NEW GREEN TECHNOLOGIES TO EXTRACT BIOACTIVES FROM *Isochrysis galbana* MICROALGA

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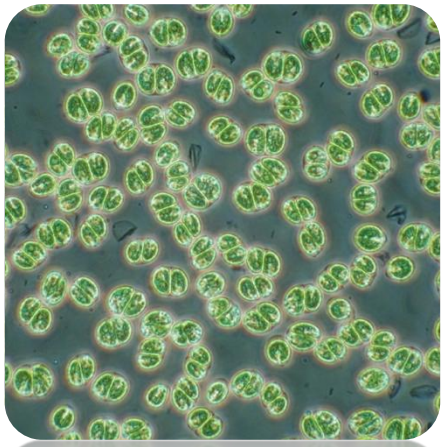

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SPECIALTIES FROM ALGAE

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 613588

Project data

- **M**ulti-product **I**ntegrated bio**R**efinery of **A**lgae: from **C**arbon dioxide and **L**ight **E**nergy to high-value **S**pecialties: **MIRACLES**
- The consortium consists of 6 Universities, 5 Research Organisations, 12 SME's and 3 MNI/end users in 6 EU countries + Norway + Chile.
- Website: **http://miraclesproject.eu/**

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Miracles WPs and Activities

Foodomics Lab. activities

WP1: Production and Harvesting

*WU, UT, UHU, **VITO**, FITO, TMUC*

Industrial strains

Harvesting

New production strains

*WU, FCPCT, UIB, UA, **UniRes**, FITO*

WP2: Bio-prospecting, metabolic optimization, cultivation at extreme locations

WP3: Development of Integrated Biorefinery

WU
DLO
CSIC
ET
DNL
DSM
VFT

Cell disruption

Extraction

Hydrophobic compounds (lipids)

Hydrophilic compounds (proteins, carbohydrates)

WP4: Product development and market assessment

NATAC, URDV, DSM

Food, incl. functional foods, nutraceuticals

EWOS, SPAROS

Aquaculture / aquafeed

BIOPOL, IMENZ, CHIMAR, NATAC, CE

Non-Food applications

VFT

Market assessment

WP5 Demonstration of integrated value chains *WU, **FITO**, DLO, CSIC, ET, DNL, EWOS, SPAROS, BIOPOL, IMENZ, CHIMAR, NATAC, URDV, DSM, VFT*

WP6 Techno-economic and sustainability assessment *WU, VFT, **NOVA**, ET*

WP7 Dissemination, exploitation and intellectual property management *WU, VFT, **IDC***

WP8 Project coordination and overall management ***WU**, IDC*

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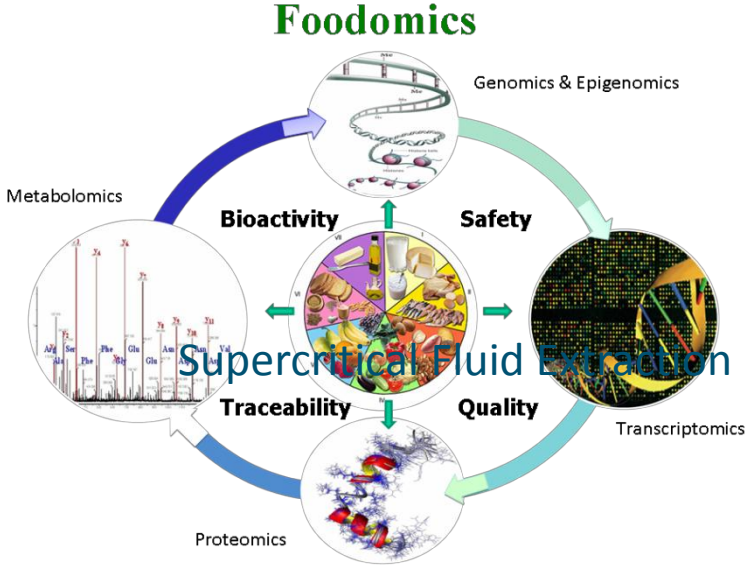
Foodomics Group

Application of advanced analytical methodologies to study the food/health relationship



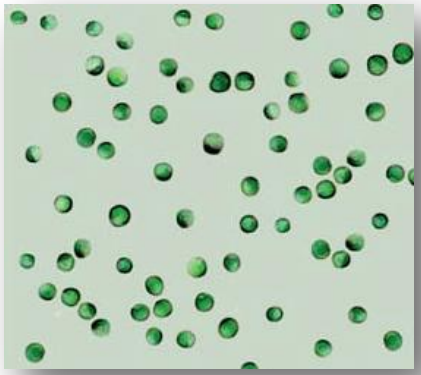
Development of green extraction processes (extraction and fractionation) based on compressed fluids (sub- and supercritical)

Supercritical Fluid Extraction

Pressurized liquid Extraction

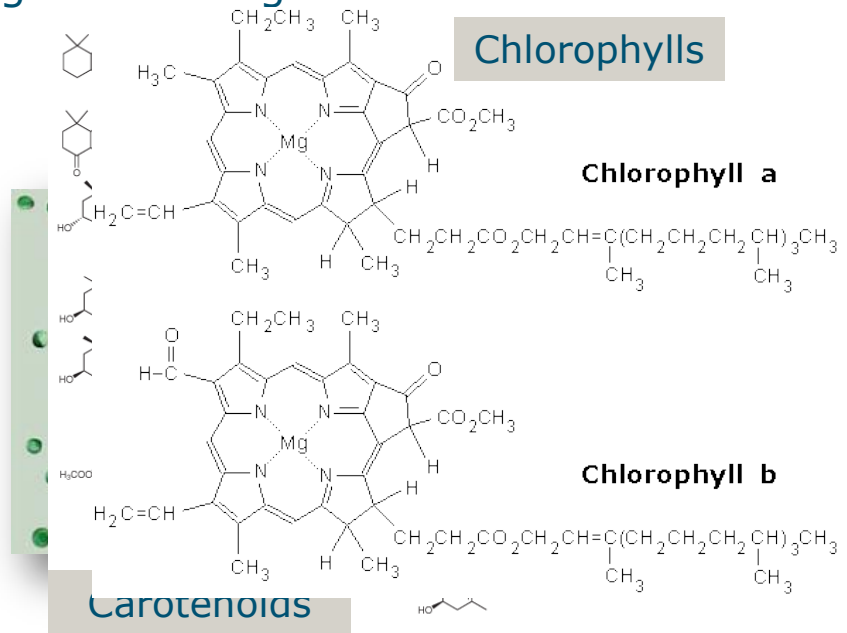
Isochrysis galbana microalga

- *Isochrysis galbana* is a marine flagellated microalga belonging to the phylum *Haptophyta*.
- This *Haptophyta* is of substantial interest in **aquaculture**, principally to feed mollusk larvae, as well as fish and crustaceans in the early stages of growth.
- Its cells are easily assimilated by larval animals because of their small size (4-7 μm) and **absence of a tough cell wall**.
- Isochrysis galbana is a marine microalga well recognized as a natural rich source of **EPA and DHA**.

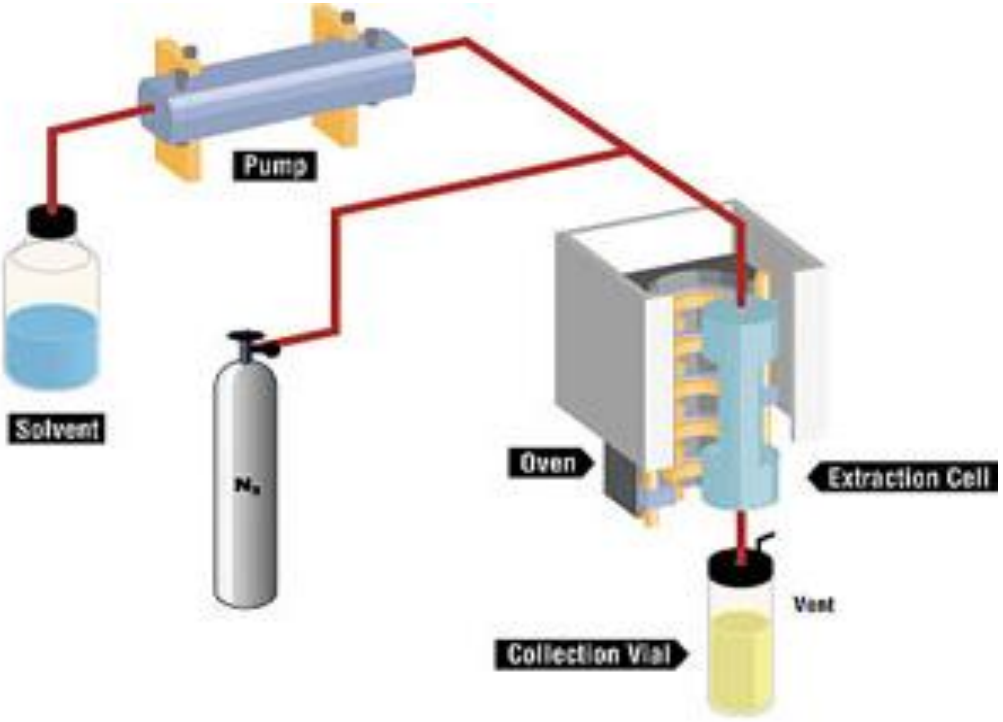


Isochrysis galbana microalga

- *Isochrysis galbana* is a marine flagellated microalga belonging to the phylum *Haptophyta*.
- This *Haptophyta* is of substantial interest in **aquaculture**, principally to feed mollusk larvae, as well as fish and crustaceans in the early stages of growth.
- Its cells are easily assimilated by larval animals because of their small size (4-7 µm) and **absence of a tough cell wall**.
- Isochrysis galbana is a marine microalga well recognized as a natural rich source of **EPA and DHA**.
- **Other bioactive compounds found**



PRESSURIZED LIQUID EXTRACTION - PLE

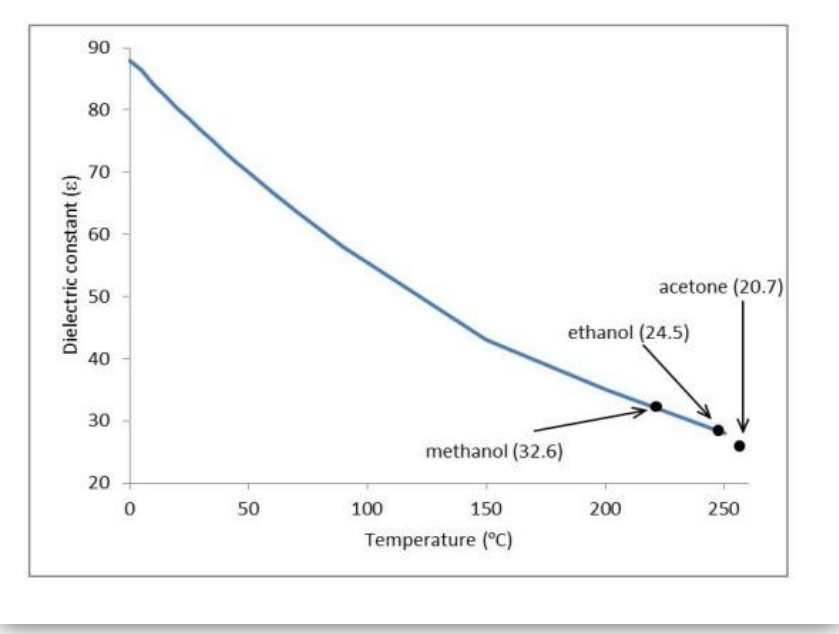


PLE main features

- Solvents maintained in **liquid state**
- Extractions with **Ethanol** and **Water** (GRAS solvents).
- Faster extraction processes
- Low volumes used of solvents compared to traditional extraction techniques.

PRESSURIZED LIQUID EXTRACTION - PLE

DIELECTRIC CONSTANT



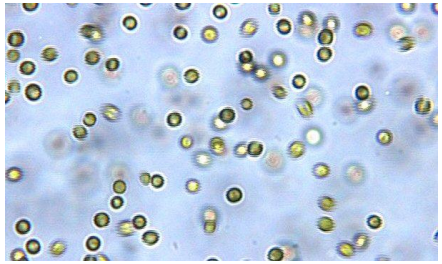
Varies significantly with increase in Temperature

What happens with water...

- Main parameter: dielectric constant (~polarity)
- Interesting alternative to the replace organic solvents in some applications

PRESSURIZED LIQUID EXTRACTION - PLE

Isochrysis galbana



EXTRACTION PROCEDURE

- *I. galbana* freeze-dried
- PLE using **water and ethanol**: experimental design.

Experimental design

Temp	EtOH
ºC	%
50	0
125	50
125	50
125	50
200	100
125	100
125	0
50	100
200	0
50	50
200	50

-Extraction conditions maintained:

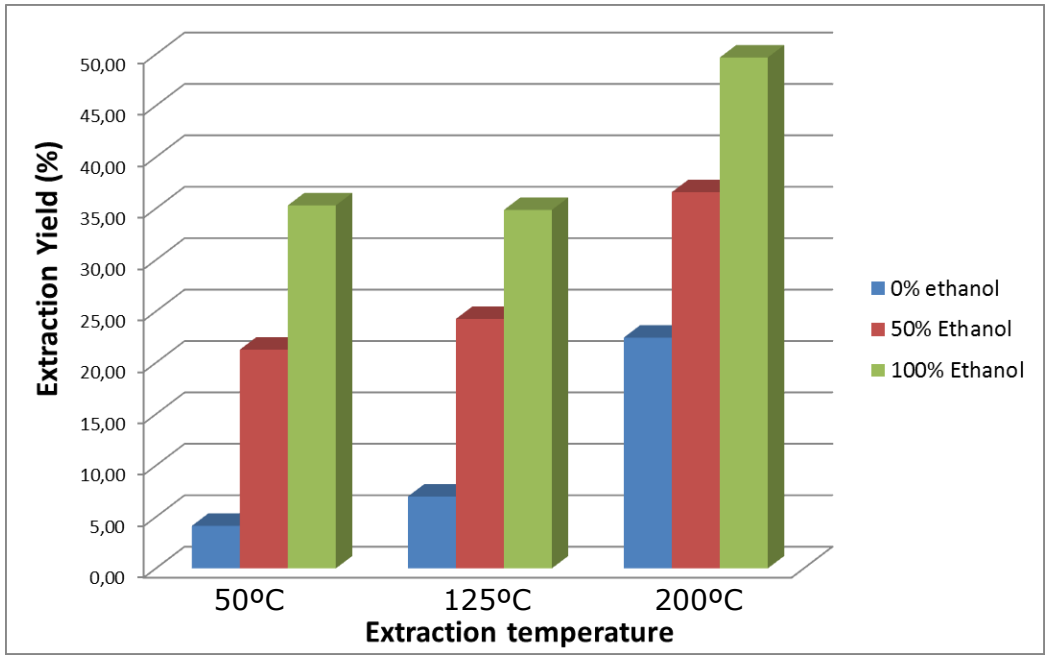
· Extraction pressure: 100 bar
· Extraction time: 20 min
· Static extraction cycles: 1 cycle.

Response variables:

- Extraction yield (%)
- Antioxidant activity (TEAC and DPPH methods)
- Total phenols amount (Folin method)

PRESSURIZED LIQUID EXTRACTION - PLE

RESULTS – EXTRACTION YIELD



- Higher T, higher yield
- Yield increased with % ethanol

Individual response optimization to maximize yield:
200 ºC, 100% ethanol

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Individual response optimization to maximize Total Phenols: 200 ºC, 0% ethanol

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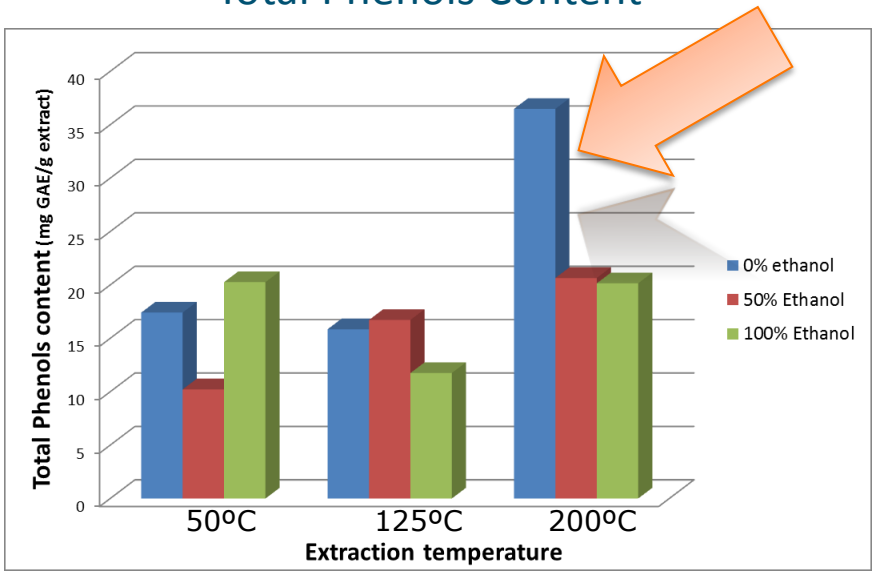
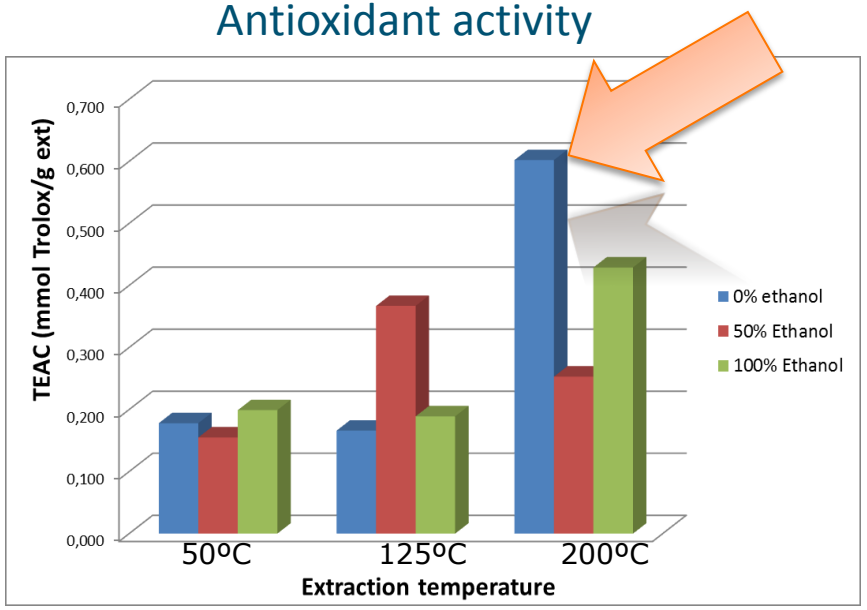
PRESSURIZED LIQUID EXTRACTION - PLE

Total Phenols Content



- Highest phenols amount using water at highest temperature

Antioxidant activity



- Highest antioxidant activity produced with water extracts at 200ºC.

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PRESSURIZED LIQUID EXTRACTION - PLE

RESULTS – Experimental design analysis

Individual optimized responses

Response	T (ºC)	% EtOH	Value
Yield (%)	200	100	49.7
Total Phenols (mg GAE/g ext)	200	0	36.48
TEAC (mmol Trolox/g ext)	200	0	0.602

Multiple Response Optimization

Factor	*Optimum*
%EtOH	15.32 %
Temp	200 ºC

Optimum	
26.9	% Yield
31.1	mg GAE/g extract
0.476	mmol Trolox/g extract

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Development of green extraction processes (extraction and fractionation) based on compressed fluids (sub- and supercritical)

Supercritical Fluid Extraction

Pressurized liquid Extraction

SUPERCRITICAL FLUID EXTRACTION - SFE

Isochrysis galbana

Sequential extractions

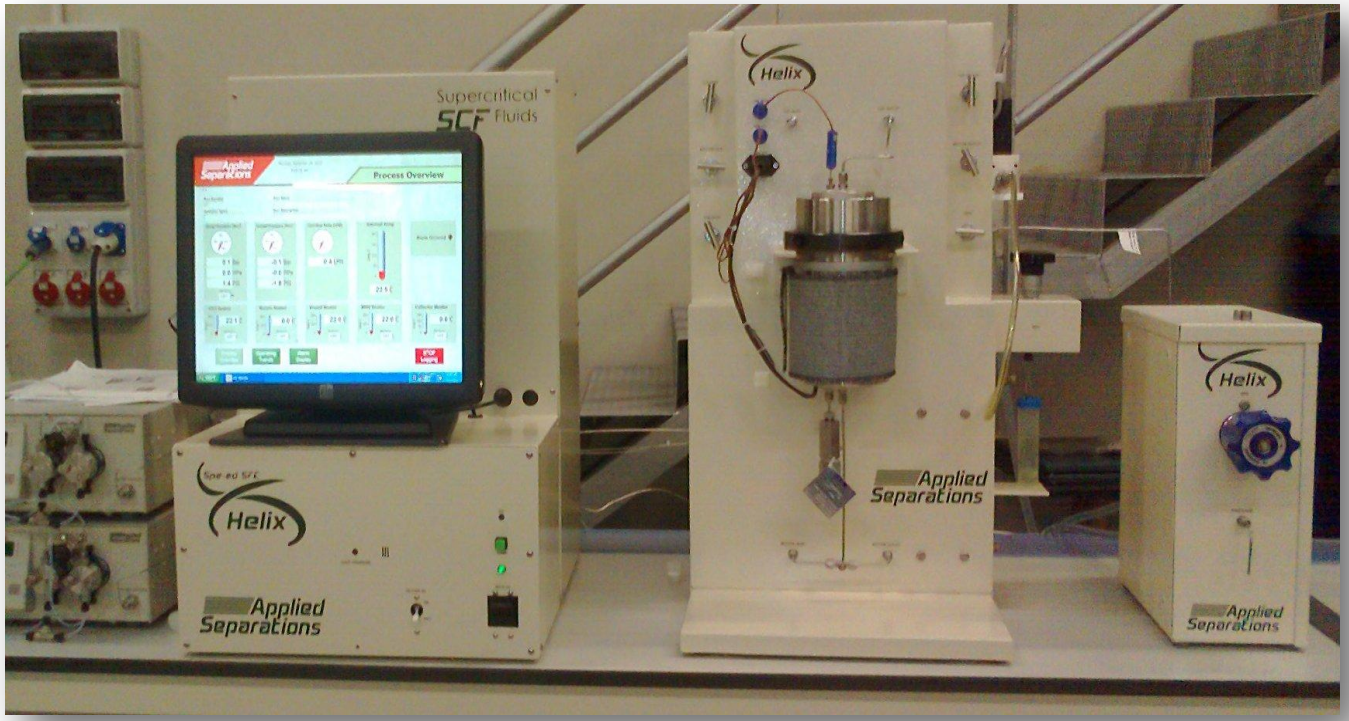
1st step: SC-CO_2 extraction to recover highly lipophilic compounds, Experimental design

2nd step: SC-CO_2+ethanol extraction to obtain extracts rich in other bioactives (more polar), Extractions performed on the residue from previous extraction step

SUPERCRITICAL FLUID EXTRACTION - SFE

Sequential extractions: 1st step optimization

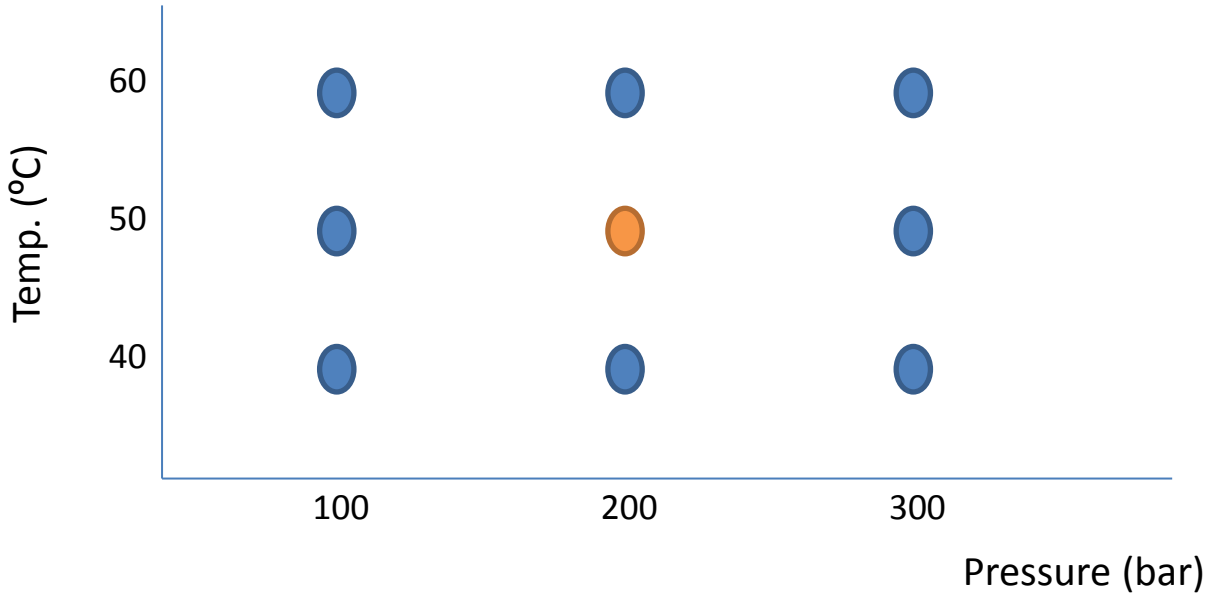
Initial extraction is performed with neat CO_2 (extremely non-polar) to extract highly lipophilic compounds



SUPERCRITICAL FLUID EXTRACTION - SFE

Sequential extractions: 1st step optimization

3-level factorial design (3^2). The studied factors were P (100-300 bar) and T (40-60 ^oC)

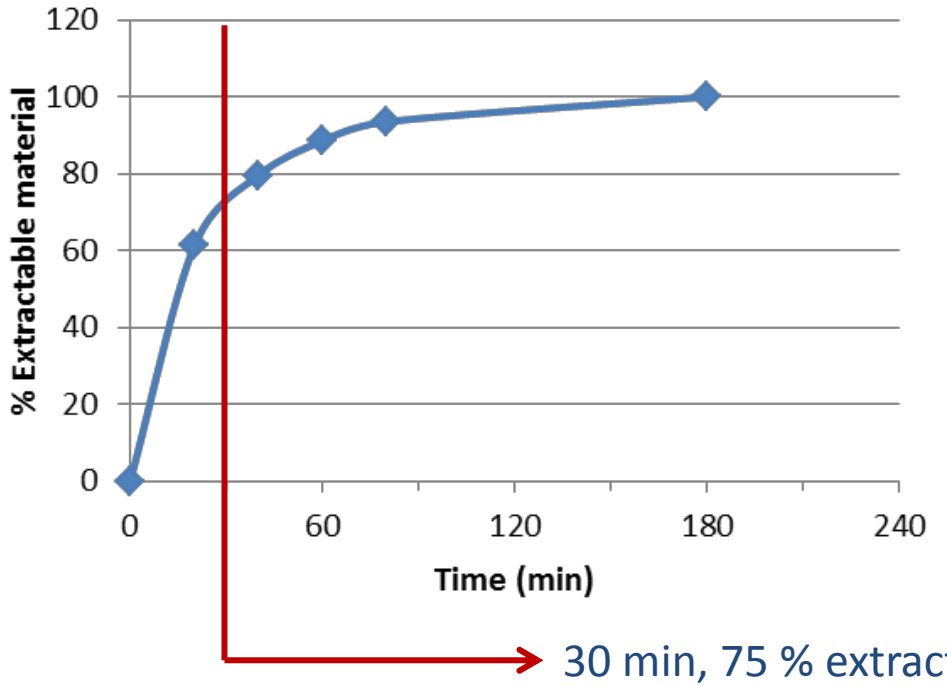


SUPERCRITICAL FLUID EXTRACTION - SFE

Sequential extractions: 1st step optimization

Extraction time optimization: Kinetic study

Extractions performed at different times in the center-point of experimental design (200 bar, 50 ºC)



30 min, 75 % extractable material reached

SUPERCRITICAL FLUID EXTRACTION - SFE

Sequential extractions: 1st step optimization

40 ºC 50 ºC 60 ºC

100 bar

40 ºC 50 ºC 60 ºC

200 bar

40 ºC 50 ºC 60 ºC

300 bar

SUPERCRITICAL FLUID EXTRACTION - SFE

Sequential extractions: 1st step optimization

Global results

Presure	Temp	Yield	Carotenoids	Chlorophyls
bar	ºC	%	mg/g extract	mg/g extract
100	40	1.21	414.29	326.10
200	40	2.58	383.03	257.30
300	40	3.06	351.54	199.97
100	50	0.45	111.16	70.63
200	50	4.30	247.88	153.65
300	50	3.98	621.88	284.82
100	60	0.45	449.48	233.91
200	60	4.08	337.94	240.79
300	60	3.92	560.81	276.57

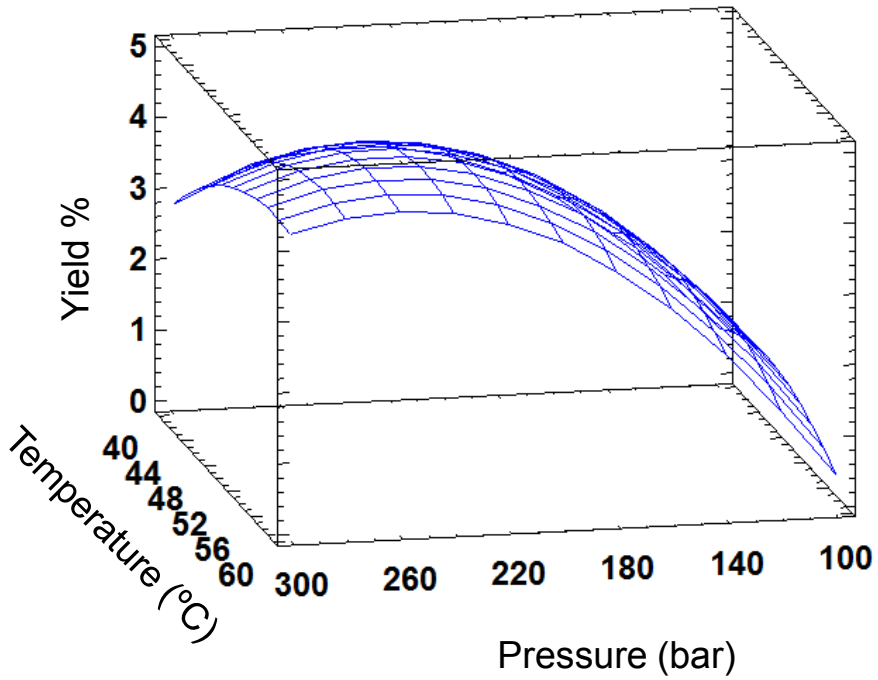
Common conditions to all extractions:

- 30 min extraction time
- 10 g of algal material used with 30 g of sea sand as dispersive agent,
- 5 L/min $CO_{2(gas)}$

SUPERCRITICAL FLUID EXTRACTION - SFE

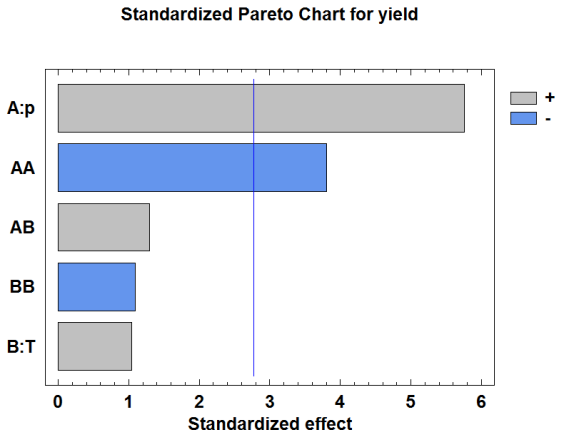
Sequential extractions: 1st step optimization

Statistical optimization of experimental design for YIELD



R^2 of mathematical model 93.2%
Significant factors: Pressure and P*P

Goal: maximize yield
Optimum value = 4.51 %
Optimum:
P= 254.2 bar
T= 55.4 °C



SUPERCRITICAL FLUID EXTRACTION - SFE

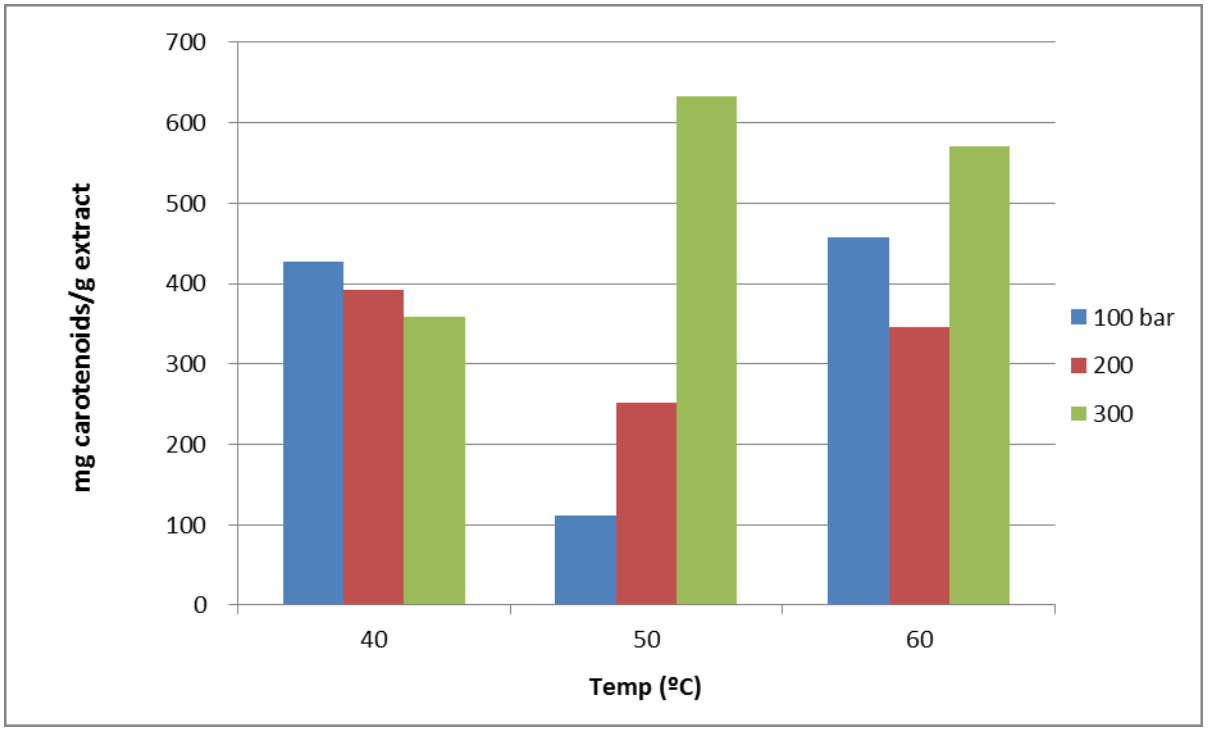
Sequential extractions: 1st step optimization

Composition of extracts:

carotenoids up to 63%

other lipids detected by GC-MS metabolomic method: PUFA

Clear trend at 50 ºC: higher pressure, higher carotenoid concentration

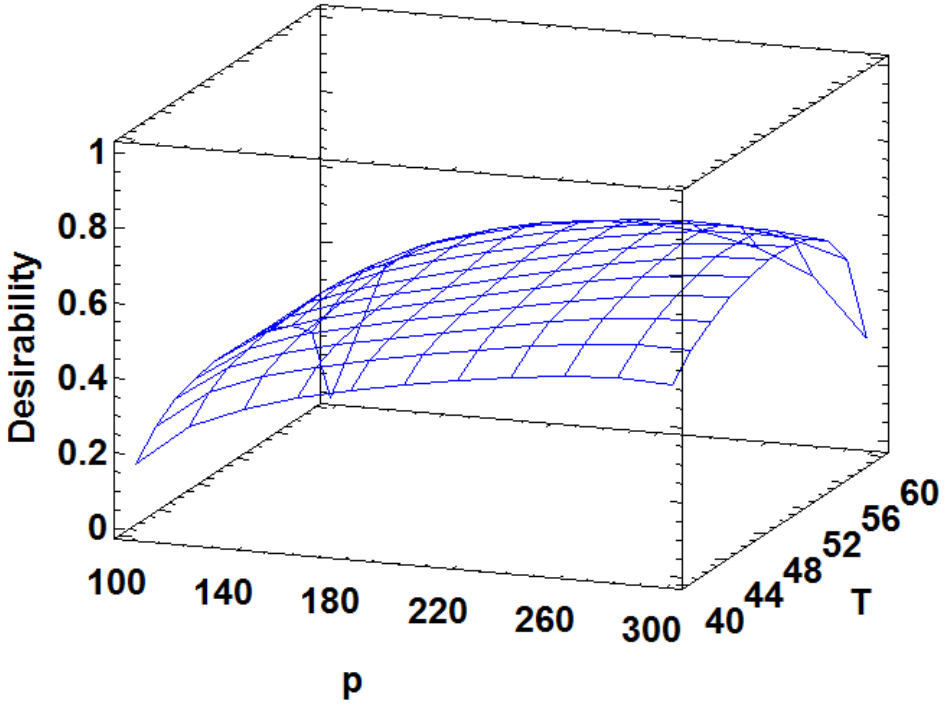


SUPERCRITICAL FLUID EXTRACTION - SFE

Sequential extractions: 1st step optimization

Multiple response optimization: Yield, Carotenoids and chlorophyls

Extraction conditions optimized for 1st extraction step to maximize yield and carotenoids and minimize chlorophylls



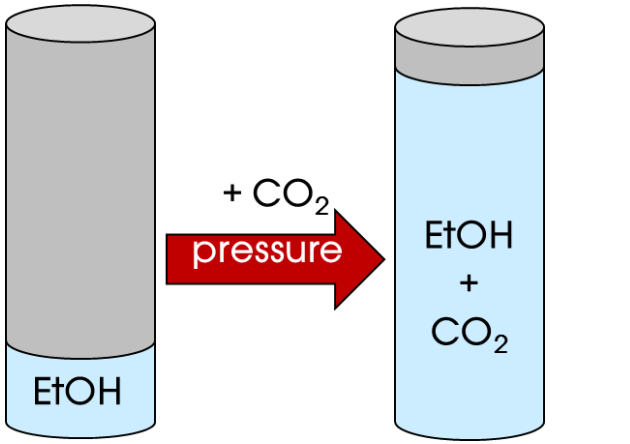
Factor	*Optimum*	
Press	300.0	bar
Temp	50.0	ºC

Optimum	
3.98	% Yield
621.8	mg carotenoids/g extract
284.8	mg chlorophylls/g extract

SUPERCRITICAL FLUID EXTRACTION - SFE

2nd step

- Extraction of polar compounds based on Gas Expanded Liquids



- Extractions performed on the residue from previous extraction step
- Experimental conditions
  - 15-70% Ethanol
  - Pressure high enough to expand EtOH, but not dissolving CO_2 (60-80 bar)
  - Same temperature as 1st step optimum (50 ºC), to reduce cooling time

SUPERCRITICAL FLUID EXTRACTION - SFE

2nd step

- Extraction of polar compounds based on Gas Expanded Liquids

Composition of extracts:

- Extracts very rich in chlorophylls
- Polyunsaturated Fatty Acids (linolenic, DHA…) detected by GC-MS metabolomic method at lower concentration than 1st step
- GC-MS metabolomic method also reported the presence of several carbohydrates

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CONCLUSIONS

- The recovery of bioactive compounds from the microalga *Isochrysis galbana* has been set up using green methodologies: PLE and SFE
- In PLE the optimized conditions to maximize yield, antioxidant activity and phenolic compounds were: 200°C and 15% etanol:85% water
- Sequential SFE was optimized. In the 1st step pure supercritical CO_2 was used and extracts rich in carotenoids were obtained. In the 2nd step the application of Gas Expanded Liquid Extraction was studied for the first time on this microalga, providing extracts with very high content in chlorophylls and carbohydrates.

THANKS

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- MIRACLES Project consortium

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- People at Foodomics laboratory

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