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# NutraMara Consortium





National University of Ireland, Galway University Road Newcastle Galway Ireland

**Teagasc Food Research** 

Centre, Ashtown & Moorepark, Ireland





University College Cork The Department of Food and Nutritional Sciences Western Road Cork Ireland

University College Dublin School of Agriculture, Food Science and Veterinary Medicine Belfield Dublin Ireland

University of Ulster Coleraine Campus Cromore Road Coleraine Northern Ireland



University of Limerick Department of Life Sciences Limerick Ireland



#### With support from and in conjunction with:



Agriculture, Food and the Marine An Roinn Talmhaíochta, Bia agus Mara







# NutraMara Conference 2012

# **Organising Committee**

Dr Maria Hayes	Dr Emma Brown
Current NutraMara Scientific Project Manager	Biomedical Sciences Research Institute
Teagasc Food Research Centre	University of Ulster
Ashtown	Coleraine Campus
Dublin 15	Northern Ireland
Ireland	BT52 1SA
T: +353 (1) 805 9957	T: +44 (0)28 70123543
E: maria.hayes@teagasc.ie	E: em.brown@ulster.ac.uk
Dr Emeir Mc Sorley	Ms Vicky Hegan
Biomedical Sciences Research Institute	Biomedical Sciences Research Institute
University of Ulster	University of Ulster
Coleraine Campus	Coleraine Campus
Northern Ireland	Northern Ireland
BT52 1SA	BT52 1SA
T: +44 (0)28 70123543	T: +44 (0)28 70123595
E: em.mcsorley@ulster.ac.uk	E: hegan-v@email.ulster.ac.uk

The NutraMara organising committee would like to express their thanks and appreciation to the following people for their assistance in organising the NutraMara Conference 2012:

- \* The staff of the office of the Irish President, Michael D Higgins
- Dr Eric Donald, PR department, Teagasc Food Research Centre
- \* Dr Catriona Boyle, Scientific Writer, Teagasc Food Research Centre
- \* Dr Alison Maloney, PR department, Teagasc Food Research Centre
- Dr Lisa FitzPatrick, PR department, The Marine Institute, Galway
- \* Ms Deirdre Fox, Teagasc Accounts Department
- \* Ms Siobhán McKenna, Teagasc Accounts Department
- \* Mrs Mary-Ellen Wall, Teagasc Accounts Department
- Mrs Margaret Hennessy, Teagasc Conference and Events Office
- Mrs Anne Heffernan, Teagasc Conference and Events Office
- \* Dr Eimear Gallagher, Teagasc Food Research Centre
- \* Dr Anastasia Ktenioudaki, Teagasc Food Research Centre
- ✤ Dr Geoffrey O' Sullivan, The Marine Institute, Galway
- \* Dr Ilaria Nardello, the Marine Institute, Galway
- \* Ms Ciara Sexton, The Castleknock Hotel and Country Club, Dublin
- \* Ms Audrey McGowan, The Castleknock Hotel and Country Club, Dublin



# **Hotel and Conference Information**

#### **Conference Venue**

The Castleknock Hotel and Country Club, Porterstown Road, Castleknock, Dublin 15, Ireland. T: +353 (0)1 640 6300

### **Conference dinner**

The conference dinner will be held in the Castleknock Hotel and Country Club on Wednesday 25<sup>th</sup> April at 8pm in the Sierra Hawthorn Suite for guests who have registered to attend.

#### Posters and dissemination activities

The posters, dissemination activities and company display stands are located in The Walnut Hawthorn Suite and can be viewed during tea/coffee and lunch breaks.

#### Breakfast times and venue

For participants staying in the Castleknock Hotel and Country Club breakfast is served from 7am – 10am in the Brasserie Restaurant.

#### Internet access

Complimentary Internet access is provided throughout the hotel. If you wish to log on in the public areas no password is required - please connect to Eircom. If you wish to log on in your bedroom please contact reception for access details.

#### Cash machine

The nearest ATM is located at the Spar supermarket in Carpenterstown which is a 2 minute drive or a 10 minute walk from the hotel.

## Checkout times and luggage storage

The final checkout time is at 12pm. If you wish the hotel to store your luggage please ask at reception upon checkout.

#### Car parking

Ample car parking is provided at the front of the hotel free of charge. Please note that a one way system is in place.



## Welcome

On behalf of the NutraMara consortium I have great pleasure in welcoming you to the NutraMara Conference 2012 in Dublin. This is an exciting time for those of us involved in marine products research which is ever expanding and constantly renewing. With the development of new technologies we face fresh challenges and exciting opportunities regarding the isolation, characterisation and utilisation of marine bioactive components. Our aim at this conference is to meet and bring together inspired people from a range of disciplines so that together we can be a driving force in marine-based functional food research.

This conference will focus on selected topics – including marine resource sustainability, marine waste and by-product utilisation and valorisation, marine algae as a source of food ingredients and marine derived ingredients as potential functional foods. We have brought together an expert panel of national and international speakers to share their knowledge in these selected areas. In addition I would like to emphasize the opportunities to view the posters from different areas of marine research and the demonstration activities provided by NutraMara partners displaying the scope of research performed within the NutraMara programme. We are delighted to have this opportunity to showcase the cutting edge research that is currently underway within the NutraMara programme partners.

I would like to thank each of you for attending our conference and for bringing your expertise to our gathering. As leaders in industry and research your vision, knowledge and experience will help shape the future of marine research. I hope that the outstanding contributions presented at this conference will help you develop new ideas and collaborations which will lead to innovative and exciting projects. Therefore, I would like to thank all contributors, in particular the invited speakers, for submission of your abstracts and your valuable contribution to this conference. In addition I am thankful to all who contributed to the organisation and running of this conference. Finally, I would like to acknowledge the sponsors of this event and the support of the Marine Institute and the Department of Agriculture, Food and the Marine, who have made this conference possible.

Decla Ina

Declan Troy

Director of NutraMara

Assistant Director of Research at Teagasc





# **Conference Biographies**

# Master of Ceremonies

### Dr Maria Hayes

Dr Hayes obtained her BSc (Hons) in Science, specialising in Industrial Microbiology and Chemistry from University College Dublin (UCD) in 2002. She carried out her PhD at the Teagasc Food Research Centre, Moorepark and University College Cork in the area of bioactive peptide isolation and characterisation from milk proteins and waste streams (whey and casein). She then carried out post-doctoral work at the Centre of Applied Marine Biotechnology Donegal, where she worked on the isolation of chitinolytic enzymes from shellfisheries crab and whelk waste streams. She is currently the NutraMara Scientific Programme Manager and the PI for work packages two and seven PI. She is responsible for supervising work regarding the isolation and chemical and biological characterisation within this work package as well as dissemination and outreach activities in work package 7. She supervises two NutraMara PhD researchers who are funded by the Teagasc Walsh Fellowship programme. Her research interests include the isolation, purification and characterisation of marine derived molecules, especially peptides and phlorotannins from marine seaweeds and by-products, bioassay development with a particular focus on heart and mental health disorders - i.e., renin, ACE-I, PAF-AH, PEP and inhibition of other enzymes with heart and mental health effects. Other interests include the generation of chitin and chitosan from marine shellfisheries waste streams, isolation and characterisation of enzymes (in particular chitinolytic enzymes) and fermentation. She has published 18 research papers in the area of functional foods research and has recently edited a book for Springer concerning the isolation, characterisation and utilisation of marine derived bioactive compounds.

#### Dr Juan Valverde

Dr Juan Valverde holds a degree in Chemistry (Organic) from University Autonoma of Madrid (Spain). Dr Valverde conducted his PhD research at the Laboratory of Molecular Interactions (College de France) and at Laboratory of Analytical Chemistry (AgroParisTech). His PhD was funded by a private multinational (UNIQ) with support of the French Research Agency (ANRT). Dr Valverde's work got awarded with the Silver Medal of the French Academy of Agriculture. In 2008, Juan Valverde became Network Scientific Officer of the Irish Phytochemical Food Network (www.ipfn.ie) developing a broad network of Irish Agro-Food Researchers and Industries. Juan is a specialist in developing analytical methods to extract, purify, characterize and quantify bioactives from different sources. Dr Valverde is member of the editorial board of the Journal of Culinary Science and Technology, member of the



Phytochemical Society of Europe (PSE) and the French Chemistry Society (Société Française de Chimie, Groupe Français de Chimie des Aliments et du gout).

# Food Research Ireland

## Dr Pamela Byrne

Pamela graduated from UCC with a degree in Zoology in 1994 and continued her education at Kings College, University of London where she received her MSc in Aquatic Resource Management in 1995. She returned to UCC in 1996 where she completed her PhD in Estuarine Ecotoxicology under the supervision of Prof. John O' Halloran. She began her career in the Irish Department of Agriculture, Food and the Marine in the Pesticide Control Service evaluating the environmental risks associated with the use of plant protection products and biocides. For the last 6 years, she has worked in the Research & Codex Division within the Irish Department of Agriculture, Food and the Marine, managing the scientific and technical elements of the Food Institutional Research Measure (FIRM) which is funded under the National Development Plan. For the last two years she has been acting as the National Contact Point for the EU FP7 Programme in the area of Food, Agriculture, Fisheries and Biotechnology. She is the Vice Chair of the Joint Programming Initiative "A Healthy Diet for a Healthy Life".

# Session 1 – Marine Resource Sustainability

## Mr Richie Flynn

As a former journalist, Richie worked for 6 years in the Irish Farmers Association press office before moving into the aquaculture sphere, where he is currently the executive secretary. Richie has been a board member of the Marine Institute since 2001 and was president of the European Aquaculture Advisory Committee from 2000 until 2010. During his career he has been involved in a number of FP6 and FP 7 projects as SME Association partner on aspects of environment and quality. Richie represents more than 200 Irish aquaculture companies at local, national and EU level. His research interests lie in aquaculture, rural development and communications.

## Dr Richard Fitzgerald

Dr Richard Fitzgerald was awarded his PhD for work on ecological interactions of fish-parasite communities but also holds qualifications in Business (MBA) and in Finance. He has been involved in Research and Development in Aquaculture for almost 30 years in a variety of roles and posts. He has published over 30 peer-reviewed papers and 100+ technical reports in areas that include projects on stock



structure, growth performance, feeds and feed supplements. In the 1980s, he worked as Technical Director with a salmon farming company owned by the State Venture Capital agency and thereafter, for a decade through the 1990s, he managed and led research efforts at the Aquaculture Development Centre in UCC. In 2000, he established and managed a specialist seafood company. Richard is currently PI of the EIRCOD aquaculture project (funded under the Sea Change Strategy with the support of the Marine Institute and Marine research Sub-programme of the NDP with ERDF funding) and manager of the Carna research facility at NUI Galway. Richard is also a founding and current director of the not for profit AquaTT consultancy (www.aquatt.ie), currently very active in EU-funded projects.

## Prof. Mark Johnson

Prof. Johnson, is the PI of work package one and supervises the work carried out by Dr Anna Soler and Alex Wan. He is a professor of marine environment at NUI Galway and obtained his BSc in Biology from the University of York and his PhD from the University of Dublin. He previously lectured at QUB, Belfast in marine ecology from 2000-2008 and was a research fellow at the University of Southampton (1997-2000) and the University of Liverpool (1995-1997). His research interests include Marine ecology, the sustainable use of marine resources, spatial ecology and Marine biodiversity and conservation.

#### **Prof. Colin Brown**

Prof. Brown is the Director of the Institute for Environment, Marine and Energy at NUI Galway. His general research interests include offshore renewable energy, seismic reflection interpretation using Biot theory to infer unconsolidated sediment engineering properties, multi-beam acoustic classification and quantification of seabed sediment properties, marine habitat mapping using ROVs and ecological modeling, electrical and electromagnetic methods for coastal hydrogeology, and numerical modeling.

#### Prof. Deniz Tasdemir

Prof. Tasdemir is originally a plant natural product chemist. After a three-year postdoctoral training on marine natural product drug discovery with Prof. Chris Ireland at the University of Utah, USA, she has been working on marine invertebrates, algae and marine microorganisms. She continued her career at the University of Zurich and then London School of Pharmacy before joining the NUI Galway in 2011. She has written about 80 publications, several reviews, book chapters and holds several patents. She is the recipient of many prestigious awards, including ETH-Zurich Silver Medal, Pierre Fabre Prize of the Phytochemical Society of Europe (PSE) and Egon Stahl Silver Medal of the Society for Medicinal Plant and Natural Product Research



(GA). She is a member of the editorial/advisory editorial board of journals *Marine Drugs, Planta Medica, Phytochemistry Letters* and *Frontiers in Ethnopharmacology.* She is also the member of BoDs of the largest learned natural product societies, the Society for Medicinal Plant and Natural Product Research (GA) and Phytochemical Society of Europe (PSE). Her research interests lie in the fields of marine natural product chemistry, drug discovery from marine invertebrates, seaweeds and microoragnisms against cancer and infectious diseases and marine functional foods.

#### Declan Troy, NutraMara Director

Declan Troy is the Assistant Director of Research, Teagasc, the Irish Agriculture and Food Development Authority with special responsibility for science based knowledge transfer to the food sector. He is a Chartered Chemist of the Royal Society of Chemistry and completed his post graduate studies at University College Dublin in 1986 investigating the biochemistry of muscle proteases as a function of pH. He later became Head of the Meat Science Department in The National Food Centre, Dublin. During this time he has published over 100 scientific peer reviewed publications, book chapters and scientific articles, mainly in the area of food quality. The main focus of his research was on the biochemistry of muscle proteins and their effects on meat tenderness. Declan has always encouraged the up-take of science based innovations by the food industry and has interacted widely with the sector to this end. His work has contributed to the introduction of new technologies at industrial level particularly in Irelands competitive beef sector. He has coordinated numerous EU meat science projects and is currently coordinating ProSafeBeef, a €20 million project with 41 transnational partners aimed at advancing beef safety and quality through research and innovation. This landmark project includes close interaction with the meat science and industry community. He coordinates two EU Framework Marie Curie Training Sites for early stage career meat science PhD students in meat biochemistry and functional meat products. Currently he is the Director of the Marine Functional Food Research Initiative (NutraMara), a multidisciplinary programme aimed at discovering bioactive components from Irish marine sources for use in added value functional food products. He has collaborated in his research programme with many different research groups from all around the world including Australia, Korea and USA. He has been invited to speak at many international scientific conferences and industry seminars. He has supervised numerous PhD and M.Sc. students to completion. Declan sits on many national and international committees formulating research priorities in food science and technology and advising state agencies and companies. Declan holds the post of Vice President of the Institute of Food Science and Technology Ireland and is Chairman of the IUFoST 2014 Scientific Committee. Declan believes that there is huge potential for exploitation by the international food sector to innovate based on sound science, firm knowledge and key entrepreneurial skills for the betterment of the global economy.



### **Dr Dagmar Stengel**

PhD in seaweed biology from Queen's University Belfast after which she undertook several postdoctoral research positions at the Biologische Anstalt Helgoland, Germany, and NUI Galway before appointment to Lecturer in Marine Plant Science (Botany and Plant Science and Ryan Institute) in 2003. She is Research Streamleader for Environment and Biotechnology within the School of Natural Sciences at NUI Galway and is the Theme Leader for Algal BioSciences, NUI Galway. Her research interests focus on metabolic responses of algae (seaweeds and microalgae) to their environment and natural variability in bioactive compounds of commercial importance; the effects of climate change and anthropogenic influences, harvesting regimes and deterioration of water quality that impact on sustainable utilisation of algal resources; and the targeted cultivation for optimised production of seaweed and microalgal biomass and of primary and secondary metabolites with industrial potential.

# Session 2 – Marine Waste and By-produce Utilisation and Valorisation

## Prof. Colin Barrow

Professor Colin Barrow is Chair of Biotechnology at Deakin University. He is also Director of BioDeakin. Previously he was Executive Vice President of Research and Development for Ocean Nutrition Canada (ONC) where he led the development of seafood-derived supplement and healthy food ingredients and technologies. Professor Barrow has a Ph.D. in marine natural products chemistry from the University of Canterbury in New Zealand and an MBA from Penn State in the USA. Professor Barrow has approximately 130 peer-reviewed publications, several patents, has presented at numerous conferences and workshops. Prof. Barrow research interests include a broad spectrum of natural products chemistry, biological chemistry, food biotechnology and omega-3 oil technology. His research at Deakin University is primarily in two areas: omega-3 biotechnology and amyloid fibres. His current research group at Deakin University has 8 post-doctoral fellows and 12 PhD students.

#### **Dr Jean-Pascal Bergé**

Dr Bergé was awarded his Master of Oceanography at the University of Rennes before completing his PhD in Marine Biochemistry at the University of Nantes and obtaining his Diploma of Bacteriology, Option Molecular Taxonomy and Epidemiology from the Pasteur Institute. He has extensive experience in isolation and characterisation of marine products, most notably marine lipids. His past research experience involved developing novel techniques with successful patents arising and participating in many local and European funded projects with many



publication resulting. Jean-Pascal has also been heavily involved in lecturing in the fields of biochemistry, marine pharmacology and marine biotechnology in a range of universities at Masters level. Currently he is Director of Laboratory of the Sciences and Technology of Marine Biomass, IFREMER where he is involved in work concerning the bioconversion of marine resources to valuable products.

#### **Dr Alexis Garras**

Over the past 20 years Dr Garras has completed extensive research, basic, preclinical and clinical, within the field of lipidology and cardiology. Her research background is in the field of biochemistry and molecular biology, particularly focussing on omega-3 biochemistry. Her research interests include lipid-derived substances from the marine environment that may be used within the fields of cardiovascular disease, CNS and inflammation. Currently she is working on an omega-3 derivative that is intended to reach the market place within the next 6-8 years within the field of cardiology.

#### Dr Hörður G. Kristinsson

Dr Kristinsson is the Research Director of the Matis Icelandic Food and Biotech Research and Development institute and an Adjunct Associate Professor at the Department of Food Science and Human Nutrition at the University of Florida. Dr Kristinsson obtained his PhD in Food Biochemistry at the University of Massachusetts at Amherst and his MS degree in Fisheries/Food Science from the University of Washington in Seattle. He combines practical experience of working in the marine products industry with extensive commercial and research expertise in the production of various products and ingredients. He is the holder of five patents and has published over 60 peer-reviewed papers, book chapters and books. He has led numerous research projects on increased quality, stability and healthfulness of various food products and ingredients, funded by the governmental grants and industry in the USA, Europe and Iceland, and currently manages several major national and international research projects. His research interests include seafood byproducts, marine bioactives, fish proteins and peptides, lipid oxidation and antioxidants.

#### **Dr Maria Hayes**

Dr Hayes obtained her BSc (Hons) in Science, specialising in Industrial Microbiology and Chemistry from University College Dublin (UCD) in 2002. She carried out her PhD at the Teagasc Food Research Centre, Moorepark and University College Cork in the area of bioactive peptide isolation and characterisation from milk proteins and waste streams (whey and casein). She then carried out post-doctoral work at the Centre of Applied Marine Biotechnology Donegal, where she worked on the isolation



of chitinolytic enzymes from shellfisheries crab and whelk waste streams. She is currently the NutraMara Scientific Programme Manager and the PI for work package two and seven PI. She is responsible for supervising work regarding the isolation and chemical and biological characterisation within this work package as well as dissemination and outreach activities in work package 7. She supervises two NutraMara PhD researchers who are funded by the Teagasc Walsh Fellowship programme. Her research interests include the isolation, purification and characterisation of marine derived molecules, especially peptides and phlorotannins from marine seaweeds and by-products, bioassay development with a particular focus on heart and mental health disorders – i.e., renin, ACE-I, PAF-AH, PEP and inhibition of other enzymes with heart and mental health effects. Other interests include the generation of chitin and chitosan from marine shellfisheries waste streams, isolation and characterisation of enzymes (in particular chitinolytic enzymes) and fermentation. She has published 18 research papers in the area of functional foods research and has recently edited a book for Springer concerning the isolation, characterisation and utilisation of marine derived bioactive compounds.

#### Dr Uxía Vázquez Ferreiro

Dr Ferreiro obtained her degree in marine sciences before completing her masters in environmental management. Currently she is the project manager at CETMAR Research and Development and Environmental Projects where she participates in the technical and financial management and coordination of several European projects. Since 2008, she has been working in the technical implementation of projects focusing on marine products and by-products valuation. Her work includes competitive surveillance reports, feasibility studies, drafting proposals for new projects, organization of workshops and book editing. Dr Ferreiro's research interests include fishing by-products valorisation, marine resources exploitation, water and seabed quality management and solid waste management.

#### **Ms Heather Manuel**

Heather has approximately 15 years experience conducting industrial applied research on behalf of the seafood and marine biotechnology industries primarily within the province of Newfoundland and Labrador, Canada. She is currently the director of the Centre for Aquaculture and Seafood Development, Fisheries and Marine Institute of Memorial University of Newfoundland and she was recently awarded over \$654,000 (CDN) to establish pilot scale processing systems within the centre for aquaculture and seafood development specifically to develop a demonstration bio-refinery which will convert waste fish oils into biodiesel and a chemo-enzymatic process for the extraction of chitosan from shellfish waste. Her research interests include marine biotechnology, fishery by-product utilization, chitin/chitosan, marine biofuels, shellfish extracts and marine nutraceuticals.



## Dr Maria Tuohy

Dr Tuohy completed her PhD studies in Biochemistry in NUI Galway (1986-1991), was a postdoctoral researcher in the University of Gent, BSH Institut für Holzchemie, Hamburg and NUI Galway (Joint Appointment) before her appointment to Lecturer in Biochemistry, School of Natural Sciences at NUI Galway in 1997. She is head of the Molecular Glycobiotechology group at NUI Galway, Theme co-Leader of Enzyme and Plant BioSciences (with Dr Zoe Popper), NUI Galway, a member of the Ryan Institute, NUI Galway and one of 5 PIs in the national Technology Centre for Bioenergy & Biorefining (TCBB). Dr Tuohy and her group have ~20 years experience in fungal enzyme systems and the glycobiology of terrestrial plants and algae. Dr Tuohy has supervised 32 PhD students and 16 M.Sc. students in total to completion to-date, as well as 11 post-doctoral scientists and numerous final year project students, UREKA and visiting students. Her current research group comprises 2 PhD students, 1 RA and 7 postdocs. The group's work has led to the development of two spin-out companies, one of which (formed in late 2010) currently employs 6 research scientists. Dr Tuohy is co-author of 48 peer-reviewed publications), 15 book chapters, editor of 2 books and co-author of >180 conference abstracts (posters and presentations). She is also a co-author of two patent applications which are at nationalization stage (WO/2007/091231; WO/2011/095643) and one divisional patent application (European Divisional Patent Application No. 11157751.6). Dr Tuohy's Core research interests include the production, characterization and genetic aspects of fungal carbohydrases, proteases, oxidases and oxidoreductases, the Identification of novel biocatalyts (enzymes) from fungi, especially thermophilic species, enzymes as selective tools for the extraction and functionalization of plant bioactives, biotechnological and biomedical applications for target enzymes, with emphasis on the development of environmentally-friendly, safe technologies and novel strategies for the production of bio-pharmaceuticals and nutraceuticals. The Isolation, investigation, selective modification and mechanism of action of novel bioactive substances and growth factors (especially carbohydrates) from algae as well as novel enzyme technology ('green bio-chemistry) for the solvent-free release of bioenergy feedstocks (lipids and carbohydrates), and for the recovery of valueadded co-products from terrestrial plants and algae.

## Dr Maria Cuaresma Franco

In 2006 Dr Franco obtained the Advanced Research Certificate from the University of Huelva with the project entitled "Isolation, cultivation and biotechnological potential of *Chlamydomonas acidophila* isolated from Tinto River". In May 2006 she started a PhD project cofounded by University of Huelva in Spain and Wageningen University in the Netherlands. She focused on the maximization of microalgae photosynthetic



efficiency under high irradiance conditions in a lab-scale photobioreactor. On the 27th of June 2011 she obtained a PhD degree from the University of Wageningen. Since March 2011 she has been working as a postdoctoral researcher at AlgaePARC in Wageningen. She is leading the screening process for microalgae for biofuel production. In addition, she is also working on the optimization of operational conditions for microalgae production in lab-scale photobioreactors, as well as in pilot systems outdoors (2.4 m<sup>2</sup>). Since January 2012 she has been involved in a project funded by the 7<sup>th</sup> European Framework, on the development of a modular, scalable and automatic biofilm reactor for CO<sub>2</sub> capture and algal biomass production with low operational and installation costs. Her research interests include microalgae biomass production, biodiesel, photobioreactor development, photosynthetic efficiency and high added value products from microalgae.

## Dr Freddy Guihéneuf

Dr Freddy Guihéneuf holds a PhD in Biology from the University of Maine (France) focused on PUFAs production from marine microalgae. After his PhD, Dr Guihéneuf worked for two years as a postdoctoral researcher in Israel with Prof. Sammy Boussiba on genetic engineering approaches for enhanced oil production from microalgae. Currently Dr Guihéneuf is working as a postdoctoral researcher with Dr Stengel Dagmar (NUI Galway, Ireland). His project is focused on the potential of microalgae as a source of food ingredients (NutraMara consortium). His research interests include microalgae as a source of food ingredients (PUFAs, MAAs, pigments, polysaccharides, etc.), microalgal genetic engineering for value added product, metabolism of polyunsaturated fatty acids (PUFAs) in marine microalgae and isolation, characterization and expression of genes involved in PUFAs and lipid biosynthesizes.

#### **Dr Jean-Paul Cadoret**

Dr Cadoret is the Director of the Laboratory of Physiology and Biotechnology of Algae. His research is based at the "Institut Français de Recherche et d'Exploitation de la Mer (IFREMER)" in the Laboratory of Production and Biotechnology of Algae where he is in charge of the Molecular Biology Unit. Here, the main research interest is the application of molecular biology tools in the study of algae. More specifically, transcribed phenotypes as a response to metabolic variations as well as the development of a "cell factory tool". In addition he is involved in research at the "Institut Français de Recherche et d'Exploitation de la Mer (IFREMER)" in the "unité mixte de recherche 219 DRIM (Défense et Résistance chez les Invertébrés Marins) Université de Montpellier II, IFREMER/CNRS" laboratory. Here the main area of research is the characterisation of the defence mechanisms of commercially important marine bivalves aimed at the control of diseases. This work involves genetic transformation as basic knowledge and the understanding of gene regulation



mechanisms at the developmental level, elucidation of defence mechanisms, and the pattern of reproduction. He was involved in adapting transfection systems, *in vitro* for bivalve primary cell cultures (lipofection) and *in vivo* for bivalve embryos (microinjection and biolistic). Through this work Dr Cadoret was able to identify, clone and sequence functional promoters and integration sequences (i.e. transposons) as well as various bivalve genes.

#### Dr Anna Soler-Vila

Originally from Catalonia, Anna graduated in 2001 from the University of Gran Canaria with a degree in Marine Science. She worked in a fish farm in Spain, and shortly after moved to Galway, Ireland to gain work experience in Aqua-Fact Ltd., an environmental consultancy based in Galway. In 2003 she began her PhD at the Ryan Institute in Galway. Throughout all this time, she has been involved in many projects which include macroalgae research. Currently she is a post-doctoral researcher in the marine functional food project (Nutramara). Her main research interests include chemical and/or nutritional composition of macroalgae with particular attention on applications in the areas of human food, animal feeds and health, sustainable harvesting methods of wild seaweed populations and in particular the development of integrated aquaculture in Ireland (IMTA).

#### Prof. Fergal O'Gara

Professor Fergal O'Gara is Director of the Biomerit Research Centre, a Biotechnology centre of excellence at University College Cork (UCC). He is also Emeritus Professor of Microbiology at the University. He graduated from the National University of Ireland Galway (NUIG) with a PhD in Microbiology in 1974. He spent 3 years working as a research scientist at the University of California and joined UCC in 1977. He was appointed Professor and chair of Microbiology at UCC in 2002 and served as head of Department until 2009. His research interests focus on the genetic and molecular biology of microbe-host interactions in medical and environmental biotechnology. His research programme is funded by national and international agencies and He has published widely in international molecular biology and industry. microbiology journals (230 major papers) and was awarded a Doctor of Science degree (DSc) in 1991 by the National University of Ireland (NUI) in recognition of his published works. He is an elected member of the Royal Irish Academy (RIA) and served as Vice-President from 2008-2011. Professor O'Gara serves on a number of European Science bodies. He has participated as Biotechnology advisor to UNIDO and a number of other international government organisations. He has acted as chairperson of the EU Madam-Curie training programme and chair of the European Food Safety Authority's (EFSA) panel on GMOs. He is a member of the European Environmental Research Organisation (EERO) and also acts as Editor for a number of international scientific journals.



# Session 4 – Marine Derived Ingredients as Potential Functional Foods

## **Dr Ivan Coulter**

Dr Coulter is founder and Chief Executive Office with Sigmoid Pharma Ltd. Ireland. Prior to this Dr Coulter set up, developed and managed the drug formulation and diagnostics groups at NTera (www.ntera.com), an Irish based nanotechnologyfocused drug delivery and diagnostics company. Dr Coulter has an honours degree and PhD in Pharmacology from University College Dublin, held a Post-Doctoral Fellowship at the Institut Curie, Paris and, awarded a Fulbright Scholarship, completed an MBA at Cornell University, New York. In addition, he holds a Diploma in Accounting and Finance awarded by the Association of Chartered and Certified Accountants (ACCA). Recently, Ivan was appointed to the Irish Research Council and the board of Molecular Medicine Ireland. Previously, Ivan served on the Bord Iascaigh Mhara (BIM) Irish Fisheries board. Sigmoid is focused on the development of innovative digestive disease therapeutics as well as oral delivery of peptides, vaccines and other bioactives, including plant or marine derived entities.

## Dr Thomas Smyth

Dr Smyth holds a BSc (Hons) in Biomedical Sciences and a Masters of Research (MRes) in pharmaceutical biotechnology research from the University of Ulster (UU). His PhD involved the structural elucidation of natural products with particular emphasis on Anti-MRSA components from plant sources and was carried out at UU as part of the Pharmaceutical Biotechnology Research Group. He subsequently took up a position as a Research Associate in the Microbial and Biotechnology Research Group at UU. This post investigated the directed biosynthesis of microbially derived biosurfactants including all production, downstream processing and chemical characterisations. Currently Dr Smyth is employed as a senior research officer in natural product chemistry at Teagasc Food Research centre, Ashtown. He has over a decade experience in the extraction, purification and structural elucidation of natural products from a variety of sources, including plant and marine sources. Other research interests include structural elucidation of novel bioactive compounds using a wide range of mass spec and NMR techniques, the development of rapid mass spec methods for the quantification of natural products and their degradation products within extracts and polysaccharide characterisation.

## **Prof Dick FitzGerald**

Dick FitzGerald obtained his BSc and PhD in biochemistry from University College Cork (UCC). He has worked as a research fellow at North Carolina State University and at UCC, as a senior research officer at the Chemistry Department, Teagasc Moorepark and as lecturer, senior lecturer and associate professor at the University



of Limerick (UL). Currently he is Principal Investigator at UL in the NutraMara project where his main focus is the isolation, purification and characterisation of biologically active proteins, peptides and amino acids from marine resources. His other research areas include functional food ingredients from bioactive peptides, food protein chemistry/biochemistry, food enzymology and food protein functionality. Also he is involved in the role of aminopeptidases in food protein hydrolysate debittering and the enzymatic hydrolysis and cross-linking of food proteins.

### Dr Pádraigín Harnedy

Dr Harnedy obtained her BSc and PhD in biotechnology from Waterford Institute of Technology (WIT). She worked as a forensic scientist and as a research officer in the Meat Department at Teagasc, Ashtown before commencing a postdoctoral research position on the NutraMara project at the University of Limerick. Her main focus in the NutraMara project is isolation, purification and characterisation of bioactive proteins, peptides and amino acids from marine resources. Her other research interests include the enzymatic hydrolysis of food proteins and food protein derived bioactive peptides and their roles in heart health and diabetes.

#### **Prof. Torres Sweeney**

Prof. Sweeney obtained her BSc (Hons) at University College Cork, specialising in Zoology and Physiology before completing her PhD in Reproductive Biology at University College Dublin (UCD). Following this she was awarded a European Science Foundation Postdoctoral Research Fellowship at the Medical Research Council Centre for Reproductive Biology in Edinburgh before undertaking a position as lecturer in the Faculty of Veterinary medicine at UCD where she has progressed to Associate Professor of Animal Genomics. Her research interests include the genomic regulation of physiological and disease processes in mammals. She focuses on how environmental factors (nutrients, bioactives, pathogens, toxicants, stress) interact with the genome of an animal to influence both developmental and disease traits.

#### **Dr Chris Gill**

Dr Gill received his PhD in 2000 and is a lecturer in the School of Biomedical Sciences Research Institute, University of Ulster (UU), Northern Ireland. His work within the Northern Ireland Centre for Food and Health (NICHE) is focused on the influence of diet on cancer risk, as investigated through *in vitro*, animal and human models, utilizing nutrigenomic and metabolomic tools to study the complex issues associated with diet and cancer interaction. The main areas of interest within this topic include phytochemicals, pre/probiotics (EU FP7 HYFFI, SWAFAX), and colonic microflora gut health and faecal water activity. He is a member of the strategic advisory board for



Nordforsk National Centres of excellence programme Food and Health Initiative and a member of the Danish Peer Review College.

#### **Dr Emeir McSorley**

Dr McSorley is currently a lecturer in Human Nutrition within the School of Biomedical Sciences at the University of Ulster (UU), Northern Ireland and is a registered nutritionist. She received her PhD from UU in 2002 in the area of nutrition, biochemistry and immunology specialising in omega 3 fatty acids and the autoimmune condition systemic lupus erythematosus. Dr McSorley has a BSc in Biomedical Sciences and is a registered Medical Laboratory Scientist. Following her PhD, Dr McSorley worked as a research associate within the Northern Ireland Centre for Food and Health (NICHE) at UU on an EU framework project FeMMEs and an NIH funded project - the Seychelles Child Development Nutrition Study. Dr McSorleys research interests are focused on understanding the influence of diet on aspects on cell function and human health, in particular the effects of vitamin D, n-3 PUFAs, calcium and iron in relation to inflammation and immunity, bone health and pregnancy. She is the PI for UU in the large all-Ireland based consortium 'NutraMara' which is focused on the development of Irish derived marine bioactives for incorporation into functional foods. Dr McSorley is a committee member of the Ulster Immunology Group and is a member of the Institute of Biomedical Sciences, the Academy of Medical Laboratory Sciences and the Irish and UK Nutrition Society..

### Mr Ross Campbell

Ross obtained a joint honours degree in Chemistry and Management Science from Stirling University in 1978 and has since gained a vast amount of expertise working for a number of different companies in various roles. He worked for Dow Corning as a Research Chemist in silicone polymers for the paper and textile industry before working for 3M Chemicals as a Technical Service Chemist focussing on fluorochemicals used in the paper, surfactants and fire fighting industries. Ross moved on to work as a New Ventures executive at Foseco looking for new business opportunities utilizing Fosecos capabilities in silicate chemistry. As Business Development Manager, Ross was involved in acquisitions as well as acting as Marketing Manager for Food and Pharmaceuticals for Courtaulds Chemicals Water Soluble Polymers. In 1991 Ross joined Quest as Market Development Manager to start up their carrageenan business before being promoted to Business Director responsible of all Quest's Hydrocolloid activities. Ross left Quest in 2001 and started up CyberColloids which has become a well recognized 'contract research to business development' specializing in polysaccharide chemistry for food texture and nutrition.



#### **Prof Paul Ross**

Prof. Paul Ross is Head of the Teagasc Food Research Programme (Moorepark & Ashtown Food Research Centres) which includes the role of Managing Director of Moorepark Technology Ltd. - an ultramodern Dairy Pilot Plant facility. He was appointed to his current position in July 2009. Paul is a Principal Investigator in the Alimentary Pharmabiotic Centre (APC) at University College Cork (UCC). Prof. Ross graduated with a BSc. in Microbiology/Biochemistry in 1984 and with a PhD in Microbiology in 1989, both from UCC. He has built up a close partnership with colleagues at UCC, particularly with Professors Colin Hill and Gerald Fitzgerald at the Microbiology Department and Professor Fergus Shanahan in Medicine (Director of the Alimentary Pharmabiotic Centre). His main research interests are in phage therapy, antimicrobial peptides, bacteriocins, probiotics, and anti-infectives. He has supervised approximately 50 post-graduate students. He also has coordinated or been a (co-principle) investigator on numerous national, EU and National Institute Health (US) grants. He also is a member of the Executive Management Group of the Alimentary Pharmabiotic Centre, a virtual Centre between NUI, Cork and Teagasc (devoted to the study of intestinal flora and their impact on human health. Paul was awarded the following awards the William C. Haines award by the California Research Council for his contribution to Dairy Science in 2007, The Enterprise Ireland Commercialization award in 2008, The International Dairy Federation's Elie Metchnikoff Prize in Microbiology for 2010. Paul was conferred in March 2009 with a D.Sc. based on published works. He was elected a member of The Royal Irish Academy (RIA) in May 2010 and has published 319 peer reviewed papers.

## Dr Michael O'Grady

Dr O'Grady works in the School of Food and Nutritional Sciences in University College Cork since 2001 in a research/project management capacity on a number of national and internationally funded research projects which have included ProSafeBeef (EU), Development of healthier meats and meat products (FIRM), Enhancing the healthiness, shelf-life and flavour of Irish packaged beef (FIRM) and A toolbox for rapid development of food products enabled by high pressure processing (EI). Currently he is working as a postdoctoral researcher on the NutraMara project in the areas of antioxidant activity and mechanisms of marine extracts and bioactivity of seaweed extract enriched dairy products. He focuses on the stability of seaweed extracts and marine bioactive ingredients incorporated into food products. His research interests include bioactive ingredients from marine sources, animal dietary supplementation studies, nutraceuticals and functional foods, oxidative stability and quality of muscle foods and shelf-life and sensory evaluation of foods.



#### **Dr Dermot Hurst**

Dr Hurst graduated in mechanical engineering and obtained his PhD from NUI Galway. He is a programme manager at the Marine Institute, and having worked on the development of *Sea Change - Ireland's Knowledge, Research and Innovation Strategy*, is now responsible for the development and delivery of new research initiatives designed to strengthen Ireland's marine sector. Responsibilities in this role include building initiatives to support the development of Ireland's marine foods, functional foods, marine biotechnology, seaweed, shellfish and finfish aquaculture, and transport sectors. Dr Hurst represented the Marine Institute on the Food Research Expert Advisory Group, which developed *Food Research Ireland*. Most recently he was member of the National Research Prioritisation Working Group on Health, Wellbeing and Ageing.



# Abstracts

#### Setting the Marine Foods Research Agenda

## <u>Dr Pamela Byrne</u>

Department of Agriculture, Food and Marine, Ireland

Food Harvest 2020 – Irelands vision for the agri-food and fisheries industry talks about achieving smart green and sustainable growth. Ambitious targets have been set to deliver on this Vision. By 2020, Ireland will increase the value of its primary output by  $\leq 1.5$  billion; increase the value-added by  $\leq 3$  billion and increase its exports by  $\leq 12$  billion. Specific to marine functional foods and ingredients, the subject of the NutraMara conference, Food Harvest 2020 recommends that research in marine functional foods be intensified. Delivering on these targets requires a strong research capacity and critical mass in key research areas and an innovative enterprise base to exploit the outputs of that research. This is the premise upon which Food Research Ireland is based.

Food Research Ireland – meeting the needs of Ireland's food sector to 2020 through research and innovation is the first step on a journey to ensure that the Irish food industry has access to and collaborates with the Irish food research performing organisations. Innovation is at the heart of Ireland's future economic growth. There is no doubt that Ireland's food industry is uniquely placed to lead economic growth in the export oriented indigenous manufacturing sector. However, it is recognised that this growth will only result from the development of new and innovative products, underpinned by a safe and secure food supply chain. The role of Food Research Ireland in underpinning a sustainable agri-food and fisheries industry in Ireland will be discussed.

The EU Framework Programmes have long been the main element in the internationalisation of Irish research and technological development and, building on national investments in research in recent years in both the public and private sectors, Ireland is now participating in the Framework Programme from a far stronger research base than ever before. Within Europe we are moving from the current research funding programme – FP7 – to Horizon 2020. The opportunities for Ireland's Irish research community and the seafood sector to engage is this new exciting programme will be presented.



# Session 1 – Marine Resource Sustainability

#### Sources of Marine Materials - The NutraMara Feasibility Study

#### Dr Richard Fitzgerald

National University of Ireland, Galway

The development of functional foods within NutraMara relies on the generation of novel products, either by emphasizing an underdeveloped food benefit or by adding steps to the processing of foods. For example, while seaweeds are already consumed in the west, greater demonstration of potential health benefits is likely to improve market opportunities. Similarly, existing food pathways may provide additional opportunities for generating highvalue products. The largest volume of potential material is generally associated with pelagic fisheries. This material is landed at a small number of ports. Significant inter annual variability in the volume of fishery-related material exists, reflecting changes in processing and catch volume. Whitefish and aquaculture-related material is available in lower volumes more widely around the country. With all animal material, the details of source are important, as material categorized as 'waste' cannot generally return to being considered as food. Using material as supplements for animal diets may be more flexible, but this is still regulated. The existing national resources of seaweed are broadly understood, but responses to harvesting are still quite poorly known. This causes issues with European conservation legislation, which requires a precautionary approach. Looking further along the pipeline of novel products, there are a number of steps where novelty can be generated. While a European Food Safety Authority (EFSA) human health claim remains a target, such a goal would require significant investment. Before this stage there are possibilities for high value products including food ingredients without a specific health claim, food ingredients that improve taste or product stability and improved quantities and qualities of known compounds of interest. Looking at patent and publication activity, a number of opportunities can be identified. Some groups, like peptides and certain algal polysaccharides have opportunities for novelty at levels ranging from biodiscovery and extraction methodology to product development.

#### **Natural Products: The Fundamentals of Marine Functional Foods**

<u>Prof Deniz Tasdemir</u>

National University of Ireland, Galway

Marine organisms are exceptionally rich sources of natural products with potent biological activities. Marine-derived organic molecules, particularly secondary metabolites are main interest to drug discovery research. The intensive efforts in this area in the last few decades have resulted in the approval of three marine natural products (MNPs) as anti-cancer and analgesic agents in Europe and the USA and many more are currently undergoing human clinical trials. On the other hand, marine resources have been consumed as food for thousands of years. Recently, there is a growing interest in utilization of marine material and bioactive substances



as functional foods in the prevention and management of chronic diseases. So in addition to their nutritive value, sources such as seaweeds, microalgae, bacteria, fish, shellfish and their primary and secondary metabolites (e.g. fatty acids, polysaccharides, proteins/peptides/aminoacids, polyphenolics, alkaloids etc) can offer significant health promoting benefits. The general aspects and the bottlenecks of marine drug discovery and marine functional foods research are very similar. It includes: i. sustainable supply issue ii. requirement of a) proper taxonomical identification of the source organism b) fast, modern extraction and bioactive compound fractionation, purification, characterization c) high throughput in vitro/ex vivo screening methods d) bioavailability, formulation, stability data e) in vivo animal clinical studies and human trials. Particularly the purification/profiling/characterization of the bioactives and the provision of proof of evidence for a health benefit, which is again due to natural ingredients of marine functional foods, are the most fundamental pillars upon which academic and industrial research can be developed. This presentation will present the view of a MNP chemist on both marine biodiscovery and marine functional foods. It will highlight the necessity of state-of-the-art research and close collaboration between the researchers and industrial companies to lead to the discovery and the commercial exploitation of new marine products with nutritional and functional importance.

# Variability in Bioactivity of Algal Compounds – Implications for Commercial Applications

Dr Dagmar Stengel

National University of Ireland, Galway

Marine algae (including both microalgae and seaweeds) have been identified as a valuable source of bioactive compounds with applications in the biomedical, horticultural, cosmetics and food industries. Even though the taxonomic diversity of algae is vast, globally research so far has focused on only a small number of species that are exploited commercially. Regarding applications as human foods, food ingredients and functional foods, algal compounds of interest include antioxidant, anti-inflammatory, anti-cancer and anti-diabetic activities. Families of compounds that these activities are attributed to include polysaccharides (e.g. fucoidan, laminaran), pigments (e.g. carotenoids), and phenolics (e.g. phlorotannins from brown seaweeds), but specific bioactivities are closely linked to particular chemical structures within these groups. Chemical profiles and composition are specific to different algal groups, within these to certain species, and within individual algae to specific parts. Additionally, because of their natural biological function, composition of compounds varies between habitats and seasons creating a significant chemical diversity, and thus potential, that is currently poorly characterised. Capturing this diversity and controlling variability in algal chemical composition, and linked



bioactivities, are key challenges for future commercial applications. Newly developed analytical techniques that enable separation and profiling of specific algal compounds which need to become more time-efficient and applicable to a commercial scale. An improved understanding of the metabolic function of key algal compounds and their environmental control will facilitate targeted and customised cultivation for specific markets. It will also permit the future exploitation of new species with high potential but currently limited biomass, and thus, ultimately, support the commercial development of new algal products whilst protecting natural stocks.

*Scientific Session 2 - Marine Waste and By-Product Utilisation and Valorisation* 

#### **Novel Enzymes from Marine Waste Products**

<u>Prof. Colin Barrow</u> Deakin University, Australia

The omega-3 fatty acids eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA) were originally sourced from waste fish oil that is a by-product from fish meal processing. Due to the established health benefits of oils containing these long-chain fatty acids, a billion dollar omega-3 market has been developed from this waste product. Fish oil and concentrated forms of EPA and DHA are sold as nutritional supplements, functional foods and pharmaceuticals. We and others are developing enzymatic processing methods to improve the quality of fish oil concentrates containing these unstable fatty acids. We are also developing more oxidatively stable EPA and DHA analogs and exploring alternative methods of production. Using ionic liquids and immobilization strategies we have improved the activity and selectivity of some omega-3 hydrolyzing lipases. We are also isolating and exploring the utility of new lipases from a variety of marine sources, including fish waste product and microorganisms. This presentation will describe our current results in these areas and also in the application of vegetable lipoxygenases to the formation of bioactive EPA and DHA derivatives.

# By-products: What's Up? – A French Perspective to Marine Waste Utilisation and Valorisation

Dr Jean-Pascal Bergé IFREMER, France

According to the Food and Agriculture Organisation of the United Nations, estimates for 2009 based on reporting by some major fishing countries indicate that total world fishery and aquaculture production reached 145.1 million tones with about



81.5% (117.8 million tones) that were used for direct human consumption while the remaining 18.5% (27.3 million tones) were destined for non-food products. Fishing activities and fish transformation generate big amounts of by catches and by-products. Stock rarefaction and changes in legislation modify the habits and those biomasses become more and more of interest. Fish processing by-products are commonly recognized as low value resources with negligible market value. Nowadays, they are mainly used for meal, oil or pet food mince production. Hence, there is a great opportunity here to increase the added value of these by-products. Worldwide different actors are currently working on finding solutions for a better management and upgrading of those biomasses. In France, since few years, academic teams (notably the one engaged into the scientific SEA<sup>PRO</sup> network) are involved in different programs for promoting to industrial new upgrading solutions or for improving the existing ones. The general strategy retained by those actors is the following:

- to locate and quantify the marine biomasses (raw and processed) and to establish dynamic maps in order to study different upgrading strategies. Such actions were currently part of a regional program ("Gestion Durable", PSDR-GO call);
- to transfert to industrials (bio)technological solutions for better management and use of their by-products and to reinforce links between academic and professional actors (at least 3 European projects have dealed with those aspects: Valbiomar, Biotecmar and BE-FAIR, the first two are based on networking and communication while the last one was based on demonstration sessions);
- and to conduce oriented research to find new upgrading solutions or new derived products. For example in the project Propephealth (SEAFOODplus), academic and SME have worked together to find bioactive properties in fish hydrolysates. In the SEANIOR project, research will be conduced for producing ingredients for ageing people with nutritional and sensory properties.

## By-products and Pronova Biopharma – Opportunities for Irish and EU Industries

Dr Alexis Garras

Pronova BioPharma, Norway

Pronova BioPharma is a pharmaceutical company with its headquarters in Oslo, Norway. The company research has been centred on omega-3 fatty acids, more specifically, eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) from the start. Pronova has during the course of the last 25 years developed a high concentrate omega-3 prescription pharmaceutical drug with two indications, hypertriglyceridemia and post-myocardial infarction. The drug, which is marketed under the names Omacor/Zodin/Lovaza, consists of 460 mg EPA and 380 mg DHA per one gram capsule and is dosed at one capsule/day for post-myocardial infarction and 2-4 capsules/day for hypertriglyceridemia. Omacor is sold in 56 countries



worldwide and achieved block-buster status in 2010 with sales of over one billion USD. Development of new pharmaceutical products based on omega-3 fatty acids of marine origin has been ongoing for the last 10-15 years. Pronova has a very active R&D department that has taken the work on omega-3 fatty acids several steps further and made several derivatives of EPA and DHA. These modified fatty acids exhibit effects on lipid parameters that exceed the effects seen by their parent compounds. Currently, the lead compound that is being tested is undergoing clinical Phase I trials with Proof of Concept trials being designed and expected to start by Q3 2012. There is a need for new and innovative pharmaceutical products within the field of cardiovascular disease, considering the side-effects and residual risk present with current treatment and the forecasts that metabolic syndrome and diabetes will reach epidemic proportions within the next 15-20 years.

#### Functional Surimi Seafood Developed from By-products

Dr Hörður G. Kristinsson Matis Ltd, Iceland

Significant amounts of protein rich seafood by-products can be utilized better than they are today. Due to the complex nature of many by-product this is a difficult task with many challenges. One way of utilizing protein rich by-products for human consumption is to produce surimi, which is a fish protein based product traditionally made by washing and refining fish muscle and adding cryoprotectants for stability. Surimi seafoods are popular ready-to-eat seafood products made in part with surimi. Due to its product form and high consumer acceptability, surimi seafoods are excellent vehicles to deliver health promoting marine bioactive ingredients. Our efforts in the area of surimi and surimi seafoods have been to develop new innovative processes to produce functional surimi from low value Icelandic raw materials, such as fish frame mince and species never before utilized in surimi production. A second goal has been to develop ways to incorporate bioactive marine ingredients, e.g. omega-3 fatty acids, into surimi to provide consumers with novel highly acceptable products that deliver potential health benefits. These efforts have resulted in an efficient process to make novel surimi from complex low value raw materials. Our work has also shown that addition of significant levels of highly bioactive ingredients into the novel surimi is possible with the surimi products having high consumer acceptance and a good shelf life. The demand for seafood is on the rise while wild resources are limited. Providing consumers with healthy ingredients extracted from seafood by-products in a consumer friendly form such as surimi is an excellent way to meet consumer demands while reducing pressure on wild resources.



#### **Collagen and Chitin Generation from Irish Marine Processing Waste Streams**

#### Dr Maria Hayes

Teasgasc Ashtown Food Research Centre, Ireland

Large quantities of by-products such as heads, fins, scales, shell and skin are produced each year in Ireland as a result of marine processing of white and pelagic fish as well as shellfish. These by-products may be sources of valuable ingredients including collagen, gelatine, chitin and chitosan for use in the food, chemical, pharmaceutical and horticultural industries. Chitin is the second most abundant polymer in nature and differs from cellulose due to the presence of the acetamido group on the C2 atom of the glucose subunit. Within NutraMara, various methods were developed to extract chitin from crustacean shells, specifically from the shell of the brown crab, Cancer pagurus and prawns. To date, the most efficient method for extraction and generation of chitin and chitosan involves demineralisation using acid (0.25 M HCL), deproteinisation using base (0.25 M NaOH) and deacetylation using strong base at 60°C for 6 hours. Chitin and chitosan generated within NutraMara is currently under assessment for its anti obesity properties at UCD. Collagen is a major structural protein in the connective tissue of skin and bone, giving strength and support to tissues. The collagen molecule is a triple-helix, with three  $\alpha$ -chains that adopt a three dimensional structure. It contains a high percentage of glycine, hydroxyproline and proline which make up the most common repeating triplet in the molecule, with glycine always occupying every third position. Gelatine is the hydrolyzed form of collagen and is a high value functional protein. NutraMara has also developed extraction methods for the generation of collagen and gelatine from salmon fish skins. Hydrolysed collagen was generated using the food grade enzyme papain. RP-HPLC fractions were tested for their heart health activities using bioassays targeted at inhibiting the actions of two key enzymes namely, platelet activating factor acetylhydrolase (PAF-AH) and renin.

#### An Approach to the Valorisation of Fishing By-products in Galicia

# Dr Uxia Vazquez Ferrerio

Centro Tecnológico de Mar, Spain

Galicia is a coastal region located in the northwest of Spain. Traditionally, it has a strong dependence on fishing. Nowadays, the marine industry of Galicia is still a social and economic strategic sector, having a turnover of about 4.750 million Euros per annum and directly employing almost 46.000 people. The most competitive subsectors are extractive fishing, processing industry, shellfish gathering and aquaculture. Galicia counts on the 44% of the national fishing fleet, mainly small-scale fishing fleet. There are 113 frozen fish processing plants and 65 canning factories. Mussel culture is developed in 3.388 rafts and more than 25 turbot farms



are located along the coastline. Discards and fish by-products are basically generated at three steps of the value chain: onboard, at fishing markets and at processing plants. Marine organisms discarded by the Galician fleet could represent 16.9% of the total capture. These discards are essentially linked to trawling and they are currently thrown away. Regarding fish by-products, they are generated both onboard and onshore and they are usually linked to fish processing. These byproducts mainly consist of heads, viscera, skins and bones. At the present, several factories in the region profit those generated onshore to obtain fish meal and fish oil. It appears that sustainable raw materials supplies are available and that the technology for obtaining value-added products is quite developed. Many research groups are looking at marine resources and many projects have been carried out to take advantages of this marine material. However, valorisation processes have not been implemented at an industrial scale in Galicia. Why? Among other reasons, the causes could be attributed to the dispersion of the raw material sources, which entails logistical complexity and heterogeneity in the quality of raw material, the lack of marine-based biotechnological companies in the region and the non-competitive price of raw material.

# Valorisation of Shellfisheries Wastes in Newfoundlands – Valorisation of Resources by Canadian Industries

#### <u>Ms Heather Manuel</u>

St. John's Newfoundland, Canada

Canada produces over 400,000 tonnnes per year of waste from wild capture fisheries and aquaculture. The Maritimes, Newfoundland and Labrador (NL), and the Gulf regions have the largest volumes of waste material generated from the fishing and aquaculture industries. Collectively these three regions account for ~323,055 MT (78%) of the national total waste generated. The use of seafood by-products has been identified by Canadian industry, academia and government as a potential source of raw material for the production of aquaculture feed ingredients (e.g. fish meal and fish oil), alternative fuels (e.g. biodiesel), pharmaceutical and biomedical products. NL produces in excess of 100,000 tonnes of fisheries waste per year. Crustacean processing discards account for more than 55% of this waste. The commercial production of chitin/chitosan, astaxanthin, and industrial enzymes derived from crustacean discards has enormous potential value for new market niches in the pharmaceutical and biomedical industries. However, there are currently no processors in the province with processing technologies in place to cost effectively deal with this waste stream. The estimated value of lost revenues to the Newfoundland and Labrador economy in 2010 due to crustacean processing discards was in the range of \$500,000 CDN; more than 50% of the total market value of seafood production in the province for the 2010 season. To address the fisheries waste issue in NL, the CASD has undertaken a series of industrial research initiatives



to capitalize on a number of opportunities to enhance the value of the NL seafood industry, namely theevaluation of new technologies for the production of fish meal and fish oil from waste streams from the wild capture fisheries and aquaculture; thedevelopment of Eco-Friendly Technologies for the Extraction and Production of Chitin and Chitosan from crustacean shell waste and the development of biodiesel from waste fish oil.

# Scientific Session 3 - Marine Algae: A Source of Food Ingredients

## AlgaePARC: Algae Production and Research Centre – Potential Benefits for Industry

## Dr Maria Cuaresma Franco

Wageningen University, Netherlands

Algal production needs to develop from a craft to a major industrial process. Major challenges are to reduce production costs and energy requirements and increase production scale. Although microalgae are not yet produced at large-scale for bulk applications, recent advances - particularly in the methods of systems biology, genetic engineering, process control, and biorefinery – present opportunities to develop this process in a sustainable and economical way within the next 10 to 15 years. AlgaePARC, a new applied research centre on microalgae has been set up at Wageningen UR to compare present technology, and to develop new reactor concepts and process control strategies to achieve lower production costs and energy requirements and to gain knowledge for the design and process control of large-scale microalgae facilities. The goal of AlgaePARC is to fill the gap between fundamental research on algae and full-scale algae production facilities. This is done in flexible pilot scale facilities in order to perform applied research and obtain direct practical experience. The set-up is fully automated and allows for fast changes between photobioreactor types, layout, and process control strategies. The initial systems chosen for reflect the present development of several reactor concepts at laboratory scale, by different research groups and companies, and will enable a rigorous comparison between systems, selection and, ultimately, the development of a more efficient system and optimized operational concepts. A five year research program started at AlgaePARC in 2011 and is presently supported by 19 companies in the food, oil, chemical and technology development sectors.

## The Potential of Microalgae as a Source of Food Ingredients

# Dr Freddy Guiheneuf

National University of Ireland Galway, Ireland

There has been a growing interest in functional food development from natural sources including algae because of their beneficial health effects. Due to their



taxonomic ( $\approx$  40.000 species) and biochemical diversities, microalgae and Cyanobacteria have the potential to become a novel source of bioactive molecules. Polyunsaturated fatty acids (PUFA), carotenoids, phycobiliproteins, phenolic compounds, polysaccharides and vitamins are the major molecules of interest from microalgae, particularly due to their capabilities to enhance the nutritional and functional quality of foods. Nevertheless, only few microalgal species are successfully produced, commercialized and used in human diets today. Microalgae are more commonly used in aquaculture industry as live feed for larvae of bivalves, crustaceans and marine fish: food for rotifers and shrimps. Algal cultivation, induction and accumulation of bioactive high-value products is a complex problem. Algae grow in diluted culture media and require large areas and water volumes, causing high cultivation and harvesting costs, posing contamination problems and variable productivities due to climate variability. On the other hand, the metabolic plasticity of microalgae and cyanobacteria allows them to adapt quickly to changing environmental factors such as light, temperature, salinity and nutrients. Their production requires therefore the development of algal culture techniques appropriate to obtain constant levels in molecules of interest and improve their nutritional value. In addition to applications of microalgal biotechnology in food industries, in recent years, investigations into their suitability as a potential source of biofuel has attracted much attention; several academic and industrial projects are focused on strain selection, genetic improvement and cultivation technologies to reduce high production prices for algal biomass.

#### Microalgae: Identification and Selection for Biotechnological Applications

Dr Jean-Paul Cadoret IFREMER, France

The term microalgae covers a group of single-celled photosynthetic organisms, with very dissimilar characteristics. Depending on ambient conditions such as salinity, light, nutrient concentrations, the size and appearance of microalgae can profoundly change, making their identification difficult. Information regarding the number of described species reports hundreds of thousands of species spread over the globe both in marine, freshwater or brackish water. Through their presence on the surface of oceans covering 70% of the planet, they play a major role in the global climate to transform  $CO_2$  into organic matter. Microalgae have significant growth and productivity, higher than those of land plants. This biological diversity, and an exceptional adaptability, let suppose a proportional originality in molecules and then new applications in the broad field of biotechnology. This means food, aquaculture, environment with an understanding of mechanisms and tools for toxinogenesis abatement, applications in industry with the recovery of silica, enzymes or pigments. In the future, work on the valorization of marine by-products will benefit from modern advances in scientific techniques for genome sequencing which are now



available at cost effective prices. Automated screening will offer a complementary way to search for novel molecules as well as selected recombinant molecules by genetic engineering.

#### Integrating Aquaculture, A Source of Marine Molecules

#### Dr Anna Soler-Vila

National University of Ireland Galway, Ireland

Aquaculture continues to be the world's fastest growing food producing sector, with per capita supply increasing from 0.7 kg in 1970 to 7.8 kg in 2008. This would be equivalent to an average annual growth rate of 6.6 percent. During 2008, aquatic plant production stood at 15.8 million tonnes (wet weight), representing in terms of weight an annual growth rate of almost 8 percent since 1970. Aquaculture is an essential industry to meet food demand in a world of ever increasing human population. However, rapid development of intensively fed aquaculture systems (e.g. finfish) throughout the world has often led to an array of environmental impacts such as eutrophication, decreasing local biodiversity or increasing sea floor sedimentation. Moreover, production of waste materials (e.g. offal, bones, skins and shells) from aquaculture is usually associated with expensive discharge/disposal solutions. The development of integrated multi-trophic aquaculture (IMTA) could provide a solution by creating a balanced culture system. For example the production of organic and inorganic nutrient waste from fed cultured animals (e.g. finfish, shrimp) could be a food source for extractive species (e.g. seaweed and shellfish), when cultured adjacent to each other. As such, this could lead to minimising waste outputs, while diversifying the species cultured. Furthermore, the utilization of marine waste products as a raw material for future industries (e.g. source of marine bioactives) would increase the economic value by reducing waste from aquaculture. However, development of integrated aquaculture systems will only happen in Europe, if there is a major change in attitude in political, social, and economic reasoning by seeking sustainability, long-term profitability, and responsible management of coastal waters.

# Genomic and Metagenomic Approaches to Identify Novel Bioactive Molecules with Utility in Functional Foods

<u>Prof. Fergal O'Gara</u> University College Cork, Ireland

Infections caused by food borne pathogens, such as *Salmonella* spp. *Campylobacter jejuni*, and *Escherichia coli* are a major public health problem worldwide. The problem of antimicrobial resistance is also increasingly relevant to the food industry. The microbial flora of farm animals, ultimately the source of most food pathogens,



are becoming resistant to antimicrobials, providing both a problem for the food industry and a reservoir of antimicrobial resistance that could spread into the wider community. Therefore, there is an urgent need to identify novel antibiotics to combat these food borne pathogens. The marine environment and marine sponges in particular, have been shown to be a rich source of novel bioactive compounds. Microbial symbionts in the sponge are thought to be the source of many of these bioactive compounds, but the majority of these microbes remain uncultured, making much of the microbial diversity inaccessible to traditional microbiology. Notwithstanding this we have isolated seventy-three bacterial isolates from the marine sponges Polymastia boletiformis, Axinella dissimilis and Haliclona simulans which following phylogenetic analysis of 16S rRNA gene sequences were identified as Pseudovibrio spp. Sixty-two (85%) of the isolates showed activity against at least one of the pathogens tested, including *Escherichia coli*, *Salmonella enterica* serotype Typhimurium, methicillin-resistant Staphylococcus aureus (MRSA), and Clostridium difficile. PCR screens of the Pseudovibrio isolates also revealed the presence of potential antibiotic-producing polyketide synthase genes. Genome sequencing and analysis based approach are currently being employed to identify gene clusters that direct the biosynthesis of these novel anti-food pathogen metabolites, thereby allowing the rapid identification and targeting of novel compound classes. Heterologous expression of gene clusters which will be identified will then be performed in phylogenetically related hosts to functionally express these pathways.

# Scientific Session 4 – Marine Derived Ingredients as Potential Functional Foods

#### SmPill<sup>™</sup>: Delivering Complex Innovation

#### <u>Dr Ivan Coulter</u> Sigmoid Pharma, Dublin City University, Ireland

For centuries phytochemicals have formed the basis of traditional or pharmaceutical medicines. Traditionally, most phytochemicals have been derived from plant. Marine life diversity represents and untapped mine of potential phytochemicals. The opportunities to identify and test marine derived phytochemicals is significant. In common with plant derived phytochemicals, many marine extracts are and lipophilic. Lipophilicity presents not only extraction, but also formulation issues. In the past molecules may have been selected based on solubility in water. This may have resulted in many bioactive phytochemicals being discarded with the waste water. Over the past number of decades the pharmaceutical industry has supported the development of drug delivery technologies to enhance the solubility and permeability of poorly soluble molecules. The result in an increased number of lipophilic molecules progressing through clinical trials and into the market. Today, more than 40% of all molecules on the market and in clinical development have



limited water solubility. Sigmoid has developed and integrated oral drug delivery technology, SmPill<sup>™</sup>, that not only enhances solubility, but also enables better absorption of molecules from the intestine into the bloodstream. The SmPill<sup>™</sup> technology is equally applicable to enhance the solubility, permeability and stability of marine derived bioactive molecules.

#### Strategies for the Chemical Characterisation of Marine Natural Products

#### Dr Thomas Smyth

Teasgasc Ashtown Food Research Centre, Ireland

Marine macroalgae generally contain a diverse range of chemical compounds derived from a variety of different structural groups such as carotenoids, fatty acids, polyphenols, proteins, sugars and polysaccharides. Following validation of a biological activity for a particular macroalgae species, it is imperative that the chemical structures responsible for these activities are determined. Unfortunately, in many studies this is not always the case as bioactivities are often linked to particular compound groups, in a speculative manner, without chemical characterisation. Due to the complexity involved in the purification of these compounds the characterisation of these molecules is often overlooked. In this study, a variety of different techniques used to characterise marine natural products will be demonstrated. In addition, the significant information that can be obtained from each technique will be discussed. Case studies will be used to demonstrate the chemical characterisation of two complex types of molecules found in seaweed, namely, phlorotannins from Fucus vesiculosus and laminarin polysaccharide from Laminaria digitata. Phlorotannins are usually isolated and biologically screened as a complex mixture with limited assessment of the individual structures present. A variety of chromatographic steps, along with MS and NMR, have been used in this study to characterise and quantify individual phlorotannins. The presence of Laminarin (a  $\beta$ -glucan) is generally determined using a Laminarase enzyme, which converts the polysaccharide into glucose. However, it is well known that the biological activity of Laminarin is linked to its structural characteristics and is widely known to vary according to species, geographical location and season of harvest. The techniques required for structural characterisation of the size and branching pattern of Laminarin from Irish Laminaria digitata will be demonstrated.



# Proteins and Peptides from Marine Sources: Their Potential as Functional Food Ingredients

<u>Prof. Dick FitzGerald and Dr Padraigin Harnedy</u> University of Limerick, Ireland

Marine organisms such as macroalgae, fish, shellfish and marine processing waste represent abundant sources of structurally diverse bioactive proteins, peptides and amino acids. Activities associated with blood pressure lowering, anti-oxidant, antimicrobial, anti-viral, anti-fungal, anti-protozoan, anti-coagulant, anti-diabetic, anticancer, anti-tyrosinase, opioid, immunostimulatory, calcium binding, cholesterol lowering and appetite suppression functions have been reported from these sources. А number of products containing marine-derived protein hydrolysates/peptides are currently on the market with a limited number having Foods for Specified Health Use (FOSHU) approval. As some macroalgal species found in Irish waters are rich sources of protein (10-47% (w/v dry weight) they have potential to act as valuable substrates for bioactive peptide mining. The same applies to fish and shellfish waste/by-products arising from onboard and on-shore processing plants. The applicability of macroalgae and marine processing waste as substrates for cardioprotective, anti-diabetic and antioxidant activity mining will be outlined. The in vitro and in vivo evidence for such biofunctional activities will be reviewed. Preliminary data on the in vitro cardioprotective, anti-diabetic and antioxidant activity of protein hydrolysates generated from a macroalgal species harvested off the coast of Ireland will be presented. Protein fractions derived from Palmaria palmata were subjected to digestion with food-grade proteolytic enzyme preparations. These protein hydrolysates were assessed *in vitro* for their potential cardioprotective (angiotensin converting enzyme and renin inhibitory), anti-diabetic (dipeptidyl peptidase IV inhibitory) and anti-oxidant (ferric reducing antioxidant power and oxygen radical absorbance capacity) activities. Selected Palmaria palmata protein hydrolysates display significant activity in the above bioassays. Further work is required on the bioavailability, gastrointestinal stability, sensory properties, processing and storage stability, and in vivo efficacy are needed to realise the functional food potential of these promising ingredients.

# Marine Polysaccharides – Potential Functional Foods against Obesity and Inflammation

<u>Prof. Torres Sweeney</u> University College Dublin, Ireland

Diets in developed countries are often highly calorific with inadequate content of complex carbohydrates and dietetic fibre. A spectrum of modern day diseases such as obesity and inflammatory bowel disease are strongly influenced by inadequate



dietary quality. In an attempt to halt the development of these dietary related diseases, consumers are now requesting natural functional food products that promote health and well-being. It is anticipated that the search for bioactives from marine derived macroalgae offers enormous potential considering their enormous taxonomic diversity and vast evolutionary modifications to cope with varying environmental conditions. The objectives of our research are to identify marine polysaccharides that inhibit the synthesis of inflammatory cytokines in the gastrointestinal tract and inhibit the maturation of pre-adipocytes into lipid accumulating mature adipocytes. Extracts from a variety of seaweed species and crustacean shells have been generated as part of the Nutramara consortium (see Hayes et al., for further details) and provided for bioactivity screening. Initially, all extracts are screened for bioactivity in-vitro. The *in-vitro* anti-obesity screen consists of 3T3 pre-adipocte cells that are stimulated to differentiate into mature – lipid accumulating mature adipocyte cells. Anti-obesity lead functional compound (LFC) status is granted to compounds which inhibit the cells from differentiating into mature lipid accumulating adipocytes. The *in-vitro* anti-inflammatory screen consists of Caco-2 cells that are stimulated with TNF-alpha to produce inflammatory cytokines. LFC status is granted to compounds which inhibit the pro-inflammatory cytokine profile in TNF-alpha stimulated Caco-2 cells. Compounds that identified as LFCs in-vitro are subsequently tested in ex-vivo tissue. Finally, a select few compounds will be tested for bioactivity *in-vivo*. This presentation will describe our current results, whereby extracts of the macroalgal species: Fucus serratus, Ascophyllum nodosum and Fucus vesiculosus have been identified with both antiobesity and anti-inflammatory bioactive potential.

# Anti-Cancer Properties of Irish Seaweed on Colon and Breast Carcinogenesis In Vitro

#### Dr Chris Gill

Northern Ireland Centre for Food and Health, University of Ulster, Northern Ireland

Globally, breast and colorectal cancer are among the leading causes of mortality from malignant disease in the western world. Epidemiological studies provide support for a role of diet in the aetiology of these cancers .There is a wealth of historical and anecdotal evidence for the nutritional and health benefits of eating and using seaweed, essentially based on Asian cultures and traditions where seaweed has always featured strongly in the diet and medicine. Therefore given the potential value of incorporating seaweed into the Irish diet from a chronic health perspective, we assessed extracts derived from Irish seaweeds for putative anticancer activity using colonic (HT29, HT115, CaCo2) and breast (MCF7, MCF10A MDAMB231) cell lines. The investigation utilised a series of *in vitro* systems to model key stages of carcinogenesis; namely 1) initiation, events giving rise to DNA damage; 2) promotion, events that affect cell proliferation and 3) metastasis, events that



allow the carcinoma to invade other tissues ultimately causing mortality. The results indicated that extracts had variable effects which were dependent upon the seaweed species and the lineage of the cell line tested.

#### Health Benefits from the Incorporation of Irish Seaweed in the Diet

#### Dr Emeir Mc Sorley

Northern Ireland Centre for Food and Health, University of Ulster, Northern Ireland

Seaweeds, rich in bioactive compounds, may have an important role in the prevention or modulation of disease due to their purported anti-viral, anti-cancer, anti-inflammatory and anti-microbial activity. The use of marine derived bioactives, particularly from seaweed sources, in the promotion of health through its incorporation into the habitual diet, whilst common in Asian countries, is still fairly novel in Westernised countries. Evidence from epidemiological studies in Asia have found a lower incidence of chronic disease (cancer, cardiovascular disease, diabetes and osteoporosis) as well as beneficial effects to digestive health, weight management and cognition in those who frequently consume seaweed as part of their diet. Although still an emerging research field, the data provided for the health benefits of seaweed consumption or supplementation in humans are lacking in terms of quantity and quality. In particular there is an absence of consistent, reproducible data from human studies with regard to the beneficial effects of the incorporation of Irish seaweeds directly in the diet or via the medium of its incorporation into a functional food. Within NutraMara, the anti-inflammatory, antioxidative and immune enhancing effects of two Irish seaweeds, Palmaria palmate and Laminaria digitata were investigated. In the first study pork meat produced from pigs fed a laminarin & fucoidan rich diet (see O'Grady for more details) was consumed by healthy humans in a double blind placebo controlled intervention study. The second randomised placebo controlled study supplemented the diet with a Palmaria palmata fortified bread. Although exploratory, results from these studies contribute to the literature that seaweed are important resources of novel and biologically active metabolites with valuable health promoting potential and emphasises that further research is warranted in this area.

#### Food Ingredients from the Marine – An Industrial Perspective

<u>Mr Ross Campbell</u> Cybercolloids Ltd., Ireland

CyberColloids is a private contract research to business development company specialising in polysaccharide chemistry focussed on food texture and nutrition. Over the past years CyberColloids has developed a client base from China to USA and Norway to India, developing and evaluating novel food ingredients and



technologies. One area of expertise within CyberColloids has been the use of seaweeds as a source of marine hydrocolloids, carrageenan, agar and alginates, which are used as predominately as natural thickeners and gellants in the food industry. Back in 2007, CyberColloids started to consider why Asians treat seaweeds with much more respect than people in the west. In fact should seaweeds not be referred to as sea vegetables? What is being thrown away after hydrocolloid extraction? To start answering such questions CyberColloids have been successful with several FP7 projects looking at using seaweed as a source of novel prebiotics, antioxidants and flavour molecules.

#### **Prebiotic Potential of Marine Derived Ingredients**

#### Prof. Paul Ross

#### Teasgasc Moorepark Food Research Centre, Ireland

In the past decade, considerable research has been conducted on the use of prebiotics as functional food ingredients so as to manipulate the composition of colonic microflora and improve health. By definition, a prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora, that confer benefits upon host well-being and health. The most important bacterial genera targeted for selective stimulation are the indigenous bifidobacteria and lactobacilli. Many polysaccharides from various sources have displayed prebiotic activity both in vitro and in vivo. In its natural form, the seaweed plant contains a high concentration of polysaccharides of varying structure and functionality making it a suitable choice of investigation as a prebiotic. Moreover, oligosaccharides, which have established prebiotic activity, can be produced naturally or may be derived from algal polysaccharides after chemical, physical or biochemical degradations. However, while algal polysaccharides have great potential as prebiotic agents, definitive criteria need to be achieved in order to classify a food ingredient as a prebiotic. These criteria are: 1) resistance to gastric acidity, to hydrolysis by mammalian enzymes, and to gastrointestinal absorption; 2) fermentation by intestinal microflora; and 3) selective stimulation of the growth and/or activity of those intestinal bacteria that contribute to health and well-being. The objective of this research was to investigate the prebiotic potential of different species of seaweeds (macroalgae) gathered from numerous locations along the Irish coastline. To this end, the ability of seaweed extracts to supplement the growth of bifidobacteria and lactobacilli was assessed in parallel with a well-established prebiotic, fructo-oligosaccharide. As part of our future work, structure-function studies will also be carried out to examine the effects of purified seaweed polysaccharides on gut morphology and intestinal microbiota.



#### Pork Meat Enhanced with Seaweed Derived Ingredients

<u>Dr Michael O'Grady</u> University College Cork, Ireland

Brown seaweeds are rich in polysaccharides such as laminarin, fucoidan and alginic acid. Health benefits of laminarin and fucoidan (L/F) include antitumor, antiviral, antibacterial and antioxidant activities. A brown seaweed extract containing L/F was manufactured from Laminaria digitata. Pigs (n = 30) were divided into groups of 6 and fed one of 2 diets containing L/F extract for 3 and 6 weeks pre-slaughter: Control group (basal diet); basal diet + 250 mg L/F kg feed for 3 and 6 weeks and the basal diet + 500 mg L/F kg feed for 3 and 6 weeks. *M. Longissimus dorsi* (LD) steaks were stored in modified atmosphere packs (MAP) (75% O<sub>2</sub> : 25% CO<sub>2</sub>) for up to 14 days at 4°C. The pH, surface colour (L\* lightness, a\* redness and b\* yellowness values, Minolta colorimetry) and microbiology (psychrotrophic and mesophilic counts, log CFU/g pork) of LD steaks were unaffected by dietary L/F over the 14 day storage period. In LD steaks stored in MAP, increased levels of lipid oxidation (TBARS, mg MDA/kg pork) were observed on day 11 and 14 of storage. Dietary L/F reduced levels of lipid oxidation compared to controls indicating deposition of antioxidant components in LD muscle. On day 14 of storage, the lowest (P < 0.05) levels of lipid oxidation occurred in the LD steaks from pigs fed 250 or 500 mg L/F kg feed for 3 weeks pre-slaughter. 'Visual' (pinkness, whiteness, drip, package quality, purchasing appeal and overall acceptability descriptors) and 'eating quality' (appearance, tenderness, oxidation flavour, liking of flavour and overall acceptability descriptors) sensory analyses was carried out on fresh and cooked LD steaks, respectively. Statistical analysis (APLSR) indicated that sensory scores were unaffected by dietary L/F. Overall results demonstrate potential for the incorporation of marine-derived bioactive antioxidant components into muscle foods via the animals' die



## **Poster Abstracts**

### P1

### Adaption and Optimisation of a Protein Extraction Protocol for Salmon Processing By-products

<u>Neves, A.</u>, Harnedy, P. and FitzGerald, R.J. Life Sciences Department, Schrodinger Building, University of Limerick

Large quantities of marine processing by-products such as viscera, heads, trimmings and fish frames are generated annually. Currently these components are discarded or used as fertilizers, animal feed or sold on for fish oil extraction. However, it is becoming more evident that marine processing by-products are rich reservoirs of structurally diverse biofunctional components such as proteins, peptides and amino acids<sup> $\perp$ </sup>. The by-products can contain significant levels of protein (10-23% (w/w)) and may act as substrates for the generation of bioactive peptides. Biological activities such as hypotensive, antioxidant, anti-microbial, anti-coagulant, anti-diabetic, anticancer, immunostimulatory, calcium-binding, hypocholesteremic and appetite suppression effects have been linked to these components. With the above health benefits in mind therefore the marine by-products have significant promise as functional food ingredients. The objective of this study was to examine a range of parameters associated with the extraction of protein components from fish (salmon)-based waste with the view to developing an industrially relevant protein extarction protocol. Parameters such as weight:volume ratio, number of sequential extractions, homogenization intensity and duration, pH and agitation time were assessed. The optimum conditions identified for extraction of protein from farmed salmon trimmings were a weight:volume of 1:5, homogenization for 30 sec at 16,000 1rpm/min, pH 11 with agitation for 15 min. Furthermore, it was found that a single extraction was sufficient as the quantity of protein extracted in second and third sequential extracts was low. Using the optimized protein extraction method  $305.09 \pm$ 7.38 mg protein/gram wet weight was extracted from frozen minced salmon trimmings. In addition, 32% (v/v) lipid was extracted during the procedure adopted. The extraction protocol optimized in this study will be used to extract protein from other salmon processing by-products such as liver and roe. In addition the process will be applied to the by-products associated with other fish/shellfish species.

Harnedy, P. A. & FitzGerald, R. J. (2012). Bioactive peptides from marine processing waste and shellfish: A review. *Journal of Functional Foods*, 4(1), 6-24.



# Influence of the Extraction Solvent on the DNA-protective Compounds Extracted from Brown Seaweed

<u>O'Sullivan, A.M.<sup>1</sup></u>, O'Callaghan, Y.C.<sup>1</sup>, O'Grady, M.N.<sup>1</sup>, Hayes, M.<sup>2</sup>, Kerry, J.P.<sup>1</sup> and O'Brien, N.M.<sup>1</sup>

- <sup>1</sup> School of Food and Nutritional Sciences, College of Science, Engineering and Food Science, University College Cork, Ireland.
- <sup>2</sup> Teagasc Food Research Centre Ashtown, Ireland

Seaweed contains a range of potential health promoting compounds such as polyphenols, pigments, tocopherols, peptides and vitamins. Brown seaweed is an exclusive source of phlorotannins and fucoidans<sup>1</sup>. Extraction conditions and solvent type influence the nature of compounds extracted from seaweed<sup>2</sup>. The solvents utilised in the present study (water, ethanol and methanol) are polar and can extract a range of hydrophilic compounds including phlorotannins from seaweed. The objective of the present study was to determine the potential protective effects of solvent extracts of Ascophyllum nodosum (AN), Fucus servatus (FS) and Fucus vesiculosus (FV) against hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and tert-butylhydroperoxide (tert-BOOH) -induced DNA damage in Caco-2 cells. Extracts were prepared using 100% water (H<sub>2</sub>O), 60% ethanol (EtOH), 80% ethanol (EtOH) or 60% methanol (MeOH). Caco-2 cells were pre-treated with each extract for 24 hours followed by exposure to either 50 $\mu$ M H<sub>2</sub>O<sub>2</sub> or 200 $\mu$ M tert-BOOH for 30 min. DNA damage was determined using the comet assay. Addition of H<sub>2</sub>O<sub>2</sub> and tert-BOOH increased DNA damage in Caco-2 cells from a control level of 8% to 55% and 30%, respectively. AN (60% EtOH), AN (80% EtOH), FS (100% H<sub>2</sub>O), FS (80% EtOH) and FV (60% MeOH) extracts significantly (P < 0.05) protected against *tert*-BOOH-induced DNA damage while AN (100% H<sub>2</sub>O) and AN (80% EtOH) reduced H<sub>2</sub>O<sub>2</sub>-induced DNA damage. The MeOH extracts of both AN and FS did not protect against either H<sub>2</sub>O<sub>2</sub> or tert-BOOH -induced DNA damage. Extracts from AN were the most effective at reducing oxidant-induced DNA damage.  $H_2O$  and 80% EtOH appeared to be the most effective solvent at extracting DNA-protecting compounds from seaweed.

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- 2. Shanab, S.M.M. (2007). Antioxidant and antibiotic activities of some seaweeds (Egyptian isolates). *International Journal of Agriculture and Biology*, 9, 220-225.



# Isolation and Characterisation of Bioactive Peptides with *In Vitro* Renin Inhibitory Activities from the Macroalga *Palmaria palmata*

Fitzgerald, C.<sup>1</sup>, Mora, L.<sup>1, 2</sup>, Gallagher, E.<sup>1</sup>, Prieto, J.<sup>3</sup>, Soler-Vila, A.<sup>4</sup> and Hayes, M.<sup>1</sup>

- <sup>1</sup> Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland
- <sup>2</sup> Centre for Systems and Synthetic Biology, School of Biological Sciences Royal Holloway, University of London, Egham, Surrey
- <sup>3</sup> Department of Pharmaceutical and Biological Chemistry, School of Pharmacy, University of London, 29-39 Brunswick Square, London
- <sup>4</sup> Ryan Institute, National University of Ireland Galway, Ireland

Marine macroalgae are a rich source of macronutrients such as protein and polysaccharides. The red macroalga Palmaria palmata, has a long tradition of consumption in coastal European and North American regions. P. palmata protein content can be as high as 25% of its dry weight depending on the time of year harvested. To combat hypertension, various stages of the renin angiotensin system (RAS) can be positively affected. Renin has long been recognised as the initial rate limiting enzyme in the RAS. Other stages of the RAS have recently been inhibited by bioactive peptides sourced from macroalgae. However, to date renin inhibitory peptides have yet to be discovered in macroalgae. The aim of this study is to isolate and characterise renin inhibitory peptides from P. palmata for the possible inclusion in heart healthy functional foods. Following proteolytic hydrolysis of crude P. palmata protein, reverse phase- high performance liquid chromatography (RP-HPLC) was employed to enrich for peptides with renin inhibitory activities. One RP-HPLC fraction inhibited renin activity by 58.97 % (± 1.26) at a concentration of 1mg/mL. This fraction was further characterised using nano electrospray ionization quadropole/time of flight mass spectrometry (ESI-Q/TOF MS). A number of novel peptide sequences were elucidated and the parent protein from which they were derived determined using MS in tandem with protein database searches. All sequences were confirmed using de novo sequencing. Microwave-assisted Solid Phase Peptide Synthesis (MW-SPPS) of the identified peptides was carried out on the elucidated peptides to identify the active sequences and determine their half maximal inhibitory concentration ( $IC_{50}$ ). A peptide with the sequence IRLIIVLPILMA demonstrated considerable renin inhibitory activity giving an IC<sub>50</sub> of 3.34 mM. Thus far this study shows that macroalgae such as P. palmata can be considered a source of heart healthy bioactive peptides for possible inclusion in functional food.



## P4 Screening and Analysis of Irish Seaweed Extracts for Prebiotic Potential

<u>Collins, K.</u>, Lordan, S., Stanton, C. and Ross, P. Teagasc Food Research Centre Moorepark, Ireland

Marine environments are home to approximately 13% of all living species and cover 70% of the Earth's surface. Consequently, they represent an underutilized source of bioactive compounds for the food, pharmaceutical and cosmetic industries<sup>1</sup>. Seaweeds are highly bioactive, rich in non digestible polysaccharides and have high fibre content. In addition, seaweeds have been suggested as a possible source of prebiotics<sup>2</sup>. A prebiotic is a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health. Prebiotics are resistant to degradation by human gut enzymes, hydrolysis and stomach acid<sup>3</sup> and pass through the gastrointestinal tract intact, where they can then be acted upon by the endogenous bacteria present there. The aim of this study was to investigate the prebiotic potential of 15 different seaweed species found along the Irish coast. A highthroughput bioassay was designed to screen different strains of bifidobacteria and lactobacilli for their ability to utilize complex carbohydrates. An extract concentration of 2.5 mg/ml was used with OD readings at 600 nm taken at 0, 20 and 28 h. A known prebiotic, fructo-oligosaccharide and a minimal media without extract were included as controls. The brown seaweeds, Ascophyllum nodosum, Pelvetia canaliculata, Fucus serratus, Fucus spiralis and Fucus vesiculosis, significantly (P<0.05) stimulated the growth of Bifidobacterium breve 8807, Bifidobacterium lactis Bb12 and/or Bifidobacterium longum 6205 when compared to growth in a minimal media under the same conditions. Little or no supplementary growth was seen with the red and green extracts. As a result of these findings, we intend to further characterise and analyse the bifidogenic potential of the brown seaweed extracts.

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# Marine Microalgae as Potential Sources of Nutritionally Important Polyunsaturated Fatty Acids (PUFA's)

#### Guiheneuf F. and Stengel D.

Ryan Institute, National University of Ireland Galway, Ireland

Eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) are important omega-3 polyunsaturated fatty acids (PUFA). They are known for their beneficial effects on human health, especially in the treatment of atherosclerosis, cancer, rheumatoid arthritis, psoriasis and diseases of old age, such as Alzheimer's and age-related macular degeneration. DHA are of major importance for pre- and post-natal brain and retina development. Several studies suggest that western diets are deficient in omega-3 with a ratio omega-6 to omega-3 fatty acids around 15:1. The diet recommendations for early humans evolved a ratio of approximately 1:1, and therefore an omega-3 supplementation. Today, in food processing, marine lipids are mainly obtained from fish oil. The accumulation of pollutants, odour problems, and the depletion of fisheries resources require researchers to develop other sources of production for PUFA-rich oils. Lipid production by microalgae can achieve over 50-60% of their dry weight under certain conditions, with PUFA content up to 50% of total fatty acids. Microalgae are consequently a potential alternative food ingredient to enrich the human diet in omega-3 fatty acids; they may also have superior lipid stability because of their naturally rich antioxidant carotenoid and vitamin content. As part of the Marine Functional Food Research Initiative (NutraMara), the main objectives of this work are to investigate profiles and production of PUFA (EPA & DHA) and pigments in marine microalgae, and their potential as functional food ingredients. A first screening of some bioactive compounds (PUFAs, carotenoids and phycobilins) has been undertaken using HPLC and GC-MS, before investigating their growth performance for biomass production and optimisation of particular interesting compounds. The final aim will be to supply a biomass rich in bioactive molecules with applications in functional foods.



# Assessment of Brown algal lipids and phlorotannins from Irish and Canadian Phaeophytaceae as inhibitors of enzymes important in heart health

Hayes, M.<sup>1</sup>, Soler-Vila, A.<sup>2</sup>, Mathieu, A.<sup>3</sup>

- <sup>1</sup> Teagasc Food Research Centre Ashtown, Ireland
- <sup>2</sup> Ryan Institute, National University of Ireland Galway, Ireland
- <sup>3</sup> Oceans Ltd., Newfoundland, Canada

A quarter of the world's adult population is afflicted by hypertension and this is predicted to increase to over 29 % by 2025<sup>1</sup>. The pathogenesis of high blood pressure is thought to be due to several factors including; increased activity of the renin angiotensin aldosterone system (RAAS), the sympathetic nervous system, genetic influences and increased activity of the kalikerenin system. Phlorotannins are polyphenols consisting of polymers of phloroglucinol that are found only in the brown algae. They have been reported previously to possess a myriad of bioactivities including ACE-inhibitory and antioxidant activities. Macroalgae are not rich in lipids but are a novel source of essential fatty acids, in particular PUFAs. The aim of this research was to isolate marine lipids and phlorotannins from seaweeds native to Ireland and Newfoundland and to characterise their potential heart health bioactivities using in vitro bioassays prior to chemical analysis of bioactive extracts.

The extraction of marine lipids and phlorotannins from several Irish and Newfoundland seaweed species including Fucus spiralis, Ascophyllum nodosum, Fucus vesiculosus, Alaria esculenta and Fucus serratus was carried out using accelerated solvent extraction and previously reported phlorotannin extraction methods<sup>2</sup>. Algal lipids were extracted from each seaweed species in triplicate using an automated Dionex 200 accelerated solvent extraction system and Chloroform:methanol (2:1 v/v ratio). Phlorotannins were extracted following removal of lipids using a mixture of acetone:water (7:3 v/v ratio, which contained 0.3 % ascorbic acid). All fractions were dried using nitrogen. Generated lipid and phlorotannin extracts were screened for their abilities to inhibit enzymes important in the development of artherosclerosis and heart health problems. These enzymes included platelet-activating factor acetylhydrolase, renin and Factor Xa. Further work includes purification of these extracts using GC-MS and MS techniques.

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## Application of Extracted Chitosan from Irish Shellfish Waste to Preserve Fresh-cut Vegetables: New Alternative to Chlorine

#### Khouya K.

Dublin Institute of Technology, Ireland

Seafood sales in Ireland are expected to grow by 30% over the period of 2005 to 2015; this will produce an increase between €702m to €911m for the export sales and for domestic sales from €311m to €379m. In Ireland, marine processing waste is estimated to be 64x10<sup>3</sup> tonnes annually. Currently, valorisation of wastes from seafood processing, in particular, shellfish is of great importance. Chitin is the main constituent of shellfish, it is a polymer of  $\beta(1-4)-2-N$ -acetamido-2-deoxy- $\beta$ -D-glucose. In terms of availability, chitin is considered the most widespread polysaccharide second to cellulose. In this study, chitin was extracted from brown crab (Cancer pagurus) wastes by demineralisation and deproteinisation. Response surface methodology (RSM) was applied to determine the optimum conditions (time, temperature, and concentration) for chitin extraction using lactic acid. Demineralised chitin was deproteinised using commercial protease from Bacillus licheniformis. The final chitin product was deacetylated into chitosan using sodium hydroxide (NaOH). Extracted chitosan was applied as alternative to chlorine to preserve fresh-cut carrots. Peeled carrots were immersed in different chitosan (degree of deacetylation: DDA 75%) solutions (5g/L, 10g/L, 150g/L). After processing, carrot slices were packaged in 35µm oriented polypropylene (OPP) bags (200x320mm). Processed and packaged carrots were stored at 4°C for 10days. Quality parameters: total phenols, FRAP, DPPH, texture, pH, gas headspace, and microbial load were analysed over storage. Better preservation effect was observed on samples that were treated with chitosan compared to those treated with chlorinated water.



# Antimicrobial-producing Marine *Bacillus* Isolated from Sand, Seawater and Seaweeds

Prieto, M.L.<sup>1</sup>, O'Sullivan, L.<sup>1</sup>, Tan S.P.<sup>1</sup>, McLoughlin, P.<sup>1</sup>, Hughes, H.<sup>1</sup>, O'Connor, P.M.<sup>2,3</sup>, Cotter, P.D.<sup>2,3</sup>, Lawlor, P.G.<sup>4</sup> and Gardiner, G.E.<sup>1</sup>

- <sup>1</sup> Eco-Innovation Research Centre, Waterford Institute of Technology, Waterford, Ireland
- <sup>2</sup> Teagasc Food Research Centre Moorepark, Ireland
- <sup>3</sup> Alimentary Pharmabiotic Centre, University College Cork, Ireland
- <sup>4</sup> Teagasc, Pig Development Department, Animal and Grassland Research and Innovation Centre, Moorepark, Ireland

Marine bacteria represent a potentially enormous source of novel antimicrobial compounds. However, relatively few antimicrobials produced by seaweed-derived bacteria have been fully characterised and bacteriocin production has not been reported to date. The objective of this study was to mine culturable bacteria from Irish marine sources, mainly seaweed, for antimicrobial activity and to characterise the antimicrobials produced. A total of 303 bacterial isolates were obtained from a range of Irish seaweeds, as well as sand and seawater and subsequently screened for antimicrobial activity against a range of Gram-positive and –negative pathogenic and non-pathogenic indicator bacteria. In parallel, more than 6,000 bacterial colonies from the same sources were screened against the same targets using a high throughput, combined isolation and antimicrobial detection assay. In total, fifteen isolates with antimicrobial activity against at least one of the pathogens tested (i.e. Escherichia coli, Salmonella Typhimurium, Listeria monocytogenes, methicillinresistant Staphylococcus aureus) were recovered. These were identified as either Bacillus licheniformis or pumilus by 16S rRNA gene sequencing. Characterisation of the antimicrobial compounds produced with respect to spectrum of activity, cross sensitivity, heat and pH stability and enzyme sensitivity suggested that some at least were bacteriocins. A set of PCR primers was designed for Bacillus bacteriocins and screening with these and other primers revealed that three of the marine isolates harboured genes encoding the two-peptide lantibiotic lichenicidin. The production of lichenicidin was then confirmed by mass spectrometry. This is the first definitive proof of bacteriocin production by seaweed-derived bacteria and the first report of lichenicidin production by marine bacteria. These data provide further evidence of the value of marine bacteria as a source of antimicrobial compounds.



# Large-scale Macroalgal Biomass Production for Biofuel – Overview of the EnAlgae Project

#### Edwards, M.

National University of Ireland Galway, Ireland

The Interreg IVB Strategic Initiative project Energetic Algae ('EnAlgae') aims to establish the conditions within which algal biomass production can reduce greenhouse gases of North-West Europe (NWE). This 4-year transnational project (2011-2015) will develop a network of pilot facilities for both micro- and macroalgae to combine technical information with economic, policy and LCA data. A computerbased knowledge decision support tool will be created to evaluate this information for future use by policy makers and investors across the NWE region. Standard operating procedures are being developed for the production of biomass at macroalgal pilot facilities based in Ireland, Northern Ireland and France. Production data for seaweed biomass grown at sea (on a scale of approximately one hectare per facility) will be assessed alongside environmental data for the first time. Optimum seeding, deployment and harvesting techniques will be defined, in order to maximise the biomass production for biofuel requirements. In addition, each site will form part of a demonstration network, where interested investors can attend workshops and learn state-of-the-art algal cultivation techniques that will enable them to develop seaweed farms in other areas across NWE.

#### P10

# Irish Seaweeds as a Source of Pigments and Fatty Acids as Bioactive Compounds with Potential Applications in Functional Foods

<u>Schmid, M.</u>, Guiheneuf, F. and Stengel, D. Ryan Institute, National University of Ireland Galway, Ireland

Seaweeds are considered a value source of bioactive compounds with potential uses in functional foods. For example, they are naturally rich in polysaccharides, phenolics, polyunsaturated fatty acids (PUFAs) and carotenoids which have been shown to have numerous beneficial effects on human health. However the composition and quantity of functional compounds in seaweeds can be highly variable and is dependent on abiotic conditions including temperature, light and nutrient availability in their environment. Single and synergistic effects of those factors on the chemical composition of macroalgae are still not fully understood. In order to achieve optimised levels and preferable compositions of bioactives an improved knowledge of the effects of extrinsic factors is necessary. To address this scientific gap, in this project seasonal and spatial variation in chemical composition, and thus potential bioactivity, in field-collected Irish seaweeds of commercial



interest is quantified using high pressure liquid chromatography (HPLC) and gaschromatography (GC). At the collection sites abiotic factors are monitored to establish the source of potential variability in seaweed chemical composition. The screening of bioactives in samples collected from the wild is complemented by laboratory cultures of macroalgae that aim to achieve high levels of bioactives in target species under specific culture conditions. This study will provide a comprehensive overview of the bioactive profiles of key species and a first detailed inventory of polyunsaturated fatty acids in Irish seaweeds. Initial PUFA and pigment profiles are presented here. The results of this study can be used in the future by industry to select specific harvesting sites and seasons, and will provide a baseline for future optimised production of species of commercial interest in culture.

## P11

#### Extraction and Enrichment of Bioactive Phlorotannins in Irish seaweeds

Tierney, M.S.<sup>1, 3</sup>, Smyth, T.<sup>1</sup>, Hayes, M.<sup>1</sup>, Soler-Vila, A.<sup>2</sup>, Croft, A.K.<sup>3</sup> and Brunton, N.<sup>4</sup>

- <sup>1</sup> Teagasc Food Research Centre Ashtown, Ireland
- <sup>2</sup> Ryan Institute, National University of Ireland Galway, Ireland
- <sup>3</sup> School of Chemistry, University of Wales Bangor, Wales
- <sup>4</sup> School of Agriculture and Food Science, University College Dublin, Ireland

Phlorotannins, marine polyphenols consisting of polymers of phloroglucinol, are found in abundance in many species of brown seaweed. Phlorotannins are the fundamental cell wall components of brown seaweed and have been suggested to possess secondary roles in response to changes in UV radiation and nutrient availability to minimize oxidative stress. Phlorotannins have been reported to have a range of bio-activities, including anti-hypertensive and antioxidant, and therefore could be applied as therapeutics in the future. Characterisation of the complexity of phlorotannins from Irish seaweed species to date has been limited. This research involved the extraction of Irish seaweed species, namely brown Fucus spiralis, Pelvetia canaliculata, Ascophyllum nodosum and green Ulva intestinalis using the food-friendly solvents of water and aqueous ethanol. Crude extracts possessing high phenolic content and *in vitro* antioxidant activity were enriched in a food-friendly manner using molecular weight cut-off (MWCO) dialysis to generate fractions less than 3.5 kDa, 3.5-100 kDa and greater than 100 kDa in size. Aqueous ethanol extracts were partitioned into a hydrophilic and hydrophobic portion prior to dialysis. Reverse-phase flash chromatography was employed to purify the low molecular weight (LMW) phlorotannins in the less than 3.5 kDa MWCO fractions for further antioxidant activity testing. Brown seaweed species displayed significantly higher phenolic contents, radical scavenging abilities and ferric reducing power compared to the green Ulva species. Following MWCO dialysis of these brown seaweed extracts, most of the 3.5 kDa-100 kDa fractions showed significantly



increased phenolic content and antioxidant activities relative to their respective crude extracts, highlighting the abundance of phlorotannins in this size range. Purified LMW phlorotannins from less than 3.5 kDa MWCO fraction also exhibited enhanced antioxidant activity. Further analysis is currently being carried out to characterise the individual phlorotannin structures present.

## P12a

# Preliminary Investigation of the Structural Characteristics of Fucoidan Isolated from Irish Laminaria digitata

<u>Heffernan, N.<sup>1</sup></u>, Smyth, T.<sup>1</sup>, Soler-Vila, A.<sup>2</sup>, McLoughlin, P.<sup>1</sup>, Fitzgerald, D.<sup>3</sup>, Brunton, N.<sup>4</sup>

- <sup>1</sup> Teagasc Food Research Centre Ashtown, Ireland
- <sup>2</sup> National University of Ireland, Galway
- <sup>3</sup> University College Dublin, Ireland
- <sup>4</sup> University of Limerick, Ireland

Fucoidans are highly sulphated cell wall polysaccharides found mainly in brown algae. The main skeleton of fucoidans consists of  $\alpha$ -1, 3-linked sulphated L-fucose. A repeating sequence of alternating  $\alpha$  (1 $\rightarrow$ 3) and  $\alpha$  (1 $\rightarrow$ 4) glycosidic bonds is also possible. Recently algal fucoidans have attracted interest due to their numerous biological properties including anticoagulant, antithrombotic, anti-inflammatory, antitumour, contraceptive and antiviral activity. The biological activity of fucoidan has been linked to its structure and is known to vary according to species, geographical location and season of harvest. Fucoidans have potential applications in medicine and functional food products and thus their production, structure and properties are of great interest. The present study is an investigation of the structural characteristics of high molecular weight fucoidan extracted and purified from Laminaria digitata harvested from Irish shores. Molecular weight was determined using size exclusion chromatography with Evaporative Light Scattering Detection (ELSD). Monosaccharide composition was determined using (GCMS) Gas Chromatography Mass Spectrometry after hydrolysis and permethylation of the polysaccharide. Linkage positions were determined by permethylation of the intact fucoidan which was then hydrolyzed and the free hydroxyl group's peracetylated. Ratios between linkage positions were determined using NMR analysis providing vital information on the structural features of fucoidans present in Irish Laminaria digitata.



## P12b Bioactivity Guided Isolation of Phlorotannins and UPLC-MS Profiling

Heffernan, N.<sup>1</sup>, Smyth, T.<sup>1</sup>, Soler-Vila, A.<sup>2</sup>, Fitzgerald, D.<sup>3</sup>, Brunton, N.<sup>4</sup>

- <sup>1</sup> Teagasc Food Research Centre Ashtown, Ireland
- <sup>2</sup> National University of Ireland Galway, Ireland
- <sup>3</sup> University of Limerick, Ireland
- <sup>4</sup> University College Dublin, Ireland

Antioxidants play an important role in biological systems including defence against oxidative damage and participation in major signalling pathways of the cell. Irish origin macroalgae are largely unexplored as a source of antioxidants despite the fact they are know to produce them as a coping mechanism in stressful marine environments. Phlorotannins are a type of tannin found only in brown macroalgae and have been reported to exhibit good antioxidant potential. To date however, Irish origin phlororannins have yet to be profiled to investigate their complexity and to evaluate their isomeric content. In this study the antioxidant potential and phlorotannin content of brown algae Fucus serratus harvested from Irish shores was investigated. Extracts prepared using food friendly solvent ( cold water, hot water and ethanol/water) were fractionated using dialysis tubing with molecular weight cut offs of 3.5KDa, 8KDa and 100KDa. The in-vitro antioxidant activity of these molecular weight fractions was measured using the, 2-Diphenyl-1-Picrylhydrazyl (DPPH) Ferric Reducing Antioxidant Power (FRAP) assays. Levels of total phenols in these fractions were also measured using the Folin-Ciocalteu assay. Fractions exhibiting good antioxidant potential and a high phenol content were then further fractionated using a reverse phase flash chromatography system. These fractions were then analysed using UPLC-MS to identify the phlorotannins and to also provide information of the complexity and isomeric content of these phlorotannins.

## P13

# Supplementation of Salmon Diets with Red Macroalgae (*Palmaria palmata*): Effects on Fresh Salmon Fillet Quality

Moroney, N.C.<sup>1</sup>, O'Grady, M.N.<sup>1</sup>, Wan, A.<sup>2</sup>, Soler-Vila, A.<sup>2</sup>, Johnson, M.<sup>2</sup>, Fitzgerald, R.<sup>2</sup> and Kerry, J.P.<sup>1</sup>

- <sup>1</sup> Food Packaging Group,College of Science, Engineering and Food Science, University College Cork, Ireland
- <sup>2</sup> Ryan Institute, National University of Ireland Galway, Ireland

Red macroalgae (seaweed) contains a range of bioactive compounds with multifunctional bioactivities (e.g. antioxidant carotenoids). Phycoerythrin is a major light



harvesting protein pigment present in red seaweed (*Palmaria palmata*)<sup>1</sup>. The aim of this study was to assess the effect of supplementation of salmon diets with *Palmaria* palmata (PP) on the quality and shelf-life of fresh salmon fillets stored in modified atmosphere packs (MAP). Salmon were fed control, positive control (commercial astaxanthin) and PP-based diets (5%, 10% and 15% PP inclusion levels) for 12 weeks pre-slaughter. Salmon fillets were stored in MAP (60% N<sub>2</sub> : 40% CO<sub>2</sub>) for up to 15 days at 4°C. The surface colour (CIE 'L\*' lightness, '+ a\*' redness, '- a\*' greenness and ' $b^{*'}$  yellowness values) of salmon fillets was measured using Minolta colorimetry. Negative '- $a^*$ ' values as a result of PP addition were observed compared to positive controls. Surface 'b\*' values increased with increasing PP level. Levels of lipid oxidation (TBARS, mg MDA/kg muscle/salmon) and microbiology (psychrotrophic counts, log CFU/g salmon) of salmon fillets were unaffected by dietary PP. In fresh salmon fillets, 'visual' sensory analysis descriptor scores for pinkness, purchasing appeal and overall acceptability were lower than positive controls. In general, 'eating quality' sensory descriptors (texture, odour, oxidation flavour and overall acceptability) in cooked salmon fillets (180°C for 12 min) were not influenced by dietary PP. Additional experimental work (iron-induced oxidation of salmon fillet homogenates) will investigate muscle deposition of bioactive antioxidant components as a result of PP inclusion in salmon diets. Future research will examine the functionality of different macroalgae species in salmon diets and their effects on salmon fillet quality and shelf-life.

Harnedy P.A. and Fitzgerald R.J. (2011). Bioactive proteins, peptides, and amino acids from macroalgae. Journal of Phycology, 47, 218-232.

#### P14

#### Evaluation of Irish seaweed Extracts as Inhibitors of Starch-digestive Enzymes

#### <u>Lordan</u>, S., Stanton, C. and Ross, P. Teagasc Food Research Centre Moorepark, Ireland

Hydrolysis of dietary starch is the major source of glucose in the blood and  $\alpha$ amylase and  $\alpha$ -glucosidase are the key enzymes involved in starch breakdown and intestinal absorption, respectively. It is believed that inhibition of these enzymes can significantly decrease the postprandial increase of blood glucose level after a mixed carbohydrate diet and therefore can be an important strategy in the management of hyperglycaemia linked to type II diabetes<sup>1</sup>. As well as being sources of polyunsaturated fatty acids, dietary fibre, minerals and certain vitamins, seaweeds contain appreciable amounts of polyphenols which have been suggested to influence responses relevant to diabetes through inhibition of starch-digestive enzymes. The objective of this study was to compare cold water and ethanol extracts of different seaweeds for their ability to inhibit the activity of  $\alpha$ -amylase and  $\alpha$ -glucosidase. To this end, 15 cold water extracts and 15 ethanol extracts of brown, red and green



seaweeds were tested using a high-throughput bioassay. Both the cold water and ethanol extracts of the brown seaweeds, *Ascophyllum nodosum, Fucus serratus, Fucus vesiculosis* and *Pelvetia canaliculata*, significantly (P<0.01) inhibited  $\alpha$ -amylase and  $\alpha$ -glucosidase activity. *Himanthalia elongata* and *Laminaria digitata* cold water extracts also had a significant (P<0.01) inhibitory effect on  $\alpha$ -glucosidase, while the ethanol equivalent had no effect. Moreover, only the ethanol extract of *Fucus spiralis* significantly (P<0.01) decreased the activity of the starch-digestive enzymes. These findings have shown that both the type of seaweed and the method of extraction are determining factors in the ability of the extract to inhibit pancreatic  $\alpha$ -amylase and intestinal  $\alpha$ -glucosidase. In conclusion, further investigations into the use of seaweed extracts, particularly brown seaweed extracts, as a potential therapy for postprandial hyperglycaemia are warranted.

Kwon, Y.I., Apostolidis, E., Shetty, K. (2008). *In vitro* studies of eggplant (*Solanum melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. *Bioresour. Technol.* 99: 2981-2988.

## P15

## Anticancer Activity of Raw and Digested Irish Seaweed Extracts on Colorectal Cancer Cell Lines *In Vitro*

<u>Nitecki, S.S.<sup>1</sup></u>, Allsopp, P.J.<sup>1</sup>, Brown, E.M.<sup>1</sup>, Magee, P.J.<sup>1</sup>, McSorley, E.M.<sup>1</sup>, Soler-Vila, A.<sup>2</sup>, Strain, J.J.<sup>1</sup>, Gill, C.I.R<sup>1</sup>

- <sup>1</sup> Northern Ireland Centre for Food and Health, University of Ulster, Northern Ireland
- <sup>2</sup> Ryan Institute, National University of Ireland Galway, Ireland

Purified seaweed compounds have been shown to reduce cancer cell proliferation and induce apoptosis *in vitro*<sup>1,2</sup>, however the anti-cancer characteristics of raw or colon available crude seaweed have not been well studied. The aim of this study is to investigate the anti-proliferative activity of crude and digested extracts from Irish seaweed on colorectal cancer cell lines in vitro. Seaweed specimens, harvested from the coast of Galway, included *Ascophyllum nodosum, Laminaria digitata, Palmaria palmata* and *Ulva intestinalis*. A "crude" homogenate of fresh seaweed was prepared in addition to a "digested" fraction, representing colon available material after *in vitro* simulation of human gastro-intestinal digestion. The anti-proliferative activity of the extracts was tested *in vitro* using three colorectal cancer cell lines: HT29 early adenocarcinoma, CaCo2 adenocarcinoma and HT115 metastatic carcinoma. An MTT cytotoxicity assay was used to establish a sub-lethal dose range of seaweed treatments on all cell lines. The sub-cytotoxic treatments were further tested using a 7-day cell growth experiment to estimate the effect of the treatments on cell growth and proliferation. It was shown that both crude and digested extracts



were toxic to cells at doses greater than 20mg/ml dry weight. The sub-cytotoxic treatments of all seaweed extracts, both crude and digested, exhibited antiproliferative activity on tested cell lines in a dose dependent manner. The strongest response was observed with *Palmaria palmata* and *Laminaria digitata* seaweed extracts, which significantly inhibited proliferation of all cell lines at doses as low as 3-6mg DW/ml. All seaweed extracts had a strong antiproliferative activity on colorectal cancer cell lines *in vitro*. The mechanisms of the seaweed induced cell death e.g. inhibition of cell cycle events, induction of apoptosis or necrosis pathways are currently being investigated.

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- 2. Smit, A.J. Medicinal and pharmaceutical uses of seaweed natural products: A review. *Journal of Applied Phycology*. 2004;16(4):245-62.

#### P16

## Seasonal and Geographical Variation of Total Nitrogen, Non-Protein Nitrogen and Protein Nitrogen Content of Macroalgae Native to the Irish Coastline

<u>Flaherty, T.</u>, Harnedy, P. and FitzGerald, R.J. University of Limerick, Ireland

To date there is no clearly defined method for the determination of the level of protein nitrogen (PN) or non-protein nitrogen (NPN) in macroalgae. Traditional the kjeldahl method is used for quantification of total nitrogen (TN) in macroalgae. This method does not take into account that TN in macroalgae comes from both protein and non-protein sources. Research has shown that macroalgal TN levels can vary with season and geographical location and because this does not distinguish between PN and NPN it is difficult to determine which one or if both parameters vary with season and geographical location. The objective of this study was to develop a method for extraction and quantification of NPN in macroalgae and to use this method to determine if the NPN and PN levels vary with season and geographical location. For this study 5 macroalgal species, Palmaria palmata, Ulva rigida, Fucus serratus, Laminaria digitata and Ascophylum nodosum, were harvested from two different locations, Spiddal in Co. Galway and Finavarra, Co Clare, in February, April, July and October 2011. Preliminary results show that Palmaria palmata NPN and PN levels vary to a large extent from one season to the next with  $15.97 \pm 2.63$  mg of protein nitrogen/g dry weight recorded in July and  $35.63 \pm 0.23$ mg of protein nitrogen/g dry weight recorded in February. The same trend was seen with the NPN where  $1.85 \pm 0.30$  and  $7.07 \pm 0.26$  mg of non-protein nitrogen/g dry weight was recorded in July and February, respectively. Furthermore, geographical variations in NPN and PN levels were seen in some seasons but not in others.



Harnedy, P. A., & FitzGerald, R. J. (2012). Bioactive peptides from marine processing waste and shellfish: A review. *Journal of Functional Foods*, 4(1), 6-24.

## P17

#### Valorisation of the Components of the Protein Fraction from Marine Molluscs Shells: Dermo-cosmetic Applications

Latire, T.<sup>1,2</sup>, Legendre, F.<sup>2</sup>, Bigot, N.<sup>2</sup>, Marin, F.<sup>3</sup>, Serpentini, A.<sup>1</sup>, Galera, P.<sup>2</sup>, Lebel,  $J.M^{1}$ 

- <sup>1</sup> Université de Caen Basse-Normandie, Caen, France
- <sup>2</sup> Microenvironnement cellulaire et pathologie, Faculté de Médecine, Université de Caen Basse-Normandie, Caen, France
- <sup>3</sup> Biogeosciences, Universite de Bourgogne, Dijon, France

Mollusc shells are complex structures composed mainly of calcium carbonate (CaCO<sub>3</sub>). This one is represented by various polymorphs (aragonite, calcite) whose synthesis is controlled by shell matrix proteins tightly associated, which only represent 0.2 to 5% of shell constituent. For many years, the biological effects of shell matrix proteins on vertebrate cell models have been demonstrated. Applications are diverse with opportunities in different bio-medical domains such as bone repair and osteoinduction, in dermatology (effect on tissue repair), or nutraceutical. In addition, an important source of shell by-products is available in France as a result of shellfish farming developed. In this context this study aims to evaluate the effects of protein fraction extracted from reared marine mollusc shells on human dermal fibroblasts, to add value to these by-products. To extract shell matrix proteins, three protocols were used. In the first one, shell powder is decalcified using acetic acid, which allows recovering of soluble and insoluble fractions in acetic acid (known respectively as AIM and ASM). In the second protocol, proteins are extracted directly in distilled water, which allows recovering of a water soluble matrix (WSM) fraction. In the third protocol, the protein extract is obtained by using a calcium chelating acid, ethylene diamine tetra acetic acid (EDTA). For applications in dermo-cosmetic (anti-aging strategy), we tested the effect of these different fractions on the viability and proliferation (using metabolic activity test, WST-1) of human dermal fibroblasts in primary culture. In addition, we investigated the ability of these fractions to induce synthesis of extracellular matrix components such as type I collagen in order to analyze the differentiation effects of the fractions tested on these cells in culture. Initially, for each fraction, six concentrations (0 to 1000 µg/mL) and four exposure time (24, 48,72 and 96h) were tested. Our results show that acid-soluble, acid-insoluble and water soluble matrices from three shells (shell A, B and C) modulate the metabolic activity after an incubation of 72h at 1000  $\mu$ g/mL and the type I collagen synthesis after an incubation of 96h at 500  $\mu$ g/mL. This study suggests that protein fractions modulate human fibroblasts metabolism and that shell by-products potentially could be used as dermo-cosmetic component.



# Extraction of Polyphenols from *Ascophyllum nodosum* Using Supercritical Carbon Dioxide

MacArtain, P.<sup>1</sup>, McKennedy, J.<sup>1</sup> and Matthews S.<sup>2</sup>

- <sup>1</sup> Dundalk Institute of Technology, Ireland
- <sup>2</sup> SCF Processing Ltd., Drogheda, Ireland

The use of marine biomass as a resource for energy production has garnered increasing interest in recent years. The benefits of non-competition with terrestrial resources are somewhat offset by the relative difficulty in obtaining the biomaterial. Justification of the exploitation of marine biomass as an energy source will depend on the conversion efficiency ad environmental impact of the transformation to usable energy. The anaerobic digestion of macroalgae is of interest in this context in terms of it's ability to produce biogas from it's carbohydrate content. However, the presence of polyphenols in the macroalgal matrix is inhibitory to the microbes involved in the anaerobic digestion process. With this in mind, the extraction of polyphenols from seaweed was investigated using supercritical carbon dioxide. A Supercritical Fluid is a substance which is above it's critical temperature and it's critical pressure and thus is neither a liquid or a gas but displays the properties of a fluid. Under these conditions it is possible to manipulate a material in order to extract a component from it or to impregnate it with an additional compound. In this work varying sub and supercritical conditions are investigated in order to optimise the extraction of polyphenols from Ascophyllum nodosum. A Folin Ciocalteu method was used to measure the total polyphenols extracted.

## P19

# Extracts of *Ascophyllum nodosum* have Anti-inflammatory Activity in Human Intestinal Epithelial-like (Caco-2) Cells

<u>Bojlul Bahar</u><sup>1,3</sup>, John V. O'Doherty<sup>1</sup>, Maria Hayes<sup>2</sup>, Torres Sweeney<sup>3</sup>

- <sup>1</sup> School of Agriculture and Food Science, University College Dublin, Ireland
- <sup>2</sup> Teagasc Food Research Centre Ashtown, Ireland
- <sup>3</sup> School of Veterinary Medicine, University College Dublin, Ireland

Brown seaweeds, a rich source of bioactive compounds, are demonstrated to have numerous health benefits including immunomodulatory bioactivities. This experiment was conducted to evaluate the effect of two extracts of *Ascophyllum nodosum* in suppressing the pro-inflammatory response induced by tumor necrosis factor -alpha (TNF-alpha) treatment in human intestinal epithelial-like (Caco-2) cells.



Pro-inflammatory response was induced by treating fully differentiated Caco-2 cells with DMEM containing TNF-alpha (10 ng/ml). Then, two extracts of Ascophyllum nodosum (Extract 1: 80% ethanol extract of ISCG 0023 and Extract 2: ASEM17 of ISCG 0023) were introduced to attenuate the pro-inflammatory response. The intracellular activity of NF-kB and secretion of interleukin 8 (IL-8) in the media was measured using a luciferase activation assay and an ELISA, respectively. The intracellular NF-kB activity as induced by the TNF-alpha treatment in Caco-2 cells was significantly inhibited by the presence of Ascophyllum nodosum extract 1 (P<0.001) and extract 2 (P<0.001). Relative to the control (TNF-alpha alone), treatment with 0.5, 1.0 and 2.0 mg/ml of the extract resulted in 19%, 17% and 13% NF-kB activity for extract 1 and 36%, 20% and 17% NF-kB activity for extract 2, respectively. TNF-alpha induced IL-8 secretion in the media was also significantly inhibited by extract 1 (P<0.001) and extract 2 (P<0.001). While the treatment of cells with TNF-alpha alone produced 138.8 pg/ml IL-8, treatment with 0.5, 1.0 and 2.0 mg/ml of the extract in presence of TNFa produced 15.5, 11.0 and 4.3 pg/ml IL-8 for extract 1 and 21.5, 18.4 and 9.0 pg/ml IL-8 for extract 2, respectively. It is concluded that the extract of Ascophyllum nodosum have potential to suppress the pro-inflammatory response induced by TNF-alpha in human intestinal epithelial cell.

## P20

## An investigation of the anti-genotoxicity of Irish seaweed species with high antioxidant potential in a breast cancer cell line.

<u>W.M. Crowe</u><sup>1</sup>, P.J. Allsopp<sup>1</sup>, E.M. McSorley<sup>1</sup>, C.I.R. Gill<sup>1</sup> J.J.Strain<sup>1</sup>. T. Smyth<sup>2</sup>, A. Soler<sup>3</sup>, P.J. Magee<sup>1</sup>.

- <sup>1</sup> Northern Ireland Centre for Food and Health, University of Ulster, Northern Ireland
- <sup>2</sup> Teagasc Food Research Centre Ashtown, Ireland
- <sup>3</sup> National University of Ireland, Galway, Ireland

Brown macroalgae (seaweeds) are proposed to contain an extensive catalogue of bioactive compounds, albeit their potential, especially that of Irish seaweeds, has not been fully investigated. There is accumulating evidence that antioxidant rich foods offer geno-protective effects and may significantly contribute to the prevention of cancer through reducing cellular DNA damage<sup>1</sup>. Therefore, Irish seaweeds with a high antioxidant capacity may have a role in the prevention of cancer through incorporation into the diet and warrants further investigation<sup>2</sup>. The aim of this study was to assess the anti-genotoxicity of antioxidant rich seaweeds extracts in a breast cancer cell model (MCF-7). Extracts from the brown seaweeds *Ascopyllum nodosum, Fucus serratus* and *Fucus vesiculosis* harvested from the West Coast of Ireland were selected based on their high antioxidant capacity as determined by FRAP. All extracts were investigated for their anti-genotoxic activity



on the breast cancer cell line MCF-7, using the single cell gel electrophoresis assay (comet assay). Ascophyllum nodosum exhibited a slight non-significant reduction (8%) in H<sub>2</sub>O<sub>2</sub> induced DNA damage at 200  $\mu$ g/ml, with no effect at the lower concentrations. Fucus vesiculosis (6 – 50 mg/ml) showed a consistent non-significant reduction (approx 8%) in DNA damage indicating a protective effect against DNA damage was apparent even at lower concentrations. Although our results were non-significant they have identified Fucus vesiculosis to be the best seaweed candidate for geno-protective effects albeit further research is required to truly elucidate this effect.

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## P21

## Investigation into the anti-genotoxic potential of *Fucus vesiculosis* on human colorectal adenocarcinoma cells (HT29)

Hegan, V.G.<sup>1</sup>, Brown, E.M.<sup>1</sup>, Magee, P.J.<sup>1</sup>, McSorley E.M.<sup>1</sup>, Strain, J.J.<sup>1</sup>, Smyth, T.<sup>2</sup>, Soler-Vila, A.<sup>3</sup>, Gill, C.I.R.<sup>1</sup>

- <sup>1</sup> Northern Ireland Centre for Food and Health, University of Ulster, Northern Ireland
- <sup>2</sup> Teagasc Food Research Centre Ashtown, Ireland
- <sup>3</sup> National University of Ireland, Galway, Ireland

Increasingly more research has been published on the health benefits of macroalgae which include anti-cancer<sup>1,2</sup>. One factor associated with cancer and other chronic disease development is high levels of cellular DNA damage induced through oxidative insult by reactive oxygen species; often by products of cellular metabolism such as hydrogen peroxide  $(H_2O_2)^1$ . The aim of this study is to investigate the influence of ethanolic *Fucus vesiculosis* seaweed extract in preventing the accumulation of colonocyte DNA damage, thereby reducing risk of colon cancer development. Human colorectal adenocarcinoma cells (HT29) were grown and treated with *Fucus vesiculosis* at a range of different concentrations (6 – 400µg/ml) for 24 hours before undergoing single cell electrophoresis (comet assay) allowing detection of both single and double strand breaks within the double helix<sup>3</sup>. The comet assay was used to investigate the antigenotixic effect of the extract following oxidative assault by H<sub>2</sub>O<sub>2</sub> and was also used to determining the influence of the extract on basal genotoxicity. The analysis was performed using Komet 5 software.



Comparison between the mean data for this species allows for assessment of efficacy and optimal dose range. Treatment with *Fucus vesiculosis* showed no genotoxicity at any of the concentrations tested and showed a reduction in DNA damage ranging from 14-30%, at 6, 12 and 25  $\mu$ g/ml, however these genoprotective effects were not significant (ANOVA and Dunnett T-test with *p* accepted at < 0.05.). These preliminary results would indicate that *Fucus vesiculosis* may possess some genoprotective qualities, although to truly assess its potential, further research is required to fully elucidate this finding.

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### P22

# Screening and analysis of Irish seaweed extracts for toxicity and potential anticancer activity

<u>Allsopp, P.J.<sup>1</sup></u>, Brown, E.M.<sup>1</sup>, Nitecki, S.S.<sup>1</sup>, McSorley, E.M.<sup>1</sup>, Strain, J.J.<sup>1</sup>, Soler-Vila, A.<sup>2</sup>, Smyth, T.<sup>3</sup>, Gill, C.I.R.<sup>1</sup>, Magee, P.J.<sup>1</sup>

- <sup>1</sup> Northern Ireland Centre for Food and Health, University of Ulster, Northern Ireland
- <sup>2</sup> National University of Ireland, Galway, Ireland
- <sup>3</sup> Teagasc Food Research Centre Ashtown, Ireland

Over the past few decades the marine environment has been receiving increasing interest as an exploitable source of novel bioactive compounds by the pharmaceutical and functional food industry<sup>1</sup>. Attention has been drawn to health benefits of seaweeds with accumulating evidence from observational studies and invitro research to highlight an association between the dietary consumption of seaweed and a reduced risk of a range of diseases including cancer<sup>2, 3</sup>. This study aims to explore the toxicity and putative anticancer activity of a collection of sustainable Irish seaweeds (*Ascophyllum nodosum, Laminaria digitata, Fucus vesiculosis, Fucus serratus, Chondus crispus, Palmaria palmate and Ulva intestinalis*) using a range of breast cancer cell lines that are representative of the different stages of cancer progression (MCF10a, MCF-7 and MDA-MB-231). Using the established MTT assay alongside propidium Iodide cell cycle analysis, we aim to investigate the anti-proliferative activity of these sustainable seaweed species using



4 different extraction methods (cold water extraction, hot water extraction, 80% ethanol extract and 70% Methanol extract. Significant reductions of MCF10a cell viability were noted at 400µg/ml for the ethanolic extract of *Fucus vesiculosis* and *Ulva intestinalis* which was reflected by a significant increase in subGO fraction in the cell cycle. The potent cytotoxic effect of the ethanolic extracts of *Fucus vesiculosis* and *Ulva intestinalis* and future work to determine the mechanism of cell death will provide important toxicological information with important to the role of these seaweeds in the food industry.

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