Subcritical water treatment of the industrial solid residue generated from *Gelidium sesquipedale* after agar extraction.

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1. Introduction

Phycocolloids are the main commercial seaweed extracts; however, during seaweed processing, around 70–85% of the total dry mass is rejected as waste. This is the case of *Gelidium sesquipedale* (Rhodophyta Gelidiaceae), the major seaweed resource in the Spanish agar industry; its industrial extraction process generates a solid residue that, although is used in part for fodder and fertilizer, most of it is usually disposed of. However, this solid still contains valuable compounds (an important fraction of polysaccharides, polyphenols, a high protein content with all the essential amino acids, etc.) that can be recovered reducing the environmental impact of the residue by incorporating it within the circular economy.

Previous studies on the valorization of the solid waste generated in the industrial production of agar have analyzed its potential energetic uses by pyrolysis or as precursor of activated carbon. In this work, a different approach, based on the use of subcritical water to hydrolyze and fractionate the different biomass components, has been considered to valorize this solid waste. Also, enzymatic assisted extraction (EAE) has been studied for comparison with the subcritical water treatment (SWT). Finally, fractionation and concentration of the subcritical water extracts by ultrafiltration has been studied with the purpose of obtaining isolated compounds or high-purity concentrates of bioactive compounds.

2. Materials and Methods

The raw material used in this work consisted of the *Gelidium sesquipedale* byproduct (GSB) generated after agar extraction. It has been kindly provided by Hispanagar (Burgos, Spain). GSB was characterized according to the NREL protocols. Elemental and inorganic composition and amino acid profiles were also determined. SWT was performed in a laboratory scale semi-continuous apparatus. EAE was performed by using different hydrolytic enzymes (cellulase and protease). Multichannel Filtanium ceramic membranes with an active layer of TiO2 supported on titanium with a molecular weight cut-off (MWCO) from 1 to 100 kDa were used for extracts cross-flow sequential ultrafiltration. The SWT and EAE extracts and the UF fractions were characterized by determining some of the following parameters: carbohydrates profile, peptide content, hydrolysis degree, free amino acid profile, total phenolic compounds (TPC) and reducing capacity (FRAP). The solid residues after SWT and EAE were also subjected to elemental analysis and their high heating value was calculated.

3. Results and discussion

The extraction/hydrolysis taking place during SWT favors the recovery of several bioactive compounds from GSB, providing high yields. SWT showed to be highly influenced by temperature, heating rate and residence time[1]. Reaching the desired operating temperature in the shortest time is a challenge that in the semi-continuous system used in this work has been achieved by installing a bypass to avoid the exposure of the biomass in the reactor to high temperatures during the heating procedure[2]. By working at low residence times, higher flow rates led to higher hydrolysis rates due to an enhanced diffusion of the hydrolyzed bio-compounds from the biomass into the bulk solution. Release of small molecules, such as amino acids, showed a lower dependence on increasing flow rate due to higher diffusion coefficients. It was shown no dependence on residence time for the final hydrolysis yield[1] but higher extraction yields were achieved by increasing working temperature. SWT led to an efficient extraction/hydrolysis of the protein fraction of GBP. The best experimental conditions in a semi-continuous fix-bed reactor were 200 °C and 6 mL/min with nearly 70% of the solubilized protein

content. The highest content of individual amino acids was obtained for small amino acids such as valine, alanine and glycine as well as aspartic acid and an increase in the non-polar selectivity was observed by working at high severity factors. Figure 1 shows the accumulative curve of TPC as determined by the Folin Ciocalteu assay. SWT results show that at 2 mL/min, TPC release increased by increasing temperature. Higher flow rate, 6 mL/min, led to faster release of TPC, at the operating temperature of 200 °C, but similar TPC was obtained after 240 min, 25.9-26.5 mg GAE/g dry GSB. Positive and strong correlation was obtained between the phenolics recovered and the reducing capacity of the GSB extracts. The solid residue after SWT presented lower H, N and O content, but higher ash content due to non-solubilization of this fraction, being possible its application in agriculture as fertilizers[2].

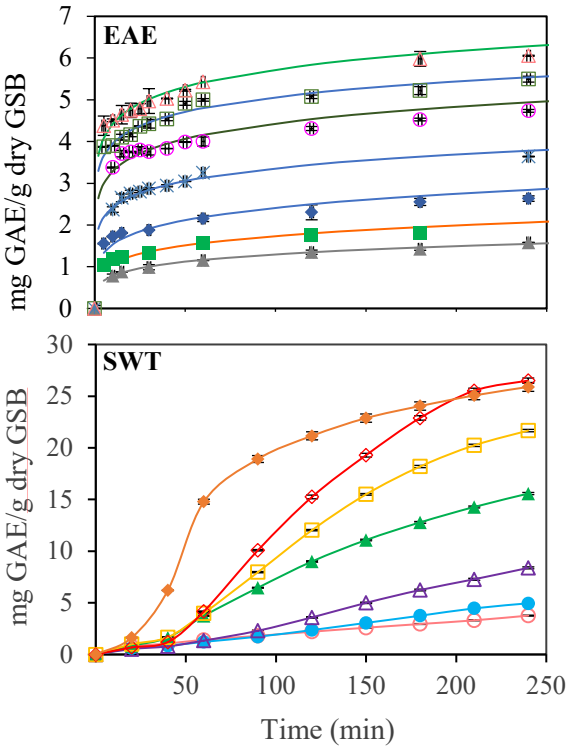

Figure 1. **EAE**, % cellulase: ▲ 0.25, ■ 0.5, ♦ 1, ∗ 2, ○ 4, □ 6, △ 8 (50 °C, pH = 5). **SWT**: F = 2 mL/min: ○ 129 °C, ● 142 °C, △ 155 °C, ▲ 171 °C, □ 185 °C, ◇ 200 °C. F = 6 mL/min: ◆ 200 °C

EAE has also been proven to be an efficient technology to valorize the GSB[3]. The use of hydrolytic enzymes allowed the release into the reaction medium of phenolic compounds bound to the cell wall of the macroalgae, the polysaccharide fraction as monomers and oligomers and the hydrolysis of the protein fraction with a higher content of hydrophobic amino acids compared to the raw material. Cellulase has been found to provide the highest hydrolytic capacity to break down the cell wall algae among the enzymes essayed.

The most influential parameter in the UF fractionation of the extracts obtained by SWT was the MWCO of the membrane. UF of the hydrolysate led to a high permeate flux reduction due to fouling. Due to the small peptides generated during SWT, selectivity of the membranes was higher for the peptide fraction than for the oligosaccharide fraction, obtaining a permeate with a high concentration of peptides.

4. Conclusions

The results obtained show that GSB constitutes a source of valuable bioactive compounds such as carbohydrates, proteins with all essential amino acids and high antioxidant activity. SWT and EAE have been proven to be green technologies capable of recovering such bioactive compounds with high yields, although EAE required longer times and provided lower yields in comparison with SWT. Extraction/hydrolysis by SWT showed to be highly influenced by temperature, heating rate and residence time and the main parameters influencing EAE were type and amount of enzyme. UF with tubular inorganic membranes has been proven to be a suitable separation technology for fractionating the extracts. Further research on the functional properties of concentrated and isolated biocompounds is needed in order to study their possible applications to be reincorporated into industrial processes.

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