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The seasonal variation of fucoidan within three species of brown macroalgae

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ABSTRACT

Fucoidan is comprised of a fucose backbone with sulphate groups, whose variation is important to the functionality of the polysaccharide. The structure of fucoidan has been reported to vary according to species, season, location and maturity; however there is currently little published data to support this. Understanding the seasonal variation of fucoidan is important for industrial applications to identify optimum harvesting times and ensure consistent product composition. This study explores the seasonal variation of three species of brown macroalgae, *Fucus serratus* (FS), *Fucus vesiculosus* (FV) and *Ascophyllum nodosum* (AN), harvested monthly off the coast of Aberystwyth, UK. Average fucoidan content is 6.0, 9.8 and 8.0 wt% respectively for FS, FV and AN, with highest quantities extracted in autumn and lowest in spring. Fucose content, varied between 18 and 28, 26–39 and 35–46 wt% and sulphate content between 30 and 40, 9–35 and 6–22 wt% for FS, FV and AN respectively, with both fluctuating inversely to the total fucoidan content. Size exclusion chromatography (SEC) has provided insight into the structural differences between the species. Based on the molecular weight (MW) distribution, and in line with previous research, it is hypothesised that fucoidan in FS has a more complex structure, with a higher degree of associated sulphate ions than in FV and AN which have a simpler, linear structure with less associated sulphate ions.

1. Introduction

Fucoidan is a sulphated polysaccharide found in brown macroalgae. Its structure is dependent on the species, season, harvest location and maturity of the plant. Its basic structure is comprised of a sulphated fucose backbone, with the most common configuration shown in Fig. 1, but which also contains small quantities of other sugars, such as xylose, uronic acids and galactose. Branched side chains are also common in some species. Reported molecular weight (MW) varies widely, with Rioux et al. quoting 43 kDa [1], and Gupta et al. 1600 kDa [2], a difference of over 1550 kDa. It was originally identified by Kylin in 1913 [3], who named it "fucoidin" and reported an extraction mainly containing fucose. Since then, fucoidan has been widely researched, with advances in both knowledge of its structure and potential properties.

The extraction of fucoidan from macroalgae has been performed by several authors in the published literature [4–9]. In general this consists of four main steps: an initial purification to remove pigments and lipids, often using an alcohol; an extraction step, often repeated several times to ensure full extraction of fucoidan and most commonly using calcium chloride, dilute hydrochloric acid (HCl) or water; further purification of the extract to remove alginate and other impurities before fucoidan is finally precipitated using ethanol [4–9]. A comparison of the three extraction solvents was carried out by Ponce et al. (2003) [10]. The results indicated that distilled water and HCl extraction gave the highest and comparable yields of 10.8 and 9.6 wt% respectively, with the structure of each extract being very similar. Zhang and Row (2015) further developed this work by identifying the best conditions for fucoidan extraction from *Laminaria japonica* [11]. Their findings suggest an extraction time of 4 h at 80 °C and 0.1 M HCl yielding the best results, giving 17 wt% fucoidan. The fucoidan extraction method used in this paper is based on these findings, making it a well-documented and reliable process.

Current research focuses primarily on fucoidan's use in the pharmaceutical industry, with the most extensively studied properties being anticoagulant, anti-thrombotic, immunomodulation, anti-cancer and anti-proliferative [12], although nutraceutical, functional food and cosmetic properties [13] have also been identified. Cho et al. [14] and Senthilkumat et al. [15] have both reported on the anticancer properties of fucoidan, showing inhibition in growth and migration of, as well as being cytotoxic to, cancer cells. Anti-inflammatory properties have been presented by Park et al. [16], who suggest that the properties seen could offer potential for the treatment of neurodegenerative diseases. Ponce at al. [10] demonstrate the antiviral properties of fucoidan

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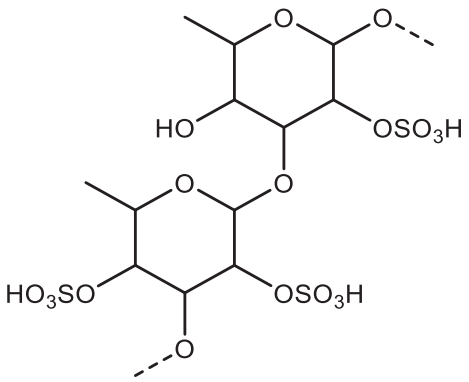

Fig. 1. Simple structure of fucoidan backbone.

fraction from *Adenocystis utricularis*, which showed high inhibition against herpes simplex virus 1 and 2. These properties have been shown by several authors to depend on the molecular weight (MW), the degree of sulphation and sulphation pattern of the fucoidans [17, 18]. Therefore knowledge about how these properties are affected by seasonal variation is vital.

The seasonal variation of fucoidan is often mentioned in the literature, although there is very little published data on the subject at present and the few references cover only a few months of the year. Rioux et al. have investigated the bioactive polysaccharides of 4 samples of *Saccharina longicruris*, from March, April, November 2005 and June 2006 [19]. The galactofucans (a type of fucoidan containing roughly equal proportions of fucose and galactose) extracted were seen to have an increase in sulphate content of 1.6% between March and November 2005, while decreasing by 7.2% between November 2005 and June 2006. A similar study by Mak et al. investigated the variation in fucoidan between July and October for *Undaria pinnatifida* [20]. They found that the fucoidan content almost quadrupled between July and September (3.6–13.7 wt%) and only dropped slightly in October. A similar trend was observed in the sulphate content of the fucoidan. The fucose content decreased significantly between July and September.

Other than the two studies mentioned [19,20], the authors were unable to find other published literature on the seasonal variation of fucoidan. Considering the change in biomedical properties due to the varying composition of fucoidan, understanding the seasonal variation of the chemical content of fucoidan is very important. The work presented here attempts to characterise fucoidan extracted from 3 species of brown macroalgae over a calendar year. Samples of *Fucus serratus* (FS), *Fucus vesiculosus* (FV) and *Ascophyllum nodosum* (AN) were collected monthly over a 12 month period between April 2010 and March 2011, fucoidan was extracted from these samples and analysed for elemental composition, fucose and sulphate content. Furthermore, size exclusion chromatography (SEC) and liquid chromatography-mass spectrometry (LC-MS) has been performed in order to gain insight into the MW of the samples and structural differences.

2. Methods

2.1. Materials

Samples of *Fucus serratus* (FS), *Fucus vesiculosus* (FV) and *Ascophyllum nodosum* (AN) were collected monthly between April 2010–March 2011 off the coast of Aberystwyth (Latitude: 52.41°N, Longitude: −4.08°W) at low tide. The samples were freeze-dried and ground using a Fritsch pulverisette 14 rotor mill through a 500 μm sieve. Whole plants were collected, frozen within two hours of collection and subsequently freeze dried for one week to ensure full lyophilisation. Dried samples were stored in sealed containers for further analysis. Standard fucoidan (F5631) was supplied by Sigma Aldrich.

2.2. Fucoidan extraction

0.5 g of ground, dried macroalgae was weighed into a 50 ml centrifuge tube and 10 ml of 85% ethanol was added and stirred overnight at room temperature. This was centrifuged and the supernatant removed. The pellet was washed once with 10 ml ethanol followed by 10 ml acetone and allowed to dry to a constant weight at room temperature. 0.3 g of the washed seaweed was weighed into a new 50 ml centrifuge tube with 7.5 ml 0.1 M HCl and stirred at 80 °C for 4 h before cooling, centrifuging and decanting the supernatant into a clean 15 ml centrifuge tube. The pH of the supernatant was determined and neutralised to pH 5–7 using a pH meter (HQ40d, Hach) if required using 1 M $Ca(OH)_2$. 1 volume (~6 ml) of 1% $CaCl_2$ was added and stored at 4 °C overnight to precipitate alginate present. The tube was centrifuged and the supernatant transferred to another clean tube, where ethanol was added to give a final concentration of 40% v/v ethanol. This was left for at least 4 h at 4 °C to precipitate the laminarin. The solution was centrifuged, the supernatant decanted into a clean tube and ethanol added again to give a final concentration of 70% v/v ethanol. It was left to precipitate fucoidan for at least 4 h at 4 °C, before being centrifuged for a final time. The extracted fucoidan was allowed to air dry to a constant weight, around 24 h.

2.3. Ultimate analysis

Analysis of the C, H, N and S content of the extracted fucoidan was carried out using a CE Instruments Flash 1112 Series analyser. Samples were prepared by weighing 2.5 ± 0.5 mg of dry fucoidan into 8 × 5 mm tin capsules, along with ~5 mg of vanadium pentoxide, required to combust the sulphur.

2.4. Analysis of fucose and sulphate content

Aqueous samples containing 2.5 wt% of the extracted fucoidan were prepared from a set of fucose standards, at 30–150 mg l^{-1}, and relevant blanks. 1 ml of each solution (either calibration, water or sample) were placed into a 15 ml Pyrex tube with 4.5 ml of 6:1 v/v H_2SO_4 (98% purity). The tube was capped, inverted several times to mix and left at room temperature for 5 min. Each tube was then placed in a boiling water bath for exactly 10 min, removed and cooled under tap water to quench the reaction. 0.1 ml of 3% aqueous cysteine hydrochloride 10 was added to each tube, inverted several times and left for 30 min before measuring at 396 and 427 nm in a UV/VIS spectrophotometer (Multiskan GO, Thermo Scientific).

Sulphate analysis was performed on the same 2.5 wt% solutions of fucoidan using the sulphate testing kit (LCK353) supplied by Hach-Lange.

2.5. Size exclusion chromatography (SEC)

SEC was performed on the fucoidan extracts using a Dionex ultimate-3000 system, fitted with a Waters 500 Ultrahydrogel column and guard column (Dionex). Aqueous, 2.5 wt% fucoidan samples were prepared and filtered through 0.2 μm syringe filters into HPLC vials. Samples were set to run with distilled water as the mobile phase, as described by Zhang et al. [11] to give the best separation for seaweed extracts, with an oven temperature of 30 °C and a flow rate of 0.5 ml min^{-1}. The high performance liquid chromatography (HPLC) software (Chromeleon v.6.8 with extension pack v.2.0) was calibrated with a set of polyethylene glycol/polyethylene glycol standards (MW 200 to 1,015,000 Da) (Fluka) and the MW of the extracted fucoidan was determined using Chromeleon integrated software.