



NEWSletter

Issue n° 4
MAR. 2020

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- Previously in ABACUS project
- Focus on WP5 & WP6

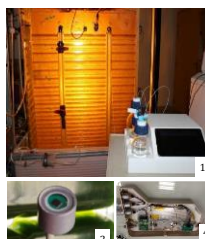
As a reminder...

Along ABACUS roadmap, market opportunities were first consolidated for terpenes and carotenoids molecules. The specifications were defined for target molecules and producing cyanobacteria and microalgae strains to select five product-strain pairs to be exploited within the project.



The most promising strains with highest productivities (biomass & products) were selected from three private microalgae collections and by genetic engineering approaches and fully characterized.

The selected strains were cultivated in closed photobioreactors at pre-pilot scale. Process parameters were optimized for high productivities in biomass and products of interest. New sensors were developed and integrated for online process monitoring and control of microalgae cultures.



Multi-kgs batches of biomass were produced at industrial scale: *Dunaliella salina* for β -carotene, *Tisochrysis lutea* for fucoxanthin, *Haematococcus pluvialis* for astaxanthin, *Porphyridium cruentum* for zeaxanthin. Genetically engineered *Synechocystis* sp. PCC 6803 was cultivated at pre-pilot scale for production of light terpenes.

WP1

Roadmap to markets

WP2

Algae selection

WP3

Process design

WP4

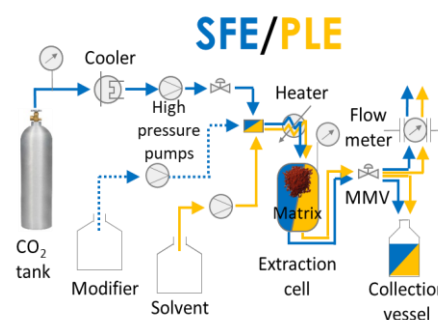
Upscaling

WP5: Fractionation





By using the **multi-kgs batches of microalgal biomass** produced at industrial scale (WP4), **WP5** develops and optimizes an **environmentally friendly extraction process** for each high-added value compound defined in the roadmap (WP1) to provide related **purified extracts** for applicability tests (WP6).

Compressed fluid extractions - **Pressurized Liquid Extraction (PLE)** and **Supercritical Fluid Extraction (SFE)** - with non-toxic solvents were applied [1,3]. Thanks to high pressure and temperature conditions, increases in extraction efficiency were observed compared to basic procedures. Purification relied on **Supercritical Antisolvent Fractionation (SAF)** and solid-liquid **adsorption** [4].

Extraction and purification conditions were optimized. Extraction yield, content and purity of carotenoids and by-products were fully characterized.



Green extraction techniques: SFE and PLE.
Modified from Gallego et al. (2018) and Gilbert-López (2017)

Microalgae	<i>Porphyridium cruentum</i>	<i>Haematococcus pluvialis</i>	<i>Tisochrysis lutea</i>	<i>Dunaliella salina</i>
Extraction	PLE x 3	PLE	PLE	SFE
Purification	biorefinery process	SAF	+ AC (adsorbent)	None
Resulting extract(s)	Zeaxanthin [2] +PE, sPS 4% carotenoids 	Astaxanthin [4] 12% carotenoids 	Fucoxanthin [5] 10% carotenoids 	β-carotene [6] 35% carotenoids 

Optimization of extraction and purification/fractionation processes for producing high added-value compounds from algae.
PE: phycoerythrin; sPS: sulphated polysaccharides; AC: activated charcoal; %: g carotenoids/100 g extract. © CSIC

- ⇒ Extracts (10-150g) from *P. cruentum*, *H. pluvialis* and *T. lutea* biomass sent for applicability tests (**WP6**).
- ⇒ Data related to extraction processes were sent for integrating downstream processes into LCA (**WP7**).
- ⇒ WP5 activities are already well valorised through 2 review articles and 3 research papers [1-5]. One additional research publication is under preparation [6] (**WP8**).

- [1] Gallego et al. (2018) Green extraction of bioactive compounds from microalgae.
- [2] Gallego et al. (2019) Development of a green downstream process for the valorization of *Porphyridium cruentum* biomass.
- [3] Gallego et al. (2019) Sub- and supercritical fluid extraction of bioactive compounds from plants, food-by-products, seaweeds and microalgae.
- [4] Gallego et al. (2019) Application of compressed fluids-based extraction and purification procedures to obtain astaxanthin-enriched extracts from *Haematococcus pluvialis* and characterization by comprehensive two-dimensional liquid chromatography coupled to mass spectrometry.
- [5] Gallego et al. (2020) Simultaneous extraction and purification of fucoxanthin from *Tisochrysis lutea* microalgae using compressed fluids.
- [6] Gallego et al. (in prep) Experimental optimization of sub- and supercritical carbon dioxide extractions of carotenoids from *Dunaliella salina*. Evaluation of the anti-acetylcholinesterase activity of the obtained extracts.

CSIC – Spanish National Research Council

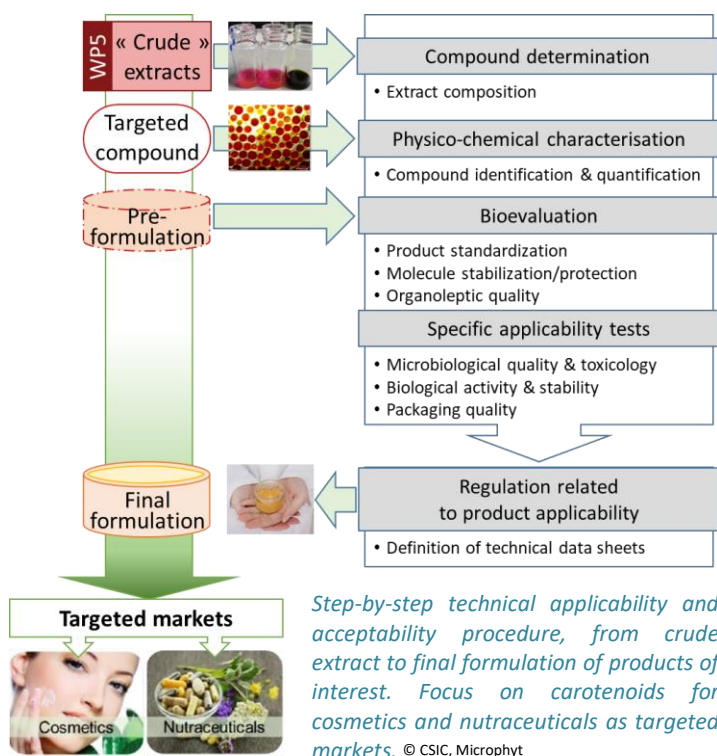
With the main objective of developing and promoting research for scientific and technological progress, CSIC is the largest public institution in Spain and the third largest in Europe with more than 13,000 employees. https://www.cial.uam-csic.es/pagperso/foodomics/index_en.html

Role in the project: As leader of WP5, the Institute of Food Science Research of CSIC (CIAL-CSIC, laboratory of Foodomics, Madrid) focuses on developing fractionation and extraction processes based on green approaches in order to provide purified compounds to WP6 from microalgal biomass (WP4).

WP6: Applicability

WP6 demonstrates the **cost-in-use acceptance** of algal fractions towards targeted markets (cosmetics, nutraceuticals and fragrances) by providing a ready-to-use formulation based on **technical acceptability and applicability tests** of targeted products. **Regulation aspects** are specifically addressed in order to inscribe the natural added-value products on the European market. In addition to targeted products, **valorising by-products** such as lipids, carbohydrates, proteins and pigments is also part of the objectives.

Respective methods with technical implementation were firstly defined to subsequently perform the **physico-chemical characterisation** of products once extracted from algal biomass ([1], D6.1, D6.2 and D6.3).



The **quantity of extracts** required for characterisation and applicability tests were defined together with WP4 and WP5. **Formulation of prototype products** to run applicability tests and check regulation criteria is ongoing.

- ⇒ Extracts (WP5) are ready to use for applicability tests.
- ⇒ Respective pre-formulations will be validated.
- ⇒ Respective technical data sheets will be completed.
- ⇒ Also by-products from extracts will be characterized.

[1] [Physico-chemical characterization report](#) of carotenoids (D6.1), terpenes (short chains, D6.2) and by-products (D6.3) produced in ABACUS.

MICROPHYT

Located in Montpellier (France), this expanding SME offers natural and sustainable solutions for nutrition and well-being. The company develops, produces and markets innovative active algal ingredients. Microphyt's experience spans from industrial production of algal biomass - with its proprietary PBR technology - to production of unprecedented bioactive ingredients. <http://www.microphyt.eu/en/>

Role in the project: By leading WP6, Microphyt provides applicability key steps for the targeted compounds. Microphyt is also involved in large-scale cultivation of microalgae (WP4).

SENSIENT COSMETICS TECHNOLOGIES

As a global leading supplier of high performance colorants and innovative ingredients, Sensient CT, a unit of Sensient Corporation, develops, produces, markets and provides its products for cosmetics industry around the world. Its headquarters are located in St Ouen l'Aumone, near Paris. <https://www.sensient-cosmetics.com/>

Role in the project: Together with Microphyt, Sensient CT tests ABACUS products for validating their respective applicability for cosmetics market.

In the next issue

Focus on WP7 activities: **Product and market acceptances.**

- ⇒ How to assess the acceptability of algal products and production processes for targeted markets? **WP7** provides the Life Cycle Assessment (LCA), the Techno-Economic Analysis (TEA) and environmental impact for the whole value chain of production.

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