Sustainable utilisation of marine resources for food use: a Bio-refinery process approach

Dr Maria Hayes
Food Biosciences, Teagasc Food Research Centre, Ashtown, Dublin 15
+353 (0) 1 8059957; Email: maria.hayes@teagasc.ie
Challenges facing stakeholders

- Legal restrictions, high costs and environmental problems regarding the disposal of marine processing wastes.


- The Marine Functional Foods Research Initiative focuses on three main marine resources (i) fish processing waste streams, (ii) seaweeds and microalgae and (iii) aquaculture sources.

- The Marine Functional Foods Research Initiative aims to exploit these marine resources for functional foods/ingredients development.

- Identification of bioactive compounds to the dietary intervention level in order to make a health claim.
# Marine Potential

<table>
<thead>
<tr>
<th></th>
<th>New derivatives</th>
<th>Development of extractions</th>
<th>Defining activities</th>
<th>Marketing and product development</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proteins, peptides and amino acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seaweed sources</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Seafood sources</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td><strong>Carbohydrates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chitin and chitosan</td>
<td>L</td>
<td>M</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Agar</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>M</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>L</td>
<td>L</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Alginate</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>M</td>
</tr>
<tr>
<td>Fucoidan</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Laminaran</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Ulvan</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td><strong>Antioxidants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td><strong>Omega-3 fatty acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>H</td>
<td>M</td>
<td>H</td>
</tr>
</tbody>
</table>

---

http://www.nutramara.ie/assets/Pdfs/feasibility_study.pdf

The Irish Agriculture and Food Development Authority
Four marine resources for techno-functional and bioactive ingredients

1. Proteins and peptides from fish by-products & underutilised fish species

2. Seaweed derived proteins and peptides

3. Seaweed derived glycine betaines and related compounds including DMSP

4. Carbohydrates from marine by-products
Marine proteins: Proteins for the future?

1. Between 1995 and 2020, about 97.5% of the global population increase will be in developing countries, by which time 84% of the world’s people (an estimated 6.3 billion) will be living in developing nations (http://www.fao.org/docrep/007/y5019e/y5019e03.htm)

2. Need to widen the choice of dietary proteins for consumers

3. Up-scaling and sustainable production and use of proteins from alternative marine sources is required
Fish processing and the composition of fish waste

- Fish frames contain muscle proteins
- Nutritious and easily digestible
- Recovery using enzymatic hydrolysis

Composition of fish "waste"
- Protein 30%
- Fat 20%
- Moisture 50%

Usual steps in fish processing

Stunning of fish → Grading of fish → Slime removal

De-heading ← Washing ← Scaling

Gutting → Cutting of fins

Meat and bone separation ← Steaks and fillets

Fish proteins ~ Advantages and Disadvantages

- Nutritionally superior to plant protein sources
- Better balance of essential amino acids
- Fish muscle proteins are heat sensitive
- Cold water species are more susceptible to denaturation by heat compared to tropical fish
Fish and shellfish species examined ~ proteins and peptides

Protein & Bioactive peptides

Salmon  Boarfish  Blue whiting  Trout  Mackerel  Prawn  Abalone

Techno-functional and potential health beneficial properties

The Irish Agriculture and Food Development Authority
Bioactive peptides sources...

Marine by-products and algal sources.....
Bioactive peptides! Blue skies research or a commercial reality?

- Sequences of between 2-30 amino acids in length
- Impart a health benefit following consumption above and beyond basic human nutrition
- Derived using fermentation, hydrolysis, high pressure

<table>
<thead>
<tr>
<th>Company Name</th>
<th>Commercial Name</th>
<th>Source</th>
<th>Bioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Senma Eisui</td>
<td>Valtrypon (sold as ingredient for supplements or foods)</td>
<td>sardine muscle - dipeptide</td>
<td>ACE inhibitor + anti-hypertensive</td>
</tr>
<tr>
<td>Nippon Supplement</td>
<td>Katsushiri oil</td>
<td>bone, heart, muscle</td>
<td>ACE inhibitor + anti-hypertensive</td>
</tr>
<tr>
<td>Natural Factors</td>
<td>PeptACE</td>
<td>breast milk, bovine milk</td>
<td>ACE inhibitor + anti-hypertensive</td>
</tr>
<tr>
<td>Metagenics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calpis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tokawa Yakuri</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copalis</td>
<td>ProBio</td>
<td>fish fillet hydrolysate</td>
<td>stress relief, anti-oxidant, lowers GI</td>
</tr>
<tr>
<td>Yalacta</td>
<td></td>
<td>fish fillet hydrolysate</td>
<td>Stress Relief</td>
</tr>
<tr>
<td>Biothalassol</td>
<td></td>
<td>fish fillet hydrolysate</td>
<td>stress relief, anti-oxidant, lowers GI</td>
</tr>
<tr>
<td>ProperNutrition</td>
<td>SeaCure</td>
<td>fish fillet hydrolysate</td>
<td>IBS, Ulcerative colitis and Crohn's disease</td>
</tr>
<tr>
<td>Copalis</td>
<td>Nutripeptin</td>
<td>white fish hydrolysate</td>
<td>lowers postprandial blood glucose</td>
</tr>
<tr>
<td>Copalis</td>
<td>Collagen HM</td>
<td>hydrolysed fish collagen</td>
<td>skin &amp; cartilage regeneration</td>
</tr>
<tr>
<td>Copalis</td>
<td>Prolastin</td>
<td>hydrolyzed fish skins</td>
<td>promotes ligament regeneration + anti-oxidant</td>
</tr>
</tbody>
</table>

*EFSA Sardine peptide product safe as a food ingredient (2010)*

Techno-functional ingredients – collagen and gelatine from salmon by-products

ACTUAL SOURCES OF COLLAGEN AND GELATINE

- Gelatine is derived mainly from bovine and pig skin (46% of world's gelatine output).
- Bones of pigs and cattle represent 23% of world output.
- Only 1% of world gelatine output comes from marine sources: skins, scales and bones of fish.

APPLICATIONS

- Skincare
- Cosmeceuticals
- Supplements
- Emulsifiers & binding agent
ADVANTAGES OF FISH SKIN COLLAGEN/GELATINE

- Fish gelatine releases aroma and shows a higher digestibility than animal gelatine.
- Lower gelling temperature: advantageous for certain uses such as in precipitation of emulsions.
- No risk of health-threatening outbreaks of bovine spongiform encephalopathy (BSE) and foot and mouth disease (FMD).
- Acceptable for Hindus and members of the Islamic and Jewish faiths.
- Most gelatine-allergic patients do not react to fish skin gelatine.
Boarfish (*Capros aper*, Linnaeus)

- Boarfish (*Capros aper*, Linnaeus) are a mesopelagic, shoaling, small and deep bodied fish species which can grow up to 23 cm in length.

- Typically, they are distributed in the eastern Atlantic from Norway to Senegal including the Mediterranean.

- Due to their size, high lipid, bone and cartilage content, boarfish are difficult to process into high quality foods.
Boarfish proteins & peptides: pH shift process

1.300 g of Minced Boarfish (Capros aper) mixed with 2000 ml of water (FRACTION I)

**Extraction I:** RPM 500, temperature 30°C, 15 min, pH 11

**Centrifugation:** 15 min, 4000 x g, 4°C

2. Supernatant

**Extraction II:** Pellet (FRACTION IV) + 2000 ml water, RPM 500, temperature 30°C, 60 min, pH 11

**Centrifugation:** 15 min, 4000 x g, 4°C

3. Supernatant

**Extraction III:** Pellet + 2000 ml water, RPM 500, temperature 30°C, 15 min, pH 2

**Centrifugation:** 15 min, 4000 x g, 4°C

Pellet washed with water and resultant bone mix

Freeze-dried (FRACTION III)

Freeze-dried

**Precipitate**

Soluble protein freeze-dried (FRACTION II)

Gelatine extraction

Boarfish bone hydrolysate with papain (FRACTION V)

Hydrolysis

**FRACTION VI**
Boarfish soluble protein
Alcalase CLEA hydrolysate

**FRACTION VII**
Boarfish soluble protein
Papain hydrolysate

**FRACTION VIII**
Boarfish soluble protein
Protease AP hydrolysate

Bioflo 110 bioreactor
Boarfish Protein – Potential heart health benefits

Stabilisation & Freeze-dried protein powders

Physical characterisation and amino acid profiling

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Mean Ash [%]</th>
<th>Mean Protein [%]</th>
<th>Water activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction I</td>
<td>20.46 (+/-0.07)</td>
<td>57.43 (+/-0.31)</td>
<td>0.055 (+/-0.008)</td>
</tr>
<tr>
<td>Fraction II</td>
<td>35.40 (+/-0.15)</td>
<td>61.90 (+/-0.31)</td>
<td>0.158 (+/-0.008)</td>
</tr>
<tr>
<td>Fraction III</td>
<td>45.80 (+/-0.41)</td>
<td>18.585 (+/-0.33)</td>
<td>0.041 (+/-0.014)</td>
</tr>
<tr>
<td>Fraction IV</td>
<td>33.63 (+/-1.35)</td>
<td>60.51 (+/-0.42)</td>
<td>0.328 (+/-0.014)</td>
</tr>
<tr>
<td>Fraction V</td>
<td>34.07 (+/-1.09)</td>
<td>53.48 (+/-0.93)</td>
<td>N/A</td>
</tr>
<tr>
<td>Fraction VI</td>
<td>36.27 (+/-0.75)</td>
<td>52.58 (+/-5.63)</td>
<td>0.342(+/-0.0005)</td>
</tr>
<tr>
<td>Fraction VII</td>
<td>13.86 (+/-0.08)</td>
<td>82.29 (+/-0.91)</td>
<td>0.507 (+/-0.00208)</td>
</tr>
</tbody>
</table>

Characterisation of peptides using MS

<table>
<thead>
<tr>
<th>Protein name</th>
<th>Observed m/z</th>
<th>Expected Mr</th>
<th>Charge</th>
<th>Calculated Mr</th>
<th>Peptide sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myosin heavy chain, fast skeletal muscle</td>
<td>1</td>
<td>734.39</td>
<td>734.32</td>
<td>G EVEDLM</td>
<td>I</td>
</tr>
<tr>
<td>M933_CYP (Cyprinus carpio)</td>
<td>2</td>
<td>761.37</td>
<td>761.37</td>
<td>L GECQNL</td>
<td>Q</td>
</tr>
<tr>
<td>Fraction I</td>
<td>387.24</td>
<td>772.44</td>
<td>772.44</td>
<td>R IEAPPHI</td>
<td>F</td>
</tr>
<tr>
<td>Fraction II</td>
<td>263.18</td>
<td>786.52</td>
<td>786.26</td>
<td>L EEECMF</td>
<td>P</td>
</tr>
<tr>
<td>Fraction III</td>
<td>394.70</td>
<td>787.37</td>
<td>787.37</td>
<td>L GECQNL</td>
<td>Q</td>
</tr>
<tr>
<td>Fraction IV</td>
<td>402.20</td>
<td>800.43</td>
<td>800.41</td>
<td>I EAPPHIF</td>
<td>S</td>
</tr>
<tr>
<td>Fraction V</td>
<td>411.22</td>
<td>820.43</td>
<td>820.40</td>
<td>G QFDQFK</td>
<td>K</td>
</tr>
<tr>
<td>Fraction VI</td>
<td>415.74</td>
<td>829.47</td>
<td>829.45</td>
<td>R ADLSRE</td>
<td>E</td>
</tr>
<tr>
<td>Fraction VII</td>
<td>403.72</td>
<td>820.43</td>
<td>820.41</td>
<td>I EAPPHIF</td>
<td>S</td>
</tr>
<tr>
<td>Fraction VIII</td>
<td>415.74</td>
<td>829.47</td>
<td>829.45</td>
<td>R ADLSRE</td>
<td>E</td>
</tr>
</tbody>
</table>

Enrichment and bioactive profiling

% ACE-I inhibition by Boarfish protein hydrolysates and filtrates

- Akazila hydrolysate
- Akazila 1% E-Lactate
- Akazila 2% E-Lactate
- Papain hydrolysate
- Papain 1% E-Lactate
- Protease AP hydrolysate
- Protease AP 1% E-Lactate
- Protease AP 2% E-Lactate
Generation of bioactive peptides from fish protein

Extraction of macroalgal, microalgal or marine by-product proteins

Hydrolysis with proteolytic enzymes

Test hydrolysates for PAF-AH, PEP, ACE-I and renin inhibition

UF 10kDa MWCO/3kDa MWCO

Purification RP-HPLC

Characterisation – UPLC & MS
Traditional use of Seaweed in Ireland and worldwide

- During Potato famine seaweed source of nourishment.
- Sluichèan- a seagrass.
- High nutrient content.
- Dulse Bread- red seaweed.

Seaweed
- in Asian countries for centuries in foods, such as sushi.
Harvesting

To the Dish

Animal products

The Irish Agriculture and Food Development Authority
Techno-functional attributes of seaweed proteins

- Algal proteins
  - Gelation
  - Water binding
  - Solubility
  - Emulsification
  - Foaming

- AlgaVia™ – Solazyme
- Lipid algal flour - Roquette
MACROALGAE PRODUCTION WORLD-WIDE

Harvested - 1,045,000 T ww
Cultivated - 15,781,159 T ww

• CHINA, top seaweed producer
  – CHINA  62.8%
  – INDONESIA  13.7%
  – PHILIPPINES  10.6%
  – REPUBLIC OF KOREA  2.8%
  – JAPAN  2.9%

• JAPAN is the second most important producer in terms of value = NORI production.

• CHILE  21,700 T

(FAO, 2010)
Open-water system in Canada (IMTA)
SEAWeed DISTRIBUTION IN IRELAND

- Two species are harvested in any large quantities, i.e., *Ascophyllum nodosum* and the calciferous algae known under the collective name of maerl.
- A third species, Laminaria is harvested in small quantities, but there is potential for larger extractions.
- A number of smaller brown, red and green algae are harvested and marketed for human food and cosmetics.

*Ascophyllum nodosum*
Techno-functional attributes of seaweed derived proteins

Emulsifying stability of protein extracted from *Himanthalia elongata* species.

---

**Essential amino acids**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Total amino acids (g/Kg DW)</th>
<th>Free amino acids (g/Kg DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threonine</td>
<td>3.25±0.04</td>
<td>0.12±0.00</td>
</tr>
<tr>
<td>Valine</td>
<td>4.28±0.18</td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>1.96±0.03</td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2.28±0.07</td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>2.05±0.07</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.28±0.08</td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>3.23±0.05</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>2.01±0.11</td>
<td>0.09±0.00</td>
</tr>
</tbody>
</table>

**Non-essential amino acids**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Total amino acids (g/Kg DW)</th>
<th>Free amino acids (g/Kg DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>5.94±0.04</td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>2.77±0.07</td>
<td></td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>7.52±0.05</td>
<td>0.32±0.01</td>
</tr>
<tr>
<td>Proline</td>
<td>2.55±0.25</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>2.98±0.03</td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>3.32±0.13</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>Cysteine</td>
<td>3.14±0.16</td>
<td>0.13±0.03</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.41±0.06</td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>3.05±0.01</td>
<td></td>
</tr>
</tbody>
</table>

**Total amino acids**

- 54.02±0.46
- 0.73±0.02

---

Marco Vaquero – visiting Post-doctoral researcher
Food Chemistry submission (2015)
Glycine Betaine and DMSP

- Glycine betaine and DMSP are osmolytic, zwitterionic compounds found in food, microalgae and seaweed.

- Formed by oxidation of bioactive choline in mammals and helps to maintain normal cell volume under osmotic stress.

- The beneficial effects of glycine betaine relate to the maintenance of normal blood concentrations of homocysteine.

EFSA health claim pursuant to article 13 of regulation (EC) No. 1924/2006. (2011)*

Generation and Extraction methodology

ASE® generation using Dionex 200 and Teagasc methodology

Enrichment using flash chromatography

Characterisation using $^1$H-NMR confirmed with MS

Load cell

Fill with solvent(s) (0.5-1 min.)

Heat and pressurise (5 min.)

Static extraction (6 min.)

Flush with fresh solvent (0.5 min.)

Purge with nitrogen (1-2 min.)

Extract ready (total time, 12-14 min.)

Centrifugation and BT AG®, 1-X8 resin purification

$^1$H-NMR

The Irish Agriculture and Food Development Authority
GB & DMSP identified in different samples

Figure 1: Comparison of the $^1$H-NMR spectra of GB (top) and the ASE® extract generated from Ulva intestinalis ISCG0356 (bottom)

Figure 2: Comparison of the $^1$H-NMR spectra generated for DMSP (top) and the ASE® extract generated from Codium fragile ISCG0029 bottom


Poster Presentation P2, page 41
Potential applications of betaine extracts

- Application in baked goods to prevent loss of moisture
- Extracts use in sports performance products
- Cognitive function applications for pregnant women

---

8 Kilibwa, United States Patent US 6,217,930 B, April 2001
10 Asia Pac J ClinNutr, 2013, 22, 2, 319-324.
Generation of GB & DMSP extracts from Irish seaweeds

In: Raw material → Stabilise → Extract → Dry

Characterise structure → Purify further → In vitro bioassay → Enrich & Purify
SHELLFISHERIES “WASTE” – CHITIN POTENTIAL

- Second most abundant natural biopolymer derived from exoskeletons of crustaceans
- Chitosan - collective name representing a family of N-acetylated chitins deacetylated to different degrees.
- Extensive applications in medicine, agriculture, food, and non-food industries as well.

Horticulture

Medical bandages

Dentistry

Functional food – weight loss
Chitin and Chitosan

- **Sources**

- **Applications**

  - Crab: 13.5 – 26.6%
  - Oyster: 3 – 6%
  - Crawfish: 29.8%
  - Krill: 41.0%
  - Shrimp: 17 – 40.4%

- EFSA concluded no cause and effect between consumption of chitosan and weight management (2011)
- Kitozyme Novel food claim and GRAS status
Generation of Chitosan from shell waste streams

Clean prawn shell (5 Kg) → Milling → Sieved to fine powder

Protein fraction

Demineralised using HCL

Protein removal using NaOH

Chitin powder (insoluble in acid and water)

Further Deacetylation using concentrated NaOH to give chitosan (slightly soluble in weak acids)

COS enzymes

Pig-trials (UCD) to assess effects on weight gain (1 Kg)

Demineralised using HCL

The Irish Agriculture and Food Development Authority
Economic feasibility of Irish chitin production

- Gaps in knowledge
- Sources
  - Discard estimates (15,141 tonnes)
  - Seasonal variation
  - Location
- Markets
- Key players
- Costs

India

Bio-Line
Thank you for your attention!