Rev. Bras. Eng. Pesca (2024) 15 (1): 34-46
DOI: 10.18817/repesca.v15i1.3600

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Arthrospira platensis CULTURED IN TILAPIA EFFLUENT WITH LOW SALINITY PRODUCES ANTIOXIDANT SULFATED POLYSACCHARIDES

Arthrospira platensis CULTIVADA EM EFLUENTE DE TILÁPIA COM BAIXA SALINIDADE PRODUZ POLISSACARÍDEOS SULFATADOS ANTIOXIDANTES

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Recebido: (10/02/2024) / Publicado: (18/07/2024)

Abstract *Arthrospira platensis* produced in tilapia effluent would yield sulfated polysaccharides (ApSPs). This study analyzed ApSPs by two extraction periods (3 or 24 h) on yield, physical-chemical features and *in vitro* antioxidant effects. Dehydrated biomass from 10‰ organic system was extracted for ApSPs, with papain, in 100 mM sodium acetate buffer (pH 5)+cysteine/EDTA, both 5 mM. After filtration, ApSPs precipited and washed with alcohol were characterized by electrophoreses in agarose/polyacrylamide gels using known glycosaminoglycans and then ApSPs stained with toluidine blue and combined or not with Stains-All. Infrared structurally evaluated the ApSPs. Antioxidant effects were *in vitro* assayed by DPPH and total antioxidant capacity (TAC) methods using BHT and ascorbic acid as references. Yields differed no between periods (4.3±1.22-3h, 4.57±0.57%-24h) and molecular analyses revealed spirulan-type ApSPs with regularity in charge/size and functional groups between samples. ApSPs showed, concentration-dependent, effects preponderant by DPPH method than TAC one, with only ~50% reduction (4 mg mL^{-1}) *vs.* BHT (100% inhibition, 4 mg mL^{-1}). Thus, extraction by 3 h optimized ApSPs from massive cultivation in tilapia salinized effluent.

Key words: organic culture; cyanobacterium; glycans; oxidative stress.

Resumo *Arthrospira platensis* produzida em efluente de tilápia renderia polissacarídeos sulfatados (ApPSs). Analisou-se dois períodos de extração (3 ou 24 h) de ApPSs sobre rendimento, características físico-químicas e efeitos antioxidantes *in vitro*. Biomassa desidratada de sistema orgânico 10‰ foi extraída para ApPSs, com papaína, em tampão acetato de sódio 100 mM (pH 5)+cisteína/EDTA, ambos 5 mM. Após filtração, ApPSs precipitados e lavados com álcool foram caracterizados por eletroforeses em géis de agarose/poliacrilamida usando glicosaminoglicanos conhecidos e, após, ApPSs corados com azul de toludina combinado ou não com "Stains-All". Infravermelho avaliou estruturalmente os ApPSs. Efeitos antioxidantes foram ensaiados *in vitro* pelos métodos DPPH e capacidade antioxidante total (CAT) usando BHT e ácido ascóbico como padrões. Redimentos não diferiram entre períodos (4,3±1,22-3h; 4,57±0,57%-24h) e análises moleculares revelaram ApPSs tipo espirulana com regularidade em carga/massa e grupos funcionais entre amostras. ApPSs mostraram, dependente de concentração, efeitos preponderantes pelo método DPPH que CAT, apenas com redução de ~50% (4 mg mL^{-1}) *vs.* BHT (inibição 100%; 4 mg mL^{-1}). Assim, extração por 3 h otimizou ApPSs de cultivo massivo em efluente salinizado de tilápia.

Palavras-Chave: cultura orgânica; cianobactéria; glicanos; estresse oxidativo.

Introduction

Sulfated polysaccharides (SPs) represent a diverse group of anionic polymers which are widely found in nature, occuring since bacteria and algae to animals (known as glycosaminoglycans) (Pomin & Mourão, 2008; Rodrigues et al., 2009, 2016; Ai et al., 2023) and some other photosyntetic organisms (Aquino et al., 2005; Silva et al., 2012). Those from seaweeds are named as galactans in Rhodophyta (Cardozo et al., 2007), as fucans or fuicodans in Ochrophyta (Pomin & Mourão, 2008, Pomin, 2012); and as hetopolysaccharides (usually ulvan) in Chlorophyta (Wang et al., 2014), naturally ocurring in the extracelular matrix playing structural and physiological roles (Pomin & Mourão, 2008). These glycans are generally of high molecular sizes (>100 kDa) and show structural and biological diversities with high content of ester goups (sulfation) on their chains leading to binding affinities in molecular interactions of specific sulfation sites (Pomin, 2012). As a consequence of their high electronegatively, SPs are biologically actives, for example, as anticoagulant (Pomin, 2012), antinociceptive, anti-inflammatory (Rodrigues et al., 2012) and antioxidant (Alencar et al., 2019) agents; and showing physical-chemical properties for industrial applications, *e.g*., as texturing agents for foods (Cardozo et al., 2007).

In recent decades, the increase of oxidative stress-associated human diseases caused by free-radicals generated from the normal cell metabolism or by exogenous sources, which the reative oxygen species (ROS) formed attack many biological molecules (Barbosa et al., 210), has indicated the need for rich sources in alternative antioxidants capable of reduzing the risks of various pathogenesis, *e.g*., carcinogenesis, atherosclerosis, DNA damage and degenerative events (Wang et al., 2014; Ai et al., 2023). Such importance is also a safe strategy to synthetic ones traditionally used against ROS and in processed foods. These agents have the ability to inhitit or scavenging the ROS formation (Cardozo et al., 2007; Wang et al., 2014). By contrast, some of them are known as toxics (butylated hydroxyanisole, butylatedhydroxytoluene-BHT and tertiary butylhydroquinone).

Considerable attention has been given to natural antioxidants from diverse origins (Wang et al., 2014; Jridi et al., 2019; Ai et al., 2023), showing safety, potency and/or production perspective. SPs act not only as free-radical scavengers *in vitro,* but also as *in vivo* antioxidants to mitigate oxidate damage (Alencar et al., 2019). For instance, Costa et al. (2010) examined SPs extracted from 11 species of topical seaweeds (including red, green and brown specimens) and observed that all they showed *in vitro* antioxidant effects considerating total antioxidant capacity (TAC), power reducing and ferrous chelating. SPs from the red seaweed *Hypnea musciformis* had the scavenging ability on hydroxyl radicals as reported by Alves et al. (2012). While Costa et al. (2012) demonstrated that the SPs from *Caulerpa cupressoides* var. *flabellata* (Chlorophyta) exhibited no *in vitro* effect in hydroxyl radical scavenging, but they showed TAC and ferrous chelating properties. Crude SPs from the vascular plant *Halodule wrightii* showed considerable antioxidant effects by TAC and free-radical scavenging tests (Silva et al., 2012). More recently, SPs isolated from the skin of Nile tilapia, *Oreochromis niloticus*, showed significant antioxidant actions, concentration-dependent, mainly in the *in vitro* ferrous chelating assay (Nascimento et al., 2021).

Arthrospira platensis ex Gomont, 1892, popular known as *Spirulina platensis*, is a blue-green cyanobacterium belonging to the Oscillatotiales order. It is morphologically characterized by a single or multicellular filamentous structure of cylindrical shape and regular spiral. This prokaryotic organism is widely found in alkaline sail lakes growing like a green cream on the surface of the water (Ai et al., 2023). Due to its rapid growth, *A. platensis* has been cultivated commercially for biotechnological and industrial purposes (Ai et al., 2023). Because of its versatility to environmental adversities, it can also be adapted to low salinity conditions with efficiency in removing ammonia compounds from water, even those generated by fish farming (Nogueira et al., 2018). Phytochemical studies demonstrated it as a rich source in diverse vital ingredients (*e.g*., SPs, proteins, vitamins, minerals and pigments) for human health-related use (Ai et al., 2023; Saboya et al., 2017; Wu et al., 2017). Majdoub et al. (2009) experimentally cultured *A. platensis* in an old

culture medium and its SPs obtained by extensively ultrafiltration were *in vitro* tested for their anticoagulant effects. Other SPs isolated from *A. platensis*, by different extraction/purification protocols, were already described with multiple bioactivities, including antioxidant, antiviral and immunomodulatory effects (Ai et al., 2023; Wu et al., 2017).

The current study was designed to obtain, by two extraction times, and physically-chemically/structurally compare antioxidant SPs extracted from *A. spirulina* biomass (ApSPs) produced in tilapia effluent with low salinity and alkaline pH, when in a boxes system during the biological treatment by this cyanobacterium.

Material and Methods

Obtaining of *A. platensis* biomass from fiber boxes system

The experimental system was based on Nogueira et al. (2008). It consisted of 500 L fiber boxes containing a *pool* of tilapia (*O. niloticus*) culture's effluent derived from regular cultivation of this fish species at the Aquaculture station of the Federal University of Ceará (FUC). *A. platensis* was inoculed and mantained in open environment under sunlight and circulation of the water induced by a submerged pump applicating a flow rate of 1,000 L h^{-1}. The effluent water of the system was renewed every 24 h and the abiotic parameters (pH 10 and 10‰ salinity) monitored daily. The cultivation structure (Figure 1A) was installed at Aquaculture Biotechnology Center, Department of Fisheries Engineering, FUC.

Figure 1. General image of the cultivation system of fiber boxes for *A. platensis* production in tilapia culture's effluent (A). Cyanobacteria biomass collected using nylon screen (B) to obtain a fresh pasta (C).

The fresh biomass was then filtered using a nylon tissue (Figure B) and the pasta sample obtained (Figure C) taken from the Planctology laboratory to the Marine Biochemical laboratory in which the material was extensivelly washed with distillated water and further frozen at – 20°C. The use of *A. platensis* was authorized through our registration with SISGEN (Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado).

Extraction of ApSPs

The dehydrated *A. platensis* biomass in air drying oven (45°C, 24 h) was obtained (granulated flour) and the SPs extraction based on Rodrigues et al. (2009)' method, as illustrated in figure 2. Briefly, 4 g sample was suspended in 100 mL of 100 mM sodium acetate buffer (pH 5.0) containing crude papain (50 mg g $tissue^{-1}$), 5 mM EDTA, and 5 mM cysteine, and incubated at 60°C for two

times (3 or 24 h). The incubation mixture was then filtered using a nylon screen and the supernatants were saved. SPsAp that were present in solution were precipitated with cold commercial ethanol in a ration of 3:1 at 4°C for 24 h. The mixture was then centrifuged at 9.560 × *g*, for 20 min. The *pellet* containing the ApSPs was washed twice with 100 mL of 80% ethanol, and once with the same volume of commercial ethanol. After each centrifugation (9.560 × *g* for 20 min), the ApSPs were finally dried using an air drying oven (60°C, 24 h) and the yields were expressed as the percentage (%, n = 3) of the dehydrated matter. The crude extracts obtained in both periods were named as ApSPs-3 and ApSPs-24, respectively.

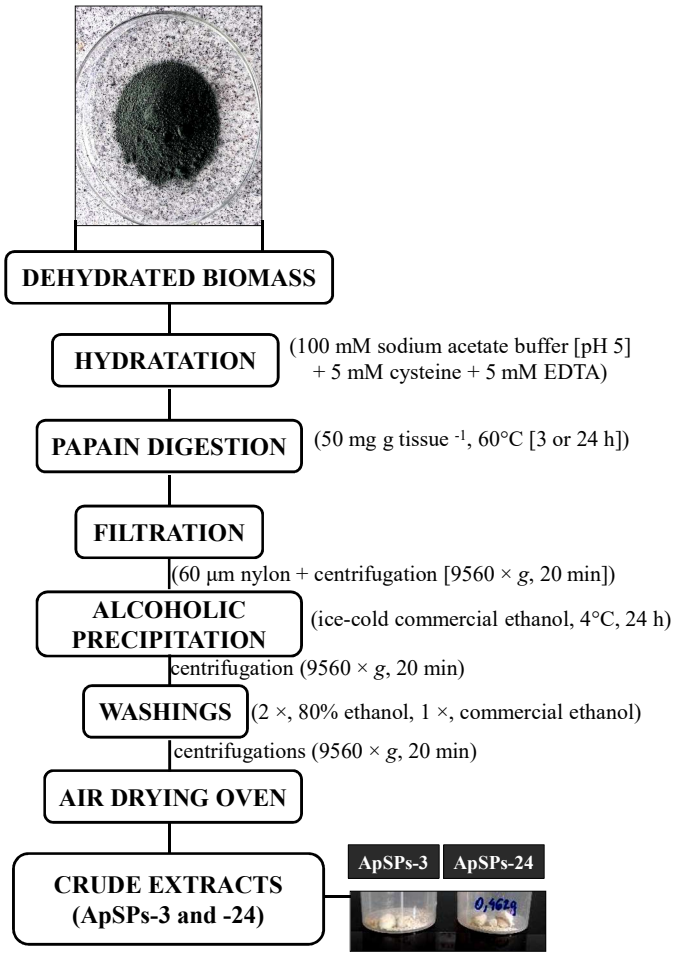

Figure 2. Scheme of obtaining of SPs from the cyanobacterium *A. platensis*.

Analysis by mean of agarose / polyacrylamide gels electrophoreses

Electrophoresis in agarose gel was conducted in order to verify the polydispersion pattern and charge density of ApSPs. In this procedure, samples were applied to a 0.5% agarose gel prepared with 0.05 M 1,3-acetate diaminopropane buffer (pH 9.0) and the run was carried out at constant voltage (100 V, 1 h). After the run, the ApSPs present in the gel were fixed with 0.1% *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide solution for 24 h and then dehydrated (Dietrich & Dietrich, 1976).

Electrophoresis in polyacrylamide gel was carried out to examine the apparent molecular mass distribution of ApSPs. For this, the samples were applied to a 6% polyacrylamide gel using 0.02 M Tris/HCl buffer (pH 8.6) and the run was performed at 500 mA for 1 h as described by Rodrigues et al. (2016).

The ApSPs present in both gels were revealed with 0.1% toluidine blue or Stains-All cationic reagent and, subsequently, the gels were destained with a solution containing absolute ethanol, distilled water and acetic acid or using distilled water only. As known markers of molecular mass, chondroitin-6-sulfate (C-6-S,~ 60 kDa), chondroitin-4-sulfate (C-4-S,~ 40 kDa), sulfated dextran (DexS,~ 8 kDa), dermatan sulfate (DS,~ 40 kDa) and/or UHEP (~ 15 kDa) were used as comparison (Andrade et al., 2017; Volpi & Maccari, 2002).

Fourier Transform Infrared (FT-IR) spectroscopy

The values of FT-IR were obtained using a spectrometer (IRPrestige-21 Shimadzu, Japan). For each measurement, 10 mg of ApSPs samples were pressed in potassium bromide (KBr) *pellets*. The measurements were performed at a resolution of 4 cm^{-1}, with 64 scans min^{-1} at 500-4000 cm^{-1}.

***In vitro* antioxidant assays**

1,1-diphenyl-2-picryl-hydrazil (DPPH) scavenging effect

The effect of ApSPs to reduce DPPH was performed according to Blois (1958), with some modifications. In this assay, different concentrations of ApSPs (0.125 to 4.0 mg mL^{-1}) were added to the methanol solution of DPPH (75 M). After 30 min, absorbance was measured at 517 nm. All reactions were performed in triplicates and BHT was used as a reference.

The DPPH scavenging effect was calculated using the following equation: scavenging activity (%) = [A0− (A − Ab)/A0] × 100, where A0 = DPPH without sample; A = sample + DPPH; and Ab= sample without DPPH.

Total antioxidant capacity (TAC)

This assay was performed by the formation of the phosphomolybdate complex, based on Prieto et al. (1999). ApSPs (0.125 to 4.0 mg mL^{-1}) were added to a solution containing ammonium molybdate (4 mM), sulfuric acid (0.6 M), and sodium phosphate (28 mM), and were incubated at 95°C for 90 min. Absorbance was measured at 695 nm. All reactions were performed in triplicate and a 200 g mL^{-1} sample of ascorbic acid (AA) was used as a positive control and considered as 100% TAC.

The data were expressed as a percentage of TAC using the following formula: TAC (%) = $[(A_{sample}-A_{blank}) / (A_{ascorbic\ ac}-A_{blank})] \times 100$.

Statistical analyses

The data were expressed as mean ± standard deviation (n = 3). Values of extraction yield of ApSPs were subjected to *t*-Studant test consideranting $p < 0.05$ as significant. For *in vitro* antioxidant assays, data were analyzed by one-way ANOVA, followed by Tukey' test, with $p < 0.05$ as statistically significant. The graphical representations were also constructed using the Origin software version 8.0 as the Statistical Analysis Software (USA).

Results and Discussion

Extraction yield of ApSPs

The extraction analysis from dehydrated *A. platensis* biomass (granulated flour) digested with nonspecific protease (papain), followed by alcoholic precipitation, led to a similar yield of crude extracts containing SPs ($p > 0.05\%$), whose values were of 4.30 ± 1.22 and 4.57 ± 0.57% after 3 or 24 h (ApSPs-3 and -24) times, respectively, as shown in table 1. It was revealed that the prolonged period to obtain its SPs was not crucial by this extraction condition, possibly reflecting a peculiarity of the method or the unicellular form of the cyanobacterium comparing to other sources and strategies (Table 1).

Table 1. Yields of SPs extracted from *A. platensis* produced in tilapia effluent *vs.* other sources.

Organism	Crude SPs	Protocol / time	Tissue	Yield	Reference
A. platensis	ApSPs-3	papain+alcohol precipitation/3 h	granulated flour	4.30±1.22%[a]	current study
	ApSPs-24	papain+alcohol precipitation/24 h		4.57±0.57%[a]	
B. occidentalis (Pacheco beach)	SPs	papain/24 h	tritured	4.00%	Farias et al. (2000)
Hypnea musciformis (Flecheiras beach)	SPs	papain+cetylpiridinium choride precipitation/24 h	tritured	49.05±0.38%	Rodrigues et al. (2011)
	SPs	hot water (80°C)+alcohol precipitation/4 h		4.28±0.26%	
Acanthophora muscoides (Pacheco beach)	SPs	papain+cetylpiridinium choride precipitation/24 h	tritured	17.00% 4.65% 1.82%	Rodrigues et al. (2016)
Oreochromis niloticus (cultivation)	SPs	Papain+cetylpiridinium choride precipitation/24 h	tritured	0.22%	Nascimento et al. (2021)

Yield was calculated as percentage (%) with basis of the dehydrated cyanobacterium biomass; Similar letters between the lines indicate no significative difference at level of 5% (*t*-Studant' test, $p < 0.05$).

The potential of *A. platensis* to yield polysaccharides varies (15-20%, w w^{-1}) with the cultivation system and/or the extraction approach (*e.g.*, alkali, water, ultrasonic, microwave and enzyme) used based on a specific review of Ai et al. (2023). Under our cultivation conditions (Figure 1), cyanobacterium-derived SPs extraction yielded ~3.48-fold lower than the respective literature. The yield of cultured ApSPs, however, was similar to that found in *B. occidentalis* (Rhodophyta) collected from natural bed; but, it importantly differed between protocols (enzyme *vs.* hot water) to obtain SPs from *H. musciformis* specimens (Rhodophyta) experimentally cultured in the littoral region (Table 1). Brazilian samples of *Caulerpa racemosa* (Chlorophyta) collected from natural habitat, digested with papain + alcoholic precipitation, yielded a greater amount of SPs than the molecules enzymatically extracted and precipitated with cetylpiridinium chloride (Rodrigues et al., 2009). Skin of tilapia (*O. niloticus*) digested with papain (24 h, 60°C), followed by precipitation with cetylpiridinium chloride, yielded~19-fold lower in SPs than *A. platensis* (Table 1).

Curiously, both periods (3 or 24 h) employed to extract ApSPs had no impact on the yield (Table 1). It was speculated that the unicellular microscopic structure of *A. platensis vs.* other multicellular organisms could be discussed (Ai et al., 2023; Rodrigues et al., 2016), since that the use of different extraction conditions and/or organic solvents are also criticals to optimize results in biotechnological processes (Rodrigues et al., 2011, 2016; Jridi et al., 2019). It has been accepted that the use of alcohol is an eco-friendly solvent of low cost to precipite raw materials (green-based method) (Ai et al., 2023), but with relatively low efficiency to recover SPs (Rodrigues et al., 2009; 2011). Based on this trend, both extracts (ApSPs-3 and -24) were further characterized by electrophoretic and FT-IR techniques.

Electrophoretic characterization of the ApSPs extracts

The analysis of ApSPs by agarose / polyacrylamide gels electrophoreses is shown in figure 3. The electrophoretic composition of both extracts obtained from 3 or 24 h extraction times was

initially observed on agarose gel, using diaminopropane buffer system, and distinct patterns of resolution, according to each staining procedure applied, was noted.

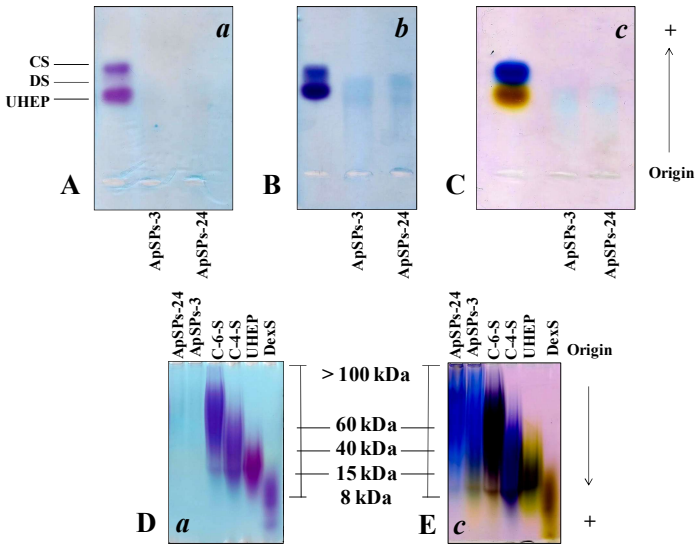

Figure 3. Agarose (A, B, C) / polyacrylamide (D, E) gels electrophoreses of *A. platensis* SPs (ApSPs-3 or -24) obtained from tilapia effluent, and standards chondroitin-6-sulfate (C-6-S,~60 kDa), chondroitin-4-sulfate (C-4-S,~40 kDa), dextran sulfate (DexS,~8 kDa), dermatan sulfate (DS,~ 40 kDa) and unfractionated heparin (UHEP,~15 kDa) present on gels were stained with 0.1% toluidine blue (*a*), combined (*b*) or not (*c*) with Stains-All.

In the treatment with the cationic dye toluidine blue (Figure 3A), the method virtually detected the presence of polydisperse spots on agarose gel; therefore, suggesting relatively *poor* samples in ester groups in their structures *vs.* other sources (Rodrigues et al., 2016; Nascimento et al., 2021). It supported two hypotheses: 1) the low efficacy of the alcohol solvent to concentrate SPs (Rodrigues et al., 2009; 2011) because its addition to material could be alter the dielectric constant of water (Costa et al., 2012); or 2) *A. platensis* cultivation in tilapia effluent water with low salinity impacted its biosynthesis of SPs based on Aquino et al. (2005) and Silva et al. (2012) discussing Brazilian seagrasses.

Combined toluidine/Stains-All staining particulary revealed blue polydisperse bands with high more sensivities on agarose gel, showing electrophoretic mobility as UHEP (Figure 3B). This behaviour suggested that both ApSPs samples were able to complex with the diamine contained in run buffer due to their close vicinal charges of the here extracted compounds and, possibly, they having the same charge/mass ratio (Costa et al., 2012; Dietrich & Dietrich, 1976). Addictionally, it allowed us to note that other non charged sugars (at high level of carboxylated groups) were also presents in both analyzed preparations, revealing a more stable and visible complex (Volpi & Maccari, 2002), as in animals-derived (glycosaminoglycans) SPs which present uronic acid residues in their structures (Dietrich & Dietrich, 1976; Nascimento et al., 2021). By contrast, both samples on agarose gel stained with Stains-All alone exhibited bands as a color-code for cyan, suggesting the preponderance of uronic acid residues making part of the polymer based on review of Ai et al. (2023). For plant, Souza et al. (2015) detected blue polydisperse bands regarding the presence of hexuronic acid in *Geoffroea spinosa* polysaccharides, when revealed with Stains-All alone.

Majdoub et al. (2009) laboritorially produzed *A. platensis* in a 30 days old culture medium and analyzed for SPs. Electrophoresis in acetate cellulose separated the material in two main bands of crude SPs. Crude exopolysaccharide sample (PUF) comigrated as CS, whereas its fractions (PUF1

and PUF2), obtained by ion-exchange chromatography, showed mobility as less far than hyaluronic acid and as CS, respectively.

On the basis of our results, it was speculated, at initial level, that the cyanobacterim *A. platensis*, when cultured in a tilapia effluent-euriched system with low salinity (Figure 1), produzed uronic acids-rich SPs as visualized in cyan and with electrophoretic profile similar to that of UHEP (Figure 3C).

From polyacrylamide procedure both samples (ApSPs-3 and -24) were also examined regarding their molecular distribution using different standard glycosaminoglycans of known molecular masses. As expected, the results showed that both ones were almost invisible on gel stained with toluidine blue *vs.* standards that revealed strong metachromatic properties (Figure 3D). These observations confirmed those from the agarose gel analysis, as already mentioned (Figure 3A). On the other hand, only use of Stains-All staining reacted with all SPs by a particular level of color on gel (Andrade et al., 2017), as visualized in figure 3E. Based on each SP with specific colors was useful in identifying of the ApSPs extracted in this study revealing intense cyan like hyaluronic acid (Andrade et al., 2017).

The samples of ApSPs-3 and -24 were characterized as a heterogeneous system to be of molecular distribution from ~40 to 100 kDa in comparison with CS standard (Figure 3E). Majdoub et al. (2009) estimated a molecular mass of 165 kDa, by size exclusion chromatography, for SPs obtained from *A. platensis*. Wu et al. (2017) used three separation approaches (sevage deproteinization and column chromatography, ethanol-ammonium sulfate aqueous two-phase system and column chromatography, and one-step high speed counter-current chromatography with ethanol-ammonium sulfate) for water-soluble SPs from *A. platensis* and compared their efficiencies. The authors found a molecular weight SP of 12.33 kDa through gel chromatography. Structurally, seaweeds-derived SPs are usually > 100 kDa, therefore, high molecular sizes (Pomin & Mourão, 2008; Pomin, 2012; Wang et al., 2014).

Under our experimental conditions (Figure 1) and considerating both electrophoretic investigations (Figure 3), *A. spirulina* would also express acid SPs of intermediate molecular masses (< 100 kDa). Focusing on structural aspect, the ApSPs were then evaluated regarding their structural features by FT-IR analysis.

Structural characterization by FT-IR spectroscopy

In order to identify the functional groups present in each sample (APSPs-3 and -24), FT-IR study was carried out using KBr *pellets*. It has proved as a useful tool to offer data regarding structural similarities between SPs from diferent organisms (Cardozo et al., 2007; Alves et al., 2012; Costa et al., 2012 Jridi et al., 2019).

In this sense, structural features were identified and compared with other authors that explored *A. platensis* SPs (Majdoub et al., 2009; Wu et al., 2017; Ai et al., 2023), as well as between the spectra of ApSPs-3 and -24, obtained by both extraction times, respectively, as illustrated in figure 4.

FT-IR spectra of the crude samples (ApSPs-3 and -24) suggested acid polysaccharides of sugar chains containing low intensity signals of ester radicals making part of both structures (Figure 3). Thus, characteristic absorptions of major bands were attributed at 3417-3419 cm^{-1} and 1637-1647 cm^{-1} for O-H stretching and OH bend, respectively (Silva et al., 2012; Wu et al., 2017; Jridi et al., 2019). Addictionally, around 2929-2933 cm^{-1} both samples evidenced bands from the C-H (Costa et al., 2012).

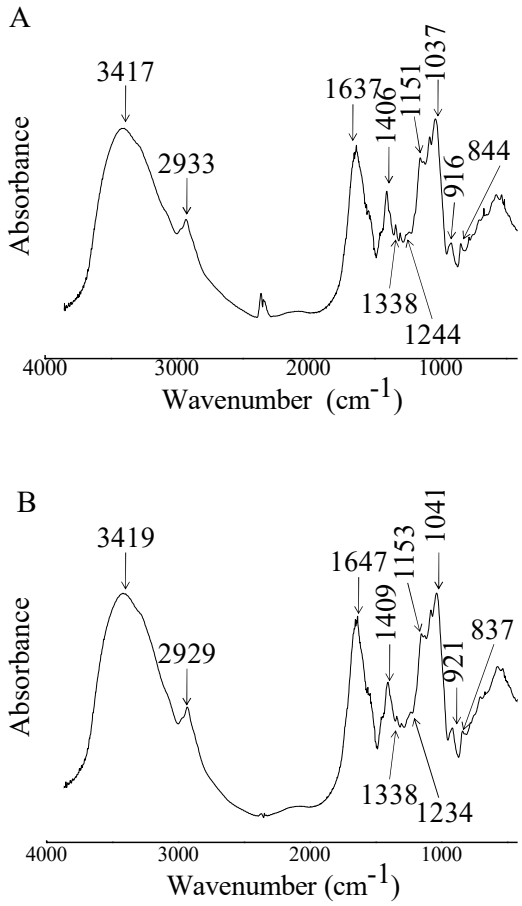

Figure 4. FT-IR spectra of the *A. platensis* SPs (ApSPs-3 [A] and -24 [B]) at 500-4000 cm^{-1}.

More relevant was the signals at 1406-1409 cm^{-1} (O-C=O bending) (Majdoub et al., 2009; Wu et al., 2017) and 1234-1244 cm^{-1} (S=O asymmetric stretching) (Costa el., 2012; Wu et al., 2017; Alencar et al., 2019; Jridi et al., 2019) corresponding to a high concentration for uronic acids than ester groups from the analyzed polymer samples. It confirmed, therefore, those previous results by electrophoreses before mentionated (Figure 3) and supported a particular structural level of the ApSPs obtained, when the cyanobacterium was cultured using organic medium with low salinity (Figure 1), contrasting other reports (Majdoub et al., 2009; Wu et al., 2017; Ai et al., 2023).

The abundance of uronic acids determined by FT-IR has been reported in *A. platensis* SPs composition (Majdoub et al., 2009; Ai et al., 2023). Still important, it was observed at 1037-1041 cm^{-1} (C-O-C), 1078-1080 cm^{-1} (C-OH) and 1151-1153 cm^{-1} (C-C) ring vibrations in the osidic cycles (Majdoub et al., 2009), as well as at 837-844 cm^{-1} (4-sulfate) related to the occurence of sulfated rhamnose residues as substituents (Majdoub et al., 2009; Costa et al., 2012; Wu et al., 2017).

Collectivelly, our findings partially indicated that the ApSPs extracted with papain (3 or 24 h) + alcoholic precipitation were similar to a spirulan-type SP based on Majdoub et al. (2009), but with lower sulfate levels in their structures. Furthermore, these results evidenced a structural regularity between both samples of the sugar chain of polysaccharides as important for *in vitro* antioxidant studies.

In vitro antioxidant effects of ApSPs

The *in vitro* antioxidant properties of living organisms-derived SPs have been explored by different methods (Ai et al., 2023), measuring their effects under three stages (initiation, propagation and termination) (Silva et al., 2012). Current study used only two *in vitro* assays (DPPH-scavenging and TAC) which are related to initial phase of the oxidation process (Alves et al., 2012; Alencar et al., 2019; Costa et al., 2010; Costa et al., 2012; Silva et al., 2012; Nascimento et al., 2021), whose antioxidant responses are shown in figures 5 and 6.

DPPH assay

In the DPPH-scavenging assay (Figure 5), in which both samples of ApSPs- and -24 were tested at various concentrations (0.125 to 4 mg mL^{-1}), it showed that the acid SPs-rich extracts scavenged the DPPH radical in a similar dose-dependent manner between them, but the profile of antioxidant action differed ($p < 0.05$) over > 50% at 4 mg mL^{-1} for APSPs-3, although less potent than BHT systhetic antioxidant (100% scavenging, at 1-4 mg mL^{-1}) used as standard.

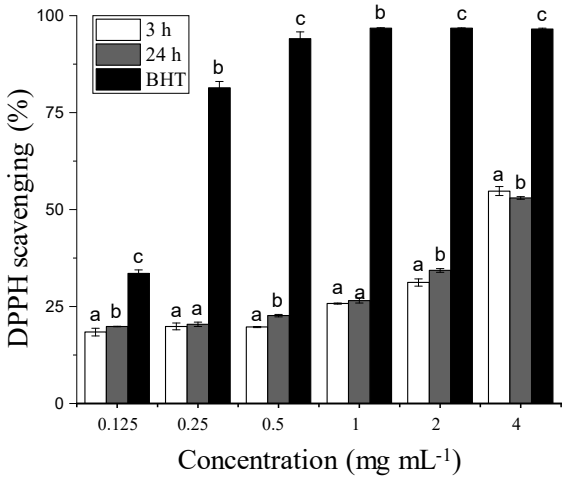

Figure 5. Effects of *A. platensis* SPs extracted after 3 or 24 h (ApSPs-3 or -24) with scavenge ability on DPPH free radicals. Different letters among the bars indicate significative difference at level of 5% (ANOVA, followed by Tukey' test, $p < 0.05$).

Although revealing that both *A. platensis* crude extracts were rich in acid polysaccharides with low sulfate content (Figures 3 and 4), our results were consistents with other studies where SPs isolated from different origins inhibited the free radicals by DPPH test, including extract and/or fraction (Alencar et al., 2019; Costa et al., 2012; Silva et al., 2012; Jridi et al., 2019; Nascimento et al., 2021). This property is displayed by a mechanism by which the sulfate radicals of the polysaccharide donate electrons and produce a reducing power (Jridi et al., 2019). As the electronegativity (sulfation degree) and molecular weight of the SPs have a positive correlation in some bioactiviteis (Pomin & Mourão, 2008; Pomin, 2012); however, the antioxidant effects may not always be linked to such issues (Alves et al., 2012).

In fact, different *A. platensis* source or extraction method would determine conditions to obtain different antioxidant molecules, as herein described. It is possible that specific sites of uronic acid residues present in ApSPs would contribute for antioxidant action, as reviewed by Ai et al. (2023). On this view, there was a report on bioactive nonSP, rich in hexuronic acid, isolated from a higher plant that reduced thromboembolic events without hemorrhage *in vivo* (Souza et al., 2015). The ApSPs were more effectives at high concentration (Figure 5), similary to those (uronic acid-containing polysaccharides) of *O. niloticus* skin when in a dose-dependent relationship, but they reduced only in 30.26% using DPPH method (Nascimento et al., 2021).

TAC assay

Regarding the TAC test, ApSPs were able to virtually reduce Mo to form a green phosphate/Mo complex total (Prieto et al., 1999), as shown in figure 6. Both extracts (ApSPs-3 and -24 h) exhibited lower TAC potential, with reduction rate only up to ~10% at concentration of 4 mg mL^{-1} than ascorbic acid (AA) standard (100% inhibition, at 2 mg mL^{-1}) and other sources in SPs, such as those from *O. niloticus* skin (25% inhibition, at 4 mg mL^{-1}) (Nascimento et al., 2021) and from Rhodophyta *G. caudata* (~90% inhibition, at 4 mg mL^{-1}) (Alencar et al., 2019). Costa et al. (2010) also detected *in vitro* effects of TAC reduction in SPs isolated from 11 macroalgae, when examined at concentration of 2 mg mL^{-1}.

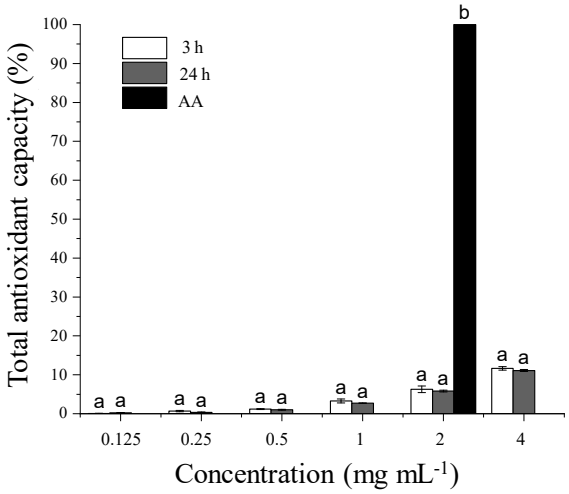

Figure 6. Effects of *A. platensis* SPs extracted after 3 or 24 h (ApSPs-3 or -24) on TAC. Different letters among the bars indicate significative difference at level of 5% (ANOVA, followed by Tukey' test, $p < 0.05$).

Comparing both antioxidant tests, it was concluded that the ApSPs had specific ability in the DPPH-scavenging assay than TAC one. This behaviour has been found for some SPs (Costa et al., 2010; Nascimento et al., 2021). Therefore, these variations in the bioactivity of these polymers are the result of their structural heterogeneities found in nature (Pomin & Mourão, 2008, Costa et al., 2010; Pomin, 2012; Wang et al., 2014). Factors such as sterospecific features, monossacaridic composition, glycosylation sites, aromericity, and spartial conformation are important and their biological actions not occur as a mere consequence of their charges (Pomin, 2012). However, we can not discard that the presence of contaminant with ApSPs structure may influence their bioactivities (Rodrigues et al., 2009, 2011), since that phenolic compounds displaying antioxidation can also be found (Ai et al., 2023) to support our partial conclusions. It has been accepted that protease removed proteins during preparation of ApSPs-3 and -24 (Rodrigues et al., 2011; 2016).

Given the yield, structural and bioactivity analyses of SPs, it is possible to experimentally produce the cyanobacterium *A. platensis* in tilapia effluent water with low salinity and alkaline medium to obtain antioxidant SPs from the dehydrated raw biomass. More relevant was the fact that, regardless of the extraction condition, these molecules presented almost identical structural regularity and antioxidant action of uronic acid-rich SPs. It can be speculated that the same sites of action may be involved in the reducing potential, which could favor the massive production of this cyanobacterium in less favored regions as a nutritional source with benefits to human and animal health.

Conclusion

The study revealed that the cultivation model in the open environment using a fiber boxes system for *Arthrospira platensis* massive production in tilapia culture' (10‰) salinized effluent contributed to the knowledge of its biochemistry of carbohydrates, revealing a spirulan-type particular acidic sulfated polysaccharide with antioxidant action and optimal extraction required only 3 h according to the chemical analyses.

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