LWT - Food Science and Technology 230 (2025) 118239

Contents lists available at ScienceDirect

LWT

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

Extraction and characterization of cushuro (*Nostoc sphaericum*) polysaccharides

N. Chasquibol [a,*], A. Sotelo [a], M. Tapia [a], Y. Paredes [a], A. Sajid [b], S. Sahin [b], M. Wu [b], F.M. Goycoolea [b,c,**]

[a] Universidad de Lima, Instituto de Investigación Científica, Grupo de Investigación en Alimentos Funcionales, Ingeniería Industrial, Av. Javier Prado Este, 4600, Fundo Monterrico Chico, Surco, Lima, Peru
[b] School of Food Science & Nutrition, University of Leeds, LS2 9JT, Leeds, United Kingdom
[c] Faculty of Biology, Department of Cell Biology and Histology, Universidad de Murcia, Campus de Espinardo, Murcia, 30100, Spain

ARTICLE INFO

Keywords:
Polysaccharide
Cushuro
Nostoc sphaericum
Extraction optimization
Rheology

ABSTRACT

Cushuro (*Nostoc sphaericum*) is a blue-green edible freshwater microalgae of ancestral use as a traditional food in the Andean regions of Peru. Knowledge of the extraction process conditions and characteristics of cushuro biomass polysaccharides are instrumental to expand its range of applications and appraise its potential utilization as a hydrocolloid ingredient in food and other applications. Polysaccharides acid extract (PCAcE) and polysaccharides aqueous extract (PCAqE) with hot water at 90 °C were extracted from cushuro (*Nostoc sphaericum*) biomass collected from the Department of Ancash, Peru. PCAcE showed higher extraction yield (49.0 ± 0.5 %), purity (83.3 ± 0.3 %), emulsifying capacity (55.6 ± 1.0 %), foaming capacity (8.3 ± 0.3 %), water holding capacity (3.0 ± 0.11 g/g) and DPPH radical scavenging activity (73.0 %) than PCAqE. Also, FTIR spectra showed a typical polysaccharides' fingerprint. PCAcE showed a Mw of 1.0 ± 0.4 × 10^5 Da, dispersity index (Đ) of 3.5 ± 0.4, radius of gyration (Rg) of 22 ± 5 nm and a branched-type conformation, characterized by multi-detection HPSEC. The monosaccharide composition of PCAcE consisted mainly of glucose, xylose and galactose, and lower amounts of rhamnose. The rheological properties in aqueous solution, investigated under sensitive small-deformation oscillatory rheometric, revealed that the polysaccharide acidic extract (PCAcE) showed "weak gel" properties, unexpected on a typical highly branched polysaccharide. These results suggest potential applications of cushuro (*Nostoc sphaericum*) polysaccharides as a viscosity enhancer and natural emulsifier to stabilize oil-water emulsions in foods and other applications.

1. Introduction

Polysaccharides have emerged as an important class of biopolymers due to their potential applications in different fields such as foods, pharmaceuticals, or the remove of heavy metal in wastewater from wastewater (Shen et al., 2019). These are a consequence of their techno-functional properties (water-holding capacity, oil-holding capacity) and biological activities (*e.g.*, anticancer, antioxidant, antitumor, immunomodulatory, etc.) Soua et al., 2020. Polysaccharides form gels or viscous solutions of great industrial value and are incorporated in cooked processed products to increase their yield, improve water holding capacity, modify texture, reduce formulation costs and improve the stability of emulsions (Shen et al., 2019). These applications of polysaccharides are attributed to their physicochemical properties (Ktari et al., 2017; Wang et al., 2017), such as the molecular weight, rheological properties, monosaccharide composition and other properties.

Cushuro (*Nostoc sphaericum*) is a spherical gelatinous blue-green alga of diameter ~10–25 mm, with appearance similar to grapes that forms microscopic and macroscopic colonies that bloom in freshwater aquatic environments in the high Andean areas of Peru, over ~3000 m altitude. In Peru, cushuro is harvested and in the lagoons of the Departments of Ancash, Junín, Cajamarca, Huánuco, Cusco, and Puno, where there are lagoons with crystalline waters rich in nitrogen, which favors its development; it is also known as murmunta, llullucha or llayta (Chasquibol et al., 2025). It has been consumed by people mainly in Peru

* Corresponding author.
** Corresponding author. School of Food Science & Nutrition, University of Leeds, LS2 9JT, Leeds, United Kingdom.
E-mail addresses: nchasquibol@ulima.edu.pe (N. Chasquibol), fmartin.goycoolea@um.es (F.M. Goycoolea).

https://doi.org/10.1016/j.lwt.2025.118239
Received 16 May 2025; Received in revised form 24 July 2025; Accepted 26 July 2025
Available online 28 July 2025

and Bolivia for a thousand years. The neutral flavor makes cushuro a good ingredient of Peruvian gastronomy and for development of food products. (Pérez-Lloréns & Vergara, 2023; Pérez-Lloréns & Vergara, 2023).

Chasquibol et al., 2025, *Nostoc sphaericum* confirmed that cushuro (*Nostoc sphaericum*) is a rich source of calcium (~567–~1357 mg/100 g), iron (~13.5–~26 mg/100g), magnesium (~95–~126 mg/100 g), and protein (~32.4–~36.9 g/100 g) too. In two , complementary papers, the potential applications of cushuro polysaccharide in food formulations have also been shown. On the one hand, cushuro (*Nostoc sphaericum*) polysaccharide was co-microencapsulated with sacha inchi (*Plukenetia huayllabambana*) oil, natural antioxidant extracts from the oleoresin of charapita chili peppers (*Capsicum frutescens* L.) and grape orujo (*Vitis vinifera* L.) extract. This approach resulted in better oxidative stability and longer shelf-life than microcapsules stabilized by commercial antioxidant BHT (Chasquibol et al., 2024). On the other hand, the rheological properties of mixed hydrogels of purple corn (*Zea maiz* L.) extracts, red prickly pear (*Opuntia ficus-indica*) pulp, cushuro (*Nostoc sphaericum*) polysaccharide and tara (*Caesalpinia spinosa*) gum were evaluated (Arenas & Chasquibol, 2024). The results showed that cushuro (*Nostoc sphaericum*) polysaccharide can modify the rheological properties of mixed food hydrogels.

The aim of this paper is to extract polysaccharides from cushuro (*Nostoc sphaericum*), and study their physicochemical properties, antioxidant activity, FTIR spectroscopy, techno-functional properties, molecular weight distribution, monosaccharide composition, and rheological properties. The results provide valuable information for the extraction of polysaccharides from cushuro (*Nostoc. sphaericum*) and their potential use in food and other applications.

2. Materials and methods

2.1. Raw materials

Cushuro (*Nostoc sphaericum*) alga was collected from the lakes of in the district of Cotaparaco, province of Recuay, Department of Ancash-Peru, as detailed in our previous work (Chasquibol et al., 2025). The fresh cushuro was transported to our laboratory at Universidad de Lima, where it was washed and dried at 60 °C for 12 h in an infrared dehydrator (IRD D18, Spain), then ground in a food grinder (Grindomix GM200/Restch, Germany) to obtain cushuro flour and stored in aluminized bags at 25 °C. All reagents used were classified and supplied by Merck and Milli-Q water was used.

2.2. Polysaccharide extraction

The solubilization of cushuro (*Nostoc sphaericum*) flour and the optimal ultrasound-microwave-assisted extraction (UMAE) (CW-2000, Nade, Shanghai, China) conditions were determined according to Chasquibol et al., 2025. After cooling, the mixture with pH 1.5 was filtered under vacuum and separated into a supernatant and a residue 1 (R1). The supernatant was deproteinized by adjusting the pH to 4 and after centrifugation the supernatant was adjusted to pH ~7 4 with NaOH 2 mol/L, followed by concentration at a rotary evaporator (Buchi B-100, Switzerland).

The concentrate solution was precipitated by adding two volumes of absolute isopropanol (99 %, v/v) and incubation overnight at 4 °C. After centrifugation at 10,000 g × 10 min, the pellet was successively washed with isopropanol (70, 80, 90 %, v/v), and centrifugated at 10,000 g × 10 min obtaining the polysaccharide acid extract (PCAcE).

To obtain the polysaccharide aqueous extract, the cushuro flour was dispersed in hot water (90 °C) at a solid/solvent ratio (1 g/64 mL) by (UMAE) (CW-2000, Nade, Shanghai, China) (Chasquibol et al., 2025). After cooling the mixture with pH~7 was filtered under vacuum and separated into a supernatant and a residue 1 (R1). The supernatant was concentrated in a rotary evaporator (Buchi B-100, Switzerland) and continued according to the procedure described above until the aqueous polysaccharide extract (PCAqE) was obtained. Finally, the extracts (PCAcE and PCAqE). were dried at 50 °C for 3 h, ground in a food shredder (Grindomix GM200, Restch, Haan, Germany) and stored in aluminized bags at 25 °C for further analysis.

2.3. Physicochemical characterization

The proximal composition was conducted according to Chasquibol et al., 2025. The moisture content was determined at 110 °C to constant weight using a moisture analyzer Sartorius (MA 30, Got tingen, Germany). The total protein content was determined as g/100 g nitrogen × 6.25 using a Kjeldahl analyzer (UDK 139, VELP, Usmate Velate, Italy). The ash content was determined by incineration at 550 °C for 72 h, in a muffle furnace (Linn High Therm, VMK-80, Germany). The fat content was determined with hexane for 9 h.

2.4. Purity

The purity of the polysaccharide extracts was determined by evaluating the total carbohydrate content according to the method of Dubois et al. (1956) with some modifications. 780 μL of a aqueous solution of polysaccharide (0.1 mg/mL) were mixed with 780 μL of phenol solution (5 g/100 g, w/v), and 4 mL of concentrated H_2SO_4 in a 15 mL test tube. The mixture was vortexed for 30 s and kept in a water bath for 20 min at room temperature. Calibration curve was constructed with D-Glucose standard solutions (10–150 μg/mL), The absorbance of samples and standards were measured at 490 nm using a spectrophotometer (Shimatzu UV-1280, Kyoto, Japan). Purity was calculated using equation (1):

$$\text{Purity (\%)} = (\text{Weight D-Glucose of dry sample})/(\text{Weight of dry sample}) \times 100 \quad (1)$$

2.5. Determination of antioxidant activity via radical DPPH

Antioxidant activity was determined by the DPPH method Chasquibol et al., 2024 was used with some modifications. 4.5 mL of PCAcE and PCAqE solutions (0.5–2.5 mg/mL) were mixed with 3.9 mL of DPPH radical solution at 25 ppm, The absorbance at 517 nm was recorded after 1 h of incubation in the dark using a spectrophotometer (Shimatzu UV-1280, Kyoto, Japan). Ultrapure water (4.5 mL) was used as a control sample and butylated hydroxytoluene (BHT) (1 mg/mL) was used as a benchmark of a commercial antioxidant. The scavenging activities (SA) (%) of free radicals was calculated according to Equation (2):

$$\text{SA (\%)} = [(\text{Abs517 Control sample}) - (\text{Abs517 Sample})/(\text{Abs517 Control sample})] \times 100 \quad (2)$$

2.6. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of polysaccharides were determined using a Shimadzu spectrometer (IRTracer-100, Kyoto, Japan). A portion of samples were placed directly on a QATR10 fixture and pressed with a diamond/ZnSe prism in transmittance (%) mode and the spectra were recorded in the 400-4000 cm^{-1} region with a resolution of 4 cm^{-1} and 32 scans. Software LabSolutions IR (Shimadzu, Japan) was used to analyze the spectra.

2.7. Functional properties

2.7.1. Water holding capacity (WHC)

The WHC was carried out as previously described by Chasquibol

et al., 2025 with same modifications. The WHC was calculated using equation (2):

WHC (g/g) = (Weight of the swollen sample – Weight of the dry sample)/(Weight of the dry sample) (3)

2.7.2. Emulsifying capacity (EC)

The EC was evaluated by the method of Coorey et al. (2014). Samples (1 g/100 mL) were mixed for 10 min with 100 mL of corn oil using a Silverson homogenizer (L5M-A, Silverson, Chesham, Buckinghamshire, UK) at 10,000 g for 10 min. Samples were centrifuged at 1600 g for 15 min, the volume of the emulsion layer was measured, and the EC was determined using equation (3):

EC (%) = (Volume of the emulsion layer) / (Total volume of the mixture) x 100 (4)

2.7.3. Foaming capacity (FC)

The FC was carried out as previously described by Chasquibol et al., 2025 with same modifications. The FC was calculated using equation (4):

FC (%) = (Foam volume after homogenization) / (Total volume of the sample) x 100 (5)

2.8. Characterization of molecular weight distribution by multidetection size exclusion chromatography (SEC) and asymmetric flow field-flow fractionation (AF4)

The determinations of the molecular weight distribution and conformation of cushuro (*Nostoc sphaericum*) polysaccharides PCAcE and PCAqE were carried out on a POSTNOVA AF2000 Multiflow instrument from Postnova Analytics (Postnova Analytics GmbH, Landsberg, Germany), equipped to operate in both modes, namely asymmetric field flow fractionation (AF4) and size exclusion chromatography (SEC). The system is coupled with an online 21-angle multi-angle light scattering detection detector, MALS (PN3621), refractive index detector, RI (PN3150), and dual UV light spectroscopy (SEC-MALS-RI-UV/Vis). The system had an analytical AF4 asymmetric channel (Postnova Z-AF4-CHA-611) with a spacer of 350 μm. The temperature was controlled using a thermostat (PN4020) at 30 °C and the membrane used was a regenerated cellulose membrane with a cutoff 10 kDa (Z-AF4-MEM-612-10 kDa). For this purpose, the polysaccharide samples were solubilized in water 3 mg/mL (added with 0.2 mg/mL of NaN_3) and filtered through a nitrocellulose membrane with pore size 5 μm. The conditions for the fractionation were as reported in a previous study on locust bean gum polysaccharide (O'Connell et al., 2023).

Since only (PCAcE) sample presented water solubility such that it could be filtered through a 0.45 μm pore size membrane, it was the only sample amenable for study by SEC-MALS-DRI. To this end, a TSK gel PWH Guard column (13 μm 7.5 × 7.5 mm, Tosoh Bioscience, Germany) and two columns OHpak SB-802.5 HQ (exclusion limit 1×10^4 Da) and OHpak SB-806M HQ (exclusion limit 2×10^7 Da) from ShodexTM HPLC (Showa Denko Europe GmbH, Germany) were used. The mobile phase was 10 mmol/L $NaNO_3$ + 0.20 mg/mL NaN_3 filtered through membranes of pore size 0.1 μm, flow rate 0.5 mL/min, and temperature 30 °C. The dn/dc value used was 0.148 mL/g, and 50 μL were injected for each determination. All measurements were carried out in triplicate. The molecular weight results were analyzed using the NovaMALS program (v. 1.5.0.7).

Table 1
Physicochemical characterization and functional properties of PCAcE, and PCAqE from cushuro (*Nostoc sphaericum*) polysaccharides.

	PCAcE	PCAqE
Yield (%g/100 g)	49.0 ± 0.5[a]	12.9 ± 0.7[b]
Humidity (%g/100 g)	8.5 ± 0.3[a]	13.1 ± 0.3[b]
Ashes (g/100 g)	6.4 ± 0.1[a]	2.4 ± 0.2[b]
Protein (g/100 g)	1.4 ± 0.4[a]	16.8 ± 0.5[b]
Purity (%) (g D-GlucoseGalactose/100 g sample)	83.3 ± 0.3[a]	65.8 ± 0.8[b]
Emulsifyng capacity (EC) (%)	55.6 ± 1.0[a]	54.8 ± 1.1[a]
Foaming capacity (FC) (%)	8.3 ± 0.3[a]	11.0 ± 1.0[b]
Water holding capacity (WHC) (g/g)	3.0 ± 0.1[a]	3.6 ± 0.3[a]

Results are expressed as means ± SD (n = 3). a,b values in the same file with different letters vary significantly at $p < 0.05$.

2.9. Monosaccharide composition

The monosaccharide composition was studied only on the (PCAcE) sample. To this end, hydrolysis was carried out at 100 °C with 4 mol/L trifluoroacetic acid for 4 h. Subsequently, the samples were evaporated in a SpeedVac apparatus and resuspended in DMSO for chromatographic analysis. The neutral monosaccharide content was studied in the (PCAcE) sample by high performance anion exchange chromatography with pulse amperometric detection (HPAEC-PAD) on a Dionex equipment using a Carbopac PA20 column. Standardization of the method was carried out using high purity monosaccharide standards (López-Franco et al., 2008).

2.10. Rheology

The rheological behavior of (PCAcE) and (PCAqE) samples was studied using an Anton Paar rheometer (MCR 302, Graz, Austria) with RheoCompass software (version 1.24). Both steady shear and small-amplitude oscillatory determinations (recorded within the linear viscoelastic region) were carried out using a stainless-steel truncated cone (cone diameter 49.951 mm, cone angle 0.987°, truncation 0.099 mm, cone part no. 79040) and stainless-steel plate geometry (part no. 16222), which allowed the viscoelastic behavior of these materials to be examined. For this purpose, the polysaccharide samples dissolved in 0.1 mol/L NaCl (~50 mg/mL) were used. In the case of (PCAcE), rheological determinations were also carried out at lower concentration dilutions in the range 0.08–50 mg/mL.

2.11. Statistical analysis

Results were expressed as mean ± standard deviation. All measurements were determined in duplicate or triplicate. Analysis of variance (ANOVA), which was used at $P < 0.05$ followed by Tukey's post hoc test to identify significant differences between groups. All analyses were performed using Minitab 19.0 software (Minitab Inc. USA).

3. Results and discussion

3.1. Physicochemical characterization and techno-functional properties

As shown in Table 1, the extraction yield resulted greater for PCAcE (49.0 ± 0.5 g/100 g) than PCAqE (12.9 ± 0.7 g/100 g), due to a better solubilization of the polysaccharide fractions with HCl than with hot water method (Wassie et al., 2021). Liu, Su, Xu, Chen, Zhang, Zhou, and Wang (2018) reported high extraction yield (36.0 g/100 g) from *Enteromorpha prolifera* for polysaccharide under acid extraction method. In addition, Chi et al. (2018), compared hot water and alkali extraction methods, reporting a better yield for *E. prolifera* with acid extraction method.

The ash content of PCAcE (6.4 ± 0.1 g/100 g), was higher than some commercial polysaccharides, such as agar and carrageenan, ranged from

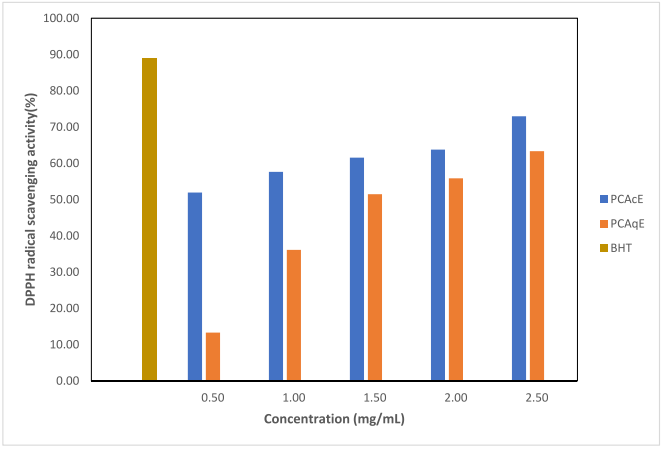

Fig. 1. Dependence of DPPH radical scavenging activity on the concentration of PCAcE and PCAqE polysaccharides from cushuro (*Nostoc sphaericum*). Butylated hydroxytoluene (BHT) (1 mg/mL) is shown as a benchmark of a commercial antioxidant.

2.5 ± 0.03 g/100 g to 2.7 ± 0.031 g/100 g (Jayasinghe et al., 2016). The protein content of PCAqE (16.8 ± 0.45 g/100 g) was higher than commercial polysaccharides: agar (4.3 ± 0.08 g/100 g), carrageenan 5.6 ± 0.22 g/100 g) and alginic acid (6.2 ± 0.74 g/100 g) (Jayasinghe et al., 2016).

PCAcE sample resulted with the highest purity (83.3 ± 0.2 g/100 g) than PCAqE (65.8 ± 0.8 g/100 g). Shen et al. (2018) (80.04 ± 2.47 g/100 g to 85.75 ± 3.08 g/100 g) reported similar values from *Nostoc flagelliforme Nostoc commune* polysaccharide extracts. With respect to functional properties, the study of the EC of PCAcE and PCAqE samples showed very similar EC values (~55 %). These results were lower than polysaccharide extracts from *Nostoc flagelliforme* reported by Shen et al. (2019) (~64–74 %). Differences in emulsification capacity may be influenced by several factors, including the amount of protein linked to their structure (Bach & Chen, 2017), suggesting that structural aspects other than net protein content are involved in the ability of cushuro polysaccharides to emulsify o/w. Gum arabic, with protein contents <2 g/100 g similar to PCAcE, is a known natural emulsifier composed of a highly branched arabinogalactan bound to a protein structure that is responsible for its ability to stabilize o/w emulsions (Randall et al., 1989).

The foam capacity (FC) of PCAqE sample was higher (11.0 ± 1.0 %) than PCAcE (8.3 ± 0.3 %), but lower than FC obtained for polysaccharide from *N. flagelliforme* (17 %–35 %) *N. commun*e Shen et al., 2019).

Finally, PCAcE (3.0 ± 0.1 g/g) and PCAqE (3.6 ± 0.3 g/g) showed WHC values similar to those recorded for xanthan gum (3.1 g/g), and guar gum (3.6 g/g) (Bojorges et al., 2025); but lower than the results obtained for *N. flagelliforme* polysaccharides (Shen et al., 2019) (27.82 g/g). High WHC values is important to contribute with the viscosity and desirable texture of food products such as custards, sausages, baked goods and doughs (Bojorges et al., 2025).

3.2. DPPH radical-scavenging activity

Fig. 1 shows the DPPH radical scavenging activity of PCAcE and PCAqE samples at different concentrations (0.5–2.5 mg/mL). PCAcE showed higher DPPH radical scavenging activity (73.0 %) than PCAqE (63.3 %), but much lower than BHT included as a benchmark of a commercial antioxidant (88.91 %). Furthermore, DPPH radical scavenging activity were higher than the polysaccharide fraction reported by Wang et al., 2022 (52.72 %). According to Peasura et al. (2015), the molecular weight of polysaccharides plays an important role in their bioactivity, and polysaccharides with low molecular weight (PCAcE) have more reductive OH terminals available for reacting with radical species than PCAqE. This is consistent with the results of Mw characterization discussed further below.

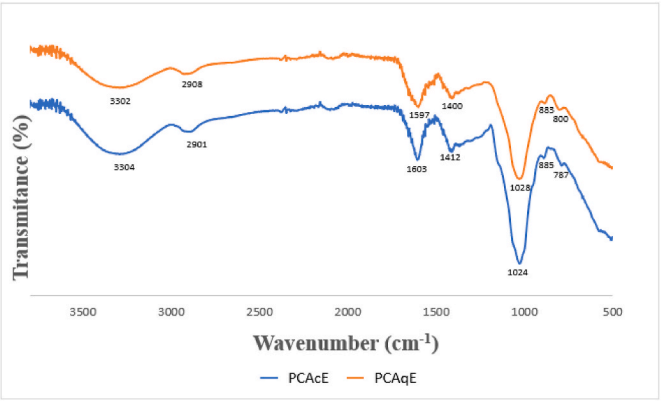

Fig. 2. FTIR spectrum of PCAcE, and PCAqE from cushuro (*Nostoc sphaericum*) polysaccharides.

In addition, the antioxidant activity in both polysaccharides may be related to the monosaccharide composition, polysaccharides extraction with UMAE and other factors that reducing their molecular weight and increasing their antioxidant activities (Shen et al., 2018; Wang et al., 2022; Yang et al., 2024).

3.3. Fourier transform infrared (FTIR) spectroscopy analysis

FTIR spectra (Fig. 2) of PCAcE and PCAqE samples showed typical polysaccharide peaks at ~3304, ~2901, ~1603, ~1412, ~1024, ~885, and ~787 cm^{-1}, characteristic of a polysaccharide FTIR fingerprint (Perlin & Casu, 1982). A broad and intense peak was observed at ~3304 cm^{-1}, assigned to polymeric ν(O-H) stretching vibrations, peaks at ~2901 cm^{-1}, which can be attributed to ν(C-H) stretching (Li et al., 2018; Shen et al., 2018). The peaks (~1597-1600 cm^{-1}) and (~1412 cm^{-1} and ~1400 cm^{-1}) are assigned to the ν_{as}(C=O) stretching vibrations are diagnostic of the presence of carboxyl groups (Shen et al., 2018; Wang et al., 2022). A strong absorption and intense peaks were observed at ~1024-1028 cm^{-1}, corresponding to ν_{as}(C-O-C) or ν(C-O-H) stretching vibrations (Wang et al., 2022), also at ~1035 cm^{-1} was reported a strong absorption of D-Glucose (Kacurakova et al., 2000). The small band observed in both spectra at ~883-885 cm^{-1} is diagnostic with the presence of β-type glycosidic linkages (Rodriguez et al., 2017), while the band observed on both spectra at ~787-800 cm^{-1} is consistent with the presence of α-type glycosidic bonds. Thus, suggesting the presence of both types of linkages in the polysaccharide structure.

3.4. Characterization of molecular weight distribution

In the present study, HPSEC and AF4 with multidetection, UV and MALS were used. The AF4 elugrams are shown in Fig. 3 Correspond to the samples of PCAcE (panel a) and PCAqE (panel b) extracted polysaccharide. The red line in the elugrams corresponds to the UV detector signal, which is proportional to the concentration and the blue line represents the light scattering detector signal (MALS 90°) which is proportional to the size of the eluting fragments. It is observed in the left elugram of PCAcE the presence of a peak that elutes at t = 30 min corresponding to a population of small size, and a second peak that elutes at a longer time (t = 45 min), corresponding to another population of larger size. Similarly, the right elugrams of PCAqE sample presents a second peak eluting at approximately t = 40 min and is more prominent than the first peak (t = 30 min).

The results agree with previous reports by Wang et al. (2022), who studied the effect of different extraction methods on *Nostoc commune*

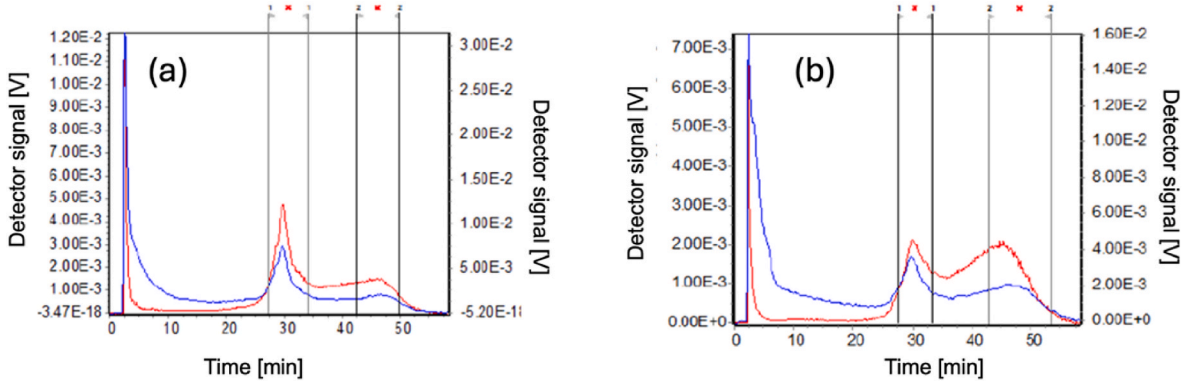

Fig. 3. Representative elugrams from AF4-MALS-RI-UV/VIS of PCAcE (a), and PCAqE (b) from cushuro (*Nostoc sphaericum*) polysaccharides (both at 3 mg/mL in NaN_3 0.2 mg/mL at 25 °C). MALS 90° (red traces) and UV (blue traces) signals are shown in both panels. Also shown are the regions of interest used to perform peak analysis for the quantitative analysis of the molecular weight distribution.

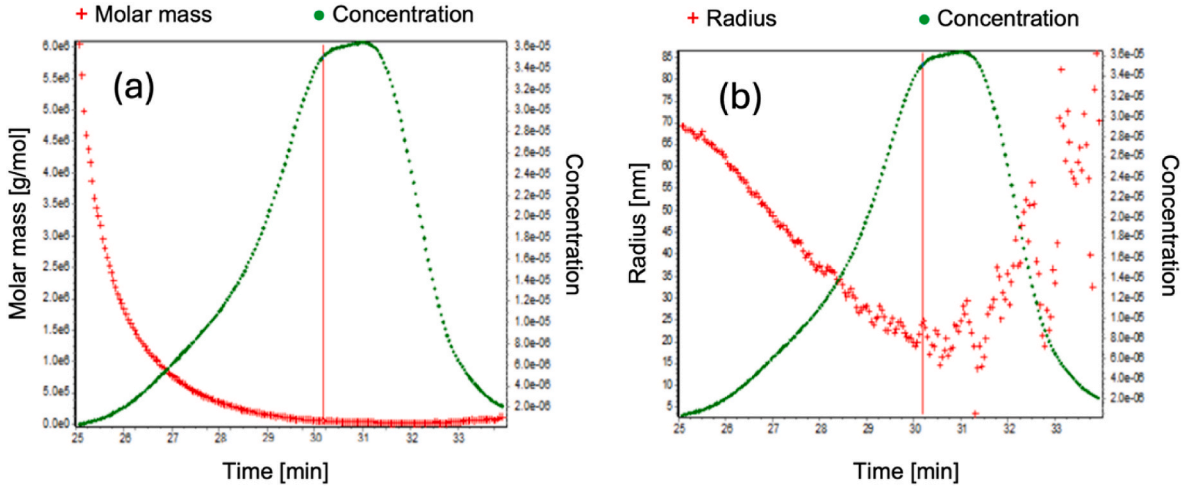

Fig. 4. Molecular weight distribution (panel a) and radius of gyration (panel b) of polysaccharide acidic extraction (PCAcE) (1.6 mg/mL in 100 mM NaCl at 25 °C; mobile phase 10 mM $NaNO_3$ + 0.2 mg/mL NaN_3).

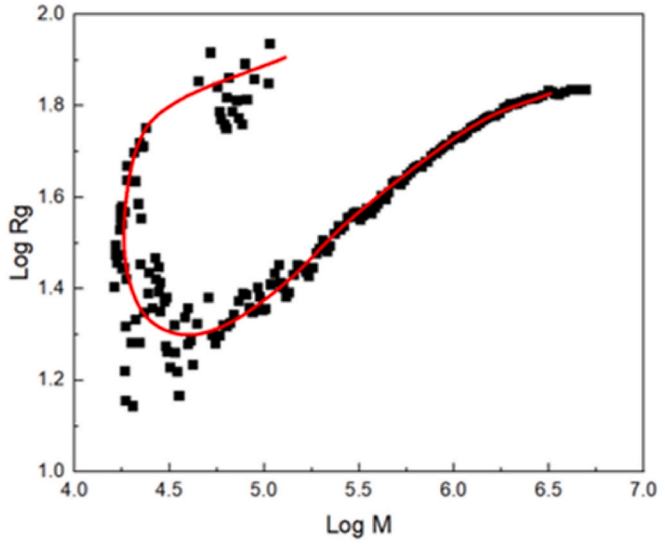

Fig. 5. Double-logarithmic conformation plot shows the dependence of radius of gyration (log Rg) and molecular weight (M) of polysaccharide acid extraction (PCAcE) (1.6 mg/mL in 100 mM NaCl at 25 °C; mobile phase 10 mM $NaNO_3$ + 0.02 % NaN_3).

heteropolysaccharides.

Fig. 4 Shows the molecular weight (panel a) and radius of gyration (panel b) distributions as determined by SEC-MALS-RI-UV/Vis. This characterization was only performed on the polysaccharide acid extraction (PCAcE) sample given that this technique involves filtration through a 0.45 μm filter, whereas the PCAqE sample did not pass through the filter.

Note that a broad peak corresponding to the concentration elution profile is shown (green symbols) with a Gaussian distribution though slightly skewed shape. The molecular weight distribution profile shows the expected elution of the larger molar mass fractions and the gradual monotic decrease as the elution proceeds. Panel b, in turn, shows the corresponding distribution of the radius of gyration (*Rg*). Of note, as expected, the larger species elute first, however, after ~30 min, an upward increase in the size is observed denoting the subsequent elution of larger fractions. This anomalous shape of the curve indicates the presence of a branched polysaccharide species whose branches are long enough to get trapped in the pores of the column and thus delay their partitioning during elution.

This anomalous elution behavior can be further characterized by the conformational plot representation of Log *Rg vs.* Log Mw shown in Fig. 5. Inspection of the plot reveals the up tailed curve described by the data at low molecular weights where it can be noted that there is a fraction of species sharing the same molar mass but either low or high *Rg*. This is reasoned as the consequence of the presence of two types of structures of

Table 2
Summary of characterization parameters of molecular weight distribution and protein content of (PCAcE) and (PCAqE) from cushuro *(Nostoc Sphaericum)* polysaccharides.

	PCAcE [a]	PCAcE [b]	PCAqE [a]
	Characterization Method		
Parameters	AF4-MALS-UV/Vis	SEC-MALS-UV/Vis	AF4-MALS-UV/Vis
Fit Model	Random coil	Random coil	Random coil
Mn (Da)	$(3.0 \pm 0.5) \times 10^{7a}$	$(3.00 \pm 0.8) \times 10^{4b}$	$(2.3 \pm 0.7) \times 10^{7ac}$
Mw (Da)	$(4.6 \pm 0.6) \times 10^{7a}$	$(1.0 \pm 0.4) \times 10^{5b}$	$(5.1 \pm 1.7) \times 10^{7ac}$
Mz (Da)	$(6.5 \pm 1.0) \times 10^{7a}$	$(5.2 \pm 3.4) \times 10^{5b}$	$(1.0 \pm 0.4) \times 10^{8c}$
Đ (Mw/Mn)	1.5 ± 0.1^{a}	3.5 ± 0.4^{b}	2.2 ± 0.3^{ac}
Rg (nm)	145 ± 3^{a}	22 ± 5^{b}	50 ± 4^{c}
Recovery performance (%)	17.1 ± 2.2^{a}	83.4 ± 1.8^{b}	13.8 ± 1.5^{ac}

Results are expressed as means ± SD (n = 3). a,b,c values in the same file with different letters vary significantly at $p < 0.05$.
[a] 5 μm pore size membrane-filtered samples.
[b] 0.45 μm pore size membrane-filtered samples.

the polysaccharides present, a linear one of large *Rg*, and a branched one of lower *Rg*. The degree of branching was quantified according with the Zimm-Stockmayer equation by analyzing both average *Rg* values (Eq (5)) (Zimm & Stockmayer, 1949).

$$g = \left(\frac{R_{br}^2}{R_{ln}^2}\right)_M = 0.0907 \quad (5)$$

Where R_{br} represents the radius of gyration of the branched fractions and R_{ln} that of the linear ones. The calculated g parameter value (g = 0.0907), using average values at the minimal (R_{br}) and maximal (R_{ln}) points at Mw ~5.6×10^4 Da (log Mw ~ 4.75), corresponds with that of a highly branched structure (Phélippé et al., 2019; Jensena et al., 2013). In agreement with this result, Wang et al. (2022) performed the structural characterization of cushuro (*Nostoc commune*) polysaccharide by ultrasound-assisted extraction, observing the presence of a main backbone chain of → 6)-D-Glcp (1 → and → 2, 6)-D-Glcp, bearing branches of 1,2,6 -Gal and 2,3 -Me2-D-Ara. Earlier studies, by Hough et al. (1952), have already mentioned that *Nostoc* polysaccharide presents branched glucose and galactose units. Hence, the results offer a first approximation to the macromolecular structure of the polysaccharide of *N. sphaericum*.

Table 2 summarizes the results of the molecular weight distribution parameters of PCAcE and PCAqE samples determined by AF4- and SEC-MALS-UV/Vis. Techniques. Note that the PCAcE sample characterized by AF4, filtered through a 5 μm mesh, had average molecular weight distribution parameters (Mn, Mw and Mz) that lied in the range ~$3.0 \pm 0.5 \times 10^7$ to ~$6.5 \pm 1.0 \times 10^7$ Da, while for the same sample filtered through a 0. 45 μm and by SEC-DRI-MALS technique, these parameters were more than two orders of magnitude lower in the range ~$3.00 \pm 0.8 \times 10^4$ to ~$5.2 \pm 3.4 \times 10^5$ Da, as the expected consequence of removal of aggregates by the nanopore filtration step. The latter values compare well with those reported by Wang et al. (2022), who determined molecular weight values of ~1×10^4 Da using SEC-DRI-MALS analysis for the polysaccharide of cushuro (*Nostoc commune*) extracted by ultrasound-assisted extraction. Guo et al. (2015, 2019) reported a molecular weight ranging between ~1.90 and ~2.18×10^5 Da for the polysaccharide extracts from *Nostoc commune* Vaucher.

In turn, PCAqE analyzed by AF4-MALS-UV/Vis technique and filtered through a 5 μm mesh, had average molecular weight values ranging between ~$2.3 \pm 0.7 \times 10^7$ and $1.0 \pm 0.4 \times 10^8$ Da. Table 2 also provides the average *Rg* values obtained by AF4 for the polysaccharide acid extraction is ~145 ± 3 nm; while the *Rg* obtained by AF4 for the polysaccharide aqueous extraction is ~50 ± 4 nm, these values correspond to nanoparticles, due to the molecular aggregation that occurs in the polysaccharide. SEM and AFM analysis showed that the polysaccharide presented a colloidal type structure (Wang et al., 2023).

3.5. Monosaccharide composition

Fig. 6 Shows the HPAEC-PAD chromatographic profile of a mixture of neutral sugar standards composed of rhamnose (Rha), arabinose (Ara), galactose (Gal), glucose (Glu), mannose (Man), xylose (Xyl), and fructose (Fru), showing six peaks with retention times equivalent to 6. 57, 6.85, 8.13, 8.95, 9.56, 9.56, and 10.23 min corresponding to Rha, Ara, Gal, Glu, Man/Xyl and Fru, respectively.

Table 3 Shows the results expressed as mole fraction of each monosaccharide derived from the analysis of the area under the curve of the peaks corresponding to each one. The polysaccharides obtained are mainly composed of glucose (31.1 %), xylose (25.9 %), galactose (16.1 %), rhamnose (13.0 %) and arabinose (1.9 %) and the absence of mannose and fructose. This composition is comparable to that reported by several authors of polysaccharides extracted from *Nostoc comume* and *N. sphaericum* (Huang et al., 1998; Jensena et al., 2013, Helm et al., 2000; Hu et al., 2003, Yanfang et al., 2018, Li et al., 2023). Heteropolysaccharides from microalga such as *Chlorella* from the spiral group have been documented to also be comprised by rhamnose, arabinose,

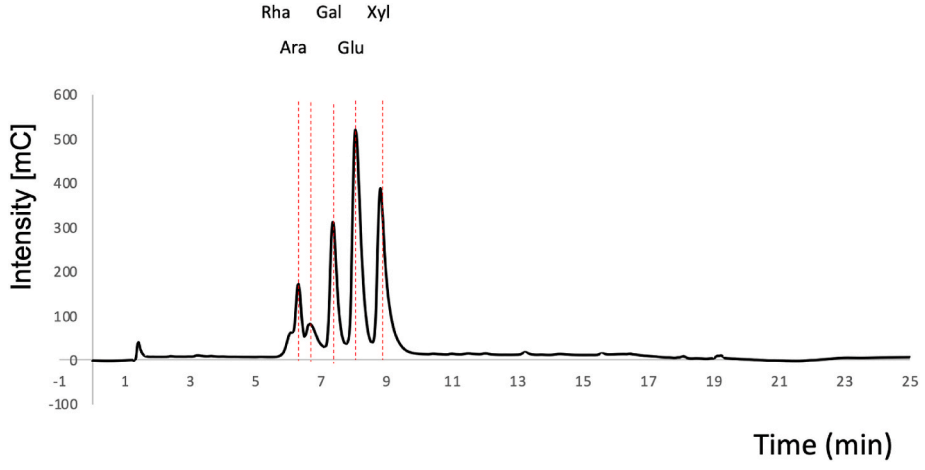

Fig. 6. HPAEC-PAD chromatogram (Dionex) for PCAcE (10 μg/mL). Labels indicated the assignment of the peaks as per the monosaccharide standards elution times. Mannose and xylose co-elute together at 9.56 min.

Table 3
Molar composition (%) of PCAcE from cushuro (*Nostoc sphaericum)* polysaccharide, determined by HPAEC-PAD.

Glc	Xyl	Gal	Man	Rha	Uronic acids	Ara	GlcN	Reference
31.1	25.9	16.1	n.d. [a]	13	n.d.[a]	1.9	——[b]	This study
49.6–50.8	25.9–28.3	20.3–23.6	0–1.8	–	——[b]	——[b]	——[b]	Huang et al. (1998)
44.0	20.9	21.5	1.6	3.5	——[b]	——[b]	——[b]	Hu et al. (2003)
44.56	16.48	19.75	4.20	0.76	10.17[c]	1.21	1.74	Guo et al. (2019)
49.55	16.40	21.42	1.85	n.d.[a]	8.64[c]	0.67	1.47	Guo et al. (2019)

[a] Not detectable.
[b] Not reported.
[c] GlcA + GalA.

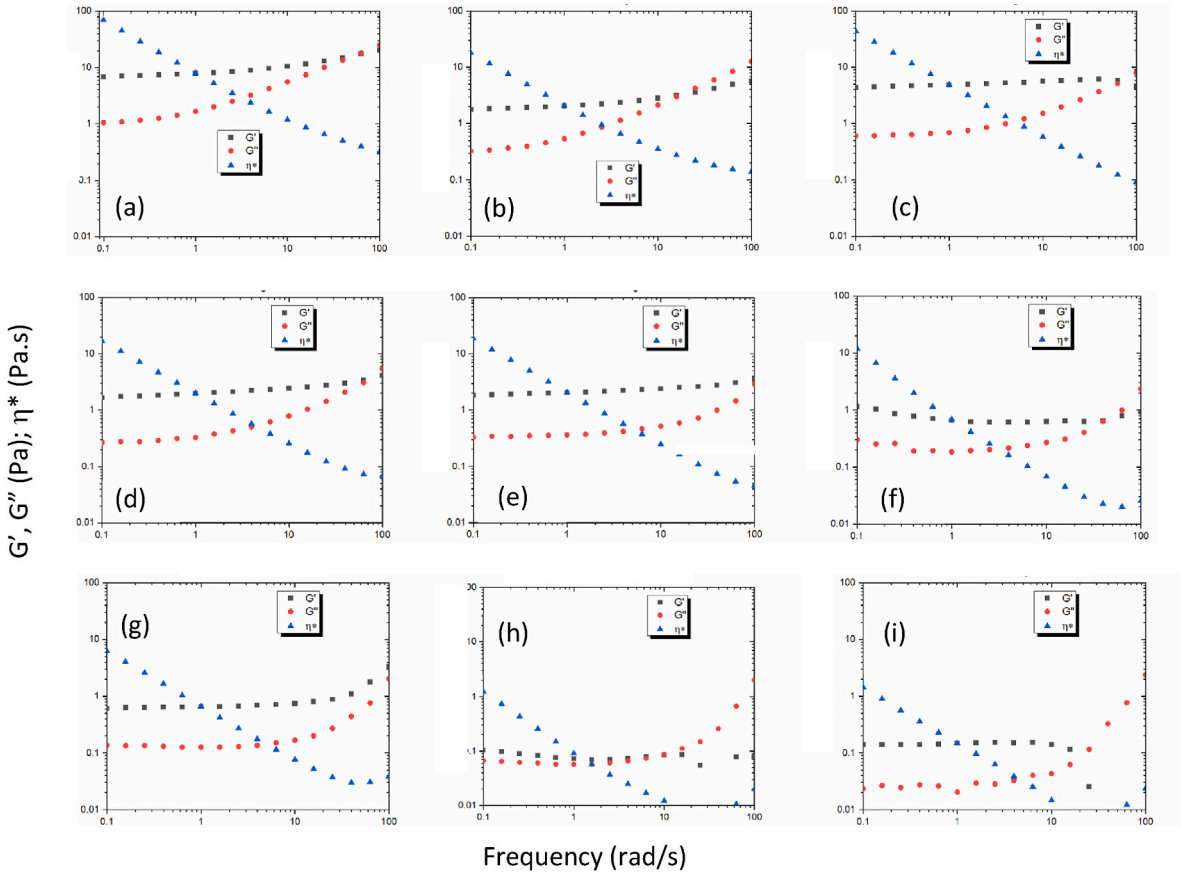

Fig. 7. Dependence of the storage (G' ■) and loss (G''●) viscoelastic moduli and of the complex viscosity (η* ▲) on the oscillation frequency for PCAcE aqueous solutions of varying concentration: (a) 49.6 (mg/mL), (b) 39.6 (mg/mL), (c) 30.3 (mg/mL), (d) 24.7 (mg/mL), (e) 12.7 (mg/mL), (f) 8.18 (mg/mL), (g) 6.32 (mg/mL), (h) 2.74 (mg/mL), and (i) 1.65 (mg/mL) (strain 1 %, 20 °C, 0.1 M NaCl).

glucose and galactose (Dolganyuk et al., 2020). Also, it has been documented that exopolysaccharides from cyanobacteria are constituted mainly by high molecular weight heteropolysaccharides made up of glucose, galactose, xylose and uronic acids (Pereira et al., 2009). Overall, there is a close agreement of the monosaccharide composition of the polysaccharide from *N. sphaericum* with previously reported data. A greater content of Rha, and lower amount of Gal and Glc can be highlighted though.

Helm et al. (2000) described *Nostoc* polysaccharide as a structure composed of repeating units of six monosaccharides, consisting of β-D-glucose, α -D-galactose and β -D-xylose linked at 1–4, and with α-L-ribose substituents on the D-xylose units, and β-nosturonic acid substituents on some D-glucose units.

3.6. Rheology

The viscoelastic mechanical properties of PCAcE in aqueous solution was studied by dynamic oscillatory rheology. The dependence of the storage (G′) and loss moduli (G″) and complex viscosity (η*) on the oscillation frequency for acidic polysaccharide solutions at concentrations between 1.65 and 49.6 mg/mL are shown in Fig. 7. A predominance of G′ over G″ and a negative dependence of complex viscosity (η*) on oscillation frequency is observed through the tested oscillation frequencies and throughout the studied range of concentrations. Of note, at concentrations greater than 8.18 mg/mL, the values of both G' and G'' show a slight dependence on the oscillation frequency, which is characteristic of a weak gel system (Barzic, 2021). This rheological behavior was rather unexpected, and do not correspond with that of a highly branched polysaccharides such as gum Arabic or mesquite gum, which behave as Newtonian fluids (Goycoolea et al., 1995). Either the adoption of an ordered conformation or of a highly swollen covalently crosslinked structure in cushuro polysaccharide may account for this discrepancy.

Previous studies have found the viscosity of *Nostoc sphaericum* polysaccharide solutions to be higher than *Nostoc commune*

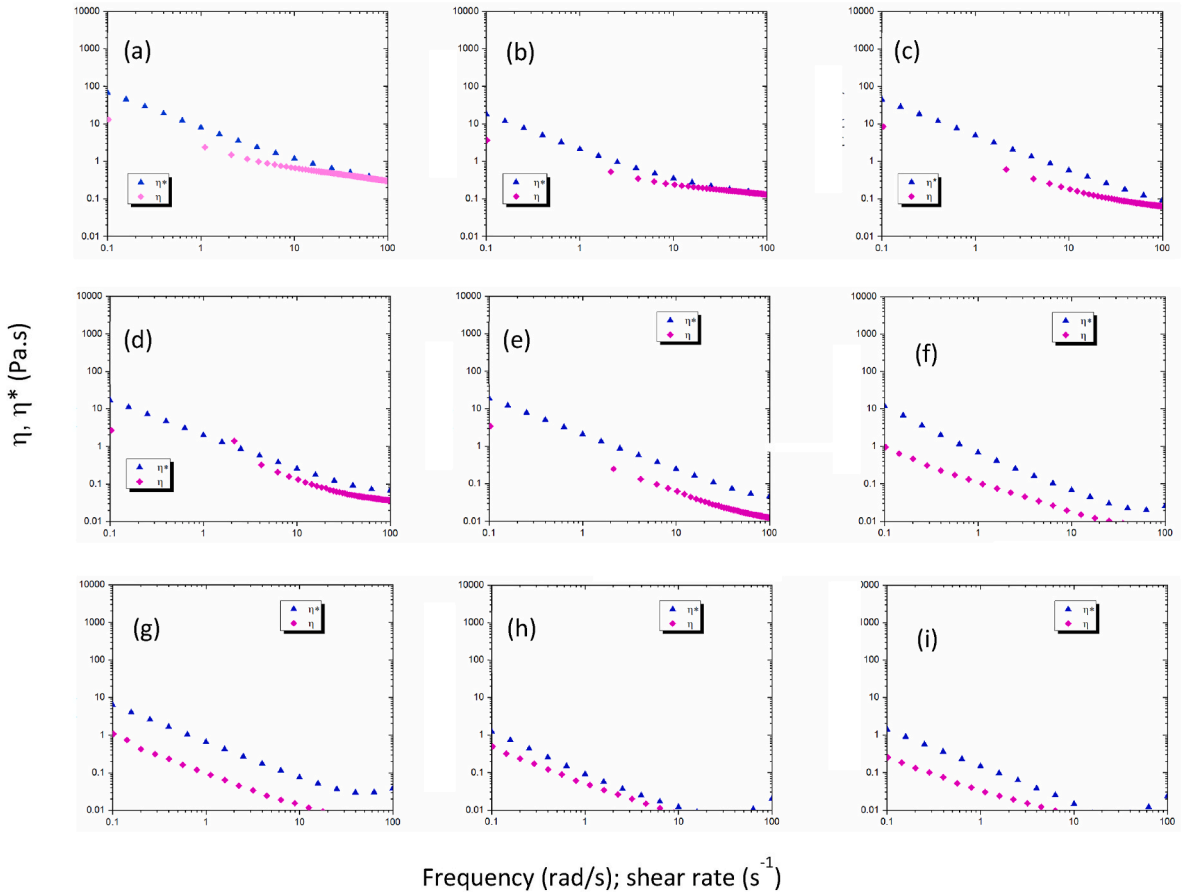

Fig. 8. Cox-Merz plots of the dependence of complex (η* ▲) and steady-shear dynamic (η ◆) viscosity on oscillation frequency and shear rate, respectively, for PCAcE solutions of varying concentrations of: (a) 49.6 (mg/mL), (b) 39.6 (mg/mL), (c) 30.3 (mg/mL), (d) 24.7 (mg/mL), (e) 12.7 (mg/mL), (f) 8.18 (mg/mL), (g) 6.32 (mg/mL), (h) 2.74 (mg/mL), and (i) 1.65 (mg/mL) (strain 1 % on oscillatory measurements, 20 °C, 0.1 M NaCl).

(Torres-Maza et al., 2020), however, these differences may stem not on the biological source nor structure of the two polysaccharides, but on the molecular weight associated to the extraction method. The aqueous neutral extracted batch at high concentration showed the typical behavior of a strong gel. This material did not dissolve upon subsequent dilution nor on heating as recorded on visual observations (not shown).

To gain further insight into the rheological profile of PCAcE in aqueous solution, steady-shear viscosity measurements were recorded and compared with the complex viscosity ones obtained by dynamic oscillatory rheology to construct well-known Cox-Merz superposition plots of the dependence of complex viscosity (η*) and steady-shear dynamic viscosity (η) on the oscillation frequency (ω) and shear rate ($\dot{\gamma}$), respectively, as shown in Fig. 8. Inspection of the plots reveals that at concentrations of 12.7 and below, the values of η* are greater than those of η through the ω and $\dot{\gamma}$, respectively. At concentrations of 24.7 and greater, this behavior is less pronounced, but it is still evident. Overall, these results can be taken as diagnostic of the formation of a "weak gel" type structure, similar in kind to the behavior documented for other polysaccharides such as xanthan gum (Morris et al., 1981), which combine gel-like response to small deformations with the ability to flow freely under shear. Xanthan gum is a bacterial exopolysaccharide comprised by a linear cellulose backbone with trisaccharide substituents every other glucose unit, and is known to adopt a conformationally ordered helical conformation which explains, to some extent, its weak-like properties. By contrast, cushuro PCAcE is suggested to be highly branched and not known to adopt an ordered conformation. Hence, the weak-gel character can be attributed to the presence of large molecular aggregates, consistent with the results from AF4 fractionation (Fig. 3 and Table 2.).

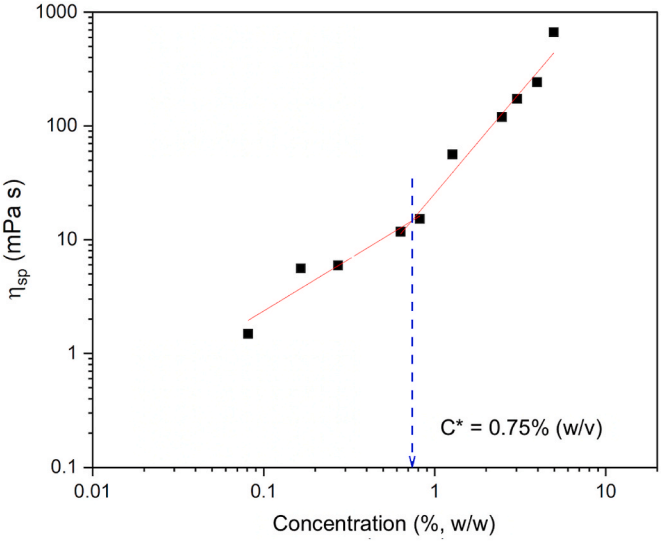

Fig. 9. Double-logarithmic plot of the dependence of the specific viscosity (η_{sp}) on the concentration of PCAcE (1 %, 20 °C, 0.1 M NaCl).

The double logarithmic concentration dependence of specific viscosity ($\eta_{sp} = (\eta - \eta_{solvent})/\eta_{solvent}$) calculated from the steady-shear viscosity (measured at $\dot{\gamma} = 1\ s^{-1}$) is shown in Fig. 9. Two regimes are clearly distinguished, the dilute and semi-dilute that converge at below and above a critical concentration, denoted as C* at ~0.75 % w/v,

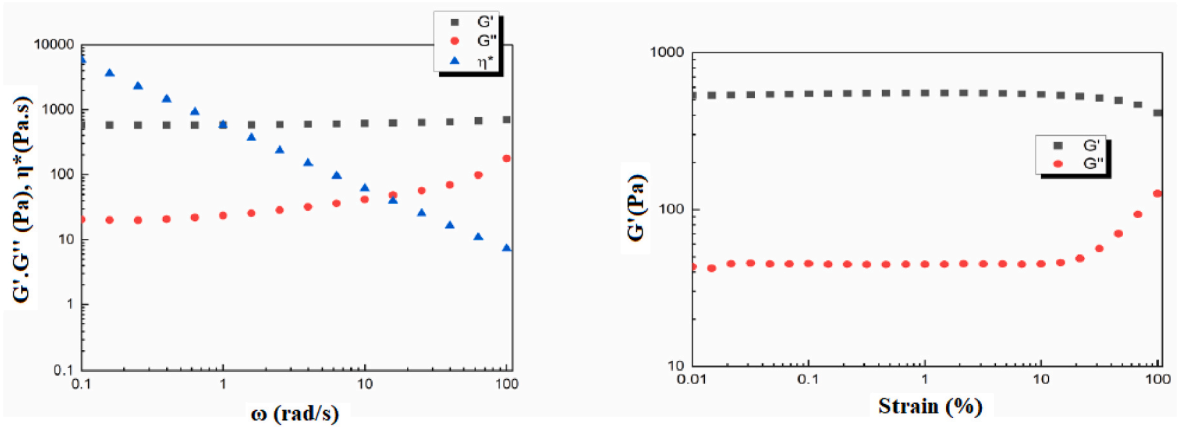

Fig. 10. Dependence of the of the storage (G' ■) and loss (G"●) viscoelastic moduli and of the complex viscosity (η* ▲) on the oscillation frequency (ω) (left panel) (γ = 1 %), and of the of the storage (G' ■) and loss (G"●) viscoelastic moduli on the strain (ω = 1 rad/s) (right panel) for PCAcE solutions (20 °C, 0.1 M NaCl).

respectively. The two regimes show a linear dependence with calculated slopes of 0.9 and 1.8, respectively. This change in the concentration dependence of η_{sp} pinpoint the point at which the polymer chains in the dilute regime have occupied the entire volume fraction of the solution and transition from the dilute to the semi-dilute regime, from which point, at higher concentration, they pass to a concentrated state, in which the chains pass from being isolated entities to an entangled state. This behavior was established for random coil disordered of PCAcE in aqueous solution since the early studies in these systems (Morris et al., 1981). Further future studies should aim to obtain viscosimetric measurements for more dilute solutions than those used in the present study for the rheometer determinations and extend the data set to lower polymer concentrations. This will also enable to determine the intrinsic viscosity of the polysaccharide and construct the well-known double logarithmic plot of η_{sp} *vs.* coil overlap (c[η]), and from it, determine with greater precision the slope of the dilute regime data while comparing it with previous data for other linear and branched polysaccharides in aqueous solution (Goycoolea et al., 1995; Morris et al., 1981). Knowledge of the concentration dependence of the viscosity of a polysaccharide is essential to establish conditions for preparing hydrogels or nanofibers, which must be obtained from solutions in the concentrated regime. The rheological behavior of PCAqE sample was also studied. However, it was only possible to study the behavior of the swollen gel obtained at the highest concentration, given that it was not possible to dilute it homogeneously due to its insolubility. Fig. 10. (left panel) shows the frequency dependence of the viscoelastic G′ and G″ moduli and complex viscosity (η*) behavior of this gel, which was examined using a serrated parallel plate geometry to prevent possible slippage of the sample once loaded and sandwiched between the two parallel plate geometries and ensure to conduct the measurements at strain values within the linear viscoelastic regime.

Fig. 10 (right panel) shows that G′ and G″ moduli values did not vary in the range of strain 0.01–~10 %, thus ensuring that at 1 % strain, measurements were well within this regime. The data on both panels show all the rheological features characteristic of a firm gel. Finally, it should be noted that neither of the two cushuro (*Nostoc sphaericum*) polysaccharides showed an obvious dependence on temperature when heated to 80 °C and subsequently cooled.

4. Conclusions

Cushuro polysaccharides extracts from *Nostoc sphaericum* presented purity of ~83 and ~66 %, respectively, with a greater extraction yield on PCAcE than PCAqE, due to combined ultrasound cavitation effect and the high extraction temperature in acidic conditions. The established protocol can be scaled up in future pilot plant and fully fledged industrial scale trials. AF4 and HPSEC allowed the determination of the average molecular weight distribution parameters (Mn, Mw and Mz) and the Rg for the PCAcE both unfiltered and filtered, thus revealing the presence of large aggregates in the former. In addition, by AF4, it was possible to analyze the soluble fraction of PCAqE, showing a branched-type conformation, in agreement with cushuro (*Nostoc commune*) polysaccharides reported previously. The sugar composition comprised glucose, xylose and galactose, in agreement with previous reports. The viscoelastic properties of PCAcE examined by small-deformation oscillatory and steady-shear rheology were consistent with a weak-gel system, even at low concentrations (6.3 mg/mL). This was not the expected behavior of a highly branched polysaccharide but pointed to the role of highly aggregated fractions. The techno-functional properties obtained would anticipate the potential use of cushuro (*Nostoc sphaericum)* polysaccharides in the stabilization of foams and emulsions, as well as a thickening and potentially gelling agent in jams, and in the formulation of functional foods.

CRediT authorship contribution statement

N. Chasquibol: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **A. Sotelo:** Methodology, Investigation, Formal analysis. **M. Tapia:** Writing – original draft, Investigation, Formal analysis. **Y. Paredes:** Methodology, Investigation, Formal analysis. **A. Sajid:** Investigation, Formal analysis. **S. Sahin:** Investigation, Formal analysis. **M. Wu:** Investigation, Formal analysis. **F.M. Goycoolea:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank the Programa Nacional de Investigación en Pesca y Acuicultura (PNIPA) belonging to the Ministerio de la Producción of Peru for financing the collaboration project PNIPA-PES-SIADE-PP-000277 (2020) between University of Leeds in the United Kingdom and the Universidad de Lima – Peru.

Data availability

Data will be made available on request.

References

Arenas, C., & Chasquibol, N. (2024). Development of food hydrogels with andean purple corn (Zea mays L.) extracts and cushuro (*Nostoc sphaericum*) polysaccharide: Rheological characterization. *Biology Life Science. Forum, 37*, 1. https://doi.org/10.3390/blsf2024037001

Bach, Q. V., & Chen, W. H. (2017). Pyrolysis characteristics and kinetics of microalgae via thermogravimetric analysis (TGA): A state-of-the-art review. *Bioresource Technology, 246*, 88–100. https://doi.org/10.1016/j.biortech.2017.06.087

Barzic, A. I. (2021). Rheology and structural properties of polysaccharides. *Polysaccharides: Properties and Applications*, 367–383. https://doi.org/10.1002/9781119711414.ch17

Bojorges, H., López-Rubio, A., Martínez-Abad, A., & Fabra, M. J. (2025). Functional and bioactive properties of the protein-polysaccharide extracts from brown algae: Exploring novel functional ingredients. *Food Hydrocolloids, 162*, Article 110967. https://doi.org/10.1016/j.foodhyd.2024.110967

Chasquibol, N., Sotelo, A., Tapia, M., Alarcón, R., Goycoolea, F. M., & Hernández-Álvarez, A. J. (2025). Evaluation of cushuro (*Nostoc sphaericum*) as an alternative source of minerals, functional protein and bioactive peptides. *LWT - Food Science and Technology*. , Article 117426. https://doi.org/10.1016/j.lwt.2025.117426

Chasquibol, N., Sotelo, A., Tapia, M., Alarcon, R., Goycoolea, F. M., & Pérez Camino, M. C. (2024). Co-Microencapsulation of Cushuro (*Nostoc sphaericum*) polysaccharide with Sacha Inchi oil (*Plukenetia huayllabambana*) and natural antioxidant extracts. *Antioxidants, 13*, 680. https://doi.org/10.3390/antiox13060680

Chi, Y., Li, Y., Zhang, G., Gao, Y., Ye, H., Gao, J., et al. (2018). Effect of extraction techniques on properties of polysaccharides from *Enteromorpha prolifera* and their applicability in iron chelation. *Carbohydate Polymers, 181*, 616–623. https://doi.org/10.1016/j.carbpol.2017.11.104

Coorey, R., Tjoe, A., & Jayasena, V. (2014). Gelling properties of Chia seed and flour. *Journal of Food Science, 79*(5). https://doi.org/10.1111/1750-3841.12444

Dolganyuk, V., Belova, D., Babich, O., Prosekov, A., Ivanova, S., Katserov, D., Patyukov, N., & Sukhikh, S. (2020). Microalgae: A promising source of valuable bioproducts. *Biomolecules, 10*, 1153. https://doi.org/10.3390/biom10081153

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, E. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*, 350–356. https://doi.org/10.1021/ac60111a017

Goycoolea, F. M., Morris, E. R., Richardson, R. K., & Bell, A. E. (1995). Solution rheology of mesquite gum in comparison with gum arabic. *Carbohydrate Polymers, 27*, 37–45. https://doi.org/10.1016/0144-8617(95)00031-2

Guo, N., Bai, Z., Jia, W., Sun, J., Wang, W., Chen, S., & Wang, H. (2019). Quantitative analysis of polysaccharide composition in *Polyporus umbellatus* by HPLC–ESI–TOF–MS. *Molecules, 24*(14), 2526. https://doi.org/10.3390/molecules24142526

Guo, M., Ding, G. B., Guo, S., Li, Z., Zhao, L., Li, K., & Guo, X. (2015). Isolation and antitumor efficacy evaluation of a polysaccharide from *Nostoc commune* Vauch. *Food & Function, 6*(9), 3035–3044. https://doi.org/10.1039/c5fo00471c

Helm, R. F., Huang, Z., Edwards, D., Leeson, H., Peery, W., & Potts, M. (2000). Structural characterization of the released polysaccharide of desiccation-tolerant *Nostoc commune* DRH-1. *Journal of Bacteriology, 182*(4), 974–982. https://doi.org/10.1128/JB.182.4.974-982.2000

Hough, L., Jones, J. K. N., & Wadman, W. H. (1952). An investigation of the polysaccharide components of certain fresh-water algæ. *Journal of the Chemical Society*, 3374–3381. https://doi.org/10.1039/jr9520003393

Hu, C., Liu, Y., Paulsen, B. S., Petersen, D., & Klaveness, D. (2003). Extracellular carbohydrate polymers from five desert soil algae with different cohesion in the stabilization of fine sand grain. *Carbohydrate Polymers, 54*(1), 33–42. https://doi.org/10.1016/S0144-8617(03)00135-8

Huang, Z., Liu, Y., Paulsen, B. S., & Klaveness, D. (1998). Studies on polysaccharides from three edible species of *Nostoc* (Cyanobacteria) with different colony morphologies: Comparison of monosaccharide compositions and viscosities of polysaccharides from field colonies and suspension cultures. *Journal of Phycology, 34*(6), 962–968. https://doi.org/10.1046/j.1529-8817.1998.340962.x

Jayasinghe, P. S., Pahalawattaarachchi, V., & Ranaweera, K. K. D. S. (2016). Effect of extraction methods on the yield and physiochemical properties of polysaccharides extracted from seaweed available in Sri Lanka. *Poultry, Fisheries & Wildlife science. Poult fish wildl science, 4*(150), 1–6. https://doi.org/10.4172/2375-446X.1000150 (150).

Jensena, S., Petersen, B. O., Omarsdottir, S., Paulsen, B. S., Duus, J. O., & Olafsdottir, E. S. (2013). Structural characterisation of a complex heteroglycan from the cyanobacterium *Nostoc commune*. *Carbohydrate Polymers, 91*(1), 370–376. https://doi.org/10.1016/j.carbpol.2012.08.063

Kacurakova, M., Capek, P., Sasinkova, V., Wellner, N., & Ebringerova, A. (2000). FT-IR study of plant cell wall model compounds: Pectic polysaccharides and hemicelluloses. *Carbohydrate Polymers, 43*(2), 195–203. https://doi.org/10.1016/S0144-8617(00)00151-X

Ktari, N., Feki, A., Trabelsi, I., Triki, M., Maalej, H., Slima, S. B., Nasri, M., Ben Amara, I., & Ben Salah, R. (2017). Structure, functional and antioxidant properties in Tunisian beef sausage of a novel polysaccharide from *Trigonella foenum-graecum* seeds. *International Journal of Biological Macromolecules, 98*, 169–181. https://doi.org/10.1016/j.ijbiomac.2017.01.113

Li, H., Liu, Y., Zhou, J., Liu, S., Liu, Y., Yang, Y., … Guan, L. (2023). The protective mechanism of a novel polysaccharide from Lactobacillus-fermented *Nostoc commune Vauch.* on attenuating cadmium-induced kidney injury in mice. *International Journal of Biological Macromolecules, 226*, 1444–1454. https://doi.org/10.1016/j.ijbiomac.2022.11.256 (2023).

Li, H., Su, L., Chen, S., Zhao, L., Wang, H., Ding, F., … Huang, Z. (2018). Physicochemical characterization and functional analysis of the polysaccharide from the edible microalga *Nostoc sphaeroides*. *Molecules, 23*(2), 508. https://doi.org/10.3390/molecules23020508

Liu, Y., Su, P., Xu, J., Chen, S., Zhang, J., Zhou, S., … Wang, Y. (2018). Structural characterization of a bioactive water-soluble heteropolysaccharide from *Nostoc sphaeroids kütz*. *Carbohydrate Polymers, 200*, 552–559. https://doi.org/10.1016/j.carbpol.2018.08.034

López-Franco, Y. L., Calderón de la Barca, A. M., Valdez, M. A., Peter, M. G., Rinaudo, M., Chambat, G., & Goycoolea, F. M. (2008). Structural characterization of Mesquite *(Prosopis velutina)* gum and its fractions. *Macromolecular Bioscience, 8*, 749–757. https://doi.org/10.1002/mabi.200700285

Morris, E. R., Cutler, A. N., Roos/Murphy, S. B., & Rees, D. A. (1981). Concentration and shear rate dependence of viscosity in polysaccharide solutions. *Carbohydrate Polymers, 1*, 5–21. https://doi.org/10.1016/0144-8617(81)90011-4

O'Connell, A., González-Espinosa, Y., Goycoolea, F. M., Schuetz, P., & Mattsson, J. (2023). Characterisation of locust bean gum with asymmetric flow field-flow fractionation (AF4) and light scattering. *Carbohydrate Polymers, 322*, Article 121286. https://doi.org/10.1016/j.carbpol.2023.121286

Peasura, N., Laohakunjit, N., Kerdchoechuen, O., & Wanlapa, S. (2015). Characteristics and antioxidant of Ulva intestinalis sulphated polysaccharides extracted with different solvents. *International Journal of Biological Macromolecules, 81*, 912–919. https://doi.org/10.1016/j.ijbiomac.2015.09.030

Pereira, S., Zille, A., Micheletti, E., Moradas-Ferreira, P., De Philippis, R., & Tamagnini, P. (2009). Complexity of cyanobacterial exopolysaccharides: Composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly. *FEMS Microbiology Reviews*. https://doi.org/10.1111/j.1574-6976.2009.00183.x

Pérez-Lloréns, J. L., & Vergara, J. J. (2023). Microalgae in phycogastronomy. In *Handbook of food and feed from microalgae* (pp. 349–355). Elsevier. https://doi.org/10.1016/B978-0-323-99196-4.0002.

Perlin, A. S., & Casu, B. (1982). Spectroscopic methods. In G. O. Aspinall (Ed.), *The polysaccharides* (Vol. 1, pp. 133–193). NEW York: Academic Press.

Phélippé, M., Gonçalves, O., Thouand, G., Cogne, G., & Laroche, C. (2019). Characterization of the polysaccharides chemical diversity of the Cyanobacteria *Arthrospira platensis*. *Algal Research, 38*. https://doi.org/10.1016/j.algal.2019.101426

Randall, R. C., Phillips, G. O., & Williams, P. A. (1989). Fractionation and characterization of gum from *Acacia Senegal*. *Food Hydrocolloids, 3*(1), 65–75. https://doi.org/10.1016/S0268-005X(88)80011-0

Rodriguez, S., Torres, F. G., & López, D. (2017). Preparation and characterization of polysaccharide films from the Cyanobacteria *Nostoc commune*. *Polymers from Renewable Resources, 8*(4), 133–150. https://doi.org/10.1177/204124791700800401

Shen, S., Jia, gang, ru, S., Wu, Y., kai, Y., rong, R., Lin, Y. H., Zhao, D. xue, & Han, P. pei (2018). Effect of culture conditions on the physicochemical properties and antioxidant activities of polysaccharides from *Nostoc flagelliforme*. *Carbohydrate Polymers, 198*, 426–433. https://doi.org/10.1016/j.carbpol.2018.06.111

Shen, S. G., Lin, Y. H., Zhao, D. X., Wu, Y. K., Yan, R. R., Zhao, H. B., Tan, Z. L., Jia, S. R., & Han, P. P. (2019). Comparisons of functional properties of polysaccharides from *Nostoc flagelliforme* under three culture conditions. *Polymers, 11*(2), 263. Vol. 11, Page 263 https://pubmed.ncbi.nlm.nih.gov/30960247/.

Soua, L., Koubaa, M., Barba, F. J., Fakhfakh, J., Ghamgui, H. K., & Chaabouni, S. E. (2020). Water-Soluble polysaccharides from *Ephedra alata* stems: Structural characterization, functional properties, and antioxidant activity. *Molecules, 25*(9), 2210. https://doi.org/10.3390/molecules25092210

Torres-Maza, A., Yupanqui-Bacilio, C., Castro, V., Aguirre, E., Villanueva, E., & Rodríguez, G. (2020). Comparison of the hydrocolloids *Nostoc commune* and *Nostoc sphaericum*: Drying, spectroscopy, rheology and application in nectar. *Scientia Agropecuaria, 11*(4), 583–589. https://doi.org/10.17268/sci.agropecu.2020.04.14

Wang, C., Gao, X. D., Chen, Z. Q., Chen, Y., & Chen, H. X. (2017). Preparation, characterization and application of polysaccharide-based metallic nanoparticles. *A Review. Polymers, 9*, 689. https://doi.org/10.3390/polym9120689

Wang, J., Shang, M., Li, X., Sang, S., McClements, D. J., Chen, L., Long, J., Jiao, A., Ji, H., Jin, Z., & Qiu, C. (2023). Polysaccharide-based colloids as fat replacers in reduced-fat foods. *Trends in Food Science & Technology, 141*, Article 104195. https://doi.org/10.1016/j.tifs.2023.104195. ISSN 0924-2244.

Wang, X., Yang, Z., Liu, Y., Wang, X., Zhang, H., Shang, R., & Wang, S. (2022). Structural characteristic of polysaccharide isolated from *Nostoc commune*, and their potential as radical scavenging and antidiabetic activities. *Scientific Reports, 12*(1), Article 22155. https://doi.org/10.1038/s41598-022-26802-x

Wassie, T., Niu, K., Xie, C., Wang, H., & Xin, W. (2021). Extraction techniques, biological activities and health benefits of marine algae *Enteromorpha prolifera* polysaccharide. *Frontiers in Nutrition, 8*, Article 747928. https://doi.org/10.3389/fnut.2021.747928

Yang, Y., Ren, P., Sun, Y., Li, J., Zhou, X., Zhang, H., … Guan, L. (2024). Structure elucidation and molecular mechanism of an immunomodulatory polysaccharide from *Nostoc commune*. *International Journal of Biological Macromolecules, 283*, Article 137435. https://doi.org/10.1016/j.ijbiomac.2024.137435

Zimm, B. H., & Stockmayer, W. H. (1949). The dimensions of chain molecules containing branches and rings. *The Journal of Chemical Physics, 17*(12), 1301–1314. https://doi.org/10.1063/1.1747157