

Brown macro-algae are rich in polysaccharides that are potential therapeutic agents. Particularly interesting is the unique presence of fucoidan, a family of polysaccharides that have distinct antibacterial, antiparasitic and antiviral properties. This article reviews the bioactive properties of fucoidan and presents facts on an algal product that is currently developed as a practical feed ingredient.

Brown Macro-algae

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Today, there is an increasing interest in the identification of natural therapeutic products for the control of diseases in animal production to avoid the problems associated with excessive use of antibiotics and other bio-accumulating chemicals. The cell walls of brown macro-algae contain a range of different polysaccharides including alginic acids (alginates), laminarins (laminarans) and fucoidans (sulphated fucans). Alginic acids are used as thickeners, emulsifiers, stabilisers, binding and gel forming agents in the food, cosmetics, textile, construction and pharmaceutical/biomedical industries. Laminarins and fucoidans are currently subjects of much research interest to characterize their structure and understand their biological activities and potential applications. Fucoidans, in particular, are showing much promise in disease control and other feed applications. Among the algae, fucoidans are unique to the brown macro-algae or Phaeophyceae. They are present in intercellular junctions, as well in droplets that exude from the algal fronds. In the marine environment, fucoidans have been described from certain invertebrate sources, in particular sea cucumbers, *Ludwigothurea grisea* and several sea urchin species.

Oileán Glas Teoranta (OGT) is a new Irish company seeking to develop novel value-added products from Irish macro-algal resources. OGT is currently collaborating with the Department of Biochemistry of National University of Ireland, Galway (NUIG), to study fucoidan polysaccharides in key brown macro-algal species abundant on the western seaboard of Ireland. A key interest is to quantify the natural variation (seasonal and spatial) of fucoidan polysaccharides in the species as well as to understand their anti-microbial and antiviral properties.

What are fucoidans?

The term fucoidan is generally used to describe a family of polysaccharides, containing of L-fucose as one of the major monosaccharides, which are generally highly sulphated and contain varying amounts of glucuronic acid and xylose. While the first fucoidan ('fucoidin') was isolated from brown algae at the beginning of the 20th century (Killing, 1913), detailed studies on the structure of fucoidans started only in the mid-1960s by the eminent carbohydrate chemist, Elizabeth Percival. An extensive review of the structure of fucoidans has recently been published by Berteau and Mulloy (2003). Data to date indicate that significant variation exists in fucoidan structure particularly with respect to species, local climatic and environmental factors and extraction/purification methods. Whether such variations result in differences in the bioactivity of fucoidans is an aspect investigated by our institutions.



Ascophyllum sp.

There is a lack of consistency in the terms used to define various fucoidan fractions. In the past, fucoidan fractions were often named according to the species from which they were derived, e.g. sargassan from *Sargassum* sp. or ascophyllan from *Ascophyllum* sp. or after the sugars they contained, e.g. Xylofucoglycuronan (ascophyllan) or glycorunogalactofucan (sargassan). Berteau and Mulloy (2003) have suggested that the term 'sulphated fucan' be used to describe L-fucose rich polysaccharides with $\geq 90\%$ L-fucose (e.g. marine invertebrate fucoidans), while the term 'fucoidan' would describe L-fucose-containing polysaccharides from algal sources which have varying fucose levels.



A harvest field on the Connemara coast of Ireland from which the brown macro-algae *Ascophyllum sp.* are sourced.

Bioactive properties of fucoidans

It should be noted that most bioactive properties of fucoidans have only been determined *in vitro*. Bioactive properties that have been identified thus far for fucoidans include anti-angiogenesis activity, anticomplementary activity, antiproliferative effects on tumor cells, as well as anti-inflammatory, anticoagulant and antioxidant properties. Fucoidan fractions have demonstrated strong anticoagulant activity virtually equivalent to heparin. Heparin is a well-known highly sulphated polysaccharide traditionally used in medicine as an anticoagulant but with many other important bioactive properties. Even though heparin is derived from animal sources and fucoidans are obtained from plant sources, there is close structural similarity between both sulphated



Ascophyllum sp.

polysaccharides which may explain their similar bioactive properties. The need for safe, natural, non-animal heparin analogues as a precaution against possible BSE-like incidences makes fucoidan from algae as potential alternative to animal-derived heparin.

The antimicrobial properties of some fucoidan fractions are perhaps the most relevant for feed applications. These properties have been demonstrated both *in vitro* and *in vivo*. Certain fucoidan fractions are also thought to stimulate the immune system of living organisms, including invertebrates.

Antibacterial properties

Fucoidans have been shown *in vitro* to inhibit the adhesion of staphylococcal bacteria to biomaterials mainly through hydrophobic interactions. They inhibited the binding of a wide range of enterococci and *Streptococcus bovis* strains to the extracellular matrix proteins of animal cells. A commercial product based on macro-algal meal was recently shown to have positive effects on growth and the immune response of weaning pigs challenged by *Salmonella* infection. Extracts containing fucoidans from different brown algal species have been shown to inhibit the attachment of *Helicobacter pylori*, a bacterial pathogen of the gastric tract. The effectiveness depended on the type of fucoidan and the algal source. It is proposed that the inhibition occurs as a result of the coating the bacterial surface by the charged polysaccharides.

Effects on parasites

Commercial products based on seaweed meal have been recommended for limiting coccidial infections in poultry. The inhibition of host infection by *Plasmodium falciparum*, the causative agent of Malaria, has been documented. The effect is attributed to the prevention of the invasion of human erythrocytes by *Plasmodium* merozoites by the negatively charged fucoidan molecules. There are indications that the sulphate groups on fucoidan play a major role in preventing the parasite infection. There are also a number of studies on the inhibition of other parasites by fucoidans.

Antiviral properties

The antiviral potential of sulphated fucans (polysaccharides comprised mainly of L-fucose) has been demonstrated *in vitro* for Human Immunodeficiency Virus, Herpes Simplex Virus and human cytomegaloviruses. Tasmanian kelp extracts (*Undaria pinnatifida*) were found to inhibit *in vitro* mitogenic effects of HSV on Human T cells. Fucoidan from *Fucus vesiculosus* inhibited HIV *in vitro*. The proposed mechanism suggests that fucoidans interact with binding sites on the target cell surface, thus preventing viral binding with the cell. All *in vitro* studies on the anti-viral effect of fucoidans have shown that the sulphate group is necessary (at least for anti-HIV activity) and potency increases with the degree of sulfation.

A recent study has shown that sulphated fucans, at concentration of 100 $\mu\text{g/ml}$, almost completely inhibited syncytium (or large cell body) formation that is essential for the spread of human T-cell lymphotropic virus type 1. Fucoidans inhibit infection by the Respiratory Syncytial Virus infection, both *in vitro* and in a mouse model. However, all fucoidan fractions may not possess antiviral activity. For example, while galactofucans display high inhibitory activity against herpes simplex viruses 1 and 2 with no cytotoxicity, uronofucoidans exhibit no antiviral activity.

Effect of fucoidans in aquaculture species

Several studies evaluating the effect of sugars extracted from macroalgae on the general health of fish have been published. Satoh et al., (1987) observed that alginates can stimulate the immune system of Red Sea bream and increase their resistance to pathogens. Gabrielsen et al., (1998) reported the positive effect of *Ascophyllum nodosum* extracts on haemolytic and lysozymic activity in Salmon. These authors also noticed improved resistance to the bacterium, *Aeromonas salmonicida*, in fish fed a diet supplemented with a macro-algal extract rich in polysaccharides. The positive effects were attributed to the β -glucan (laminaran) present in the algae. Dalmo et al. (1998) observed enhanced resistance to *A. salmonicida* and *Vibrio salmonicida* in Atlantic salmon fed with pellets containing *Laminaria hyperborea* extracts. Castro et al. (2004) tested the effect of different macro-algal (green, red and brown algae) extracts on turbot's immune system and found an increase of the respiratory burst activity of the phagocytes. They concluded that most of the stimulatory capacity of the water soluble extract was associated with the presence of the polysaccharides.

There have been a few investigations on the effects of algal preparations on the shrimp immune system. Rivera et al., (2002) observed bactericidal activity of kelp preparations against *Vibrio* sp. at concentrations as low as 0.1%. Chotigeat and colleagues (2004) have observed *in vitro* that a crude fucoidan preparation from *Sargassum polycystum* inhibited growth of *Vibrio harveyi*, *Staphylococcus aureus* and *Escherichia coli* and that when orally administered, it reduced the impact of White Spot Syndrome Virus (WSSV) in black tiger shrimp. Takahashi et al., (1998) has observed the ability of fucoidan-containing meals and extracts to control WSSV in the Japanese kuruma shrimp. Cruz-Suarez et al., (2000) noted the same effects using Mexican kelp (*Macrocystis pyrifera*), and are currently evaluating the antiviral properties of fucoidans on Pacific white shrimp.

Including brown macroalgae in aqua feeds: practical considerations

Cruz-Suarez et al. (2002) stated that the inclusion rate of *Ascophyllum nodosum* meal in shrimp feed should be around 3.5 to 4%. The average level of fucoidan in *A. nodosum* is around 5% (on dry weight basis), so the inclusion rate of 3.5-4% means a fucoidan concentration of about 0.2% in the feed. Takahashi et al., (1997) also provided a similar dosage recommendation for effective antiviral activity.



A green paste product made from *Ascophyllum* sp. through a proprietary cold production process.

In the preparation of macro-algal crude extracts for oral delivery of fucoidan, the high variability in fucoidan levels should be taken into consideration. Significant variation exists in the amount and the composition of fucoidan, depending on species, age or part of the plant, the season, location and various climatic and environmental factors, as well as extraction procedures (Tables 1-5). We have noted high, seasonal variation in the fucoidan content of *A. nodosum* plants collected from four different sites along the Irish West coast. The fucoidan content varied from <2% of the dry weight in January 2004 to over 5% in June 2004. Differences between the sites are also clear. Plants from two sites sampled in June 2004 had a fucoidan content of approximately 5%, while the other two sites exhibited a fucoidan content of >10% at the same sampling time. We have also developed a reliable, reproducible extraction protocol that results in high yields of fucoidan. The OGT cold production process yields a green paste with fucoidan content almost as high as that observed in the harvested macro-algae. Results to date show that other procedures using heat clearly result in a noticeable drop in the fucoidan content of the final product.

Brown macro-algae have other nutrients that also need to be considered while formulating with the ingredient. The protein content of brown macro-algae (5-15% DW-basis) is lower than that of red (35-40% DW) or green (15-20% DW) macroalgae. However, betaine levels are quite high, which may impart a certain attractant value to the ingredient. While lipids represent approximately 1-5% of the total dry matter, they are rich in 18- and 20- C-atom fatty acids, particularly eicosapentanoic acid and arachidonic acid. We have observed Vitamin C levels up to 140 ppm and Vitamin E levels of 45 IU/kg in OGT products. The cold production process used by OGT allows conservation of heat-sensitive nutrients at levels naturally occurring in the seaweed, which may not be the case with a meal produced at high temperatures. The products also contain high concentrations of iron (900 ppm), calcium (0.61%), manganese (11.27 ppm), cobalt (3.33 ppm) and selenium (1.9 ppm).

Brown macro-algae represent a naturally concentrated source of diverse polyphenols, which comprise a wide range of molecules with different biological activities including antioxidant properties. However, some of these phenolic components are potent anti-nutritional factors. Polyphenols extracted from brown macro-algae are currently being studied by the Department of Biochemistry and Botany, and the Irish Seaweed Centre, NUI Galway, to understand the full spectrum of biological activity of these molecules, their interaction with other non-phenolic biomolecules in algal extracts and to develop extraction methods for selective removal of some of these phenolic fractions from the products.

Conclusions

The potential of brown macro-algae as a valuable resource of bioactive compounds, particularly fucoidan, based on past research work and our own investigations, is high. From an aquaculture standpoint, the antiviral properties of fucoidan products will be most beneficial. The shrimp farming industry loses many millions of dollars every year due to viral disease problems.

Our research program plans to conduct *in vivo* trials in fish and shrimp with OGT products. A joint project with the University of Caen (Laboratory of Marine Biology and Biotechnology) has just completed an investigation in turbot that shows that the product is safe for the fish at 5% inclusion levels. More trials on the effectiveness of the product are planned. We also intend to study the stability of fucoidan during

Table 1: Interspecies variation in fucoïdan levels in brown macro-algae

Species	Fucoïdan fractions, % of the dried seaweed weight	Sources
"Fucoïds"	4-9	Chapman, 1970
<i>Ascophyllum nodosum</i>	6-8 4-10 11	Chapman, 1970 Cruz-Suarez <i>et al.</i> , 2000 Sakai <i>et al.</i> , 2002
<i>Bifurcata bifurcate</i>	17	Mian & Percival, 1973
<i>Chorda filum</i>	0.27-11	Kuda <i>et al.</i> , 2002
<i>Cladosiphon okamuranus</i>	2.0	Sakai <i>et al.</i> , 2003
<i>Desmarestia aculeata</i>	2.2-4.8	Percival & Young, 1973
<i>Durvillaea antarctica</i>	2.4	Sakai <i>et al.</i> , 2002
<i>Ecklonia maxima</i>	4.6	Sakai <i>et al.</i> , 2002
<i>Ecklonia radiata</i>	0.4-1.4	Chapman, 1970
<i>Eisenia bicyclis</i>	7.0	Sakai <i>et al.</i> , 2002
<i>Fucus evanescens</i>	4.8-12	Zvyagintseva <i>et al.</i> , 1999, 2003
<i>F. spiralis</i>	9-11	Chapman, 1970
<i>F. vesiculosus</i>	7	Sakai <i>et al.</i> , 2002
<i>Himanthalia lorea</i>	18	Mian & Percival, 1973
<i>Laminaria cichorioides</i>	0-7.8	Zvyagintseva <i>et al.</i> , 1999, 2003
<i>L. japonica</i>	1.1-2.9	Zvyagintseva <i>et al.</i> , 1999, 2003
<i>Laminaria sp.</i>	1.5	Sakai <i>et al.</i> , 2002
<i>Lessonia nigrescens</i>	5-20	Chapman, 1970
<i>Macrocystis pyrifera</i>	4.0	Sakai <i>et al.</i> , 2002
<i>Nemacystis decipiens</i>	0.5-8	Cruz-Suarez <i>et al.</i> , 2000
<i>Padina pavonia</i>	10.2	Kuda <i>et al.</i> , 2002
<i>Pelvetia caniculata</i>	5	Mian & Percival, 1973
<i>Sargassum horneri</i>	20	Chapman, 1970
<i>Undaria pinnatifida</i>	5.17 6.77-15.1	Kuda <i>et al.</i> , 2002 Kuda <i>et al.</i> , 2002

Table 2: Variation in fucoïdan levels with respect age and part of brown macro-algae

Species	Fucoïdan fractions, % of the dried seaweed weight		Sources
	Stage in the life cycle		
<i>Laminaria cichorioides</i> <i>L. japonica</i>	Young	2 Years-old	Zvyagintseva <i>et al.</i> , 2003 Zvyagintseva <i>et al.</i> , 2003
	7.8 1.1	3.5-7.2 2.8	
<i>Undaria pinnatifida</i>	Sporophyte	Blade	Kuda <i>et al.</i> , 2002 Sakai <i>et al.</i> , 2002
	15.1 8.0	6.77	

Table 3: Effect of seasonality on fucoïdan level in brown macro-algae

Species	Fucoïdan fractions, % of the dried seaweed weight			Sources
	Periods			
<i>Fucoïd</i>	Spring	Autumn	Chapman, 1970	
	4	9		
<i>Ecklonia radiata</i>	Average Year	Mid-winter	Chapman, 1970	
	0.4	1.4		
<i>Desmarestia aculeata</i>	March	August	Percival & Young, 1974	
	4.8	2.2		
<i>Macrocystis pyrifera</i>	May	August	Cruz-Suarez <i>et al.</i> , 2002	
	4.1	3.8		
		September		5.9

Table 4: Impact of harvesting location on fucoïdan levels in brown macro-algae

Species	Fucoïdan fractions, % of the dried seaweed weight			Sources
	Locations of Collections			
<i>Laminaria sp.</i>	Lower Sub-littoral	Upper Sub-littoral	Chapman, 1970	
	5	20		
	Kratemaya Bay	Paramushir Isld.		Iturup Isld.
<i>Fucus evanescens</i>	12.0	4.8	5.3	Zvyagintseva <i>et al.</i> , 2003

Table 5: Effect of the extraction process on fucoïdan levels in brown macro-algae

Species	Fucoïdan fractions, % of the dried seaweed weight		Sources
	Differences in Extraction or Process		
<i>Fucus evanescens</i>	Cold Extraction	Hot extraction	Zvyagintseva <i>et al.</i> , 1999 2003
	5.3	9.7	
<i>Laminaria cichorioides</i>	4.8-12	8.8-9.7	1999 2003
	7.2	6.5	
<i>L. japonica</i>	3.5-7.8	0-6.5	1999 2003
	3.4	2.7	
<i>Chorda filum</i>	1.1-2.8	2.4-2.9	2003
<i>Chorda filum</i>	Dried	Washed	Kuda <i>et al.</i> , 2002
	11	0.27	



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long-term storage, particularly when present in crude algal preparations. The use of selective enzymes as tools to modify fucoidan fractions, for structural characterization and to correlate structure with biological activity is an aspect currently under development. The interaction and possible synergy between fucoidan and other biomolecules present in brown macro-algal extracts also need to be investigated. The potential of brown macroalgal extracts as anti-fungal agents also requires investigation. Fungal infections are notoriously difficult to treat and often develop into chronic infections. For example, black gill disease caused by *Fusarium* sp. in crustaceans may result in significant pre-harvest mortality. A single, environmentally

safe treatment strategy to combat all microbial infections with a natural product such as a brown macro-algal extract or meal, would certainly be most advantageous. The nutrients supplied by the algal product would be an added bonus. ■

References:

Contact the authors or download from the extended version of the paper which will be available at <http://www.feedware.com> in late January 2005.

Mr. Franck Hennequart is a Project Manager and Scientific Officer with Oilean Glas Teoranta (OGT), an Irish company, with a core business based on the harvesting and processing macro algae from the Donegal coast in North West Ireland, for applications in the horticulture, animal health, cosmetics, human nutrition, biopharma sectors and other value-added markets. Franck is a Marine Biologist with extensive international experience, and is currently coordinating the scientific collaboration with NUI, Galway, funded by Enterprise Ireland, on investigating fucoidan levels and bioactivity in brown macro-algae from harvest sites along the west coast of Ireland.




Dr. Maria Tuohy is a lecturer in the Department of Biochemistry, NUI, Galway, and research PI of the Molecular Glycobiotechnology Group, which has significant expertise in the structure-function analysis, molecular genetics, regulation and biotechnological application of eukaryotic glycoproteins and novel biocatalysts, including carbohydrate-modifying enzymes and proteases, many of which are derived from fungal sources. In addition the group is investigating the bioactive potential of key algal polysaccharides, i.e. laminaran and fucoidan, and in parallel the cell biology and function of important cell-surface carbohydrate-binding proteins (lectins), using proteomics and genomics approaches.

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