Microalgae Biorefineries and Sustainability: The Role of Compressed Fluids

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ABSTRACT

New strategies of integrated green processes to obtain valuable products from different microalgae (*Isochrysis galbana*, *Scenedesmus obliquus*, and *Nannochloropsis gaditana*) using compressed fluids are described in the present work. In fact, a compressed fluids processing platform is suggested based on the use of carbon dioxide at medium-high pressures to modify the chemical and/or physical properties of the solvents. In this sense, innovative extraction technologies such as Pressurized Liquid Extraction, Gas-Expanded Liquids, Supercritical Fluid Extraction with modifier and Supercritical Fluid Extraction using neat carbon dioxide can be seen as easily interchangeable sustainable technologies in which CO_2 is employed to tune solvent characteristics related to viscosity, diffusivity and polarity.

INTRODUCTION

At present there is an enormous interest in providing new answers to one of the main societal challenges: sustainability. Therefore, strategies directed towards a reasonable use of resources, increasing the production and minimizing the environmental impact are of high interest. Embedded in this idea, the modern concept of biorefinery is receiving attention since it involves from biomass conversion processes to equipment to produce fuel, power, and added-value chemicals from organic material. Considering this framework, the extraction of high added-value products from different natural sources is of high interest since it can allow consolidating the idea of sustainable processes.
Nevertheless, for the development of these sustainable processes the 12 principles of Green Chemistry have to be closely examined, considering that effectively provide a framework for designing and/or improving materials, products, processes and systems from an environment protection perspective. New challenges researchers are facing are the development of fast, selective, efficient, sustainable, green (without using toxic organic solvents) processes, providing also with high yields and at lower costs. Processes able to meet these requirements are, among others, those based on the use of compressed fluids such as supercritical fluid extraction (SFE), gas-expanded liquids extraction (GXLs), pressurized liquid extraction (PLE) and subcritical water extraction (SWE) [1]. These processes can be integrated and intensified while designing sustainable natural

products extraction processes. In any case, both alternatives allow reducing energy consumption, cost as well as waste and by-products. Main differences account for the efficient coupling of different unit operations involving the best conditions for each one (process integration) or performing different unit operations within the same equipment (process intensification) [2]. Thus, the development of a compressed fluids processing platform able to move from extraction of non-polar/medium polarity bioactives by SFE (with neat CO_2 or CO_2 + small amount of modifier) to the extraction of polar compounds by GXLs or PLE (using green polar solvents) can be of high interest. These technologies can be seen as easily interchangeable in which CO_2 is employed to tune solvent characteristics related to viscosity, diffusivity and polarity [3].

Among marine sources, microalgae are one of the most promising feedstocks for sustainable supply of commodities for both food and non-food products; they use light energy and carbon dioxide with higher photosynthetic efficiency than plants for the production of biomass; moreover, they do not need to be grown on arable land thus alleviating food versus fuel conflicts. Microalgae can thus become an excellent source for oils, proteins, polysaccharides, carotenoids, pigments, antioxidants, lipids, etc. [4, 5]. The algae-based biorefinery concept relies on the complete process optimization from biomass production to generation of different products through the development of a platform able to offer a multitude of different products, from bulk chemicals, food supply (proteins, fibre), bioactives to be used for food ingredients and oil for biofuel.

In the present work, some examples developed under the framework of the EU Project MIRACLES, and using the compressed fluids processing platform, are presented for multi-product biorefinery of different microalgae strains (*Isochrysis galbana*, *Scenedesmus obliquus* and *Nannochloropsis gaditana*).

MATERIALS AND METHODS

Samples

Freeze-dried samples of *Isochrysis galbana*, *Scenedesmus obliquus* and *Nannochloropsis gaditana* and frozen samples of *Isochrysis galbana* (wet biomass) were obtained from Fitoplancton Marino S.A. (Cadiz, Spain), each of them grown under standard conditions of the Company.

Compressed fluids processing platform

Extractions were carried out in a “Spe-ed Helix” supercritical fluid extractor from Applied Separations (Allentown, PA, USA). This equipment can be used to perform SFE (with or without a co-solvent), GXLs extraction and PLE. Depending on the sample to be extracted, different sequential steps were performed in increasing (ScCO2 < CXE < PLE with ethanol < PLE with water) or decreasing order of polarity (pure water (PLE) > $ScCO_2$/ ethanol (GXL) > supercritical CO_2 (ScCO2)), to exhaust the microalgae biomass of extractable compounds, fractionating its components in order to give valuable isolated fractions.

Total carotenoids, chlorophylls, lipids, sugars, proteins and antioxidant activity

Methodologies employed for analyzing the different fractions obtained have been described elsewhere [6]. Recoveries were determined by measuring the initial content in the microalgae biomass and were expressed as %.

RESULTS

Different unit operations based on the use of compressed fluids have been optimized and combined in order to develop different biorefinery strategies for valorization of the different microalgae biomass.

Isochrysis galbana dry and wet biomass- Process comparison

A full sequential procedure (4-steps) was set up for *Isochrysis galbana* dry biomass, in which each step used the residue of the previous extraction. The steps of the process were (1) SFE using ScCO2, (2) Gas Expanded Liquids (GXL) using a mixture ScCO2:EtOH, (3) PLE using EtOH, and (4) PLE using water [6]. A summary of the results obtained are shown in Figure 1, in which % of recovery (% of extracted material compared to the total amount present in the initial biomass, left) and composition of the extracts (right) can be observed.

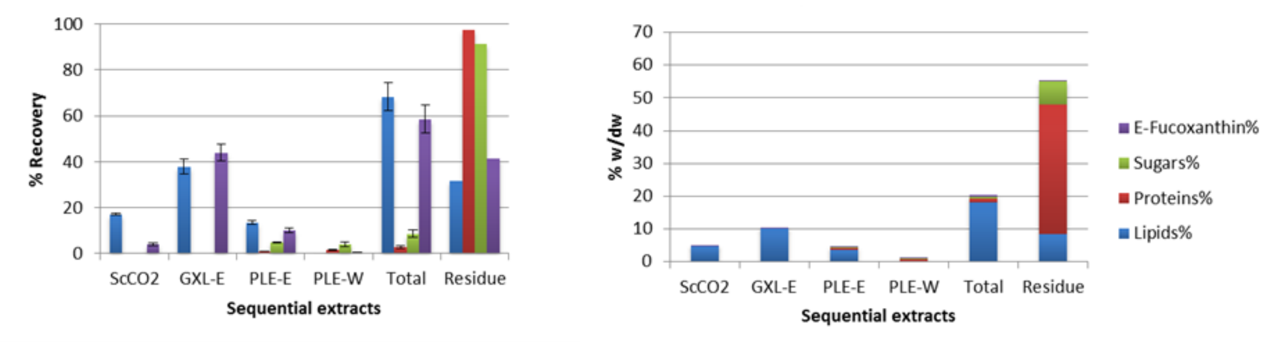

Figure 1. Percentage recovery and composition (% weight extract/dry weight algae) obtained considering the 4 step sequential process for *I. galbana* dry biomass.

Considering the similarities between the extracts obtained in steps 2 and 3 of the process, step 3 was suppressed and step 2 was re-optimized. This way, a three-step re-optimized process was performed to obtain sequential extracts. The starting amount of biomass was 100 g and the extraction conditions were: (1) $ScCO_2$, 300 bar, 50 ˚C, 60 min; (2) $ScCO_2$ + 50% EtOH, 70 bar, 50 ˚C, 120 min; (3) Water, 100 bar, 80 ˚C, 30 min.

After succeeding in the downstream processing of freeze-dried *I. galbana*, starting from non-polar and ending with polar solvents, a green platform in the reverse order of polarity was developed. This reverse process involves some advantages regarding energy saving and reducing costs, as the freeze-drying step can be avoided and algae biomass can be directly used after harvesting. Because of logistic reasons, the biomass was used frozen. The extraction procedure is described in Figure 2, while a summary of the results obtained for the reverse process are shown in Figure 3, including % of recovery (left) and composition of the extracts (right) [7].

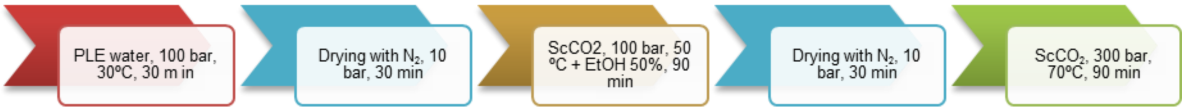

Figure 2. Reverse extraction process for *I. galbana* wet biomass.

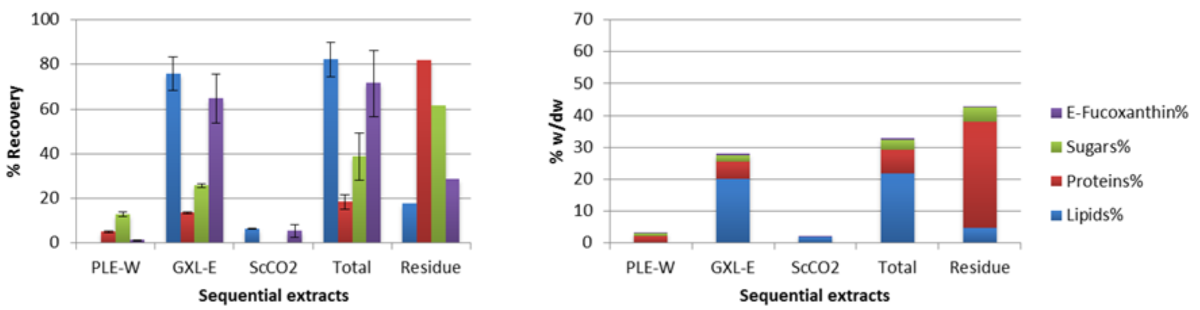

Figure 3. Percentage recovery and composition (% weight extract/dry weight algae) obtained considering the 3 step sequential process for *I. galbana* wet biomass (reverse process).

By comparing the results obtained by both processes using dry (Figure 1) and wet (Figure 3) biomass, it can be seen that the recovery of lipids and fucoxanthin is higher in the reverse process (starting with wet biomass), reaching up to 80% and 70%, respectively. Some valuable sugars and proteins are obtained in the first fraction (Figures 2 and 3) although most of them remain in the residue and therefore, can be submitted to other sequential processes for their fractionation.

<u>*Scenedesmus obliquus* dry biomass</u>

The development of a sequential process for *S. obliquus* started with the evaluation of different treatments for cell-wall disruption (cryogenic grinding, basic hydrolysis with NaOH 0.1M and freeze-thaw cycles), which did not improve the results obtained by PLE of the untreated biomass. On the other hand, high-pressure homogenization (HPH) was evaluated for cell disruption, which provides much better yields in the different steps of the process. Thus, a sequential green process was developed using HPH freeze-dried algae biomass. Three pressurized steps were established, using solvents in increasing order or polarity, as: (1) $ScCO_2$, 360 bar, 50 ˚C, 120 min; (2) $ScCO_2$ + 75% EtOH, 70 bar, 50 ˚C, 150 min; (3) Water, 100 bar, 50 ˚C, 45 min. The recoveries (% of extracted material compared to the total amount present in the initial biomass) of the studied compounds in each of the steps of the process are shown in Figure 4.

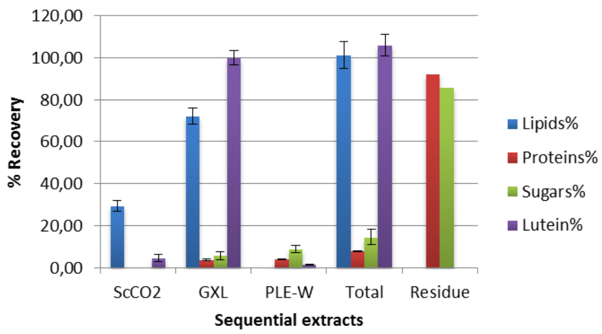

Figure 4. Percentage recovery obtained considering the 3 step sequential process for *S. obliquus* dry biomass.

Lutein and β-carotene were the main carotenoids identified by HPLC in this species, of which the first one is more valuable. Results showed almost 100% extraction of lipids of different polarity: non-polar (first step, around 30%) and polar (second step, 70%). Lutein was also mainly extracted under GXL conditions. Residues contained, almost exclusively, sugars and proteins [8].

Nannochloropsis gaditama dry biomass

A first step using SC-CO_2 as extracting solvent at 400 bar and 55 °C was performed, according to the literature [9]. HPLC analysis of the extract revealed 8% recovery of the carotenoid violaxanthin and the presence of non-polar (triacylglycerols) and mildly polar (partial glycerides and free fatty acids).
After SFE using SC-CO_2, an experimental design (3-level factorial design (3^2)) was carried out, using the residual biomass, using PLE and considering as factors: Extraction solvent (Ethanol/water from 0 to 100% ethanol) and Temperature (from 40 to 170 °C) and as response variables: Yield, Total phenolic content, antioxidant activity (TEAC), total carotenoids and total lipids. The experimental design was performed on *N. gaditana* treated at high pressure (1200 bar) and subsequently freeze-dried. The optimum extraction conditions according to the experimental design were the use of ethanol (100%) at 170 °C. Three replicate extractions in optimum conditions were performed and the results were comparable to those predicted by the mathematical model. In summary, extracts obtained under optimum conditions showed certain antioxidant activity (0.72 mmol trolox eq g-1 extract) and contained around 8.3 mg carotenoids g^{-1} extract, and 70% of polar lipids. GC-MS analysis revealed that palmitoleic, palmitic, myristic and the PUFA eicosapentanoic (EPA) acids were the predominant fatty acids.

CONCLUSION

In the present work, different examples concerning biorefinery of dry microalgae (*Isochrysis galbana*, *Scenedesmus obliquus*, and *Nannochloropsis gaditana*) biomass and wet *I. galbana* biomass are presented. In these examples, the use of a compressed fluids processing platform is suggested based on the use of carbon dioxide at medium-high pressures to tune solvent characteristics related to viscosity, diffusivity and polarity. Different unit operations have been developed based on innovative extraction technologies such as Pressurized Liquid Extraction, Gas-Expanded Liquids, Supercritical Fluid Extraction with modifier and Supercritical Fluid Extraction using neat carbon dioxide and later optimized for a sequential extraction able to extract valuable components from microalgae biomass. Advantages of these processes are related to enhancement of mass transfer and improvement of energy and environmental costs, among others.

ACKNOWLEDGEMENTS

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