Innovative Food Product Development Cycle: Frame for Stepping Up Research Excellence of FINS

European Commission

By-product and resource utilisation in the marine sector

Dr. Maria Hayes, TEAGASC



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DEFINITION OF A BY-PRODUCT

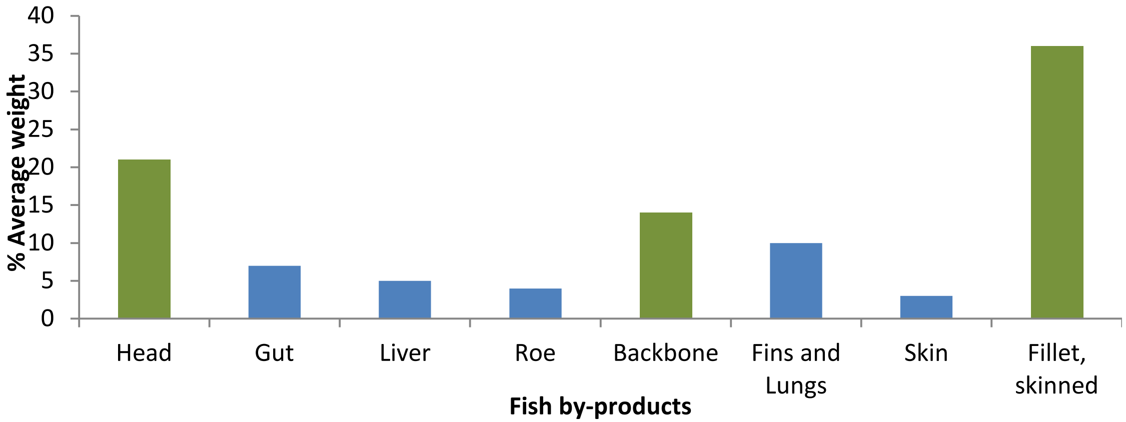
- Waste includes products that cannot be used for feed or value-added products but which have to be composted, burned or destroyed.
- The EU regulation on animal by-products (EC Nr. 1774/2002) adopted on the 3rd of October 2002, defines animal by-products as whole carcasses or parts of animals not intended for human consumption. Marine by-products intended for human consumption are not included in this definition.

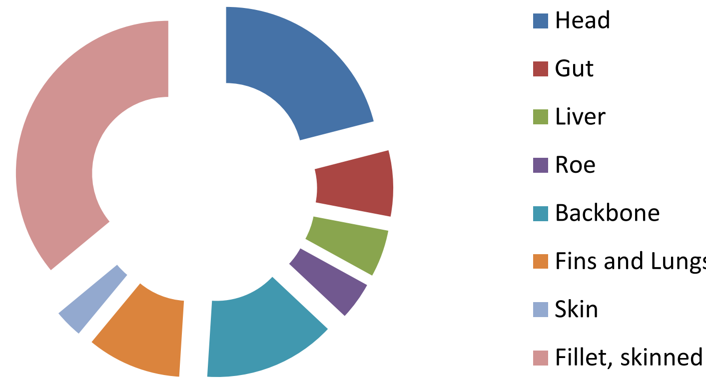
Need for change in use of food processing by-products?

- **FOOD SECURITY:** 2030 50% more food required by the planet
- **MARINE:** Reformed common fisheries policy (CFP)- new discards ban, landing obligation, achieving maximum sustainable yield (MSY) by 2020
- **ORIGIN GREEN:** 15% reduction in general waste by 2017*
- **Resources are limited** – growing populations

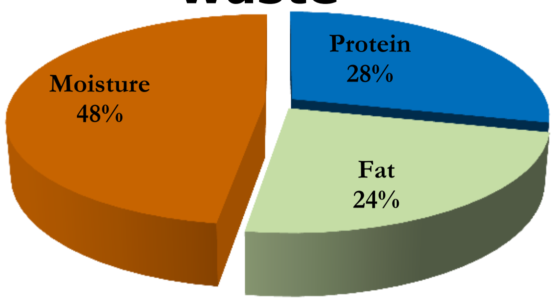
* Bord Bia Sustainability report 2015

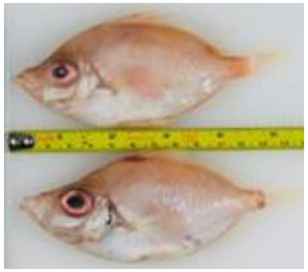
Fish composition & derived by-products





Composition of fish "waste"

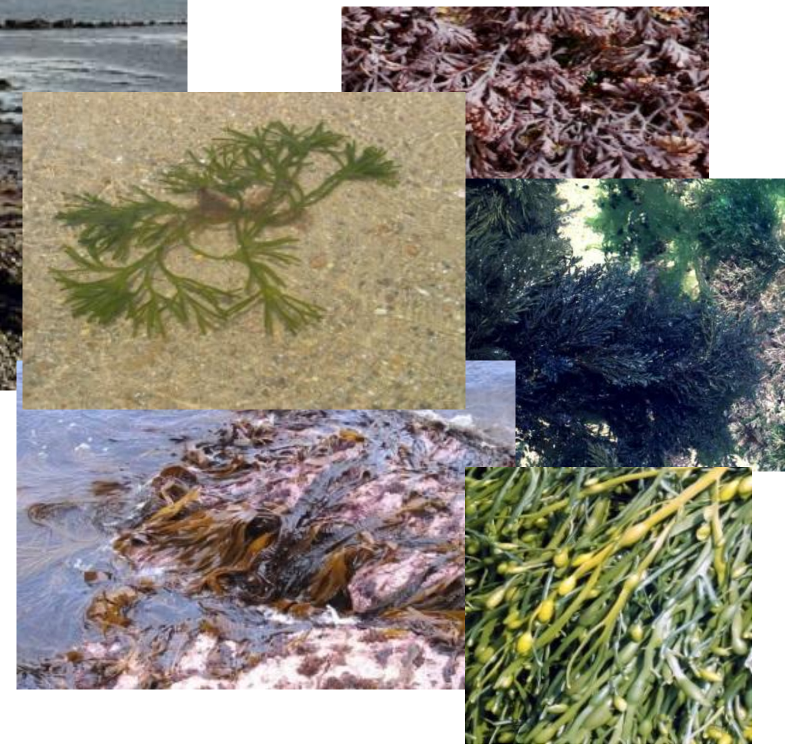


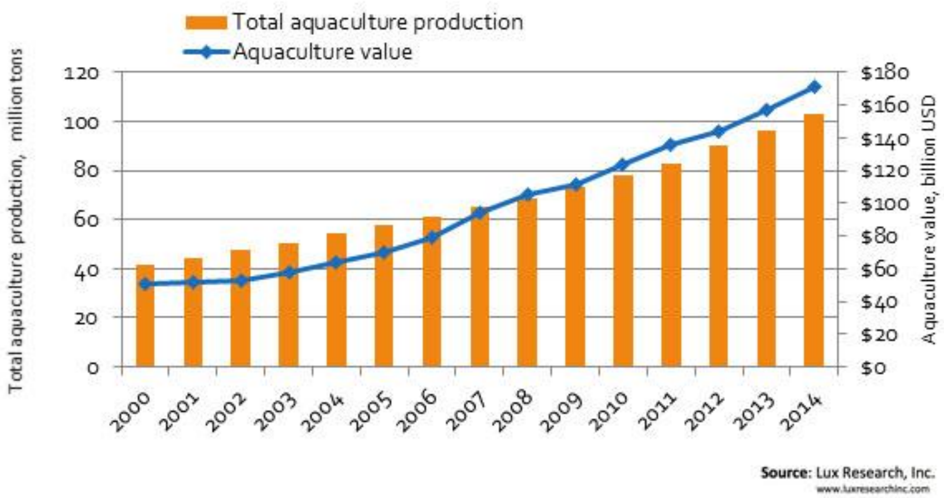


* Ghaly, A. E., Ramakrishnan, V. V., Brooks, M. S et al., 2013

Natural resources & potential aquaculture

- **Over 300 species found from Kinvarra Co. Galway to Co. Clare**

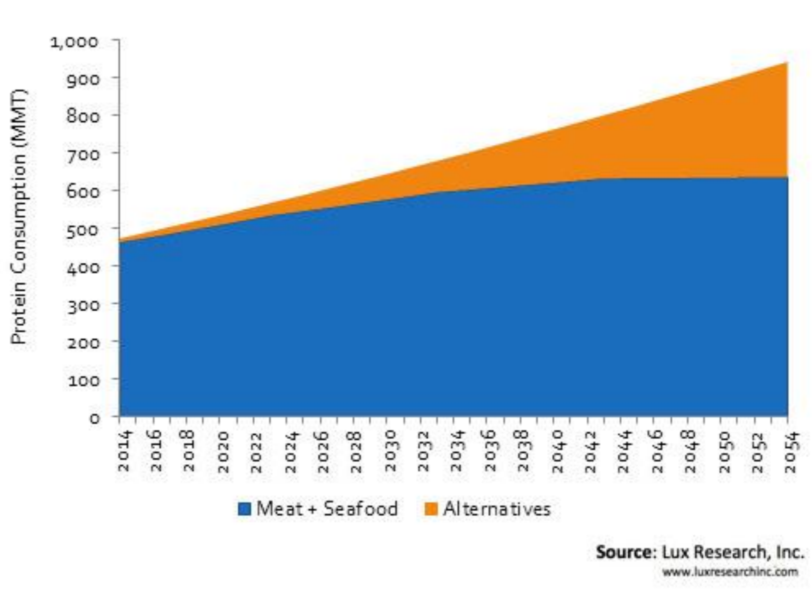


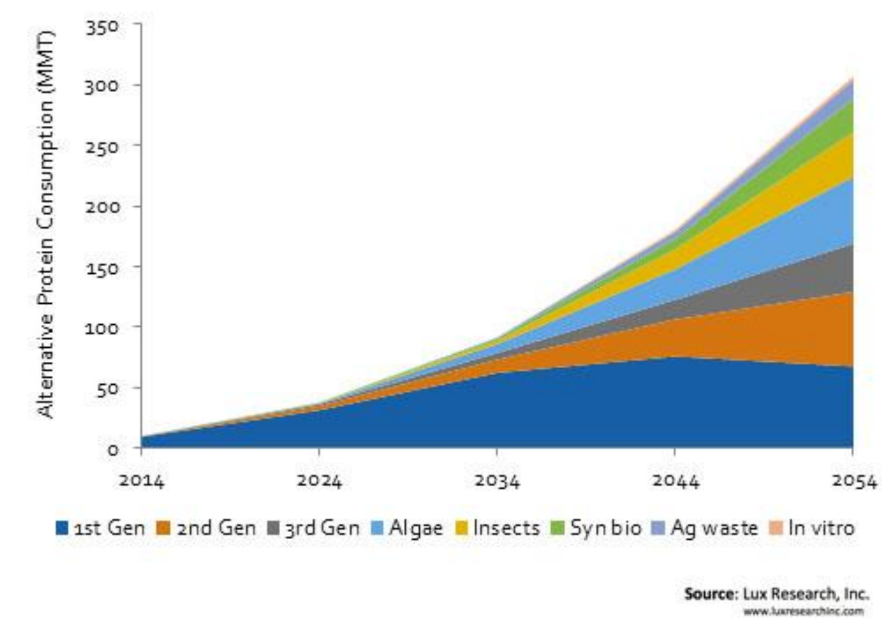


eagasc
Agriculture and Food Development Authority

The Irish Agriculture and Food Development Authority

Demand



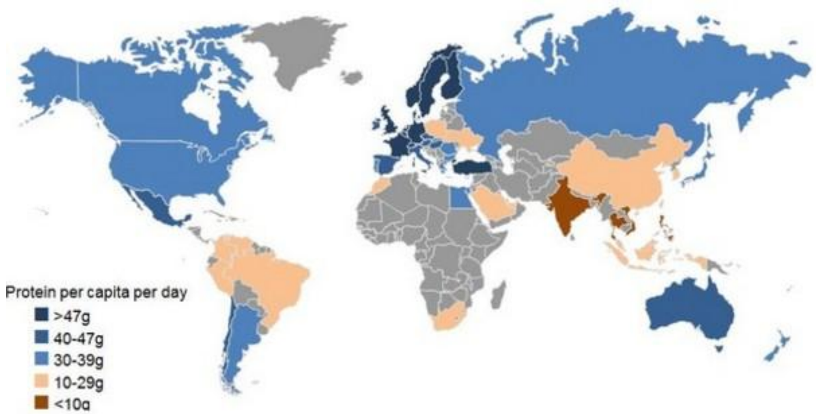


- Increases in global population and prosperity levels heighten protein demands. The UN predicts population growth to 9.6 billion by 2050, and rising global prosperity drives increased protein consumption as affluent consumers seek a better diet.

- Predicted that global protein consumption will rise from 470 MMT in 2014 to more than 940 MMT by 2054.

* The World Bank, Fish to 2030 Prospects for fisheries and aquaculture December 2013

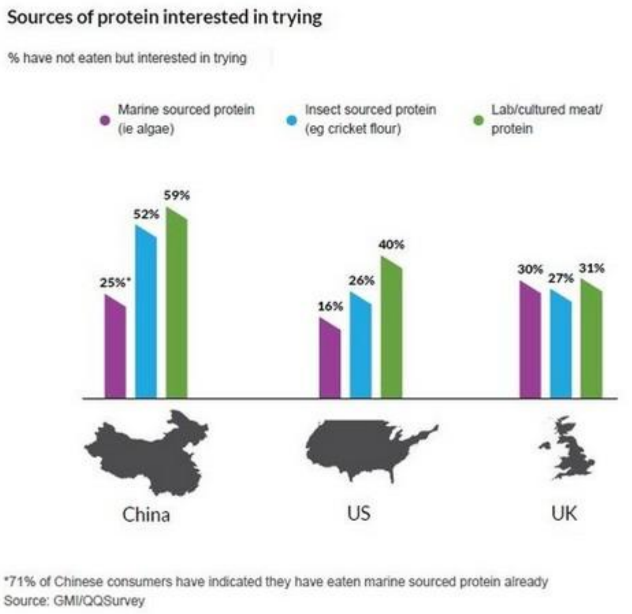
Global Protein trends



Big regional differences in protein consumption

Emerging markets India and China

Healthy aging and sports nutrition



- **Chinese most adventurous**
- **30 % of Europeans would try marine proteins**

Foodnavigator.com accessed on the 16th of September 2015 at: http://www.foodnavigator.com/Market-Trends/Zooming-into-protein-trends-region-by-region

By-products from fish and shellfish : potential uses

Protein & Bioactive peptides

Salmon

Boarfish

Blue whiting

Trout

Mackerel

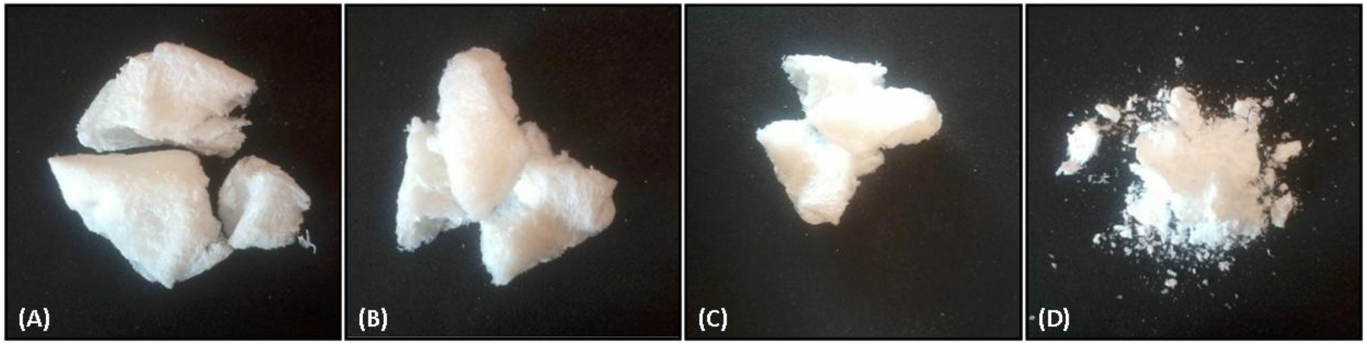
Abalone

Prawn

Protein & Bioactive peptides

Techno-functional and potential health beneficial properties

Techno-functional ingredients – collagen and gelatine from salmon



Gelatine generated with the NaCl method: (A) Salmon Bones, (B) Salmon Skin, (C) Salmon Heads and (D) Salmon Offal

ADVANTAGES OF FISH SKIN COLLAGEN/GELATINE

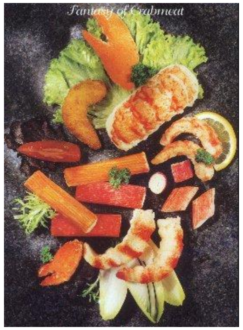
- Fish gelatine releases aroma and shows a higher digestibility than animal gelatine.
- Lower gelling temperature: advantageous for certain uses such as in precipitation of emulsions.
- No risk of health-threatening outbreaks of bovine spongiform encephalopathy (BSE) and foot and mouth disease (FMD).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

NutraMara

Techno-functional attributes of proteins

Marine proteins

Gelation



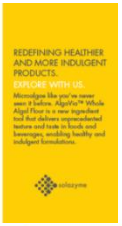
Water binding

Solubility

Foaming

Emulsification

- AlgaVia ™ – Solazyme
- Lipid algal flour - Roquette

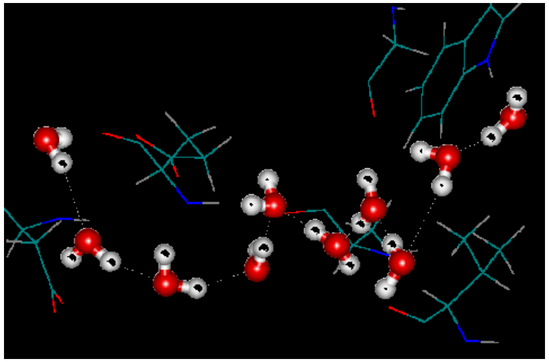


Techno-functional attributes of FPHs

- "Those physical & chemical properties that influence the behaviour of proteins in food systems during processing, storage, cooking and consumption"
- Physicochemical properties include: size, shape, amino acid composition, sequence, net charge, distribution, hydrophobicity, hydrophilicity, structures, molecular flexibility, rigidity in response to external environment (pH, temperature, salt concentration…)

Division of techno-functional properties

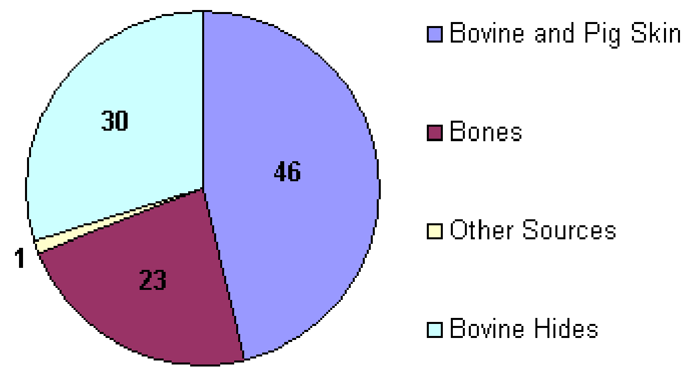
- Mechanism of action
- Hydration (absorption of water/oil, solubility, thickening, wettability)
- Protein structure and rheological characteristics (viscosity, elasticity, adhesiveness, aggregation, gelification)
- Protein surface (emulsifying, foaming, protein-lipid film formation, whippability)



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 worlds 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.



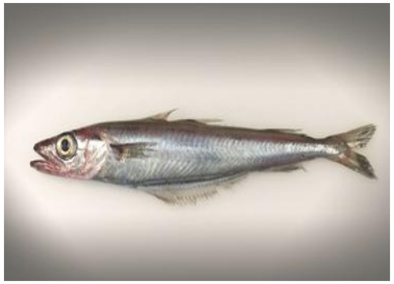
APPLICATIONS

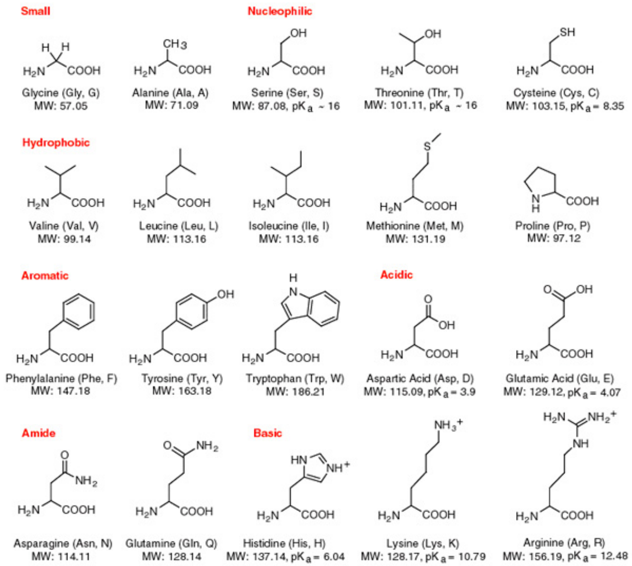
- Skincare
- Cosmeceuticals
- Supplements
- Emulsifiers & binding agent

Marine proteins ~ Advantages and Disadvantages

- Nutritionally superior to plant protein sources if from fish
- 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

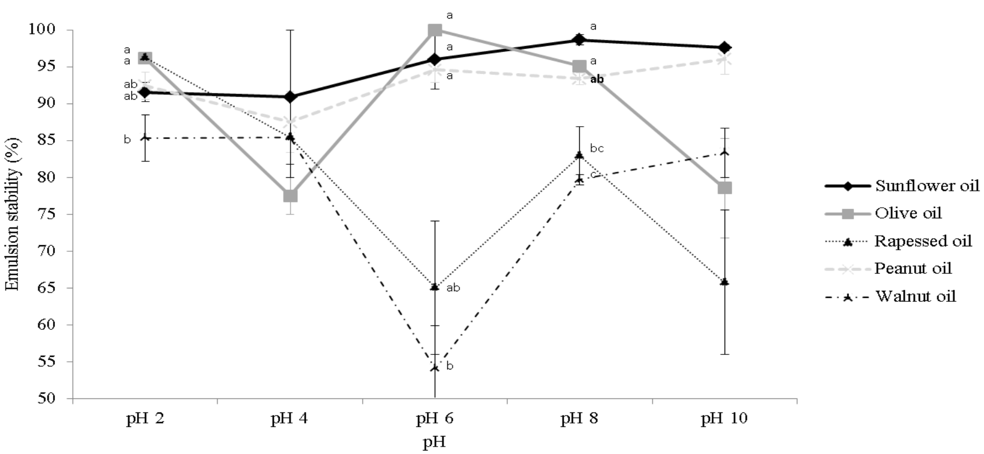
V's





Seaweed proteins

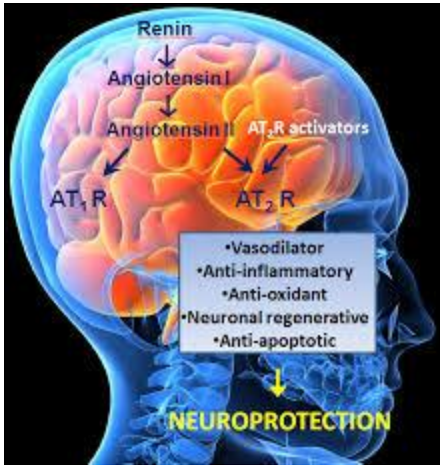
Emulsifying stability of protein extracted from *Himanthalia elongata* species.



Essential amino acids	Total amino acids (g/Kg DW)	Free amino acids (g/Kg DW)
Threonine	3.25±0.04	0.12±0.00
Valine	4.28±0.18	
Methionine	1.96±0.03	
Isoleucine	2.28±0.07	
Leucine	2.05±0.07	
Phenylalanine	2.28±0.08	
Lysine	3.23±0.05	
Histidine	2.01±0.11	0.09±0.00
Non-essential amino acids		
Aspartic acid	5.94±0.04	
Serine	2.77±0.07	
Glutamic acid	7.52±0.05	0.32±0.01
Proline	2.55±0.25	
Glycine	2.98±0.03	
Alanine	3.32±0.13	0.11±0.01
Cysteine	3.14±0.16	0.13±0.03
Tyrosine	1.41±0.06	
Arginine	3.05±0.01	
Total amino acids	54.02±0.46	0.73±0.02

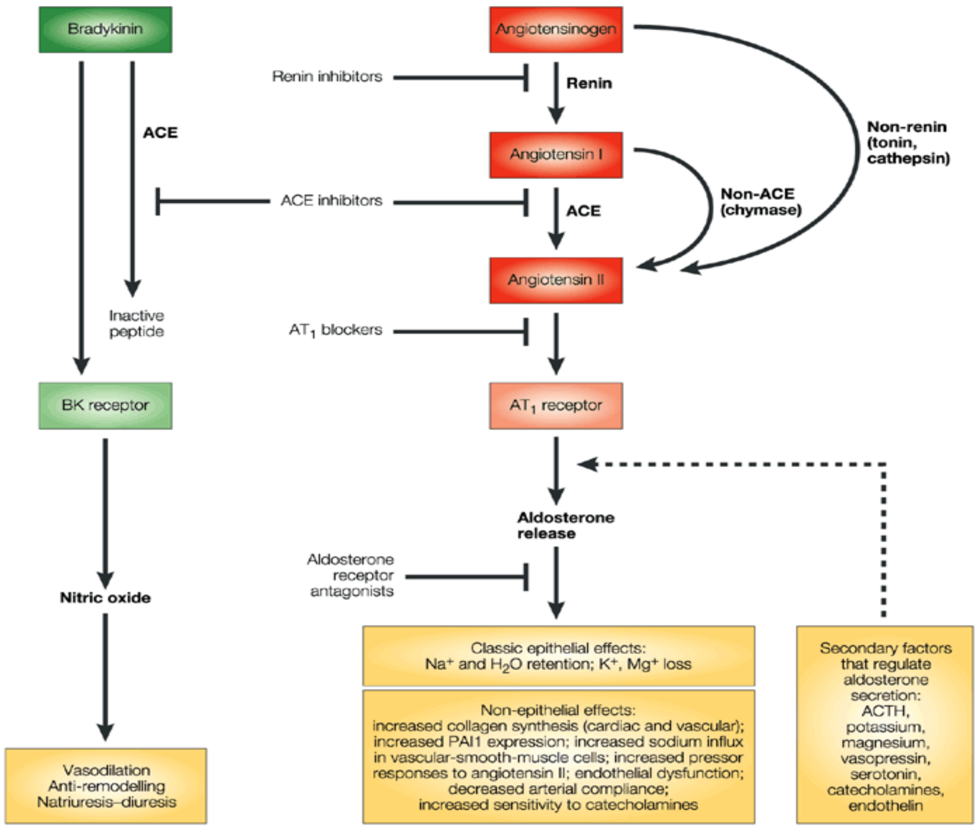
Bioactive or physiological activities of proteins/peptides

- Heart health (renin, ACE-I, DPP-IV inhibitory)
- Diabetes (DPP-IV and GLP-1 inhibitory)
- Satiety (Cholecystokinin (CCK))
- Neuro-protective effects

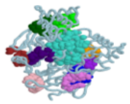


ACE-I inhibition (Heart health)

The Rennin-Angiotensin-Aldosterone System



Angiotensin I



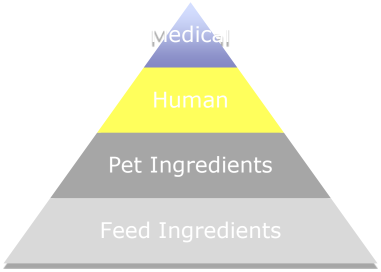
ACE

Angiotensin II

- Fish
- Meat
- Milk
- Egg
- Plant
- Seaweed
- Algae

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**



Company Name	Commercial Name	Source	Bioactivity
Senmie Ekisu	Valtyron (sold as ingredient for supplements or foods)	sardine [illegible]uscle - dipeptide ([illegible]	ACE inhibitor + anti-hypertensive
Nippon Supplement	Katsuabushi ol[illegible]ide	bo[illegible] [illegible]ctive	[illegible] inhibitor + anti-hypertensive
Natural Factors	PeptACE	[illegible]	[illegible]nti-
Metagenics	[illegible]	[illegible]	[illegible] inhibitor + anti-[illegible]ertensive
Calpis	[illegible]	[illegible]	[illegible]-hypertensive
Tokiwa Yaku[illegible]	[illegible]	[illegible]	[illegible]ertensive
Copalis	Proti[illegible]	[illegible]	stress reli[illegible]
Yalacta	[illegible]	[illegible]	Stress Relief
Biothalassol	[illegible]	[illegible]fish hy[illegible]e (Molva Molva)	stress relief, anti-oxidant, lowers GI
ProperNutrition	SeaCure	fish fillet hydrolysate	IBS, Ulcerative colitis and Crohn's disease
Copalis	Nutripeptin	white fish hydrolysate	lowers postprandial blood glucose
Copalis	Collagen HM	hydrolysed fish collagen	skin & cartilage regeneration
Copalis	Prolastin	hydrolysed fish skins	promotes ligament regeneration + anti-oxidant

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

***http://www.efsa.europa.eu/en/efsajournal/pub/1684.htm**

Generation of bioactive peptides from protein sources (e.g. milk)

Milk

↓

Extraction of different casein and whey proteins

↓

Hydrolysis with chosen proteolytic enzyme (Thermolysin, papain) (Duration in hours, specific temperature, agitation)

↓

Full hydrolysate

↓

Bioassay
1) Protein content
2) Water activity (A_w)
3) Antioxidant assays
- DPPH
- FRAP
- Metal chelating activity
1) Renin inhibition
2) ACE inhibition
3) PAF-AH inhibition
4) AChE inhibition
5) PEP inhibition

10-kDa MWCO filtrate

↓

Bioassay
1) Protein content
2) Water activity (A_w)
3) Antioxidant assays
- DPPH
- FRAP
- Metal chelating activity
4) Renin inhibition
5) ACE inhibition
6) PAF-AH inhibition
7) AChE inhibition
8) PEP inhibition

3-kDa
1-kDa MWCO filtrate

↓

Bioassay
1) Protein content
2) Water activity (A_w)
3) Antioxidant assays
•DPPH
•FRAP
•Metal chelating activity
4) Renin inhibition
5) ACE inhibition
6) PAF-AH inhibition
7) AChE inhibition
8) PEP inhibition

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)*

* Valverde, J., Hayes, M., et al., (2015), Planta Medica, 81, 679-684.

http://www.efsa.europa.eu/en/efsajournal/doc/2052.pdf

Generation of GB & DMSP extracts from Irish seaweeds

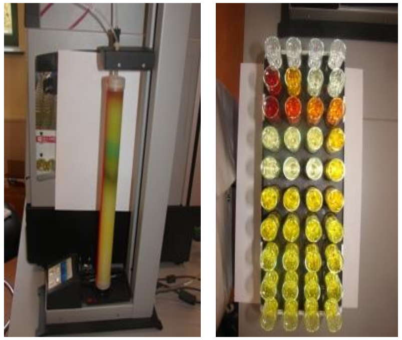
In: Raw material

Stabilise

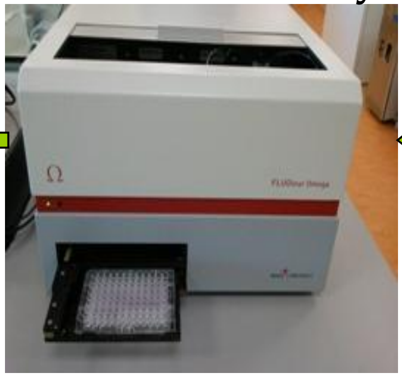
Extract

Dry

Enrich & Purify



***In vitro* bioassay**



Purify further

Characterise structure

* Valverde, J., Hayes, M., et al., (2015), Planta Medica, 81, 679-684.

Chitin and Chitosan

- Sources






Crab 13.5 – 26.6 % **Oyster 3-6%** **Crawfish 8%** **4** **p 4%**

- Applications


Biotechnology Journal

Review

- EFSA concluded no cause and effect between consumption of chitosan and weight management (2011)
- Kitozyme Novel food claim and GRAS status

*Hayes et al., (2008), Biotechnol. J. 3, 7, 878-889..

Chitin and chitosan from marine shell by-products

Clean prawn shell (5 Kg)

Protein fraction

Milling

Sieved to fine powder

Demineralised using HCL

Protein removal using NaOH

Chitin powder (insoluble in acid and water)

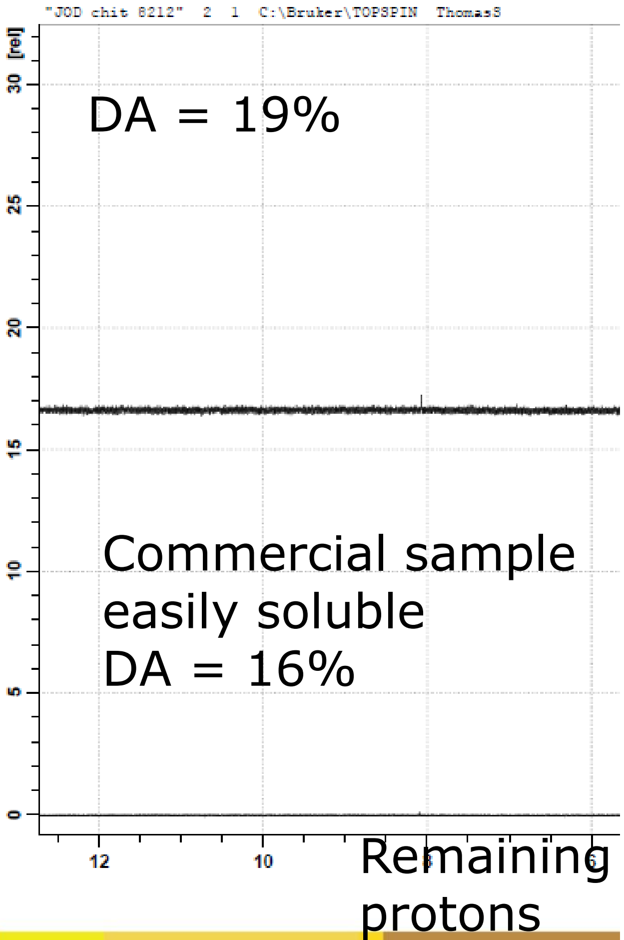
Further Deacetylation using concentrated NaOH to give chitosan (slightly soluble in weak acids)

COS

enzymes

*Egan, AM, Sweeney, T., Hayes, M. et al., (2015) PLOS ONE, 10 (12):00144127

Chitosan analysis method development



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Hydrodynamic characterisation of chitosan and its interaction with two polyanions: DNA and xanthan

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ABSTRACT

Chitosan, a soluble polycationic derivative of insoluble chitin, has been widely considered for use in the food, cosmetic and pharmaceutical industries. Commercial ("C") and in-house laboratory ("L") prepared chitosan samples extracted from crustaceous shells with different molecular weight and degrees of acetylation (25% and 15%) were compared with regards to (i) weight-average molecular weight (M_w); (ii) sedimentation coefficient ($s^0_{20,w}$) distribution, and (iii) intrinsic viscosity ($[\eta]$). These parameters were estimated using a combination of analytical ultracentrifugation (AUC), size exclusion chromatography coupled to multi-angle laser light scattering (SEC–MALS) and differential pressure viscometry. Polydisperse distributions were seen from sedimentation coefficient distributions and elution profiles from SEC–MALS. M_w values obtained for each sample by sedimentation equilibrium measurements were in excellent agreement with those obtained from SEC–MALS. Mark–Houwink–Kuhn–Sakurada (MHKS) and Wales van Holde analyses of the data all suggest a semi-flexible conformation.

The principle of co-sedimentation was then used to monitor the interactions of the two different molecular weights of L chitosans with two polyanions, DNA and xanthan (another double helical high molecular weight molecule). Interactions were clearly observed and then quantified from the changes in the sedimentation coefficient distribution of the mixture compared to unmixed controls using sedimentation velocity. The interactions appeared to show a strong dependence on molecular weight. The relevance of this for DNA condensation applications is indicated.

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1. Introduction

Chitosan is the generic term for a family of linear, copolymeric polysaccharides derivatives of chitin extracted from the exoskeletons of crustaceans or the cell walls of Basidomycete fungi. The basic structure of chitosan consists of 2-amino-2-deoxy-β-D-glucopyranose units linked through a β (1 → 4) linkage, with a high degree of *N*-acetylation. The variation of chitosans in solution depends on 3 degrees of freedom, namely their molecular weight, degree of acetylation (DA) and also the distribution of acetyl groups along the chain (Venugopal, 2011).

Chitosan has many distinctive properties including biocompatibility, biodegradability, hemocompatiblity, antibacterial activity, nontoxic, antitumor, fungistatic and anticholesteremic (Rinaudo, 2006). As a result of these properties, chitosan has received a great deal of interest for use in the food (No, Meyers, Prinyawiwatkul, & Xu, 2007), cosmetic (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004), and pharmaceutical industries (Morris, Kök, Harding, & Adams, 2010). Additionally, specific complexes of chitosan with an oppositely charged polyelectrolyte yield three-dimensional

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FOOD stars *Innovative Food Product Development Cycle: Frame for Stepping Up Research Excellence of FINS*

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