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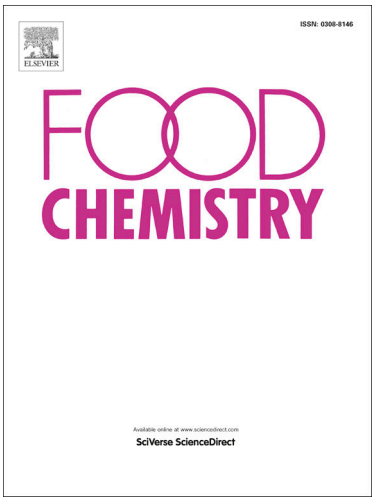

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Perennial halophyte *Salicornia neei* Lag.: cell wall composition and functional properties of its biopolymers

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Abstract

Salicornia neei halophyte extends in Argentina seashores. To envisage potential applications, cell wall sequential extraction performed on dry plant yielded 1.1, 2.4, 0.3 and 0.9% of pectin fractions respectively extracted by room temperature water, 90ºC-water, CDTA and Na_2CO_3. They contained 21-33% uronic acids (UA) with low degree of methylation and 0.5-1.2 molar ratios of neutral sugars to UA. High arabinose level suggests that long arabinan side-chains maintain cell wall flexibility in water deficit. Fractions also contained 10-36% of proteins. The KOH-soluble fractions (4.3%) were mainly arabinoxylans. At 2.0% w/v, pectin fractions developed "weak gel"-type networks with Ca^{2+}, while arabinoxylans generated "dilute solutions". Cellulose (28%) and lignin (45.1%) were the main biopolymers in the final residue, which showed low water swelling capacity (3.6 mL/g) due to lignin, increasing when arabinoxylans were also present. Phenolics (9.8%) were mainly water-extractable. *Salicornia* is a source of biopolymers and antioxidants potentially useful for food applications.

Keywords**:** *Salicornia neei*; cell wall polysaccharides; phenolics; lignin; cellulose; dynamic rheology; hydration properties.

1. Introduction

Salicornioideae (Amaranthaceae/Chenopodiaceae) family comprises 11 genera and ca. 100 species of succulent halophytes distributed worldwide in coastal and inland saline habitats. Most species have peculiar articulated, seemingly leafless stems and spike-like sessile thyrses with highly reduced flowers hidden by fleshy bracts (Piirainen, Liebisch & Kadereit, 2017). Jume or sea asparagus is a species of succulent and halophyte plant of the Salicornioideae that grows on seashores or salty soils of South America (de Souza, Mendes, Doncato, Badiale-Furlong, & Costa, 2018). It has been botanically defined as *Salicornia ambigua* (Michaux), *Sarcocornia ambigua* or *Sarcocornia perennis* (Alonso & Crespo, 2008; Cervellini & Angeletti, 2015), but nowadays it was molecularly classified as *Salicornia neei* Lag., which includes the species found in the seashores of the Patagonia of Argentina (Piirainen et al., 2017; Souza et al., 2018; Doncato & Costa, 2018; Costa, Kadereit, & Peres Moraes de Freitas, 2019). According to Costa et al. (2019), *S. ambigua* (Michaux) is restricted to the northern hemisphere, whereas *Sarcocornia perennis* is restricted to Eurasia.

Based on their ability to thrive in seawater-flooded and saline soils, the small succulent shrubs with leafless stems and branches of the genera *Salicornia* L. and *Sarcocornia* A. J. Scott (Salicornioideae) are characterized as halophytes (Costa et al., 2019). Halophytes need a high-salt soil composition to grow properly. This quality is related mainly to the ability to control ion uptake and the vacuolar compartmentalization of Na^+, K^+, and Cl^-, to maintain the osmotic balance between vacuoles and cytoplasm by the synthesis of osmotic active molecules (Loconsole, Cristiano, & De Lucia, 2019). The coasts between southern Brazil (Costa et al., 2019) and Argentinean Patagonia (Piirainen et al., 2017) are covered by salt marshes dominated by *S. neei*, which support large populations of polychaetes, pelecypods, and crustaceans. These organisms are

subject to predation by oystercatchers, gulls, and terns (Bianciotto, Pinedo, San Roman, Blessio, & Collantes, 2003; Freitas & Costa, 2014).

Plants from the genus *Salicornia* have a wonderful nutritional potential. They contain high amounts of proteins, sulfur, and minerals (Loconsole, Cristiano, & De Lucia, 2019). Also, they have a significant lipid content with a healthy profile of fatty acids. *Salicornia* plants produce antioxidant metabolites, which are desirable in the human diet (Cervellini & Angeletti, 2015; Loconsole, et al., 2019). The reddish color of *Salicornia* shoots can indicate the accumulation of anthocyanins and other phenolic compounds, which are antioxidants used by the plants to tolerate stresses (water deficits, high soil salinization, high ultraviolet radiation) (Freitas & Costa, 2014). *Salicornia* is being used in the gourmet cuisine, as well as a substitute of salt after drying, being cultivated for these purposes in Western Europe (Cervellini & Angeletti, 2015; Lopes, Cavaleiro, & Ramos, 2017; Diaz, 2019; Loconsole et al., 2019). The edible parts of glassworts have tender leaves and shoots which can be used in a fresh salad, or boiled like spinach without salt. The color, after cooking resembles seaweed, and the flavor and texture are similar to young spinach or asparagus (Loconsole et al., 2019). *Salicornia* plants also show a high capacity for water filtration (Diaz, 2019). They are currently considered an excellent resource in areas with low or null economic value, and a novelty in the field of agriculture. A crop irrigated with seawater was developed in marshes of Tierra del Fuego (Argentina), which demonstrated that lambs fed with *Salicornia* grasslands were leaner for human consumption. At the same time, the crop reduced in 50% the cholesterol level of the animals (Bianciotto, 2014). Also, *Salicornia* is being considered in Argentina to yield a second-generation biofuel (Cervellini & Angeletti, 2015).

We suggest that *S. neei* could be also applied as a source of biopolymers mainly coming from the cell walls of leaves and stems, which can be useful for different applications. For example, pectins, the cell wall polysaccharides characterized by a backbone of 1,4-linked α-D-Gal*p*A (galacturonan chains) (Scheller & Ulvskov, 2010), can be used as thickener and gelling agent in the food and pharmaceutical industries. The European Food Safety Authority (EFSA) just recognized in 2010 the pectin as a health promoting ingredient (Ciriminna, Fidalgo, Delisi, Ilharco, & Pagliaro, 2016). Hemicelluloses, composed mainly of cell wall polysaccharides carrying β-(1→4)-linked backbones with equatorial configurations at C-1 and C-4 (Scheller & Ulvskov, 2010), can be used as additives and dietary fiber supplements, and as biomaterials for pharmaceutical use (da Silva et al., 2012). Cellulose, with β-(1→4)-linked glucan chains, high tensile and compressive strength, has widespread uses in various fields such as nanotechnology, food industry, cosmetics, textile and paper industries, and in pharmacy to form drug-delivery systems (Gupta et al., 2019). Thus, through the present study, the potential of *Salicornia neei* as a source of useful substances was determined by the analysis of the chemical composition. We hypothesize that the cell wall polysaccharides of salt-stressed plants could be somehow different from those of regular land plants. The knowledge of the composition of cell wall polymers, which determines also functional properties such as rheology, hydration capabilities and antioxidant potential of the fractions obtained from *S. neei*, will be relevant to envisage the usefulness of the polymers contained.

2. Materials and Methods

2.1. Material

Vegetative shoots of *S. neei* Lag. were collected in the Atlantic coast of Bahia Bustamante (Chubut province), Argentina. The plants were sorted, air dried, cleaned manually, and the bark and other debris were removed from some stems. The remaining dried cylindrical leafless shoots with branches were then dried and milled to a fine powder (mesh #40) before extraction. All chemical reagents were of analytical grade from Sigma-Aldrich (St. Louis, USA) and Merck (Argentina Branch).

2.2. Analytical determinations

Total carbohydrates, and uronic acids (UA) were determined as reported by Basanta, de Escalada Pla, Stortz, & Rojas (2013), using D-galactose and D-galacturonic acid (GalA) as standards, respectively, with GalA expressed as anhydro units. For both methods, for insoluble materials, the pretreatment of Ahmed and Labavitch (1977) was utilized. Soluble proteins were determined as explained by Lowry et al. (1951). The proportion of neutral sugars (NS) was determined after subtracting the UA content from that of total carbohydrates. The degree of esterification was calculated as the molar ratio between the methanol and UA contents (Basanta et al., 2013).

In order to determine the NS composition, each fraction (ca. 3 mg) was hydrolyzed with 2 M trifluoroacetic acid (TFA, 1 mL) for 90 min at 120 ºC in closed-cap vials, reduced using $NaBH_4$, converted to alditol acetates and then analyzed as described by Fissore et al. (2011). The identity of the UA was evaluated through the carboxyl reduction with a soluble carbodiimide [N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride] as reported by Fissore et al. (2011).

Total phenolics' (free phenolics, cell wall phenolic esters, and conjugated phenolic acids) determination was carried out as reported by Basanta et al. (2013), with gallic acid as standard. Results were expressed as g of gallic acid per 100 g of sample.

148

149 *2.3. Extraction and fractionation*

150 The milled material from *S. neei* was extracted with chloroform-methanol (1:1)
151 for 2 h at room temperature to remove the lipids. The residue was separated after
152 filtration, then washed several times with acetone and dried (fraction **FLM**).
153 Afterwards, a sequential extraction was carried out as shown in Fig. 1. Shortly, it was
154 extracted with water at room temperature for 24 h (**CWSF**), the residue reextracted
155 twice with water at 90 ºC for 8 h and the extracts were joined to yield fraction **HWSF**.
156 Following, extraction proceeded with CDTA 0.05M in NaAcO / HAcO 0.05M (pH=6)
157 for 24 h (**CSF**), Na_2CO_3 0.1M (**NSF**), KOH 4% (**4KSF**) and 24% (**24KSF**) (Basanta et
158 al., 2013), in agreement with the usual procedures (Fry, 1986; Brett & Waldron, 1996;
159 Brummel et al., 2004; Marry et al., 2006). All of these three last extractions were carried
160 out in the presence of 0.01% $NaBH_4$ at room temperature for 24 h. In all cases, the
161 insoluble material was separated from the supernatant by centrifugation (8600 x *g*), and
162 the supernatant was lyophilized (water extractions) or dialyzed (MWCO 3,500) and
163 lyophilized to obtain the extracts named in each case.

164 The chemical composition of each solubilized fraction isolated as above
165 mentioned (Fig. 1) was determined through the corresponding method described in the
166 subsection 2.2 “Analytical determinations”.

167

168 *2.4. Determination of cellulose and lignin in the final residue (KIR)*

169 Cellulose, lignin, and non-cellulosic carbohydrates (pectins and hemicelluloses), UA
170 and proteins were separately determined in **KIR** (Fig. 1) by selective extraction from
171 0.0100 g of material with different concentrations of sulfuric acid (1 M or 72% w/w), as
172 reported by Basanta et al. (2014). According to this method, cellulose and lignin are

gravimetrically quantified. The UA content, total carbohydrates, and the composition of the NS in the non-cellulosic carbohydrate component of **KIR** was determined in the supernatants as explained in subsection "Analytical determinations". The NS content was calculated as the arithmetical difference between the contents of non-cellulosic polysaccharides and UA.

2.5. Antioxidant capacity

The antioxidant capacity was determined on the dry plant powder as the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity with L-(+)-ascorbic acid (AA) as the standard. Results were expressed as mg AA/100 g (Basanta et al., 2013).

2.6. Rheological oscillatory assays in soluble fractions

Dynamic mechanical spectra (frequency sweeps at linear viscoelastic conditions) were recorded from the 2.00% w/v aqueous systems developed with the respective lyophilized cold water (**CWSF**), hot water (**HWSF**), Na_2CO_3 (**NSF**), 4%-KOH (**4KSF**) and 24%-KOH (**24KSF**) polymeric fraction. A sample of 0.04 g of each suitable fraction was suspended in water under vortexing. After that, the aqueous systems were finally dissolved by heating into a water bath at 80ºC, and left for 24 h at room temperature for complete hydration. The aqueous system was then heated again into a water bath at 80ºC, and 200 μL of a $CaCl_2$ solution were then added with vortexing in order to reach a final concentration of 15 mM $CaCl_2$ in the 2.00% w/v polymeric solution finally obtained.

A sample solution was transferred to the 25-mm-diameter serrated parallel plate of an MCR300 Paar Physica rheometer (Austria) with a gap of 1000 μm and a constant temperature of 20.0 ºC maintained through a peltier unit (Viscotherm VT2 Physica,

198 Austria). Shear storage (*G*') and loss (*G*") moduli as well as the *tan*δ (*G*"/*G*')
199 viscoelastic parameters were recorded at stationary state as a function of the angular
200 frequency (0.1-100 rad/s range) for each polymeric fraction, using a constant strain
201 selected from the linear viscoelastic range determined in the previous assay of
202 amplitude sweep. Measurements were performed in triplicate.

203 Amplitude (stress versus strain) sweeps were first performed at a constant frequency
204 of 1 Hz in order to determine the linear viscoelastic range of each aqueous system
205 developed, from which a value of strain was chosen for the subsequent record of the
206 mechanical spectra (frequency sweeps).

207

208 *2.7. Hydration properties of **FLM**, **NIR** and **KIR***

209 Swelling capacity (SC), water holding capacity (WHC), and water retention capacity
210 (WRC) were determined in triplicate for the *Salicornia* powder (**FLM**), **NIR** and **KIR**
211 (Fig. 1), as described previously (Basanta et al., 2013).

212

213 *2.8. Statistical analysis*

214 Results were reported as the average and standard deviation for three sample
215 replicates. Analysis of results was performed through ANOVA (α: 0.05) followed by
216 multiple comparisons evaluated through the least significant difference (LSD) test,
217 using the Statgraphic package (Statgraphic Plus for Windows, version 5.0, 2001,
218 Manugistic Inc., Rockville, MD, USA).

219

220 *3. Results and discussion*

221 *3.1. Pectin extraction and fractionation of S. neei*

The dried shoots were constituted by a 7.1% of lipidic material, as determined by their extraction with chloroform-methanol. This result was similar to the 5.2% obtained by Costa et al. (2014) after applying a similar extractive method.

The lyophilized residue obtained after removal of lipids (**FLM**) was submitted to the sequential extractive procedure shown in Fig. 1 to determine the polymer composition of *S. neei*. The yields obtained for the soluble fractions subsequently isolated by cold and hot water (**CWSF**, **HWSF**), CDTA (**CSF**) and Na_2CO_3 (**NSF**) are reported in Fig. 2a, expressed on dry mass of **FLM**. Their total amounts conform less than 5% of the original material. Therefore, a very low proportion of pectins constitutes the cell walls of *S. neei*. The hot water extraction gives the highest yield of pectins (2.4%, **HWSF**), whereas CDTA extracts barely 0.3% (**CSF**) (Fig. 2a).

In the sequential extraction of cell wall polymers, water extracts those pectins loosely bound in the cell wall matrix, while CDTA extracts pectins with their homogalacturonan (HG) smooth regions physically crosslinked by calcium ions, with formation of "egg-box-like" structures (Fry, 1986; Braccini & Perez, 2001). Calcium-crosslinked pectins are especially located in the middle lamella, being mainly responsible for cell-cell adhesion (Marry et al., 2006). The Na_2CO_3 solution extracts pectins anchored in the cell wall matrix through covalent bonds like diester bridges of ferulate at the arabinan side chains of the rhamnogalacturonan I (RG-I) hairy regions of neighboring pectin macromolecules (Fry, 1986; Marry et al., 2006). Pectins are basically constituted by GalA in their HG regions, chains that can be interrupted by alternating monomers of L-rhamnose (Rha), constituting the RG-I short regions, where the Rha is laterally substituted by chains containing arabinose (arabinans) and/or galactose (arabinogalactans, galactans). These side substituents give to the RG-I a hairy

246 appearance. Hence, L-arabinose (Ara), D-galactose (Gal) and Rha constitute the main
247 NS of pectins (Vincken et al., 2003).

248 In the sequentially isolated pectin fractions **CWSF**, **HWSF**, **CSF** and **NSF**, the UA
249 (GalA) contents were respectively 30.9, 21.4, 27 and 34% (Table 1). The UA originally
250 showed low degree of methyl esterification (DM) (16-25%), with the exception of **NSF**
251 (Table 1), where the extractive procedure (Fig. 1) was which hydrolyzed the methyl
252 esters. It was determined by Le Gall et al. (2015) that under salt stress, increased
253 demethylesterified pectins, mediated by pectinmethylesterase activity, tend to crosslink
254 with the calcium ions, leading to a stronger gel character of the cell wall matrix. Taking
255 into account the NS contents that we determined in **CWSF**, **HWSF**, **CSF** and **NSF**
256 (Table 1), the corresponding NS/UA molar ratios were calculated, and the values
257 obtained (0.8, 1.3, 0.5, and 1.0, respectively; Fig. 2b) are those expectable for pectins
258 (Basanta et al., 2013). In **CSF**, the high UA/NS ratio is remarkable, expectable in
259 calcium crosslinked pectins, which are essentially constituted by HG chains, and with
260 low proportion of RG-I regions (NS) because they usually hinder the calcium
261 crosslinking. HG segments constituted by at least 10 to 14 GalA units are reported as
262 needed to form “egg box-like” structures through calcium ions between neighboring
263 pectin macromolecules (Braccini & Perez, 2001; Vincken et al., 2003).

264 Beyond the main pectin components (UA and the NS: Rha, Ara and Gal), high
265 proportions of proteins were strikingly co-extracted in **CWSF**, **HWSF**, **CSF** and **NSF**,
266 with values ranging from 10 to 36% (Table 1). One intriguing possibility is that pectin
267 domains might be linked to polypeptides that serve as nuclei for their biosynthesis.
268 Crosslinks between RG-I and extensins, which are hydroxyproline-rich glycoproteins
269 located in the cell walls, have been reported for years (Fry, 1986; Jackson et al., 2001;
270 Pereira et al., 2011). Extensins are self-assembling amphiphiles that generate

scaffolding networks, where electrostatic extensin-pectate interaction may template the assembly of the pectic matrix, whose HG chains are negatively charged at physiological pH (Pereira et al., 2011). On the other hand, a peptidoglycan structure in which HG, RG-I, galactan and xylan domains were linked to arabinoxylan pectin arabinogalactan protein 1 (APAP1) was reported by Anderson (2016). Primary cell wall sensors for salt acclimation are wall-associated kinases, present in halophyte plants. The cell wall plays an important role in salt tolerance, especially in the detection of salt stress. In halophytes, cell homeostasis and osmotic adjustment are maintained, with consequences on the synthesis of proteins such as extensin and cell wall pectins (Le Gall et al., 2015).

The NS profile obtained for **CWSF**, **HWSF**, **CSF** and **NSF** is reported in Fig. 3a. Arabinose is always the main monosaccharide, and other components of pectins like Rha and Gal also appear in significant amounts. There are minor contents of Xyl and Man in all fractions, and also of Glc, especially in fraction **HWSF** (Fig. 3a), where it reaches 5.7g per 100g of the solubilized fraction (**HWSF**). Glucose could arise from the cell cytoplasm, but it could also arise from small proportions of starch, especially considering that the hot-water extract is the richest in this monosaccharide. The UA/Rha molar ratios observed for the pectins were always above 10, reaching a value of 20 for **CWSF** and **NSF**, and a value of 37 for the HG-enriched **CSF** (Fig. 2c). Hence, the isolated pectins are constituted by a high proportion of HG regions. The RG-I short segments are highly branched by long arabinan side chains, as also demonstrated by the high Ara/Rha (8-19) and Ara/Gal (3.6-14) molar ratios, as well as by a Gal/Rha molar ratio below 2.5 (Fig. 2c). Hence, the longest arabinan side chains belonged to the calcium (**CSF**) and covalently (**NSF**) crosslinked pectins.

The plant cell walls must be both flexible and strong to enable and constrain cellular, tissue and organ growth (Anderson, 2016). Moore, Farrant and Driouich (2008)

296 suggested that arabinan side chains of the RG-I of pectins are responsible for
297 buffering/replacing the water loss during desiccation and, hence, in preventing the
298 formation of tight junctions (e.g. calcium egg-boxes, strong H-bonding interactions).
299 Therefore, long arabinan side chains maintain the cell wall flexibility in spite of the low
300 levels of water in the environment, by hindering the formation of calcium-egg-box
301 interactions between scarcely methylesterified HG regions (Jones, Milne, Ashford, &
302 McQueen-Mason, 2003; Moore et al., 2008) like those found in our work (Table 1).
303 Alassali, Cybulska, Ríos Galvan, & Thomsen (2017) also found important levels of
304 arabinose in their study of the freshly green biomass of *Salicornia sinus-persica*
305 collected at Umm Al Quwain shores in United Arab Emirates for bioethanol production.
306 It was fractionated into the juice and the pulp. A content of 1.0-1.5% of free sugars was
307 found in the fresh juice, consisting of glucose (8.78 g/L), fructose plus xylose (3.90
308 g/L), and arabinose (0.35 g/L). On the other hand, the raw pulp of fresh *S. sinus-persica*
309 contained 15.63 g of glucose, 10.68 g of fructose plus xylose, and 11.08 g of arabinose
310 per 100 g dried mass.
311
312 *3.2. The presence of phenolic compounds in the pectic fractions*
313 Phenolic compounds were found in **CWSF** and **HWSF** at levels of 4.4 and 2.2%,
314 respectively (Table 1), corresponding probably to intracellular phenolics, since they
315 were water soluble. Flavonoids and other phenolics concentrated in the vacuoles of the
316 epidermal cells of *Salicornia* were reported as responsible for the absorption of the UV-
317 B radiation (280-320 nm spectral range), which increased in intensity because of the
318 Antarctic ozone hole and general depletion of the ozone layer in Tierra del Fuego and
319 Patagonia coast of Argentina. An increase in pigment absorption at 305 nm was
320 determined in the *Salicornia* salt-marsh of San Sebastián Bay, which was related to the

increase in the concentration of methanol-extractable absorbing pigments (Bianciotto et al., 2003). Costa, Chaves, Rombaldi, & Souza (2018) studied fresh vegetative and reproductive segments of shoots (with seeds) of three biotypes of *S. ambigua* cultivated in a field plot irrigated with saline effluent from a shrimp tank, and under significantly high NaCl soil contents. The 80% acidified aqueous methanol extracted the highest total phenolic content in shoots, which varied between 0.745 and 1.586 g/100 g fresh mass, expressed as gallic acid. However, most of the phenolic compounds in *S. ambigua* were extractable with water in all samples (0.678-1.325 g/100 g fresh mass). Kaempferol and gallic acid were the major phenolics, followed by hydroxybenzoic acid and quercetin. Coumaric, ferulic and caffeic acids, as well as catechin and epicatechin were also found in minor amounts (1.2-56 mg/100 g fresh mass). Bertin et al. (2014) extracted phenolics through methanol at 10 ºC from the aerial parts of the *S. ambigua* obtained from two regions of the Santa Catarina, Brazil. The methanolic extract obtained, treated with NaOH/pH 12 and finally acidified, contained scopoletin, syringaldehyde, eight phenolic acids (*p*-coumaric, cinnamic, vanillic, ferulic, caffeic, syringic, sinapic, and chlorogenic acids) and five flavonoids (galangin, quercetin, naringin, kaempferol and isoquercitrin). The *S. ambigua* samples collected from the natural marsh showed a higher number of flavonoids and of phenolic acids, being ferulic and caffeic acids the major phenolic compounds in both regions. Pinheiro et al. (2017) evaluated the integrated culture of *S. ambigua* and Pacific white shrimp in a seawater aquaponic system with biofloc. The final total phenolic content expressed as gallic acid in the aerial parts of *Sarcocornia* was 41.34 mg/100 g of fresh mass, as determined in the methanolic extract through the Folin Ciocalteau assay. These low levels of phenolics and, hence, of antioxidant activity was ascribed to the very low stress involved when plants are grown in an aquaponic system. The apical branches of the 100 days old *S. ambigua* shoots with fertile segments

(with seeds) were utilized after harvesting at Aracati (Brazil) and freeze-drying by de De Souza, Da Silva, Costa, & Badiale-Furlong (2018). Samples were watered with the saline effluent from a shrimp tank. Despite of the high proportion of lignin in the cell walls, the high-power ultrasound (30 min; 315 W; 5.89 W/cm^2) was able to extract with 80%-ethanol the highest yield of free phenolics (24.4 mg gallic acid/g dry mass; Folin Ciocalteau method). De Souza, Mendes, Doncato, Badiale-Furlong, & Costa (2018) analyzed 34 weeks old plants of the Brazilian *S. neei* in greenhouse and field conditions. BTH2 genotype mitigated photooxidative stress induced by salt exposition by increasing the relative content of chlorophyll b and the shoot content of flavonoids such as quercetin to enhance the antioxidant activity. All above-cited results from southern Brazil have been obtained with *S. neei* plants (previously wrongly named *S. ambigua*). Differences in yields and profiles of the extracted phenolic compounds can be accounted by the different solvents used for extraction (methanol or water), pH (acidic or alkaline medium), the origin and genotypes of the plants studied, and growth conditions.

*3.3. Extraction of **4KSF** and **24KSF** fractions and of final residue (**KIR**)*

After pectin extraction with Na_2CO_3 aqueous solution, only hemicelluloses, cellulose and lignin are expected to remain in the cell wall residue obtained at this step (**NIR**; Fig. 1) (Fry, 1986; Brett & Waldron, 1996). Strong alkaline solutions like KOH (4% and 24% w/v) act as chaotropic agents minimizing strong hydrogen-bonding between hemicelluloses and cellulose. Hence, they successively extract the hemicelluloses less and more strongly associated to cellulose (Fry, 1986).

As observed in Fig. 2a, the yields of **4KSF** and **24KSF**, expressed on dry mass of **FLM**, were around 3% for these hemicelluloses-enriched fractions. Thus, as occurred in

371 the case of pectins, a low proportion of hemicelluloses was also obtained in the cell
372 walls of *S. neei* (≈6.5%).

373 On the other hand, the largest proportion of the **FLM**, 58.3% w/w (dry basis),
374 corresponded to the final insoluble residue, **KIR** (Fig. 1), which is normally ascribed to
375 cellulose and lignin (Fry, 1986; Brett & Waldron, 1996). After analysis through sulfuric
376 acid (1M and 72%), we determined that **KIR** is mainly constituted by lignin (45%) and
377 cellulose (28%), but important contents of non-cellulosic carbohydrates (22%) and
378 proteins (4.5%) remained (Table 1). The non-cellulosic carbohydrates included a small
379 UA content (1.8%; Table 1). Evidently, the cellulose microfibrillar framework of *S. neei*
380 entangled some proteins of the cell wall as well as an important proportion of non-
381 cellulosic carbohydrates. The later corresponded to a very small amount of HG chains
382 (UA) (Table 1) and to a higher proportion of remaining RG-I (Fig. 2e), with Rha, Gal,
383 and part of the Ara content shown in Fig. 3c. The main components of the non-
384 cellulosic carbohydrates of **KIR** (Table 1) were Ara (8.4%) and Xyl (9.1%) (Fig. 3c),
385 which can be ascribed to arabinoxylans that remained entangled with cellulose after
386 extraction with 24% KOH (Fig. 1). The Ara/Xyl molar ratio was below 0.8 (Fig. 2e).
387 All these compounds were released after dissolution of the cellulose by 72% sulfuric
388 acid (section 2.4). Similarly, Alassali, Cybulska, Ríos Galvan, & Thomsen (2017) found
389 that the contents of fructose plus xylose and of arabinose in the fresh juice of *Salicornia*
390 *sinus-persica* biomass increased from 3.90 g/L and 0.35 g/L to 5.83 g/L and to 3.32 g/L,
391 respectively, after a 72% sulfuric acid treatment.

392 Cell wall modifications in halophytes in response to salt stress only involve
393 alteration of the secondary cell wall structure. The high lignin content found in
394 *Salicornia* can then be a characteristic of halophytes, as they might need cell wall
395 strengthening. (Le Gall et al., 2015). Lignin replaces water into the cell wall network

396 transforming the hydrophilic, hydrated gel of the cell wall matrix into a hydrophobic
397 environment, increasing the strength of hydrogen bonds between polysaccharides (Brett
398 & Waldron, 1996).

399 In the sequentially isolated hemicellulosic material **4KSF** and **24KSF**, the UA
400 contents were low (≈15%) in comparison to those of the pectin fractions previously
401 isolated (Table 1). It has been demonstrated that the identity of the UA is GalA (by
402 reduction of the UAs, hydrolysis and GC) and not D-glucuronic acid (GlcA), indicating
403 that it corresponds to pectic material. Therefore, there are pectins that remain entangled
404 in the hemicellulose network, being only released after strong alkaline treatment. On the
405 other hand, the NS contents were the highest observed (65-68%, Table 1). As expected,
406 NS/UA molar ratios of 5 were calculated (Fig. 2b), which are higher than ratios
407 expected for pectins. The NS composition of these hemicellulosic materials is highly
408 dominated by the presence of Xyl and Ara, suggesting that arabinoxylans are the main
409 hemicellulosic material found in *S. neei*. The Ara/Xyl molar ratios calculated were of 1
410 (**4KSF**) and 1.8 (**24KSF**), respectively (Fig. 2d). Although part of these Ara contents
411 can be ascribed to the side substitution of Rha in the RG-I of the pectins found
412 entangled with the arabinoxylans, the low levels of Rha (1.6-2.8%) and Gal (3-
413 3.9%)(Fig. 3b) suggests that most of the Ara is part of the arabinoxylans. The UA/Rha
414 molar ratio was higher in the pectins extracted in **4KSF** (9) than in those isolated in
415 **24KSF** (5) (Fig. 2d).

416 Besides the arabinoxylan component of **4KSF** and **24KSF**, moderate proportions of
417 proteins were again co-extracted in both solubilized fractions (7.6-13%, Table 1).

418 The total protein content of the whole *S. neei* material was 5.2% w/w (Table 2),
419 similar to the value of 6% reported previously (Cervellini & Angeletti, 2015). It is an

420 important value to be considered at the moment of *Salicornia* evaluation, for example,
421 as a forage resource.

422 Biomass composition of *S. neei* was in some measure comparable to traditional
423 lignocellulosic biomass used as substrate for biofuel production (Cybulska et al., 2014,
424 Bañuelos et al., 2018). In a different species, Bañuelos, Velazquez-Hernandez, Guerra-
425 Balcazar, and Arjona (2018) studied the dried and milled crop residues (plants without
426 seeds) of *Salicornia bigelovii* grown in Mexico (Ensenada, Baja California) for the
427 production of bioethanol. The biomass composition was comparable to traditional
428 lignocellulosic biomasses, and included 46.22% of cellulose, 14.93% of hemicellulose
429 and only 1.96% of lignin. However, Cybulska et al. (2014) also analyzed the straw
430 without seeds (dried stems, inflorescences, and branches) of *S. bigelovii* grown in the
431 United Arab Emirates by irrigation with saltwater (40 ppt) as the feedstock for
432 bioethanol production. The seedless *S. bigelovii* showed an extremely high ash content
433 (43.08 g/100 g dry mass). The washed biomass composition was comparable to
434 traditional lignocellulosic biomasses, showing relatively high glucan and xylan content
435 (26 and 22% dry mass, respectively) but with lower lignin content (7% dry mass). Our
436 result suggests that this low lignin content of Salicornia species is concentrated in the
437 **KIR** fraction.

438

439 *3.4. The presence of phenolic compounds in the hemicellulose fractions*

440 Phenolic compounds were determined at levels of ≈1.6% in **4KSF** and **24KSF** after
441 alkaline treatment (Table 1), which can then be ascribed to esterified feruloyl or
442 coumaroyl units. Feruloyl can be the pending group in the Ara lateral substituents of
443 xylan backbones (Scheller & Ulvskov, 2010). As reported above, Bertin et al. (2014)

found ferulic and caffeic acids as the major phenolic compounds in extracted through methanol at 10ºC from the aerial parts of the *S. ambigua*.

3.5. Rheological behavior of soluble fractions

The rheological performance of **CWSF**, **HWSF**, **NSF**, **4KSF** and **24KSF** aqueous soluble fractions was studied after their chemical characterization. It is a very important quality in relation to the potential utility of the polymeric fractions as additives in food and pharmaceutical formulations, where they can act as thickeners or gelling hydrocolloids, modifying the flow behavior of the developed systems.

The solid extracts of **CWSF**, **HWSF**, **NSF**, **4KSF** and **24KSF** (Fig. 1) were dissolved in water at a concentration of 2.0% w/v for rheological characterization and, as the pectins contained in these soluble fractions were of low DM (Table 1), calcium ion was added (15 mM $CaCl_2$). The mechanical spectra obtained are shown in Fig. 4 (a-b), which were recorded between 0.1 and 100 rad/s of angular frequency. For **CWSF**, **HWSF** and **NSF** (pectins), "weak gel"-type systems were found in the presence of calcium (Fig. 4a). This was explained by the fact that the elastic modulus (*G*') was always above the viscous one (*G*'') but in less than one logarithmic cycle, and showing some frequency dependence of moduli. The **NSF** spectrum presented the lowest dependence on frequency, and showed the highest value of *G*' (160 Pa at 2 rad/s of angular frequency). It was followed by those recorded from the **CWSF** (*G*' = 63 Pa at 2 rad/s) and **HWSF** (*G*' = 45 Pa at 2 rad/s) aqueous systems, with lower *G*' values and some higher frequency dependence (Fig. 4a).

On the other hand, the aqueous systems of the hemicelluloses **4KSF** and **24KSF** produced spectra typical of dilute solutions at the same concentration, as demonstrated from the respective mechanical spectrum (Fig. 4b). The viscous modulus *G*'' was above

469 *G*', being both strongly dependent on frequency, with a crossing point at a high
470 frequency value (≈10 rad/s).

471 As observed through the mechanical spectra obtained (Fig. 4a), the pectins were
472 able to interact enough in the water medium by entanglements between arabinan side
473 chains as well as by "egg-box-like" junctions through calcium ions at the HG segments
474 of neighboring macromolecules. As longer arabinan side chains at the RG-I can hinder
475 the formation of the "egg-box-like" structures between the HG segments of neighboring
476 macromolecules (Moore et al., 2008), not true-gel frameworks were formed then by
477 these pectins in calcium presence, in spite of the low degree of methylation and high
478 UA contents (Fig. 4a). The high proportion of proteins may also probably contribute to
479 disturb the "egg-box-like" structures' formation.

480 The arabinoxylans of **4KSF** and **24KSF** did not interact between macromolecules,
481 as demonstrated by the spectra obtained at the same concentration, which are
482 characteristic of "dilute solutions" (Fig. 4b). This is coherent with the assumption that
483 the side substitution of the xylan backbone occurs by short Ara chains (one Ara per Xyl
484 monomer in the backbone for **4KSF**, and less than two Ara units per Xyl monomer in
485 the backbone for **24KSF**) (Fig. 2d), instead of long chains of arabinan as side
486 substituents of the main xylan chain. As demonstrated by the dilute solution spectra
487 recorded (Fig. 4b), the proportion of pectins co-extracted in both fractions was too low
488 (Table 1) as to produce any thickening effect (Fig. 3b).

489

490 *3.6. Hydration properties of S. neei powder and residues **NIR** and **KIR***

491 The hydration properties of fibers are important parameters in, for example, a food
492 system, because they affect the physiological behavior of fibers in the gut and also the
493 texture of the food product and the processing conditions. A higher WRC, SC, and

494 solubility are attributed to the presence of high soluble fiber components. The total *S.*
495 *neei* powder and the final residue of the sequential extraction (**KIR**) (Fig. 1) showed
496 comparable low SC values (Fig. 4c), whereas the **NIR** residue (Fig. 1), which contains
497 all arabinoxylans, proteins and some entangled pectins in addition to lignin and
498 cellulose, presented the highest SC value (6.2 mL water/g dry mass) (Fig. 4c). The
499 WHC values of *Salicornia* powder, **NIR** and **KIR** were similar, of ≈1 g of water per
500 gram of dry residue (Fig. 4c). The WRC values were lower than the respective WHC
501 value, and below 1 g of water per g of residue, especially for **NIR** and **KIR** (Fig. 4c).
502 The very low levels of remaining pectins in **NIR** and **KIR** can be responsible for the
503 low nominal values of WHC and WRC and also of SC. In addition, the relative high
504 content of lignin in the cell walls of *Salicornia* powder creates a hydrophobic
505 environment and, hence, low hydration (Brett & Waldron, 1996).

506 Ye, Tao, Liu, Zou, & Zao (2015) obtained insoluble fiber from orange pomace by
507 elimination of the soluble fraction with 60ºC-water. The insoluble fiber residue, dried
508 (60ºC, 48 h), grinded and micronized showed SC and WHC values of 7.14-6.17 mL/g,
509 and 7.33-5.74 g water/g fiber, respectively. In another case, a wheat bran dietary fiber
510 powder was prepared through ultrafine grinding (Zhu, Huang, Peng, Oian, & Zhou,
511 2010). The insoluble fiber product, free of phytic acid, starch and proteins, dried and
512 micronized gave low values of SC (5.79 mL water/g), WHC (5.89 g/g) and WRC (4.61
513 g/g). In other works, a WHC value of 3.6 g/g was obtained from rice bran (Wen, Niu,
514 Zhang, Zhao, & Xiong 2017), 6.1 g/g from wheat bran, 2.32 g/g from maize hulls, 2.48
515 g/g from wheat hulls and 4.9 g/g from soybean fiber (Vazquez-Ovando, Rosado-Rubio,
516 Chel-Guerrero, & Betancur-Ancona, 2009; Wen et al., 2017). All these hydration values
517 reported were similar to those shown by *Salicornia* powder, **NIR** and **KIR** (Fig. 4c).
518

519 *3.7. Potentiality of S. neei as a source of biopolymers and co-extracted antioxidants*

520 As a result of the chemical analysis performed, the final composition of the dry *S.*
521 *neei* powder can be summarized in Table 2. According to the phenolic composition,
522 mainly in aqueous extractable phenolics (6.6%), it is expectable an antioxidant capacity.
523 The antioxidant capability of the *S. neei* powder determined in the present work through
524 the DPPH radical scavenging activity was equivalent to 222 mg of AA per 100 g of dry
525 plant (Table 2). For comparison, a DPPH radical scavenging capacity between 27.4 and
526 61.1 mg of AA equivalents per 100 g was reported for total phenolic contents between
527 42 and 109 mg per 100 g of fresh plums from California (Gil, Tomas-Barberan, Hess-
528 Pierce, & Kader, 2002).

529 A whole fraction enriched in pectins and arabinoxylans was separated with a total
530 yield of almost 9%, which can be investigated as dietary fiber supplements as well as
531 thickeners, for example, in food formulation (Basanta et al., 2018). Pectins
532 demonstrated their ability to constitute “weak gels” in water in the presence of calcium
533 ions. The alkali-extractable arabinoxylans are of particular interest as they could have
534 bread-improving properties in cereal flours (Ebringerová, Hromádková, & Heinze,
535 2005). The co-extracted proteins and the ferulic acid contents of pectins (**NSF**) and
536 arabinoxylans can contribute with emulsifying properties to the polysaccharide fractions
537 (Siew & Williams, 2008).

538 The main components of *S. neei* biopolymers were cellulose and lignin (42.6%;
539 Table 2). They demonstrated some swelling and WRC capacity in water. Cellulose can
540 be analyzed for development of nanofibers (Bernhardt et al., 2019). Lignin and cellulose
541 can be also investigated as food ingredients, with some antioxidant capability of lignin
542 (Lu et al., 2012).

543

4. Conclusion

By sequential extraction of cell wall biopolymers it was determined that *S. neei* contains a low level of calcium gelling pectins (4.5%) with low RG-I proportion (NS/UA=0.5-1.2), and of arabinoxylans (4.3%), together with proteins (5.2%) and 7.1% of lipidic material. The RG-I of pectins was characterized by long chains of arabinans, which have been related to the maintenance of cell wall flexibility under water deficit, and with an important role in salt tolerance, with consequences on the synthesis of cell wall proteins such as extensin. Beyond these components, cellulose (16.3%) and specially lignin (26.3%) were by far the main biopolymers found in *S. neei* (dry plant) and, hence, a relevant potential for their use as bulking (antioxidant) active food ingredients can be inferred. The final residue of the sequential extraction of cell wall polymers (**KIR**) was rich in cellulose and especially in lignin, which determined a rather low hydration capability. Despite of the poor rheological effect of arabinoxylans (**4KSF** and **24KSF**) when dissolved at 2.0% w/v concentration in water with calcium ion, they increased the hydration level of the cellulose and lignin fibers when present in the insoluble residue (**NIR**). Phenolics (9.8%) were mainly water-extractable (6.6%), related probably to their protective function against UV radiation in the plant, while the rest were ester bridges and/or pendant groups in arabinoxylans and probably also in some pectins. After this study, it is concluded that *S. neei* constitute a source of biopolymers and co-extracted antioxidants that can be evaluated as natural food additives/ingredients as well as for material development. Knowledge about what crosslinkings have to be considered at the moment of the extraction of the components of interest by mean of enzymes, for example, was also gained after this study.

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794 **Figure Captions**

795 **Fig. 1.** Scheme of sequential extraction of defatted *S. neei* powder (**FLM**).

796

797 **Fig. 2.** Yield of soluble fractions obtained by sequential extraction. Different letters
798 indicate significant differences ($n = 3$) based on an LSD test ($p < 0.05$) (**a**). Molar ratios
799 calculated for neutral sugars (NS) /uronic acids (UA) (**b**), UA/rhamnose (Rha),
800 arabinose (Ara)/Rha, galactose (Gal)/Rha, and Ara/Gal (**c**), UA/Rha and Ara/xylose
801 (Xyl) (**d**), and for the last residue obtained through the sequential extraction (**KIR**) (**e**).

802

803 **Fig. 3.** Neutral sugars' composition of the soluble fractions sequentially extracted from
804 *S. neei* (**a**, **b**), and of the final residue (**c**). Different letters for a given monosaccharide
805 indicate significant differences ($n = 3$) based on an LSD test ($p < 0.05$).

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807 **Fig. 4.** Mechanical spectra recorded at 20ºC from 2.00% w/v aqueous solutions (15 mM
808 $CaCl_2$) of **CWSF**, **HWSF**, **NSF** (**a**), **4KSF** and **24KSF** (**b**). Hydration properties of
809 *Salicornia* powder, and of **NIR** and **KIR** residues: swelling (SC), water holding (WHC)
810 and water retention (WRC) capacities. Different letters for a given property indicate
811 significant differences ($n = 3$) based on an LSD test ($p < 0.05$) (**c**).

812 **Table 1.** Analysis of the extracted fractions from *S. neei* and of the final residue (**KIR**).

	Uronic acids (g/100 g dry fraction)[1]	DM[2] (% molar ratio)	Neutral sugars (NS) or non-cellulosic carbohydrates (g/100 g dry fraction)[1]	Proteins (g/100 g dry fraction)[1]	Phenolics (g /100 g dry fraction)[1,3]	Cellulose (g/100 g dry fraction)[1]	Lignin (g/100 g fraction
SF	30.9 ± 0.3	16	22 ± 1	35.9 ± 0.5	4.4 ± 0.1	—	—
SF	21.4 ± 0.8	25	26 ± 3	25 ± 6	2.2 ± 0.3	—	—

T	27 ± 3	21	13 ± 1	10.1 ± 0.3	ND	—	—
T	34 ± 1	1.8	31 ± 4	28.6 ± 0.6	ND	—	—
SF	16 ± 1	—	68 ± 4	13 ± 1	1.7 ± 0.9	—	—
ISF	14.8 ± 0.4	—	66 ± 0.8	7.6 ± 0.4	1.5 ± 0.5	—	—
R	1.8 ± 0.1	—	22 ± 6	4.5 ± 0.8	ND	28 ± 5	45 ± 8

[1]Mean and standard deviation (SD) for *n*=3 are reported.
[2]DM: degree of methylation.
[3]Phenolics' content determined after alkaline hydrolysis, and expressed as grams of gallic acid per 100 g of dry fraction.
ND: non detectable.

818 **Table 2.** Biopolymer composition, phenolics' content and antioxidant capacity of dry *S.*
819 *neei*.

	g/100 g dry plant
Lipids	7.1
Aqueous extractable phenolics[1,2]	6.6
Bound phenolics[2,3]	3.2
DPPH[4¶] (mg AA/100g dry plant)	222
Proteins	5.2
Pectins	4.5
Arabinoxylans	4.3
Cellulose	16.3
Lignin	26.3
Non-cellulosic carbohydrates of KIR	13.1

820 [1]Phenolics fraction extracted in CWSF and HWSF.
821 [2]Expressed as grams of gallic acid per 100 g of dry plant.
822 [3]Phenolics fraction extracted in 4KSF and 24KSF.
823 [4]DPPH scavenging antioxidant activity expressed as mg of
824 L-(+)-ascorbic acid (AA) per 100 g of dry plant.
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828 **CRediT author statement**
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830 **Perennial halophyte Salicornia neei Lag.: cell wall composition and functional properties**
831 **of its biopolymers**

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HIGHLIGHTS

Sequential extraction of *Salicornia neei* halophyte gave 4.7% of pectin and protein

These water and Na_2CO_3 soluble pectin fractions gave weak-gel type systems in calcium

KOH-soluble fractions (4.3%) were arabynoxylans that gave dilute solutions in calcium

Cellulose and lignin were major components (42.6%); phenolics (9.8%) were also found

Joined to high antioxidant activity made *S. neei* a source of useful food additives