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Microalgae as Alternative Source of Nutraceutical Polyunsaturated Fatty Acids

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The use of sustainable biobased resources is the basis of the European strategy for bioeconomy development. Microalgae represent one of the most interesting biological source, since they are relatively simple microorganisms containing high-value compounds with potential applications in numerous industrial sectors. The global nutraceutical market is well developed and with an enormous potential for growth: suffice to say that it has shown considerable growth with a value in 2016 between 168 and 174 billion euros, with a growth forecast that will allow it to reach between 285 and 313 billion euros in 2024.
In accordance with the National Center for Biotechnology Information, by 2050, microalgae will constitute ~18% of protein source among the more diverse market. Healthy properties and excellent nutritional values have been conferred to microalgae, with particular regard to the high content of carbohydrates, vitamins, carotenoids and polyunsaturated fatty acids (PUFAs). The omega-3 PUFAs are extremely important throughout life and are a dietary need found predominantly in *Nannochloropsis gaditana* oleaginous microalga. In fact, although fish contain long-chain omega-3, they acquire PUFAs mainly from their microalgal diet. Additionally, considering depleting fish supplies, the use of microalgae as alternative raw material might be considered of increasing importance in the near future. Herein we report the assessment of the economic feasibility of an integrated process which allows to extract PUFAs from *Nannochloropsis gaditana*, by taking into account the main operational parameters able to influence the final products yield.

1. Introduction

Microalgae are well known to be a precious source of bioactive compounds with healthy properties and excellent dietary values (Foley et al., 2011). In fact, these micoorganisms are characterized by a high content of nutritional substances such as proteins, carbohydrates, or compounds with important healthy properties such as vitamins, pigments and PUFAs (Foley et al., 2011). Due to the increasing awareness among consumers about beneficial properties of natural compounds and the importance of a diversified diet rich in omega-3 together with a well-balanced ratio between them and omega-6, the global omega-3 market produced sales volume of about 2.99 billion euros in 2018 and it has been estimated it will increase up to 8.91 billion euros at 2025 (www.verifiedmarketresearch.com, 2019).
The global demand of Eicosapantenoic/Docosahexaenoic acid (EPA/DHA) in 2013 was estimated to be 124 thousand tonnes, with a predicted increase up to be 241 thousand tonnes by 2020 (van der Voort et al., 2017). Market region analysis for EPA/DHA production highlights a contribution of 22% for Europe, 30% for Asia Pacific, 40% for North America and 3% for other regions (van der Voort et al., 2017).

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Algae-based EPA/DHA market value corresponds to about 18%, 79% ofwhich extracted from fish oil (mainly anchovy and sardines that, combined, represented over 75% feedstock share for oil extraction) and 3% of EPA/DHA market is covered by krill oil (van der Voort et al., 2017).However, theoil contained in selected microalgae strains, which could substitute both fish and krill EPA/DHA natural sources, manifests several advantages in terms of smell and taste together with other characteristics, such as the vegetarian origin and sustainability, thus becoming a very attracting field in the bioeconomy and sustainability context. In addition, the use of microalgae as unconventional raw material might be considered of growing importance given the near-future depletion of fish supplies. As a confirmation of this, the Food and Agriculture Organization evaluates that the 1000 tons of fish oil supplied in the last few years, will not significantly increase (van der Voort et al., 2017), hence this will confirm as alternative markets can offer enormous opportunities for the microalgae-based EPA/DHA production. EPA and DHA exhibit anti-inflammatory and healthy properties thus contributing to prevent cardiovascular diseases; they are also useful in the treatment of high blood pressure or rheumatoid arthritis (Tomic-Smiljanic et al., 2019). One of their most important ability is the reduction in fasting and post-prandial serum triglycerides; they positively alter lipoprotein particle sizeand contribute to maintain the normal brain function and adequate eye vision (Molino et al., 2019).

1.1 Regulatory issues

The use of microalgae for the supply of EPA and DHA is gaining great attention in the food and feed sectors (Molino et al., 2019; Iovine et al., 2019). However, at European level only a few species have been authorized and they are listed in the "novel foods" catalogue, according to the list of new European foods established by theCommission Implementing Regulation (EU) 2017/2470 of 20 December 2017 (www.eur-lex.europa.eu, 2019a).

The microalgal biomass/extract/metabolites authorized as novel foods fall into the category of “food products and ingredients consisting of or isolated from microorganisms, fungi or algae” as defined by Regulation (EU) 2015/2283 (www.eur-lex.europa.eu, 2019b). Hence, following the European legislation, actually EPA and DHA can be commercialized in form of oil derived from *Ulkenia* sp. and *Schizochytrium* sp. as shown in Table 1.

Table 1: Microalgae-based novel food for the production of food supplements. Adapted from Regulation (EU) 2017/2470 (www.eur-lex.europa.eu, 2019b)

Authorised Novel Foods	**Specification**	**Conditions under which the novel food may be used**	
	Description/Definition:	**Specified food category**	**Maximum levels**
Algal oil from the microalgae *Ulkenia* sp.	Oil from the micro-algae *Ulkenia* sp. Acid value: ≤ 0.5 mg KOH/g Peroxide value (PV): ≤ 5.0 meq/kg oil Moisture and volatiles: ≤ 0.05 % Unsaponifiables: ≤ 4.5 % Trans-fatty acids: ≤ 1.0 % DHA content: ≥ 32 %	Bakery products (breads, rolls and sweet biscuits)	200 mg DHA/100 g
		Cereal bars	500 mg DHA/100 g
		Non-alcoholic beverages (including milk based beverages)	60 mg DHA/100 ml
***Schizochytrium* sp. oil rich in DHA and EPA**	Acid value: ≤ 0.5 mg KOH/g Peroxide value: ≤ 5.0 meq/kg oil Oxidative stability: All food products containing *Schizochytrium* sp. oil rich in DHA and EPA should demonstrate oxidative stability by appropriate and recognised national/international test methodology (e.g. AOAC) Moisture and volatiles: ≤ 0,05 % Unsaponifiables: ≤ 4.5 % Trans-fatty acids: ≤ 1 % DHA content: ≥	Food Supplements as defined in Directive 2002/46/EC for adult population excluding pregnant and lactating women	3000 mg/day*

22.5 % EPA content: ≥ 10 %		
	Food Supplements as defined in Directive 2002/46/EC for pregnant and lactating women 450 mg/day	450 mg/day*
	Total diet replacement for weight control as defined in Regulation (EU) No 609/2013 and meal replacements for weight control 250 mg/meal	In accordance with the particular nutritional requirements of the persons for whom the products are intended*
	Milk-based drinks and similar products intended for young children	250 mg/meal*
	Processed cereal based food and baby food for infants and young children as defined in Regulation (EU) No 609/2013	200 mg/100 g*
	Foods intended to meet the expenditure of intense muscular effort, especially for sportsmen	Not specified
	Foods bearing statements on the absence or reduced presence of gluten in accordance with the requirements of Commission Implementing Regulation (EU) No 828/2014	Not specified
	Bakery Products (Breads, Rolls and Sweet Biscuits)	Not specified
	Breakfast Cereals	200 mg/100 g*
	Cooking Fats	500 mg/100 g*
	Dairy Analogues except drinks	360 mg/100 g*
	Dairy Products except milk-based drinks	600 mg/100 g for cheese; 200 mg/100 g for soy and imitation milk products (excluding drinks)*
	Non-alcoholic Beverages (including dairy analogue and milk-based drinks)	600 mg/100 g for cheese; 200 mg/100 g for milk products (including milk, fromage frais and yoghurt products; excluding drinks)*
	Cereal/Nutrition Bars	80 mg/100 g*
	Spreadable Fats and Dressings	500 mg/100 g*
		600 mg/100 g*

** Maximum levels of DHA and EPA combined*

Nannochloropsis microalga, belonging the *Eustigmatophycea estramenopile* family, is a specie characterized by the ability to accumulate high content of lipids and fatty acids (Molino et al., 2019; Iovine et al., 2019). *Nannochloropsis* is a coccoid, unicellular microalga found in fresh, brackish, and seawater sources. In particular, *Nannochloropsis gaditana* cells show the highest amount of PUFAs and could represent an extremely valuable source of EPA, that can reach values above 4.3% wt/wt; therefore, its biomass and oil extract, represent a promising novel food. Even though *Nannochloropsis gaditana* and/or its metabolites/extracts have not been authorized by European Commission to be placed on the market, an application has been initiated by a Spanish Company in 2011 and an initial assessment has been carried out by the Agencia española de seguridad alimentaria y nutrición (ES) (Applications under Regulation (EC) N° 258/97 of the European Parliament and of the Council, 2019). This work explores the feasibility of EPA production from *Nannochloropsisgaditana*by considering the extraction step as a part of a biorefinery in which every step has been developed with a view of sustainability.

2. Materials and methods

The preliminary economic evaluation of EPA extraction from the *Nannochloropsis gaditana* microalgae takes into consideration the individual contribution of each operation mentioned in Figure 1 and included in the VALUEMAG project (Valuable Products from Algae Using new Magnetic Cultivation and Extraction Techniques, Horizon 2020-Grant Agreement No 745695; www.valuemag.eu, 2019).

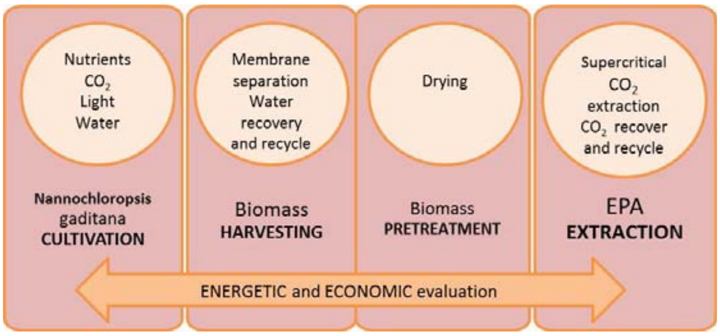

Figure 1: Process steps taken into consideration for the energetic and economic evaluation

The overall process involves microalgae cultivation into ad hoc developed reactor with a conical form (named SOMAC; Savvidou et al., 2019) which might allow to optimize the cells growth, a subsequent biomass harvesting with the concomitant water recovery and recycle. This step mainly consist in a membrane separation by means polymeric hollow fibers having the pore size in the microfiltration range (~0.2 µm) allowing to collect water in the permeate (Marino et al., 2019), and to concentrate algae biomass in the opposite side of the membrane, i.e. into the retentate. The obtained biomass should be pretreated, by a lyophilizer-based drying and ball-mill disruption, and finally used as substrate for EPA extraction. The use of supercritical CO_2as green solvent implicates a downstream gas recover and reuse for additional extraction steps. The optimization of the operational conditions for EPA production have been recently reported by Molino et al. (2019) and Iovine et al. (2019) and they have constituted the starting point for the energetic and economic preliminary assessment of the entire VALUEMAG biorefinery (Figure 1).

Table 2: Optimized operative conditions for PUFAs extraction from Nannochloropsis gaditana at bench scale Molino et al. (2019) and extract quality evaluation

Main operational condition			***Biomass***	**Extract**
Biomass loaded (g)	21.2	Ash	10.12%	2.04%
Pressure (bar)	350	Protein	47.37%	78.93%
Temperature (°C)	50	Carbohydrates	21.89%	4.44%
CO_2 flow rate (Kg/min)	0.35	Total dietary fiber (TDF)	4.05%	1.44%
Extraction time (min)	135	Lipids	16.57%	13.15%
		of which FAMEs:	69.84%	89.89%
		FAME Composition:		
		SFA	28.55%	31.06%
		MUFA	26.08%	28.25%
		EPA	36.80%	33.03%
		Other PUFA	8.57%	7.65%

Table 2 shows the main operative conditions for the extraction stage optimized on the basis of the results at bench scale and evidencesthe extract quality obtained after thesupercritical CO_2 utilization. The extraction

yield of the process has been 19.19% and performing a comparison with the biomass characterization it is possible to observe an increase of proteins and a decrease of ashes content, TDF and carbohydrates.
At the end of the extraction process, EPA recovery has been 17.2% by working under a CO_2 pressure of 350 bar at 50 °C with a flow rate of 0.35 Kg/min (2229 $kgCO_2/kg_{biomass}$). Operating potential costs for the VALUEMAG plant has been estimated on the average costs of the installed power (kW), compressed air (l/s), and energy consumption (kWh/day) and have been expressed as euros/day.

3. Results and discussion

The cost index for microalgae cultivation as well as for the other steps necessary for producing algal EPA, indicate as ~73.56% is related to the extraction step based on the utilization of supercritical carbon dioxide.
The remaining 17.45% cost is attributed to the drying stage which makes use of a lyophilizer. More precisely, by considering that after the dewatering-harvesting step the obtained biomass should be around 10wt% (100 g/l) on the basis of a potential initial production of 5-10 l/day, the energy demand for the Ice Condensing is in the range 30-60 kWh/day for the complete lyophilization and production of 0.5-1.0 kg/day of dry biomass. The mechanical pretreatment which allows to perform cell disruption, should affect the process economy to a lesser extent, i.e. 0.56%, since the energy demand for this operation is estimated to be in the range of 1-2 kWh/day (0.5-1.0 kg/day of dry biomass to treat).The projected cost for growth phase plant, mainly depending on the use of light irradiation source required for the correct microalgae survival and growth,should contribute for ~7.13% and nutrients for around 1.29%. The average cost evaluated for the overall process is around40 euros/day (euros/Kg_{dry}).

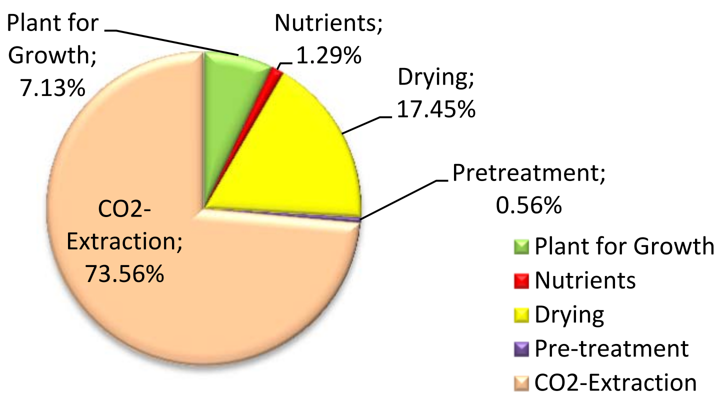

Figure 2. Operating cost index for microalgae growth and EPA extraction via supercritical CO2 extraction calculated on the basis of the VALUEMAG technology (www.valuemag.eu, 2019).

The cost influence related to the growth stage is represented in Figure 3.

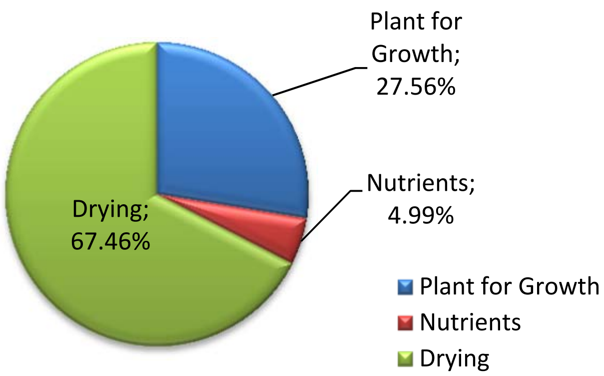

Figure 2. Operating cost index for microalgae growth step calculated on the basis of the VALUEMAG technology (www.valuemag.eu, 2019).

Microalgae cultivation planned according theVALUEMAG technology which average cost is estimated to be ~10 euros/day, highlights as ~67.46% of the cost is related to the drying stage, while the cost for the growth plant is around 27.56% and is mainly correlated with the use of LED light irradiation sources. Nutrients consumption cost is estimated to affect the economy for 4.99%.

4. Conclusions

The global omega-3 market has promoted sales volume of about 2.99 billion euros at 2018 and it has been estimated it will increase up to 8.91 billion euros at 2025. EPA/DHA market value is correlated to 18% with microalgae as natural source, 79% derives from fish oil (anchovy and sardines combined constitute >75% feedstock share for oil extraction) and 3% from krill oil. The oil recovered from algae might be advantageous in view of the pleasant smelll and taste together with the vegetarian origin and sustainability in comparison to fish oil.The supercritical CO_2 extractionhas underlined a product composed by 78.9 wt% of proteins and 13.1wt % of lipids, while the rest (about 8%)has been constitutedbycarbohydrated, TDF and ashes. Lipids content in the extracted biomass has an EPA content of about 33%. Herein it has been performed a preliminary economic and energetic assessment for an integrated process which involves *Nannochloropsisgaditana* cultivation, biomass harvesting-dewatering followed by pretreatment with a lyophilizer and a planetary ball-mill for cell disruption, and finally, EPA extraction by supercritical CO_2. The average cost for the growth phase and biomass drying shows an average value of ~10 euros/day, while including also the CO_2 extraction stage, with the production target of 1 Kg of dry biomass for day, the cost amounts to ~40 euros/Kg.

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