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From Haematococcus Pluvialis Microalgae a Powerful Antioxidant for Cosmetic Applications

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The global cosmetic market is promptly growing, showing a strong boost with the growth of economic well-being. In this context, the demand of innovative, more and more specific cosmetic ingredients has been the key for searching alternative, preferably naturally-based, active components. Microalgae represent one of the most attracting microorganisms and natural deposit for bioactive compounds for their peculiar compositions and properties. In the cosmetic sector, their efficient application has recently been highlighted by the placing on the market of different cosmetic preparations. A powerful antioxidant, 550 times more effective than vitamin E, with enormous potential for cosmetic and pharmaceutical applications, is astaxanthin. Although high-quality astaxanthin can be obtained from microalgae, particularly from *H. pluvialis* specie, actually the major part of the market is occupied by the synthetic form (99%), conferring substantial differences between their price. In fact, algal-based astaxanthin costs >6000 €/Kg, while the synthetic form 600 €/kg. Thus, along with novel natural product launch, the development of cost-effective technologies able to match the existing ones, represents the major challenge for the microalgae application. This study aims to determine the feasibility of microalgal-based astaxanthin production, by exploring both procedural issues and costs evaluation.

1. Introduction

In accordance with the Federal Food, Drug & Cosmetic Act of the US FDA and article L5131-1 of the French Public Health Code, a cosmetic product is defined as any substance or preparation that is to be rubbed, poured, sprinkled, or sprayed on, introduced into or applied to external parts of the body, in particular the epidermis, hair and capillary systems, nails, lips and external genitals, or to the teeth and the mucous membranes of the oral cavity as the product cleans, perfumes, protects them, modifies their appearance, keeps them in good condition or helps to reduce body odours (Cosmetics DGCIS 2012). In short, its function is to assure personal hygiene and beautification.The global cosmetic market is promptly expanding, and it had a strong growth with the advance of economic well-being. Recently it has been evidenced as the use of cosmetic in human daily life has become a necessity. In fact, more than 70% of the population considers cosmetic and personal care products essential for their daily lives (www.cosmeticseurope.eu, 2019).In particular, facial cosmetics might contribute to increase the perceptions of attractiveness, by improving appearance. Etcoff et al. (2011) evidenced as the characteristics of a beautiful face can constitute relevant bio-signals, proposing a potential correlation with judgments of social attributes, proposing a potential correlation with judgments of social attributes. According to some estimates, the global cosmetics market in 2017 has reached 475 billion euros with a Compound Annual Growth Rate (CAGR) between 7% and 7.14%, and with a forecast that could raise more than 625 billion euros in 2022 (www.alliedmarketresearch.com, 2019; www.globenewswire.com, 2019).

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The increasing demand for cosmetics is directly linked to the search of innovative, preferably natural, functional ingredients to meet the customer requests, that is improving personal hygiene, facial, body skin and hair health.

Nowadays consumers prefer to use biological and environmentally sustainable cosmetics respect to products with chemical ingredients fossil derived. There is a crescent demand, for example, for microalgal biomass/extracts, which could significantly affect the cosmetic market in the near future (Chew et al., 2017; Molino et al., 2019a, Molino et al., 2018a; Savvidou et al., 2019). This is due to the outstanding microalgae properties and the possibility to easily modulate their growth driving it towards the production of selected antioxidant/pigments or other nutrients and secondary metabolites. Until now, the known algal species are more than 20000 (Christaki et al., 2012), and they are able to mitigate greenhouse gas emissions providing for about 40–50% global photosynthesis each year (Wang et al., 2015). Both whole algae biomass and algae extracts are present in some cosmetic formulations, mainly in skin and body creams, lotions, gels, soaps, shampoos and they show protective actions against UV rays and as anti-aging, conferring hydration and protection of the skin (Ariede at al., 2017). The extracts mainly derive from coccoid and filamentous microalgae, *Phaeodactylum tricornutum* species, *Chlorella* sp. or from macroalgae like *Fucus*. Proteins and peptides are also extracted from Porphyra, Wakame and microalgae such as *Arthrosira platensis* and *Chlorella*. *Haematococcus pluvialis, Isochrysis galbana, Nannochloropsis gaditana, Phaodactylum tricornutum, Tetrasemis chuii* and *Dunaliella salina* are present attracting microorganisms with enormous potential (Marino et al., 2019; Molino et al., 2018b; Molino et al., 2018c) and their use as ingredient in cosmetics is allowed at European level since 2006 when 2006/257/CE Commission Decision has been issued for the establishment of an inventory of cosmetics (www. eur-lex.europa.eu, 2019) . This Regulation introduced the Cosmetic ingredient database or CosIng (www. eur-lex.europa.eu, 2019) mentioning ingredients that have been authorized for the production of cosmetics. The CosIng database is available and can be consulted on the European Commission website by simply entering the necessary information such as the full name of the selected compound. Among the microalgae extracts, oil and biomass present in the CosIng database, those of *Haematococcus pluvialis*, which represents the richest natural xanthophyll carotenoid astaxanthin (3,3′-dihydroxy-β, β′-carotene-4,4′-dione) source, is one of the most interesting cosmetic ingredient due to the outstanding metabolite properties. In fact, the red-pink pigment astaxanthin is a potent anti-inflammatory and has more antioxidant capabilities than vitamin A and vitamin E. The United States Food and Drug Administration has already accepted the use of astaxanthin as food colorant in animal feed and the European Commission considers natural astaxanthin as a food dye and the *Haematococcus pluvialis* oleoresin as food supplement as evidenced by the Commission Implementing Regulation 2017/2470/EU (Casella et al., 2019). In the cosmetic sector the CosIng reports the following forms: *Dunaliella salina*/*Haematococcus pluvialis* extract, *Haematococcus pluvialis* extract/oil/powder with the related functions reported in Table 1.

Table 1: Cosmetic ingredients (EC number, International Nomenclature of Cosmetics (INCI)) of interest for this work and authorized to be used in cosmetic formulation (CosIng search, 2019)

Name	EC number	INCI	Description	Function(s)
Haematococcus pluvialis	919-412-5	*Haematococcus pluvialis* extract	*Haematococcus pluvialis* Extract is an extract of the Alga, *Haematococcus pluvialis*, Stephanosphaeraceae	Antioxidant
	Unavailable	*Haematococcus pluvialis* Oil	*Haematococcus pluvialis* Oil is the oil expressed from the Alga, *Haematococcus pluvialis*, *Stephanosphaeraceae*	Antioxidant Skin conditioning
	Unavailable	*Haematococcus pluvialis* Powder	*Haematococcus pluvialis* Powder is the powder derived from the Alga, *Haematococcus pluvialis, Stephanosphaeraceae*	Antioxidant
	unavailable	*Dunaliella salina*/*Haematococcus pluvialis* Extract	*Dunaliella Salina/Haematococcus pluvialis* Extract is the extract of the alga, *Dunaliella salina* and *Haematococcus pluvialis*	Antioxidant Skin protecting

The aim of this work was to evaluate the economic feasibility of a natural astaxanthin production based on the cultivation of *Haematococcus pluvialis* microalgae and the subsequent antioxidant extraction via supercritical CO_2 utilization.

2. Materials and Methods

The research work was performed in the context of VALUEMAG project (Horizon 2020-Grant Agreement No 745695). More precisely the presented algal refinery is composed by three main components: 1) the algae production system, 2) the biomass pretreatment and 3) CO_2 extraction of bioactive compounds. The Block Flow Diagram of the VALUEMAG bioefinery is represented in Figure 1.

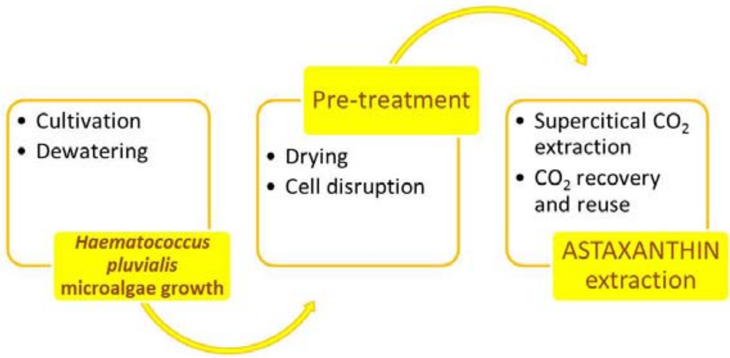

Figure 1. Natural astaxanthin production via Haematococcus pluvialis microalgae cultivation, dewatering,biomass drying and pretreatment, pigment extraction and carbon dioxide recovery and recycle.

For microalgae cultivation within the VALUEMAG project a specific conical reactor (called SOMAC), for an optimal cells growth, has been developed. Moreover, the pioneering approach is based on the microorganisms modification by means the incorporation of superparamagnetic iron oxide nanoparticles which could enhance the biomass production (Savvidou et al., 2019). The correct cultivation of magnetic modified microalgae (MAGMA) is assured by their immobilization on the a soft magnetic conical surface of SOMAC reactor and a thin water layer. After the algal growth phase, biomass is harvested through a dewatering system which allows at the same time, to recover and recycle water, hence perfectly integrated in the sustainability context. For this purpose, an hydraulic pressure difference applied across the two sides of a polysulfone microfiltration membrane permits to obtain a permeate that is reused as a cultivation medium and a retentate that contains the harvested microalgae biomass (Marino et al., 2019). Algal cells biomass are dried by using a lyophilizer and after drying process, biomass is mechanically pretreated for enhancing solvent penetration during the subsequent extraction process of bioactive compounds. The resulting biomass enters in the supercritical CO_2 unit in which gaseous carbon dioxide, after passing through a condenser subcooling device, is sent by means of a pump to the extractor filled with the microalgal matrix. Downstream of the extractor, a separator is located to isolate the desired bioactive compounds from the carbon dioxide used during the process. Finally, the gas is sent again to the condenser subcooling apparatus which allows to recycle for other extraction step.

On the basis of the above mentioned overall process, a preliminary economic assessment of the astaxanthin production by *Haematococcus pluvialis* cultivation has been carried out considering the contributes of the different steps. The costs, expressed in terms of euros/day, have been calculated by considering the previously optimized operational conditions related to the astaxanthin extraction.

3. Results and discussion

For an optimal microalgae growth, considering the overall biorefinery described above (Section 2) and taking into account a productivity of 0.5-1 Kg/day on dry basis, the content range of the various nutrients necessary for *Haematococcus pluvialis* cells, have been estimated and the related costs calculated (Table 2).

Table 2: Culture medium composition required for Haematococcus pluvialis cultivation

Nutrient	**Content (g/day)**	**Cost (eurocents/day)**
$NaNO_3$	150 ÷ 225	7.50 ÷ 11.25
$MgSO_4*7H_2O$	7.50 ÷ 11.25	0.075 ÷ 0.1125
$CaCl_2*2H_2O$	3.6 ÷ 5.4	0.122÷0.184
$K_2HPO_4*3H_2O$	4.0 ÷ 6.0	28.4 ÷ 42.6
$C_6H_8O_7$	0.6 ÷ 0.9	0.036 ÷ 0.054
$C_6H_8FeNO_7$	0.6 ÷ 0.9	8.8 ÷ 13.2
H_3BO_3	0.286 ÷ 0.429	2.12 ÷ 3.18
Na_2CO_3	2 ÷ 3	0.056 ÷ 0.084
Na_2*EDTA	0.10 ÷ 0.15	2.58 ÷ 3.87
Traces including: ($CuSO_4*5H_2O$, $ZnSO_4*7H_2O$ $MnCl_2*4H_2O$, $Na_2MoO_4*2H_2O$, $Co(NO_3)_2*6H_2O$)	69.28 ÷ 103.92($x10^{-3}$)	6.63 ÷ 9.94
TOTAL	**169-253**	**56.32 ÷ 84.47**

Before astaxanthin extraction, a mechanical ball-mill pretreatment (operational conditions as reported by Molino et al., 2018b,c) stage was performed in order to break down the cellular wall of microalgae strain.
The optimized operational conditions which allowed to recover astaxanthin by using supercritical carbon dioxide, are summarized in Figure 2.

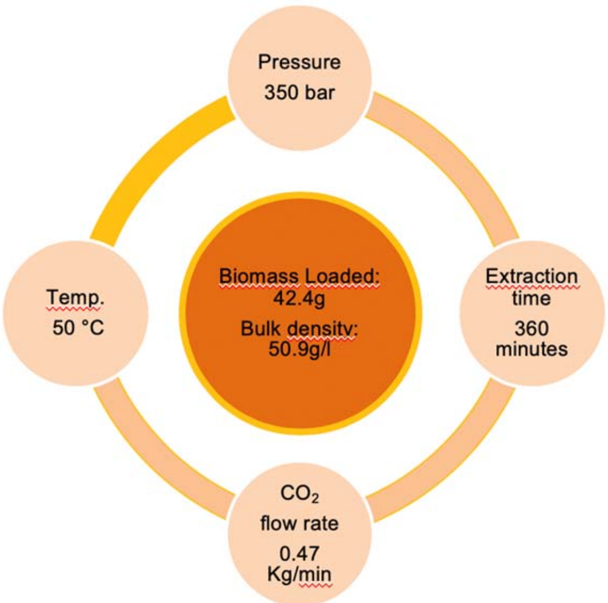

Figure 2. Optimized operational conditions adopted for extracting astaxanthin from Haematococcus pluvialis via supercritical CO_2 operation (Molino et al., 2018b, c).

Supercritical CO_2 pressure of 350 bar, temperature of 50 °C, CO_2 flow rate of 0.47 Kg/min are optimized conditions to obtain 23.58wt% of extract that contains the 48.4 of astaxanthin recovered, 97.2% of β-carotene recovered as well as 53.3% of lutein respect to the biomass content. Table 3 reports the initial biomass and the extract composition. The proteins content passed from 25.69% to 55.37% and similarly, the carotenoids increased from 2.87% to 6.38% and lipids from 2.60 to 8.72% in the biomass and the extract, respectively. On the contrary, carbohydrates and TDF amount reduced after the extraction operation.

Table 3: Haematococcus pluvialis red phase and extract composition

	Biomass	**Extract**
Ash	4.02%	3.61%
Protein	25.69%	55.37%
Carbohydrates	6.30%	0.26%
Total dietary fiber (TDF)	58.52%	25.66%
Carotenoids	2.87%	6.38%
Lipids	2.60%	8.72%
of which FAME:	88.38%	88.84%
Carotenoids composition:		
Astaxanthin	69.71%	65.67%
β-carotene	3.45%	6.51%
Lutein	26.84%	27.82%
FAMEs Composition:		
SFA	28.14%	26.47%
MUFA	23.66%	26.07%
PUFA	48.20%	47.46%

Starting from these results, the calculated energy demand and the costs associated with the VALUEMAG biorefinery approach, have allowed to estimate that the most expensive operation is represented by the supercritical fluid extraction, which implies an average cost of 31.33 euros/day, followed by the drying step, the microalgae growth (mainly due to the use of LED lights), the nutrients and finally the mechanical pretreatment.

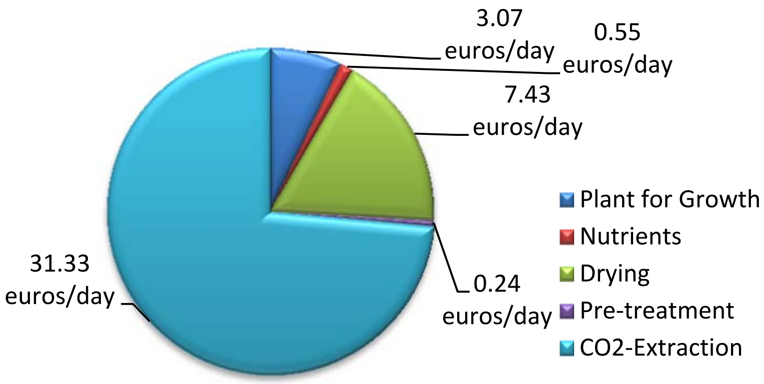

Figure 3. Economic estimate of astaxanthin production from microalgae

The energy consumption mirrors the economic impact. In fact, to perform the supercritical CO_2 extraction about 190.4 kWh/day are required, that is a value higher the sum of the remaining employed steps. However, the overall biorefinery offer precious advantages for the sustainable astaxanthin production, since it guarantees to preserve the pigment integrity without any contamination due to possible solvent residues; in addition, it avoids the application of any downstream purification which might imply additional costs for astaxanthin recovery prior its placing on the cosmetic market.

4. Conclusions

In this study, the feasibility of astaxanthin production from microalgae has been evaluated in a biorefinery context which, starting from *Haematococcus pluvialis* cultivation, allows to obtain the powerful antioxidant by means of supercritical CO_2 extraction. Within this biorefinery concept a dewatering step enables to reduce algal cultivation costs together with a carbon dioxide recovery and reuse for the environment protection.

Characterization of the extract after the use of supercritical CO_2 revealed that the content of proteins, lipids and carotenoids increased in comparison to that of the biomass. On the contrary, after the extraction TDFs amount was lower than initial biomass. A preliminary cost analysis, performed on the basis of previously optimized operational conditions, have evidenced that, considering the potential productivity of 0.5-1 Kg/day on dry basis, an average cost of ~42.60 euros/day is required for the efficient astaxanthin recovery. It is possible to assume that a high impact is due to astaxanthin extraction step. However, the supercritical CO_2 utilization constitutes a valid eco-friendly alternative to the traditional techniques. In fact, it guarantees the production of high-stability algal metabolite and assures CO_2 recycle without any dangerous emission in the environment.

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