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Green extraction of microalgae components for incorporation in food and feed supplements

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Microalgae have been long recognized as potential food and feed solution, since they are able to meet the population growth on demand for a more sustainable food and feed, especially with respect to protein production. In addition, bioactive components, such as eicosapentaenoic acid (EPA), with well-known antioxidant and anti-inflammatory properties, can be extracted and incorporated in food supplements. Microalgae cultivation and processing becomes even more sustainable if simultaneously coupled to green technologies for the environmental protection. One of the most promising techniques is the supercritical fluid extraction which allows to extract bioactive compounds without loss of their activity and free from extraction solvents. In this work, a pilot scale supercritical CO_2 ($ScCO_2$) extraction plant was exploited for performing the extraction of active components from microalgae biomass potentially usable in the food and the feed sectors. *Nannochloropsis gaditana* microalga was selected as natural producer of EPA. The extract obtained after $ScCO_2$ tests was enriched of EPA and protein, therefore suitable for food applications. The exhausted biomass, having a high content of carbohydrates and total dietary fiber, might be proposed as feed supplement

1. Introduction

The numerical growth of the world population and the increasing depletion of resources has led to a search for new sources of food. Microalgae have been very attractive for their biological characteristics and chemical composition as potential source for food and feed (Milldege et al., 2012). Several microalgae species were identified for the capability to produce protein (*Artrhospira platensis* and *Chlorella sp.*), carbohydrates, lipids (*Nannochlorpsis* sp., *Crypthecodinium cohnii, Chlamydomonas reinhardtii*) in particular poly-unsaturated fatty acids (PUFAs). The chemical composition of several microalgae species as *Arthrospira platensis*, *Chlorella sp.*, *Nannochlorpsis oculata*, *Nannochloropsis gaditana*, was deeply investigated highlighting an interesting content of protein (40-50% dry weight), carbohydrates (~20%) and lipids (10-20%), total dietary fibers (5-15%) (Matos et al., 2016). Some of these species (*Arthrospira platensis*, *Chlorella vulgaris*, *Chlamydomonas* *reinhardtii*, *Auxenochlorella protothecoides*, *Dunaliella bardawil*, and *Euglena gracilis* were currently recognized as safe (GRAS) for the application as food source (Torres-Tiji et al., 2020). Other species were studied for the production of high-value compounds as carotenoids, beta-carotene produced by *Dunaliella salina*, astaxanthin produced by the red cysts of *Haematococcus pluvialis* and lutein produced by *Scenedesmus almeriensis* that can be constituted around 1-2% dry weight of the total cellular content (Molino et al., 2019a).

In addition, bioactive components, such as eicosapentaenoic acid (EPA), with well-known antioxidant, anti-inflammatory properties and preventive properties against cardiovascular diseases, can be extracted from *Nannochlorpsis gaditana* and incorporated in food supplements for nutraceutical applications (Molino et al., 2020a). The production of eicosapentaenoic acid from microalgae have more advantages than fish-source but the cost production is currently not advantageous. The exploitation of green technologies is a solution for the more sustainable and advantageous development of cultivation and processing of microalgae. Supercritical fluid extraction using carbon dioxide (CO_2) is a promising unconventional green extraction technology for the production of high-value compounds from microalgae (Mezzomo and Ferreira 2016, Catchpole et al., 2018).

Eicosapentaenoic acid was extracted by *Nannochlorpsis gaditana* using carbon dioxide supercritical fluid extraction as reported in previous works (Li et al., 2019, Molino et al., 2020b). Andrich et al., 2005 investigated carbon dioxide supercritical fluid extraction of fatty acids from *Nannochloropsis sp.* obtaining an extract with an EPA content equal to around 30% of total fatty acids.

In this work, a pilot scale supercritical CO_2 ($ScCO_2$) extraction plant was exploited within the bio-refinery approach of VALUMEAG plant for performing the extraction of active components from microalgae biomass potentially usable in the food and the feed sectors. *Nannochloropsis gaditana* microalga was selected as natural producer of EPA. The extract obtained after $ScCO_2$ tests was enriched of EPA and protein, therefore suitable for supplement applications as previously reported by Molino et al., 2020a. The exhausted biomass, having a high content of carbohydrates and total dietary fiber, might be proposed as feed supplement within a circular economy approach.

2. Materials and methods

The production of eicosapentaenoic acid (EPA) was investigated in VALUEMAG plant trough the cultivation of *Nannochloropsis gaditana* biomass in a magnetic photobioreactor unit till to the extraction by carbon dioxide supercritical fluid extraction (CO_2-SFE). The complete diagram of the entire process is shown in figure 1. The cultivation system was composed of a cultivation unit in the photobioreactor combined with a dewatering unit allowing water reuse. The produced biomass was dried and pre-treated in the pre-treatment unit for the following extraction step in the CO_2-SFE unit.

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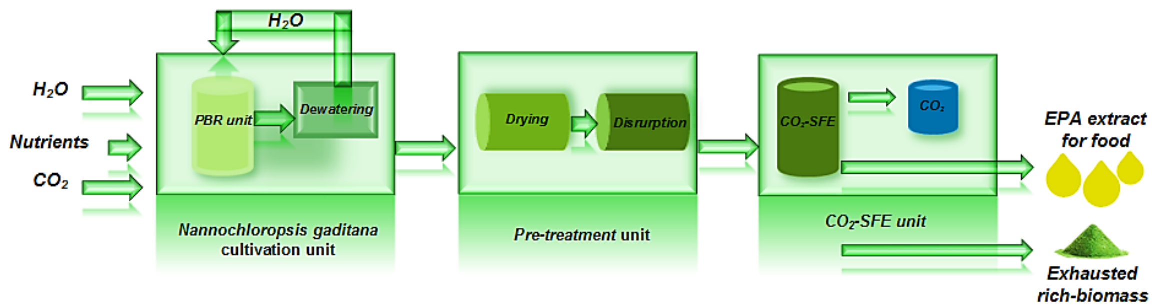

Figure 1: EPA and exhausted biomass production from Nannochloropsis gaditana in VALUEMAG plant

2.1 Cultivation system

In VALUEMAG plant, *Nannochloropsis gaditana* was cultivated in a magnetic photobioreactor developed by the National Technical University of Athens (NTUA) as project coordinator and NOMASICO LTD Company as a project partner. The magnetic photobioreactor is a conical steel structure called SOMAC (SOft Magnetic Cone) with 6 m base diameter and around 6 m height, and a working volume of 15.7 liter. The SOMAC unit is located in Cyprus in an appropriate structure for the development of the integrated system. The microalgae *Nannochloropsis gaditana* was cultivated using f-medium following the recipe reported by Guillard and Ryther 1962. In table 1 the composition and the daily quantity of each macronutrients, trace elements and vitamins are listed.

Table 1: Culture medium composition for the growth of Nannochloropsis gaditana used in the VALUEMAG plant

Macro-nutrients	Content (g/day)
NaCl	50 ÷ 10
$NaNO_3$	7.5 ÷ 11.25
NaH_2PO_4*H2O	0.50 ÷ 0.75
Na_2SiO_3*9H_2O	3.0÷4.5
Trace elements	**Content (g/day)**
Na_2*EDTA	0.44 ÷ 0.65
$FeCl_3$*6H_2O	0.31 ÷ 0.47
$CuSO_4$*5H_2O	1 ÷ 1.5 x10^{-3}
$ZnSO_4$*7H_2O	2.2 ÷ 3.3 x10^{-3}
$CoCl_2$*6H_2O	1 ÷ 1.5 x10^{-3}
$MnCl_2$*4H_2O	18 ÷ 27 x10^{-3}
Na_2MoO_4*2H_2O	0.6 ÷ 0.9 x10^{-3}
Vitamins	**Content (g/day)**
Thiamine*HCl	0.05 ÷ 0.075 x10^{-3}
Biotine	0.05 ÷ 0.075 x10^{-3}
B12	0.05 ÷ 0.075 x10^{-3}
Total Nutrients	61.7 ÷ 27.6

The dewatering section was designed using a hollow fiber membrane in polysulfone (PS) that is contained in a cartridge 39.4 cm long and with an external diameter of 10.4 cm. The main characteristics of the membrane are a pore size of 0.2 μm and a total area equal to 2.5 m^2. The membrane surface avoids a volume – lumen side of 750 ml and a hold-up volume (shell side) of 675 ml. The dewatering unit was designed to operate once a day a maximum concentration of around 100 g/l to obtain 5-20 l/day of dewatered biomass. The quantity of water that can be reused is equal to around 80-95 l/day.

2.2 Carbon dioxide supercritical fluid extraction system

The extraction process was performed after drying and pre-treatment of the biomass. For the economic assessment, drying process was obtained using a spray dryer and pre-treatment optimized through steam explosion. SFE using CO_2 was carried out by means of a pilot scale plant. The characteristic are described in a previous work (Molino et al., 2019b). Operative CO_2-SFE conditions were optimized at bench scale as reported by Leone et al., 2019. In table 2 the optimized operative conditions that were performed using the pilot scale CO_2-SFE are reported. The extraction was performed loading in the extractor 21.2 g of pre-treated biomass. The loaded biomass had a bulk density equal to 50.9 g/L. The extraction was carried out at 350 bar, 50 °C. The CO_2 flow rate was fixed at 0.35 kg/min. The extraction was performed for 135 minutes.

Table 2: Optimum operative conditions for supercritical CO_2 extraction of Nannochloropsis gaditana

Operative conditions	Unit of measure	
Loaded biomass	g	21.2
Bulk density	g/L	50.9
Pressure	bar	350
Temperature	°C	50
CO_2 flow rate	Kg/min	0.35
Extraction time	min	135

2.3 Economic evaluation

The entire process for the production of EPA through VALUEMAG plant was economically evaluated by investigating the cost contribution of each unit. The energy demand and cost were evaluated for each facilities from cultivation to extraction unit and expressed as cost of installed power (kW), compressed air (L/s), and energy consumption (kWh/day). These preliminary costs were calculated on the basis of the experimental conditions optimized in a first instance at laboratory scale and subsequently correlated with the VALUEMAG plant (Marino et al., 2020). The obtained results took into account the contribute of supercritical CO_2 extraction by considering 1 kg/day as the biomass produced through the SOMAC plant.

3. Results and discussion

The composition of the initial biomass of *Nannochloropsis gaditana* that can be obtained after the cultivation and pre-treatment (drying and disruption) units in VALUEMAG plant is shown in figure 2. The biomass is rich of protein that represents the 47% of total cellular content. Carbohydrates and lipids are equal to 22% and 17% respectively. Total dietary fibers (TDFs) were equal to 4%.

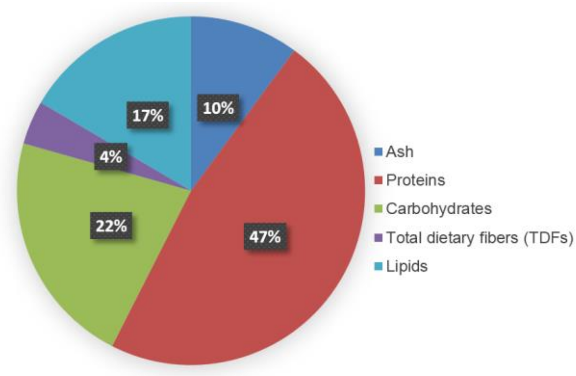

Figure 2: Nannochloropsis gaditana biomass characterization

Fatty Acids Methyl Esters (FAMEs) constituted a percentage of lipids equal to 69.84%. The percentage of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) respect to total FAMEs were 28.55%, 26.08% and 8.57% respectively. EPA constituted the highest percentage of FAMEs that was equal to 36.80%. *Nannochloropsis gaditana* biomass was extracted using $ScCO_2$ extraction following the optimized operative conditions reported in table 2. The obtained extract contained EPA that represented the 29.69% of the total lipid content. The extract was already evaluated for nutraceutical and food applications for the protein content around 78%.
After CO_2-SFE, the exhausted biomass was characterized to investigate the remained compounds content. The evaluation of exhausted biomass was explored within the application of circular economy approach. The chemical composition of exhausted biomass revealed a great content of proteins and carbohydrates equal to 42.73% and 27.67% as shown in table 3. TDFs content resulted equal to 4.96% and lipids are equal to 11.83%. In the exhausted biomass, the quantity of ashes was equal to 12.80%. The characterization of exhausted biomass revealed a great quantity of FAMEs that corresponded to 88.96% of total lipids. Furthermore, EPA was contained in exhausted biomass and corresponded to 33% of FAMEs.

Table 3: Nannochloropsis gaditana exhausted biomass characterization

Compounds	Exhausted biomass (%)
Ash	12.80
Proteins	42.73
Carbohydrates	27.67
Total dietary fibers (TDFs)	4.96
Lipids	11.83
Of wich FAMEs	88.96
FAMEs composition	
SFAs	32.20
MUFAs	27.21
EPA	33
Other PUFAs	7.59

Nannochloropsis gaditana is an interesting microalgae species for aquaculture application for the nutritional value and the EPA content. Ayala et al., 2020 demonstrated as the inclusion of *Nannochloropsis gaditana* hydrolysed biomass in the diet of the well-known Gilthead Seabream (*Sparus aurata*) can affect positively on the growth of juvenile and can accelerate accelerated the acquisition of discoid body shape. The diet was

supplied with 2.5% and 5.5% of dry biomass, fish meal and oil (15 and 10% respectively), wheat meal (around 10%), krill meal (2%) and others vitamins. The chemical composition of the experimented diets was equal to: protein 47.6%, lipids 15.9%, ashes 7.4%, fiber 3.3% and nitrogen free-extract 25.8% (Ayala et al., 2020). The composition is not far from the composition of the exhausted biomass that can be a potential additive for fish diets in aquaculture sector. Sales et al., 2021 tested the effect of *Nannochloropsis gaditana* extracts as a substitutive ingredient of fish oil in diets for Gilthead Seabream (*Sparus aurata*). The extracts were obtained after bead milling pre-treatment and extraction by ultrasonication. EPA content was recorded equal to 22% of total saponifiable lipid extract. The amendment of *N. gaditana* extracts in the diets highlights the possibility to the partial substitution of fish oil with a more sustainable ingredients that affect positively the growth and the content of EPA in muscle fatty acids composition.
In addition, the economic assessment of *Nannochloropsis gaditana* production by VALUEMAG plant was evaluated, highlighting that the highest cost were due to CO_2-SFE unit that accounted for 20.4 euro/kg dry biomass for an operational time of 1 day. The less expensive cost contribution were due to drying and pre-treatment (Table 4). Cultivation unit and dewatering contributed with a cost equal to 3.55 euro/kg dry biomass.

Table 4: Economic assessment of Nannochloropsis gaditana production within VALUEMAG plant

Contribute	euro/kg_{dry}
Nannochloropsis gaditana cultivation unit and dewatering	3.55
Drying	1.19
Pre-treatment steam explosion	0.01
CO_2-SFE	20.4
Total cost	25.12

The total cost for the production of *Nannochloropsis gaditana* within VALUEMAG plant resulted equal to 25.12 euro/kg dry weight biomass for a daily production process.

4. Conclusions

The presented work demonstrated that the chemical composition of the *Nannochloropsis gaditana* exhausted biomass remaining after CO_2-SFE in the VALUEMAG plant can be a potential source of nutrients, hence might be adapted to the circular economy needs. The protein content and the composition of FAMEs, with particular emphasis to EPA, in the exhausted biomass might be exploited in the feed sector and, in particular, in aquaculture market where sustainable feed will be necessary to reduce the impact on the fish resource. Further studied will surely be necessary to further investigate the effect of the exhausted biomass use as feed ingredient in fish diet. However, as starting point, the use of green and safe CO_2-SFE technology for obtaining in one step both the solvent-free extract and the exhausted biomass,can guarantee their safety for living beings.

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