## Ultrasound assisted and ultrasound assisted enzymatic extraction of seaweed valuable compounds: potential, challenges and guidelines

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Seaweeds are important sources of polyphenols, polyunsaturated fatty acids, proteins, vitamins and polysaccharides which grant them applications in various industries, including food, cosmetics, pharmaceuticals, and agriculture (Jung *et al.*, 2013). Seaweed is a very complex biomass. Its composition is highly variable between species, season harvesting time and location. This poses a challenge to its efficient utilization, specifically to the efficient and selective extraction of its components (Kraan, 2012). The current traditional extraction techniques are time consuming and rely on high temperature and high amounts of organic solvents (Garcia-Vaquero *et al.*, 2017). Hence, developing novel greener and more efficient extraction methods is necessary to boost the development of this growing industry.

Among the non-conventional extraction methods that have been investigated for biomass treatment, enzymatic and ultrasound techniques gain attention for their various advantages, namely high efficiency, shorter and easier operation, sustainability and scalability potential (Terme et al., 2020). Lately, ultrasound assisted enzymatic extraction (UAEE), which combines both enzymatic and ultrasound techniques, receives more and more attention from researchers for the treatment of biomass (Le Guillard et al., 2016; Yachmenev et al., 2009). This technique involves the use of carbohydrases, proteases and ultrasound waves to break down or puncture the seaweed cell wall and allow the release of the compounds of interest. This process is characterised by a great variability given by the high number of factors affecting the ultrasonic and enzymatic process, including wave frequency (kHz), amplitude ( $\mu$ m), power (W), sonotrode tip area (cm<sup>2</sup>), treated mass (kg), treatment time (s), pH, solid-liquid ratio and the application of batch or continuous processes (Córdova et al., 2022). These variables, together with the ultrasound generator brand and model, are often mistakenly omitted or unclearly reported in many publications, hindering proper reproducibility of the process, of the results and hence, of their comparison. Therefore, this study is focused on drawing guidelines and fair principles for designing and reporting experiments and processes involving ultrasound and ultrasound-assisted enzymatic treatment of biomass, focusing on seaweed. Two experimental - ultrasound specific - parameters have been put forward (i.e., ultrasonic intensity (W/ cm<sup>2</sup>) and energy input (W·s/g)) to enable the comparison between different processes and subsequently identify the most promising ones. Based on the results of this work, further experimental studies will be designed and conducted to develop ultrasound assisted enzymatic extraction methods of valuable compounds from seaweed.

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## Keywords

Seaweed; Sustainability; Extraction; Ultrasound; Enzymes; Guidelines