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Enhanced Carrageenan Extraction from Chondrus crispus Using Pulsed Electric Field Pretreatment

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Abstract

Chondrus crispus is a major commercial source of carrageenan, a sulfated polysaccharide widely used in food and biotechnological applications. However, conventional hot water extraction methods often require prolonged heating and intensive processing, which can reduce extraction efficiency, increase energy consumption, and partially degrade carrageenan structure, thereby compromising functional properties and overall product quality. Improving extraction efficiency while preserving carrageenan structural integrity therefore remains a key challenge for sustainable macroalgal processing. In this study, pulsed electric field (PEF) pretreatment was evaluated as a non-thermal strategy to enhance carrageenan extraction from *C. crispus* harvested from the Norwegian coast. Biomass was subjected to PEF treatment at electric field strengths of 2.5 and 3.0 kV cm^{-1} using pulse numbers ranging from 100 to 1500, followed by aqueous extraction. The results were compared with conventional hot water extraction (HWE).

PEF pretreatment significantly influenced polysaccharide recovery, with the highest yield obtained at 3.0 kV cm^{-1} and 1500 pulses, exceeding that of the HWE ($p < 0.05$). Protein content in the solid residues was not significantly affected by PEF intensity, indicating minimal protein solubilization or degradation. Monosaccharide analysis revealed galactose as the dominant sugar in all extracts, consistent with carrageenan-rich polysaccharides, with enhanced galactose recovery under optimized PEF conditions. Structural characterization by ^{1}H and ^{13}C NMR spectroscopy confirmed the presence of mixed κ- and ι-carrageenan motifs across all treatments, with no evidence of major structural degradation.

Overall, PEF-assisted extraction represents an effective and mild approach to improve carrageenan recovery from *C. crispus* while preserving polysaccharide structure, highlighting its potential for application in sustainable macroalgal biorefineries.

Introduction

Red macroalgae is an important renewable resource for the production of structurally diverse sulfated polysaccharides with extensive applications in food, pharmaceutical, cosmetic, and biotechnological industries. Among these polysaccharides, carrageenans — linear galactans composed of alternating β-D-galactopyranose and α-D-galactopyranose residues — are of particular commercial relevance due to their gelling, thickening, and stabilizing properties (Campo et al. 2009; Usov 2011; Necas and Bartosikova 2013). The physicochemical properties of carrageenans, including gel strength, viscosity, and biological activity, are strongly influenced by their molecular weight, degree of sulfation, and the relative abundance of κ-, ι-, and λ-type structural motifs (Velde and Ruiter 2002; Pereira and Van De Velde 2011).

Chondrus crispus (Stackhouse), commonly known as Irish moss, is one of the most extensively studied and commercially exploited red algae for carrageenan production. Its polysaccharide fraction is typically rich in κ- and ι-carrageenan, making it a valuable raw material for industrial extraction (Campo et al. 2009; Pereira and Van De Velde 2011). Conventional carrageenan extraction relies primarily on hot water

or alkaline treatments, which are effective but energy-intensive and may induce partial depolymerization or structural modification of the polysaccharide chains (Rioux et al. 2007; Deniaud-Bouët et al. 2014). In addition, thermal extraction often lacks selectivity and may compromise the integrity of other valuable biomass components, such as proteins, thereby limiting opportunities for full biomass valorization.

In recent years, non-thermal and energy-efficient technologies have gained increasing attention as sustainable alternatives to conventional extraction methods. PEF technology has emerged as a promising pretreatment technique capable of enhancing mass transfer through electroporation of cell membranes while minimizing thermal and chemical degradation (Toepfl et al. 2007; Parniakov et al. 2015). PEF has been successfully applied to improve the extraction of intracellular compounds from various biological matrices, including plant tissues, microalgae, and macroalgae, primarily by enhancing cell membrane permeabilization and mass transfer (Parniakov et al. 2015; Robin et al. 2020). In seaweed systems, this electroporation effect has been reported to facilitate the recovery of hydrocolloids and other intracellular polysaccharides from several carrageenophyte red macroalgae, suggesting its potential as a mild and energy-efficient alternative to thermal extraction. However, despite this growing application, systematic studies addressing the influence of PEF parameters on carrageenan yield, composition, and structural integrity in *Chondrus crispus* remain limited.

Beyond extraction yield, preservation of carrageenan structural features is critical for determining functional performance. Nuclear magnetic resonance (NMR) spectroscopy is a powerful analytical tool for elucidating carrageenan fine structure, including glycosidic linkage configuration, sulfation patterns, and the relative abundance of κ- and ι-carrageenan motifs (Knutsen et al. 1990; Velde and Ruiter 2002). Coupling advanced extraction strategies with detailed structural characterization is therefore essential to assess whether emerging technologies such as PEF can enhance extraction efficiency without compromising polysaccharide quality.

The aim of the present study was to evaluate the effectiveness of PEF pretreatment for the extraction of carrageenan-rich polysaccharides from *C. crispus* harvested from Norwegian coastal waters. The effects of electric field strength and pulse number on polysaccharide yield, protein retention, and monosaccharide composition were systematically investigated and compared with conventional hot water extraction to assess PEF processing's potential role in cascade biorefinery approaches for this species. The structural features of the extracted polysaccharides were characterized by ^{1}H and ^{13}C NMR spectroscopy to assess potential differences in carrageenan composition and integrity. The results provide new insights into the potential of PEF-assisted extraction as a mild and sustainable strategy for carrageenan recovery and support its integration into future macroalgal biorefinery concepts.

Material and methods

Raw material and harvesting

C. crispus was harvested from Hafrsfjord (58.94546, 5.67160), Stavanger, Norway, in September 2023. At the time of sampling, the tidal water level ranged from 38 from 49 cm, and the seawater temperature was 15.6°C. The harvested samples were thoroughly rinsed with tap water followed by distilled water to remove epiphytes, sand and other debris. The cleaned wet biomass was homogenized using an IKA T25 Digital Ultra TURRAX homogenizer (IKA, China). For each experimental condition, three biological replicates were prepared, each consisting of 20 g (wet weight) of *C. crispus* suspended in 200 mL of distilled water.

Pulsed electrical field treatment

PEF treatment was conducted using a PEF Pilot™ Dual system (Elea GmbH, Quakenbrück, Germany) equipped with an 8 dL batch chamber and an electrode gap of 8 cm. Samples were treated at two applied voltages of 20 kV and 24 kV, corresponding to effective electric field strengths of 2.5 kV cm^{-1} and 3.0 kV cm^{-1}, respectively. For each field strength, pulse numbers of 1500, 1200, 800, 400 and 100 were applied. The pulse frequency and pulse width were maintained at 30 Hz and 6 µs, respectively, for all treatments. Following PEF treatment, the samples were centrifuged at 10 000 × g for 20 min at 25°C using a Multifuge X3 FR centrifuge (Thermo Fisher Scientific, Waltham, MA, USA). The resulting supernatant was first filtered through a coffee filter (10–15 µm) to remove coarse particulate matter, followed by fine filtration using a Whatman No. 4 filter paper. Polysaccharides were precipitated by adding three volumes of cold ethanol (99.5%, v/v), and the mixtures were incubated at 4°C overnight. The precipitated polysaccharides were subsequently recovered for further analysis. A schematic overview of the experimental workflow is shown in Fig. 1.

Conventional hot water extraction

For conventional hot water extraction, 20 g (wet weight) of freshly chopped *C. crispus* was mixed with 200 mL of distilled water and heated at 95°C for 2 h under continuous mechanical stirring. After extraction, the mixture was centrifuged at 10 000 × g for 20 min at 25°C (Multifuge X3 FR, Thermo Fisher Scientific, Waltham, MA, USA) to separate the algal residue. The supernatant was subsequently filtered through Whatman No. 4 filter paper then mixed with 3-fold volume of cold ethanol (99.5%, v/v). The mixture was incubated at 4°C overnight to precipitate the polysaccharides, following a previously reported protocol (Atya et al. 2023).

Composition analysis

The dry matter (DM) content of untreated *C. crispus* raw material was determined by oven drying the biomass at 105°C for 24 h. The remaining residue was dried at 550°C for 24 h to determine the ash content in a muffle furnace (Carbolite-Gero AAF 11, Neuhausen, Germany).

The protein content in the solid fraction was determined using the Kjeldahl method with a nitrogen-to-protein conversion factor of 6.25. Approximately 0.10 g of freeze-dried material was digested with 15 mL

of concentrated sulfuric acid (Merck KGaA, Darmstadt, Germany) in the presence of two copper catalyst tablets (Kjeltabs Cu/3.5, Nerlien Meszansky, Oslo, Norway). Digestion was performed at 420°C for 1 h using a Kjeltec digestion system (Tecator Inc., Herndon, VA, USA). After digestion, 30 mL of distilled water was added, and the samples were analyzed in triplicate using a Kjeltec™ 8400 analyzer (FOSS Analytics, Hillerød, Denmark).

The monosaccharide composition of the ethanol-precipitated polysaccharides was analyzed by gas chromatography with flame ionization detection (GC–FID; Agilent Technologies, Santa Clara, CA, USA). Arabinose, fucose, galactose, glucose, rhamnose (Sigma-Aldrich, St. Louis, MO, USA), and xylose (Merck KGaA, Darmstadt, Germany) were used as external standards. Sample preparation and analytical procedures were performed according to previously published methods, with minor modifications, as described in (Englyst et al. 1994; Maribu et al. 2024).

Before NMR measurements, the polysaccharide molecular weight was reduced by ultrasonic treatment using an ultrasonic homogenizer (Qsonica Q125, Newtown, CT, USA). A 1.5% (w/v) polysaccharide solution was sonicated in a cooled water bath at 80% amplitude for 3 h. The sonicated solution was subsequently centrifuged, filtered, and lyophilized. The dried polysaccharide was redissolved in D_2O to a final concentration of 1.5% (w/v). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 600 MHz spectrometer for all samples at 90 °C. ^{1}H NMR spectra were acquired using a standard single-pulse experiment with water suppression (Bruker pulse program *zgpg30*), employing pulsed field gradients to attenuate the residual water signal. A total of 512 scans were collected for ^{1}H NMR and 20,000 scans for ^{13}C NMR, with a relaxation delay of 5 s. Sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS, $\delta = 0$ ppm) was used as an internal chemical shift reference.

Results

Chemical characterization

The untreated *C. crispus* biomass exhibited a dry matter (DM) content of 22.3 ± 1.4%, while the ash content accounted for 15.9 ± 1.1% of the DM. These values are consistent with previously reported data for red macroalgae, which are characterized by relatively high mineral contents due to their marine origin (Rioux et al. 2007; Holdt and Kraan 2011).

Polysaccharide yields varied significantly depending on the applied pretreatment and extraction conditions (Fig. 1). Among all treatments, PEF pretreatment at 24 kV combined with 1500 pulses resulted in the highest polysaccharide yield, which was significantly higher than those obtained at lower pulse numbers under the same electric field strength and higher than that of the HWE ($p < 0.05$). This enhancement can be attributed to PEF-induced electroporation of algal cell membranes, which increases permeability and promotes improved mass transfer during subsequent extraction (Toepfl et al. 2007; Barba et al. 2015). The untreated control also exhibited a comparatively high polysaccharide yield, indicating that conventional extraction remains effective for *C. crispus*. Similar observations have been

reported for carrageenan-rich red algae, where hot water extraction alone can yield substantial polysaccharide recovery (Campo et al. 2009). Nevertheless, the superior yield achieved under high-intensity PEF conditions demonstrates the added value of electrical pretreatment in enhancing extraction efficiency.

At both 20 kV and 24 kV, a general decrease in polysaccharide yield was observed with decreasing pulse number. Treatments employing ≤ 400 pulses resulted in significantly lower yields, with no marked differences among these low-intensity conditions. This suggests the existence of a threshold for PEF intensity below which cell permeabilization is insufficient to markedly enhance polysaccharide release. Similar threshold-dependent effects of PEF intensity on extraction efficiency have been reported for both microalgal and macroalgal systems (Parniakov et al. 2015; Robin et al. 2020). Statistical groupings indicated by different letters in Fig. 2 confirm significant differences between treatments ($p < 0.05$).

Overall, these results demonstrate that both electric field strength and pulse number are critical parameters governing polysaccharide recovery from *C. crispus*. High-intensity PEF pretreatment appears necessary to achieve meaningful improvements over conventional extraction, supporting its potential application in sustainable macroalgal biorefinery processes.

The protein content of the solid fractions obtained after the different processing treatments ranged from 14.2 to 16.2% on a dry weight (DW) basis (Figure. 3). No statistically significant differences were observed among the PEF-treated samples across the investigated electric field strengths (20 kV and 24 kV) and pulse numbers (100–1500 pulses) ($p > 0.05$), indicating that variations in PEF processing intensity did not affect protein retention in the solid residue.

The highest protein content was observed in the raw material, followed by the hot water extraction (HWE) treatment. In contrast, all PEF-treated samples exhibited comparable protein levels, which remained close to those of the untreated biomass. This suggests that PEF pretreatment did not promote substantial protein solubilization or degradation during subsequent extraction steps. Similar observations have been reported for PEF-treated macroalgal and microalgal biomass, where electroporation primarily enhances polysaccharide or metabolite release while largely preserving protein structures within the solid matrix (Parniakov et al. 2015; Barba et al. 2015; Maribu et al. 2024).

The limited impact of PEF on protein content further indicates that the applied electric field strengths and pulse durations were sufficiently mild to avoid protein denaturation or excessive leaching into the liquid phase. This is consistent with previous studies demonstrating that PEF is a non-thermal technology capable of selectively disrupting cell membranes without causing significant thermal or chemical damage to intracellular proteins (Toepfl et al. 2007; Robin et al. 2020). In contrast, thermal treatments such as hot water extraction are more likely to promote partial protein solubilization or structural modification, which may explain the slightly lower protein content observed after HWE. Overall, these results demonstrate that PEF-assisted processing enables efficient polysaccharide extraction from *C. crispus* while maintaining protein integrity in the residual biomass. This selective behaviour highlights

the potential of PEF as a gentle pretreatment strategy in macroalgal biorefineries, where simultaneous valorization of polysaccharides and protein-rich residues is desirable.

The monosaccharide composition of the polysaccharide precipitates obtained under different extraction conditions is presented in (Fig. 4). Across all samples, galactose was the dominant monosaccharide, followed by glucose, whereas xylose and mannose were detected only in minor amounts.

Galactose contents ranged from 102 to 208 g per 100 g sample, with the highest values observed for treatments performed at 24 kV using 1200 or 400 pulses. In contrast, the lowest galactose content was obtained by conventional hot water extraction (HWE). The predominance of galactose is in agreement with the chemical structure of carrageenan, which consists mainly of alternating 3-linked β-D-galactopyranose and 4-linked α-D-galactopyranose units. Similar monosaccharide profiles have been reported for carrageenan-rich extracts from red macroalgae, confirming galactose as the principal constituent sugar (Campo et al. 2009; Pereira and Van De Velde 2011; Necas and Bartosikova 2013). The enhanced galactose recovery observed under PEF conditions suggests more efficient disruption of the algal cell wall and improved solubilization of carrageenan compared to HWE.

Glucose was consistently the second most abundant monosaccharide detected in all extracts. Although its concentration varied among treatments, no clear correlation with extraction conditions was observed. The presence of glucose is likely associated with the co-extraction of storage polysaccharides or minor glucan components embedded within the algal cell wall matrix, as previously reported for red algae polysaccharide extracts (Lahaye and Robic 2007; Rioux et al. 2007).

Xylose and mannose were detected at relatively low levels in all PEF-treated samples, and no statistically significant differences were observed among the extraction conditions. Mannose was not detected in the HWE sample, indicating that its extraction may require more intensive cell disruption, which is more effectively achieved by PEF treatment. Minor amounts of these sugars have been attributed to hemicellulosic polysaccharides or glycoprotein-associated carbohydrates in red algae (Deniaud-Bouët et al. 2014).

Overall, the monosaccharide profiles demonstrate that extraction conditions strongly influence galactose recovery while having a limited effect on minor sugar components. The higher galactose yields obtained under optimized PEF conditions highlight the potential of this technique for the selective extraction of carrageenan-rich polysaccharides, offering a promising alternative to conventional thermal extraction methods.

Nuclear magnetic resonance (NMR) spectroscopy was employed to elucidate the structural features of polysaccharides extracted from *C. crispus*, including monosaccharide composition, glycosidic linkage configuration (α- and β-anomers), and sulfation patterns. To enhance spectral resolution and minimize aggregation effects commonly associated with high-molecular-weight polysaccharides, all samples were subjected to ultrasonic pretreatment prior to analysis and measured at elevated temperature (90°C) using a 600 MHz NMR spectrometer.

The ^{1}H NMR spectra of all polysaccharide extracts exhibited characteristic carbohydrate proton resonances in the range of 3.0–5.7 ppm (Figure. 5). The spectral profiles were consistent with carrageenan-type polysaccharides and indicated the presence of mixed ι- and κ-carrageenan structures across all extraction conditions. Notably, a distinct and reproducible resonance at 5.17 ppm was observed in all spectra and assigned to the anomeric proton of 4-linked-D-galactose-6-sulfate (D6S). This structural unit is recognized as a biosynthetic precursor of both κ- and ι-carrageenan (Knutsen et al. 1990). The persistence of this signal confirms that both PEF–assisted extraction and conventional hot water extraction preserved the fundamental carrageenan backbone without inducing major structural degradation.

The spectrum obtained from the hot water extracted sample displayed sharper and better-resolved resonances compared to those of the PEF-treated fractions. This observation likely reflects differences in molecular weight distribution and structural heterogeneity between the extracts. Hot water extraction may promote partial depolymerization or preferential extraction solubilization of lower-molecular-weight fractions, resulting in reduced signal broadening. In contrast, the broader peaks observed in the PEF-treated samples suggest the retention of higher-molecular-weight carrageenan chains and possible intermolecular aggregation effects.

Furthermore, a gradual decrease in peak sharpness was observed with decreasing electric field strength and pulse number, indicating a potential relationship between PEF treatment intensity and the degree of polysaccharide fragmentation or solubilization. These findings suggest that higher-intensity PEF conditions may enhance extractability while preserving the structural integrity of carrageenan polymers.

^{13}C NMR spectroscopy provided further insight into the detailed chemical structure of the extracted carrageenan fractions (Fig. 6). Characteristic anomeric carbon signals corresponding to 3,6-anhydro-D-galactose and sulfated D-galactose residues were observed in the range of 95–102 ppm, in agreement with reported chemical shift assignments for κ- and ι-carrageenan (Velde and Ruiter 2002; Pereira et al. 2009).

Detailed analysis of the chemical shifts and relative signal intensities of ring carbon atoms revealed distinct resonances attributable to carrageenan repeating units. As summarized in Table 1, signals at 74.04, 74.73, and 74.70 ppm were assigned to the C-4, C-5, and C-6 positions of 3-linked galactose-4-sulfate units, respectively. These assignments are consistent with the presence of mixed κ- and ι-carrageenan structural motifs, characterized by alternating β-D-galactose-4-sulfate and 3,6-anhydro-α-D-galactose residues.

Comparative analysis of the ^{13}C NMR spectra revealed differences in chemical shift patterns and signal intensities between PEF-treated and hot water–extracted samples. These variations likely reflect differences in sulfation degree, linkage distribution, and molecular organization induced by the extraction method. These structural features suggest that PEF treatment better preserves the native carrageenan architecture, potentially maintaining higher molecular weight and sulfation heterogeneity. Such preservation is expected to translate into improved functional properties, including enhanced gel

strength, viscosity, and overall techno-functional performance, compared with polysaccharides obtained by hot water extraction, which may promote partial depolymerization.

^{13}C NMR spectroscopy provides valuable information on the detailed chemical structure of polysaccharides particularly those composed of repeating or related oligomeric blocks. In ^{13}C NMR spectra Figure. 6, the anomeric signals characteristic of d-anhydro galactose residue in carrageenan were observed within the range of 95–102 ppm.

To assess the structural composition of the carrageenan fractions, the chemical shifts and relative signal intensities of the anomeric and ring carbon atoms were analyzed. As summarized in Table 1, distinct signals were detected at 74.04, 74.73, and 74.70 ppm, corresponding to the C-4, C-5, and C-6 positions of 3-linked galactose-4-sulfate units, respectively. These signals are indicative of the presence of ι- and κ-carrageenan structural motifs.

The comparison of ^{13}C NMR spectra from PEF - treated and hot water extracted samples revealed differences in chemical shift patterns and signal intensities, reflecting variations in sulfation and linkage configurations between extraction methods.

Table 1

Chemical shifts (ppm) of the ^{13}C NMR spectra of carrageenan fractions extracted by PEF and hot water extracted from *C crispus*.

Repeating unit	Sugar	Carbon atom					
		C-1	C-2	C-3	C-4	C-5	C-6
G4S-DA	3-linked	102.41	69.57	78.88	74.04	74.73	61.22
	4-linked	95.21	69.81	79.08	78.26	76.73	69.39
G4S-DA, 2S	3-linked	102.41	69.37	77.04	71.71	73.49	71.71
	4-linked	91.97	75.69	78.24	72.06	77.03	70.58

The units used in the present study to annotate signals are in the following abbreviations:

G4S-DA

β-D-galactose, 4-sulfate and 3,6 anhydro-α-D-galactose

G4S-DA, 2S

β-D-galactose, 4-sulfate and 3,6 anhydro-α-D-galactose, 2- sulfate.

Discussion

The chemical characterization of *C. crispus* highlights the influence of pulsed electric field (PEF) pretreatment on polysaccharide recovery while preserving other biomass components. The dry matter and ash contents of the untreated biomass fall within the typical range reported for red macroalgae, reflecting their naturally high mineral content associated with the marine environment(Rioux et al. 2007; Holdt and Kraan 2011). These baseline values confirm the suitability of the raw material for carrageenan extraction.

The variation in polysaccharide yield across treatments demonstrates that both electric field strength and pulse number are key factors governing extraction efficiency. The significantly higher yield obtained at 24 kV and 1500 pulses indicates that high-intensity PEF conditions effectively enhance cell membrane permeabilization through electroporation, thereby facilitating the release of intracellular and cell wall–associated polysaccharides (Toepfl et al. 2007; Barba et al. 2015). The presence of a threshold effect, below which no significant improvement is observed (≤ 400 pulses), suggests that a minimum energy input is required to achieve sufficient disruption of the algal cellular structure. This behavior is consistent with previously reported PEF-assisted extraction mechanisms in algal systems (Parniakov et al. 2015; Robin et al. 2020).

Despite the improvements achieved with PEF, the relatively high yield obtained from the untreated control and hot water extraction confirms that *C. crispus* is inherently amenable to conventional extraction. However, the added value of PEF lies in its ability to enhance extraction efficiency without relying solely on prolonged thermal treatment, supporting its application as a more energy-efficient and potentially sustainable alternative (Barba et al. 2015).

The protein content results further emphasize the selectivity of PEF processing. The absence of significant differences among PEF-treated samples, along with values comparable to the untreated biomass, indicates that PEF does not promote extensive protein solubilization or degradation. This suggests that the applied conditions primarily target membrane permeability without disrupting protein integrity within the solid matrix (Parniakov et al. 2015; Barba et al. 2015). In contrast, the slightly lower protein content observed after hot water extraction likely reflects partial protein solubilization or thermal denaturation. These findings highlight an important advantage of PEF as a mild, non-thermal technology that enables selective fractionation of biomass components (Toepfl et al. 2007; Robin et al. 2020).

The monosaccharide composition provides additional insight into the effectiveness and selectivity of the extraction process. The dominance of galactose across all samples confirms that the extracted polysaccharides are primarily carrageenan, consistent with the known composition of *C. crispus* (Campo et al. 2009; Pereira and Van De Velde 2011; Necas and Bartosikova 2013). The enhanced galactose content observed under optimized PEF conditions indicates improved recovery of carrageenan-rich fractions, likely due to more efficient disruption of the cell wall matrix. In contrast, the relatively minor variations in glucose, xylose, and mannose suggest that these components are less sensitive to extraction conditions and may originate from structurally distinct polysaccharides or minor cell wall constituents (Lahaye and Robic 2007; Rioux et al. 2007).

The absence of mannose in the hot water extract, compared to its presence in PEF-treated samples, further supports the role of PEF in enabling the release of less accessible or more tightly bound components within the biomass. However, the consistently low levels of these minor sugars indicate that the extraction process remains largely selective toward carrageenan (Deniaud-Bouët et al. 2014).

The observed chemical shifts in both the anomeric and ring carbon regions confirm that all extraction methods yielded carrageenan-type polysaccharides with preserved primary structural features. The presence of signals corresponding to both sulfated galactose and 3,6-anhydrogalactose residues indicates a mixed κ-/ι-carrageenan composition, in agreement with the ^{1}H NMR results (Velde and Ruiter 2002; Pereira et al. 2009).

Differences in signal intensity and peak sharpness between treatments likely reflect variations in molecular weight distribution, sulfation degree, and structural organization. The relatively well-defined signals observed in PEF-treated samples suggest that this method preserves the native polysaccharide architecture more effectively, maintaining structural heterogeneity and potentially higher molecular weight (Parniakov et al. 2015; Barba et al. 2015). In contrast, the spectra of hot water–extracted samples may indicate partial structural modification, such as depolymerization or desulfation, resulting from prolonged thermal exposure (Campo et al. 2009). Such changes can influence the physicochemical properties of carrageenan, including gelation behavior and viscosity(Pereira and Van De Velde 2011).

Overall, the results demonstrate that PEF pretreatment enhances polysaccharide extraction efficiency while preserving protein content and maintaining selectivity toward galactose-rich carrageenan fractions. This selective behavior is particularly advantageous in macroalgal biorefineries, where the integrated valorization of multiple biomass components is desired (Barba et al. 2015; Robin et al. 2020).

Furthermore, PEF-assisted extraction represents a mild alternative to conventional thermal methods, enabling efficient carrageenan recovery while better preserving its structural integrity. Such preservation is likely to contribute positively to the functional properties of the extracted polysaccharides in food and biotechnological applications.

Conclusions

This study demonstrates that pulsed electric field (PEF) pretreatment is an effective and mild strategy for improving carrageenan extraction from Chondrus crispus. Compared with conventional hot water extraction, optimized PEF conditions significantly enhanced polysaccharide recovery, with the highest yields obtained at 3.0 kV cm^{-1} and 1500 pulses. The improved extraction efficiency is attributed to electroporation-induced membrane permeabilization, which facilitates mass transfer and promotes selective release of intracellular carrageenan without the need for intensive thermal processing.

Importantly, PEF treatment preserved the biochemical quality of the biomass. Protein content in the residual solids remained largely unchanged, indicating minimal protein solubilization or degradation and supporting the suitability of PEF for cascade biorefinery applications where multiple biomass fractions

are valorized. Monosaccharide analysis confirmed galactose as the dominant sugar, with higher recovery under PEF conditions, consistent with enhanced carrageenan extraction. Structural characterization by ^{1}H and ^{13}C NMR spectroscopy verified the presence of intact κ- and ι-carrageenan motifs across all treatments, with no evidence of major depolymerization or structural damage.

Overall, PEF-assisted extraction offers a non-thermal, energy-efficient, and selective alternative to conventional methods, enabling higher carrageenan yields while maintaining structural integrity and functional quality. These findings highlight the strong potential of PEF technology for sustainable macroalgal processing and its integration into future green biorefinery systems.

Declarations

Authorship contribution statement

Marwa E. Atya: Conceptualization, Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Ingrid Maribu:** Conceptualization, Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Shingo Matsukawa:** Funding acquisition, Project administration, Writing – Reviewing and Editing. **Marthe Jordbrekk Blikra:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Conceptualization.

Data availability statement

Data available on request from the author.

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.

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Figures

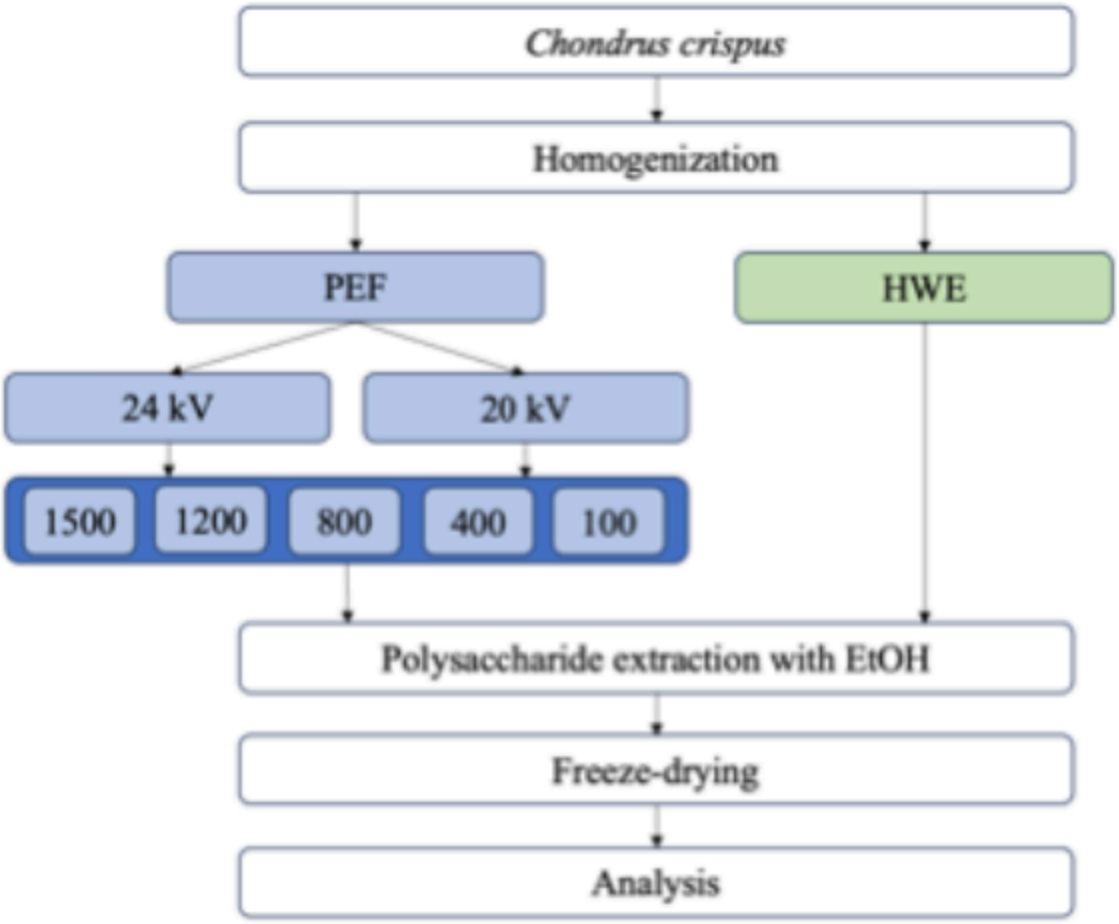


Figure 1

Experimental workflow for carrageenan extraction.

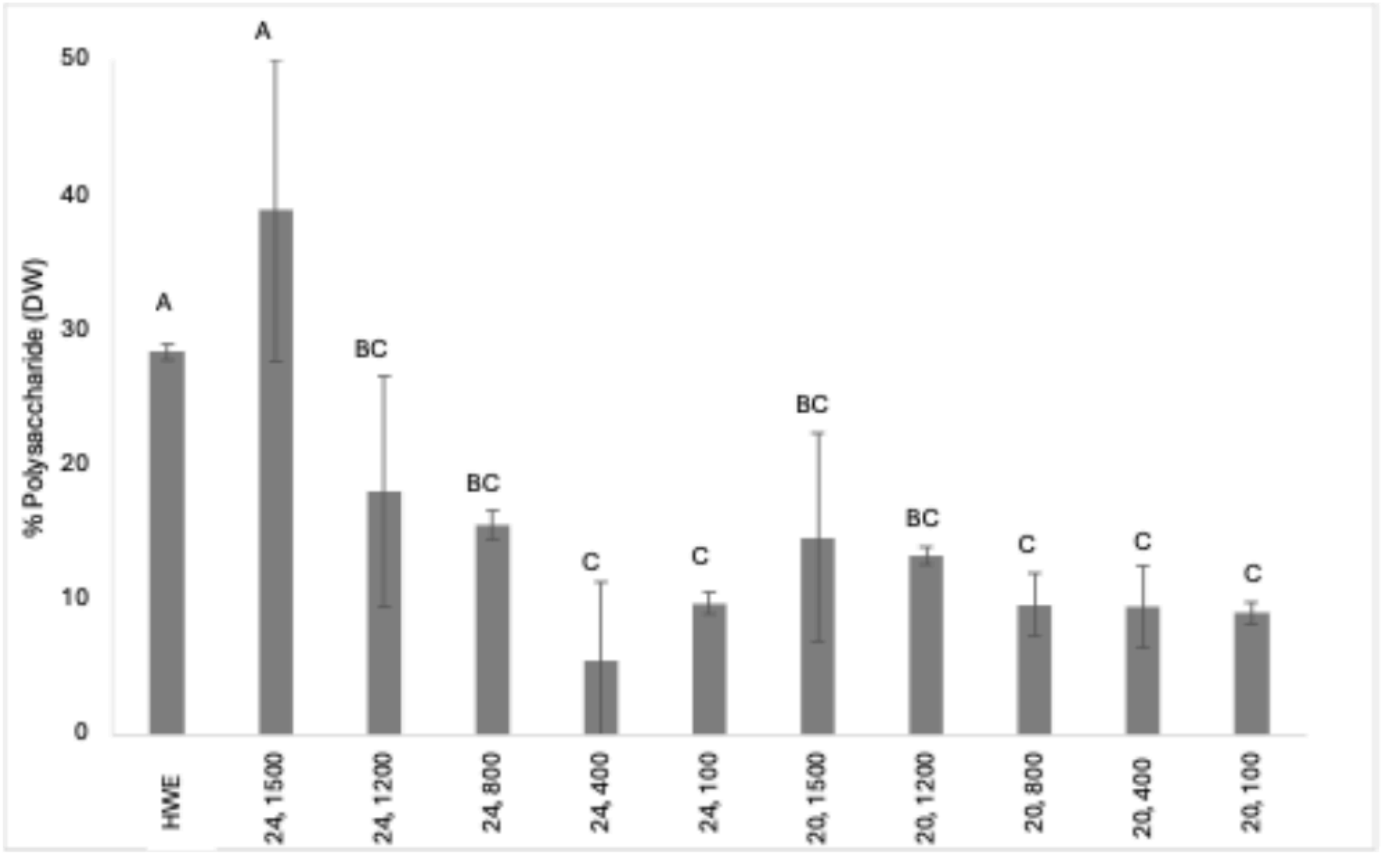


Figure 2

Polysaccharide yield from the different treatments presented as the percent yield of the total DW. Different letters indicate statistically significant differences between treatments ($p < 0.05$).

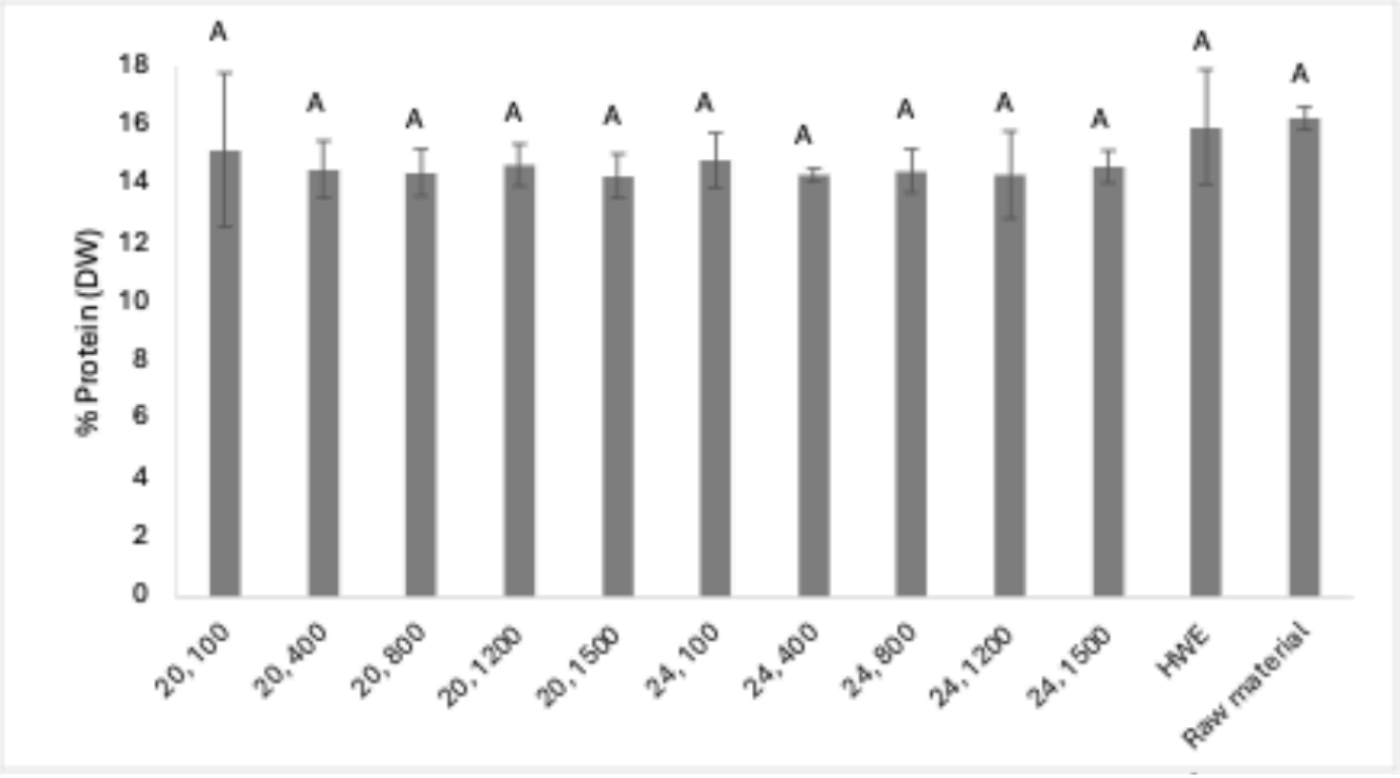


Figure 3

Protein content of the solid fractions obtained after different treatments. The results are expressed as percent of dry weight ± standard deviation (n = 9). Capital letters indicate statistically significant differences ($p < 0.05$).

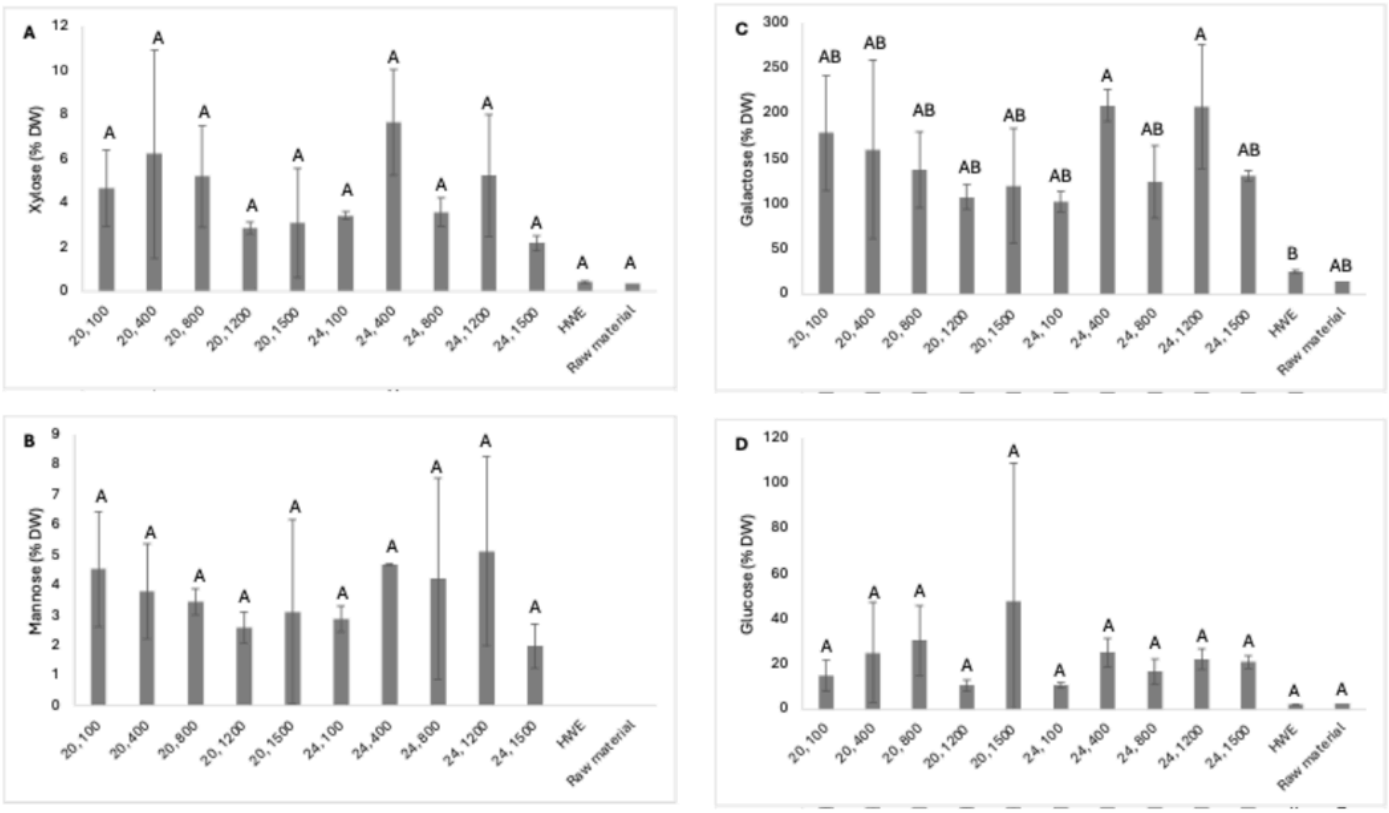


Figure 4

Monosaccharide composition of polysaccharide precipitates obtained under different extraction conditions: xylose (A), mannose (B), galactose (C), and glucose (D). Results are expressed as g monosaccharide per 100 g sample ± standard deviation (n = 2). Capital letters indicate statistically significant differences ($p < 0.05$).

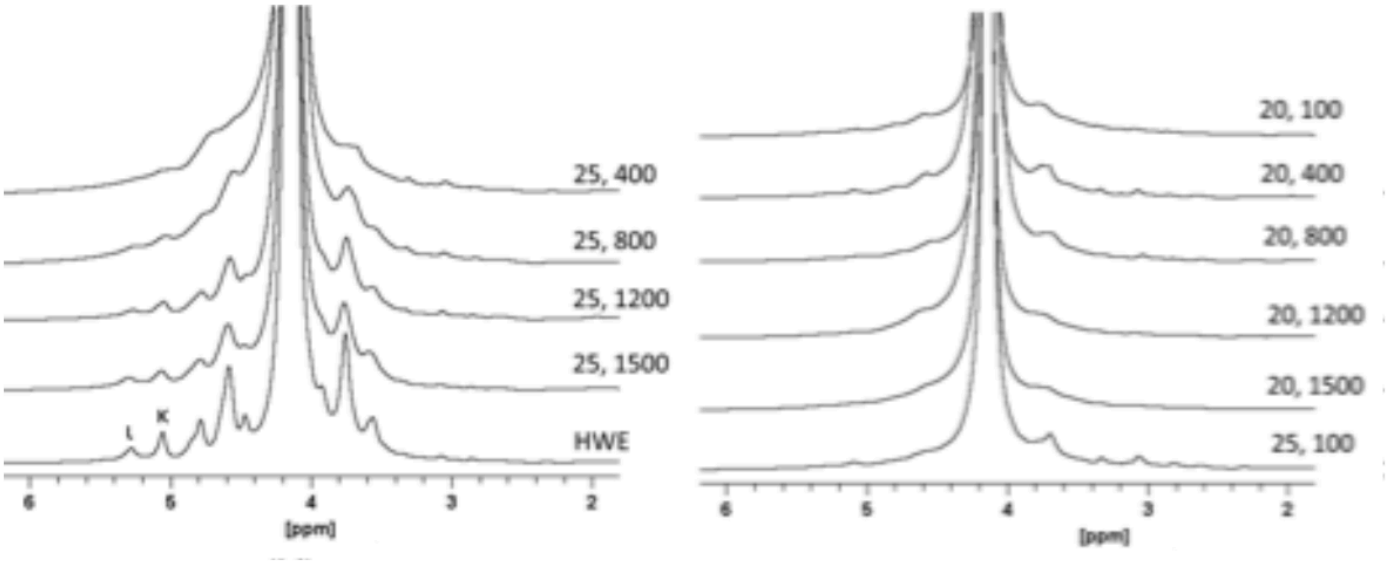


Figure 5

^{1}H NMR spectra for carrageenan fractions extracted by pulsed elecrtic field (PEF) and hot water extraction method. Samples were dissolved in D_2O and analyzed at 65 °C.

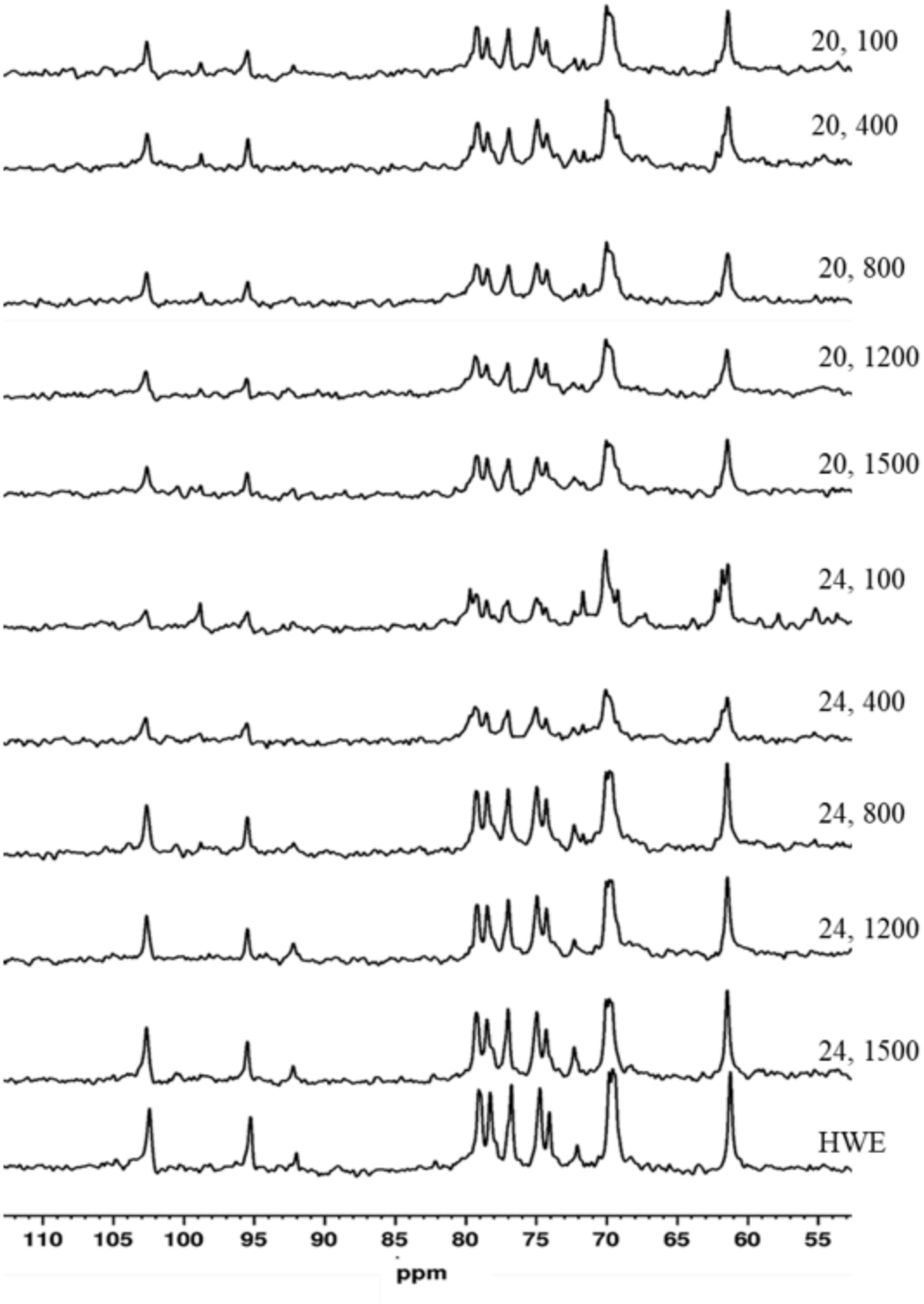


Figure 6

^{13}C NMR spectra for carrageenan fractions extracted by pulsed electric field (PEF) and conventional method. Samples were dissolved in D_2O and analyzed at 65 °C.