

EXTRACTION AND PURIFICATION OF MONOSACCHARIDES FROM MARINE SEAWEEDS

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Submitted December 5, 2024; Revised January 8, 2024; Accepted January 8, 2024; Published January 20, 2025

ABSTRACT

Seaweeds are abundant in bioactive compounds, and their unique polysaccharide content, which includes rare sugars with potential industrial applications, has garnered significant attention in recent years. This review aims to illuminate the structural diversity and biotechnological significance of rare sugars by exploring their occurrence and biosynthesis in seaweeds. Understanding the presence of rare sugars in these marine organisms requires a detailed analysis of the biosynthetic pathways involved in their formation. The study emphasizes the importance of precise structural characterization for the identification and quantification of rare sugars, detailing advanced analytical techniques employed in this process. It also addresses challenges in extraction and purification, underscoring the need for scalable and cost-effective methods for industrial production. The potential applications of rare sugars span the food, pharmaceutical, and other industries, highlighting their growing significance in health-related applications and their biological and medicinal properties. Additionally, the review discusses issues such as low yields, storage stability, and regulatory concerns, providing a comprehensive overview of the current state of rare sugars research and application.

Keywords: Extraction, purification, seaweed, sugars, monosaccharide

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INTRODUCTION

Marine macroalgae and other seaweeds have emerged as promising sources of valuable nutrients and bioactive compounds, with applications in food ingredients, nutritional supplements, and cosmetics. Despite covering over 70% of the Earth's surface, relatively few marine organisms have been extensively studied. The cell walls of seaweeds contain commercially significant polysaccharides that serve diverse biological functions.

Marine macroalgae possess a variety of polysaccharides that play essential roles in their structural integrity and energy storage [1].

Different types of algae feature distinct polysaccharides: brown algae primarily contain alginates, which provide structural support and flexibility while storing energy as laminarin; red algae are characterized by carrageenans and agar, which contribute to cell wall structure and gel-like properties;

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and green algae mainly utilize cellulose as their primary structural component, akin to land plants. Storage polysaccharides such as laminarin and floridean starch act as energy reserves that can be metabolized as needed. Beyond their biological functions, these polysaccharides have attracted considerable interest for human applications in food production, pharmaceutical research, biomaterial development, and potential nutraceutical innovations [2].

The complexity and versatility of marine macroalgal polysaccharides illustrate the remarkable biochemical adaptations of these organisms, revealing that these compounds are not merely passive cellular components but active molecules with critical functions. Fucoidan, predominantly found in brown algae, varies in molecular characteristics including length, branching patterns, monomer fractions, and sulfate levels. These structurally diverse fucoidans, also known as fucose-containing sulfated polysaccharides, exhibit bioactive properties influenced by species, thallus age, seasonal variations, and extraction methods. While fucose is the predominant sugar, other monomers such as galactose, mannose, glucose, and uronic acids are also present. In some cases, such as sulfated galactofucans, the ratio of galactose may rival that of fucose.

Seaweeds are rich in bioactive compounds, including vitamins, minerals, antioxidants, and rare sugars, which demonstrate anti-inflammatory and immune-supporting properties. These components are currently being explored for their potential health benefits. In addition to basic nutrition, seaweeds provide essential proteins, fiber, and omega-3 fatty acids that contribute to overall health and well-being when included in diets. The development of sustainable cultivation techniques, such as offshore farming and integrated multitrophic aquaculture, is in progress [3].

However, challenges remain in market development, environmental impact assessment, and regulatory frameworks. Addressing these challenges necessitates collaboration among governments, businesses, and the scientific community, alongside innovative technologies and sustainable practices.

Cultivating seaweeds for rare sugar production aligns with sustainable practices, requiring minimal resources and allowing for rapid growth. Conducting environmental impact assessments and implementing sustainable practices are essential for minimizing ecological effects. Recognizing the potential of rare sugars can benefit industry stakeholders by highlighting opportunities for market expansion, product innovation, and economic development. The commercial landscape for rare sugars is undergoing significant transformation, driven by increasing market interest in biotechnological and pharmaceutical applications [4].

Seaweed-derived rare sugars represent an emerging field with substantial potential, evidenced by their diverse applications across multiple industries. This manuscript emphasizes the growing commercial interest in these compounds, noting their potential uses in functional foods, nutraceuticals, pharmaceuticals, and specialized chemical sectors. Although current commercial products are limited, ongoing research and advancements in extraction technologies suggest promising future developments.

Rare sugars are a unique class of compounds that differ from common sugars like glucose and sucrose. These molecules, characterized by their distinct molecular structures, show promise in various applications, including pharmaceuticals, skincare, and wound dressings. Their potential also extends to biotechnological

processes such as enzyme synthesis and biofuel production. Ongoing research is focused on understanding their chemical structures, biosynthesis pathways, and physiological roles [5-8].

The versatility of rare sugars derived from marine macroalgae, including their potential as low-calorie sweeteners, bioactive compounds, and precursors for various industrial applications, positions them as a strategic area for biotechnological innovation. This review aims to address the critical need for an in-depth exploration of rare sugars from seaweeds, their occurrence, biosynthesis, and potential biotechnological applications.

EXTRACTION AND PURIFICATION TECHNIQUES

The extraction of macroalgal polysaccharides typically involves several phases. To extract the polysaccharides of interest and assess their biological activity and potential industrial uses, the process includes pre-treating the macroalgae, preparing the macroalgal biomass, employing extraction techniques (comparing traditional solvent extraction with innovative methods such as ultrasound, microwave, and enzyme-assisted extractions), and applying purification techniques.

To extract polysaccharides from macroalgae, the algae must first be cleaned of sand and epiphytes using either seawater or distilled water. After cleaning, the algae are dried through either oven drying or freeze drying. The dried biomass is then ground to maximize efficiency in the subsequent extraction processes. An alternative method, patented as the exudate technique, allows for the production of laminarin and fucoidan in industrial quantities from living macroalgal tissue, bypassing the need for drying. In this method, fresh macroalgae is chopped into pieces no larger than 1 cm and placed in a

dark, humid environment. The resulting exudates can then be used to extract and purify the polysaccharides laminarin and fucoidan [9].

Common pre-treatments documented in the literature include washing the dried biomass with either acetone alone or a mixture of methanol, chloroform, and water. More recent studies have investigated the use of a combination of acetone and ethanol at varying temperatures, involving either single or multiple ethanolic pre-treatments. These alcohol treatments aim to eliminate strongly attached molecules that contaminate the target compounds, such as mannitol, chlorophyll, fats (defatted), proteins (deproteinated), and phenols (dephenolated). Additionally, innovative pre-treatments recently documented in the literature include the compressional-puffing hydrothermal process. This technique involves heating macroalgae to atmospheric pressure (at 140°C, 180°C, and 220°C) and then rapidly reducing the pressure in a vessel containing superheated water. This process alters the cellular structure of the macroalgae prior to fucoidan extraction.

EXTRACTION METHODS

Enzyme-Assisted Extraction

EAE utilizes enzymes to break down algal cell walls and release target compounds. To improve extraction efficiency, critical factors such as pH, temperature, and treatment duration should be optimized for each enzyme used [10]. Enzymes that catalyze the breakdown of structural components in cell walls, such as cellulose, β -glucan, and hemicellulose, typically enhance the extraction of target molecules. Conventional chemical extraction

There are distinct methods for extracting materials using hot or cold water, as well as acids or alkalis. The disruption of hydrogen bonds between polysaccharides by hydrogen ions and hydroxyl ions typically facilitates extraction under acidic or

alkaline conditions. Conventional extraction methods rely on the solubility of target chemicals and often involve pre-treatment stages that use solvents to eliminate lipids, pigments, proteins, and other contaminants.

Microwave-Assisted Extraction

MAE employs non-ionizing electromagnetic radiation with frequencies ranging from 300 MHz to 300 GHz to break hydrogen bonds and encourage the migration of dissolved ions. This process allows the solvent to penetrate the cell matrix more effectively, facilitating the extraction of desired components. Various parameters, including temperature, pressure, time, and the ratio of algae to water, can be adjusted to optimize the yield of the intended product [11].

Ultrasound-Assisted Extraction

UAE operates by exposing biomass to high-frequency sound waves exceeding 20 kHz. In this process, vapor bubbles form in liquids under high pressure and implode when subjected to strong ultrasonic fields. When these bubbles burst near liquid-solid interfaces, such as cell walls, the intense pressure causes the cells to break down. The low temperatures used in UAE enable the extraction of thermosensitive target molecules.

Supercritical Fluid Extraction

A material that exists at a temperature and pressure above its critical point, where the distinction between gas and liquid phases disappears, is referred to as a supercritical fluid. By adjusting temperature and pressure, the fluid's solubility can be modified. Carbon dioxide is commonly used for this purpose because it has a relatively low critical point compared to other compounds, requiring less energy to reach a supercritical state. Supercritical fluids possess superior transport properties compared to liquids due to their high diffusivity and low viscosity [12-14].

Furthermore, since supercritical fluid extraction does not involve solvents, it is considered an environmentally friendly method. However, the costs associated with this technique can be high compared to other extraction methods, making it primarily suitable for extracting highly valuable compounds.

PURIFICATION

Membrane Separation

Membrane filtration techniques, such as ultrafiltration and nanofiltration, utilize size exclusion to remove contaminants. These methods are effective in eliminating undesirable materials and concentrating rare sugars. Membrane filtration, particularly nanofiltration, is an attractive technology in sugar production as it reduces chemical usage, produces high-quality clarified juice, and minimizes the need for chemical pretreatment [15].

Spiral Agitation

Centrifugation methods based on density differences, such as isopycnic centrifugation, can separate rare sugars effectively. This approach is particularly useful for differentiating sugar fractions with varying densities. Additionally, spiral agitation can enhance mixing and interaction between phases during the crystallization process. For instance, in the separation of glucose and fructose through freezing crystallization, spiral agitation can help maintain a uniform distribution of sugars and promote effective mass transfer between the solid and liquid phases [16-18].

Chromatography

Column chromatography is a refinement technique that separates the components of a mixture based on their differing mobilities in a mobile phase, using a stationary phase. Silica gel and alumina are frequently chosen as the stationary phase due to their strong affinity for certain sugars. A solvent or a combination of solvents acts as the mobile phase to elute the sugars from the stationary

phase. Sugars that have a high affinity for the stationary phase elute last, while those with a lower affinity elute first. A sequence of columns with various stationary phases can achieve a high level of purity for the rare sugars. For a more detailed description of the column chromatography procedure, either methanol or ethanol serves as an appropriate solvent to dissolve the seaweed extract [19]. The solution is then introduced into a column packed with alumina or silica gel. The rare sugars are sequentially eluted from the column by pumping the mobile phase through it. The fractions containing the rare sugars are collected and tested to determine purity. The purified rare sugars can then be utilized for various applications or further analysis. Column chromatography is an effective and straightforward method for extracting rare sugars from seaweed, and it is also cost-efficient.

High-performance liquid chromatography (HPLC) is another method commonly employed to isolate rare sugars from seaweed. In HPLC, a sample containing rare sugars is dissolved in a solvent and passed through a column filled with a stationary phase. This stationary phase consists of particles coated with a material that has a strong affinity for specific sugars. A high-pressure pump propels the mobile phase (solvent or solvent mixture) through the column. The rare sugars in the sample interact with the stationary phase based on their chemical composition. Sugars that are more compatible with the stationary phase remain on the column longer, while those with lower affinity elute more quickly. Ultimately, the rare sugars exit the column in the order of their retention times, with those having longer retention times eluting last [20].

Either methanol or ethanol serves as a suitable solvent for dissolving the seaweed extract. Next, a filter removes any particles

from the solution that could clog the HPLC column. The filtered solution is then introduced into the HPLC apparatus, which consists of a pump, a column, a detector, and a data collection system. The pump applies high pressure to drive the mobile phase through the column. The rare sugars in the sample interact with the stationary phase to varying degrees. Their retention times on the column depend on their affinity for the stationary phase; those with lower affinity will elute more quickly. As the mobile phase progresses, the rare sugars elute from the column in order of increasing retention time. A detector, such as a UV or refractive index detector, identifies the eluted sugars by measuring their absorbance or refractive index. The data collection system then compiles these measurements into a chromatogram, where the peaks represent the eluted rare sugars. After purification, these sugars can be collected for various applications, including further analysis.

These extraction methods vary in sustainability, scalability, and efficiency. While solvent extraction is the most effective technique, it is neither sustainable nor scalable. Enzyme-assisted extraction, though less effective, offers greater scalability. Although both solvent extraction and enzyme-assisted extraction are more effective than supercritical fluid extraction, it remains the most scalable and environmentally friendly option. The choice of method for extracting and purifying rare sugars from seaweeds will depend on the intended application and desired purity level. Sustainability is a critical factor in this process; since seaweeds are a renewable resource, extraction methods should not harm the ecosystem.

Filtration and Precipitation

In many cases, precipitation serves as the initial stage of purification. A suitable precipitating agent, such as acetone or

alcohol, is added to the crude extract, resulting in the separation of solid precipitate from the solution containing rare sugars. Filtration is then employed to remove the precipitates from the liquid, with vacuum filtration and centrifugation being common techniques. Nanofiltration is utilized to separate and purify saccharides from biomass. Nanofiltration has gained significant attention in recent decades due to its high selectivity, low energy consumption, and minimal environmental impact. Hybrid membrane techniques that combine nanofiltration with ultrafiltration can effectively eliminate undesirable solutes, leading to purer saccharides [21, 22].

Crystallization

One method to further purify rare sugars is crystallization. This process encourages crystal formation by regulating variables such as concentration and temperature. Once crystals form, they are extracted from the remaining solution, and the purity can be enhanced by repeating the procedure. While rare sugar crystallization is not widely discussed in the literature. A study investigated the overexpression, crystallization, and preliminary X-ray crystallographic analysis of d-ribose-5-phosphate isomerase from *Clostridium thermocellum* to determine its three-dimensional structure.

CONCLUSION

The discovery of rare sugars in seaweeds presents new opportunities with potential impacts across various industries. Comprehensive studies of their occurrence and biosynthesis have revealed the complex biochemistry of seaweed polysaccharides. To fully harness the potential of these sugars, it is essential to adopt biotechnological approaches, including advanced extraction techniques and scalable production processes.

Author(s) Contribution: resources data, S. Zainab; review, Z. Anum; proof-reading, S. Amber.

Funding: No funding involved.

Ethical Approval: Not applicable

Conflict of Interest: Nil.

Consent for Publication: All authors approved the manuscript for publication.

REFERENCES

1. Dai, Y.; Zhang, J.; Jiang, B.; Zhang, T.; Chen, J. New Strategy for Rare Sugars Biosynthesis: Aldol Reactions Using Dihydroxyacetone Phosphate (DHAP)-Dependent Aldolases. *Food Biosci.* 2021, 44, 101377. DOI: 10.1016/j.fbio.2021.101377.
2. Zhang, Y.; Xu, M.; Porkelsson, G.; Aðalbjörnsson, B. V. Comparative Monosaccharide Profiling for Taxon Differentiation: An Example of Icelandic Edible Seaweeds. *Biochem. Syst. Ecol.* 2022, 104, 104485.
3. Mijailovic, N.; Nesler, A.; Perazzolli, M.; Aït Barka, E.; Aziz, A. Rare Sugars: Recent Advances and Their Potential Role in Sustainable Crop Protection. *Molecules.* 2021, 26(6), 1720.
4. Dörschmann, P.; Mikkelsen, M. D.; Thi, T. N.; Roeder, J.; Meyer, A. S.; Klettner, A. Effects of a Newly Developed Enzyme-Assisted Extraction Method on the Biological Activities of Fucoidans in Ocular Cells. *Mar. Drugs.* 2020, 18(6), 282. DOI: 10.3390/md18060282.
5. Ale, M. T.; Mikkelsen, J. D.; Meyer, A. S. Important Determinants for Fucoidan Bioactivity: A Critical Review of Structure-Function Relations and Extraction Methods for Fucose-Containing Sulfated Polysaccharides from Brown Seaweeds. *Mar. Drugs.* 2011, 9(10), 2106–2130. DOI: 10.3390/md9102106.
6. Li, Z.; Gao, Y.; Nakanishi, H.; Gao, X.; Cai, L. Biosynthesis of Rare Hexoses Using Microorganisms and Related

- Enzymes. *Beilstein J. Org. Chem.* 2013, 9(1), 2434–2445. DOI: 10.3762/bjoc.9.281.
7. Liu, X.; Liu, B.; Wei, X. L.; Sun, Z. L.; Wang, C. Y. Extraction, Fractionation, and Chemical Characterisation of Fucoidans from the Brown Seaweed *Sargassum pallidum*. *Czech J. Food Sci.* 2016, 34(5), 406–413. DOI: 10.17221/322/2015-CJFS.
 8. Yuan, Y.; Macquarrie, D. Microwave Assisted Extraction of Sulfated Polysaccharides (Fucoidan) from *Ascophyllum nodosum* and Its Antioxidant Activity. *Carbohydr. Polym.* 2015, 129, 101–107. DOI: 10.1016/j.carbpol.2015.04.057.
 9. García-Vaquero, M.; Rajauria, G.; O'Doherty, J. V.; Sweeney, T. Polysaccharides from Macroalgae: Recent Advances, Innovative Technologies and Challenges in Extraction and Purification. *Food Res. Int.* 2017, 99, 1011–1020. DOI: 10.1016/j.foodres.2016.11.016.
 10. Li, C.; Wang, C.; Wang, S.; Qian, G.; Zhu, Q.; Liu, Y.; Wang, W. Optimization of Ultrasonic-Assisted Extraction Technology of *Sargassum fusiforme* Polysaccharides and Evaluation of Their Antioxidant Activity. *Food Sci. Technol. Res.* 2013, 19(2), 157–162. DOI: 10.3136/fstr.19.157.
 11. Rafiquzzaman, S. M.; Rahman, M. A.; Kong, I. S. Ultrasonic-Assisted Extraction of Carrageenan. In *Seaweed Polysaccharides* Jayachandran Venkatesan Sukumaran Anil Se-Kwon Kim; Amsterdam, The Netherlands: Academic Press, Elsevier, 2017; pp 75–81.
 12. Li, X.; Tan, S.; Luo, J.; Pinelo, M. Nanofiltration for Separation and Purification of Saccharides from Biomass. *Front Chem. Sci. Eng.* 2021, 15(4), 837–853. DOI: 10.1007/s11705-020-2020-z.
 13. Jung, J.; Yeom, S. J.; Kim, J.; Kim, J. K.; Natarajan, S.; Ahn, Y. J.; Kang, L. W. Overexpression, Crystallization and Preliminary X-Ray Crystallographic Analysis of D-Ribose-5-Phosphate Isomerase from *Clostridium thermocellum*. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* 2009, 65(11), 1141–1144.
 14. Maneein, S.; Milledge, J. J.; Nielsen, B. V.; Harvey, P. J. A Review of Seaweed Pre-Treatment Methods for Enhanced Biofuel Production by Anaerobic Digestion or Fermentation. *Fermentation.* 2018, 4(4), 100.
 15. Xia, Y.; Cheng, Q.; Mu, W.; Hu, X.; Sun, Z.; Qiu, Y.; Wang, Z. Research Advances of D-Allulose: An Overview of Physiological Functions, Enzymatic Biotransformation Technologies, and Production Processes. *Foods.* 2021, 10(9), 2186.
 16. Beerens, K.; Desmet, T.; Soetaert, W. Enzymes for the Biocatalytic Production of Rare Sugars. *J. Indus. Microbiol. Biotechnol.* 2012, 39(6), 823–834. DOI: 10.1007/s10295-012-1089-x.
 17. Olsson, J.; Toth, G. B.; Albers, E. Biochemical Composition of Red, Green and Brown Seaweeds on the Swedish West Coast. *J. Appl. Phycol.* 2020, 32(5), 3305–3317. DOI: 10.1007/s10811-020-02145-w.
 18. Jaulneau, V.; Lafitte, C.; Jacquet, C.; Fournier, S.; Salamagne, S.; Briand, X.; . . . Dumas, B. Ulvan, a Sulfated Polysaccharide from Green Algae, Activates Plant Immunity Through the Jasmonic Acid Signaling Pathway. *Biomed Res. Int.* 2010, 2010, 525291.
 19. Li, B.; Lu, F.; Wei, X.; Zhao, R. Fucoidan: Structure and Bioactivity. *Molecules.* 2008, 13(8), 1671–1695. DOI: 10.3390/molecules13081671.

20. Athukorala, Y.; Lee, K. W.; Kim, S. K.; Jeon, Y. J. Anticoagulant Activity of Marine Green and Brown Algae Collected from Jeju Island in Korea. *Bioresour. Technol.* 2007, 98(9), 1711–1716. DOI: 10.1016/j.biortech.2006.07.034.
21. Costa, L. S.; Fidelis, G. P.; Cordeiro, S. L.; Oliveira, R. M.; Sabry, D. A.; Câmara, RBG.; Rocha, HAO.; Costa, MSSP.; Almeida-Lima, J.; Farias, E. H. C. Biological Activities of Sulfated Polysaccharides from Tropical Seaweeds. *Biomed. Pharmacother.* 2010, 64(1), 21–28. DOI: 10.1016/j.biopha.2009.03.005.
22. Damonte, E.; Neyts, J.; Pujol, C. A.; Snoeck, R.; Andrei, G.; Ikeda, S.; Witvrouw, M.; Reymen, D.; Haines, H.; Matulewicz, M. C. Antiviral Activity of a Sulphated Polysaccharide from the Red Seaweed *Nothogenia fastigiata*. *Biochem. Pharmacol.* 1994, 47(12), 2187–2192. DOI: 10.1016/0006-2952(94)90254-2.