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Marina råvaror i utveckling av hållbara foder

Foto: Ilar Gunilla Persson

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Fish feed

- **Larvae & early growth:** Live feed; mikroalgae, artemia etc
- **On-growth:** Formulated feed; traditionally fish meal & fish oil, nowadays ↑ vegetable sources like soy

- **Fish meal/fish oil:**
  - \+ Resembles ”wild” feed with respect to DHA & EPA-content, protein/oil, high nutrition/density
  - \- Aquaculture calls for high production of fish meal/fish oil → risk for ”fodder fish” fish depletion!!

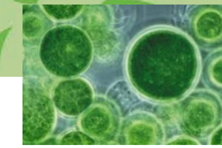
- **Vegetable fats & proteins**
  - \+ Saves marine resources
  - \- Antinutritional factors (fibers), poorer digestion, increased pollution, ↓n-3 PUFA content in the fish, negative environmental impact (soy).

Reduction in fishmeal inclusion in compound aquafeed of different fish species and species groups

Species/species group	Fishmeal inclusion in compound aquafeed		
	1995	2008	2020*
	(Percentage)		
Fed carp	10	3	1
Tilapias	10	5	1
Catfishes	5	7	2
Milkfish	15	5	2
Miscellaneous freshwater fishes	55	30	8
Salmons	45	25	12
Trouts	40	25	12
Eels	65	48	30
Marine fishes	50	29	12
Marine shrimps	28	20	8
Freshwater crustaceans	25	18	8

* Projected.

Source: Adapted from Tacon, A.G.J., Hasan, M.R. and Metian, M. 2011. *Demand and supply of feed ingredients for farmed fish and crustaceans: trends and prospects.* FAO Fisheries and Aquaculture Technical Paper No. 564. Rome, FAO. 87 pp.

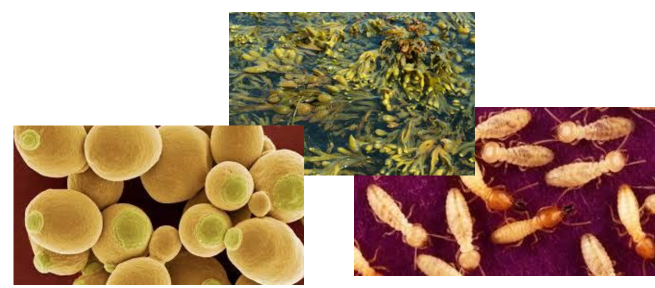


Examples of alternative oils

- Vegetable oils containing biosynthetic precursors to LC n-3 PUFA , e.g. stearidonic acid (C18:4, n-3)
- Single-cell oils with n-3 LC-PUFA (algae, modified yeast..)
- Oils from genetically modified oil-seed crops, i.e. with n-3 LC-PUFA.
- Poultry oil
- Oils from seafood byproducts

Examples of alternative proteins

- Soybean
- Barley
- Rice
- Peas,
- Canola
- Lupine
- Wheat gluten
- Corn gluten
- Yeast
- Insects
- Algae/seaweed
- Seafood byproducts

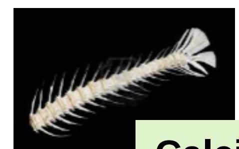


6 141 900 ton fish produced in EU (28 countries)

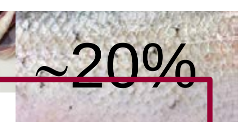
5 220 615 ton "consume fish" → **Only~2 610 308 ton for food!**

921 285 ton "fodder fish" → **~1 312 830 ton underutilized muscle**

- Fish mince
- Functional proteins
- Peptides



- Fish meal
- Calcium
- Phosphate



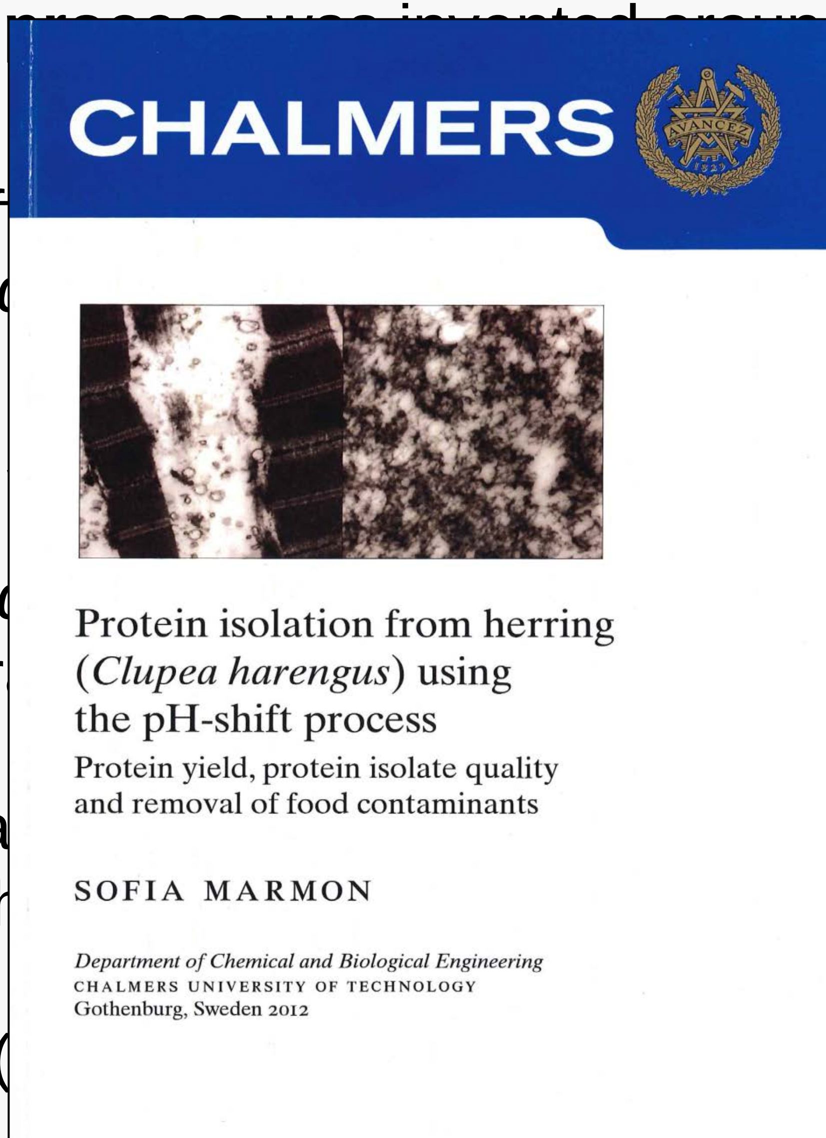
~20%

- Oil
- Phosphilipids
- Nucleic acids (e.g. DNA)/nucleotides
- Peptides

Sources: FAO, RUBIN, VRF, Sintef, Nofima, Havs och Vattenmyndigheten, Eurostat

The **pH-shift** d 2000, and since then we have with:

- Herring of dif
    - *Isolate*
    - *Fillets*
    - *Whole*
    - *Whole*
    - *By-proc*
- Cod heads/fr
- Blue whiting
- Whole venda
- Mussels (with
- Microalgae
- Macroalage (



*Most trials done in **lab scale**, but 2010-2011 also **pilot scale***

Classic steam-driven fish oil/meal production

Heat (95-100°C, 15-20 min) proteins coagulate, fat deposits open up, oil and chemically bound water is released

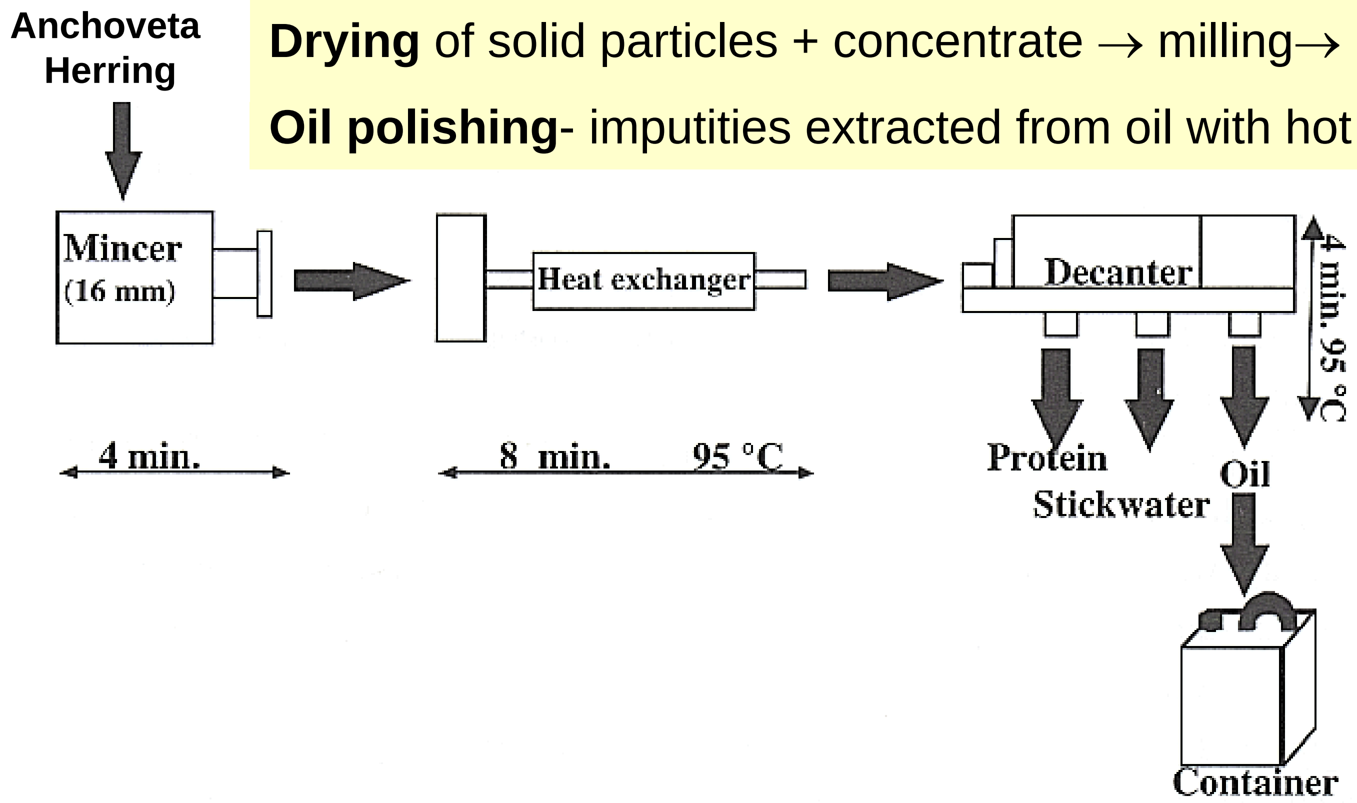
Press/g-force: Separates liquid from solid particles (press cake)

Separation of liquid → water (”stickwater”) and oil phase

Evaporation of ”stickwater” → concentrate of soluble compounds

Drying of solid particles + concentrate → milling→ meal

Oil polishing- imputities extracted from oil with hot water

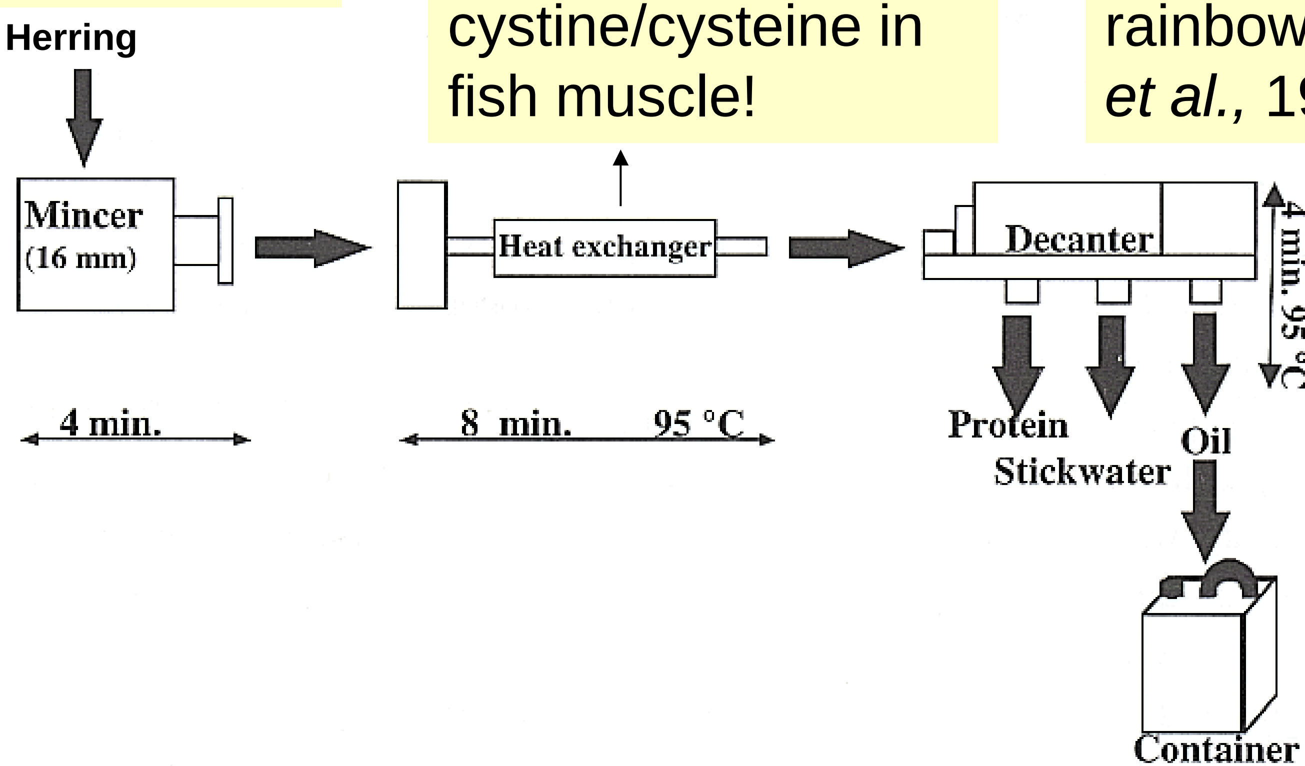


Classic steam-driven fish oil/meal production

Fish meal from byproducts of ”poorer quality”

Heat (95°C) can induce S-S bonds and losses of cystine/cysteine in fish muscle!

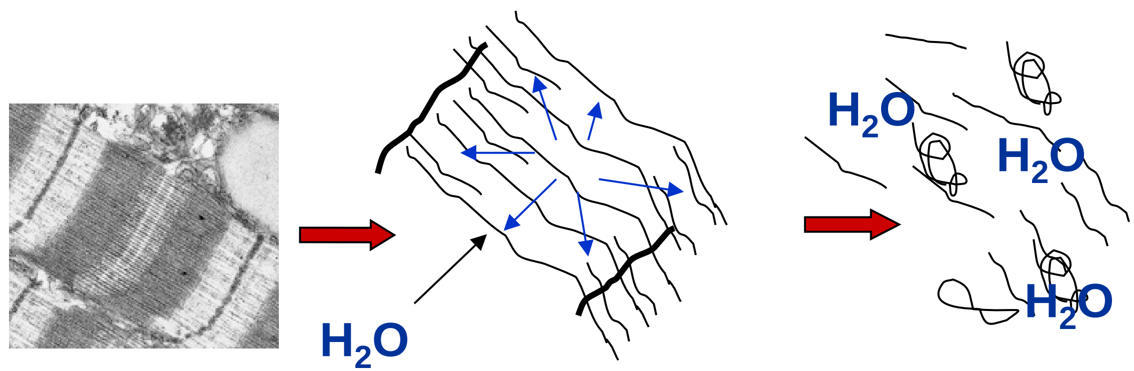
Reduced protein and amino acid digestibility in feeding studies with rainbow trout (Opstvedt *et al.*, 1984)



Utgångshypotes:

pH-skiftprocessen ett intressant alternativ för att tillverka foderingredienser från fisk- & skaldjursbiprodukter →bättre digererbarhet, mer essentiella aminosyror, bibehållen funktionalitet

How can extreme pH dissolve proteins?



Expansion & Exposure of charged groups

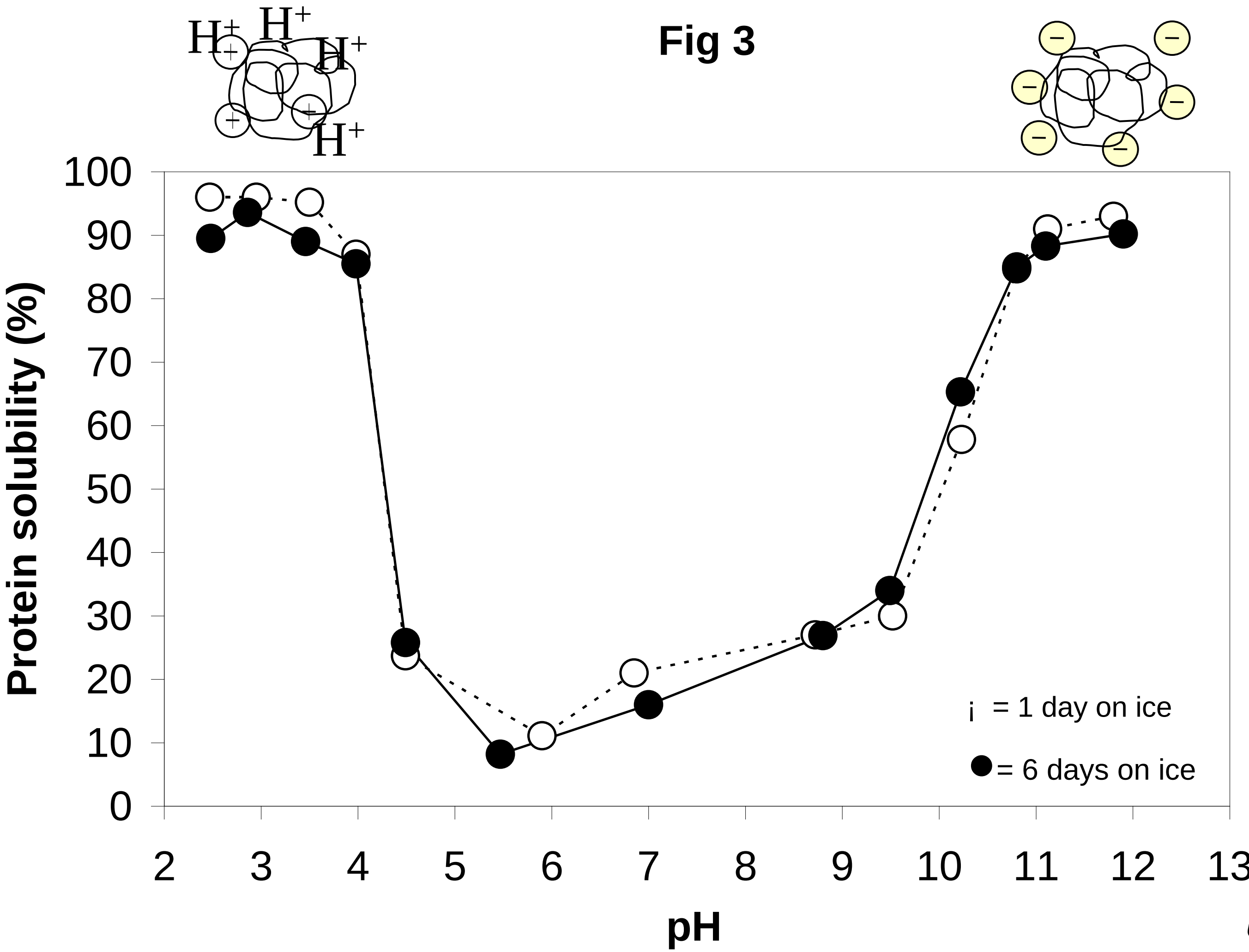
Expansion caused by creating repulsion between proteins

Solubilization

↓

Basis for isolation

Protein solubility vs. pH



Undeland, et al., 2003

Oxidative stabil

Bioactivity

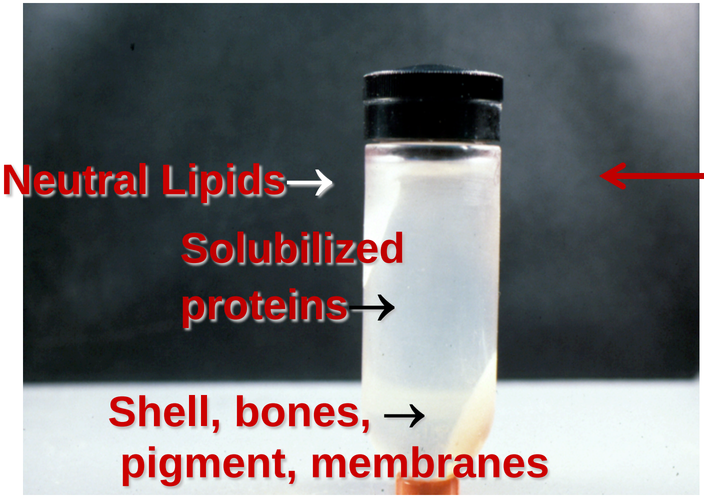
Homogenization
raw material + water (≥1:3)

↓

Protein solubilization
pH → ~3 or 11

↓

Separation
(e.g. ≤10 000g)



↓

Protein precipitation
pH → ~5.5

↓

Protein dewatering
(≤10 000g)

↓

Final protein isolate

↓

Gelation
(pH →7.1, 2% salt
20 min 90°C)

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Homogenization
raw material + water (≥1:3)

- Water consuming

↓

Protein solubilization
pH → ~3 or 11

↓

Separation
(e.g. ≤10 000g)

↓

Protein precipitation
pH → ~5.5

↓

Protein dewatering
(≤10 000g)

- Dewatering challening in large scale

↓

Final protein isolate

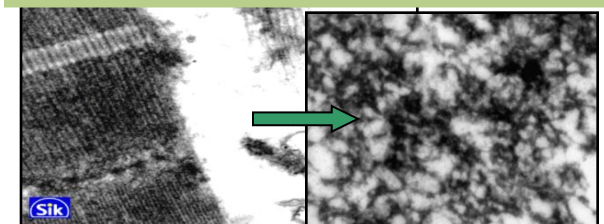
- Requires antioxidants with blood rich materials

Compared to the actual **fishing step**, the pH shift process is not of major importance for neither the climate gas emissions nor the energy consumption coupled to the protein isolate

- 50-75% Protein recovery

- ≤ 70% ↓ fat
- ≤ 70-80% ↓ in dioxins & PCB´s
- ≤ 80 % ↓ DSP-toxins
- ↑ essential amino acids
- Retained protein digestibility

- Good gelation capacity



Composition of mussel raw material and isolates

Process	**Moisture**	**Ash (% dw)**	**Lipid (% dw)**	**Protein (% dw)**
Mussel meat	800±19[a]	90±1.5[a]	108±17	467±52
Acid process (1:6)	900±16[b,c]	n.a.	110±70	763±151
Alkaline process (1:6)	867±22[b,c]	n.a.	188±83	639±138
Acid process (1:9)	925±14[b]	41±1.2[b]	143±33	903±58
Alkaline process (1:9)	884±7[c]	42±8.5[b]	134±9	730±94
Acid process whole mussels (1:9)	941±7[b]	n.a.	83±4	749±73
Alkaline process whole mussels (1:9)	886±8[b,c]	n.a.	122±21	802±99

Good quality fish meals contain crude protein levels above 66%, fat content around 8 to 11%, and ash generally below 12%.

Amino acids

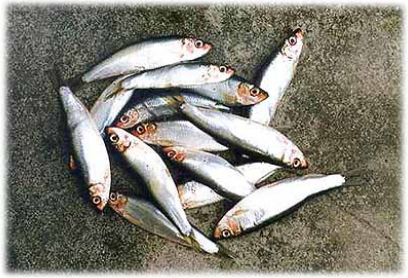
Cysteine + Methionine/total protein,
Lysine/total protein
Methionine/total protein

- *higher in protein isolates than in the mussel meat*

- *higher than the minimum ratios regarded as acceptable in industrial fodder/feed products (41, 45 and 22 g kg−1 respectively)*

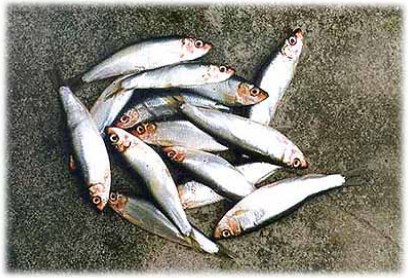
Composition of gutted herring raw material and isolates

	Herring mince	Alkali-made isolate	Acid-made isolate
Water (%)	78.0 ± 0.4 (n=3) [a]	89.3 ± 0.9 (n=8) [b]	89.8 ± 0.7 (n=6) [b]
Lipids (%, dw)	35.9 ± 1.5 (n=3) [a]	17.7 ± 2.2 (n=7) [b]	22.2 ± 6.9 (n=5) [b]
Protein (%, dw)	56.5± 3.2 (n=3) [a]	81.0 ± 6.4 (n=5) [b]	81.3 ± 6.5 (n=6) [b]
Ash (%, dw)	8.8 ± 1.0 (n=5) [a]	1.1 ± 0.3 (n=5) [b]	1.5 ± 0.4 (n=5) [b]



Minerals (µg/g dw)

	Herring mince	Alkali-made isolate	Acid-made isolate
Copper	1.6 ± 0.1 [a]	5.0 ± 0.9 [b]	7.3 ± 2.2 [b]
Zinc	46 ± 6 [a]	93 ± 12 [b]	165 ± 32 [c]
Iron	26 ± 1 [a]	43 ± 12 [ab]	46 ± 4 [b]
Calcium	$12 \cdot 10^3 \pm 0.6 \cdot 10^3$ [a]	$0.15 \cdot 10^3 \pm 0.03 \cdot 10^3$ [b]	$1.6 \cdot 10^3 \pm 0.54 \cdot 10^3$ [c]
Magnesium	960 ± 58 [a]	85 ± 11 [b]	170 ± 46 [c]



Recovery of proteins with pH-shift processing of trout by products & krill

Protein recovery trout frames/heads	78-89%
Change in ash	14 → 1.4-2.1
Protein recovery whole krill	47-50%
Change in ash	17 →5%

Chen & Jaczynski, J Agric Food Chem, 2007, 55, 9079-9088
Chen, Tou, Jaczynski, JFS, 2009, 74, H31-39

Earlier projects carried out on the pH-shift process have been funded by **Formas and Fiskeriverket/Jordbruksverket**

Ongoing projects where this process is involved are:

-Nomaculture (Mistra -*James H*)

-MareValue (Vinnova -*Mehdi A*)

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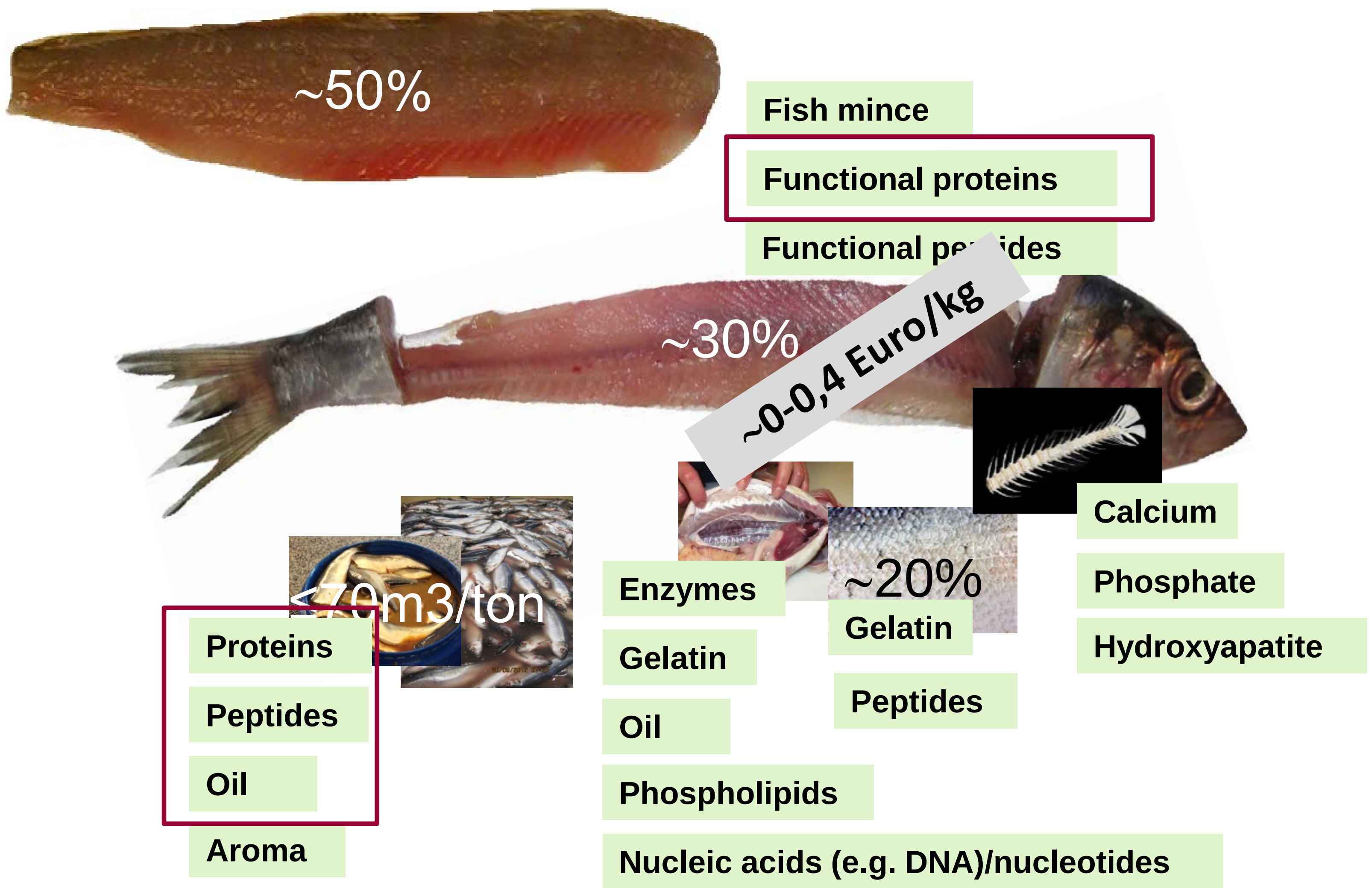
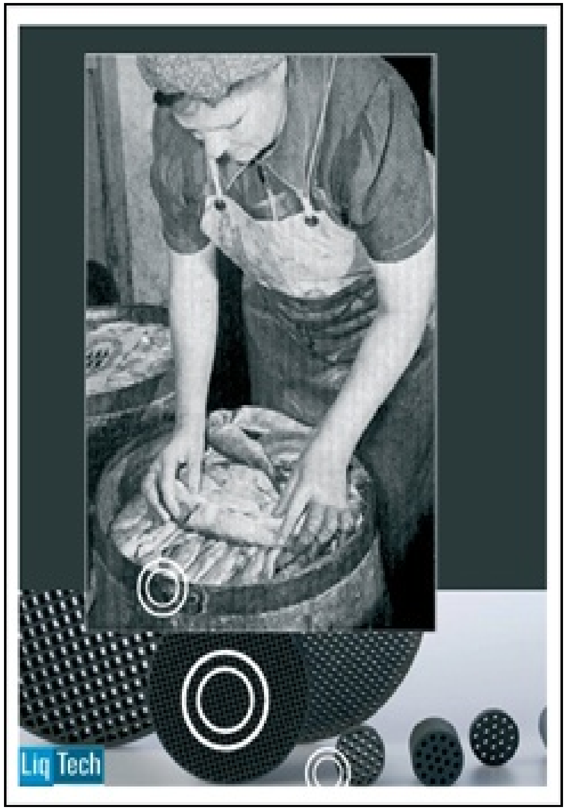


Sources: *FAO, RUBIN, VRF, Sintef, Nofima, Havs och Vattenmyndigheten, Eurostat*

The PIPE project

➢ **P**elagic **I**ndustry **P**rocessing **E**ffluents: Innovative and sustainable solutions



Partners:

- *DTU, Denmark*
- *Chalmers, Sweden.*
- *Univ of Iceland*
- *Fish and Food Expert (FFE), Denmark.*
- *Paul Mattsson AB, Sweden: Herring processor.*
- *Lykkebergy A/S, Denmark: Herring processor*
- *Liqteq A/S, Denmark Ceramic membranes*
- *A-factory A/S, Denmark: Electrochemistry*

Project period: January 2012-April 2015
Budget: 9 Million NOK

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Nordic Innovation
DTU Danmarks Tekniske Universitet
LiqTech INTERNATIONAL
UNIVERSITY OF ICELAND
AVANCEZ 1829
FISKBEREDNING PAUL MATTSSON AB
LYKKEBERG

The fish industry, especially the marinated herring industry consumes a lot of water….

….and genererates interesting side streams with high levels of biomolecules..

… but currently ending up as effluents which are sent to the local sewage plants!

PIPE Objectives

1. Test **ceramic membrane and electrochemistry** separation technologies either alone or in combination on different process waters (WP1)

2. Quantify the waters´ organic matter and document their **composition before and after application of the technology** (WP2)

3. Characterize and define the **quality** as well as the **properties** of the recovered organic material and investigate **new applications**. (WP3)

4. Investigate the **potential market** for both the tested technologies and the obtained bio-molecules and **perform cost/benefit analysis** (WP4)

WP1: Waters addressed

RSW-water

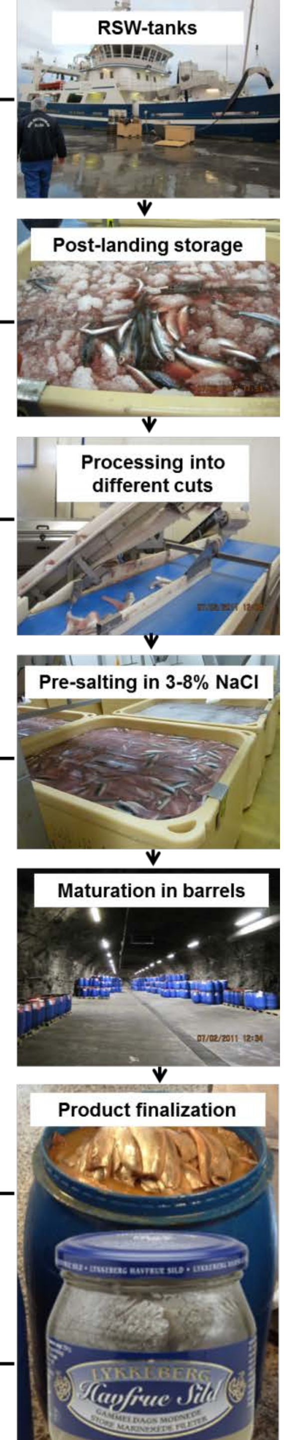
Storage water

Filleting water

Salt brines

Maturation brines (SC, TSp)

Desalting brines



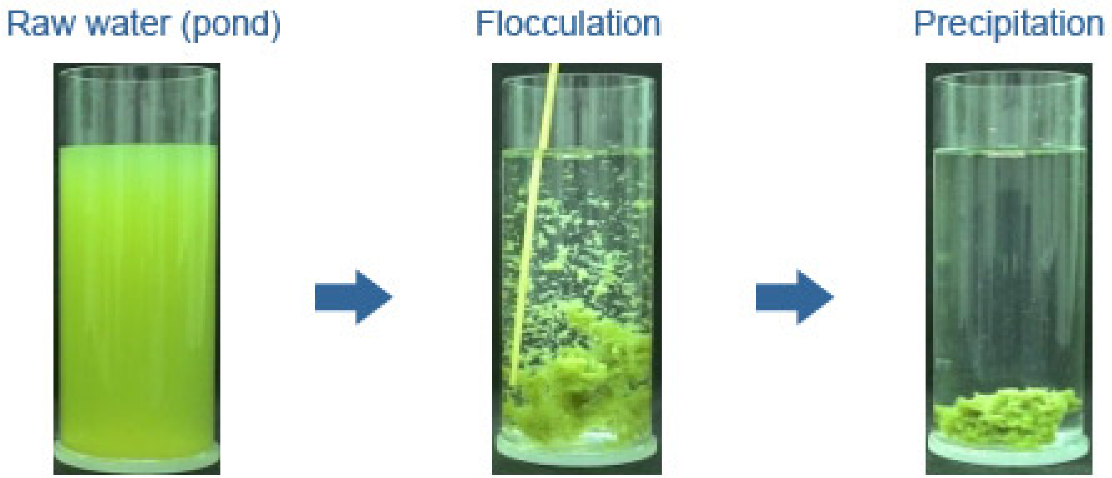
Boat to barrel -Chalmers

Barrel to Jar -DTU

WP1: Techniques addressed

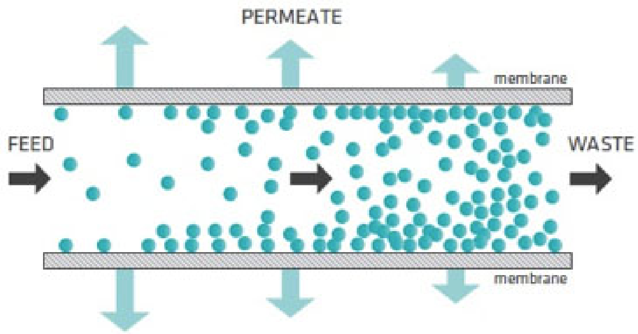
Electroflocculation

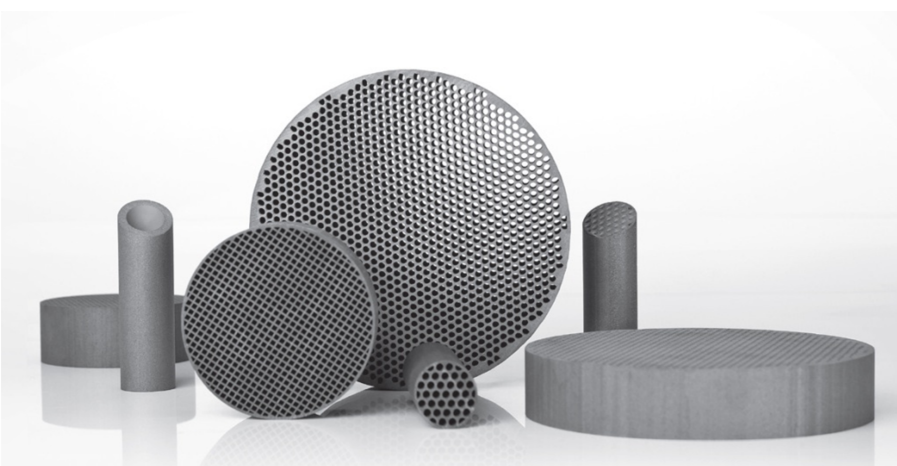
- Coagulation using either Al or Fe electrodes
- Applying a current, leading to release of Al or Fe ions
- Combination of positively charged metal ions with negatively charged particles in the water causes flocculation



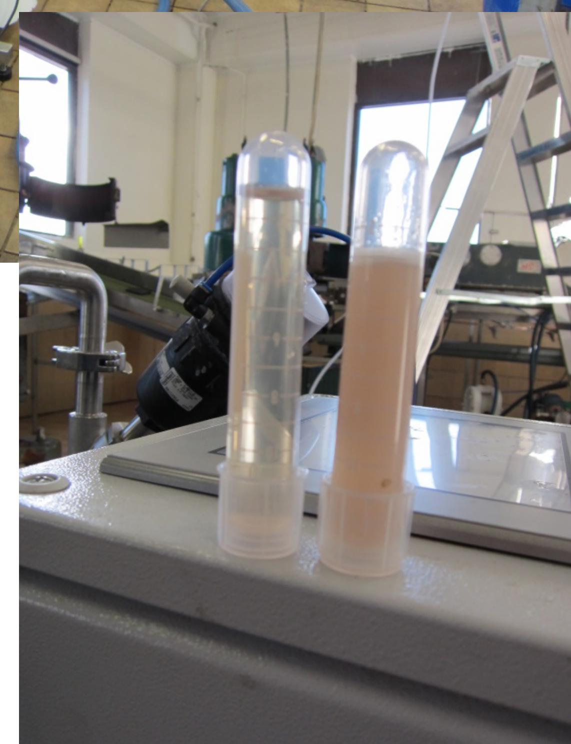
WP1: Techniques addressed

Membrane filtration

- Size separation
- 0.04 um nominal pore size
- Crossflow filtration
- 



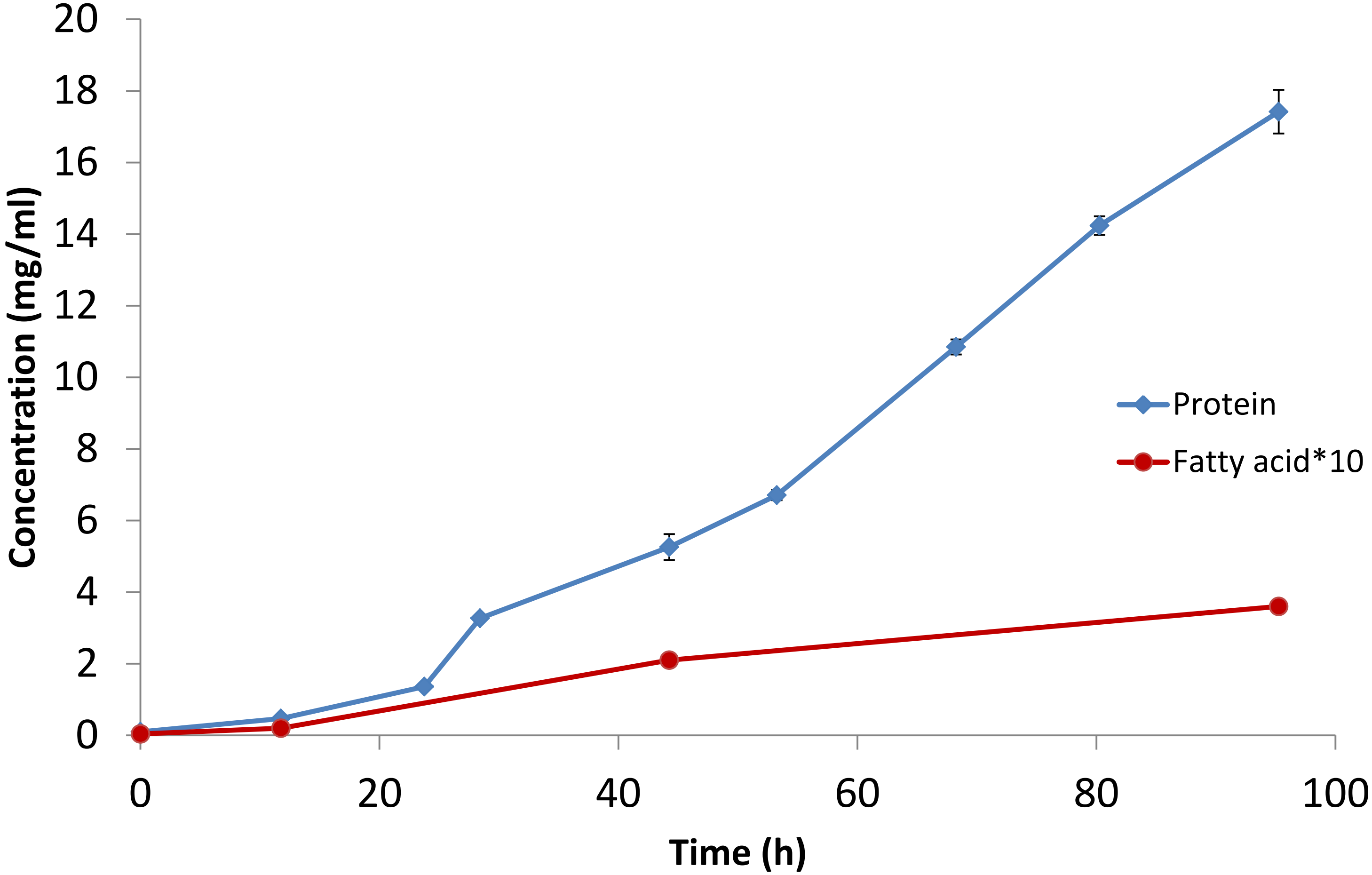


WP2 Characterization of waters and Fractions

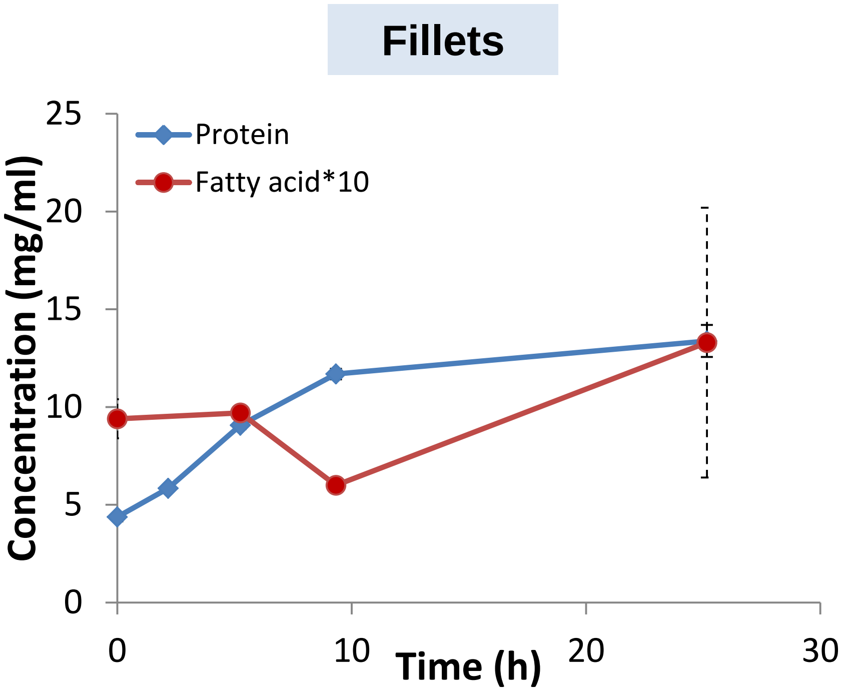
We have analyzed: Dry matter, minerals, protein, polypeptide profile, amino acids, fatty acids, COD

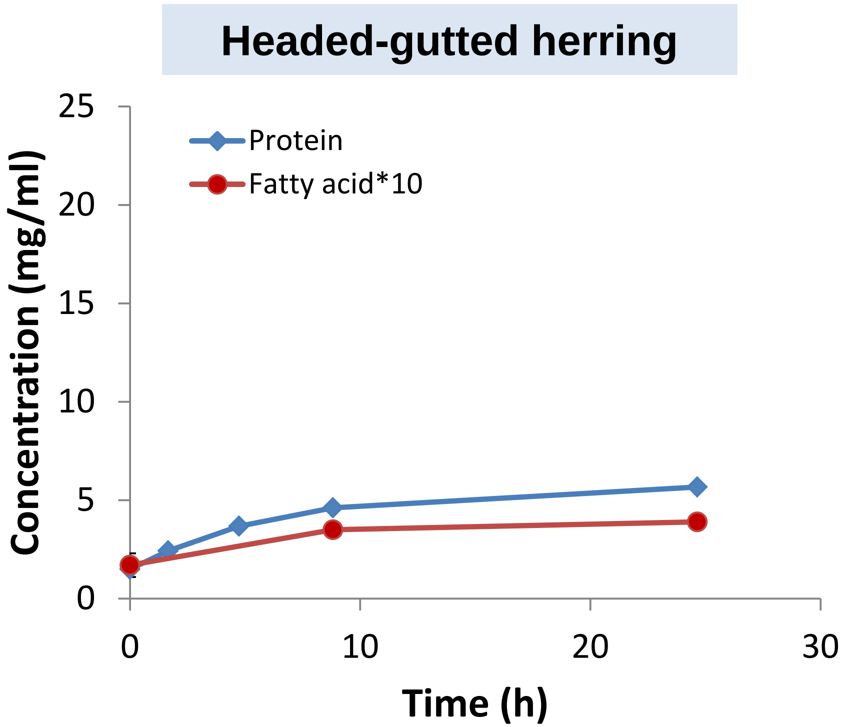
Protein and fatty acids: Effect of incubation time



Concentration of total proteins and fatty acids in Storage water (SW);
herring size 3 March 2012 incubated up to four days (Hedlund et al., 2012).

Protein and fatty acids: Effect of product type

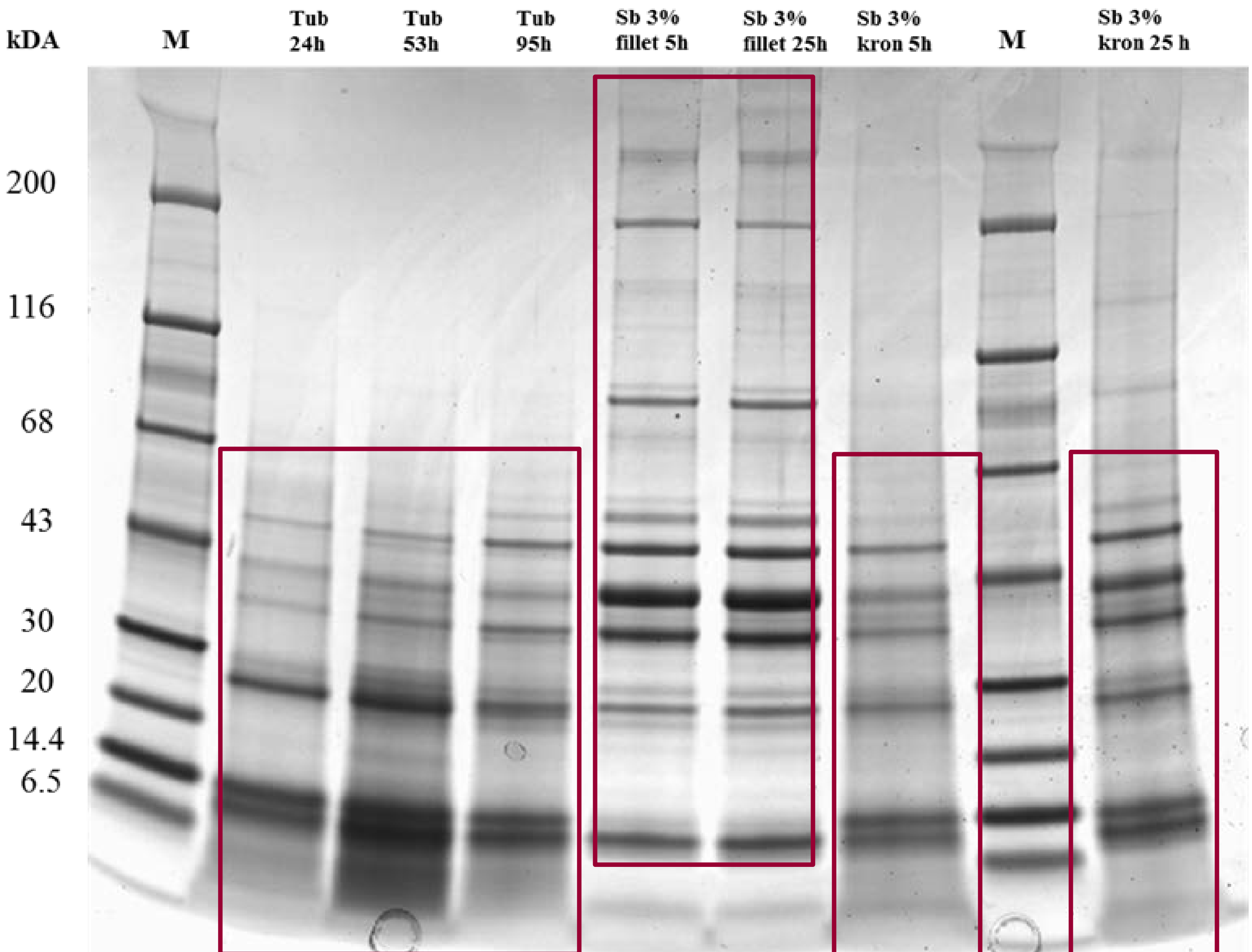




Total proteins and fatty acids in SB (3%); *fillets* or *headed/gutted* herring from March 2012 incubated up to 25h

(Hedlund et al., 2012)

Polypeptide profile of samples



From boat to barrel -sum up

•Up to 8% dry matter recorded (SB)-salt also responds!

•Calcium and magnesium dominating trace elements

•Highest protein and fatty acid content found in SB´s, up to 12.7±0.3 and 2.5±0.1 g L^{-1}, respectively.

•Time, increased muscle exposure and salt increased release

•Long chain n-3 polyunsaturated fatty acids represented up to 44.5% of total fatty acids.

•Smaller polypeptides in “early waters”; larger ones (myofibrillar proteins) in SB

•Stability must be considered; TVB-N and TBARS raises with time

from barrels to jars

- High dry matter, salt and protein content in the different brines, up to **2.5% fatty acids and 7% proteins!**

WP 2: Total Losses in herring marination

- ***From boat to barrel:***

For 1 t herring 10 kg Protein
4 kg pure Fatty acids

Amount per year (estimated)

Protein	105 670 kg
Lipid	65 253 Kg
Water	*34 650 m3*

- ***From barrels to Jars***

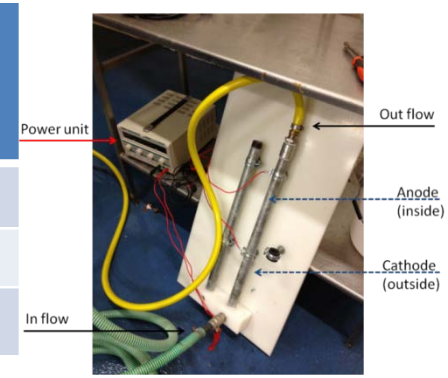
From 1t herring 110 kg protein
40 kg pure fatty acids

Amount per year (estimated)

Protein	4 540 kg
Lipid	1 880 Kg
Water	*235 m3*

WP 2: Recovery with techniques -examples

Electro-flocculation	Protein conc. (mg/mL)	Total Protein distribution (%)	Fatty acid conc. (mg/mL)	Total fatty acid distribution (%)
Salt brine (5%)	12.30	**100**	2.70	**100**
EF concentrate	19.10	**30.4**	7.50	**62.1**
EF-outlet	9.90	**69.6**	0.90	**37.9**



Ultra-filtration (0.04 µm)	Protein conc. (mg/mL)	Total Protein distribution (%)	Fatty acid conc. (mg/mL)	Total fatty acid distribution (%)
Salt brine (3%)	10.6	**100**	2.96	**100**
UF retentate	12.0	**93.7**	3.59	**101**
UF permeate	0.42	**0.69**	0.80	**4.74**

Flotation	Protein conc. (mg/mL)	Total Protein distribution (%)	Fatty acid conc. (mg/mL)	Total fatty acid distribution (%)
Salt brine (3%)	15.7	**100**	2.96	**100**
FL concentrate	38.6	**33.6**	15.8	**73.0**
FL outlet	10.4	**57.5**	1.81	**52.8**

WP 2: Conclusions regarding the techniques

- EF is efficient, but not applicable for recovery of high value molecules due to the high **AL concentration** in the treated water – other coagulants may be possible

- UF is working, however further optimisation is needed for pre-treatment step to obtain a **efficient separation**.

- Much **lower pore sizes** are needed to separate the low molecular weight bioactive compounds from the process water

- **Flotation** has large potential as pre-treatment step or even alone

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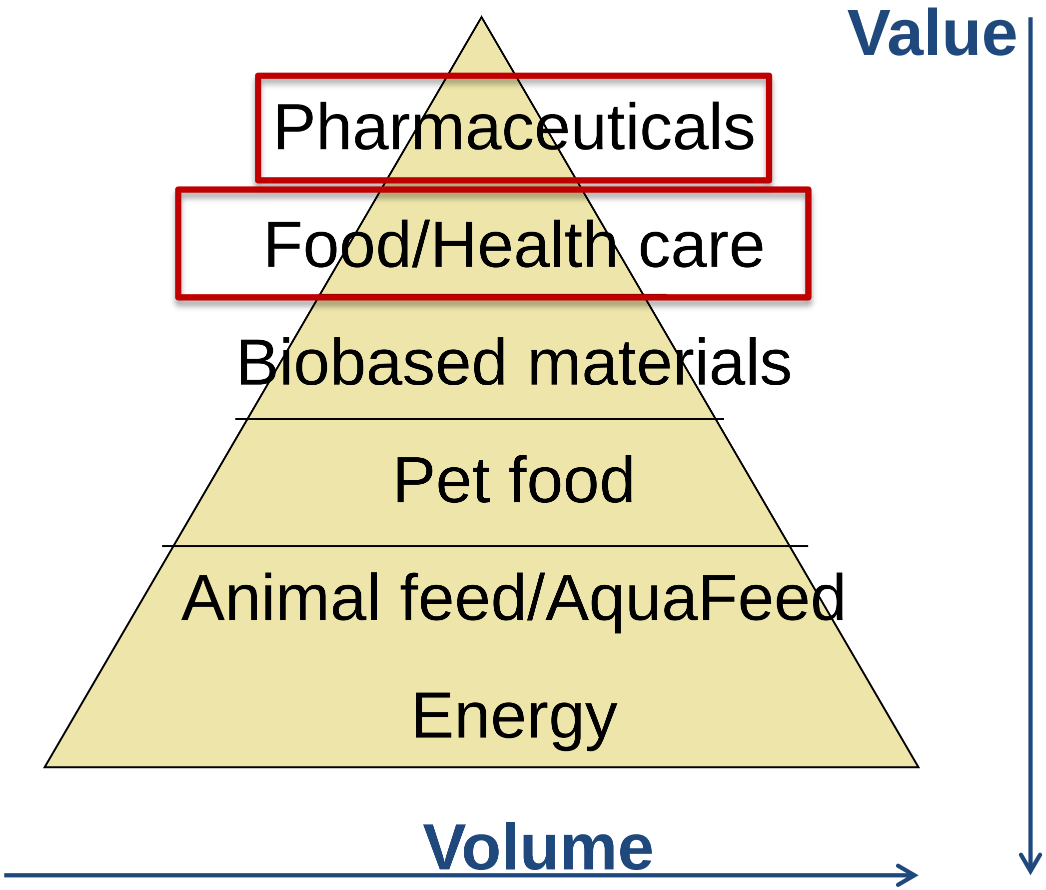
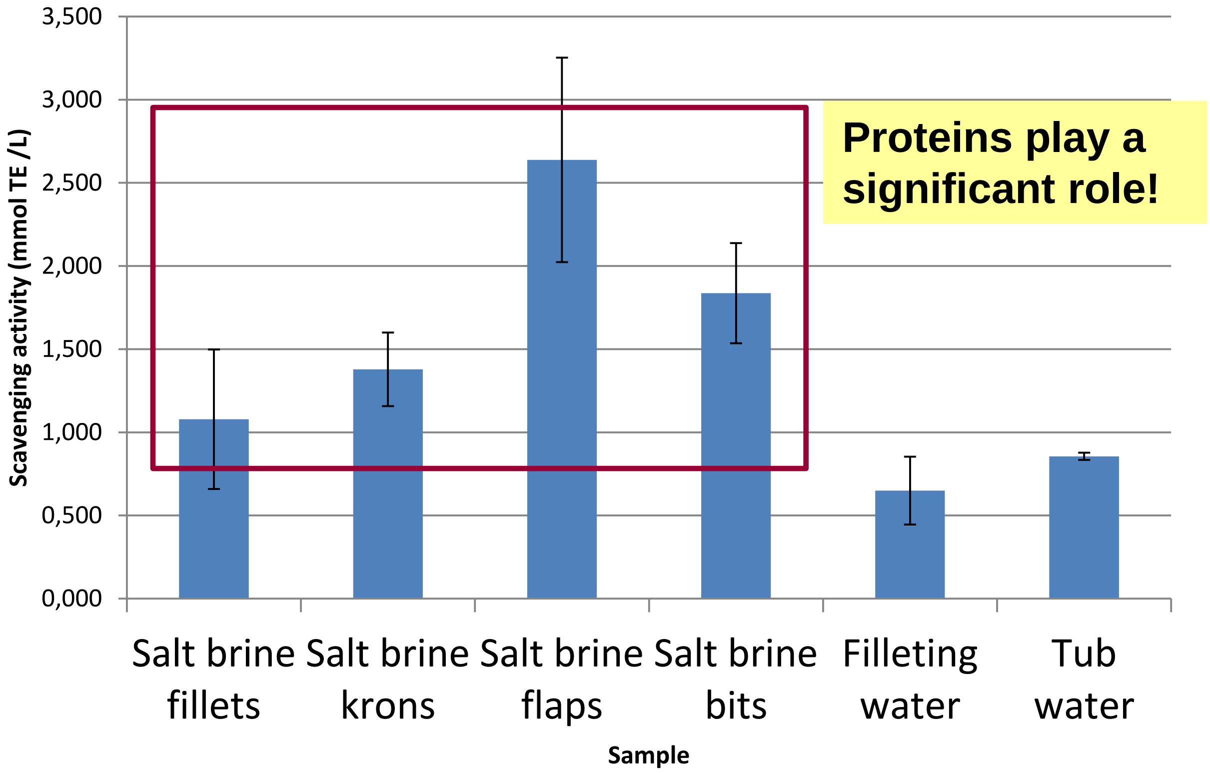
WP3 Valorization of the fractions



From boat to barrel

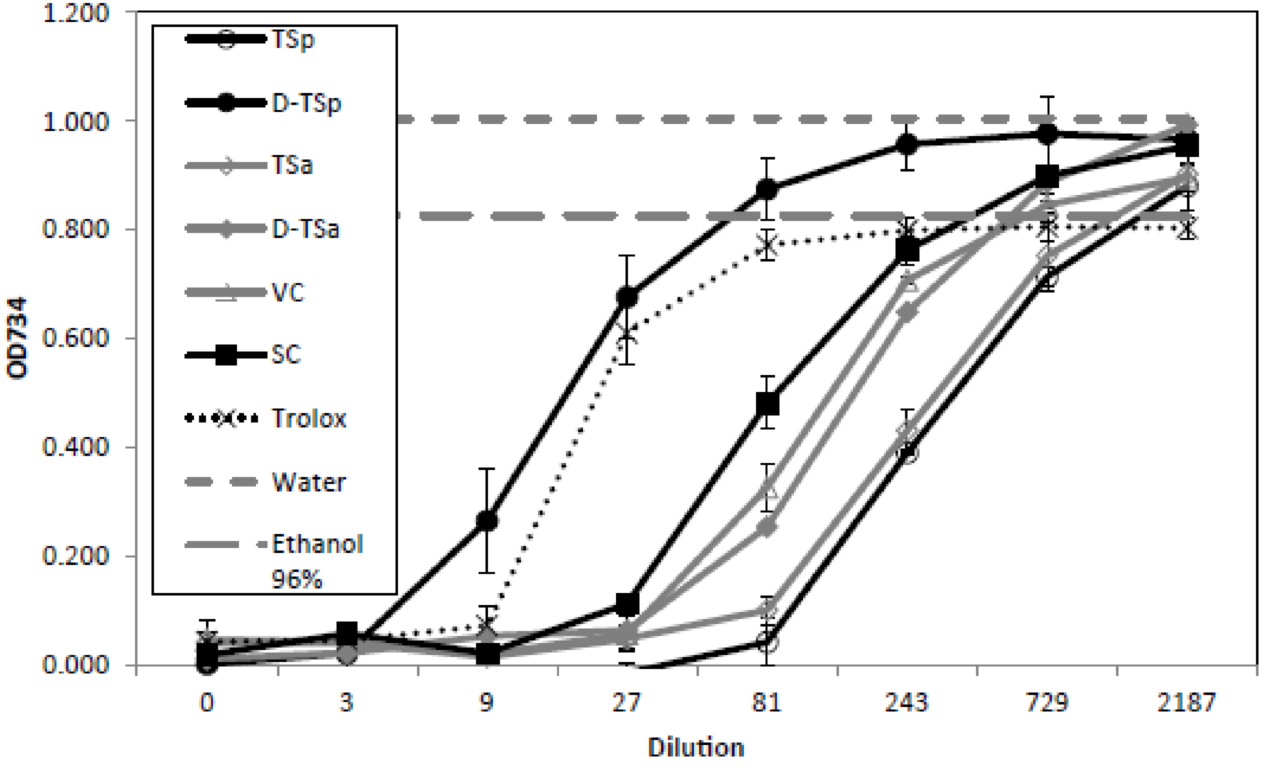
Antioxidant: Peroxyl radical Scavenging (ORAC)



Early process waters has an added value as source of antioxidants but require further characterisation

From barrel to jars

Antioxidant: Radical Scavenging



Brine also has added value as source of antioxidants –both **peptides and phenolic compounds from added spices** play a role. MALDI MS and MS/MS analysis used to identify peptides

From barrel to jars Enzyme Activity

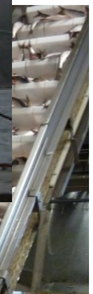
Samples	Peroxidase Abs/min/ml	Protease RFU/min/ml
VC	Nd	6.81
SC	Nd	4.23
Tsa	2,20	11.51
Tsp	0,96	3.75
D-Tsa	Nd	11.24
D-Tsp	Nd	4.4

Brine has added value as source of enzymes but may requires further purification

WP3 Conclusions

Possibilities to valorize process waters as they are or after fractionation

- Documented in vitro antioxidat activity
- Demonstration of technical functionality (foaming and emulsification)
- Detected enzymatic activity
- Possibilities to cultivate certain microorganisms in UF permeates



Significant amounts of compounds with nutritional, bioactive and functional properties in water streams….

….but techniques to use and recover them must be simple to make it economically feasible!

norden

Nordic Innovation

Extracting Novel Values from Aqueous Seafood Side Steams -NoVAqua

Project period: 2015.04.15 - 2018.04.14

Total Budget: 6 044 042 NOK

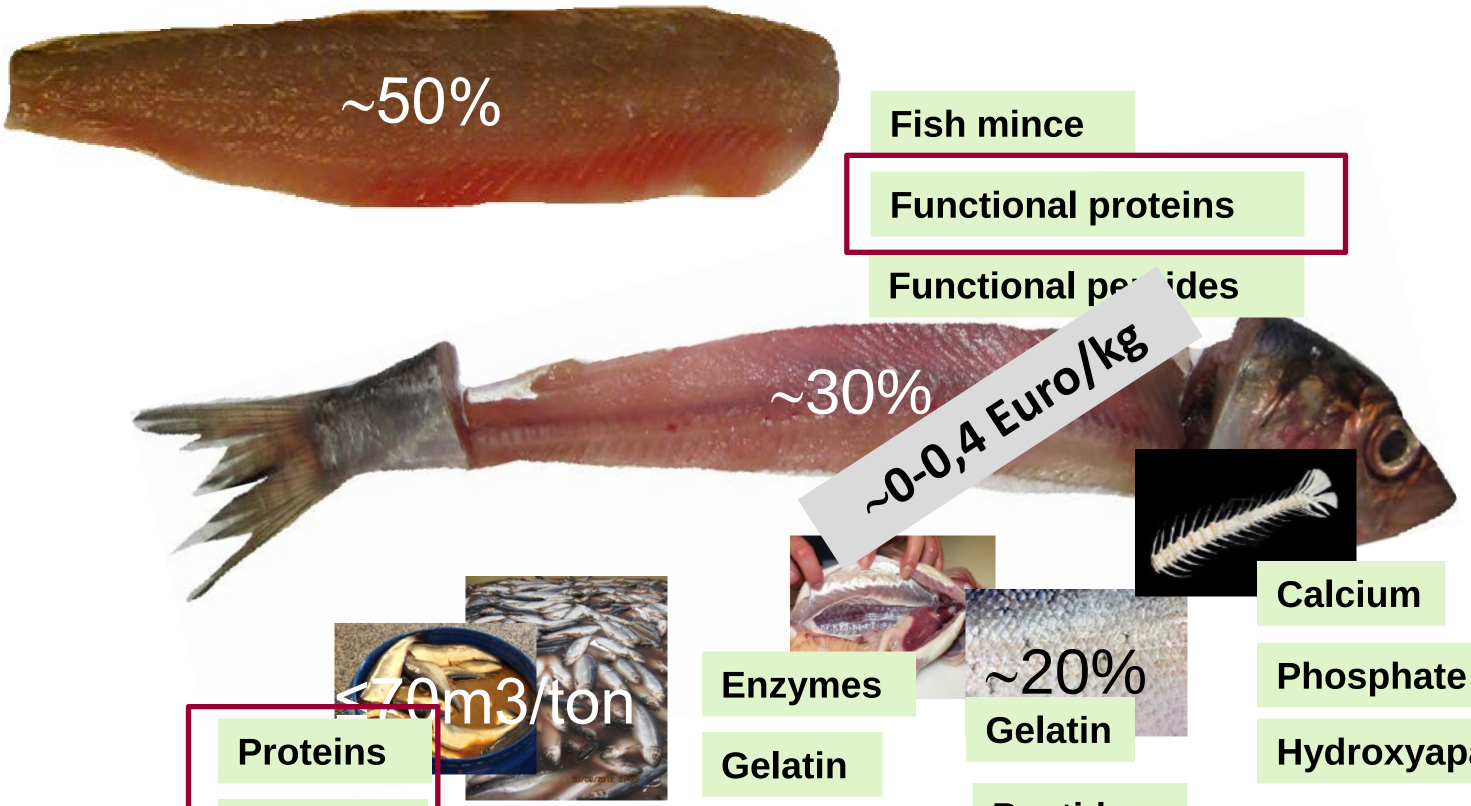
- 50% financed by Nordic Innovation, Marine Innovation 2.0
- 50% financed through in kind by industrial participants

Team NoVAqua

Leader: Ingrid Undeland, Biology and Biological Engineering –Food and Nutrition Science, Chalmers University of Technology

Participants:

- Johan Johannesson & Lennart Fransson, Räk & Laxgrossisten, Sweden
- Erik Jessen Jurgensen & Tore Svendsen, Bio-Aqua, Denmark
- Wolfgang Koppe & Gunvor Baardsen, Skretting Aquaculture Research Station, Norway
- Rikard de Man Lapidot & Berit Wallén, Fisk Idag, Sweden
- Caroline Baron *et al.*, Technical University of Denmark (DTU)
- Kristina Sundell *et al.*, Gothenburg University, Sweden
- Bita Forghani & Eva Albers, Chalmers University of Technology



Fish mince

Functional proteins

Functional pe ides

Enzymes

Gelatin

Oil

Phospholipids

Nucleic acids (e.g. DNA)/nucleotides

Gelatin

Peptides

Calcium

Phosphate

Hydroxyapatite

Proteins

Peptides

Oil

Aroma

Sources: *FAO, RUBIN, VRF, Sintef, Nofima, Havs och Vattenmyndigheten, Eurostat*

Table 3. Amino acid profile (g kg^{-1} dw) of mussel meat and protein isolates (PI) made thereof using 6 volumes of water.

	Blended mussel meat	**PI from acid processing**	**PI from alkaline processing**
Cystein	9±0.3	23.5±3.5	18±1
methionine	12±1.7	37.2±5.7	32±0.1
lysin	39±4	117±19	109±0.5
(Cys+Met)/protein (%)	3.4±0.2	7.4±0.3	4.9±0.4
Met/protein (%)	25.7±3.5	48.8±15	50.0±7.0
Lys/protein (%)	6.3±0.5	14.3±0.3	10.6±0.9

Table 4. Amino acid composition of herring mince and protein isolates.

	Herring mince (mg/g protein)	Alkali-made isolate (mg/g protein)	Acid-made isolate (mg/g protein)
Cysteine	10	11	10
Methionine	31	36	34
Aspartic acid	89	102	98
Threonine	40	46	44
Serine	41	42	39
Glutamic acid	130	128	143
Proline	43	33	32
Hydroxyproline	11	1	1
Glycine	65	35	38
Alanine	61	53	58
Valine	51	56	56
Isoleucine	43	51	50
Leucine	70	82	81
Tyrosine	32	33	38
Phenylalanine	39	40	40
Tryptophane	11	14	12
Histidine	25	26	27
Lysine	83	86	95
Arginine	59	64	60
Total amino acids	935	939	958

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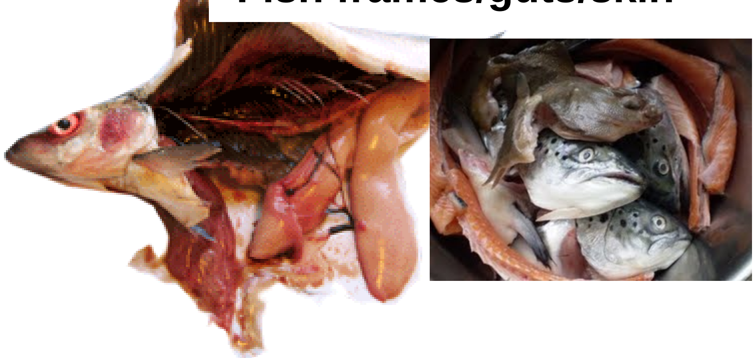
	Blended mussel meat	**PI from acid processing**	**PI from alkaline processing**
Cystein	9±0.3	23.5±3.5	18±1
methionine	12±1.7	37.2±5.7	32±0.1
asparagine	57±8	160±25	128±1
threonine	26±3	72±12	61±0.5
serine	27±3	71±11	62±1
glutamine	73±7	216±38	172±1
proline	22±3	50±5	44±1
glucine	40±4	59±8.4	55±1
alanine	29±2	72±12	62±1
valine	26±4	68±6.5	60±1
isoleucine	24±3	71±8.7	60±1
leucine	36±6	112±16	92±1
tyrosine	19±1	63±13	54±0.5
Phenylalanine	21±3	58±7	51±0.3
histidine	11±1	30±3	28±1
ornitin	1.6±0.6	1.2±0.01	1±0.04
lysin	39±4	117±19	109±0.5
arginine	36±8	105±15	92±1
hydroxyproline	2.9±0.9	1.2±0.01	0.9±0.04
Taurine*	21	4.8	4.3
Total detected amino	532.5	1292	1186

Vision of today: Use more of the aquatic raw materials to food production!

Fish frames/guts/skin



Small/crushed mussels, shells

The concept of ”Finishing diet”

“When plant oil-based diets are fed during the growing phase and replaced by a fish oil-based diet during a period prior to slaughter, most of the beneficial lipid composition of fish in terms of human dietary recommendations is restored. “

Mörköre & Pickova. *Alternate oils in fish feeds. Eur. J. Lipid Sci. Technol. 109 (2007) 256–263*