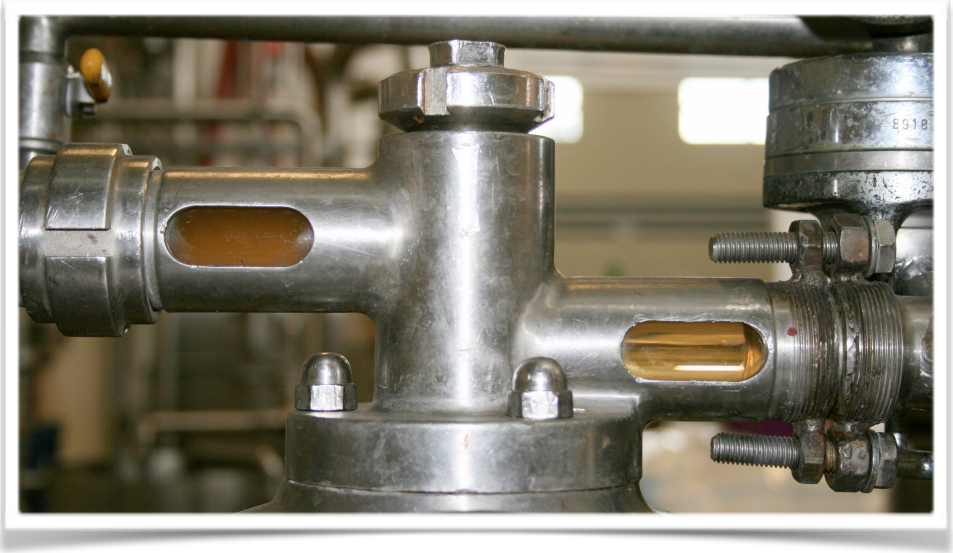
The SWAFAX Project



Seaweed derived anti-inflammatory agents and antioxidants

Many seaweeds contain a wide range of bioactive compounds, including polyphenols, with potential antioxidant and anti- inflammatory activity. Although polyphenols from land plants are widely used as functional food ingredients and food supplements, seaweed sources have been little studied or exploited. The current project was therefore designed to address a commercial opportunity to obtain bioactive polyphenols from seaweeds for application in food and health & wellness products. The consortium comprised 3 SME partners from the seaweed and health & wellbeing sectors and 3 centres of excellence for health & nutrition research and biopolymer & polysaccharide chemistry.

Brief overview of the project

During the initial stages of the project seaweed polyphenol extracts (SPEs) were prepared from a range of European seaweeds including the brown seaweed *Ascophyllum nodosum*. The antioxidant and anti-inflammatory activities of these extracts were then investigated *in vitro*. One food grade extract was also prepared. This extract was assessed *in vivo* in humans in (i) a short-term dietary intervention that addressed bioavailability and metabolism of the component polyphenols and (ii) a 24 week dietary intervention study, the end points of which included prevention of oxidative DNA damage in lymphocytes and modulation of anti-inflammatory and pro-inflammatory cytokines in plasma.

Hebridean Seaweed Co., Scotland.

The largest seaweed processor in UK, manufacturing products for animal feed, soil enhancement and nutraceuticals.

www.hebrideanseaweed.co.uk

Marigot Ltd., Ireland.

Specialists in the development and manufacture of human supplements and animal feed based on calcareous marine algae.

www.aquamin.com

Mesosystem S.A., Portugal.

Market leaders in the manufacture of high quality products and equipment for medical cosmetics, beauty, health & wellness.

www.mesosystem.com

CyberColloids' role in the project

CyberColloids were responsible for the development of novel extraction processes to produce SPEs at laboratory scale, for the demonstration of scaled up production at semi-commercial scale and for the tech transfer element of the project in which the new project knowledge was transferred to the SME partners.

Key project findings

The SWAFAX study was the first to investigate the gastrointestinal modifications of seaweed phlorotannins from *A. nodosum in vitro* and their bioavailability and effect on inflammatory markers *in vivo* in humans.

***Ascophyllum nodosum* seaweed**

In the upper GI tract, dietary polyphenols act as substrates for a number of enzymes, they are subjected to extensive metabolism by glucosidase enzymes, phase I enzymes (hydrolysing and oxidising) and phase II enzymes (conjugating and detoxifying) to yield glucuronides and sulphate derivatives. Further transformations can also occur in the colon, where gut microbiota breakdown the complex polyphenolic structures to smaller units, which may also be absorbed and further metabolised.

In the SWAFAX study, gastrointestinal digestion and fermentation of SPEs was simulated *in vitro* using a model to mimic gastric and ileal digestion and colonic microbial fermentation, followed by dialysis to simulate absorption into the circulation. LC-MS was used for a comparative characterisation of the end products (phlorotannin metabolites). A number of phlorotannin oligomers were identified after simulated digestion and colonic fermentation and seven metabolites corresponding to *in vitro*-absorbed metabolites were also identified.

The fate of polyphenols in the human body is typically assessed by measuring plasma concentrations and/or urinary excretion of polyphenols and their metabolites at certain time points following intake. Samples may or may not be further treated with different enzymes to release conjugate moieties (*e.g.* glucuronic acid and sulphate groups).

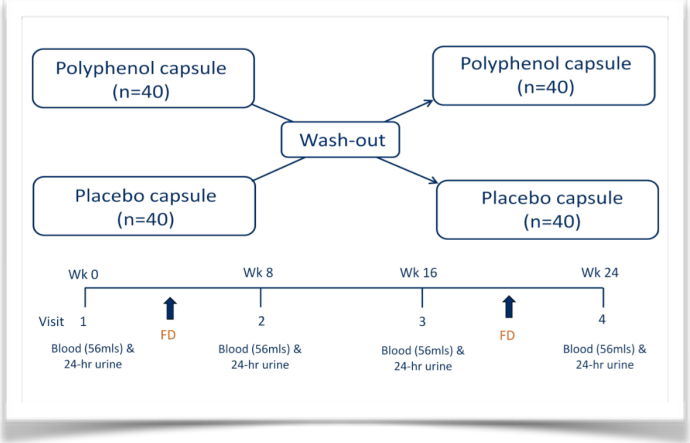
Levels of phenolic compounds and conjugated metabolites can increase rapidly after intake. Evidence in plasma and urine at time points 1-2h typically indicates absorption in the small intestine whilst at 4–8h in plasma and 8–24h in urine indicates metabolism in the large intestine. In the SWAFAX study, the majority of phlorotannin metabolites were found in plasma and urine samples collected 6–24h after intake, indicating limited small intestinal absorption followed by gut microbial metabolism in the large intestine. Thus reflecting the HMW range of phlorotannins that were present in the SPE.

Urine and plasma samples contained a variety of metabolites that could be attributed to both unconjugated and conjugated metabolites (glucuronides and/or sulphates). Using LC-MS, phlorotannin oligomers were detected in urine samples, two of which had been previously identified in the *in vitro*- digested samples. This represents the first demonstration for seaweed derived polyphenols in humans.

This project received funding from the European Seventh Framework Program managed by REA – Research Executive Agency FP/2007-2013

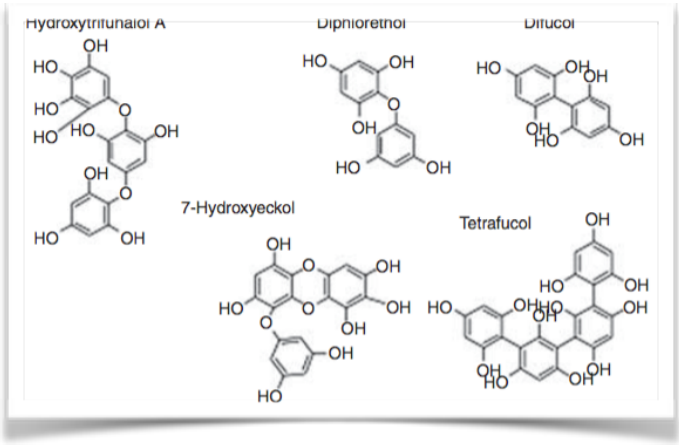
Substantial differences in the patterns of polyphenol metabolites were seen between individual volunteers. Such differences have been observed for other polyphenols and have been attributed to differences in gut microbiota composition and the expression of metabolic enzymes. These difference were also observed in the 24 week dietary intervention study that was run to investigate the longer term effects of the SPE on oxidative stress and inflammation. In this study, polyphenols were only detected in the plasma and urine of 42/78 subjects and were highly variable.

The longer term study was a randomised double-blind placebo-controlled crossover trial with 78 subjects that were considered at risk/ overweight. The subjects who were aged 30-65 years with a BMI ≥25 kg/m^2 consumed either a 400mg SPE capsule containing 100mg of polyphenols or a 400mg placebo daily for an 8-week period. Analysis of diet, health and lifestyle factors revealed that consumption of the SPE did not affect the habitual food consumption patterns in the intervention population.



Study design: 24 week intervention

The bioavailability of seaweed phenolics was determined by analysis of urine and plasma samples as before and bioactivity was assessed with a panel of blood-based markers including lymphocyte DNA damage (primary outcome), plasma oxidant capacity, C-reactive protein (CRP) and inflammatory cytokines (secondary outcomes).



Some of the phlorotannins identified in the study (Corona *et al.* 2016)

The 8-week intervention with the SPE did not have any significant effects on any of the blood based markers at a population level however some significant effects were seen in higher risk subsets. In obese subjects (BMI >30 kg/m^2), a significant reduction (P=0.048) in basal DNA damage was observed (measured using the comet assay) as was a significant difference in the total oxidative capacity of plasma (P=0.025). In all subjects and subsets, there were no significant effects on the cardiovascular marker (CRP) or any of the inflammatory cytokines .

In summary

The SWAFAX study showed for the first time that seaweed phlorotannins are metabolised and absorbed by humans, predominantly in the large intestine, and there is a large inter-individual variation in their metabolic profile. In addition, seaweed phlorotannins can exert beneficial anti oxidative effects in obese individuals

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CyberColloids Ltd., Ireland.

Private R&D company, experts in polysaccharide and hydrocolloid chemistry and developing novel processing methodology for seaweed and other polysaccharide rich biomasses.

www.cybercolloids.net

NICHE, University of Ulster, N. Ireland.

N.I. Centre for Food & Health, pre-eminent university department in the UK and Ireland in the area of nutrition and health, specific expertise in conducting human intervention studies.

www.ulster.ac.uk

University of Reading, UK

Department for Food & Nutritional Science. Internationally recognised centre of excellence specialising in research on role of dietary components on human health and risk of chronic disease.

www.reading.ac.uk/food

Scope for future research

This study has significantly added to our understanding of the fate of seaweed derived phlorotannins *in vivo* and for the the first time started to shed light on the role of colonic biotransformation and bioavailability. However, as with any study that utilises a dietary intervention approach and yields results that demonstrate high variability between individuals, the work needs to be confirmed using more and different groups of volunteers.

Phlorotannins are known to complex with other nutritional components such as carbohydrates and proteins and their bioactive potential can also be affected by exposure to certain factors such as temperature and pH. This study assessed the bioaccessibility of polyphenols in the form of a capsule/ extract but further work is required to address the potential impact of other components when consumed as part of a complex food matrix and also the potential effects of cooking and processing.

The SPE investigated here was derived from one type of seaweed - *Ascophyllum nodosum* but it is well documented that phlototannins derived from different brown seaweeds are highly variable, in addition extraction methods and type of source material used can affect the quantity, type and molecular weight range of phlorotannins in any given extract. There is significant scope for the further development and optimisation of extraction and analytical techniques in order to move towards the production of standardised and better characterised extracts, Such extracts are essential in order to carry out meaningful comparative studies in the future.

***Fucus vesiculosus* seaweed**

For more detail see:

Corona, C., Ji, Y., Anegboonlap, P., Hotchkiss, S., Gill, C., Yaqoob, P., Spencer, J.P.E. and Rowland, I. (2016). Gastrointestinal modifications and bioavailability of brown seaweed phlorotannins and effects on inflammatory markers. British Journal of Nutrition. 115(7):1240-53.

Look out for:

Baldrick, F,.R., *et. al.* (2016). The impact of consumption of a polyphenol rich extract from the brown seaweed *Ascophyllum nodosum* for 8 weeks on DNA damage and antioxidant activity in an at-risk population. *In prep.*