

Facilitating Ulvan Extraction from *Ulva lactuca* via Deep Eutectic Solvent and Peracetic Acid Treatment

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Abstract. *Ulva lactuca* is a green seaweed commonly called sea lettuce and contains sulphated polysaccharides that have biological activity. Despite its lack of ecological sustainability, strong acids (HCl) and strong bases (NaOH) are widely used as solvents in the conventional extraction process for ulvan. Deep eutectic solvent (DES) is an alternative to ulvan extraction solvent, which is more environmentally friendly and has low toxicity. This study aims to assess the efficacy of peracetic acid (PAA) pretreatment and post-treatment in enhancing the quality of extracted ulvan using a DES-based solvent in the extraction process. Ulvan extraction using DES with a temperature of 85-95°C for 1 hour and adding 2% PAA pretreatment, 0.1%, 0.5%, and 2% PAA post-treatment were conducted. PAA pre- and post-treatment was carried out with a 1:10 (w/v) ratio at room temperature for 30 minutes. The analysis included moisture content, yield, functional groups, sulphate content, and color. The test results revealed that ulvan treated with 2% PAA pretreatment had the highest moisture content and sulphate content, at 18.71% and 33.39%, respectively, while ulvan treated with 0.1% PAA post-treatment had the highest yield, at 41.96%. Adding peracetic acid concentration can increase the color quality of the ulvan. PAA pre- and post-treatment had a significant effect on all ulvan quality parameters.

Keywords: DES, Extraction, Peracetic Acid, Polysaccharide, *Ulva lactuca*, Ulvan

INTRODUCTION

Ulvan, a sulphated polysaccharide and complex hydrocolloid, plays a significant role

as the main component of the cell wall in *Ulva* seaweeds, constituting 9-36% of their dry weight (Mo'o *et al.*, 2020). Its constituent components include rhamnose, glucuronic

acid, iduronic acid, and xylose (Lakshmi *et al.*, 2020). Extensive research has highlighted the potential utilization of Ulvan in functional food applications due to its nutritional value and bioactive components. Moreover, Ulvan exhibits beneficial biological activities such as antioxidant (Guedes *et al.*, 2013), immunomodulating (Peasura *et al.*, 2016), anticancer (El Azm *et al.*, 2019), anti-inflammatory (Liu *et al.*, 2019), anticoagulant (de Carvalho *et al.*, 2020), antiviral (Klongklaew *et al.*, 2020), and anti-hyperlipidemia properties (Jiang *et al.*, 2020).

Ulvan is extracted through a multi-step process involving seaweed *Ulva* sp. washing, bleaching, extraction, filtration, precipitation, and drying. The extraction of Ulvan requires high temperatures, around 80-90°C, with a duration of 3-10 hours (Kidgell *et al.*, 2019). The fundamental extraction principle separates desired components from the mixture using an appropriate solvent (Mukhriani, 2014). Technically, strong acidic solvents like HCl and basic solvents like NaOH have been employed for Ulvan extraction (Ramadhan *et al.*, 2022), but these solvents pose environmental and toxicological concerns. To date, there is a growing demand for alternative solvents that are environmentally friendly, particularly to improve the quality of the extracted Ulvan. One recent solvent utilized for polysaccharide extraction is deep eutectic solvents (DES), a mixture of quaternary ammonium salts and hydrogen bond donors in precise ratios to form a eutectic point. The first DES was synthesized using choline chloride (ChCl) and urea with a molar ratio 1:2 (Abbott, 2005).

DES presents itself as an environmentally friendly, economically viable, non-reactive with water, biocompatible, and biodegradable solvent (Jhong *et al.*, 2009). Das *et al.* (2016) reported the use of DES as

an extraction solvent for carrageenan polysaccharides from *Kappaphycus alvarezii*, utilizing DES types such as ChCl-Urea, ChCl-Ethylene glycol, ChCl-Glycerol, 10% Hydrated ChCl-Ethylene glycol, and 10% Hydrated ChCl-Glycerol, with a solvent-to-seaweed ratio of 1:2. They reported that the hydrated form of ChCl-glycerol showed the highest yield, reaching 60.25%, compared to 100% DES, water, and conventional solvents such as HCl or NaOH. Hardiningtyas *et al.*, 2024 also reported the use of DES as an extraction solvent for Ulvan polysaccharide from *Ulva lactuca*, employing three different DES types: ChCl-Urea, ChCl-Ethylene glycol, and ChCl-Glycerol. This report found that DES ChCl-Glycerol provided the best treatment conditions for Ulvan properties. However, to extend the extracted polysaccharide quality, it is still necessary to incorporate some pre- or post-treatment of the polysaccharide during extraction. We initially explored using peracetic acid in combination with ionic liquid in the pretreatment process of seaweed biomass (Uju *et al.*, 2015). Subsequently, we successfully employed peracetic acid for the bleaching of polysaccharides, enhancing the quality of semi-refined carrageenan (SRC) derived from *Eucheuma cottonii* seaweed (Uju *et al.*, 2019).

Peracetic acid (PAA) is a strong oxidizing agent that appears as a colorless liquid with a pungent odor (Baldry *et al.*, 1991). PAA exhibits physical properties such as boiling point (1 atm) of 107°C, freezing point (1 atm) of -44°C, and density of 1.1574 g/mL (at 25°C), being highly soluble. Its chemical properties include being flammable, having a pH value of 2.5, and being produced from the reaction of acetic acid and hydrogen peroxide. The utilization of DES as a solvent for extracting Ulvan, in combination with varying amounts of PAA for post-treatment,

has not been reported. Therefore, this study aims to assess the efficacy and impact of PAA pretreatment and post-treatment on enhancing the quality of extracted Ulvan with DES solvent.

MATERIALS AND METHODS

Materials

The materials used in this study included *Ulva lactuca* seaweed harvested from the coastal area of Ujung Genteng, Ciracap Subdistrict, Sukabumi Regency, West Java, Indonesia. Deep eutectic solvent (DES) used of choline chloride (HIMEDIA PCT0203) and glycerol (EMSURE Reag. Ph Eur, Reag. USP, Germany), peracetic acid (PAA) (PT Peroksida Indonesia Pratama, Jakarta, Indonesia) at concentrations of 0.1%, 0.5%, and 2%, isopropyl alcohol (IPA) at 99%, and distilled water. The apparatus utilized in this research included an analytical balance (OHAUS), glass beakers, a magnetic stirrer (DLAB), and another glass instrument for analytical purposes.

Deep Eutectic Solvent (DES) Synthesized.

The deep eutectic solvent used in this investigation was formulated from a mixture of choline chloride and glycerol as a modification from the previous method (Abbot, 2005). Initially, these two components were blended. The choline chloride to glycerol ratio in the DES formulation was 1:2 (v/v). The mixture was then heated to 80°C and agitated with a magnetic stirrer until it achieved transparency. The appearance and viscosity of the resultant DES served as physical indicators of its successful synthesis. The previous reports by Das *et al.*, 2016 and Hardiningtyas *et al.* 2024 conducted biopolymers extractions, particularly from seaweed, using DES. These reports revealed

the high performance of hydrated ChCl-Glycerol in extracting targeted polysaccharides. Therefore, a synthesized DES with a 30% hydrated state as the main solvent was prepared to extract ulvan from *Ulva lactuca* seaweed.

Extraction of Ulvan using Deep Eutectic Solvent (DES)

Ulvan was extracted from *Ulva lactuca* using a 30% hydrated DES (ChCl-glycerol) following the method described by Hardiningtyas *et al.* (2024), with some modifications. The sample-to-solvent weight ratio was determined to be 1:20 (w/v). A 10 g sample of *Ulva lactuca* required 190 mL of hydrated DES. The extraction process was carried out using a magnetic stirrer and maintaining a temperature range of 85-95 °C for one hour. The extracted ulvan was then filtered through a 300-mesh nylon filter and precipitated for 24 hours using 99% IPA at 1:2 (v/v) at 4°C. The ulvan solution was dried in a 40°C dehydrator for 24 hours. The ulvan was analyzed for its moisture content, yield, functional groups, sulphate concentration, and color.

Pretreatment and Post-treatment Peracetic Acid (PAA)

This study used four samples, including the extraction of ulvan with pretreatment of 2% (b/v) PAA and the extraction of ulvan with post-treatment PAA concentrations of 0.1%, 0.5%, and 2% (b/v). The powdered *Ulva lactuca* seaweed was pretreated with PAA before the extraction process. At the same time, the post-treatment was applied after the extracted ulvan was obtained from the drying process and subsequent PAA treatment. The sample and peracetic acid (PAA) were maintained at a molar ratio of 1:10 (w/v). The pre- and post-treatments with PAA

were conducted for 30 minutes at room temperature.

Ulvan Quality Yield

The yield was calculated by comparing obtained ulvan's dry weight to the seaweed's total weight. Eqs. (1)-(3) were the formula for calculating the yield.

$$\text{Yield (\%)} = \frac{\text{Total weight of Ulvan}}{\text{Total weight of seaweed}} \times 100\% \quad (1)$$

$$\text{Yield in wet weight basis (\%wb)} = \frac{\text{Moisture content of Ulvan}}{100 \times \text{Yield}} \times 100\% \quad (2)$$

$$\text{Yield in dry weight basis (\%db)} = \text{Yield (\%wb)} - \text{Moisture content of Ulvan} \quad (3)$$

Moisture Content (AOAC 1995)

Using the oven procedure, the moisture content was measured. The underlying principle was water evaporation from the sample through heating, and then the sample was weighed repeatedly until a constant weight was achieved. At this constant weight, it was presumed that all the water within the sample had evaporated. The difference between the weights before and after drying indicated the quantity of evaporated water. The dish used for this analysis was oven-dried at 100-105°C for 30 minutes. The dish was weighed after cooling in a desiccator to remove moisture (A). In this pre-dried dish (B), a 2 g sample was weighed, which was subsequently oven-dried at 100-105°C. The weight of the dish plus the dry sample was calculated (C). This process was repeated until a constant weight was attained. The moisture content formula was calculated using Eq. (4)

$$\text{Moisture content (\%)} = \frac{C - A}{B - A} \times 100\% \quad (4)$$

Sulphate Content (AOAC 1995)

The sulphate content analysis was initiated by adding 0.2 g of the powdered sample. Subsequently, 5 mL of concentrated NaOH was added, and the mixture was heated until only 1 mL remained. Then, 5 mL of concentrated HCl was introduced, and the solution was heated in an acid chamber. The solution was then diluted to a total volume of 50 mL and was subsequently homogenized. A 2 mL aliquot of the solution was taken to which 2 mL of sulphate buffer and 2 mL of a 10% BaCl₂ solution were added. This mixture was further diluted to a total volume of 10 mL and was analyzed using a spectrophotometer at a wavelength of 420 nm. The subsequent formula (Eqs. (5)-(6)) represents the calculation for determining sulphate content.

$$\text{Sulphate content (\%)} = \frac{\text{Final dish weight} - \text{empty dish weight.}}{\text{Sample weight}} \times 0.4116 \times 100\% \quad (5)$$

$$\text{Sulphate content (\%db)} = \frac{\text{Sulphate content (\%wb)}}{100 - \text{Moisture Content}} \times 100\% \quad (6)$$

Functional Group Analysis

The FTIR spectrum of ulvan samples was acquired using an FTIR spectrophotometer (Perkin Elmer Spectrum One S4934, Ohio, USA). About 25 mg of the sample was placed directly on the surface of the ATR crystal and carefully pressed with a plunger with a flat tip. In the 4000–400 cm⁻¹ wavenumber range, spectra were collected with a scan speed of 0.20 cm⁻¹/s and 8 accumulations at a resolution of 4 cm⁻¹.

Color Analysis

The ulvan sample's color profile (*L**, *a**, and *b**) was evaluated with the RGB-1002 (Lutron, Taiwan) device, which generated a color spectrum consisting of R (red), G

(green), and B (blue) values. Subsequently, the color was converted to HunterLAB using the color-hex application on the Colour Converter website (colormine.org).

Statistical Analysis

The data of yield, moisture content, and sulphate content tests for the Pre-treatment PAA 2% (Pre2%), Post-treatment PAA 0.1% (Post0.1%), Post-treatment PAA 0.5% (Post0.5%), and Post-treatment PAA 2% (Post2%) treatments were subjected to normality tests to determine if the error distribution in the data was normal. Once the data passed the normality test and were calculated to be normally distributed, an analysis of variance (ANOVA) was performed. The ANOVA used a Completely Randomized Design with a single factor. Data analysis was done using Microsoft Excel and the SPSS software, version 22.0.

RESULTS AND DISCUSSION

Moisture Content

Moisture content analysis was conducted for all treatments, including Pre-treatment PAA (2%) and Post-treatment PAA (0.1%, 0.5%, and 2%). Figure 1 shows the moisture content of the ulvan extract resulting from both pretreatment and posttreatment with peracetic acid (PAA).

The ulvan extracted using DES ChCl-glycerol with a pre-treatment of 2% PAA and post-treatments of 0.1%, 0.5%, and 2% PAA showed significant differences in moisture content ($p < 0.05$). The highest moisture content in ulvan was achieved from the DES extraction with a 2% PAA pretreatment, displaying 18.71%. In contrast, the lowest moisture content was observed in ulvan subjected to a 0.1% PAA post-treatment, measuring at 10.60%. It was indicated that

the ulvan's moisture content increased with the subsequent posttreatments involving increased concentrations of peracetic acid (PAA).

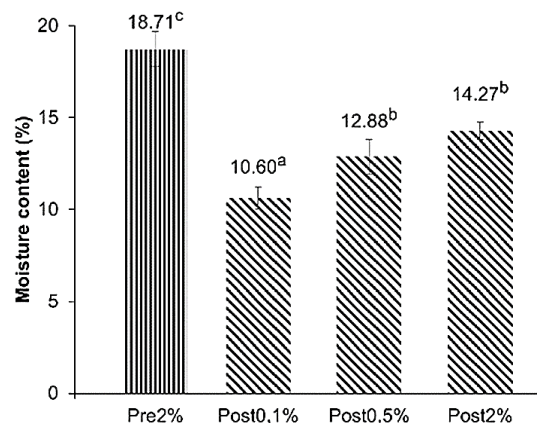


Fig. 1: The moisture content of ulvan extract, obtained using DES ChCl-glycerol with Pretreatment of 2% PAA and Posttreatments of 0.1%, 0.5%, and 2% PAA. *Data are expressed as mean \pm standard deviation ($n = 3$) and different letters in the superscript denote statistically significant ($p < 0.05$).

PAA pre-treatment and post-treatment during ulvan extraction significantly influenced its moisture content ($p < 0.05$). The ulvan generated from the extraction utilizing DES solvent, combined with PAA pre- and post-treatments, exhibited moisture content values that were lower than the standard 22% for agar flour but higher than the 12% for carrageenan flour, except the 0.1% PAA post-treatment (FAO, 2014). The moisture content of ulvan, when facilitated with a 30% DES solvent (choline chloride-glycerol), was previously reported by Hardiningtyas *et al.* (2024) to be higher at 30.73%. This phenomenon can be attributed to DES possessing a lower molar percentage than the molar percentage of water, leading to water being bound by the DES solvent (Dai *et al.*, 2013). Moreover, including PAA pre- and post-treatments in the ulvan production

process highlighted peracetic acid's capability to reduce the bound moisture content within the ulvan product. Moisture content is a primary factor that serves as a baseline for evaluating the overall quality of ulvan. In this research, subsequent assessments were calculated dryly, excluding the present moisture content to discern the effects of treatments on the observed parameters directly.

Yield

Yield serves as an initial parameter in determining the quality of ulvan. The yield of ulvan was derived from the extraction of *Ulva lactuca* seaweed using a DES choline chloride-glycerol (ChCl-glycerol) solvent combined with PAA pre- and post-treatments. A 2% PAA concentration was used for the pre-treatment, while the post-treatment involved varying PAA concentrations, specifically 0.1%, 0.5%, and 2%. The resulting percentage yields of ulvan obtained can be observed in Figure 2.

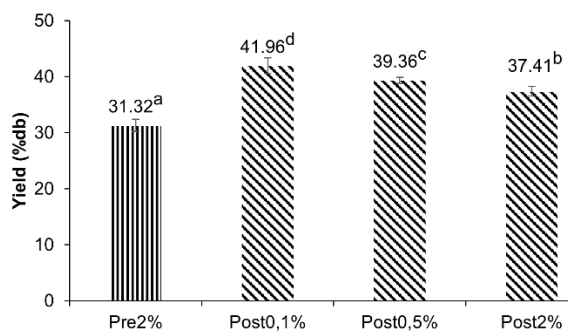


Fig. 2: The yield of ulvan extract, obtained using Using DES ChCl-glycerol with Pretreatment of 2% PAA and Posttreatments of 0.1%, 0.5%, and 2% PAA. *Data are expressed as mean \pm standard deviation ($n = 3$) and different letters in the superscript denote statistically significant ($p < 0.05$).

The highest yield of ulvan was obtained from the extraction using DES with a post-

treatment of 0.1% PAA, while the lowest was observed from the ulvan with a pre-treatment of 2% PAA. The yield of ulvan decreased significantly with the increasing concentrations of peracetic acid (PAA) during post-treatment. The pre-treatment and post-treatment with PAA during ulvan extraction significantly influenced the yield ($p < 0.05$).

The yields of ulvan varied considerably and were influenced by factors such as the type and condition of extraction. Kidgel *et al.* (2019) reported that out of the 22 *Ulva* species studied, the yield ranged between 2.7% and 40%. Previous studies on the use of peracetic acid (PAA) as a treatment for other polysaccharides, such as the Semi-Refined Carrageenan (SRC) derived from *Kappaphycus alvarezii* seaweed extraction, were conducted by Uju *et al.* (2019). Their findings indicated a reduction in yield when the PAA concentration increased. Ulvan subjected to PAA treatment experienced an enhanced hydrolysis reaction, leading to the loss of chromophore groups carrying pigments and cellulose in ulvan.

Functional Groups

Functional groups are specific groupings of atoms that dictate the properties of organic compounds. The underlying principle of the FTIR method is based on the differential absorption of infrared radiation by molecules of a material. The results of the ulvan functional group analysis using ATR FTIR are illustrated in Figure 3.

The functional group analysis results from the extraction of ulvan using DES show similar spectral patterns between the pre-treatment and post-treatment samples with PAA. The presence of hydroxyl (O-H) and C-H groups in all treatments are the main wave absorptions in polysaccharides (Tian *et al.*, 2015). The C-O-S group found in all

treatments is a primary indicator of ulvan (Koga *et al.*, 2018).

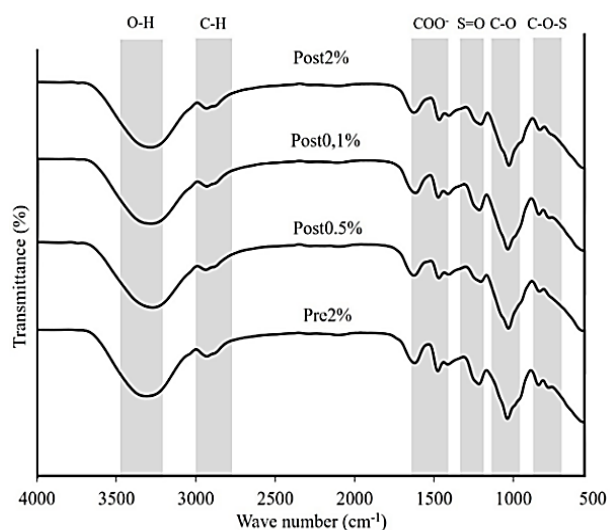


Fig. 3: Functional Groups Analysis of Ulvan Extracted Using DES ChCl-glycerol with Pretreatment of 2% PAA and Posttreatments of 0.1%, 0.5%, and 2% PAA.

The C-O-S groups between the DES solvent and the ulvan samples, both pre-treated and post-treated with PAA, suggest that peracetic acid (PAA) effectively removed the residual DES solvent bound within the ulvan. This inference was drawn from the disappearance of the DES solvent spectrum in the PAA-treated ulvan samples in the 900-700 cm^{-1} wavenumber range. Hardiningtyas's and coworker's (2024) research indicated that ulvan extracted using a 30% DES solvent retained some adhering DES solvent. Robic *et al.* (2009) noted that the FTIR results of the DES solvent synthesized from choline chloride and glycerol in the wavenumber range of 900-700 cm^{-1} is a primary marker of ulvan and exhibits a very high absorption intensity. This study's results show a lower absorption intensity in the range of 900-700 cm^{-1} compared to previous studies and the FTIR of the DES solvent synthesized from choline chloride and glycerol. The increasing

concentrations of PAA introduced during post-treatment resulted in progressively lower absorption intensities.

Sulphate Content

The sulphate content is a crucial parameter in ulvan and serves as an indicator of its biological activity. The biological activity of ulvan is determined based on the percentage of sulphate contained within it (Lakshmi *et al.*, 2020; Ramadhan *et al.*, 2022). The sulphate content analysis aims to identify the constituting compounds of ulvan. Moreover, the sulphate content influences the purity level of ulvan and affects its primary structural composition. The results of the sulphate content analysis are presented in Figure 4.

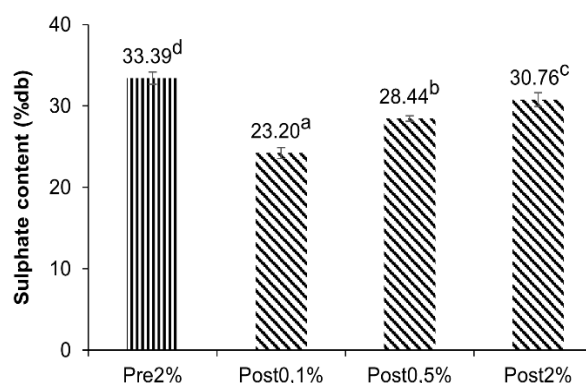


Fig. 4: The sulphate content of ulvan extracted Using DES ChCl-glycerol with Pretreatment of 2% PAA and Posttreatments of 0.1%, 0.5%, and 2% PAA. *Data are expressed as mean \pm standard deviation ($n = 3$) and different letters in the superscript denote statistically significant ($p < 0.05$).

The analysis of sulphate content revealed that the highest level was observed in the ulvan extraction using 30% DES with a 2% PAA pretreatment, registering 33.39%. Conversely, the lowest content was detected in the posttreatment with 0.1% PAA, amounting to 23.20%. Previous research by

Hardiningtyas *et al.*, (2024) on ulvan extraction utilizing hydrated DES reported a sulphate content of 35.67%.

The sulphate content contained in ulvan extracted using DES ChCl-glycerol with a pretreatment of 2% PAA and post-treatments of 0.1%, 0.5%, and 2% PAA displayed significant differences ($p < 0.05$). Interestingly, in the post-treatment, as the PAA concentration increased, the sulfate content of ulvan also increased. This indicates the successful further purification of the extracted ulvan by PAA. Ulvan with elevated sulphate levels exhibits enhanced biological activities, including anticoagulant and antioxidant properties. The sulphate content is influenced by the primary structure of ulvan, characterized by heteropolysaccharides and disaccharides, encompassing sugars such as rhamnose, guluronic acid, and iduronic acid (Ramadhan *et al.*, 2022). The functional groups constituting the rhamnose sugar present in sulphate are S=O and C-O-S.

Color

Ulvan's color appearance, particularly its brightness level, indicates its quality; a higher brightness corresponds to superior quality. Color analysis was conducted using a Colorimeter across all samples. In the color analysis, the symbol L^* represents brightness levels ranging from 0 to 100. The symbol a^* indicates the degree of redness ($+a^*$) or greenness ($-a^*$), whereas the symbol b^* signifies the extent of yellowness ($+b^*$) or blueness ($-b^*$). The results of the color analysis are presented in Table 1.

According to the data presented in Table 1, there was a positive correlation between the concentration of PAA and the L^* value of extracted ulvan. The colorimetric measurement indicated that the ulvan sample

treated with 2% PAA during posttreatment exhibited the maximum brightness level (L^*).

Table 1. The color profile of ulvan extracted using DES under varying treatment conditions.

Sample	Color		
	L^*	a^*	b^*
Pre2%	32.09±1.49 ^b	2.22±0.48 ^{ab}	21.62±0.91 ^a
Post0.1%	22.94±0.54 ^a	4.70±1.04 ^d	25.73±1.30 ^b
Post0.5%	38.21±0.30 ^c	4.08±0.89 ^{cd}	31.33±0.54 ^c
Post2%	47.55±0.63 ^d	2.69±1.85 ^{bc}	34.23±2.21 ^{ed}

Different letters in the same column denote statistically significant ($p < 0.05$).

The lowest L^* values were observed in the ulvan extracted using 0.1% of PAA. The increase in PAA concentration resulted in the production of ulvan with enhanced whiteness ($p < 0.05$). The a^* value of ulvan with different treatment conditions showed a significant decreasing trend ($p < 0.05$), specifically when compared between the post-treatment concentrations, while b^* experienced a slight increase.

Importantly, the treatment with PAA significantly enhanced the characteristics compared to the previous report by Hardiningtyas *et al.* (2024), where ulvan extracted solely by DES exhibited L^* , a^* , and b^* values of 8.42, 0.46, and 6.64, respectively. These values indicate a shift from dark-colored ulvan to a lighter shade, potentially light brown, or off-white to creamish color, induced by the bleaching effect of PAA.

According to Uju *et al.* (2019), the utilization of peracetic acid (PAA) as a bleaching agent substantially enhances the chromatic brightness of the sample. The increased brightness of ulvan can be ascribed to the capacity of peracetic acid to eliminate pigments. Furthermore, elevated

temperatures also significantly impact the brightness of the resulting ulvan (Steiner, 1995). The ulvan-peracetic acid interaction can be described as an acid hydrolysis reaction, which involves eliminating chromophoric groups containing pigments and oxidizing cellulose inside ulvan.

Comparison of Yield and Sulphate Quality between Ulvan Extracted Using DES, DES+PAA and Conventional Methods

The quality of ulvan was determined primarily by analyzing its yield and sulfate content. The yield was the entire weight of ulvan obtained after extraction; a higher yield is typically indicative of ulvan of superior quality. The relationship between sulphate content and ulvan's biological activity and potential applications is intrinsic. Figure 5 depicts the detailed outcomes of the yield and sulphate content analyses.

The variance analysis results indicate that pre- and post-treatment applications significantly influence the yield and sulphate content of the resulting ulvan ($p < 0.05$). The ulvan treated with 2% PAA exhibited the highest yield, 37.41%, but the lowest sulfate concentration, 30.76%. In contrast, ulvan extracted with HCl had the lowest yield (16.72%) and the highest sulfate concentration (58.15%).

Ramadhan *et al.* (2022) used ultrasonic assistance with HCl as the solvent for 90 minutes at 60 °C. In contrast, the DES extraction method for ulvan was carried out at a higher temperature range (85-95°C) but for only 60 minutes. The chemical composition is significantly affected by its growth habitat, harvest age, and maturation stage (Starko *et al.*, 2018). DES has a greater binding affinity for ulvan than conventional solvents such as HCl, NaOH, and water, as evidenced by the superior yield obtained

when using DES in conjunction with 2% PAA post-treatment.

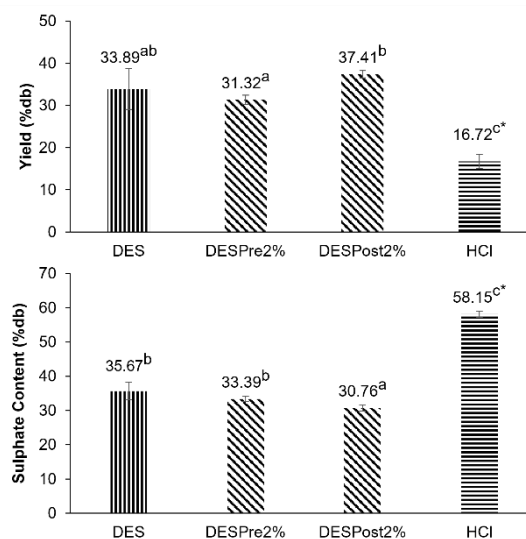


Fig. 5: Quality yield and sulphate content of ulvan extracted using DES, DES+PAA and HCl (Conventional Methods) (*Ramadhan *et al.* (2022)). *Data are expressed as mean \pm standard deviation ($n = 3$) and Different letters in the superscript denote statistically significant ($p < 0.05$).

The application of both pre-treatment and post-treatment with PAA on ulvan indicated reduced sulphate contents, compared to the previous report where DES was employed without PAA, suggesting the potential of PAA in breaking glycoside bonds within ulvan, and eventually remove the sulphate moieties. In the pretreatment condition, PAA acts as a strong acid, initiating the breakdown of seaweed cell walls, followed by the ease of interaction of the polysaccharides with DES solvents. In the posttreatment, when ulvan was treated with PAA, more reactive hydroxyl radicals ($\text{OH}\cdot$) were formed through the breakdown of CH_3COOOH . These radicals subsequently break the glycosidic bonds of polysaccharides during the depolymerization process (Uju *et al.*, 2019). This result increases

the number of monomers formed, leading to a decrease in the molecular weight of the depolymerized polysaccharide product. Hence, with the presence of PAA, the rate of acid hydrolysis of ulvan increases, with H⁺ ions aiding in the hydrolysis of glycosidic bonds, thereby reducing the sulfate content of ulvan.

However, the decreased sulfate content of ulvan extracted with DES suggests that its properties may be different from those of ulvan extracted with HCl. The sulfate content of ulvan is determined by its primary structure, which is composed of sulfate heteropolysaccharides, and its constituent disaccharides, which consist of rhamnose sugars, iduronic acid, and either glucuronic acid or xylose (Kidgel *et al.*, 2019). Importantly, the novel solvent, DES, has demonstrated its efficacy in extracting high-quality ulvan. Furthermore, the incorporation of PAA treatment not only improves the quality but also enhances the appearance and overall parameters.

CONCLUSIONS

Ulvan extracted using the DES solvent exhibited a higher yield than ulvan extracted with HCl solvents. Despite this sulfate content was 19.67% lower than the HCl solvents. Applying pre- and post-treatments with PAA significantly influenced the quality of ulvan, impacting parameters such as moisture content, yield, sulfate content, functional groups, and color profile. Introducing peracetic acid (PAA) to ulvan notably enhanced its color quality. The treatment that produced the most positive results was post-treating ulvan with 2% PAA. This treatment resulted in an extraction rate of 37.41% and a brightness (*L*^{*}) value of 47.55, surpassing the results of other treatments. Further

investigations are needed, including elemental analysis and optimization of dependent conditions in the extraction process of ulvan to establish plausible or valid mechanisms for PAA treatment in ulvan extraction using DES-based solvents. These additional studies will specifically aim to clarify and explore the intermolecular interactions among PAA, DES, and ulvan.

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