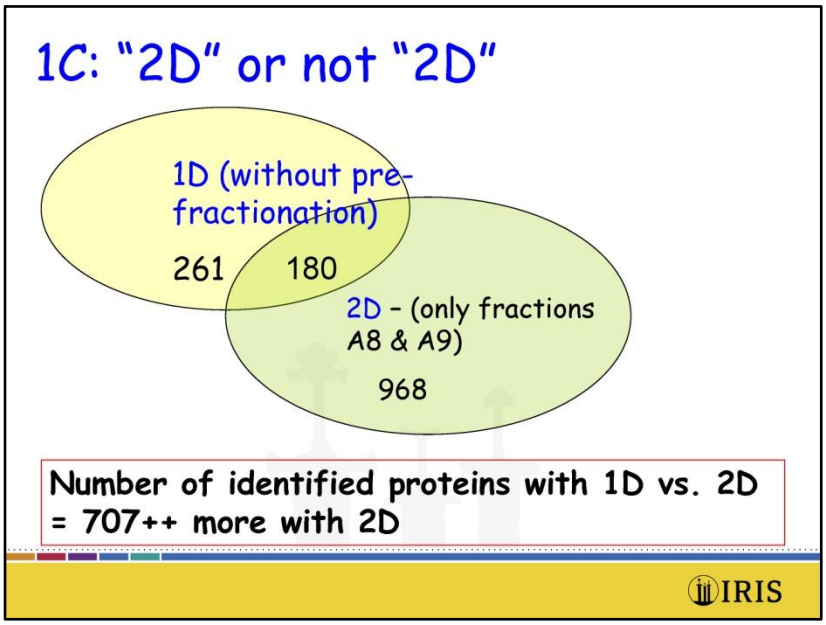
1C: "2D" or not "2D"
1D (without pre-fractionation)
261
180
2D – (only fractions A8 & A9)
968
Number of identified proteins with 1D vs. 2D = 707++ more with 2D
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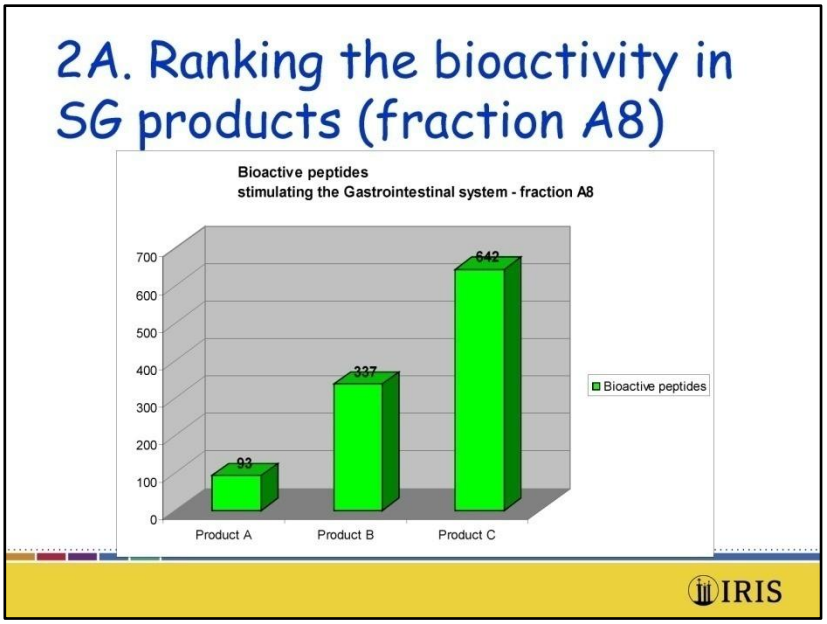
2A. Ranking the bioactivity in SG products (fraction A8)
Bioactive peptides
stimulating the Gastrointestinal system - fraction A8
700
600
500
400
300
200
100
0
93
337
642
Product A
Product B
Product C
Bioactive peptides
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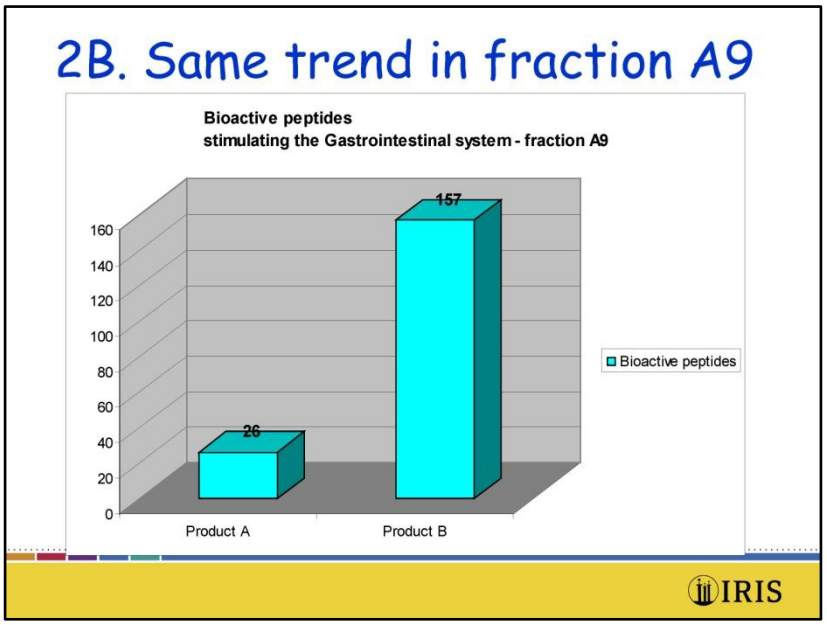
2B. Same trend in fraction A9
Bioactive peptides
stimulating the Gastrointestinal system - fraction A9
160
140
120
100
80
60
40
20
0
26
157
Product A
Product B
Bioactive peptides
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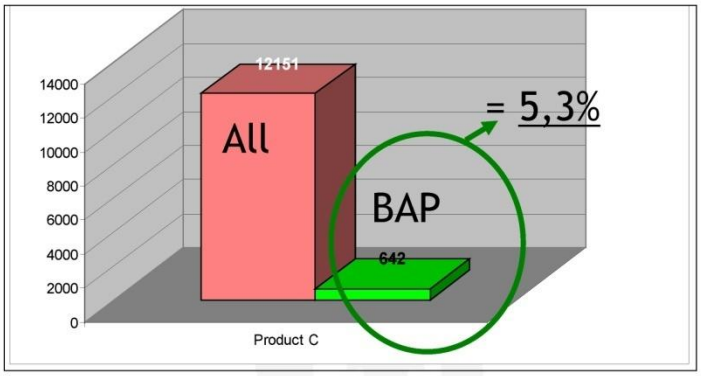
BAP (gastrointestinal system) vs. all Peptides (in A8)



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Main conclusions

- The results revealed that all products from Seagarden contain bioactive peptides that is known, from literature, to stimulate the gastrointestinal system.
- The results did also indicated that the level of bioactivity varied among products.

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Conclusions II

- Improving chromatography and MS parameters significantly increased the number of identified peptides and proteins (for both 1D and 2D).
- 2-dimensional chromatography further increases the number of identified peptides and proteins compared to 1D.
- A PERL algorithm was successfully used to determine the number of "category-specific" peptides in each product.

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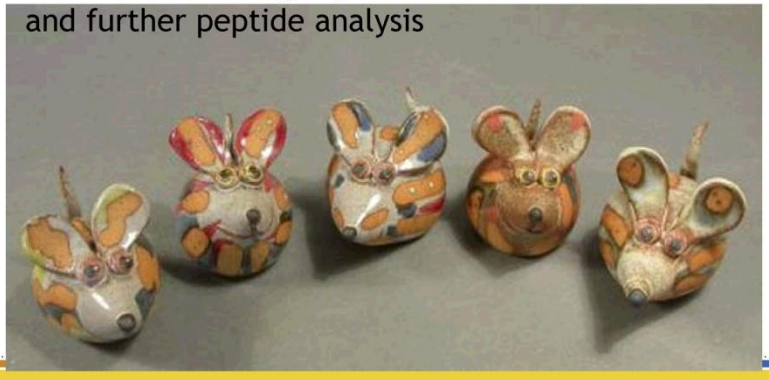
The way forward:

Clinical testing by NIFES....

and further peptide analysis



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TEAM
who did
the work
presented
Kai-Erik Uleberg, Eivind Larssen,Kjell-Birger Øysæd
IRIS

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Acknowledgement
• Innovasion Norway and Prekubator for funding.
• The rest of the project team, and particularly Gunnar Kleppe for keeping the team "on track".
• The organisers for the invitation

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..and thank you for your attention!!!

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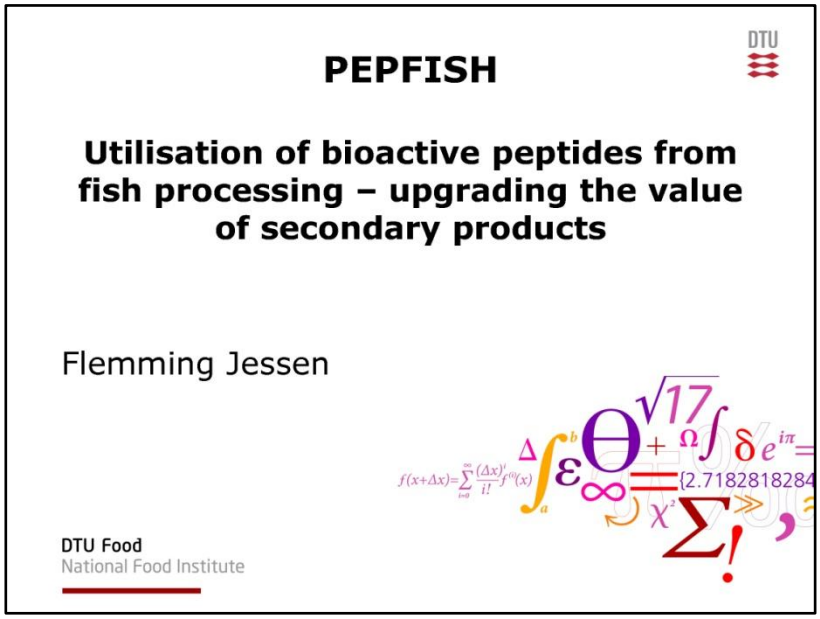
Contact:
EMail: Anne.hjelle@iris.no
Phone: 0047 97590954
Dr. Anne Hjelle
Acting Vice President
IRIS Biomiljø
Mekjarvik 12
4070 Randaberg, Norway
IRIS

Appendix 12. ”PEPFISH: Utilisation of bioactive peptides from fish processing – upgrading the value of secondary products” by Flemming Jessen

1



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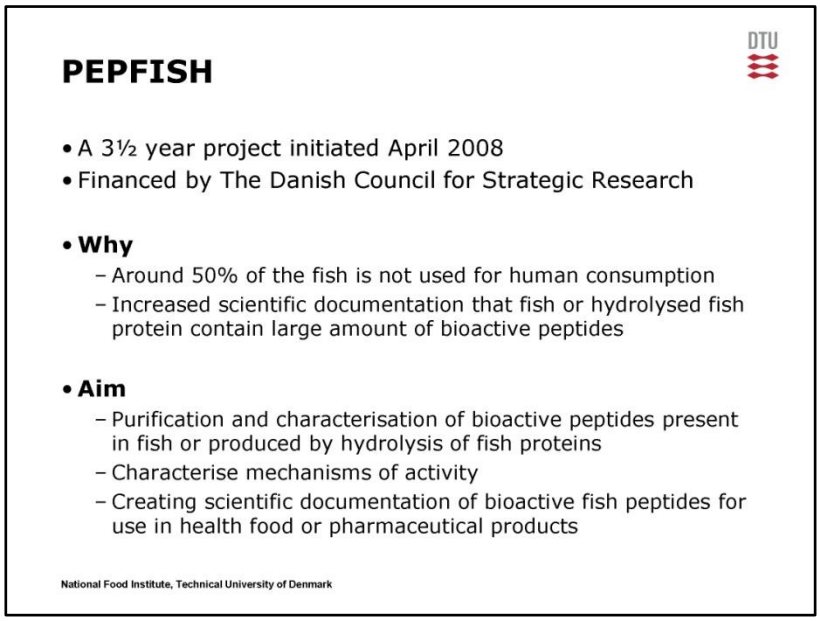
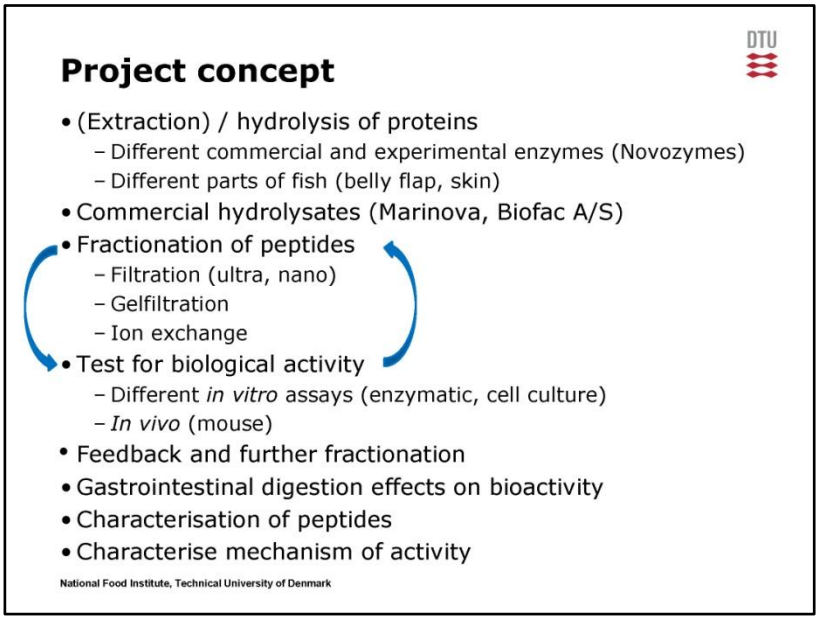


3


Project partners
• Technical University of Denmark, National Food Institute
– Lisa Lystbæk Andersen, Henrik Hauch Nielsen, Michael Engelbrecht, Flemming Jessen
• University of Copenhagen, Biological Institute
– Else K. Hoffmann, Carlo Ossum
• Rigshospitalet, Dept. of Clinical Microbiology
– Leif Percival Andersen, Anna Boschian, Lone Rasmussen
• Lund University, Division of Bacteriology
– Torkel Wadström
• University of Tromsø, Institute of Marine Biotechnology
– Edel Oddny Elvevoll
• Marinova
– Greta Jakobsen, Inez Johansson
• Biofac A/S
– Peter Rørvig, Charlotte B. Pipper
• Novozymes A/S
– Gitte Budolfsen Lynglev, Steffen Ernst

4


Project concept
DTU
• (Extraction) / hydrolysis of proteins
– Different commercial and experimental enzymes (Novozymes)
– Different parts of fish (belly flap, skin)
• Commercial hydrolysates (Marinova, Biofac A/S)
• Fractionation of peptides
– Filtration (ultra, nano)
– Gelfiltration
– Ion exchange
• Test for biological activity
– Different *in vitro* assays (enzymatic, cell culture)
– *In vivo* (mouse)
• Feedback and further fractionation
• Gastrointestinal digestion effects on bioactivity
• Characterisation of peptides
• Characterise mechanism of activity
National Food Institute, Technical University of Denmark

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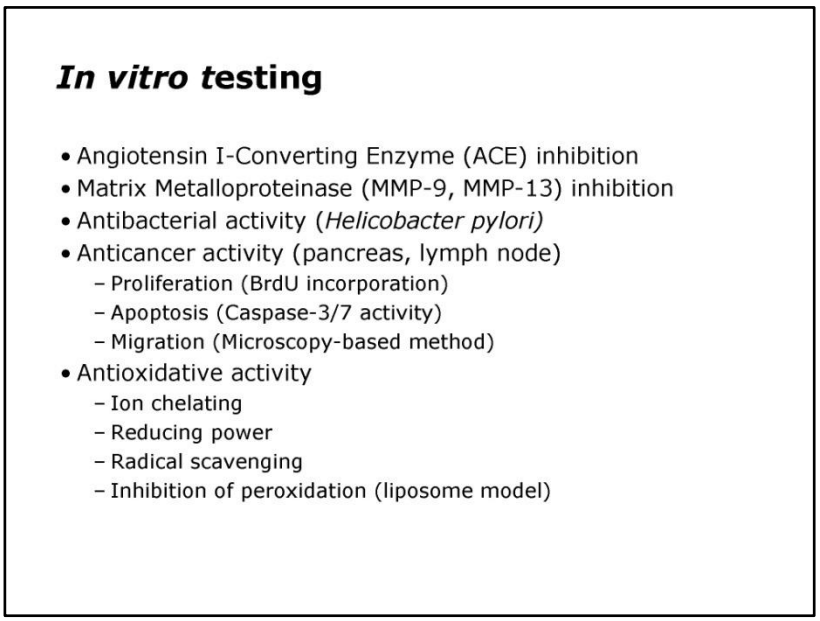
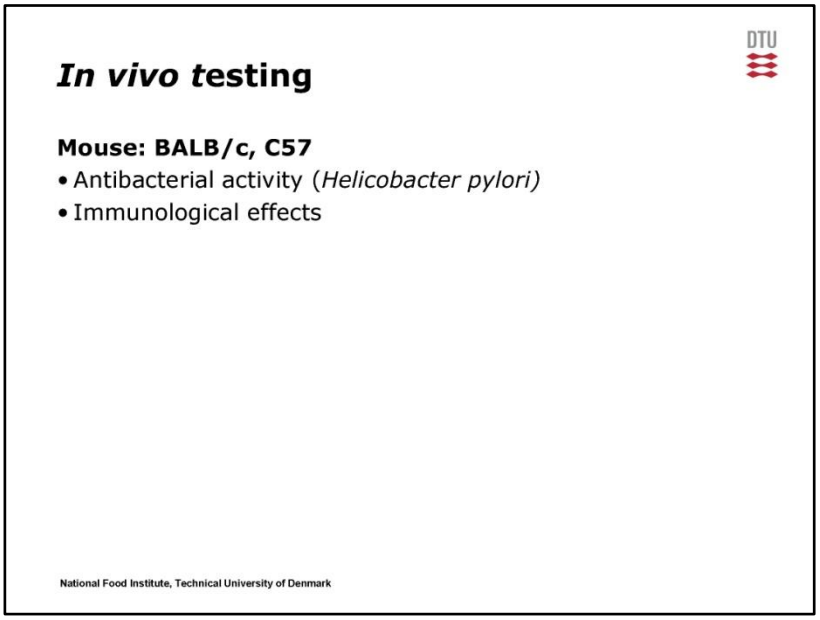

In vitro testing
• Angiotensin I-Converting Enzyme (ACE) inhibition
• Matrix Metalloproteinase (MMP-9, MMP-13) inhibition
• Antibacterial activity (Helicobacter pylori)
• Anticancer activity (pancreas, lymph node)
– Proliferation (BrdU incorporation)
– Apoptosis (Caspase-3/7 activity)
– Migration (Microscopy-based method)
• Antioxidative activity
– Ion chelating
– Reducing power
– Radical scavenging
– Inhibition of peroxidation (liposome model)

6


In vivo testing
DTU
Mouse: BALB/c, C57
• Antibacterial activity (Helicobacter pylori)
• Immunological effects
National Food Institute, Technical University of Denmark

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Preliminary results

- ACE inhibition activity
  - Found in the main part of hydrolysates or peptide fractions that have been tested.
  - Relevance?
- Anticancer activity
  - Inhibition of cell proliferation at low concentration (0.1 mg/ml) of a couple of hydrolysates and fractions from these.
  - Possible induction of apoptosis by some hydrolysates in the primary tumour cell line, but no effect on the lymph node metastasis cell line.
- Antioxidative effects
  - Activity found in commercial and experimental hydrolysates
  - Low molecular fractions have higher activity than high molecular fractions

National Food Institute, Technical University of Denmark

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www.pepfish.dk

National Food Institute
Technical University of Denmark
Mørkhøj Bygade 19
DK - 2860 Søborg

T: 35 88 70 00
F: 35 88 70 01
www.food.dtu.dk

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