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Instant Controlled Pressure Drop (DIC) as an innovative pre-treatment for extraction of natural compounds from the brown seaweed *Sargassum muticum* (Yendo) Fensholt 1955 (Ochrophytina, Fucales)

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ABSTRACT

The effects of the Instant Controlled Pressure Drop (DIC) technology on the recovery of natural compounds from the brown seaweed *Sargassum muticum* (Yendo) Fensholt 1955 (Ochrophytina, Fucales) were investigated. Fresh biomass of *S. muticum* was collected on the Brittany Coast, France. Both fresh and Air Impingement-Dried (AID) thalli of 3 and 10 cm length were submitted to DIC during 20 s or 90 s of processing time under 100 or 800 kPa of saturated steam pressure. DIC treatment significantly improved the extraction of sulphate groups at 90 s, and carotene at 20 s under 100 kPa in thalli of 10 cm. While, coupling AID to DIC, the content of neutral sugars, proteins and antioxidants were higher in AID + DIC treatments. Further, a significant increase of 20 % in fucose-containing sulfated polysaccharides and 30 % in sodium alginate under 100 kPa during 90 s, compared to oven-dried, was observed. The biomass of the invasive *S. muticum* treated by DIC and AID + DIC represented a valuable contribution for extraction of natural compounds. Advantages for industrial integration of DIC were discussed.

1. Introduction

Sargassum C. Agardh (1820) is a brown macroalgal genus of the family Sargassaceae (Fucales, Phaeophyceae) [1]. A very large number of species, varieties and forms, as well as infraspecific names, have been described (up to 360 are recorded by [2] in 2022) and spread worldwide in tropical, subtropical, and temperate environments. In Brittany (France), only the benthic *Sargassum muticum* (Yendo) Fensholt, native from northwest Pacific region, is presently represented. Considered as invasive species, *S. muticum* has acclimatized to the living conditions in the waters along the Atlantic coast where its proliferation is significant. Its propagation has increased despite the several eradication campaigns conducted in France and England, which have all failed. The valorisation of the invasive *Sargassum muticum* began after eradication failures and progress in its geographic areas have been observed [3,4]. However, except for *Sargassum* utilization as a moisturizing cream, nothing is known about valorisation of this species in animal feed or plant health uses in France and in Europe [1].

Sargassum muticum contain significant quantities of vitamins,

minerals, proteins, cell wall polysaccharides (alginates and sulphated polysaccharides rich in fucose), mannitol, laminarin and various functional polyphenols, while nutrient contents can vary with abiotic factors, such as geographical location, season, water temperature and salinity, but also with the growth and the maturity of the algal thallus, and the extraction methods [1].

A key point for the extraction of these compounds is the disruption of the seaweed cell-wall, which determines for instance their accessibility. In Fucales, the cell wall system is mostly an assembly of two polysaccharide-based networks, sulphated polysaccharide rich in fucose interlocking a β -glucan scaffold, embedded in the second made up of alginates and cross-linking polyphenols. Proteins, glycoproteins, halogenated compounds, iodine and other ions are additional components of brown algal cell walls [5] making the extraction of these compounds difficult. In addition, insoluble sulphated phenolic compounds known as phlorotannins in *Sargassum* species can be bound to cell wall polysaccharides via hydrophilic and hydrophobic bonding which protein structures are attached to [6]. Therefore, efficient disruption of the algal cell wall is essential for the extraction of seaweed compounds. The

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development of different eco-friendly technologies (*i.e.* microwave, enzyme assisted extraction or pulsed electric field...) has enabled to reduce the barrier of the seaweed cell-wall, in order to bring added value to the *Sargassum* biomass by extracting products that can be applicable to different fields, such as human, animal and plant care, cosmetic, medical or nutritional [1,7,8].

The Instant Controlled Pressure Drop, well-known as DIC (French acronym for Détente Instantanée Contrôlée), is an innovative thermo-mechanical and eco-friendly treatment. DIC is a HTST (High Temperature Short Time) treatment which involves subjecting a partially moist product (moisture content generally close to 30 %) to saturated steam pressure (100 to 800 kPa) at high temperature (between 100 and 170 °C), for a short time (10 to 60 s), followed by a sudden drop in pressure to vacuum (5 kPa in 0.02 to 0.2 s). Pressure drop thus takes place at very high velocity ($\Delta P/\Delta t > 500 \text{ kPa s}^{-1}$) and can be considered as instantaneous. Under the effect of ultra-rapid water vaporization, products are expanded and the tortuosity is reduced, leading to improved diffusivity and greater availability of active molecules [9].

Currently, with the increasing demand on natural products, food industries are increasing the interest in drying processes which could reduce energy consumption and improve the overall quality of final products [10,11], since traditional hot air-drying operations are very limited in terms of quality (nutritional, textural, and functional). For several years, a research team has been developing an innovative drying process based on the principle of high-velocity and localized air-flows, known as Air Impingement Drying (AID), to reduce drying time and enable operation at moderate temperatures [12–14]. Lately, this process has also been successfully applied to *Sargassum* [15,16].

DIC technology was designed as a solution to enhance the value of natural products, is used at both laboratory and industrial scale, and has proven its efficacy for a wide range of applications [17,18]. Even more, the coupling of DIC to drying processes, showed an improvement in the drying operation and extraction of natural compounds from plants, vegetables and fruits [9,19,20]. However, at present no scientific articles have been published showing the effects of DIC technology on the extraction of compounds from seaweeds.

In this research, the objective was to evaluate the effects of the DIC and of the AID + DIC parameters, namely saturated steam pressure and processing time, for the extraction of compounds from the brown seaweed *Sargassum muticum*.

2. Material and methods

2.1. Algal biomass

Fresh biomass of the brown seaweed *Sargassum muticum* (Ochrophytina: Phaeophyceae) was collected by hand from the beach of Kercambre in Saint-Gildas de Rhyus (47.489539, -2.820566 decimal degrees), Brittany, France. The biomass was cleaned and washed with tap water to eliminate animals and sand, the excess of water was blot-dried with absorbent paper and the fresh material was kept refrigerated (4 °C) until used in the experiments. One batch was dried in a natural convection oven (Memmert UN30, Schwabach, Germany) at 40 °C until constant dry weight, and grounded in a mill for further analysis. This batch was used as the initial reference. The dry weight/fresh weight ratio was determined by drying 1 g of fresh sample at 105 °C during 24 h in the oven.

2.2. Experimental design

2.2.1. Instant Controlled Pressure Drop (DIC) equipment

The laboratory scale reactor used in this study (CIMS company, Sablé-sur-Sarthe, France) is made up four main components: 1) A processing vessel (12L) with heating jacket, where the biomass is placed before being treated. 2) A vacuum system, which is mainly composed of a 1200 L vacuum tank equipped with a cooling water jacket and 3) a

vacuum pump suitable for maintaining a low pressure (5 kPa) in the tank. 4) A decompression system, *i.e.* an appropriate pneumatic valve that opens in a very short time (<0.2 s). This valve is the key element of the equipment, as it ensures an abrupt/instantaneous connection between the processing vessel and the vacuum tank (Fig. 1).

2.2.2. DIC and AID + DIC treatments

In a first trial, the effects of the Instant Controlled Pressure Drop (namely DIC) and the effects of the Air Impingement Drying (AID) coupled to DIC (namely AID + DIC) on thalli of *S. muticum* were evaluated. Samples dried at 40 °C in an oven (OD), freeze-dried (FD) during 72 h (Christ alpha 1–4 LSC, Osterode am Harz, Germany), and Air Impingement Dried (AID) samples were carried out (Table 1). For DIC treatments, about 100 g of fresh weight (fw) of *S. muticum* was cut into 10 cm length and placed into a metal basket, which was introduced into the processing vessel. The condition of the system was established to use a saturated steam pressure of 100 kPa and, a processing time of 20 s and 90 s, an instant vacuum released of 500 Pa s⁻¹, and finally, a released of pressure to atmospheric into the processing vessel. At the end of the cycle, the instrument outputs a liquid fraction, its analysis showed a high content of ash (>80 %) and negligible amounts of other compounds (proteins, neutral sugars, sulphates, polyphenols and pigments). No further analyzes were done. The treated solid biomass was weighed, frozen, lyophilized, then ground until powder to registered the final mass. Samples were stored at -80 °C until the extraction of compounds.

Designed for AID + DIC treatments, fresh thalli of 10 cm length were dried using the Air Impingement Dryer (AID), which is a prototype device designed by CIMS (Sablé-sur-Sarthe, France), at 35 m s⁻¹ and 40 °C, during 20–25 min to reach a moisture content of 30 %. A well-detailed, including a diagram of the device, is reported in Boy *et al.* [12–14]. Samples of 100 g fw were introduced into two plastic baskets (10 cm × 9 cm) and then placed in the centre of the drying chamber, metallic masses were installed on the baskets to avoid samples from being lost by the force of the airflow. Temperature was recorded every 10 s by an acquisition unit (SR Mini System, TC SA, Dardilly, France), equipped with two thermocouples, each one was fixed inside the basket in direct contact with the sample. Every 5 min, one of the baskets was removed from the dryer to record its weight and immediately afterwards it was re-inserted, until the final moisture content was reached. After drying, samples were subjected to DIC treatments, a sub-sample not treated by DIC was used as reference (named AID). Before DIC treatments, aliquots were dried in an oven at 40 °C to reach equilibrium mass and then, samples were dried at 105 °C until the mass was stabilized to get the dry matter. All treatments were done in triplicate (Table 1).

2.2.3. AID + DIC treatments on different thalli sizes

In a second trial, the effects of AID + DIC were investigated using two lengths of thalli. About 100 g of fresh thalli were cut into 3 and 10 cm lengths, and dried using the AID. Then, samples were subjected to DIC treatments, applying a saturated steam pressure of 800 kPa, and a processing time of 20 s and 90 s. These treatments were denoted as “3 cm, AID + DIC” and “10 cm, AID + DIC”. A sample dried in an oven (OD) was included as reference. All treatments were done in triplicate (Table 1).

2.3. Extraction of natural compounds

2.3.1. Biochemical composition

Mineral (ash) content was determined gravimetrically after incineration of samples at 550 °C for 8 h in a muffle (Carbolite CSF 1100, Bamford, England). The content of proteins, neutral sugars, and uronic acids were estimated from the acid hydrolysis of 10 mg dry weight (dw) of sample; while the sulphate groups content was estimated from an aqueous extraction of 10 mg dw. Soluble protein content was quantified with the bicinchoninic acid assay (BCA) method [22] from Pierce Kit (Thermo, Cat. no.23225), neutral sugars were determined by the phenol sulphuric acid method [23] using glucose as the standard (10–100 µg

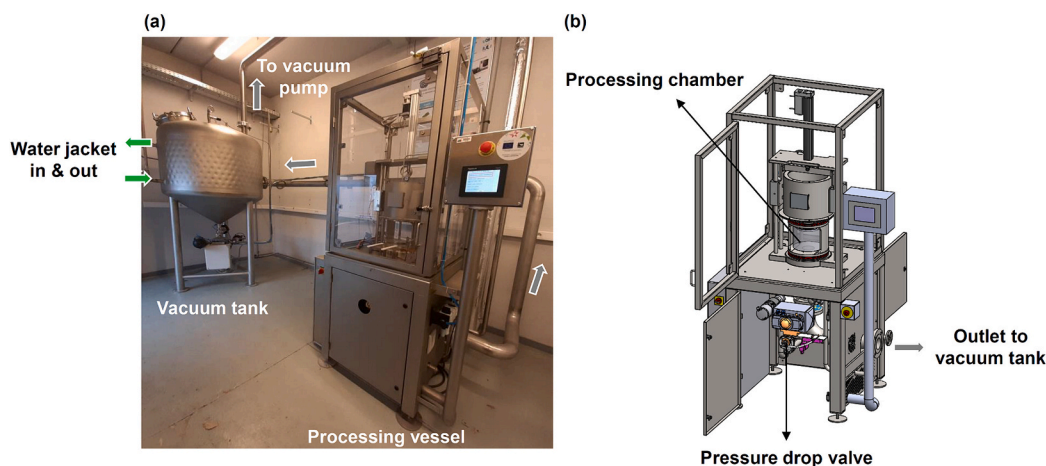


Fig. 1. Instant Controlled Pressure Drop (DIC) equipment used in this study. (a) Picture and (b) Zoom on the processing vessel of the system.

Table 1

Experimental Design for the Instant Controlled Pressure Drop (DIC) and Air Impingement Drying (AID) combined to DIC treatments, and the other drying processes: Oven-Drying (OD), Freeze-Drying (FD), and Air Impingement Drying (AID) from *Sargassum muticum*.

Assay	Code	DIC Steam pressure (kPa)	DIC Processing time (s)	Thalli Length (cm)
First assay				
1	OD	–	–	10
2	FD	–	–	10
3	AID	–	–	10
DIC				
4	20 s	100	20	10
5	90 s	100	90	10
AID + DIC				
6	20 s	100	20	10
7	90 s	100	90	10
Second assay				
8	OD	–	–	–
AID				
9	3 cm	–	–	3
10	10 cm	–	–	10
3 cm, AID + DIC				
11	20 s	800	20	3
12	90 s	800	90	3
10 cm, AID + DIC				
13	20 s	800	20	10
14	90 s	800	90	10

–: not applicable. All trials were done in triplicate.

mL^{-1}); uronic acids were measured with the meta-hydroxy-di-phenyl (MHDP) method [24] using glucuronic acid as standard ($20\text{--}100 \mu\text{g mL}^{-1}$). Sulphate groups were quantified by the Azure A method [25] adapted to a microplate, $20 \mu\text{L}$ of the extract was mixed with $200 \mu\text{L}$ of Azure A solution, using dextran sulphate as standard ($10\text{--}50 \mu\text{g mL}^{-1}$). All results were expressed as mg per g of alga dry weight (mg g^{-1} dw).

2.3.2. Pigments

The extraction of pigments from *S. muticum* samples were done using a mixture of acetone (75 mL), water (24.5 mL) and acetic acid (0.5 mL). Samples were covered with aluminium foil, shaken during 4 h at 4°C , centrifuged and filtered ($0.2 \mu\text{m}$ filter) before injected in HPLC. The

HPLC device with photodiode array detector (Dionex U3000 Thermo, Germering, Germany), was connected to a separating column in reverse phase C18 (Waters NovoPack C18, 150×3.9), preceded by a precolumn (Waters NovoPack C18). The HPLC protocol used was adapted from Silkina *et al.* [26], using solvent A consisted of methanol (51 %), acetonitrile (36 %), ammonium acetate (23 %), and Milli-Q water (13 %). Solvent B with ethyl acetate (70 %), and acetonitrile (30 %). A linear gradient (solvent A 100 % 0–27 min, solvent B 100 % 27–42 min) was used with a flowrate of 1.4 mL min^{-1} . HPLC data were collected using Chromeleon software (Thermo, Illkirch, France). The identification and quantification of the pigments were done base on the commercial standard pigments using different concentrations ($1\text{--}20 \mu\text{g mL}^{-1}$). Results were expressed as mg g^{-1} dw.

2.3.3. Antioxidants and antioxidant activity

Acetone extracts were used to estimate the concentration of the antioxidants such as Total Phenolic Compounds (TPC) and phlorotannins (Phl), as well as its antioxidant activity using the free radical scavenging assay DPPH (2,2-diphenyl-1-picrylhydrazyl). TPC was determined by the Folin-Ciocalteu method [27] adapted to microplates [28], details of the method are described in Pliego-Cortés *et al.* [29]. Phlorotannin concentration was measured by the DMAC method [30], by adding $20 \mu\text{L}$ of algal sample to $180 \mu\text{L}$ of DMAC reagent (0.05 g of 4-(dimethylamino) cinnamaldehyde in 50 mL of acidified ethanol), microplates were incubating in the dark at room temperature for 30 min and the absorbance was read at 640 nm . Both, TPC and phlorotannins used phloroglucinol as a standard ($0\text{--}1000 \mu\text{g mL}^{-1}$), and results were expressed as micrograms of Phloroglucinol Equivalents per milligram of dry weight ($\mu\text{g PGE mg}^{-1}$ dw).

DPPH method from Brand-Williams *et al.* [31], was adapted to microplates, $100 \mu\text{L}$ of 0.25 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in 100 % methanol were added to $100 \mu\text{L}$ of the algal extracts. Algal extracts concentration was 1 mg mL^{-1} , butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) were used as positive controls ($50 \mu\text{g mL}^{-1}$), a negative control was included by substituting DPPH with methanol. The percentage of inhibition was calculated as $\text{DPPH (\% inhibition)} = [(Abs_s - Abs_c) / Abs_s] \times 100$, where Abs_s is the absorbance of the algae sample and Abs_c is the absorbance of the positive control.

2.3.4. Polysaccharides

All dry samples were depigmented and defatted in an orbital shaker using 96 % ethanol (EtOH), following by methanol:chloroform (MeOH: CHCl_3 , 1:1), and 100 % acetone, during 3 h per solvent. At the end, the powder was dry under the hood at room temperate (RT) overnight. Polysaccharide extractions was adapted from Pliego-Cortés *et al.* [7], with some modifications. Briefly, using a shaking water bath on 2 g dry

weight (dw) of sample and 20 mL of acidified water (HCl at final concentration of 0.05 N), during 3 h at 50 °C. Extractions were filtrated using a cheesecloth to separate the pellet and the solubilized material. The pellet was dissolved with 3 % NaCO₃, shaking during 1 h and the soluble part was precipitated with ethanol to obtain a sodium alginate fraction. The earlier solubilized material was centrifuged (5000g, 10 min) and then, boiled 2 h at 70 °C with solid calcium chloride (CaCl₂) at the final concentration of 2 % to separate by precipitation residues of alginate. Then, soluble part was precipitated with four volumes of cold ethanol to obtain a fucose-containing sulfated polysaccharides (FCSP) fraction, generally known as fucoidan. All extractions were performed in triplicate, and results of crude polysaccharides were expressed as milligram of fucoidan or sodium alginate per gram of initial material in dry weight (mg g⁻¹ dw).

2.4. Statistical analysis

Data are presented as mean ± standard deviation in triplicate. One-way analysis of variances (ANOVA), followed by SNK's multiple comparisons test was performed. Normality of data was determined using the Shapiro-Wilk test, and homogeneity of variance using the Levene's test. If these assumptions failed, a non-parametric Kruskal-Wallis test with pair-wise comparisons was used. Spearman rank order test was used to identify the correlation between DPPH antioxidant activity and phenolic compounds. The software InfoStat/Student v.2020 (Universidad Nacional de Córdoba, Argentina) was used to performed all analysis, with a significance level α established at 5 %. Graphics were elaborated using GraphPad Software 9.0 (Boston, MA, USA).

3. Results

3.1. Biochemical composition

The effects of the DIC and the AID coupled to DIC (AID + DIC) on the

biochemical composition of *Sargassum muticum* are presented in Fig. 2. DIC technology allowed extracting a significantly ($p < 0.05$) higher content of neutral sugars and uronic groups when AID + DIC was applied for 90 s of processing time and a saturated steam pressure of 100 kPa. While, a significant ($p < 0.05$) increase was observed on the content of sulphate groups at 20 s in AID + DIC, and at 90 s in DIC treatment applied directly to fresh biomass. The protein content ranged from 91 to 189 mg g⁻¹ dw, the treatments of AID + DIC during 90 s and oven-dried samples (OD) showed the lower values. Mineral matter did not show significant differences ($p > 0.05$) among treatments, except for both 20 s of DIC and AID + DIC treatments.

In the second trial (Fig. 3), by using two different length of thalli (3 and 10 cm) and establishing the DIC steam pressure at 800 kPa, the neutral sugars in treatment AID + DIC on thalli of 10 cm showed the higher content, however, no significant differences ($p > 0.05$) were observed. The protein content showed a significant increase ($p < 0.05$) in all treatments compared to the oven-dried samples (OD). The higher extractable protein content was obtained on AID + DIC applied during 90 s in both, 3 cm and 10 cm thalli length (208.3 ± 13.8 and 194.2 ± 13.0 mg g⁻¹ dw, respectively). Despite the increase noted in sulphated groups in all AID + DIC treatments, no significant differences were observed between AID and AID + DIC treatments. On the contrary, mineral content displayed lower significant values in all AID + DIC treatments. The uronic acids ranged from 9.7 to 12.4 mg g⁻¹ dw, and the oven dried samples (OD) showed the higher content.

3.2. Pigment extractions and composition

The pigment composition of the acetone extracts of *S. muticum* based on HPLC-DAD analysis allowed to identify and quantify six pigments in all treatments according to the comparison of maximum absorption peaks (nm) and the retention time to the analytical commercial standards. The identified pigments were: chlorophyll C1 (445, 584 and 634 nm), chlorophyll C2 (446, 580, 632 nm), chlorophyll-a (432, 664 nm),

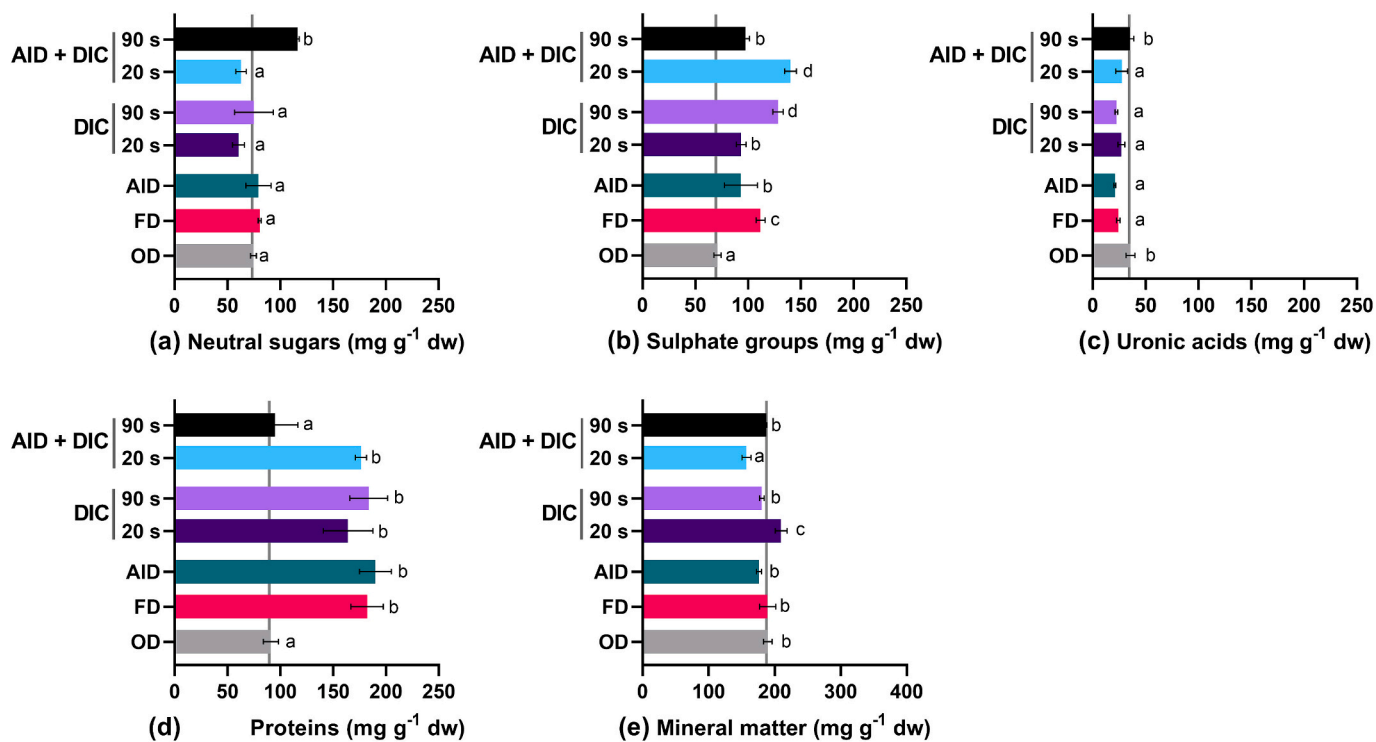


Fig. 2. Biochemical composition (mg g⁻¹ dw "dry weight") of *Sargassum muticum* according to the first assay: DIC and AID + DIC, at 100 kPa during 20 s and 90 s applied on thalli of 10 cm length. OD: Oven-Drying, FD: Freeze-Drying, and AID: Air Impingement Drying. Data are mean ± SD ($n = 3$). Different letters indicate significant differences ($p < 0.05$) between each biochemical group based on ANOVA, SNK.

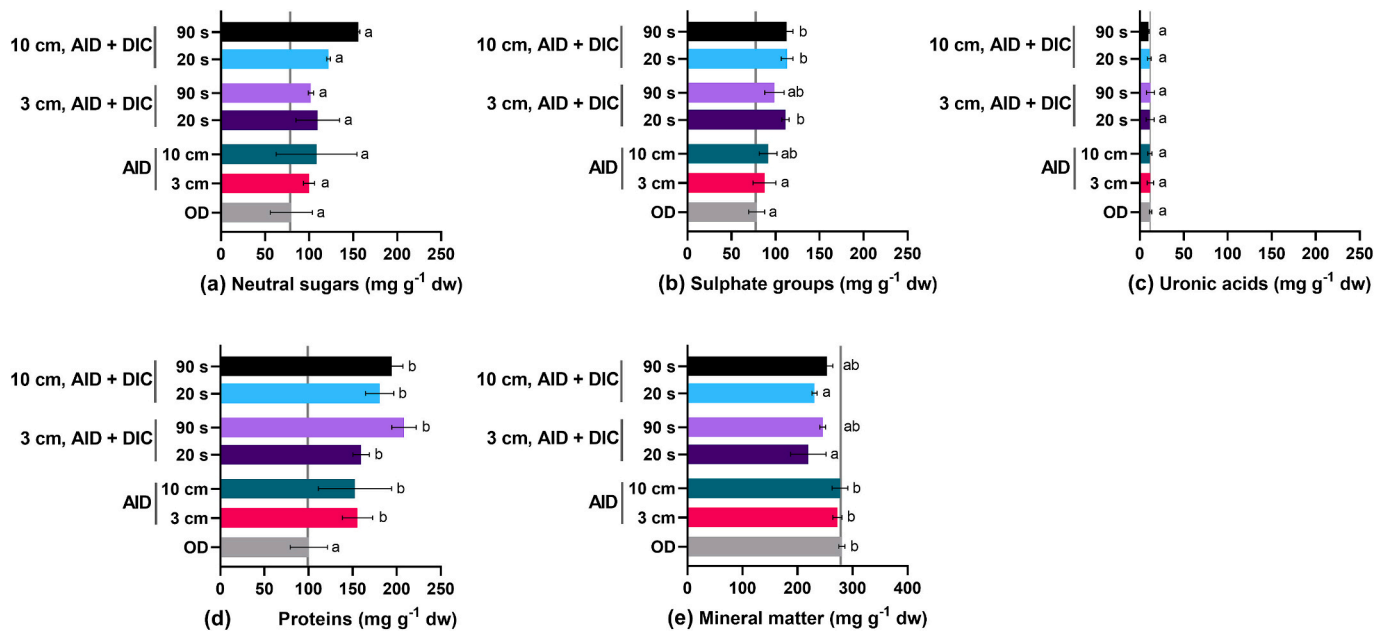


Fig. 3. Biochemical composition (mg g⁻¹ dw) of *Sargassum muticum* according to the second assay: DIC and AID + DIC, at 800 kPa during 20 s and 90 s applied on thalli of 3 cm and 10 cm length. OD: Oven-Drying, and AID: Air Impingement Drying. AID (3 cm and 10 cm) samples were subjected only to AID. Data are mean ± SD (n = 3). Different letters indicate significant differences (p < 0.05) between each biochemical group based on ANOVA, SNK (parentheses b, d) or Kruskal–Wallis, pair-wise comparisons (parentheses a, c, e).

fucoxanthin (450, 658 nm), zeaxanthin (453, 478 nm) and beta-carotene (450, 478 nm). These pigments were then grouped into three families: chlorophylls (as the sum of C1, C2 and a), xanthophylls (fucoxanthin and zeaxanthin) and carotene (beta-carotene).

The DIC system allowed to obtain a significantly higher amount of beta-carotene (3.16 ± 0.07 mg g⁻¹ dw), by applying the treatment during 20 s, under 100 kPa of steam pressure (Fig. 4b). Although, the content of xanthophylls (Fig. 4a) was the higher in the 20 s treatments (3.57 ± 0.40 mg g⁻¹ dw), no significant differences (p > 0.05) were observed. The freeze-dried samples (Fig. 4c) showed the significant higher (p < 0.05) content of chlorophylls, which represented >70 % of

the total pigments. The total content (mg g⁻¹ dw) of pigments quantified in this assay was higher in FD samples (17.87 ± 0.30 mg g⁻¹ dw) followed by DIC treatment at 20 s (12.06 ± 1.20 mg g⁻¹ dw) and 90 s (9.24 ± 0.90 mg g⁻¹ dw), this total was established as the sum of the individual pigments identified in the extracts.

The effects of the AID followed by DIC under 800 kPa of steam pressure (Fig. 4d, e, and f) performed with different thalli length (3 and 10 cm), showed that the amount of beta-carotene was significantly (p < 0.05) higher on thalli of 3 cm length applying the DIC treatment during 20 s, compared to the oven-dried samples (2.71 ± 0.58 vs 0.86 ± 0.30 mg g⁻¹ dw, respectively). Similarly, the amount of xanthophylls in this

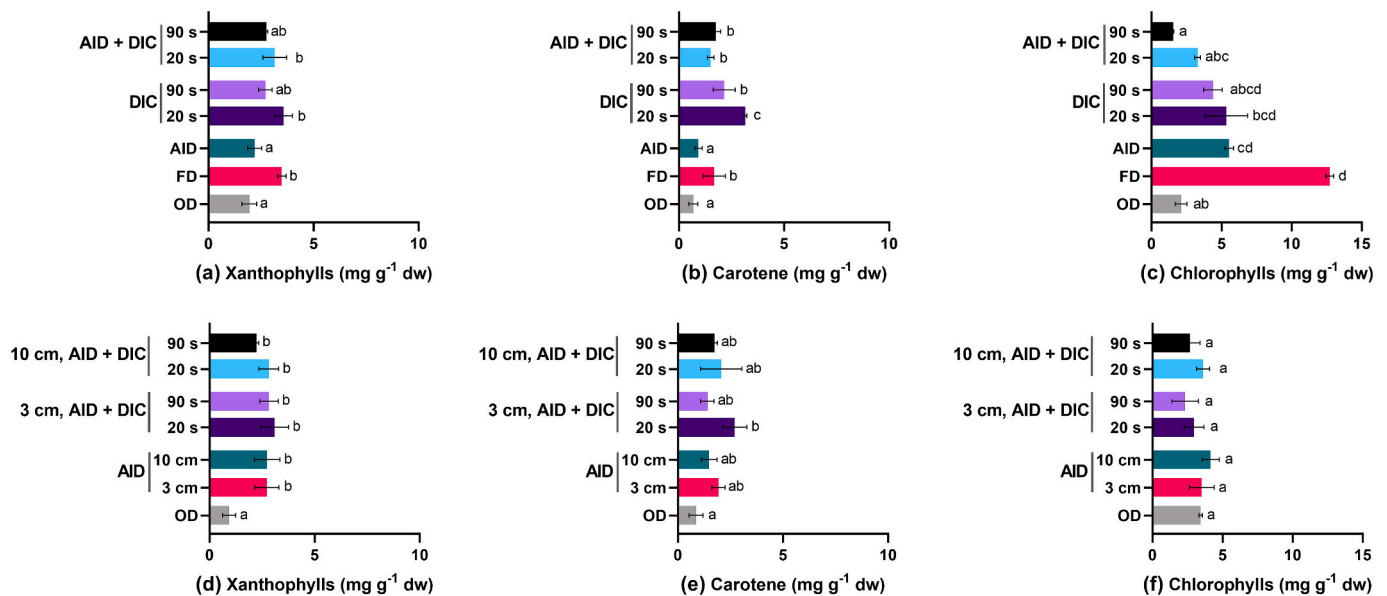


Fig. 4. Pigments content (mg g⁻¹ dw) in *Sargassum muticum* extracts. (a, b, c) DIC and DIC + AID treatments (100 kPa, 20 s and 90 s, thalli of 10 cm length), and (d, e, f) AID + DIC treatments (800 kPa, 20 s and 90 s, thalli length of 3 cm and 10 cm). OD: Oven-Drying, FD: Freeze-Drying, and AID: Air Impingement Drying. AID (3 cm and 10 cm) samples were subjected only to AID. Data are mean ± SD (n = 3). Different letters indicate significant differences (p < 0.05) between each pigment group based on ANOVA, SNK (parentheses a, b, d, e) or Kruskal–Wallis, pair-wise comparisons (parentheses c, f).

treatment was higher with $3.09 \pm 0.60 \text{ mg g}^{-1} \text{ dw}$ (composed by 97.8 % of fucoxanthin) than OD $0.93 \pm 0.31 \text{ mg g}^{-1} \text{ dw}$. The amount of chlorophylls (mainly composed of chlorophyll-a) were similar and ranged from 2.32 ± 0.94 (OD samples) to $4.14 \pm 0.61 \text{ mg g}^{-1} \text{ dw}$ (AID samples in thalli of 10 cm length), no significant differences were observed between treatments. The AID + DIC treatments applied during 20 s on thalli of 3 cm and 10 cm in length, presented the higher total pigment content (8.76 ± 0.50 and $8.48 \pm 1.00 \text{ mg g}^{-1} \text{ dw}$, respectively).

3.3. Antioxidants and antioxidant activity

The extraction of antioxidants (Table 2, first assay), such as Total Phenolic Compounds (TPC), was significantly improved by AID + DIC treatment ($p < 0.05$), by using a processing time of 90 s. TPC reached a maximum of $52.2 \pm 3.7 \mu\text{g PGE mg}^{-1} \text{ dw}$. While phlorotannins were higher in the treatments at 20 s and 90 s (44.3 ± 11.4 and $40.5 \pm 2.4 \mu\text{g PGE mg}^{-1} \text{ dw}$, respectively). The antioxidant activity (DPPH % of inhibition) in the treatment AID + DIC during 20 s, reached up to a significant ($p < 0.05$) 70 % of inhibition, which was very similar to the FD samples. On the contrary, OD samples showed the lower content of TPC, phlorotannins and DPPH activity.

For treatments with different thalli length (3 cm and 10 cm) and a steam pressure of 800 kPa, it was observed an increase on TPC compared to OD samples. However, no significant differences were found. While extraction of phlorotannins was significantly ($p < 0.05$) improved by AID + DIC applying a processing time of 20 s in thalli of 3 cm length ($40.8 \pm$

Table 2

Total Phenolic Content (TPC), Phlorotannin (Phl) content ($\mu\text{g PGE mg}^{-1} \text{ dw}$), and DPPH antioxidant activity (% of inhibition) of *Sargassum muticum* extracts according to the different treatments: Oven-Drying (OD), Freeze-Drying (FD), Air Impingement Drying (AID), and Instant Controlled Pressure Drop (DIC).

Treatment	TPC $\mu\text{g PGE mg}^{-1} \text{ dw}$	Phl $\mu\text{g PGE mg}^{-1} \text{ dw}$	DPPH % Inhibition	r DPPH-TPC
First assay				
OD	18.9 ± 3.3^a	14.9 ± 5.5^A	41.3 ± 5.2^A	-0.50
FD	26.4 ± 5.1^{ab}	20.9 ± 1.8^A	72.8 ± 1.0^C	0.99
AID	30.3 ± 6.3^{abc}	26.3 ± 4.7^{AB}	70.6 ± 0.6^{BC}	0.50
DIC				
20 s	33.5 ± 7.2^{bc}	23.9 ± 5.5^{AB}	72.4 ± 1.5^C	0.50
90 s	29.6 ± 2.9^{abc}	21.1 ± 2.1^A	65.9 ± 4.1^{ABC}	-0.50
AID + DIC				
20 s	41.5 ± 7.4^c	44.3 ± 11.4^B	73.4 ± 6.6^C	0.98
90 s	52.2 ± 3.7^d	40.5 ± 2.4^B	62.8 ± 5.5^{AB}	-0.50
Second assay				
OD	20.0 ± 1.5^a	18.1 ± 2.9^a	51.6 ± 11.9^A	-0.50
AID				
3 cm	27.8 ± 10.3^a	22.0 ± 7.8^a	56.4 ± 9.0^A	-0.50
10 cm	31.1 ± 9.4^a	27.3 ± 7.3^{ab}	55.1 ± 9.4^A	0.50
3 cm, AID + DIC				
20 s	41.6 ± 12.9^a	40.8 ± 3.8^b	69.2 ± 6.3^A	-0.50
90 s	27.5 ± 2.2^a	27.3 ± 3.7^{ab}	65.7 ± 5.6^A	-0.50
10 cm, AID + DIC				
20 s	33.0 ± 4.6^a	23.1 ± 5.9^a	60.1 ± 1.6^A	-0.50
90 s	39.8 ± 8.7^a	29.4 ± 0.7^{ab}	55.3 ± 4.1^A	0.99

First assay: DIC and DIC + AID treatments (100 kPa, 20 s and 90 s, thalli of 10 cm length). Second assay: AID + DIC treatments (800 kPa, 20 s and 90 s, thalli of 3 cm and 10 cm length). Data are mean \pm SD ($n = 3$). Different letters indicate significant differences ($p < 0.05$) between treatments and within each assay based on ANOVA, SNK (lower case letters) or Kruskal-Wallis, pair-wise comparisons (upper case letters). r Spearman's rho values for correlation between DPPH and TPC.

$3.8 \mu\text{g PGE mg}^{-1} \text{ dw}$), more than two-folds compared with OD samples. Furthermore, in the same treatment (AID + DIC, 3 cm, 20 s), the higher antioxidant activity by DPPH was observed, reaching $69.2 \pm 6.3 \%$ of inhibition, but no significant differences were observed (Table 2, second assay).

The Spearman correlation test (Table 2) showed a positive r value between TPC and DPPH in FD, AID, DIC and AID + DIC treatments applied during 20 s, but a negative r value in both DIC and AID + DIC treatments during 90 s. Similarly, the reference oven-dried sample (OD) displayed a negative correlation. In the second assay, using different thalli length, a strong correlation between TPC and DPPH activity was observed on AID + DIC treatment for 90 s on thalli of 10 cm. The AID sample (10 cm thalli) showed a positive r value about 0.50. All the remaining treatments showed negative r values.

3.4. Polysaccharide extraction

Two types of polysaccharides from *Sargassum muticum* were extracted: the crude fucoidan (formally known as fucose-containing sulfated polysaccharides, FCSP), and the crude sodium alginate. The AID + DIC treatment applied during 90 s and 100 kPa, significantly ($p < 0.05$) improved the extraction of fucoidan (Fig. 5a), registering $55.9 \pm 7.1 \text{ mg g}^{-1} \text{ dw}$, compared to the other treatments which ranged from 22.0 ± 6.2 to 34.7 ± 5.9 (FD and AID samples, respectively). Similarly, the AID + DIC allowed to significantly ($p < 0.05$) increase the sodium alginate extraction in treatment with a DIC processing time of 90 s, being >1.5 -folds compared to FD samples ($74.1 \pm 4.5 \text{ mg g}^{-1} \text{ dw}$) which showed the lower amount (Fig. 5b). Whereas, by comparing the effects of AID + DIC on the different thalli lengths, a higher amount of fucoidan was observed in the treatment of 20 s in thalli of 3 cm length (Fig. 5c), and also in sodium alginate in AID + DIC treatment applied during 90 s in thalli of 10 cm (Fig. 6d), showing values of 90.3 ± 31.7 and $90.7 \pm 15.4 \text{ mg g}^{-1} \text{ dw}$, respectively. However, no significant differences were observed. In both crude polysaccharides, the AID + DIC at 20 s in thalli of 10 cm length registered the lowest values 57.2 ± 15.7 (fucoidan) and $47.3 \pm 18.6 \text{ mg g}^{-1} \text{ dw}$ (sodium alginate).

4. Discussion

To rise an efficient recovering of natural compounds from biological products, novel and environmentally friendly methods have been developed. Among them the Instant Controlled Pressure Drop (DIC), is an innovative process and there is an abundant literature demonstrating its applications in plants, fruits, vegetables, seafoods and meat products [17,20,32]. However, as the published scientific literature lacks the effects of DIC on macroalgae, the present research provided a perspective for DIC and AID coupled to DIC as a pre-treatment for extraction of compounds from the brown seaweed *Sargassum muticum*. This species of macroalgae represents an undervalued resource, that can be considered a potential source of added-value products with low environmental impact, such as antivirals [7], plant biostimulants [8], construction materials [33]; since it is a natural renewable resource with high availability but also it could be produced through aquaculture [34].

The effect of DIC operating parameters evaluated in this study, on the biochemical composition, showed that DIC treatment applied during 90 s, at 100 kPa, allowed to obtain a significantly higher content of sulphate groups in both DIC-treated and when DIC was preceded by Air Impingement Drying (AID + DIC). Similarly, the proteins content obtained at 90 s in AID + DIC-treated at 800 kPa, in thalli of 3 and 10 cm length, was double compared to oven-dried samples. These results are in agreement with a previous observation on different DIC-treated products, as Pedrosa et al. [35], reported an increased on soluble sugar content of the legumes soybean and lentil using DIC treatments. Authors explain that it could be related to a mechanical-structure modification (a higher porosity and a rupture of the cell walls), which increased the specific surface area and improved the diffusion of solvent inside the

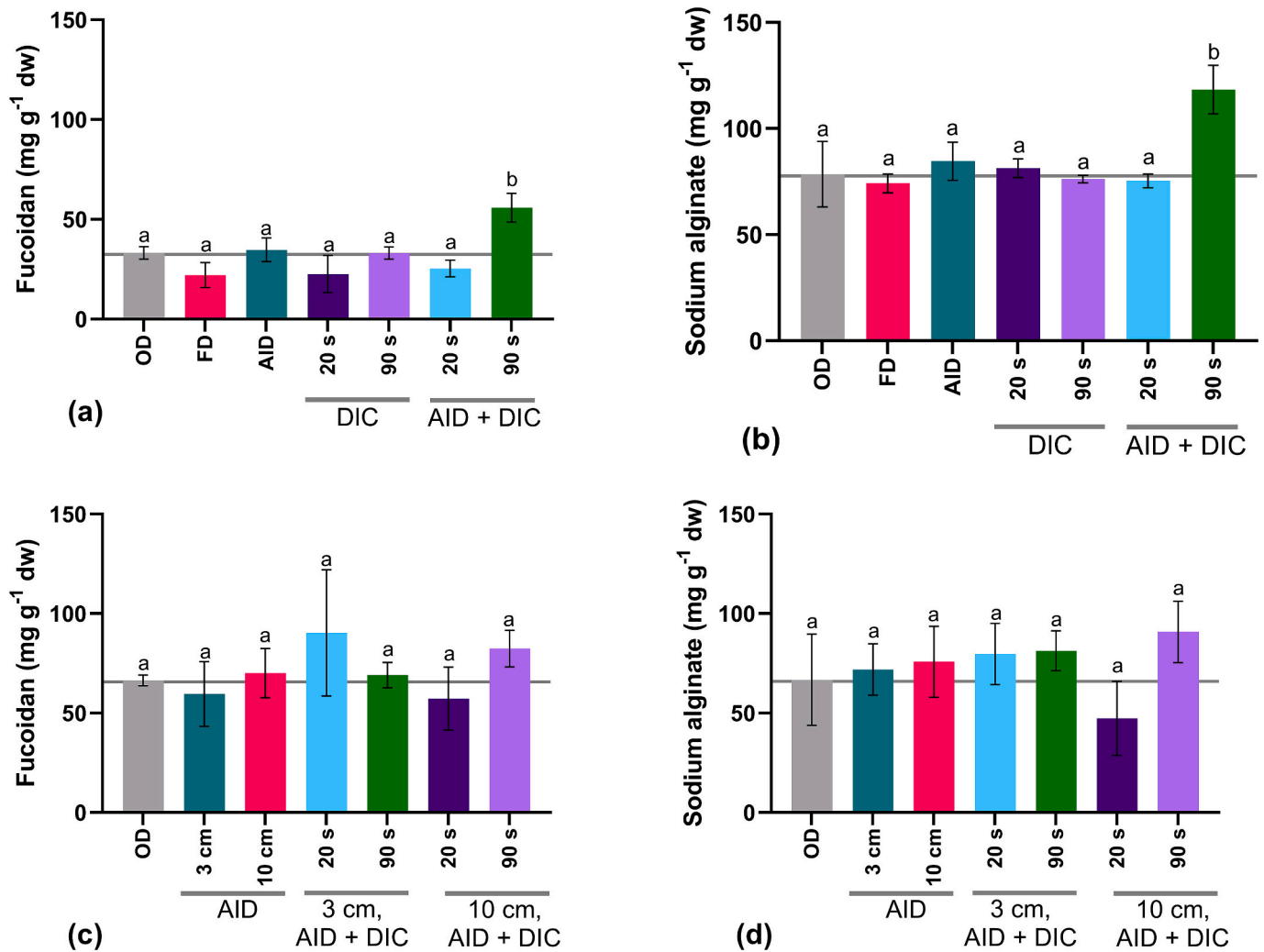


Fig. 5. Crude polysaccharides: fucooidan and sodium alginate ($\text{mg g}^{-1} \text{ dw}$), extracted from *Sargassum muticum*. (a and b) DIC and DIC + AID treatments (100 kPa, 20 s and 90 s, thalli of 10 cm length), and (c and d) AID + DIC treatments (800 kPa, 20 s and 90 s, thalli of 3 and 10 cm length). OD: Oven-Drying, FD: Freeze-Drying, and AID: Air Impingement Drying. AID (3 cm and 10 cm) samples were subjected only to AID. Data are mean \pm SD ($n = 3$). Different letters indicate significant differences ($p < 0.05$) between each polysaccharide group based on ANOVA, SNK (parentheses a, b, d) or Kruskal–Wallis, pair-wise comparisons (parentheses c).

seeds. While in the diatom *Phaeodactylum* (Bacillariophyceae), the increasing of DIC steam pressure [36], positively affected the porosity and diffusivity, allowing to increase the extraction of compounds (*i.e.* the total lipids). In our results, the steam pressure and processing time also showed a significant effect on the content of neutral sugars extracted under 100 kPa. The higher content was observed at 90 s.

Improving the extraction of pigments and antioxidants has been possible by implementing the DIC technology as a key point in the preparation of the biomass before extraction procedures. Lutein extraction, a naturally occurring carotenoid in algae, has been improved in the microalgae *Phaeodactylum* (Bacillariophyceae). DIC increased its extraction up to 6.8 mg g^{-1} compared to 3.3 mg g^{-1} from the raw material [36]. In our research, DIC treatment significantly improved the extraction of beta-carotene and xanthophylls (compared to OD) composed by fucoxanthin and zeaxanthin, all of them considered as natural compounds with high industrial value as pigments and antioxidants in food processing. By applying a DIC treatment on opuntia fruit peels to develop nutritious snacks and powder, fruit peel was pre-dried, and DIC processing parameters, improved the bioavailability of carotene and phenolic compounds using 600 kPa and 15 s [37]. While, in okra pods, DIC-texturing at 400 kPa for 50 s showed the highest values of the chlorophyll and carotenoid pigments [21]. Preserving the antioxidant compounds is also an important aspect that must be considered. The

antioxidant extraction has been improved in DIC-treated, such as orange by-products, using 490 kPa during 186 s [38]. While, an increase in TPC in carrots treated with DIC was observed in comparison to fresh samples $422.5 \text{ vs } 173.7 \text{ mg } 100 \text{ g}^{-1} \text{ dw}$ [39]. Our results showed that AID + DIC treated during 20 s and 90 s, under 100 kPa, allowed to increase the antioxidants, TPC and phlorotannins, and its antioxidant capacity (DPPH) compared to oven-dried samples. The negative correlation observed among antioxidant activity (DPPH) and TPC, could be partially explained since other compounds; such as polysaccharides, could be related to the antioxidant activity.

As reported by Mounir *et al.* [40], DIC significantly increases diffusivity by opening the structure and might crack cell walls, improving the availability of compounds. On the other hand, biomass from conventional drying has been reported to lose bioactive compounds and nutritive value. This mainly occurs during the time-consuming final period, due to the shrinkage of products, inducing a very low diffusivity. To address this problem, the Air Impingement Drying (AID) technology applied to *Sargassum muticum* showed that, at $40 \text{ }^\circ\text{C}$ and 7 m s^{-1} , a satisfactory quality of the product is maintained, with a higher apparent moisture diffusivity. Authors attributed these results to the fact that AID technology improves mass and heat transfers, thus reducing drying time and temperature levels [15]. Even by comparing to freeze-drying and oven-drying, AID allowed a good protein content and a low impact on

polyphenols and uronic acids in different species of *Sargassum* [16]. In this sense, DIC coupled to Air Impingement Drying (AID), seems to preserve and increase availability of bioactive molecules [40]. In this study, protein content was higher in AID + DIC at 800 kPa during 90 s, and during 20 s for the antioxidants TPC and phlorotannins, in thalli of 3 cm. Similar results are showed for total phenolics and flavonoids in strawberries [41], and green “Poblano” Pepper [42] compared to other drying methods.

There has always been a great interest in developing alternative techniques to improve the extraction of polysaccharides from seaweeds. In this study, the quantification of the crude polysaccharides allowed to found that DIC improved the extraction of both fucoidan and alginate under AID + DIC treatment with a processing time of 90 s and 100 kPa. These results are in agreement with other studies extracting polysaccharides, such as hemicellulose from poppy stalks (*Papaver somniferum* L.). DIC and alkaline extraction increased in hemicellulose yield by 49.7 % with the co-current saving in the amount of KOH and the precipitant solution used [43]. DIC treatments also induced a strong solubilization of *in situ* pectic polysaccharides of peach pomace, increasing the extractable pectin yield from 13.8 % to 15.3 % [44]. Likewise, the classical process of oligosaccharide extraction in *Tephrosia purpurea* seeds, was increased up to 145 % of ciceritol and 185 % of stachyose by applying DIC treatments. Authors reported that scanning electron microscopy micrographs provided evident modifications of structure of seeds due to the DIC treatment [45]. Structural modifications may be related to the increase in the extraction of polysaccharides allowing a better penetration of the solvents and acids into the cell wall, due to a higher specific surface area and higher mass transfer diffusivity. These effects have been reported on the structure of the poppy stalk for hemicellulose extraction [43] and mushrooms [46]. As reported by Sutay Kocabaş et al. [43], DIC generates similar or higher porosity in shorter time than steam explosion technology. Steam explosion, a thermo-mechanical under high pressure-high temperature process have been widely used in the pulping and textile industry, and lesser applied for extraction and modification of industrial polysaccharides, such as pectin and agar. In agar extraction from the red seaweed *Gracilaria dura* [47], the total yield was higher after steam explosion by 4.9 % compared to native extraction and up to 10.9 % in alkali treatment, with a quantity reduction on sulphate content similarly to alkali treatments. In our results, by comparing the oven-dried versus AID + DIC treated samples an increase of 20 % in crude fucoidan and 30 % in crude sodium alginate at 100 kPa was detected at 90 s, and an increase in sulphate content up to 97 % was observed at 20 s. However, the sulphate content will depend on the applications for which the polysaccharide is intended.

Finally, the potential applications of DIC at industrial level has been reported in plants and vegetables, in which it shows that industrial use is possible by reducing energy costs and contributing to preserve their nutritional and organoleptic characteristics [32]. Despite the lack of published peer-reviewed articles on DIC on seaweeds, and based on our results and previous studies reported, scaling up at the industrial level of macroalgae DIC-treated to enhance extraction of natural compound can be achievable. Further studies are needed to optimize the process and to evaluate the development for other applications and scaling to large industrial applications [48], since seaweeds, as renewable raw biomass with high diversity of compounds, could play a significant role in circular economy growth due to its biorefinery potential which is in line with FAO's Sustainable Development Goals [49]. DIC as eco-friendly technology represents a key step during the processing chain for seaweed biomass valorisation to produce high-value products. Recently research on macroalgae processing and biotechnology could held the usage of seaweed waste residues to obtain sustainable products, such as bio-oil, biochar, bioethanol, bioelectricity or biomethane [50].

5. Conclusion

This study showed the potential of the DIC, a sustainable technology,

as an appropriate strategy for the valorisation of *Sargassum muticum* biomass. The DIC and Air Impingement Drying (AID) coupled to DIC increased the extraction of many compounds, such as proteins, neutral sugars, pigments, antioxidants and polysaccharides. Further, the conventional process of oven-drying was significantly intensified by AID. *Sargassum muticum* represents a potential natural source for the extraction of many compounds. Further studies should be performed to determine the effects of the DIC technology on different species of seaweeds, as well as exploring other potential applications such as sterilization, texturization, or oil extractions.

CRedit authorship contribution statement

Hugo Pliego-Cortés: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Virginie Boy:** Writing – review & editing, Visualization, Methodology, Investigation, Conceptualization. **Nathalie Bourgougnon:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

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.

Data availability

All relevant data have been included, and will be made available on request.

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