

COMPARISON BETWEEN PRESURIZED LIQUID EXTRACTION AND MICROWAVE ASSISTED EXTRACTION OF BIOACTIVE COMPOUNDS FROM THE MICROALGA *PHAEODACTYLUM TRICORNUTUM*.

FLUCOMP
CADIZ 2015



B. Gilbert-López, A. Barranco, M. Herrero, A. Cifuentes, E. Ibáñez

Laboratory of Foodomics

Bioactivity and Food Analysis Department,
Institute of Food Science Research (CIAL-CSIC),
C/ Nicolás Cabrera 9, Campus UAM Cantoblanco
28049 Madrid, SPAIN

<http://www.cial.uam-csic.es/pagperso/foodomics/>

<http://miraclesproject.eu/>





P. tricornutum culture



*High pressure treatment (1200 bar)
to break the cell wall*



Freeze-drying



Microwave Solvent-Assisted Extraction (MAE)

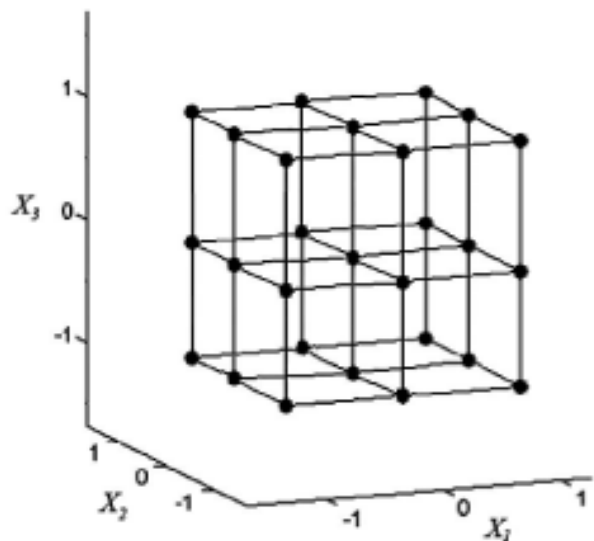


Pressurized Liquid Extraction (PLE)

**Microwave Solvent-Assisted Extraction
(MAE)**

Factorial 3^3

4 replicates in central point

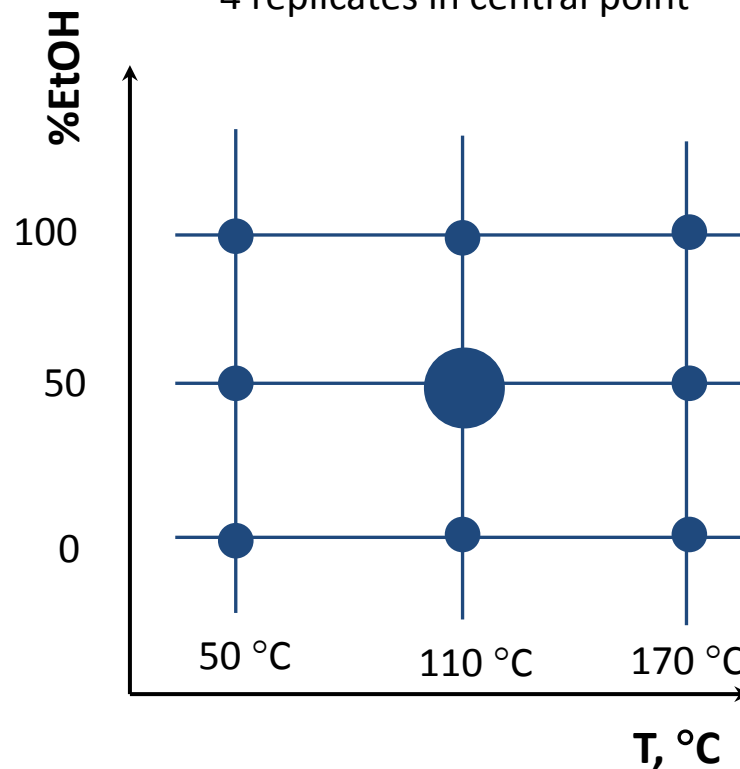


Factor	Range	
%EtOH	0-100	%
Temp	30-170	°C
Time	2-20	min

**Pressurized Liquid Extraction
(PLE)**

Factorial 3^2

4 replicates in central point

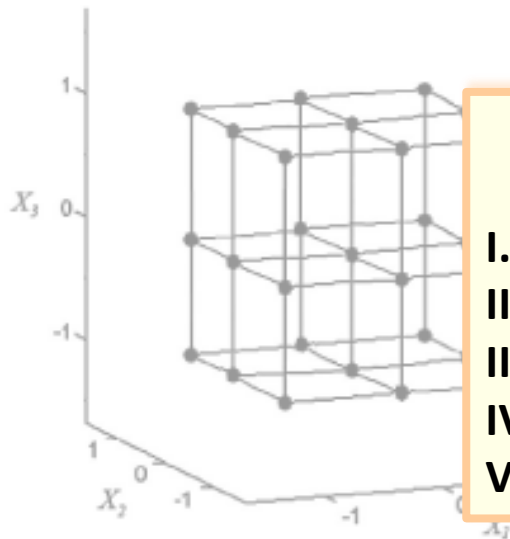


Factor	Range	
%EtOH	0-100	%
Temp	50-170	°C

**Microwave Solvent-Assisted Extraction
(MAE)**

Factorial 3^3

4 replicates in central point



Response variables

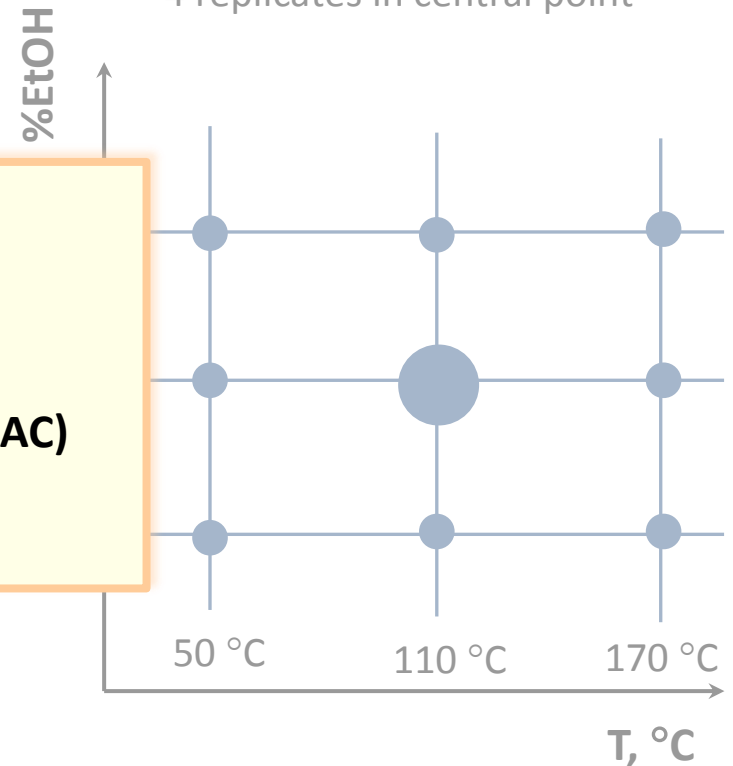
- I. Yield
- II. Total phenolic content
- III. Antioxidant activity (TEAC)
- IV. Total Carotenoids
- V. Total chlorophylls

Factor	Range	
%EtOH	0-100	%
Temp	30-170	°C
Time	2-20	min

**Pressurized Liquid Extraction
(PLE)**

Factorial 3^2

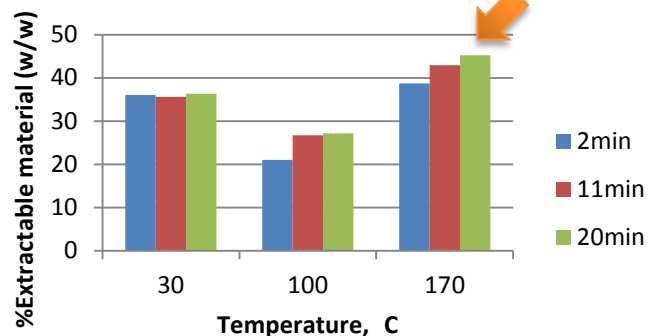
4 replicates in central point



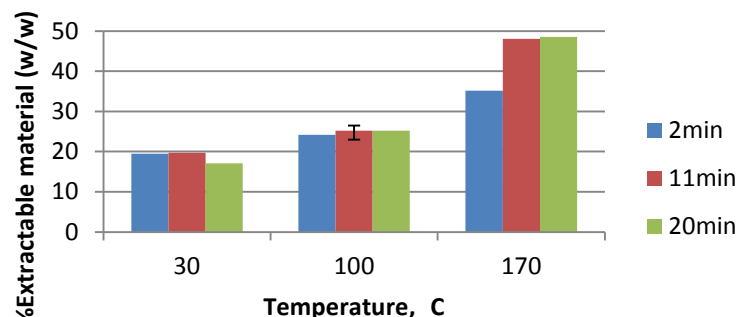
Factor	Range	
%EtOH	0-100	%
Temp	50-170	°C

Yield

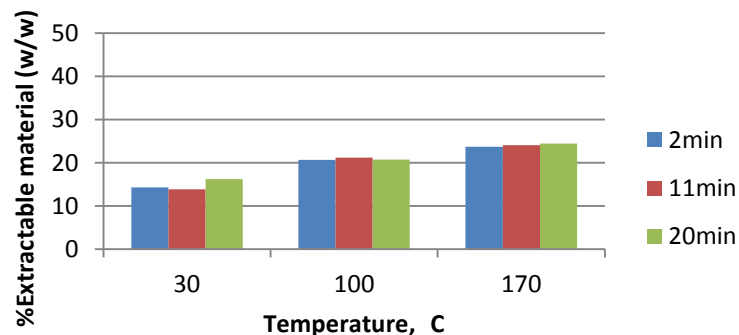
Water extraction



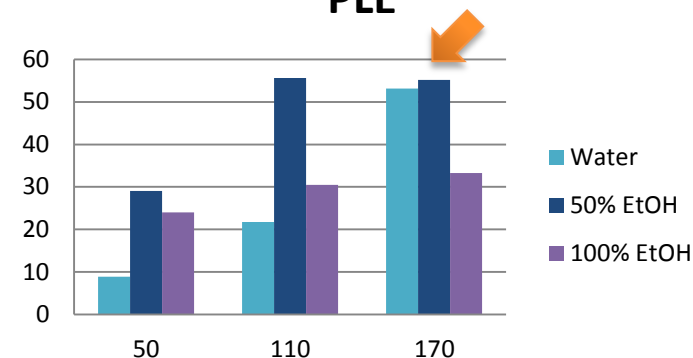
EtOH/Water 50:50 (v/v)



Ethanol extraction



PLE



Experimental Design PLE 3²

Optimum values proposed:

Extraction time: 20 min

Temperature: 170 °C

%EtOH: 40%

Yield: 61.90 %(w/w)

Experimental Design MAE 3³

Optimum values proposed:

Extraction time: 20 min

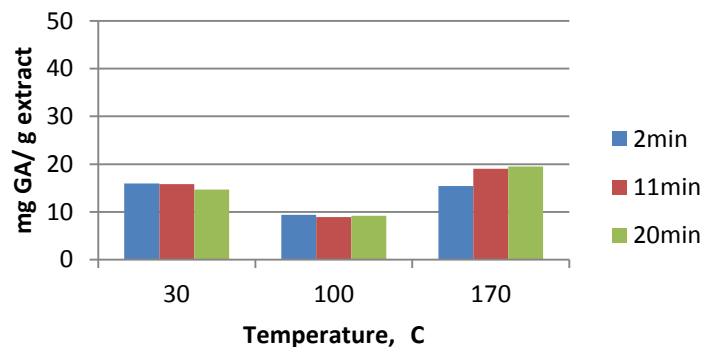
Temperature: 170 °C

%EtOH: 0%

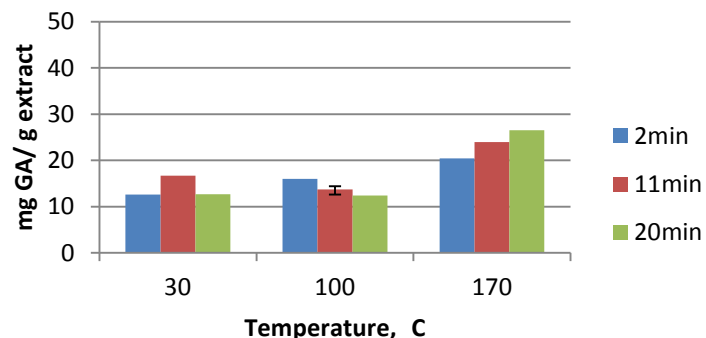
Yield: 46.52 %(w/w)

TPC

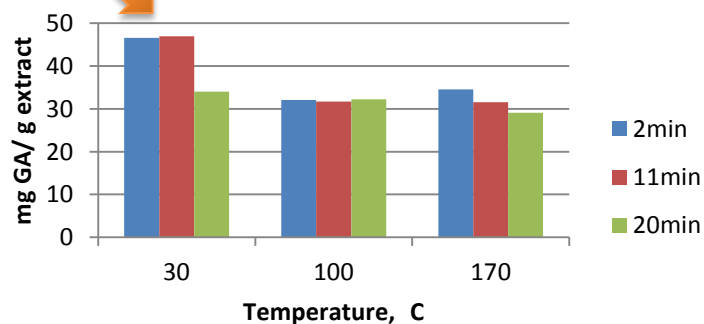
Water extraction



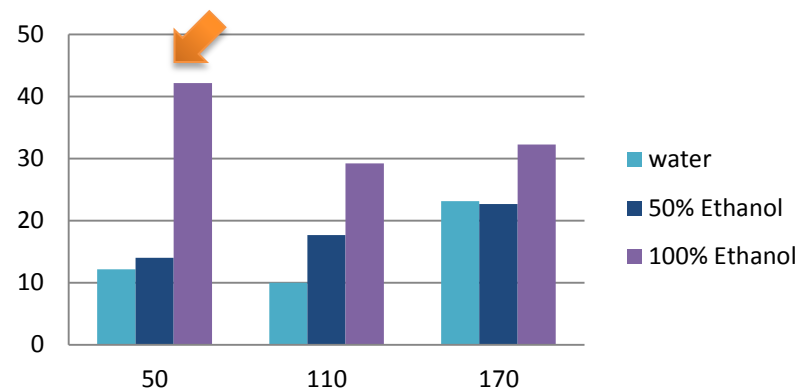
EtOH/Water 50:50 (v/v)



Ethanol extraction



PLE



Experimental Design PLE 3²

Optimum values proposed:

Extraction time: 20 min

Temperature: 50 °C

%EtOH: 100%

TPC: 39.55 mg GA/ g extract

Experimental Design MAE 3³

Optimum values proposed:

Extraction time: 2 min

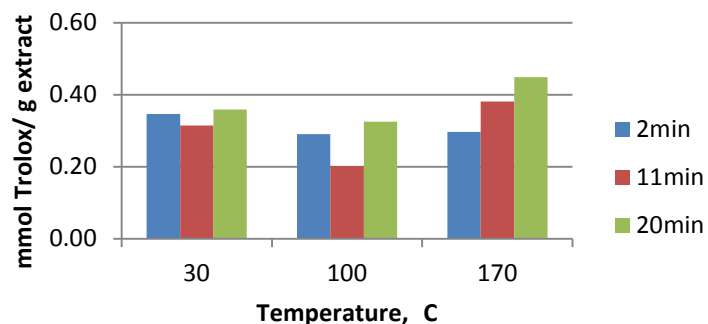
Temperature: 30 °C

%EtOH: 100%

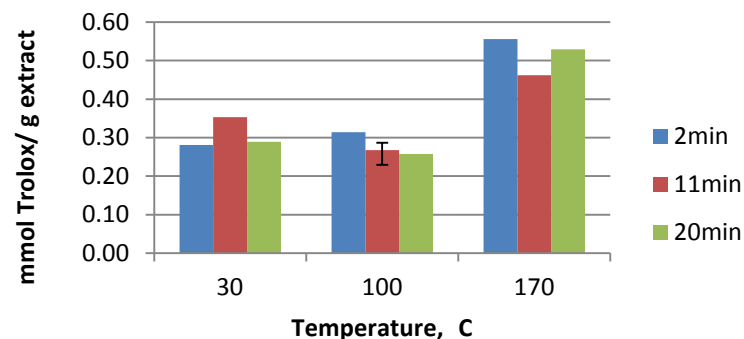
TPC: 44.04 mg GA/ g extract

TEAC

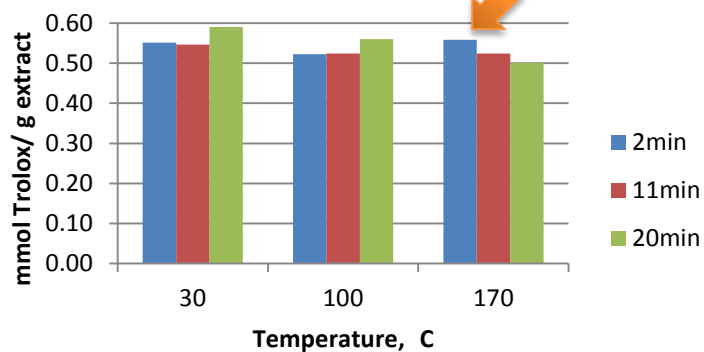
Water extraction



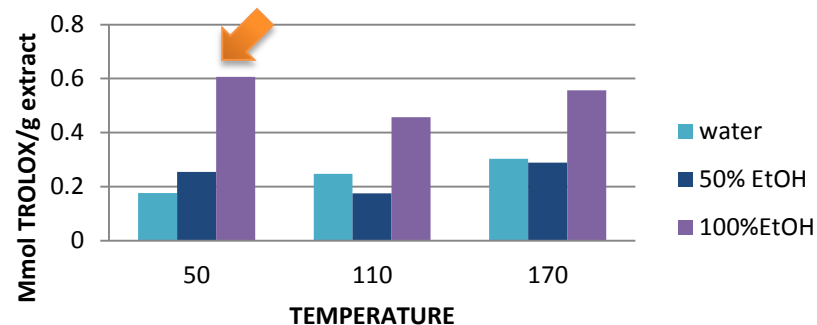
EtOH/Water 50:50 (v/v)



Ethanol extraction



PLE



Experimental Design PLE 3²

Optimum values proposed:

Extraction time: 20 min

Temperature: 50 °C

%EtOH: 100%

TEAC: 0.591 mmol Trolox/g extract

Experimental Design MAE 3³

Optimum values proposed:

Extraction time: 2 min

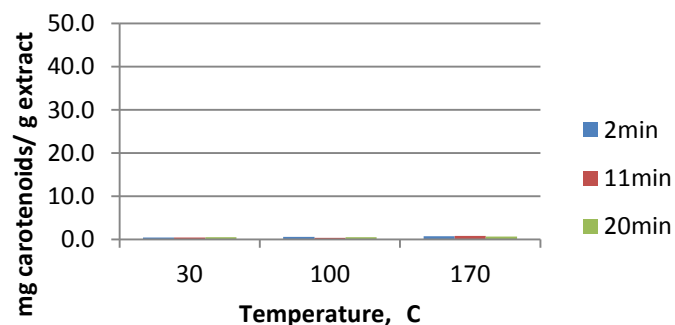
Temperature: 170 °C

%EtOH: 100%

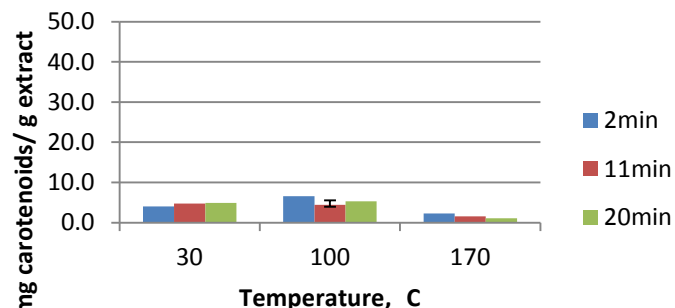
TEAC: 0.603 mmol Trolox/g extract

Total Carotenoids

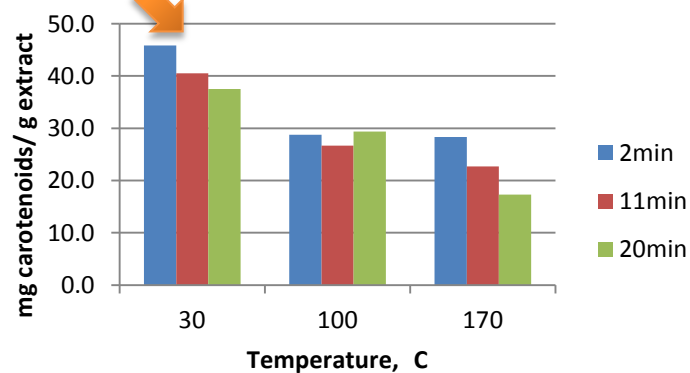
Water extraction



EtOH/Water 50:50 (v/v)

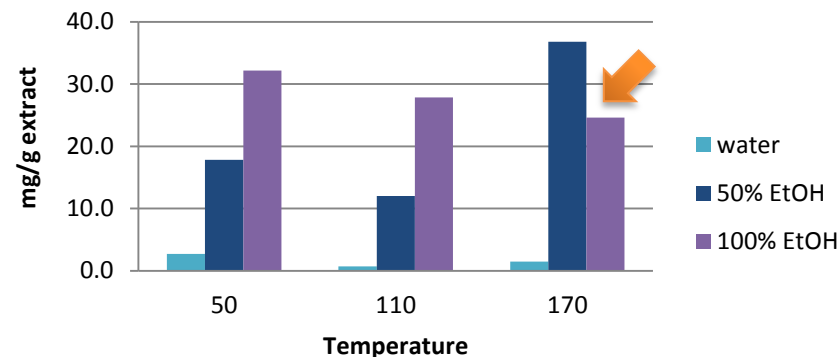


Ethanol extraction



PLE

$R^2 = 0.7983$



Experimental Design PLE 3²

Optimum values proposed:

Extraction time: 20 min

Temperature: 170 °C

%EtOH: 100%

Carotenoids: 31.02 mg/ g extract

Experimental Design MAE 3³

Optimum values proposed:

Extraction time: 2 min

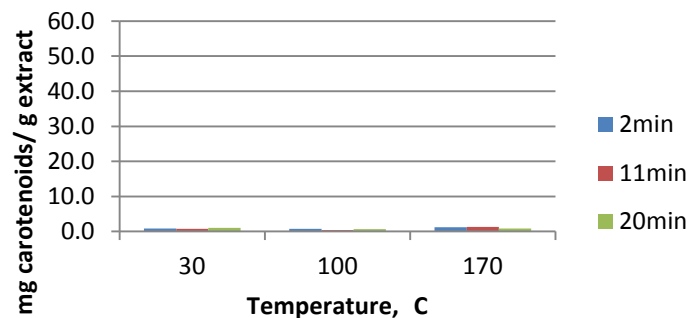
Temperature: 30 °C

%EtOH: 100%

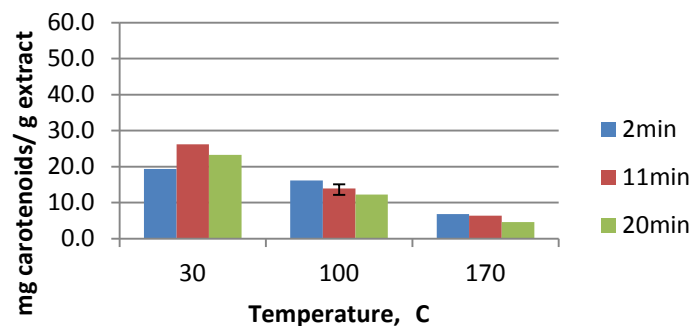
Carotenoids: 41.45 mg/ g extract

Total Chlorophylls

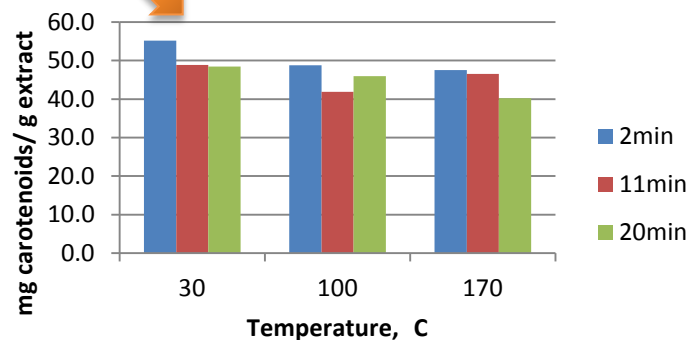
Water extraction



EtOH/Water 50:50 (v/v)

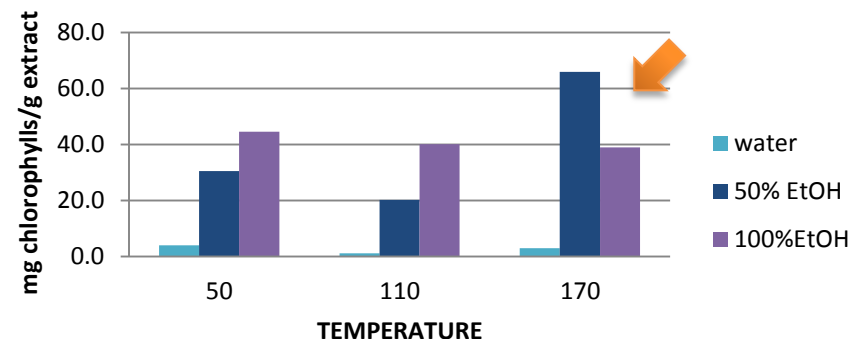


Ethanol extraction



PLE

$R^2 = 0.7651$



Experimental Design PLE 3²

Optimum values proposed:

Extraction time: 20 min

Temperature: 170 °C

%EtOH: 85.9%

Chlorophylls: 50.84 mg/ g extract

Experimental Design MAE 3³

Optimum values proposed:

Extraction time: 2 min

Temperature: 30 °C

%EtOH: 100%

Chlorophylls: 54.58 mg/ g extract

Multiple response optimization

Optimum values proposed:

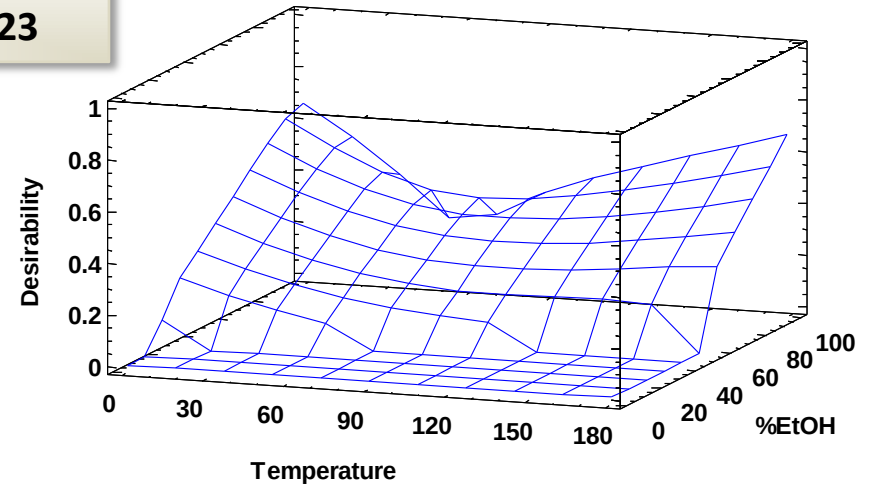
Extraction time: 5.8 min
Temperature: 170 °C
%EtOH: 100%

Yield: 28.57 %(w/w)
TPC: 35.18 mg GA/ g extract
TEAC: 0.580 mmol Trolox/g extract
Carotenoids: 24.30 mg/ g extract
Chlorophylls not considered

MAE

Desirability: 0.623

Estimated Response Surface
Extraction time=11.0



Optimum values proposed:

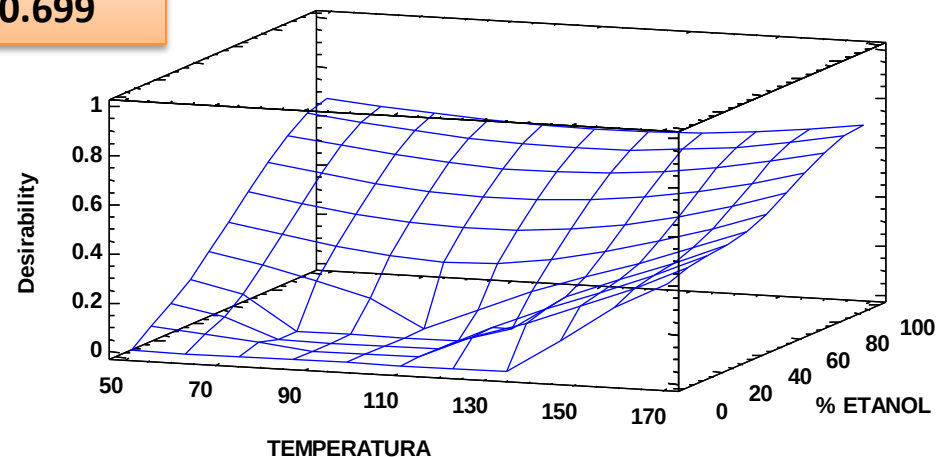
Extraction time (fixed): 20 min
Temperature: 170 °C
%EtOH: 97.7%

Yield: 34.36 %(w/w)
TPC: 31.68 mg GA/ g extract
TEAC: 0.522 mmol Trolox/g extract
Carotenoids: 30.93 mg/ g extract
Chlorophylls not considered

PLE

Desirability: 0.699

Estimated Response Surface



Multiple response optimization

Optimum values proposed:

MAE

Desirability: 0.941

Extraction time: 2 min

Temperature: 30 °C

%EtOH: 100%

Yield not considered

TPC: 44.04 mg GA/ g extract

TEAC: 0.571 mmol Trolox/g extract

Carotenoids: 41.45 mg/ g extract

Chlorophylls: 54.58 mg/ g extract

Optimum values proposed:

PLE

Desirability: 0.829

Extraction time (fixed): 20 min

Temperature: 50 °C

%EtOH: 100%

Yield not considered

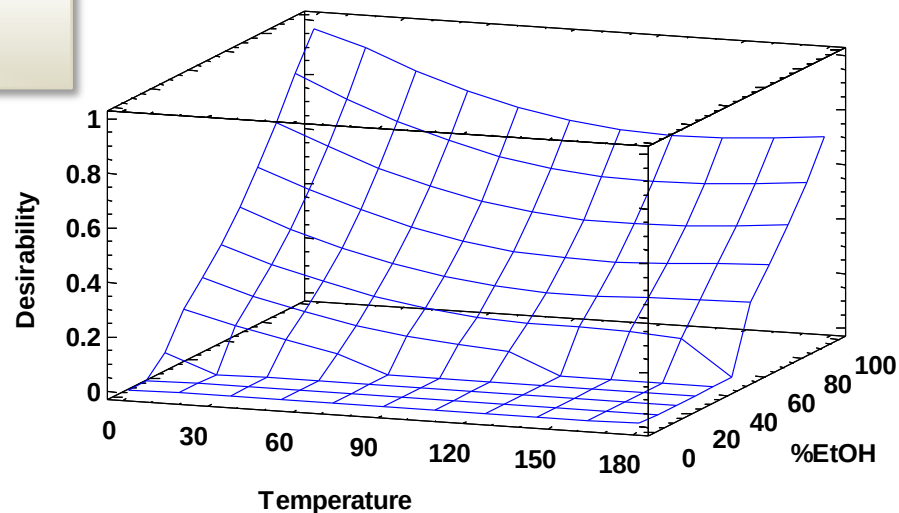
TPC: 39.55 mg GA/ g extract

TEAC: 0.592 mmol Trolox/g extract

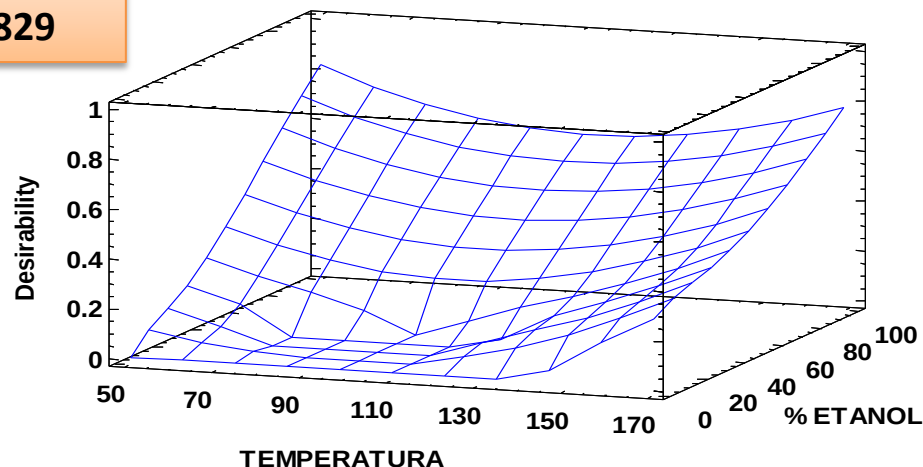
Carotenoids: 30.82 mg/ g extract

Chlorophylls: 42.48 mg/ g extract

Estimated Response Surface
Extraction time=11.0



Estimated Response Surface



Validation of proposed model – High temperature optimum

	Predicted	Experimental data	RSD(%)
MAE	Extraction time: 5.8 min	Extraction time: 6 min	
	Temperature: 170 °C	Temperature: 170 °C	
	%EtOH: 100%	%EtOH: 100%	
	Yield: 28.57 %(w/w)	Yield: 22.20	17.75 ✖
	TPC: 35.18 mg GA/ g extract	TPC: 30.50	10.09
	TEAC: 0.580 mmol Trolox/g extract	TEAC: 0.68	11.59
	Carotenoids: 24.30 mg/ g extract	Carotenoids: 24.79	1.40
	Chlorophylls not considered	Chlorophylls: 52.94	-
PLE	Extraction time (fixed): 20 min	Extraction time (fixed): 20 min	
	Temperature: 170 °C	Temperature: 170 °C	
	%EtOH: 97.7%	%EtOH: 97%	
	Yield: 34.36 %(w/w)	Yield: 38.78	8.56
	TPC: 31.68 mg GA/ g extract	TPC: 30.98	1.57
	TEAC: 0.522 mmol Trolox/g extract	TEAC: 0.54	1.97
	Carotenoids: 30.93 mg/ g extract	Carotenoids: 16.99	41.13 ✖
	Chlorophylls not considered	Chlorophylls: 37.84	-

Validation of proposed model – Low temperature optimum

Predicted

Experimental data

RSD(%)

MAE

Extraction time: 2 min

Temperature: 30 °C

%EtOH: 100%

Yield not considered

TPC: 44.04 mg GA/ g extract

TEAC: 0.571 mmol Trolox/g extract

Carotenoids: 41.45 mg/ g extract

Chlorophylls: 54.58 mg/ g extract

Extraction time: 2 min

Temperature: 30 °C

%EtOH: 100%

Yield: 14.51

TPC: 44.19

TEAC: 0.71

Carotenoids: 41.42

Chlorophylls: 58.61

-

0.25

14.95

0.20

5.04

PLE

Extraction time (fixed): 20 min

Temperature: 50 °C

%EtOH: 100%

Yield not considered

TPC: 39.55 mg GA/ g extract

TEAC: 0.591 mmol Trolox/g extract

Carotenoids: 30.82 mg/ g extract

Chlorophylls: 42.48 mg/ g extract

Extraction time (fixed): 20 min

Temperature: 50 °C

%EtOH: 100%

Yield: 23.95

TPC: 38.15

TEAC: 0.59

Carotenoids: 33.96

Chlorophylls: 54.36

-

2.56

0.55

6.86

17.34 ✖

DAD1 A, Sig=450,2 Ref=off (CAROT_BIENVE\PHAEODACTYLUM ASE\PH_170_97-1.D)

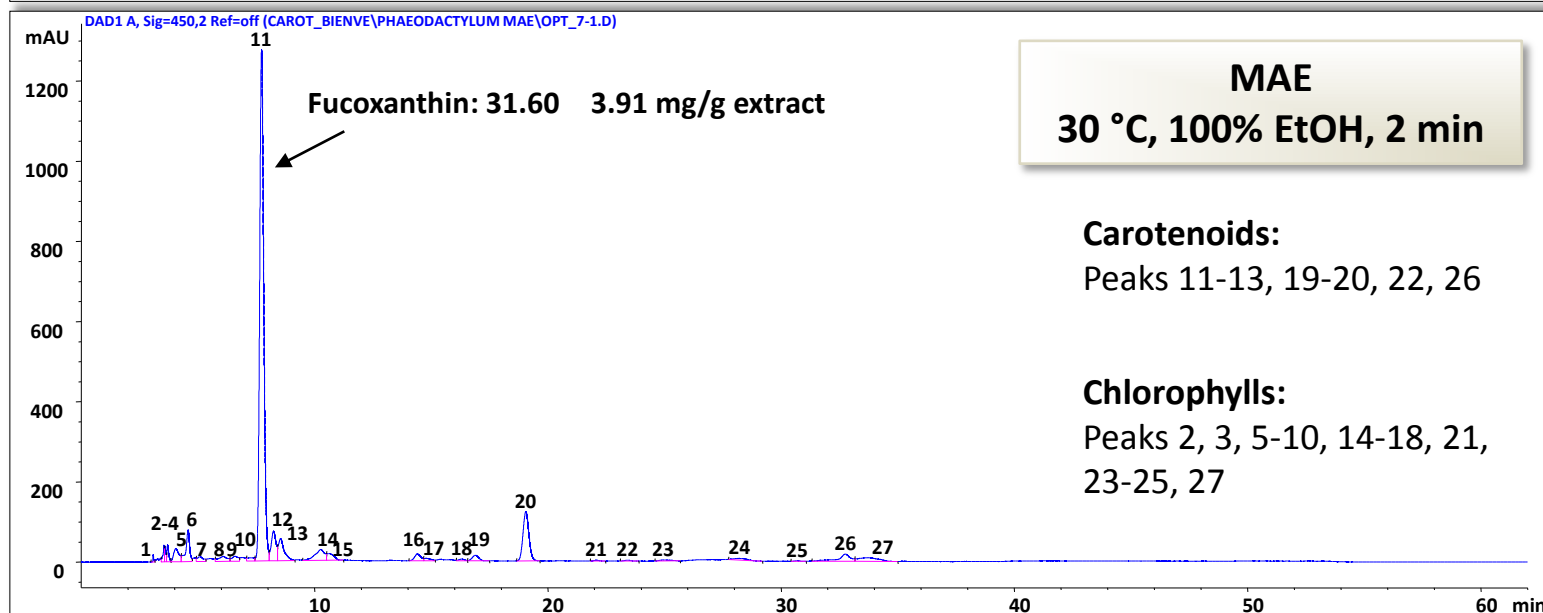
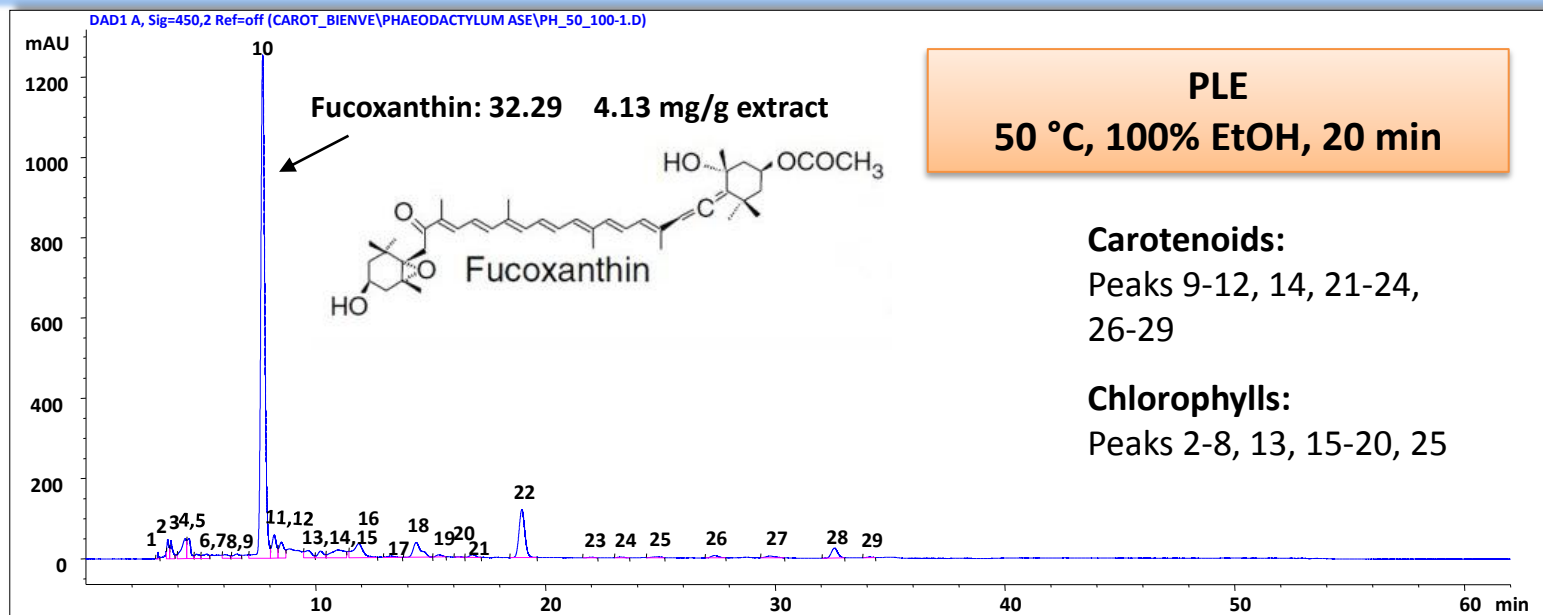
The chromatogram displays absorbance (mAU) on the y-axis (0 to 500) against time (min) on the x-axis (0 to 60). A prominent peak at 14.99 minutes is labeled 'Fucoxanthin: 14.99 0.56 mg/g extract' with an arrow. Other peaks are numbered 1 through 21. Peaks 4-7, 10-11, 14-15, and 20-21 are associated with carotenoids, while peaks 3, 8-9, 12-13, and 16-19 are associated with chlorophylls. A pink shaded region covers the baseline from approximately 10 to 35 minutes.

PLE
170 °C, 97% EtOH, 20 min

Carotenoids:
Peaks 4-7, 10-11, 14-15, 20-21

Chlorophylls:
Peaks 3, 8-9, 12-13, 16-19

Refs: M. Castro-Puyana et al., *Anal. Bioanal. Chem.* (2013), 405, 4607; B. Gilbert-López et al., *Green Chemistry* (2015), 17, 4599-4609



P. tricornutum - MAE vs. PLE

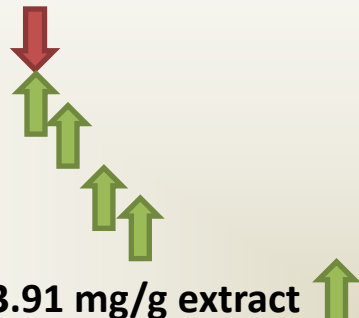
MAE

Extraction time: 6 min
Temperature: 170 °C
%EtOH: 100%

Yield: 22.20 %
TPC: 30.50 mg GAE/g extract
TEAC: 0.68 mmol Trolox/g extract
Carotenoids: 24.79 mg/g extract
Chlorophylls: 52.94 mg/g extract
Fucoxanthin: 13.38 0.54 mg/g extract

Extraction time: 2 min
Temperature: 30 °C
%EtOH: 100%

Yield: 14.51 %
TPC: 44.19
TEAC: 0.71
Carotenoids: 41.42
Chlorophylls: 58.61
Fucoxanthin: 31.60 3.91 mg/g extract



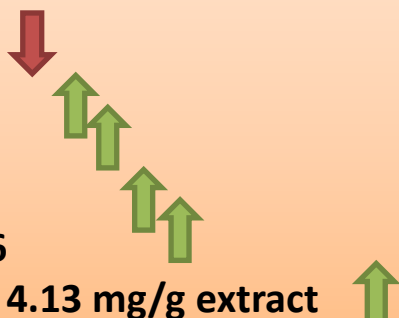
PLE

Extraction time (fixed): 20 min
Temperature: 170 °C
%EtOH: 97%

Yield: 38.78 %
TPC: 30.98
TEAC: 0.54
Carotenoids: 16.99
Chlorophylls: 37.84
Fucoxanthin: 14.99 0.56 mg/g extract

Extraction time (fixed): 20 min
Temperature: 50 °C
%EtOH: 100%

Yield: 23.95 %
TPC: 38.15
TEAC: 0.59
Carotenoids: 33.96
Chlorophylls: 54.36
Fucoxanthin: 32.29 4.13 mg/g extract



CONCLUDING REMARKS

- ✓ **Similar results** are obtained using either **MAE** or **PLE**.
- ✓ MAE: Response surface for yield do not predict well the experimental results.
- ✓ PLE: Response surface for carotenoids do not predict well the experimental results.
- ✓ **Ethanol is the best solvent** to obtain extracts with antioxidant activity and rich in fucoxanthin.
- ✓ **High temperature** to increase extraction yield lead to **1/2 concentration** of **fucoxanthin** compared to the extraction at low temperature.

Foodomics Group

Institute of Food Science Research – CIAL
Spanish Council for Scientific Research - CSIC



***THANKS FOR
YOUR
ATTENTION!!!***