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Review article

Potential of intensification techniques for the extraction and depolymerization of fucoidan

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A B S T R A C T

Fucoidans are sulfated polysaccharides from brown seaweeds with a variety of biological properties, which are dependent on both their composition and structure, and are determined by the extraction process. The molecular weight of fucoidan, associated with the biological activities and with its bioavailability, can be influenced by the extraction technologies and further depolymerization can be attained during a subsequent processing stage. The conventional technologies for extraction are surveyed and the potential of emerging techniques aiming at enhancing yields, selectivity and bioactivity is discussed.

1. Introduction

Fucoidans, composed of fucose, uronic acids, galactose, xylose, mannose, arabinose, glucose and sulfate groups, are heteropolysaccharides exclusively found in brown seaweeds. They are more highly heterogeneous, contrarily to the related compounds occurring in marine invertebrates or fucan sulfates [1,2]. Fucoidans and their lower molecular weight oligosaccharide derivatives are attracting increasing interest for their low toxicity [3–8] and broad range of actions with potential health benefits and therapeutic applications [9–14], including anticoagulant [15–19], antioxidant [20–29], antiangiogenic [16,30,31], antitumoral [29,32–38], antiviral [39–44], anti-inflammatory [16,22,45–49], immunomodulatory [3,50], antihyperlipidemic, [51], antihyperglycemic [51] and wound healing properties [52], as well as protective effects on the digestive tract [25,51].

The chemical composition of fucoidans, including the molecular weight (Mw), monosaccharide distribution, sulfate content and positions, overall conformation and biological characteristics are taxonomically dependent [1,40,53–55] and are influenced by both macroalgal biology (species, growth stage, part of the alga), geographic, seasonal and environmental conditions (water temperature, nutrients, sunlight), collecting and the extraction/purification techniques [1,12,56–58].

Low molecular weight fucoidan (LMWF) fractions are a common form of fucoidans that have enhanced biological activity [56], and more recently are being used in food supplements and pharmaceutical products, because the high molecular mass and viscous nature of some crude fucoidan have hampered their application, especially as a therapeutic agents. To obtain oligosaccharides with more diverse bioactivities, LMWF can be prepared by chemical, radical, or enzymatic means [56]. Different *in vitro* and *in vivo* studies of fucoidans are available, but no rational prediction of structure-activity relationships can be found since different fucoidan preparations were used in biological experiments. It should be emphasized that different individual components extracted from crude fucoidans can have distinct stability in depolymerization conditions and can have distinct biological activities [59].

Extraction and purification stages are necessary when the objective is the study of detailed structural analysis of fucoidans and also for industrial applications, but the potential health applications are limited by difficulties in maintaining structure, purity, and consistency of the product. Fucoidans are normally extracted from brown algae in multistep processes using hot, dilute acid, or high temperature water and a long reaction time. Since prolonged times and temperatures can be deleterious for the biological activity of sulfated polysaccharides, alternative extraction techniques to conserve their structure and biological properties were proposed [11,60]. On the other hand, an increasing health and environmental consciousness has led to the development of efficient, eco-friendly, novel and innovative technologies to reduce energy consumption, emissions and costs as well as to increase safety and product quality. Among those, some physical techniques can be highlighted, including pressurized solvents, ultrasound-assisted extraction and microwave-assisted extraction, as well as those using biotechnological aids. The novelties in the extraction and

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purification of algal polysaccharides have already been reviewed [11,61,62] but the present work deals with the fucoidan extraction and depolymerization stages using conventional and innovative technologies.

2. Extraction of crude fucoidans from seaweeds

The production of bioactive fucoidan fractions requires the extraction of crude fucoidan and, in some cases, additional depolymerization and purification. Solid-liquid extraction is a non stationary, heterogeneous mass transfer operation to separate compounds and/or fractions from a solid using a selective solvent. The most relevant stages for the extraction of natural products are a) solvent diffusion inside the solid matrix, b) hydrolysis and/or solubilization of target components in the solvent, c) diffusion of the solutes through the solid matrix and d) mass transfer to the bulk solution. Since target solutes are inside cells or can be found as polymers forming part of the cell walls, degradation of the cell walls, which act as a barrier, facilitates the release and mass transfer. The rate limiting step is usually stage c), based on internal mass transfer, and can be favored by reducing the particle size and by degrading cell walls. Seaweeds present a complex structure (Fig. 1), and the selective extraction of their components requires considerations of both solubility and accessibility.

The extraction yields and product composition and characteristics are determined by species, environment, collection, storage and preparation, including mechanical and thermal conditioning to remove water and to make the solid matrix more accessible to the solvent. The initial stage usually consists of washing the fresh seaweeds with deionized, distilled or tap water in order to remove salt, sand and epiphytes [23,41,64–67]. Sea, tap and distilled water [22,29,63] can be used individually or in sequence for rinsing.

In a mass transfer controlled process particle size is a key factor, determining the contact area and diffusion pathway. The conditioning stages include the destruction and degradation of the cell wall under mild conditions, such that the intrinsic properties of polysaccharides remain unchanged. The samples are dried and ground with the aim of destroying the cell wall and to increase the specific area [11,67]. Also direct utilization of algal pieces before water extraction [68] or before autoclaving [69] was possible. Alternatively, a simple procedure feasible for continuous production was proposed, consisting on compressional-puffing (140–220 °C, 10 s) of the dried and crumbled alga, which primarily decomposes the cellular structure of alga, decreases the bulk density of algal samples, expands the algal cellular structures, eliminates the unpleasant algal odor and facilitates the release of fucoidan by warm or hot water extraction [28,70].

In order to prevent the coextraction of other algal compounds during the aqueous extraction of fucoidan, different stages can be performed before the extraction process, to facilitate the purification stages [11,62]. Low molecular weight components, colored matter, lipids and lipophilic pigments can be selectively extracted with 70–96% ethanol for 1–24 h at 25–80 °C [19,21,29,32,35,36,40,41,49,65,66,71–81]. Alternatively [22,82–84] or successively the removal of lipids and pigments can be carried out with acetone, [15,32,67], or with a mixture of acetone and ethanol [80,85]. Extractions can be applied in a sequence, *i.e.*, with ethanol, acetone, and chloroform [85,86] or with petroleum ether and acetone [23,88].

The mixture of methanol/chloroform/water (4/2/1, v/v/v) can remove lipids, protein and colored pigments. Previous or further washing with acetone to remove lipids and phenols was also carried out [11,50,55,58,75,89–92]. A sequence of increasing temperatures with 80% ethanol first at room temperature then at 70 °C was used to extract mannitol and some salts [11,34,77,93]. This initial defatting stage can be avoided in simpler methods used at industrial scale; although the fucose and sulfate content was lower, no difference in cytotoxic and antioxidant activity occurred and the extraction yields were higher [35]. Alternatively, deoiling by sc-CO_2 extraction (25–55 MPa, 40–60 °C) [94–96] using pure solvent or 5% ethanol as modifier was proposed. The preliminary removal of pigments, lipids and phenols can provide extracts enriched in these valuable components [75,97,98], but in some cases this previous extraction step may be omitted when trying to develop simple processes [49,91].

3. Interest of depolymerization and properties of low molecular weight fucoidan (LMWF)

The large size of crude fucoidan molecules (21–1600 kDa) [20,41,93,99] is a major concern in their applications as pharmaceuticals, due to the viscosity in some highly branched molecules [93,100,101] and limited transport across the cell membrane and absorption. The polydispersity, high molecular weight (Mw) and heterogeneity also limits structural studies. A depolymerization stage can be

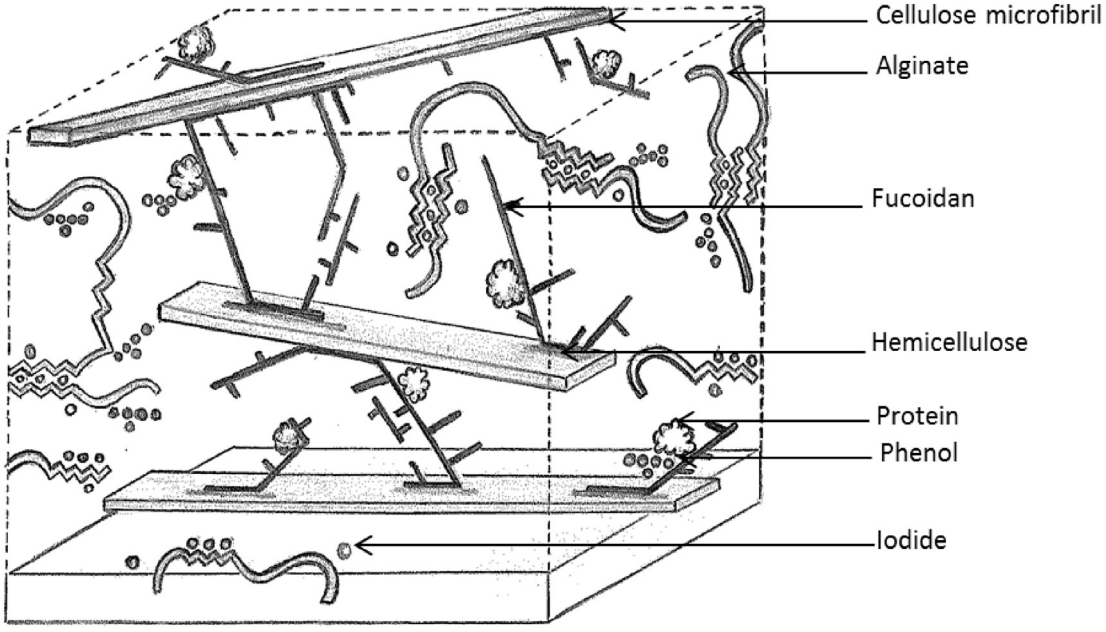

Fig. 1. Simplified scheme of brown seaweed cell wall (from the information in ref. [61]).