Optimized cascade extraction of bioactive compounds from brown seaweed via advanced extraction techniques

Garcia Peran Roberto, Sweygers Nick, Appels Lise and Dewil Raf

Chemical and Biochemical Reactor Engineering and Safety (CREaS), Process and Environmental Technology lab, Sint Katalijne-Waver, Belgium, KU Leuven, Jan Pieter de Nayerlaan 5 , 2860 Sint-Katelijne-Waver, Belgium
E-mail: roberto.garciaperan@kuleuven.be

Brown seaweed is rich in bioactive compounds such as **alginate**, **fucoidan**, **laminarin**, **polyphenols**, and **pigments**, which exhibit **antitumor**, **antiviral**, and **antioxidant properties** [1]. As such, it is a promising resource for these compounds since it does not require land or fertilizer to grow [2]. The focus of this study is **to optimize the extraction** of these compounds via **microwave** and **ultrasound** combined with a novel **biphasic liquid matrix**. *Ascophylum nodosum* (a brown seaweed species) is chosen because of its high polysaccharide content (approximately 50% wt.). Especially the sulfonated polysaccharide fucoidan is more prevalent in this species than in other species.

A **cascade extraction process** has been developed for the selective extraction of multiple components. The first step involves a novel biphasic system combined with **ultrasound** or **microwave** treatment. This biphasic system uses **MIBK** (an organic solvent immiscible in water) and an aqueous **citric acid** solution. Polyphenols, pigments, and fatty acids are extracted in the organic phase, while fucoidan dissolves in the aqueous phase. Alginate remains in the solid phase. To extract alginate, the solid phase is added to a bicarbonate solution to produce soluble sodium alginate. This solution is then mixed with ethanol to precipitate crude alginate. Similarly, ethanol is added to the aqueous phase to precipitate fucoidan. At the end of the cascade process, the following products are obtained: **pigments**, **polyphenols**, and **fatty acids dissolved in the organic phase; crude dry fucoidan**; and **crude dry alginate**. Further separation of the pigments, polyphenols, and fatty acids in the organic phase is possible with liquid-liquid separation; however, this is not the focus of this study.

By combining the biphasic extraction matrix with ultrasound and microwave, the extraction yield and selectivity are greater than those of traditional extraction methods (heating and stirring), and the number of steps necessary is lower than that of traditional methods, in which pretreatment with organic solvents is necessary to remove polyphenols and pigments, which are usually discarded [3, 4].

The experimental design used was a **Box-Behnken design** in which three factors were studied: **temperature**, **time**, and the **concentration of citric acid** in the aqueous solutions at three levels. The responses studied were the yield of **crude alginate and fucoidan**, the content of **polyphenols** in the organic phase (as gallic acid and phloroglucinol equivalents), and the content of **fucoxanthin** (brown seaweed pigment) in the organic phase. Other responses measured were the **nitrogen content** in all the products obtained and the **heavy metal content** in the aqueous solution (to elucidate if heavy metals are dissolved in the aqueous solution after extraction). Two sets of experiments were performed, one using microwave-assisted extraction and the other using ultrasound-assisted extraction, allowing for the comparison between advanced extraction techniques and for the study of how the chosen factors affect the responses under the different advanced extraction techniques. Furthermore, conventional extraction was also studied using the optimal points derived from the optimization experiments to quantify the improvement in yield and selectivity when advanced extraction techniques were used.

[1] S. L. Holdt, S. Kraan, *J. Appl. Phycol.* **2011**, *23* (*3*), 543–597. DOI: https://doi.org/10.1007/s10811-010-9632-5.
[2] G. Ó. Hreggviðsson, E. M. Nordberg-Karlsson, A. Tøndervik, F. L. Aachmann, J. M. Dobruchowska, J. Linares-Pastén, M. Daugbjerg-Christensen, A. Moenaert, T. Kristjansdottir, H. Sletta, *et al.*, in *Sustain. Seaweed Technol.* (Eds: M. D. Torres, S. Kraan, H. Dominguez), Elsevier **2020**.
[3] N. Flórez-Fernández, H. Domínguez, M. D. Torres, *Int. J. Biol. Macromol.* **2019**, *124*, 451–459. DOI: https://doi.org/10.1016/j.ijbiomac.2018.11.232.
[4] P. Digala, M. Saravanan, M. Dhanraj, J. Pamarthi, S. Muralidharan, A. Narikimelli, K. P. Dinakaran, S. Arokiyaraj, S. Vincent, *South Afr. J. Bot.* **2022**, *151*, 345–359. DOI: https://doi.org/10.1016/j.sajb.2022.03.015.

Keywords
Seaweed; Extraction; Ultrasounds; Microwaves; Cascade; Alginate ; Fucoidan; Laminarin; Polyphenols; Proteins