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Short communication

Purification of fucoidan from Bulgarian Black sea algae *Cystoseira crinita* (Desf.) Bory.

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Introduction

Fucoidans are highly complex and heterogeneous polysaccharides composed of *L*-fucose and sulfate groups, but may also contain uronic acids, galactose, xylose, mannose, arabinose and glucose. They are mainly found in brown seaweeds and marine invertebrates. In recent years, fucoidans are attracting great interest for their various activities such as anti-inflammatory, immunomodulatory, antioxidant, anticoagulant, anti-angiogenic, antihyperlipidemic, antibacterial, antitumor, prebiotic and wound healing activity (Hahn et al., 2012; Hentati et al., 2018).

Brown algae fucoidans are usually in complexes with different molecules as polyphenols, proteins, lipids and other polysaccharides (alginates). The coextraction of other algal compounds could influence the purity of the isolated fucoidan and respectively its biological activity (Hahn et al., 2012). Therefore, a pretreatment of the algae mass before the fucoidan isolation is required. The aim of this study was to optimize the extraction process of fucoidan from the Bulgarian Black sea algae *Cystoseira crinita* in order to achieve high polysaccharide yield and purity.

Materials and methods

The depigmentation/delipidization of dry powder of *Cystoseira crinita* was carried out using five different methods: *Method 1*: methanol extraction; *Method 2*: methanol:chlorophorm:water (4:2:1) extraction; *Method 3*: a consecutive acetone:chlorophorm: ethanol extraction; *Method 4*: formaldehyde:water extraction (1:50); *Method 5*: ethanol:formaldehyde:water extraction (80:5:15). The drug extragent ratio (DER) for all models was

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1:10 and the extraction time was 24 h. The fucoidan extraction was performed according to the procedure proposed by Hentati et al. (2018) using 0.1 M HCl.

The purity of the isolated fucoidan was estimated by quantifying the content of total polyphenols and proteins. Total phenolic compounds were determined by the colorimetric Folin-Ciocalteu assay using gallic acid as a standard (Singleton et al., 1999). Protein content was estimated according to the Bradford method (1976) calibrated against a standard of bovine serum albumin.

Results and discussion

The extraction yield of fucoidan from *Cystoseira crinita* was approximately 5% of the dry biomass. Other algal compounds such as phenols, terpenes, lipids and some pigments have a high affinity for fucoidan, adsorbing tightly to it during the process of extraction (Hahn et al., 2012). Therefore, an appropriate pretreatment procedure was performed to achieve high purity of the target product. Polysaccharide stability was also taken into consideration when choosing the purification agent. The results from the five different purification protocols used were as follows: *Method 1*: Fucoidan yield: 4.72%; Phenolic content: 0.97 %; Protein: 1.17%*; Method 2*: Fucoidan yield: 5.84%; Phenolic content: 1.02 %; Protein: 1.43%*; Method 3*: Fucoidan yield: 5.83%; Phenolic content: 1.39 %; Protein: 1.37%*; Method 4*: Fucoidan yield: 2.53%; Phenolic content: 0.2 %; Protein: 0.11%*; Method 5*: Fucoidan yield: 5.15%; Phenolic content: <0.1 %; Protein: 0.56%

The highest yield of fucoidan was estimated using methanol:chloroform:water mixture (method 2) and a consecutive acetone:chlorophorm:ethanol extraction (method 3). On the other hand, these models showed highest phenols and proteins content. Acetone and chloroform were used to remove fatty acids, terpenes, chlorophyll and some phenols. Alcohols as methanol and ethanol, and their aqueous solutions, were used to remove chlorophyll and the major reserve carbohydrate mannitol. Method 1, method 2 and method 3 are not expected to alter fucoidan structure but they usually result in high traces of coextracted compounds, which was also confirmed by our results (Hahn et al., 2012).

Method 4 and method 5 using formaldehyde showed the lowest content of impurities (<0.1% of phenols and <0.6% of proteins). This could be attributed to the polymerization of polyphenols by formaldehyde which linked and fixed phenols, making them insoluble (Hahn et al., 2012). The lowest yield of fucoidan when aqueous solution of formaldehyde was used, could be due to the water-solubility of the polysaccharide. Hence, in method 5 an ethanol:water solution (80:15) was applied to prevent the extraction of fucoidan during the purification procedure.

Conclusion

Water-ethanol solution of formaldehyde was determined as the optimal purification agent for the pretreatment procedure of fucoidan extraction, due to the obtained high yield and purity of the isolated polysaccharide.

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