Food Hydrocolloids 75 (2018) 229–236

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Chemical characterization and antioxidant activity of sulfated polysaccharides from *Navicula* sp.

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ARTICLE INFO

Article history:
Received 16 December 2016
Received in revised form
5 July 2017
Accepted 4 August 2017
Available online 4 August 2017

Keywords:
Navicula sp.
Sulfated polysaccharides
Chemical characterization
Antioxidant activity

ABSTRACT

Sulfated polysaccharides were extracted from *Navicula* sp. cultivated at three wavelengths: white (WSPN), red (RSPN) and blue (BSPN) with yield rates of 3.4, 3.9 and 4.4 (% *w*/*w* dry biomass basis) respectively. Analysis of these polysaccharides using gas chromatography showed that they contain glucose, galactose, rhamnose, xylose and mannose as main neutral sugars. The amount of rhamnose was higher in WSPN. The molecular weight (*Mw*) value was 17, 107 and 108 kDa for WSPN, BSPN and RSPN, respectively. The sulfate content in WSPN was higher (0.40% *w*/*w*) than in the BSPN and RSPN. The polysaccharides recovered from *Navicula* sp. presented antioxidant activity, which could be related to the molecular structural characteristics such as *Mw* and sulfate content. The scavenging activity was higher in WSPN (DPPH 49% and $ABTS^{+}$ 68 μmol Trolox/g), than in the BSPN and RSPN samples. The WSPN possess a high antioxidant capability, thus this sulfated polysaccharide might be a potential antioxidant for biotechnological applications.

1. Introduction

The bioactive compounds from microalgae have a wide range of applications such as antivirals, antimicrobials, antioxidants among others, which has been attributed to their biological activities and chemical structures (Sun, Wang, Guo, Pu, & Yan, 2014). The microalgae culture conditions are the main factor affecting their biochemical composition, being the light one of the most important variables (Markou, Angelidaki, & Georgakakis, 2012). Jungandreas et al. (2014) documented that exposition of microalgae to red light produces an increment in the carbohydrates concentration, while blue light promotes the proteins content. However, authors such as Korbee, Figueroa, and Aguilera (2005) report that the effect of white light represents the combined effect of red and blue light. The marine microalgae have been mainly used as a live food for cultivable aquatic organisms, due their rich biochemical composition; however, nowadays these microorganisms are being used to obtain bioactive compounds with high biotechnological potential in biomedical, pharmaceutical, nutraceutical and cosmetics industries, being the antioxidant activity one of the most studied aspects of these compounds. Some of the main genera of marine microalgae used for this purposes are *Dunaliella*, *Spirulina* (Abd El Baky, Hanaa El Baz & El-Latife, 2013; Hemalatha, Girija, Parthiban, Saranya, & Anantharaman, 2013; Karthikeyan et al., 2013), and recently the species from the genus *Navicula* (Affan, Karawita, Jeon, & Lee, 2007; Hemalatha et al., 2013). Some investigations about the antioxidant activity from extracts of microalgae have focused on the activity of their pigments such as carotenoids and xanthophylls; however, there are other compounds such as the polysaccharides with high antioxidant activity that should be evaluated. The microalgal sulfated polysaccharides have shown to play an important antioxidant role with effective scavenging activities on

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http://dx.doi.org/10.1016/j.foodhyd.2017.08.002
0268-005X/

different radicals (Souza et al., 2012; Sun et al., 2014). It is well known that the benthic diatom *Navicula* sp. is characterized by producing a mucilage with a high content of extracellular polymeric substances included lipids, proteins and polysaccharides (De Jesus Raposo, De Morais, & De Morais, 2013). Although there are some investigations about the properties of the sulfated polysaccharides of this microalgae, the information is incipient and there is no research on the effects of different wavelength in these organisms, particularly on the production of polysaccharides from *Navicula* and their antioxidant activity. The aim of the present study was to characterize and evaluate the antioxidant activity of sulfated polysaccharides extracted from the benthic diatom *Navicula* sp. cultivated at three wavelengths.

2. Materials and methods

2.1. Materials

The marine diatom *Navicula* sp. was obtained from the strain collection of the Laboratory of Chemical Analysis and Microbiology of the University of Sonora. All chemical reagents were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

2.2. Methods

2.2.1. Culture conditions

The experiment was carried out under indoor controlled conditions by quadruplicate, using tubs containing 10 L of culture medium. For their culture, the "F" medium from Guillard and Ryther (1962) was utilized. Cell counts were performed daily and biomass was harvested at the stationary phase. Total biomass was quantified by gravimetric methods. Microalgae were cultured at 50 μmol photon m^2 sec^{-1} of irradiance in white (400–750 nm), blue (430–480 nm) and red wavelength (595–660 nm); light was supplied by Light Emitting Diode lamps (LED) electronically controlled to the desired irradiance.

2.2.2. Extraction of sulfated polysaccharides from microalgal culture

Once finished the microalgal culture, the total biomass of each treatment was full harvested by gravity sedimentation method (Shelef, Sukenik, & Green, 1984, pp. 1–66) and lyophilized using a Freezone 6 Freeze dry System (Labconco, Kansas, MO, USA). Subsequently, the lyophilized biomass was suspended in distilled water for 1 h at room temperature to obtain the soluble fraction of sulfate polysaccharides, the suspended biomass was then centrifuged for 15 min at 20,000×*g*. The supernatant was separated and precipitate overnight on cold conditions with ethanol 96% (*v*/*v*) to allow the precipitation of sulfated polysaccharides (Fimbres-Olivarría et al., 2016) from *Navicula* sp. The resultant extracts were named as follow: WSPN (sulfated polysaccharide from *Navicula* sp. in white wavelength), BSPN (sulfated polysaccharide from *Navicula* sp. in blue wavelength) and RSPN (sulfated polysaccharide from *Navicula* sp. in red wavelength).

2.2.3. Chemical analysis

Total sugars content was estimated using the phenol/H_2SO_4 assay described in the literature (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) with a GENESYS™ 10UV spectrophotometer (Thermo Scientific). A calibration curve was utilized with glucose as the standard.

The sulfate content of the extracted polysaccharides was determined after hydrolysis with 1 N HCl at 100 °C for 1 h following the sodium-rhodizonate method proposed by Terho and Hartiala (1971). Sodium-rhodizonate forms a colored compound with the barium ion; when sulfate is present, $BaSO_4$ is formed and the intensity of the color decrease; the sulfate amount can be calculated from this reduction. Na_2SO_4 was utilized as a standard. The protein content was analyzed using the Dumas method (Leco FP-528 nitrogen analyzer, St. Joseph, MI, USA) (AOAC, 1995).

The monosaccharides content of polysaccharides was analyzed by gas chromatography (GC) (Agilent HP 6890 GC Series, Santa Clara, CA, USA) (Rouau & Surget, 1994). The samples were hydrolyzed with 3 N H_2SO_4 (98% *v*/*v*) at 100 °C, and inositol was added as the internal standard. The external standards were glucose, mannose, galactose, xylose and rhamnose (1 mg/mL, *w*/*v*), which were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Sugars were reduced to alditols with sodium borohydride, acetylated with acetic anhydride in the presence of methyl imidazole, and finally extracted with chloroform. After extraction, the alditol-acetates were injected (5 μL) in a DB 225 type column (50% cyanopropylphenyl-dimethylpolysiloxane, 30 m × 0.32 mm ID, 0.15 μm). The GC conditions were as follows: injection temperature 220 °C, detector temperature 260 °C, and oven temperature programmed to 205 °C at 10 °C/min. Nitrogen was used as the carrier gas and maintained at 1.0 mL/min. A flame ionization detector was used.

2.2.4. Fourier transform infrared (FT-IR) spectroscopy

The polysaccharide powders were pressed into KBr pellets. A blank KBr disk was used as background. FT-IR spectrums were recorded on a Nicolet FT-IR spectrophotometer (Nicolet Instruments Corp., Madison, WI, USA) and measured in absorbance mode from 4000 to 400 cm^{-1}.

2.2.5. Molecular weight determination

The molecular characteristics based on the absolute weight-average molecular weight (*Mw*) of polysaccharide were analyzed by high-performance size-exclusion chromatography (HPSEC) attached to a multiangle laser-light scattering (MALLS) and refractive index (RI) detector (mini-Dawn®, Wyatt, Milford, MA, USA). The polysaccharides (1 mg/mL *w*/*v*) were dissolved in 100 mM $NaNO_3$, filtered through a 0.2 μm membrane, and injected at 25 °C. The RI increment (*dn*/*dc*: 0.147 mL/g) utilized in the present study, was an average of different *dn*/*dc* values employed in polysaccharides from several algae (Ammar et al., 2015; Geresh, Adin, Yarmolinsky, & Karpasas, 2002; Holtkamp, Kelly, Ulber, & Lang, 2009; Sabboural et al., 2014).

2.2.6. Antioxidant activity of microalgal sulfated polysaccharides

2.2.6.1. DPPH (2,2-diphenyl-1-picrylhydrazyl radical scavenging activity assay). This assay involved the scavenging of stable DPPH radicals by the radical scavenging components of sulfated polysaccharides from *Navicula* sp. cultivated at the three wavelengths. An ethanolic DPPH solution (0.1 mM) was prepared. An aliquot of each sample (25–200 μg/mL) was added to DPPH solution (1:1 v/v) (Hou, Hsu, & Lee, 2002). The absorbance was measured at 517 nm in a GENESYS™ 10UV spectrophotometer (Thermo Scientific), after incubation for 30 min in the dark at room temperature. Vitamin C was used as a positive control. Measurements were performed in triplicate. The scavenging activity of DPPH radicals by the sulfated polysaccharides was calculated according to the next equation:

$$\text{DPPH} - \text{scavenging activity}\ (\%) = \left[1 - \left(A_{\text{sample517nm}} - A_{\text{blank517nm}}\right) \Big/ A_{\text{control517}}\right] \times 100$$