

*Unlocking the potential of
macroalgae for a thriving
European blue
bioeconomy*



CHEMICAL ANALYSES AND BENCHMARKING OF ULVAN IN CO-EXTRACTS

SEAMARK DELIVERABLE 5.5
Technical University of Denmark (DTU)



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SEAMARK DELIVERABLE 5.5: CHEMICAL ANALYSES (PURITY, PROTEIN CONTENT, CHAIN LENGTH) AND BENCHMARKING OF ULVAN IN CO-EXTRACTS

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Abstract

This document describes a pilot scale extraction trial of ulvan from the green seaweed *Ulva* sp. The protocol is based on hot water extraction followed by size fractionation using ultrafiltration. Two of the fractions (150 kDa and 15 kDa) were subsequently analysed for the content of monosaccharides, amino acids, sulfate and inorganic matter. The ulvan content was ca. 61 % for the 150 kDa fraction and ca. 49 % for the 15 kDa fraction. This range of purity is comparable to published results regarding ulvan purification from *Ulva* species.

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LIST OF ABBREVIATIONS

Abbreviation	Description
kDa	Kilo Dalton
GlcAp	D-Glucuronic acid
IdoAp	L-Iduronic acid
Rhap 3S	L-Rhamnose-3-sulfate
Xylp	Xylose
Xylp 2S	Xylose-2-sulfate
Rhap 3S	L-Rhamnose-3-sulfate
HPAEC	High-Performance Anion-Exchange Chromatography
HPLC-UV	High-Performance Liquid Chromatography with UV detection
HPSEC-RI	High-performance size-exclusion refractive index
DW	Dry weight

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1. INTRODUCTION

Species of the green macroalgae genus *Ulva* are utilized as a food source and is known under the common name sea lettuce. *Ulva sp.* contains a high amount of the polysaccharide ulvan, primarily located within the cell walls of the algae and constituting 9 to 36 % of the algal dry weight (Kidgell *et al.*, 2019). Ulvan is a sulfated heteropolysaccharide essentially consisting of repeating ulvanobiuronic acid and ulvanobiose. Ulvanobiuronic acids are disaccharides consisting of either D-glucuronic acid (GlcAp) or L-iduronic acid (IdoAp) linked to L-rhamnose-3-sulfate (Rhap 3S), while ulvanobioses are disaccharides consisting of xylose (Xylp) or xylose-2-sulfate (Xylp 2S) linked to L-rhamnose-3-sulfate (Rhap 3S) (Kidgell *et al.*, 2019) (see Figure 1). In some ulvans branching has been observed, in which glucuronic acid is (1,2)-linked to the rhamnose residue of ulvanobiuronic acid. Typically, the content of ulvanobiuronic acid is far higher than that of ulvanobiose (Kidgell *et al.*, 2019), but large variation in monosaccharide composition of ulvans has been observed depending on species, eco-physiology, season and extraction procedures. In addition to ulvan, *Ulva sp.* also contains the polysaccharides cellulose, xyloglucan and glucuronan amounting to a total content of polysaccharides of 45 % of the dry weight biomass (Lahaye, 1997).

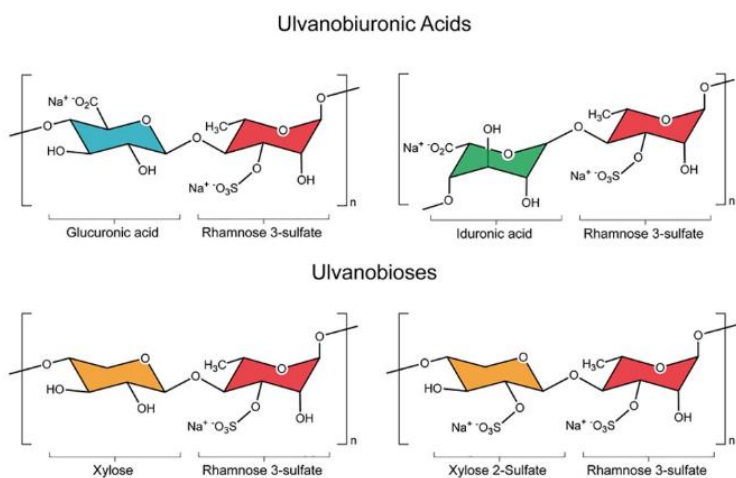


Figure 2: Molecular structure of the main disaccharides comprising ulvan (Kidgell *et al.*, 2019).

Like other sulfated polysaccharides from seaweed, ulvan has a range of beneficial properties related to human health. Ulvan has been reported to have cytotoxic-, immunomodulating-, antioxidant-, anticancerous-, anticoagulant-, antihyperlipidemic-, antiviral-, and plant immunity activities (Kidgell *et al.*, 2019). In many cases these effects have been observed using in vitro assays, and their effect in the human body after oral intake is less well investigated. Extraction of ulvan from green seaweed is a relatively simple process, and the report describes a protocol used by the company Algaia based in France to extract ulvan from *Ulva sp.*, and the resulting composition of the tested biomass.

2. METHODOLOGY

2.1 Ulvan production protocol

Following the various tests carried out in the laboratory, a first ulvan production protocol was selected to be tested in pilot scale. This protocol aimed to reduce the amount of extraction solvent and easy to transfer to industrial scale for the future.

For this purpose, 60 kilograms of fresh seaweed provided by the company AlgaPlus based in Portugal was cut using a grinder and put in a tank with 400 litres of water. The extraction was carried out at 80 °C for 5 hours. The resulting product was separated using a centrifugal decanter to obtain two fractions:

- 1) the liquid extract;
- 2) the residual biomass.

The extract was then fractionated by carrying out a series of successive ultrafiltrations with different molecular weight cut-offs (150 kDa, 15 kDa, 5 kDa, 1 kDa) using ceramic membrane filters. The first two filter sizes (150 and 15 kDa) contained two carbohydrate rich fractions. To obtain ulvans of even higher purity, an alternative procedure is to precipitate the extracted ulvan with cetyltrimethylammonium bromide (CTAB) and subsequently wash the solubilized precipitate with ethanol and use for ultrafiltration (Tran *et al.*, 2023). Those extra steps were not deemed necessary for the performed pilot scale test as it would complexify the extraction process technically and regulatory-wise with limited returns. The procedure is shown in Figure 2.



Figure 1: Diagram of the ulvan extraction protocol followed in the study.

2.2 Analytical composition of the two carbohydrate rich fractions

The two carbohydrate rich fractions were analysed for their monosaccharide composition, amino acid composition, sulfate content and content of inorganic matter. In all cases 20 mg of dry algal biomass was used for each analysis. The monosaccharide composition analysis was performed in triplicate using acid hydrolysed extract subjected to an in-house protocol based on High-Performance Anion-Exchange Chromatography (HPAEC) specific for either neutral or uronic acid sugars. The amino acid analysis was performed using the AccQ-Tag method from Waters based on High-Performance Liquid Chromatography with UV detection (HPLC-UV). The sulfate analysis was done by X-Ray Fluorescence spectroscopy.

3. RESULTS AND DISCUSSION

The results of the ulvan extraction are shown in Table 1.

Table 1: Content in percent of dry weight of monosaccharides, amino acids, sulfate and inorganic matter in two fractions of ulvan extract.

Fraction	150 kDa	15 kDa
Arabinose	0.18 %	0.39 %
Galactose	0.86 %	1.18 %
Rhamnose	22.12 %	18.92 %
Glucose	1.60 %	6.44 %
Xylose	2.05 %	3.43 %
Mannose	0.18 %	0.61 %
Total neutral monosaccharides	26.98 %	30.96 %
Glucuronic acid	13.21 %	9.62 %
Iduronic acid	13.37 %	9.95 %
Total Uronic acids	26.58 %	19.57 %
Total Carbohydrates	53.60 %	50.50 %
Histidine	0.13 %	0.00 %
Taurine	0.00 %	0.02 %
Serine	0.45 %	0.31 %
Arginine	0.46 %	0.13 %
Glycine	0.63 %	0.62 %
Aspartic acid & Asparagine	1.23 %	1.20 %
Glutamic acid & Glutamine	1.08 %	0.80 %
Threonine	0.47 %	0.43 %
Alanine	0.70 %	0.59 %
Proline	0.41 %	0.26 %
Ornithine	0.02 %	0.00 %
Lysine	0.50 %	0.11 %
Tyrosine	0.29 %	0.18 %
Methionine	0.20 %	0.11 %
Valine	0.61 %	0.39 %
Isoleucine	0.40 %	0.17 %
Leucine	0.63 %	0.25 %
Phenylalanine	0.43 %	0.15 %
Cysteine	0.16 %	0.21 %
Total Amino Acids	8.80 %	5.90 %
Sulfate (bound)	10.40 %	7.38 %
Inorganic matter	12.60 %	25.20 %

Most of the monosaccharide content consists of sugars included in ulvan (rhamnose, xylose, glucuronic acid and iduronic acid) while remaining monosaccharides only constitute a minute fraction. As ulvan is composed of disaccharides that all contain a rhamnose residue, the combined content of xylose, glucuronic acid and iduronic acid should match the content of rhamnose. This is not quite the case, which could indicate that some branching with glucuronic acid is present in the ulvan. The molar mass of sulfate is approximately half that of a monosaccharide, so the fact that the sulfate content is approximately half that of the rhamnose content, indicates that all sulfate is bound to rhamnose and none or only very little has been bound to other ulvan residues or other organic compounds. Summing the values of ulvan monosaccharides (rhamnose, xylose, glucuronic acid and iduronic acid) and sulfate gives an ulvan content of 61.15 % for the 150 kDa fraction and 49.3 % for the 15 kDa fraction. The remaining dry matter consists of inorganic matter, protein and other organic compounds such as lipids. Assuming a molar mass of an average ulvan residue of ca. 180 Da, the 150 kDa fraction has a degree of polymerization (DP) of more than 833, while the 15 kDa fraction has a DP of between 83 and 833.

The ulvan purity obtained is comparable to previous studies (e.g. Costa *et al.*, 2012). The protein content is in some protocols lowered substantially by addition of proteinase K in the extraction procedure (Costa *et al.*, 2012; Wahlström *et al.*, 2020). Alpha-amylase can also be added to decrease the content of amylose in the extract but given the low amounts of glucose found in the extract, this is hardly worthwhile.

4. CONCLUSION

Using a protocol based on hot water extraction, two fractions of ulvans were purified from *Ulva lactuca*. The purity of the ulvan was comparable to that of published studies, and the sulfation level of the ulvan was relatively high. The protocol will form the foundation for benchmarking and characterization of *Ulva sp.* derived compounds that will ultimately be included in Seamark products P10, P11 and P12 for commercial exploitation as food, feed and biomedical applications.

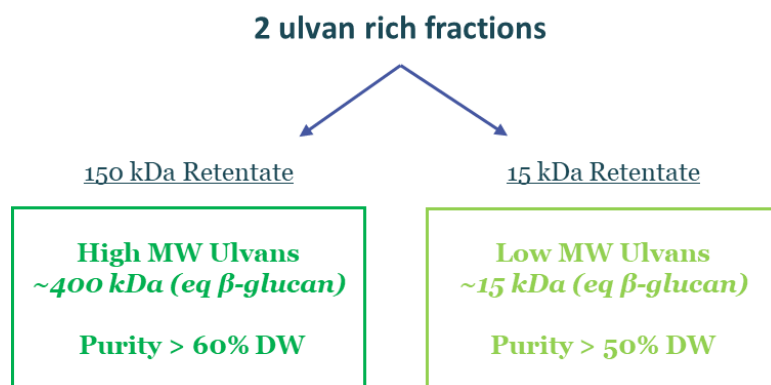


Figure 3: Outcome of the ulvan purification resulting in two size fractions containing ulvan polysaccharides.

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