CAS Key Laboratory of Analytical Chemistry for Living Biosystems
中国科学院活体分析化学重点实验室

Green Foodomics: new approaches towards the discovery of functional food ingredients with antiproliferative activity

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

Foodomics

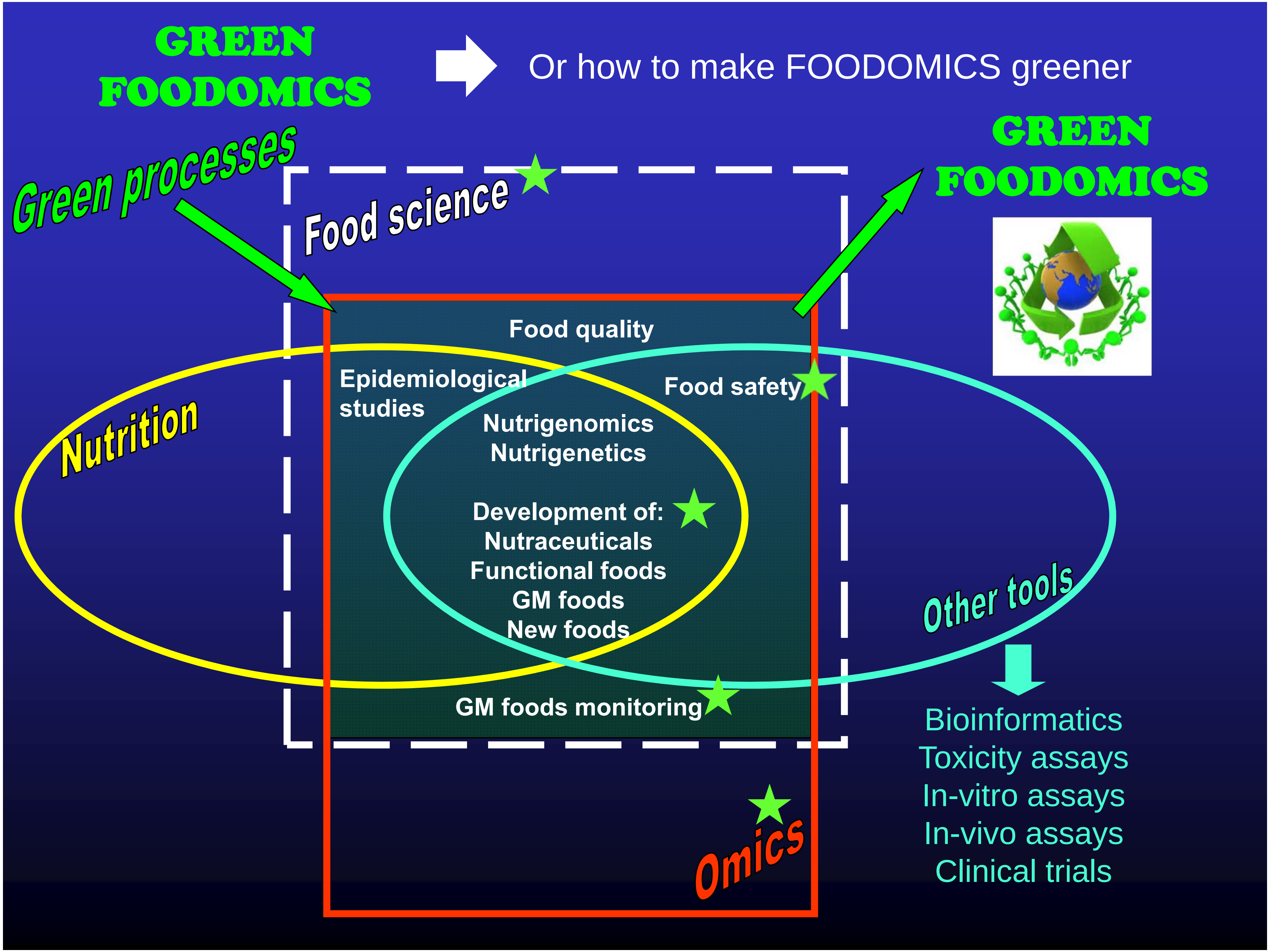
We defined **Foodomics** for the first time in a SCI journal as:

A discipline that studies the Food and Nutrition domains through the application of omics technologies.

(A. Cifuentes, *J. Chromatogr. A* 1216 (2009) 7109).

The interest in Foodomics coincides with a clear shift in medicine and biosciences toward prevention of future diseases.

IMPROVING CONSUMERS WELL-BEING AND CONFIDENCE, FULFILLING LEGISLATION


GREEN FOODOMICS
Or how to make FOODOMICS greener
GREEN FOODOMICS
Green processes
Food science
Food quality
Epidemiological studies
Food safety
Nutrition
Nutrigenomics
Nutrigenetics
Development of:
Nutraceuticals
Functional foods
GM foods
New foods
Other tools
GM foods monitoring
Bioinformatics
Toxicity assays
In-vitro assays
In-vivo assays
Clinical trials
Omics


Choice of biocompatible, renewable, safe & non-toxic reagents
Minimise byproducts & hazardouse waste
Minimise energy usage
Green Chemistry
Safer disposal & recycling
Less of certain scarce raw resources

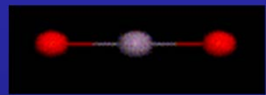
CHALLENGES IN THE DEVELOPMENT OF GREEN EXTRACTION PROCESSES

FAST

SELECTIVE

GREEN

COST-EFFECTIVE

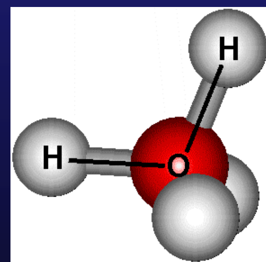


HIGH YIELDS

SUSTAINABLE

EFFICIENT

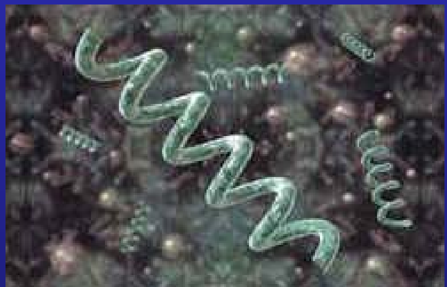
SUSTAINABLE PROCESSES



Without organic solvents

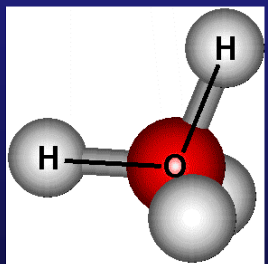
CHALLENGE

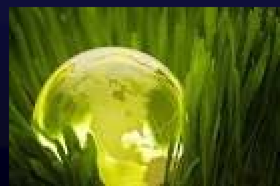
EXTRACTION BIOACTIVE COMPOUNDS FROM NATURAL SOURCES THROUGH GREEN PROCESSES



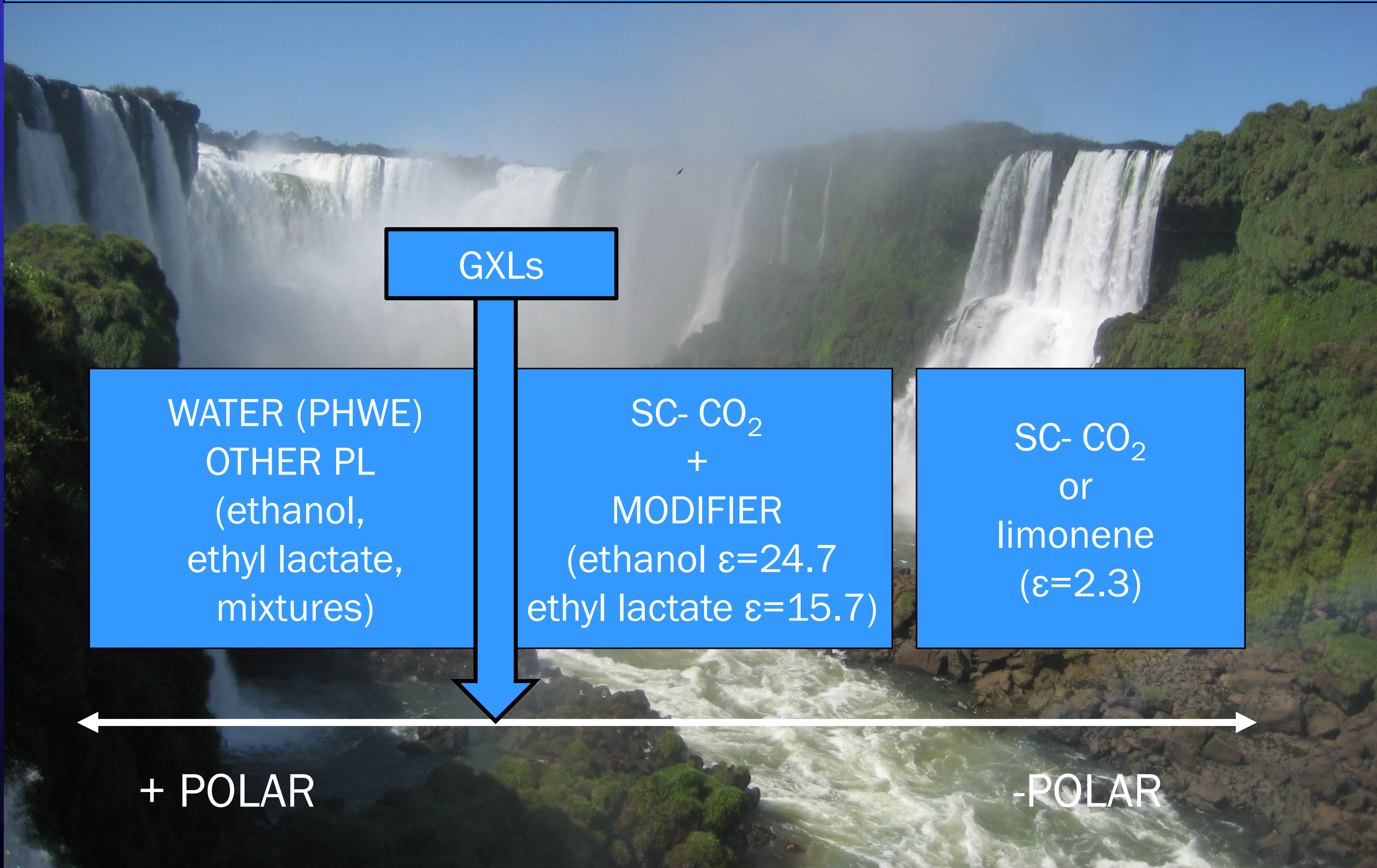
INTENSIFICATION OF PROCESSES

PRESSURE
TEMPERATURE
MICROWAVES
ENZYMES

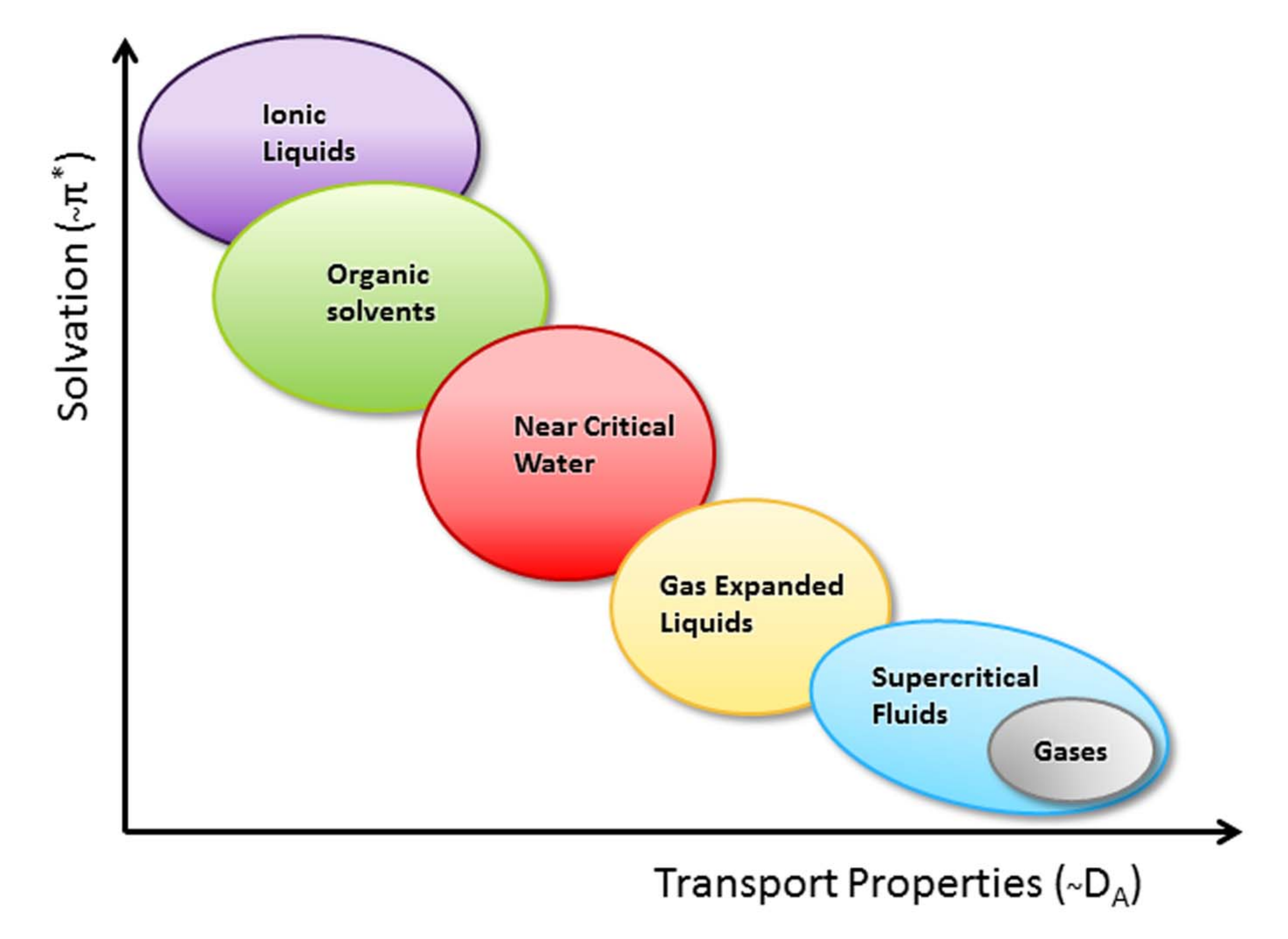




"GREEN" COMPRESSED FLUIDS AND ASSOCIATED TECHNOLOGIES



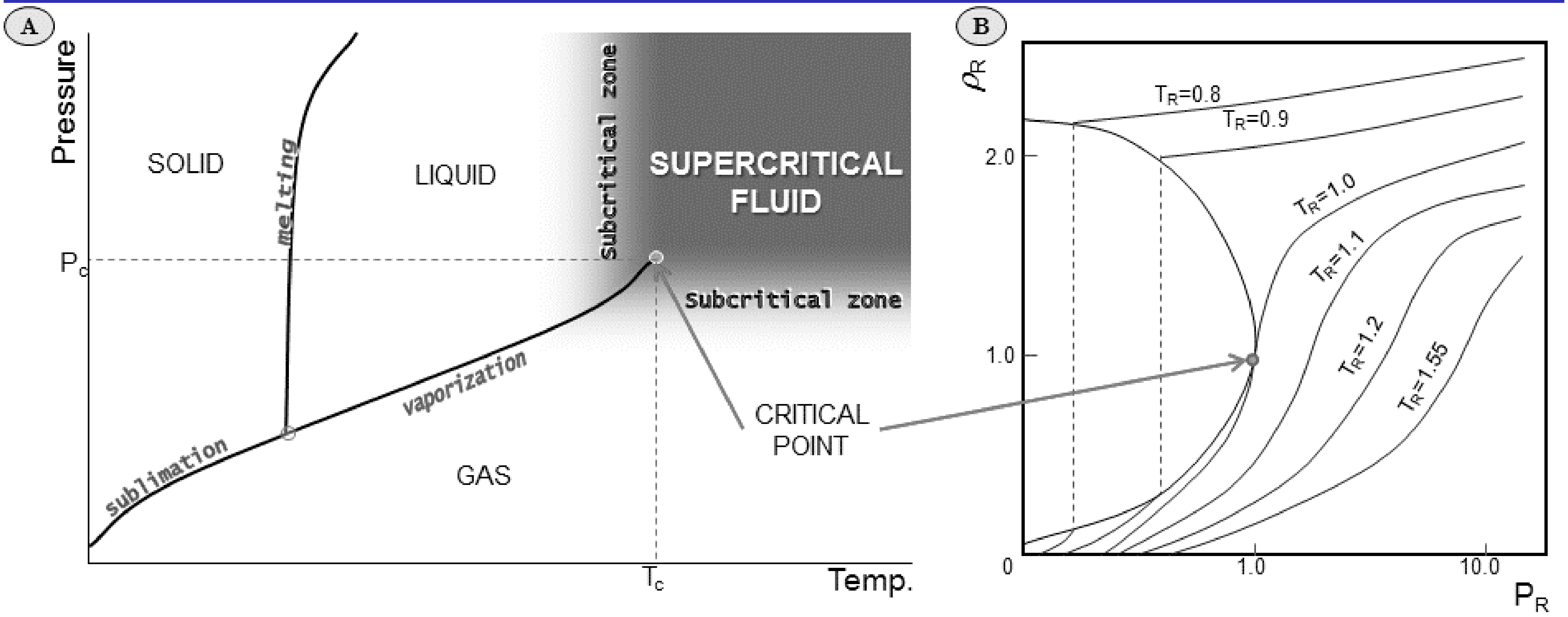
SOLVENTS FOR SUSTAINABLE CHEMICAL PROCESSES



Green Chem, 2014, 16, 1034

Supercritical fluids

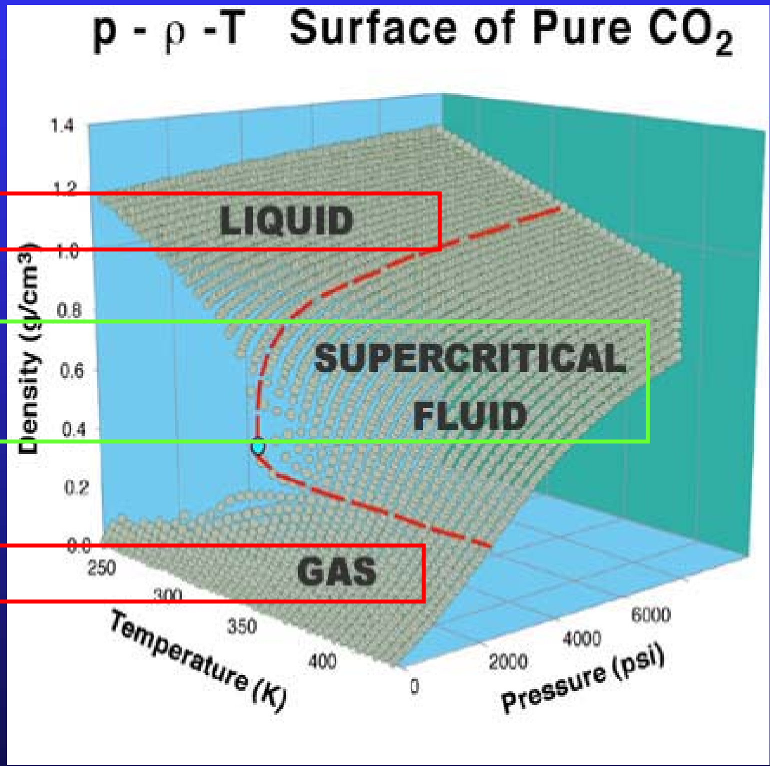
Fluids which P and T conditions are above its critical point.
Intermediate properties between L and G



Supercritical Fluids

High solvating power

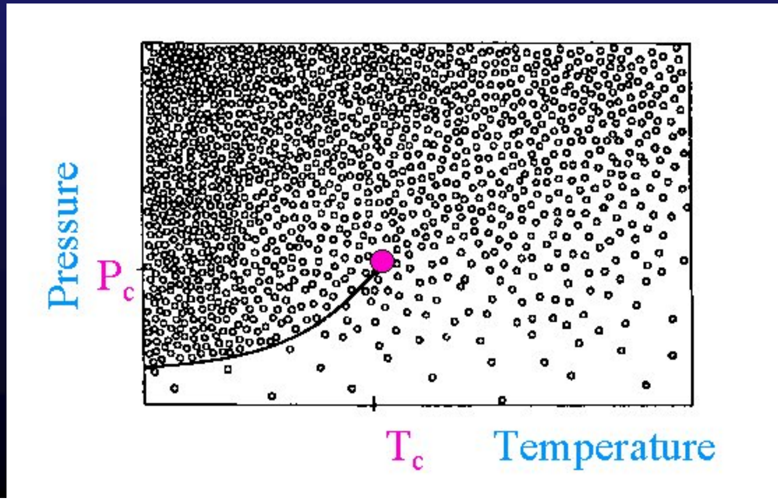
Good transport properties

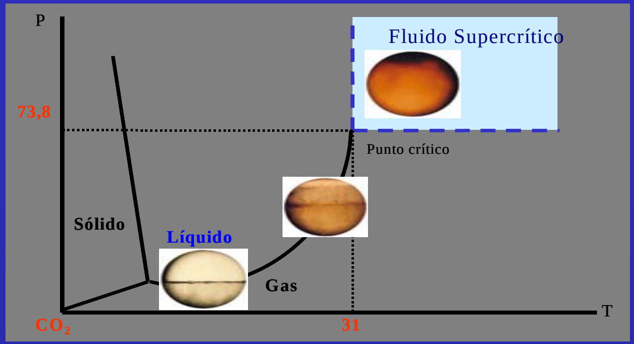


Supercritical Fluid Extraction (SFE)

- Tunable selectivity (P, δ)
- High mass transfer
- On-line fractionation
- Spontaneous removal of solvents
- The raw material is not destroyed during processing (further biomass transformation)

… others that depend on the SF employed





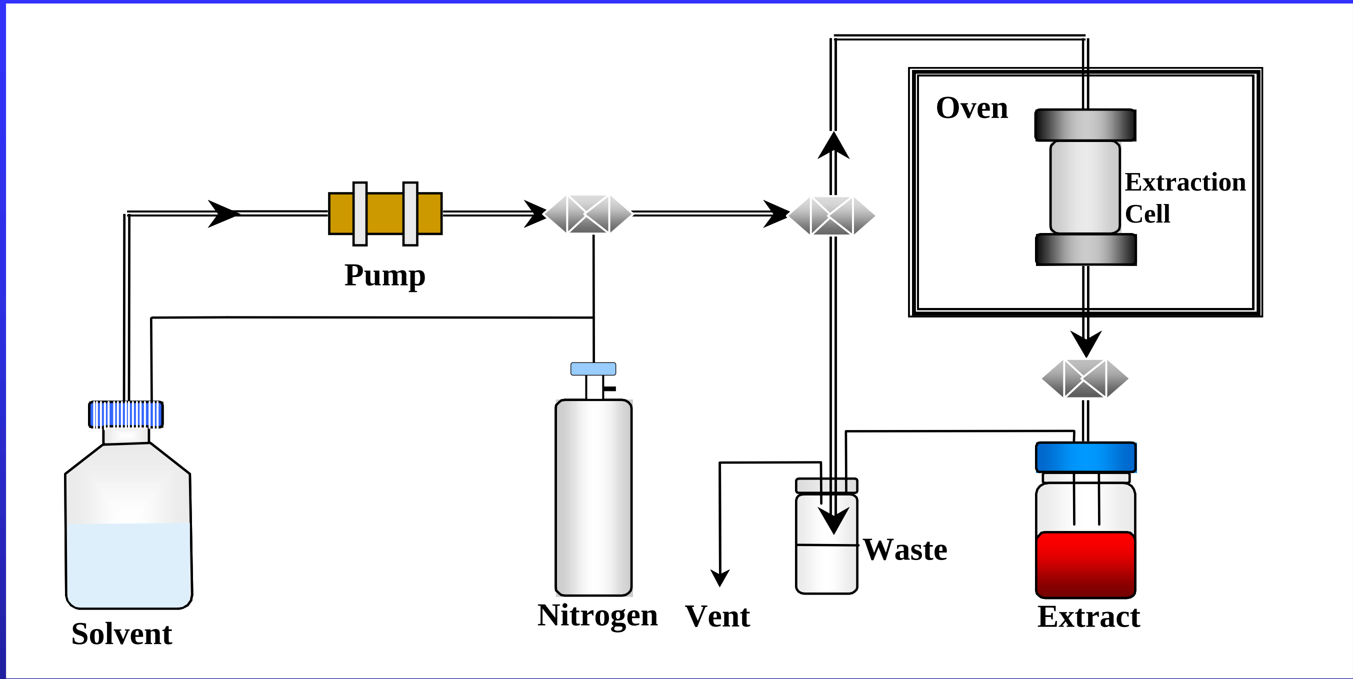
Natural products extraction using SC CO_2 - Advantages

- Favorable critical values (Tc 31.1 ºC; Pc 72.0 bar; δc 0.47 gcm-3)
- GRAS (to overcome legal restrictions applied to organic solvents and to allowed residues in products for human use)
- Ecological (CO_2 from the atmosphere, is not generated in the process)
- It is possible to obtain natural products with very low levels (or even without) residues using SC-CO_2 at low temperatures
- Non explosive
- Cheap
- CO_2 + ethanol (GRAS) can be employed to extract more polar compounds

Extraction using SC-CO_2 -Drawbacks

- **Low affinity for medium and high polarity compounds.** The main problem is that most bioactive compounds are polar.

GREEN TECHNOLOGIES-PLE



- Solvents maintained in **liquid state** and used at **T > boiling point (high pressures 10-15 Mpa)**
- **Faster** extraction processes
- **Low volumes** used of organic solvents
- Raw material placed in an **oxygen and light-free environment**
- Selectivity will depend on the solvent used (**wide range polarities**)

Pressurized Liquid Extraction (PLE)

Advantages

- PLE is faster than conventional liquid extraction processes: min vs h
- PLE uses less amount of solvents mL vs L
- High yields and recoveries independently of the matrix
- Can extract polar and non polar compounds depending on the solvent selected
- Easy to scale up

Drawbacks

- High extraction yields but lower selectivity than SFE
- PLE uses high T than SFE but the atmosphere is free of O2
- No industrial equipments for PLE although the scaling up is not difficult and the P requirements are lower than in SFE

SUBCRITICAL WATER EXTRACTION (SWE)

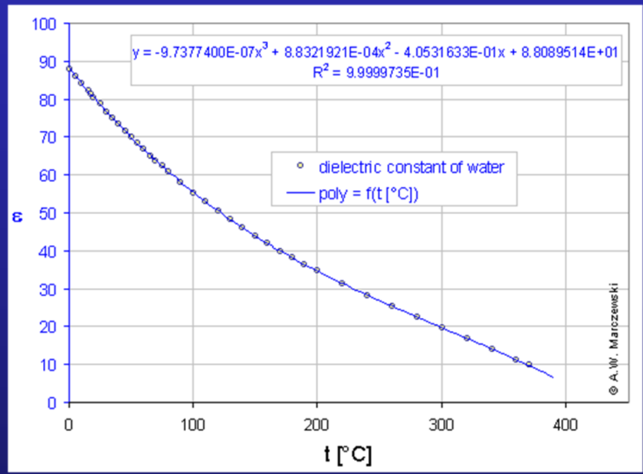
Subcritical water uses temperatures below the critical point (374º C and 218 bar) and pressures high enough to guarantee the liquid state

T ↑ ↓

Dielectric constant (ε)

Viscosity

Surface Tension



	25 ºC	300 ºC
Dielectric constant (ε)	**80**	**20**
Similar to organic solvents	**None** [illegible]	**MetOH, ACN** [illegible]
Extracted compounds	**High polarity**	**Low polarity**

SUBCRITICAL WATER AS ALTERNATIVE TO EXTRACT COMPOUNDS WITH HIGH-MEDIUM-LOW POLARITY

Subcritical water extraction (SWE)

Advantages

- Can extract polar-medium-non polar compounds depending on the temperature
- Very high selectivity with high extraction yields
- No oxidation reactions occur (no O_2 in the media)
- Abundant solvent, cheap and readily available
- No residues in the raw material or the extract
- Raw material can be re-extracted or re-used
- The greenest solvent!!!!!

Drawbacks

- The extraction mechanism is not completely understood
- Some degradation reactions can occur (mainly Maillard and caramelization reactions if carbohydrates-proteins/aminoacids are present)
- Need for development of industrial equipment
- Extracts obtained are dissolved in water although new processes are being developed to overcome this problem


OUR PROPOSAL

GREEN PROCESSING PLATFORM

Low polarity

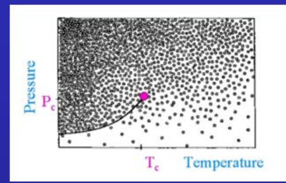
$Sc\text{-}CO_2$
Limonene
PLE, SFE

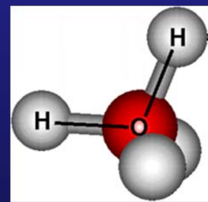
Medium polarity

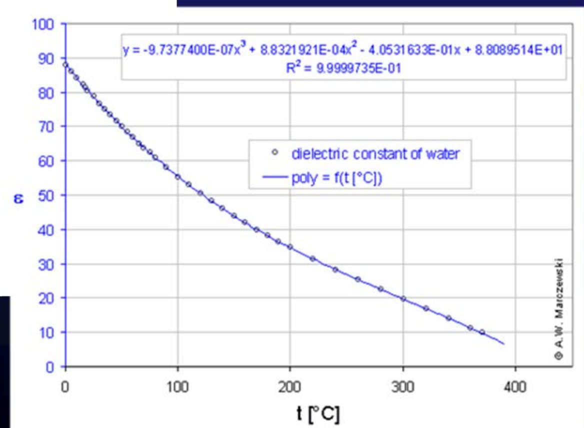
Pressurized solvents
$Sc\text{-}CO_2$+polar modifiers (EtOH)
Gas Expanded Liquids
PLE, SFE, GXL

High polarity

Water
Pressurized solvents
SWE, PLE







SUPERCRITICAL ANTISOLVENT FRACTIONATION OF ROSEMARY EXTRACTS OBTAINED BY PRESSURIZED LIQUID EXTRACTION TO ENHANCE THEIR ANTIPROLIFERATIVE ACTIVITY

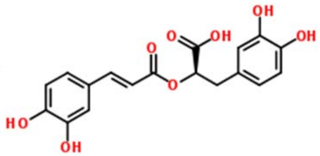
A. P. Sánchez-Camargo, J. A. Mendiola, A. Valdés, M. Castro-Puyana, V. García-Cañas, A. Cifuentes, M. Herrero, E. Ibáñez

Laboratory of Foodomics, Institute of Food Science Research (CIAL, CSIC), Madrid, Spain.

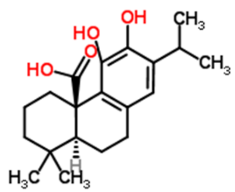
INTRODUCTION

ROSEMARY: Polyphenols with anti-proliferative effect *in-vitro* on human cancer cells

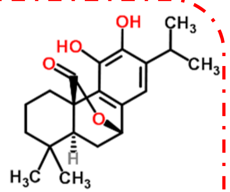
Rosmarinus officinalis



Rosmarinic Acid (RA)



Carnosic Acid (CA)



Carnosol (CS)

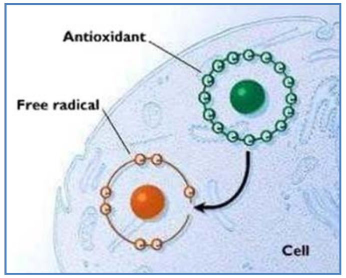
Chemoprentive Activities
Cancer cells

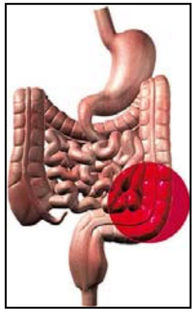
Leukemia

Skin

Breast

Potent antioxidants





Colon Cancer: 32,000 new cases in Spain (2012) (AECC, 2014[1])

[1]AECC, Asociación Española Contra el Cancer

INTRODUCTION

Our approach :

Find new strategies using green processes directed to the enrichment of polyphenols

SFE fractionation

JOURNAL OF FUNCTIONAL FOODS 11 (2014) 293–303

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jff

ELSEVIER

Two-step sequential supercritical fluid extracts from rosemary with enhanced anti-proliferative activity

Andrea del Pilar Sánchez-Camargo [a], Alberto Valdés [a], Giuseppe Sullini [a,b], Virginia García-Cañas [a], Alejandro Cifuentes [a], Elena Ibáñez [a,], Miguel Herrero [a]*

CA+CS (ca. 40-45% w/w)

Antiproliferative activity: 2-fold comparing with the single-step process

INTRODUCTION

Pressurized Liquid Extraction Vs SFE

Journal of Chromatography A, 1217 (2010) 2512–2520

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

ELSEVIER

Green processes for the extraction of bioactives from Rosemary: Chemical and functional characterization via ultra-performance liquid chromatography-tandem mass spectrometry and in-vitro assays

M. Herrero[a,b], M. Plaza[b], A. Cifuente

PLE

EtOH/H_2O

150-200°C (100bar)

RA   CA+CS

Genes Nutr (2013) 8:43–60
DOI 10.1007/s12263-012-0311-9

RESEARCH PAPER

Effect of rosemary polyphenols on human colon cancer cells: transcriptomic profiling and functional enrichment analysis

Alberto Valdés · Virginia García-Cañas · Lourdes Rocamora-Reverte · Ángeles Gómez-Martínez · José Antonio Ferragut · Alejandro Cifuentes

2314   Electrophoresis 2012, 33, 2314–2327

Alberto Valdés[1]
Carolina Simó[1]
Clara Ibáñez[1]
Lourdes Rocamora-Reverte[2]
José Antonio Ferragut[2]
Virginia García-Cañas[1]
Alejandro Cifuentes[1]

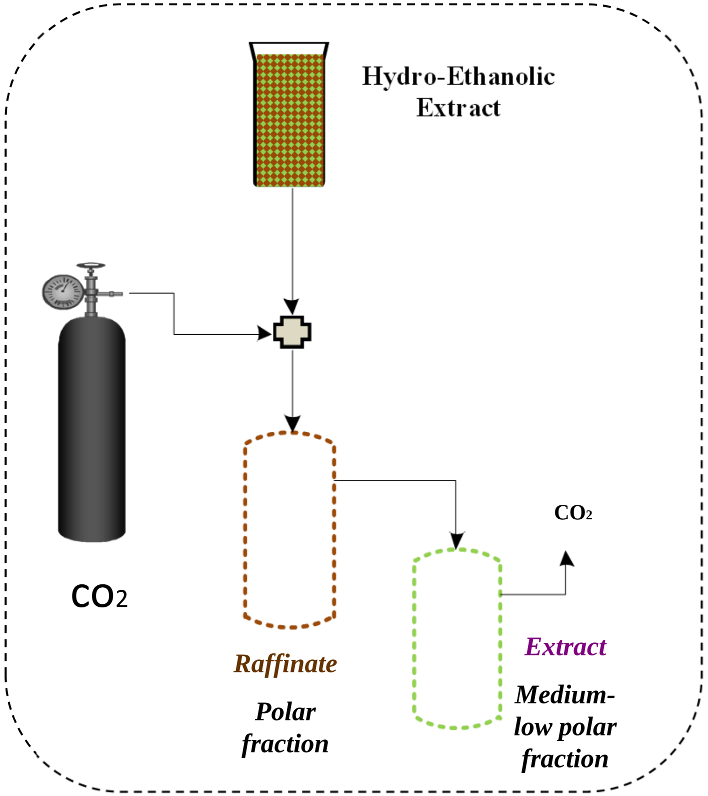
Research Article

Effect of dietary polyphenols on K562 leukemia cells: A Foodomics approach

INTRODUCTION

Supercritical Antisolvent Fractionation (SAF)

Other alternative approach



Advantages of SAF

- ✓ Able to work in continuous mode
- ✓ Relative amount of polar/non-polar compounds can be predicted (fluid phase equilibrium)
- ✓ No use of toxic solvents

INTRODUCTION

Phase equilibrium for CO_2-Ethanol-Water

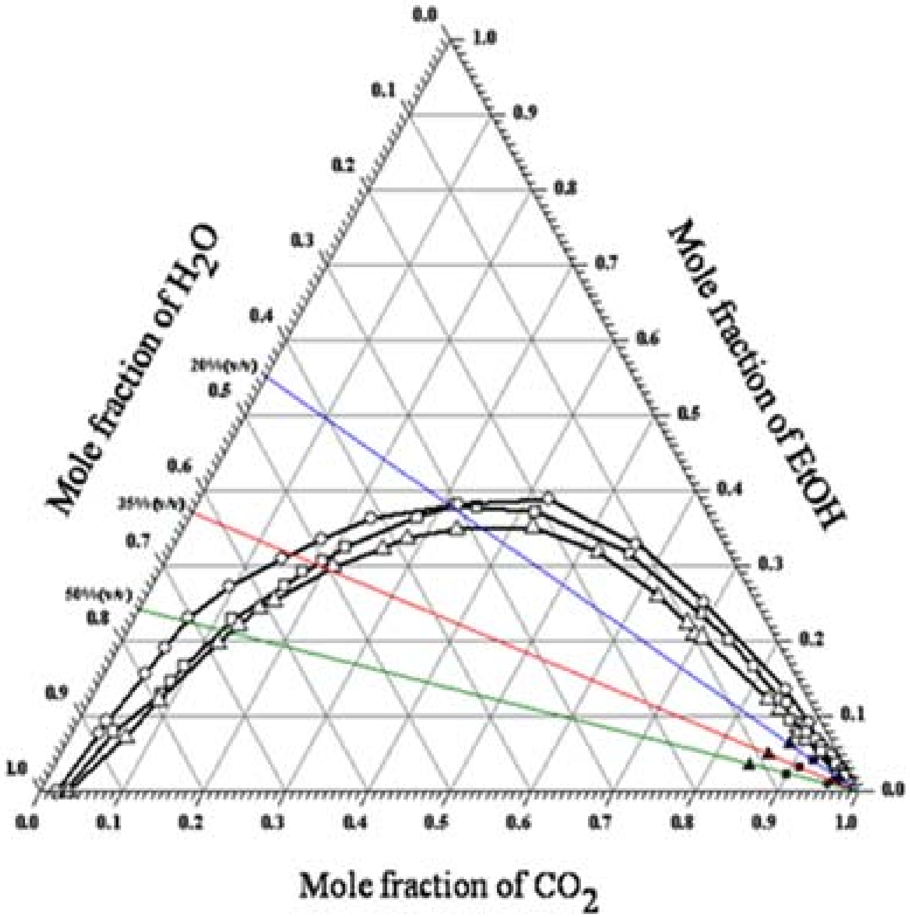
Solvent selectivity & ethanol partition coefficients

Main influencing factors involved:

Feed/SC-CO2 → Pressure → %H2O feed

↓

Optimization according the compounds' nature



OBJECTIVE

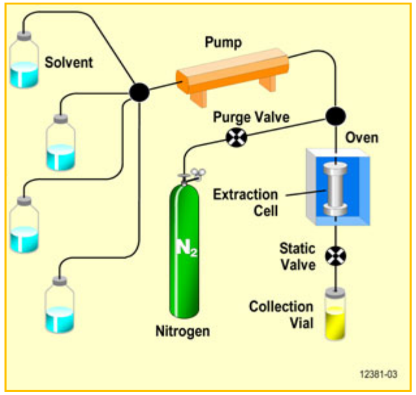
To optimize the single-step supercritical antisolvent fractionation (SAF) of a rosemary PLE extract (obtained using a mixture of ethanol and water as extracting solvent), in order to produce a CA+CS-enriched fraction which is expected to be more active against human colon cancer cells (HT-29)

Workflow

Rosemary leaves

Dryed by sunlight + Grinded (500-999µm)

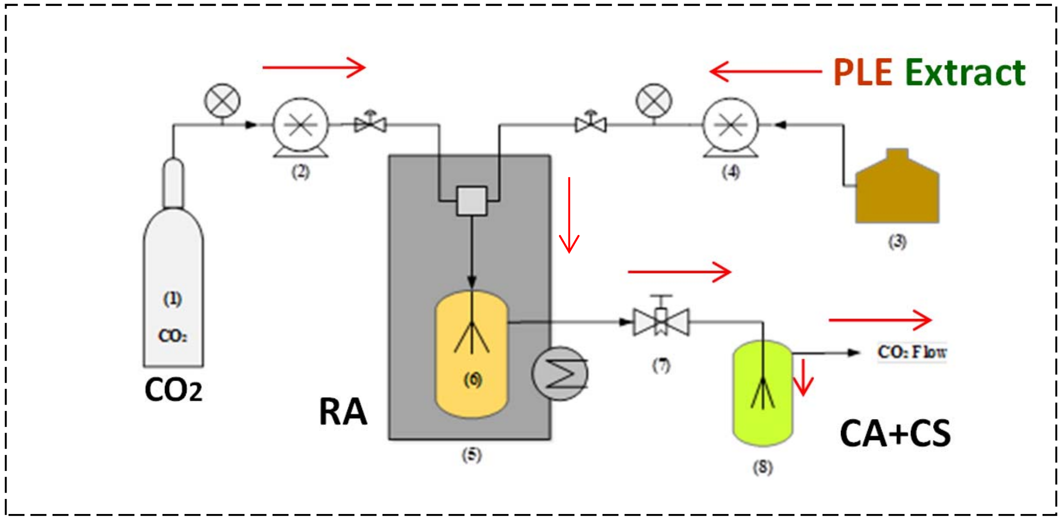
Pressurized Liquid Extraction (PLE)



T = 150°C
P = 100 bar
t= 20 min
Ethanol: water (80:20)

Supercritical Antisolvent Fractionation (SAF)

- T=40°C
- CO_2 flow = 2mL/min
- Fractionation time = 120 min



Optimization of SAF Conditions

3-level factorial design (2^3)
including 3 central points
Surface Response Methodology

Experimental Factors

Variables	Low	High	Unit
Pressure	100	300	Bar
% H_2O in feed	20	50	% (v/v)
Feed/SC-CO_2	0.025	0.1	-

Response variables

Response	Unit
RA and CA+CS	mg/g
Total Phenolic Content	mg GAE/g extract
Antioxidant Activity	mMol Trolox/g extract
EC_{50}	µg/mL
Cell Viability	%

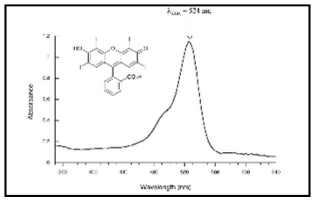
Multiple Response Optimization

Methodology

PLE Hydro-alcoholic extract – raffinate - extract

Total phenols content

Folin-Ciocalteu



Antioxidant Activity

***In vitro* assays**

TEAC Assay

DPPH radical EC50

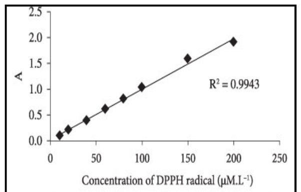
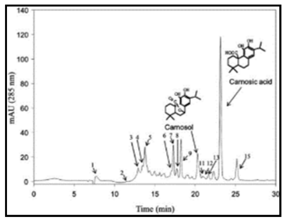


Figure 2. Absorbance of DPPH radical solutions prepared in methanol

UHPLC-MS

Quantification of phenolic compounds



Rosmarinic Acid
Carnosic Acid
Carnosol

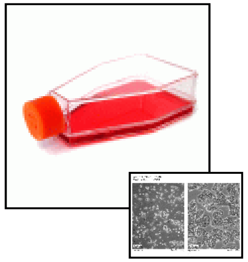
Responses

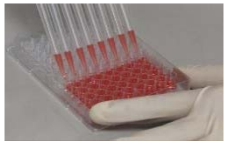
❖ **Anti-proliferative activity against human colon cancer cells.**

PLE Hydro-alcoholic extract,
Extract and **raffinate**

30 µg/mL

Colon adenocarcinoma HT-29 cells

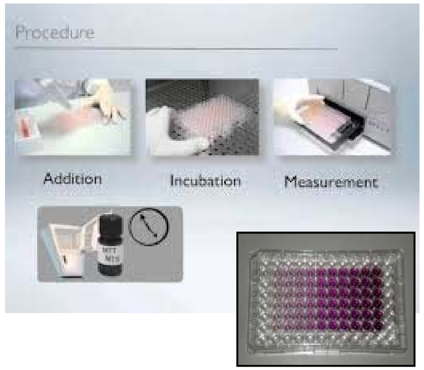




CELL CULTURE
10.000 cells/cm^2
96-well plate

24 h

MTT Assay



% Cell Viability

RESULTS

PLE

rosemary extraction:

upstream process

+

SAF

Supercritical Antisolvent Fractionation process:

downstream process

PLE Rosemary Extract Characterization

Yield: 39.86 % (w/w)

Rosmarinic Acid 25.1 mg/g

Carnosic Acid	109.0 mg/g
Carnosol	20.5 mg/g

Total Phenolic Content
208.32 mg GAE/g

Antioxidant Activity
TEAC : 2.33 mM TE/g extract d.w.b

EC_{50} = 8.51 µg/mL

%Cell Viability = 79

RESULTS

SAF

Supercritical Antisolvent Fractionation process:
downstream process

PLE

rosemary extraction:
upstream process

+

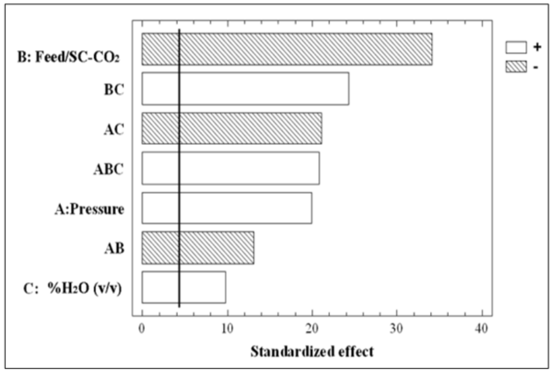
Optimization of the Supercritical Antisolvent Fractionation process: *downstream process*

RAFFINATE

Experim.	P (bar)	Feed/ $SC\text{-}CO_2$	Water in feed (% v/v)	Recovery (% wt.)	RA (mg/g)	CS (mg/g)	CA (mg/g)	TPC (mg GAE/ g extract)	TEAC (mM TE/g extract)	EC50 (mg/mL)	Cell Viability % (± 95%CI)
1	100	0.0250	50	78,7	40.03	8.94	14.83	272.46	3.06	6.29	74.2 ± 2.2
2	100	0.1000	20	93,8	**9.98**	**17.23**	**87.07**	224.27	2.52	8.52	65.0 ± 1.9
3	300	0.1000	20	76,5	14.06	5.97	19.43	239.83	2.58	9.01	72.6 ± 2.6
4	300	0.1000	50	87,7	33.67	4.40	13.74	234.59	2.71	7.95	79.2 ± 1.6
5	100	0.1000	50	96,7	29.90	13.21	49.99	245.20	2.75	8.21	86.0 ± 1.5
6	300	0.0250	50	96,0	34.92	4.58	14.34	239.14	2.64	5.89	88.4 ± 2.7
7 (CP)	200	0.0625	35	84,0	31.95	10.38	22.22	237.46	2.72	8.35	92.7 ± 2.4
8	100	0.0250	20	95,7	24.15	15.61	68.84	195.77	2.27	6.04	77.4 ± 2.7
9 (CP)	200	0.0625	35	83,1	32.82	11.39	23.80	234.11	2.68	7.86	106.0 ± 3.2
10	300	0.0250	20	84,7	**67.66**	8.64	34.80	340.67	4.16	9.32	82.1 ± 3.5
11(CP)	200	0.0625	35	85,6	31.18	11.83	22.38	243.34	2.93	7.87	94.5 ± 2.5

2.7-fold higher compared to PLE feed extract

(a) RA relative amount



Optimization of the Supercritical Antisolvent Fractionation process:
downstream process

EXTRACT

Experim.	P (bar)	Feed/ SC-CO_2	Water in feed (% v/v)	Recovery (% wt)	RA (mg/g)	CS (mg/g)	CA (mg/g)	TPC (mg GAE/ g extract)	TEAC (mM TE/ g extract)	EC50 (µg/mL)	Cell Viability % (± 95%CI)	Recovery %CA+CS (wt.)
1	100	0.0250	50	21,3	< LOQ	132.30	345.80	178.82	2.58	4.95	16.9 ± 2.3	78,5
2	100	0.1000	20	6,2	< LOQ	36.24	120.63	123.90	1.84	9.71	80.6 ± 3.7	7,6
3	300	0.1000	20	23,5	< LOQ	66.28	223.06	142.18	2.01	8.04	62.9 ± 1.9	52,5
4	300	0.1000	50	12,3	< LOQ	84.59	247.71	184.47	2.74	6.45	26.0 ± 1.6	31,5
5	100	0.1000	50	3,3	< LOQ	60.52	341.95	158.97	2.46	6.51	22.0 ± 1.9	10,3
6	300	0.0250	50	4,0	< LOQ	80.84	183.71	151.91	2.37	7.65	31.6 ± 1.9	16,5
7 (CP)	200	0.0625	35	16,0	< LOQ	144.15	197.30	148.09	2.17	8.49	45.6 ± 4.1	22,8
8	100	0.0250	20	4,3	< LOQ	24.33	108.14	117.60	1.75	11.65	36.4 ± 1.5	4,2
9 (CP)	200	0.0625	35	16,9	< LOQ	152.51	189.54	157.74	1.96	8.56	57.1 ± 3.9	24,0
10	300	0.0250	20	15,3	< LOQ	95.21	188.48	152.02	1.93	8.48	51.1 ± 1.4	31,8
11(CP)	200	0.0625	35	14,4	< LOQ	151.29	193.88	159.32	2.02	8.08	46.3 ± 2.4	20,7

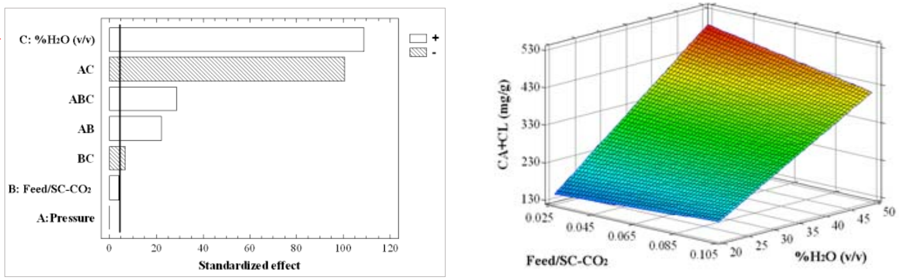
47,81% (w/w) CA+CS
3.7-fold higher compared to PLE feed extract

Improved Anti-proliferative activity :
SCFE Single-Step: 64.5% (33% CA+ CS)
Two-sequential steps: 38.7% (40% CA+CA)
Supercritical Antisolvent Fractionation: 16.9%

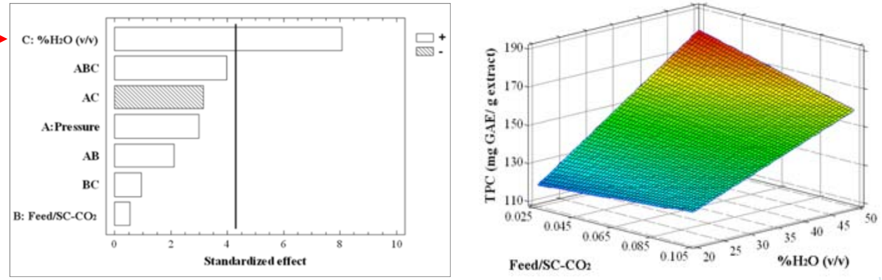
Optimization of the Supercritical Antisolvent Fractionation process:
downstream process

EXTRACT

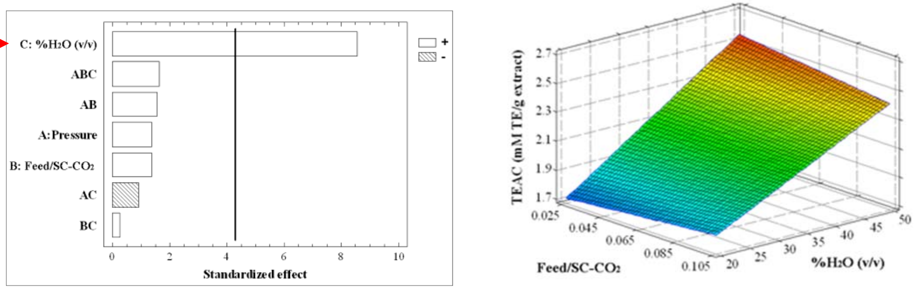
CA+CS relative amount



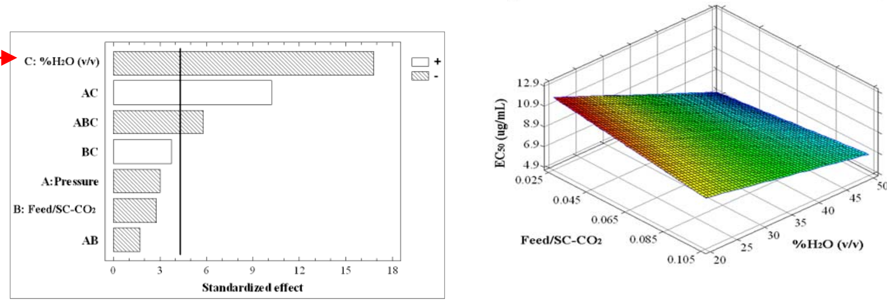
Total Phenolic Content (TPC)



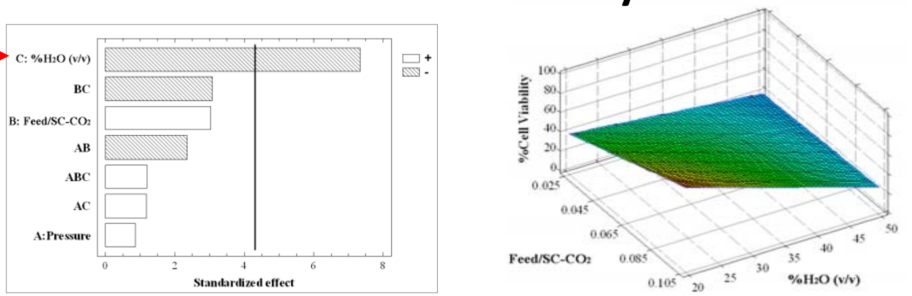
Antioxidant Activity TEAC



Antioxidant activity DPPH



%Cell Viability



Optimal conditions

P= 100 bar
Feed /SC-CO_2 =0.025
%H_2O in feed (v/v) =50

J. of Supercritical Fluids 107 (2016) 581–589

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Supercritical antisolvent fractionation of rosemary extracts obtained by pressurized liquid extraction to enhance their antiproliferative activity

A.P. Sánchez-Camargo[a], J.A. Mendiola[a], A. Valdés[a], M. Castro-Puyana[b], V. García-Cañas[a], A. Cifuentes[a], M. Herrero[a], E. Ibáñez[a,*]

[a] Laboratory of Foodomics, Institute of Food Science Research (CIAL, CSIC-UAM), Nicolas Cabrera 9, Campus de Cantoblanco, 28049 Madrid, Spain
[b] Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, Faculty of Biology, Environmental Science and Chemistry, University of Alcalá, Ctra. Madrid-Barcelona, Km. 33.600, 28871 Alcalá de Henares, Community of Madrid, Spain

Application of Hansen solubility approach for the subcritical and supercritical selective extraction of phlorotannins from brown algae *Cystoseira abies-marina*

A. P. Sánchez-Camargo, M. Herrero, L. Montero, J.A. Mendiola, V. García-Cañas, A. Cifuentes, E. Ibáñez.

Laboratory of Foodomics,

Institute of Food Science Research (CIAL, CSIC), Madrid, Spain

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

INTRODUCTION

Cystoseira abies -marina:

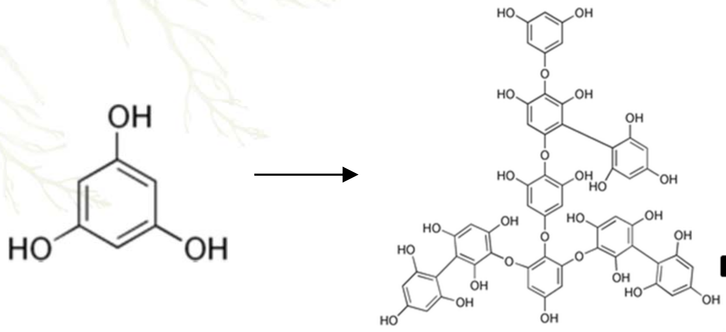
- **Most available species living on Mediterranean Sea and Atlantic Ocean ecosystems**
- **Produced to face biotic and abiotic stress conditions factors**

DEFENSIVE METABOLITES

Antioxidant, antimicrobial, immunomodulatory, anticarcinogenic, anti-inflammatory, among others

PHLOROTANNINS

- **Phlorethols & fuhalols** (ether linkages)
- **Fucols** (phenyl linkages)
- **Fucophlorethols** (ether and phenyl linkages)
- **Eckols** (benzodioxin linkages)



Phloroglucinol

MW 126 Da to 650 kDa

INTRODUCTION

Foodomics Laboratory approach

1. EXTRACTION

Food Chemistry 192 (2016) 67–74

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

ELSEVIER

FOOD CHEMISTRY

Considerations on the use of enzyme-assisted extraction in combination with pressurized liquids to recover bioactive compounds from algae

CrossMark

Andrea del Pilar Sánchez-Camargo[a], Lidia Montero[a], Valérie Stiger-Pouvreau[b], Anaëlle Tanniou[b], Alejandro Cifuentes[a], Miguel Herrero[a,*], Elena Ibáñez[a]

seaweed extract

Optimization extraction conditions

- **Enzyme-assisted extraction Vs.**

  Pressurized Liquid Extraction (PLE)

2. CHARACTERIZATION

1644

Electrophoresis 2014, *35*, 1644–1651

Lidia Montero
Miguel Herrero
Elena Ibáñez
Alejandro Cifuentes

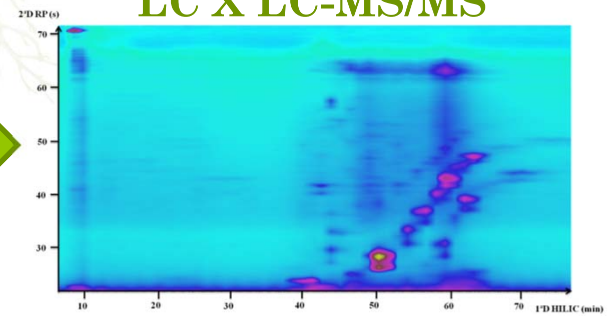
Laboratory of Foodomics, Institute of Food Science Research (CIAL, CSIC), Campus de Cantoblanco, Madrid, Spain

Research Article

Separation and characterization of phlorotannins from brown algae *Cystoseira abies-marina* by comprehensive two-dimensional liquid chromatography

Comprehensive two-dimensional liquid chromatography

LC X LC-MS/MS



X axis → D1 HILIC (diol)→Degree of polymerization

Y axis → D2 RP (C_{18})→ Hydrophobicity

INTRODUCTION

3. BIOACTIVITY ANALYSIS

Journal of Chromatography A, 1428 (2016) 115–125

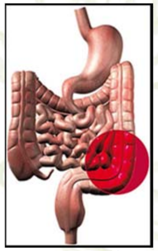
Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Anti-proliferative activity and chemical characterization by comprehensive two-dimensional liquid chromatography coupled to mass spectrometry of phlorotannins from the brown macroalga *Sargassum muticum* collected on North-Atlantic coasts

Lidia Montero[a], Andrea P. Sánchez-Camargo[a], Virginia García-Cañas[a], Anaëlle Tanniou[b], Valérie Stiger-Pouvreau[b], Mariateresa Russo[c], Luca Rastrelli[d], Alejandro Cifuentes[a], Miguel Herrero[a,*], Elena Ibáñez[a]



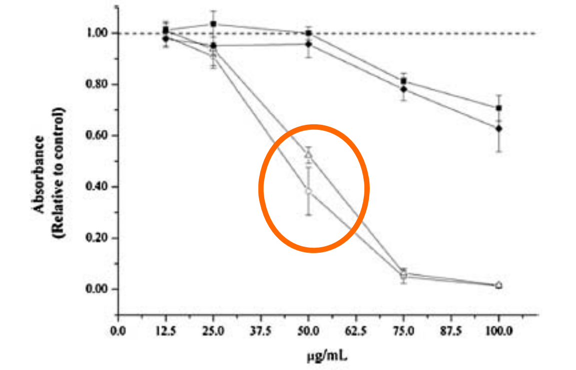


Fig. 4. HT-29 cell viability upon treatment for 24 h with different concentrations of N1 (circle), N2 (triangle), P1 (square) and P2 (diamond) extracts. Error bars are given as 95% confidence interval.

Decrease of cell viability up to 50%

4. PHLOROTANNINS PURIFICATION

Lipids

Fiber

Proteins

Carbohydrates

remove

LIQUID / LIQUID PURIFICATION

DCM

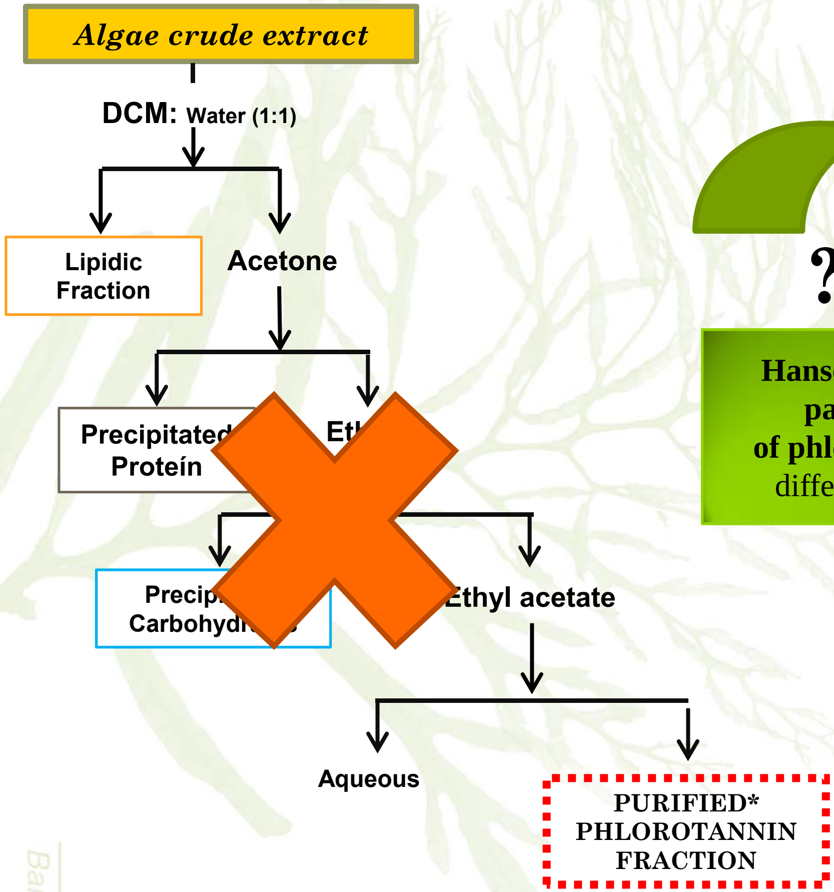
Acetone

Ethyl Acetate

INTRODUCTION

Phlorotannin Algae Purification

Step by step L/L purification



???

Hansen Solubility parameters of phlorotannins in different solvents

Green Purification - Proposal

Algae

One step/Multi-step processes

SFE

PLE

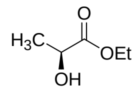
CO_2

Ethanol

Water

Ethyl Lactate

Mixtures



50-200ºC

Purified Extract

INTRODUCTION

HANSEN SOLUBILITY PARAMETERS (HSP) (1966)

Numerical estimate of the degree of interaction between materials

"like dissolves like"

$$\boldsymbol{\delta_T} = \sqrt{\delta_D^2 + \delta_P^2 + \delta_H^2} \qquad \textbf{(1)}$$

D - Dispersion or non-polar (Van der Waals)

P - Polar (Dipole moment)

HB - Hydrogen Bonds (Electron Interchange)

$$\boldsymbol{Ra} = \sqrt{4\left(\delta_{Di-}\delta_{Dj}\right)^2 + \left(\delta_{Pi-}\delta_{Pj}\right)^2 + \left(\delta_{Hi-}\delta_{Hj}\right)^2} \qquad \textbf{(2)}$$

Ra: the distance of solvents from the fixed HSP center (solutes).

INTRODUCTION

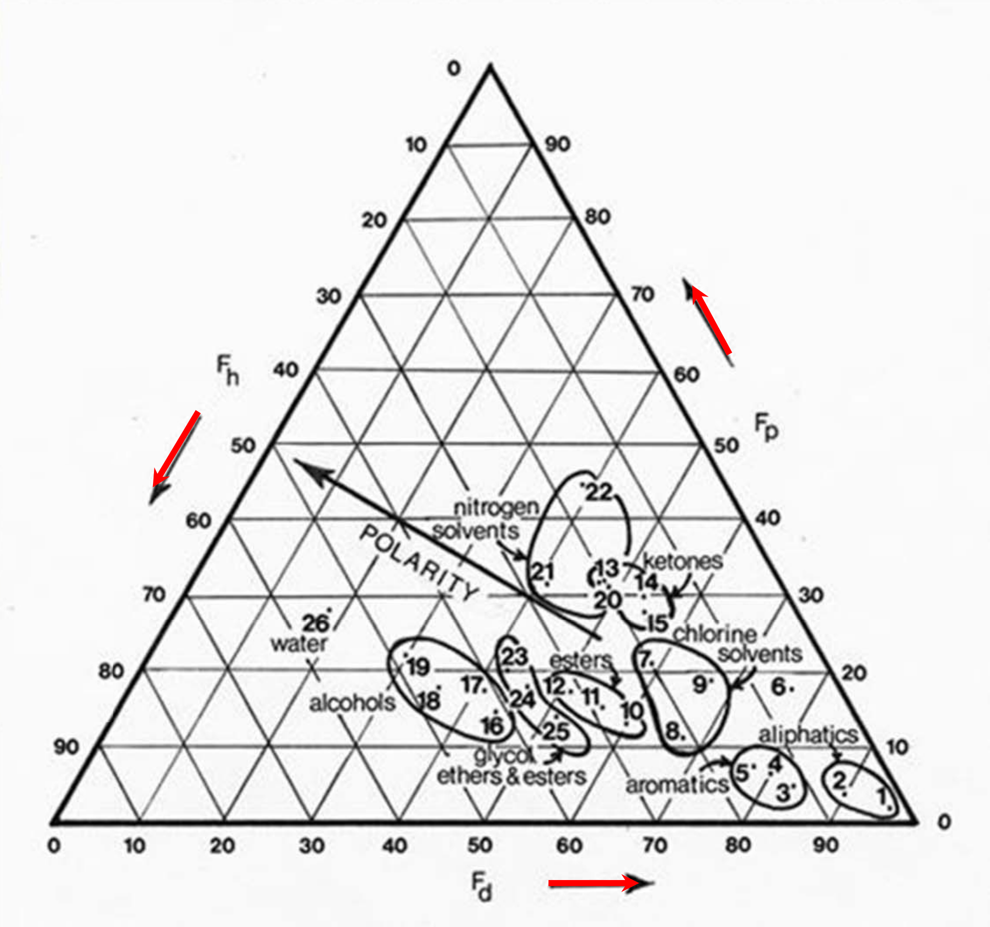
Teas (1968) : Triangular Plot for Solvent Selection

MODIFICATION OF HANSEN PARAMETERS

$$F_D = \frac{\delta_D}{\delta_D+\delta_P+\delta_H} \quad (3)$$

$$F_P = \frac{\delta_P}{\delta_D+\delta_P+\delta_H} \quad (4)$$

$$F_H = \frac{\delta_H}{\delta_D+\delta_P+\delta_H} \quad (5)$$

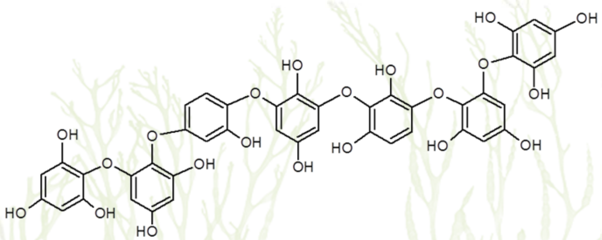


Bar = 1"

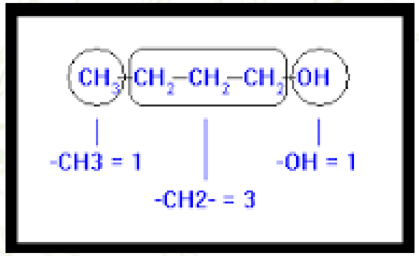
INTRODUCTION

No experimental data of

δ_D, δ_P, δ_H ???



Molecular structures using additive rules



GROUP CONTRIBUTION METHODS

Thermodynamic properties
Tc, Pc, Vm

Rackkett method (1970)
Fedors method (1974)
Joback method (1987)
Marrero & Gani (2001)

Solubility parameters

- **Hansen-Beerbower** (1966)
- Fedors (1974)
- Hoy (1989)
- Hoftyzer and Van Krevelen (1997)

Subcritical&Supercritical conditions f(Tc)

- Jayasri and Yaseen (1980)
- Williams et al. (2004)

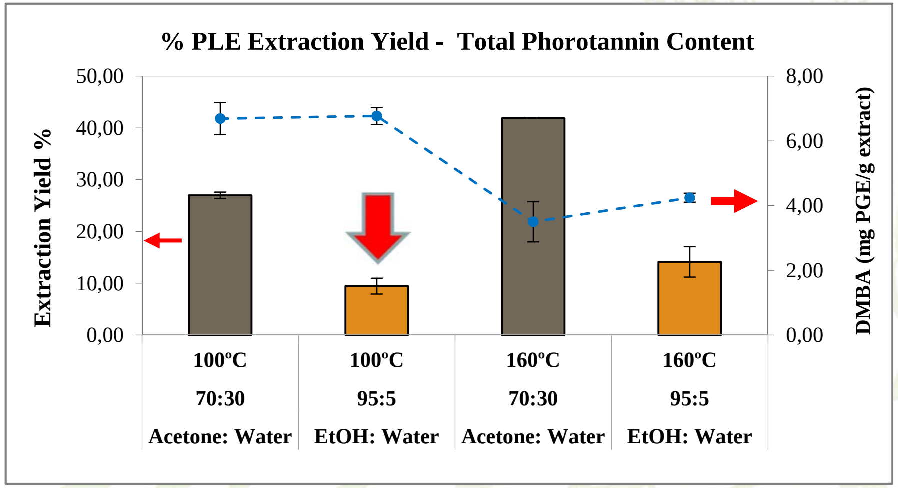
Chitin/Chitosan/Antioxidants

OBJECTIVE

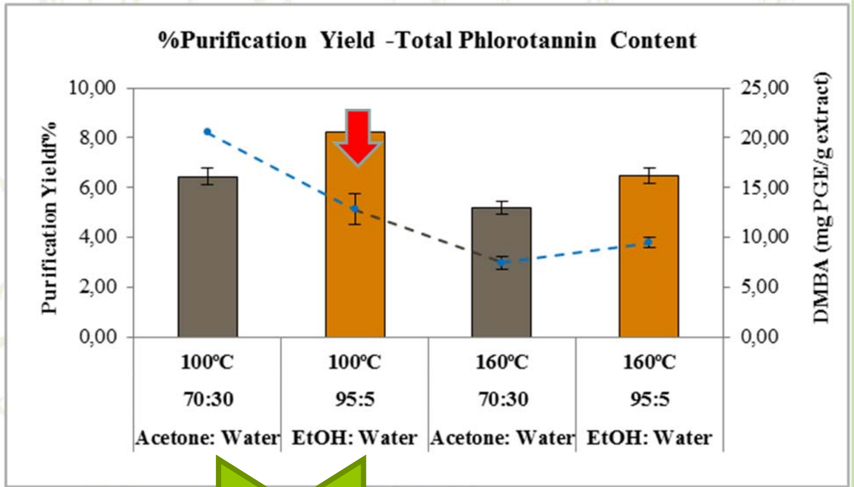
To develop a new extraction strategy using the estimation of HSP of phlorotannins in green subcritical and supercritical solvents for their selective extraction from brown algae *Cystoseira abies-marina*.

RESULTS: PLE Extraction & LCxLC Characterization

Before purification



After purification



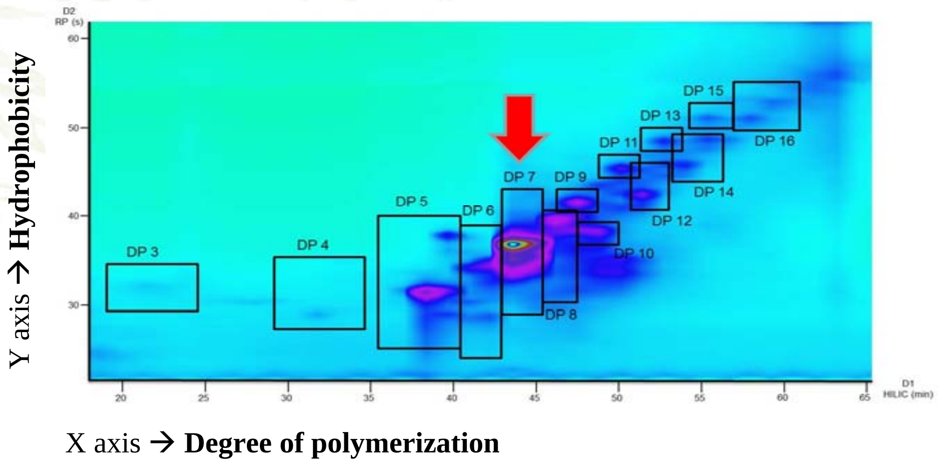
132 vs. 106
mg PGE/ g extract crude

EtOH: Water 95:5 (100ºC) sample

LCxLC-MS/MS

Phloretols/Fucols/ Fucophlorethols

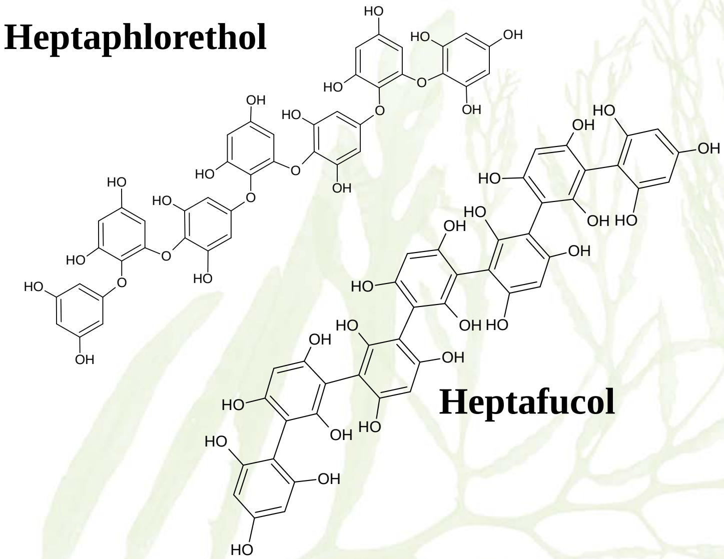
3-16 units
Polimerization Degree



Solubility parameters estimation

GROUP CONTRIBUTION METHODS: Solute

Heptaphlorethol



Heptafucol

Phlorotannin	Functional groups	# functional groups
Heptaphlorethol	Phenyl (trisubsituted)	1
	Phenyl (tretrasubsituted)	6
	-O- ether	6
	OH- (aromatic)	15
Heptafucol	Phenyl (tretrasubsituted)	2
	Phenyl (pentasubsituted)	5
	OH- (aromatic)	21

Critical property data → Marrero & Gani (2001)

One, second and Third-order contribution group

Molar Volume → Yamamoto-Molecular Break (2003)

Simplified molecular input line entry syntax (SMILES) (HSPiP Version 5.0, Denmark)

Solubility parameters → Hansen (2007)

$$\boldsymbol{\delta_T} = \sqrt{\delta_D^2 + \delta_P^2 + \delta_H^2} \qquad \textbf{(1)}$$

Temperature dependence of HSP → Jayasri and Yaseen (1980)

$$\delta_2 = \delta_1 \left(\frac{1 - T_{r2}}{1 - T_{r1}}\right)^{0.34} \qquad \textbf{(6)}$$

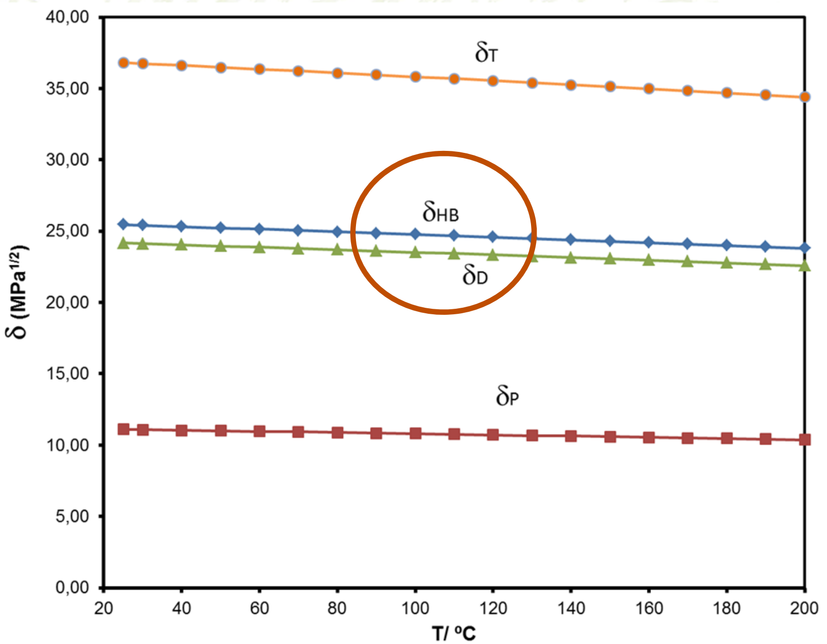
RESULTS : Hansen Solubility parameters

Solute

Heptaphlorethol
&
Heptafucol

Phlorotannin	T (^{a}C)	P (MPa)	δ_D (MPa$^{1/2}$)	δ_P (MPa$^{1/2}$)	δ_H (MPa$^{1/2}$)	δ_T (MPa$^{1/2}$)
Heptaphlorethol	25	1.0	25,45	11,10	24,17	**36,81**
	40	1.0	25,32	11,04	24,04	**36,61**
	100	1.0	24,77	10,8	23,51	**35,82**
	150	1.0	24,29	10,59	23,06	**35,12**
Heptafucol	25	1.0	28,08	12,12	28,98	**42,13**
	40	1.0	27,94	12,06	28,84	**41,92**
	100	1.0	27,36	11,81	28,24	**41,06**
	150	1.0	26,86	11,6	27,73	**40,31**

Partial solubility parameters for
Phloroethol 7DP - Heptaphorethol
Jayasri and Yaseen (1980)



No dependence of f(T)

Solubility parameters estimation

GROUP CONTRIBUTION METHODS: Green solvents

Solvents

Subcritical

- **Ethanol**
- **Water**
- **Ethyl Lactate**

Supercritical

- **CO_2**
- **CO_2+ETOH**

Physical properties f(T) =ρ, V

Rackett equation & Gunn–Yamada method

$$V(T) = \frac{f(T)}{f(T^R)} V^R \qquad f(T) = H_1(1 - \omega H_2) \qquad \textbf{(7)}$$

Solubility parameters → Hansen (1966)

$$\delta_T = \sqrt{\delta_d + \delta_p + \delta_h} \qquad \delta_{i,mix} = x_1 \cdot \delta_{i,1} + x_2 \cdot \delta_{i,2} \quad i=d,p,h \qquad \textbf{(8)}$$

Solubility parameters for subcritical & supercritical conditions → Williams et al., 2004

$$\frac{\delta_{D,ref}}{\delta_D} = \left(\frac{V_{ref}}{V}\right)^{-1.25} \qquad \frac{\delta_{P,ref}}{\delta_P} = \left(\frac{V_{ref}}{V}\right)^{-0.5} \qquad \textbf{(9)}$$

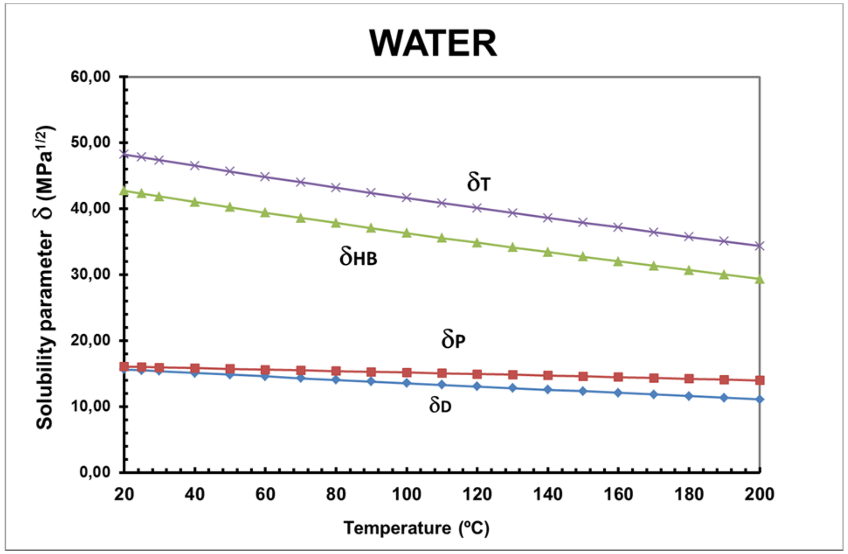
$$\frac{\delta_{D,ref}}{\delta_D} = \exp\left[-1{,}32 \cdot 10^{-3}(T_{ref} - T) - \ln\left(\frac{V_{ref}}{V}\right)^{0.5}\right]$$

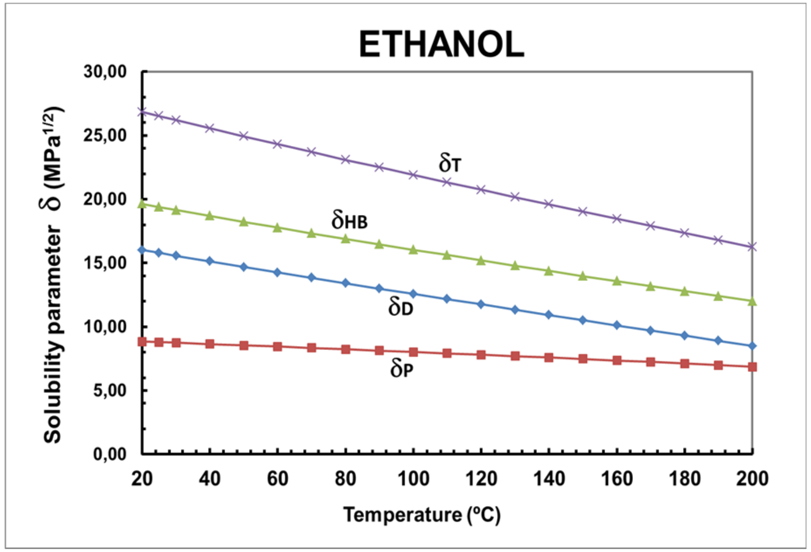
RESULTS: Hansen Solubility parameters

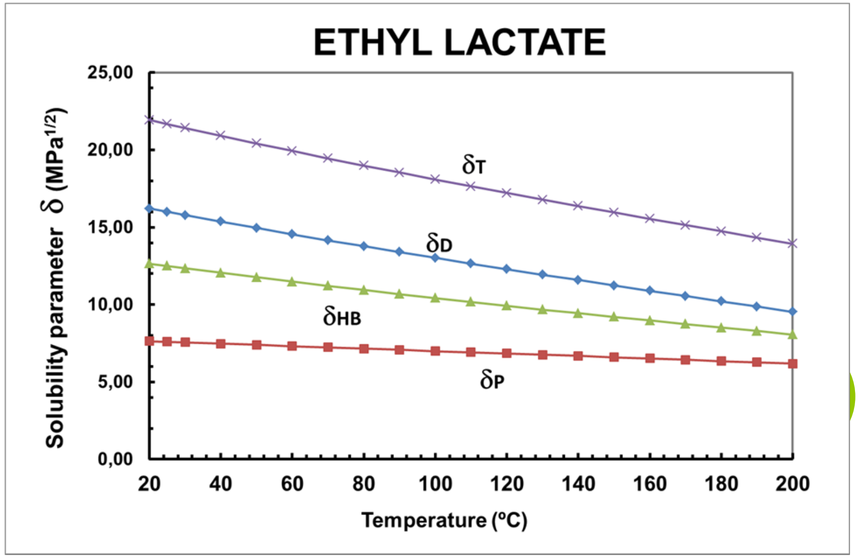
Green solvents

For **subcritical** conditions, **P** does not have a big influence on HSP.

By manipuling the subcritical fluids' **T** there is a change in the contribution of various intermolecular forces

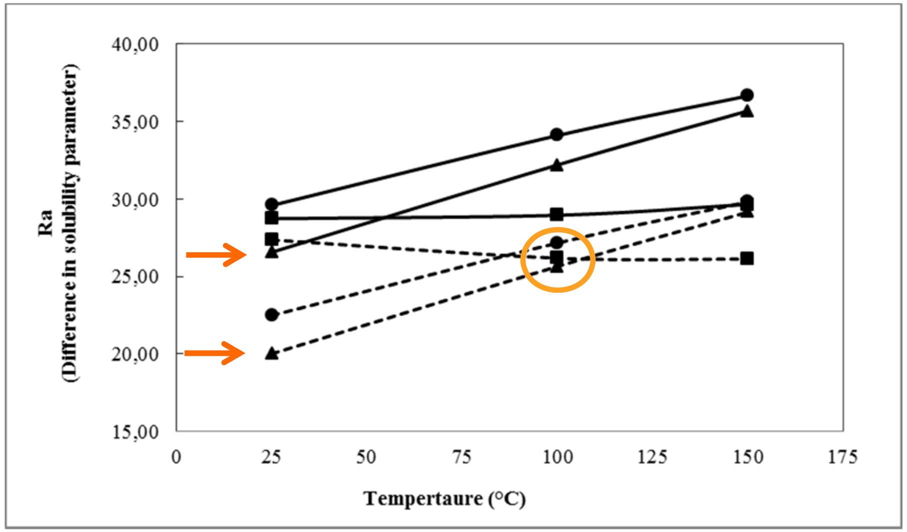




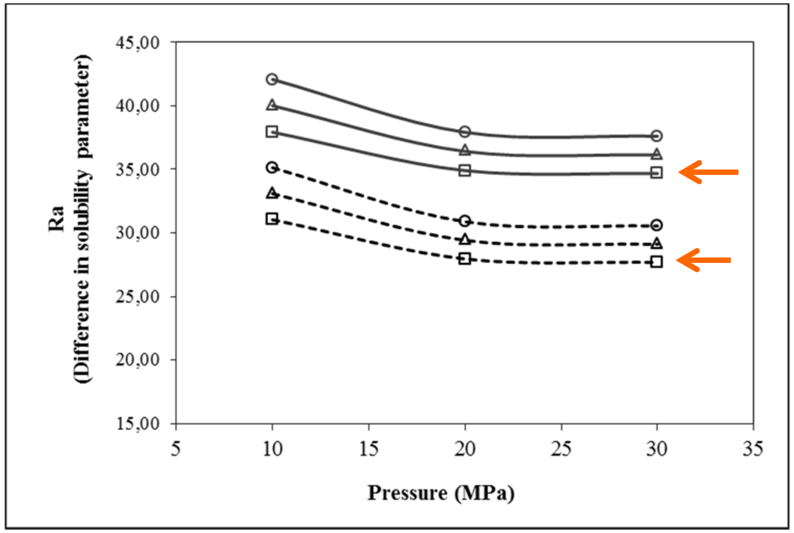


RESULTS: Hansen Solubility parameters

Subcritical conditions | *Supercritical conditions*



(a)



(b)

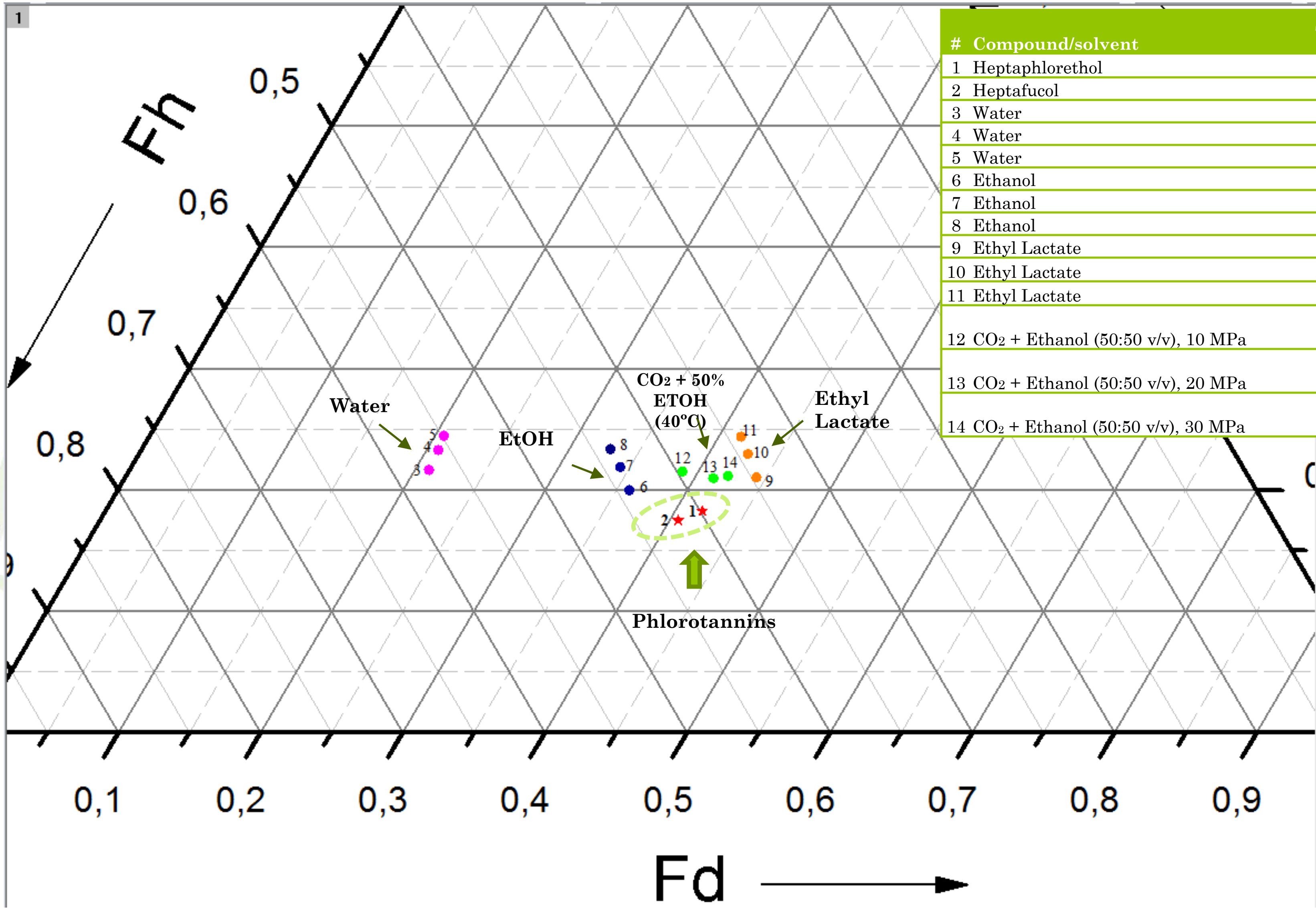
Influence of the temperature and pressure in Ra value for heptaphlorethol and heptafucol and different (a) subcritical solvents at 1.0 MPa and (b) supercritical solvents at 40ªC.

(●) Ethyl lactate, (▲) Ethanol, (■) Water,
(○) CO_2+EtOH (70:30 v/v), (△) CO_2+EtOH (60:40 v/v), (□) CO_2+EtOH (50:50 v/v).

Dashed line (- - -), Heptaphlorethol; Continous line (---), Heptafucol

Ra: distance between solute and solvent molecules. The smaller, the more likely to be compatible

RESULTS : Teas Graph (Fd, Fp, Fh)



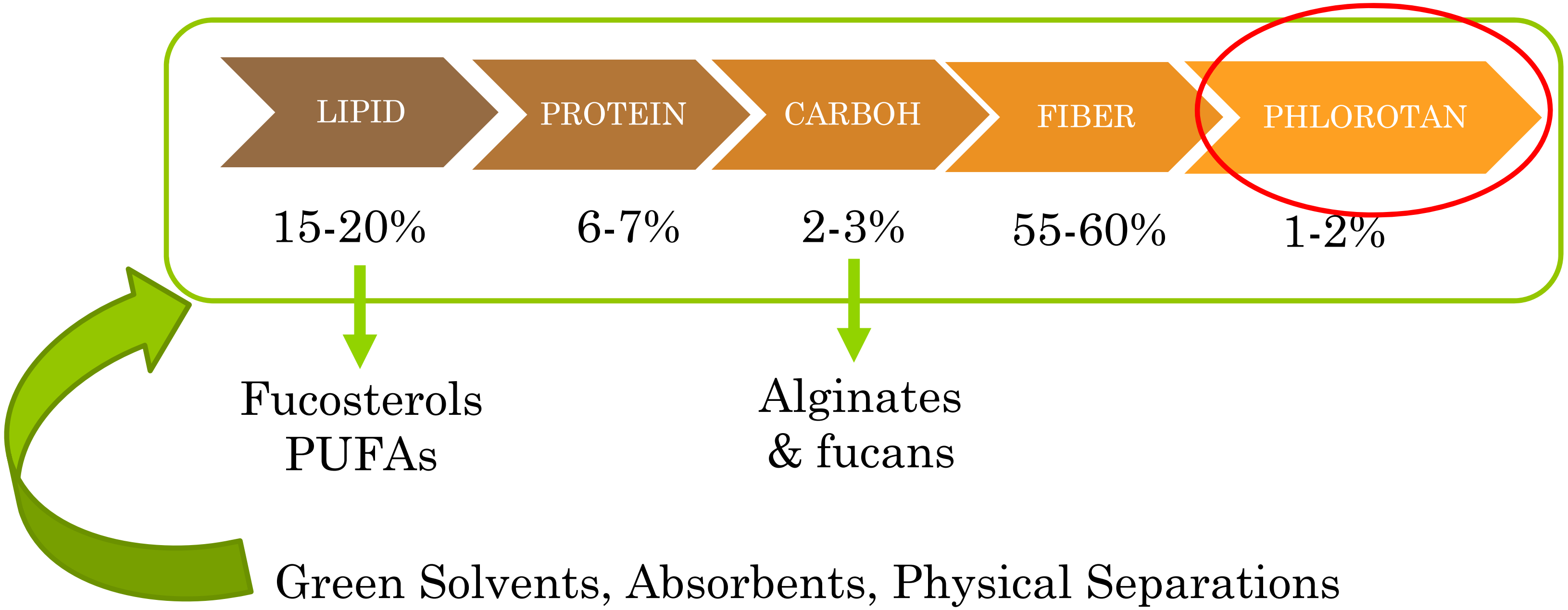
#	Compound/solvent	T (ºC)
1	Heptaphlorethol	25
2	Heptafucol	25
3	Water	25
4	Water	100
5	Water	150
6	Ethanol	25
7	Ethanol	100
8	Ethanol	150
9	Ethyl Lactate	25
10	Ethyl Lactate	100
11	Ethyl Lactate	150
12	CO_2 + Ethanol (50:50 v/v), 10 MPa	40
13	CO_2 + Ethanol (50:50 v/v), 20 MPa	40
14	CO_2 + Ethanol (50:50 v/v), 30 MPa	40

FUTURE CHALLENGES

One step process → Phlorotannins Isolation based on HSP solvents and/or their mixtures

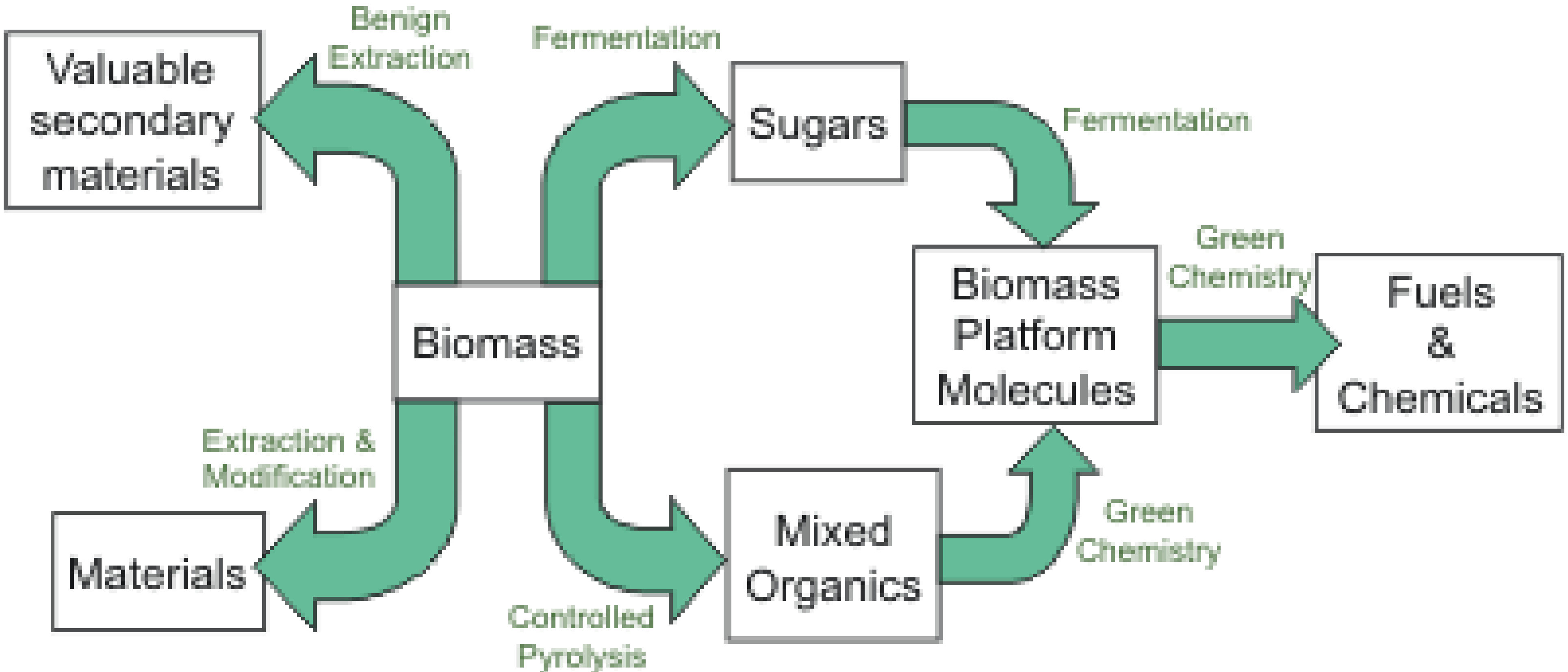
Multi-step process

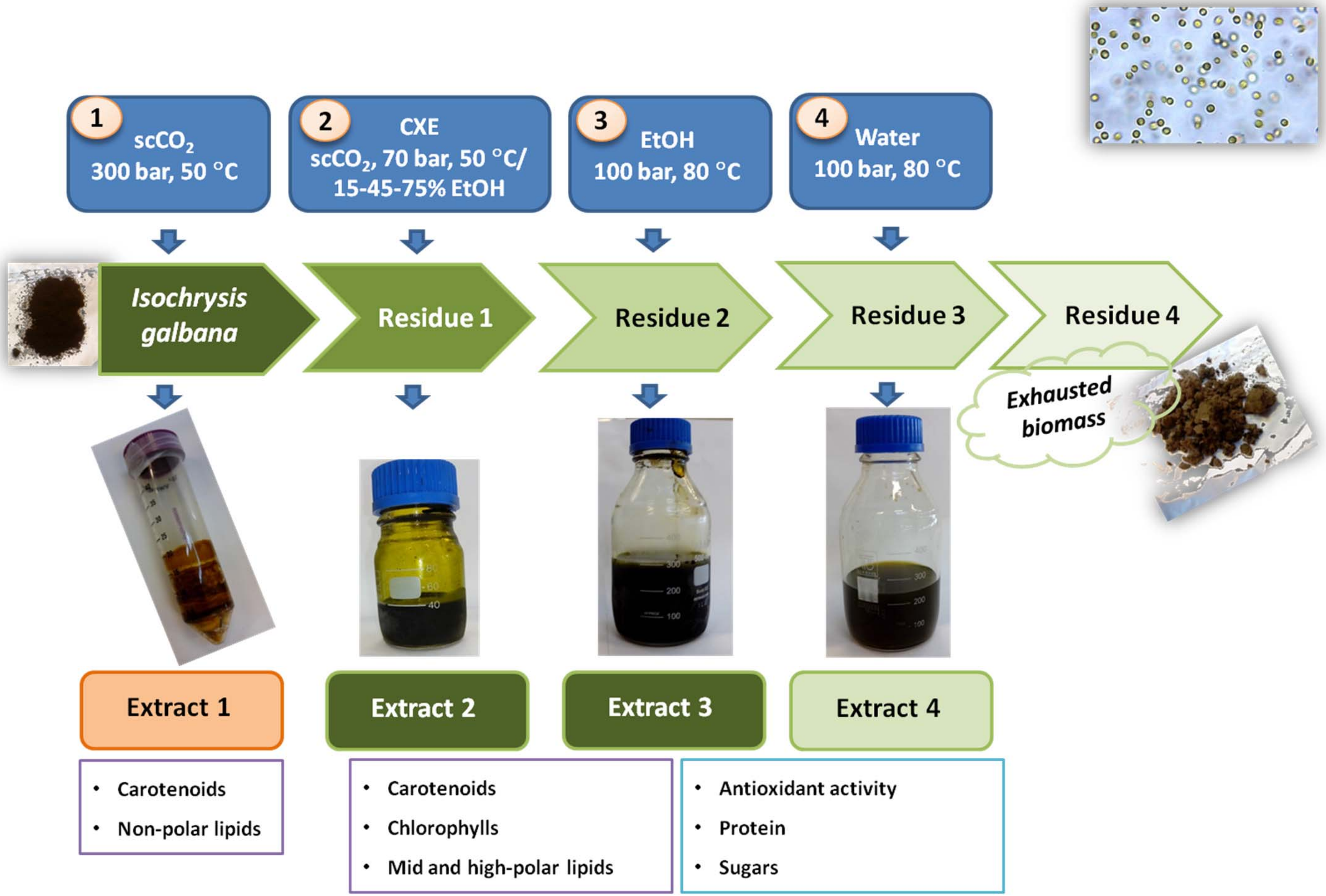
Biorefinery concept



Green Solvents, Absorbents, Physical Separations

Green Chemistry and the Biorefinery





1
$scCO_2$
300 bar, 50 °C
2
CXE
$scCO_2$, 70 bar, 50 °C/
15-45-75% EtOH
3
EtOH
100 bar, 80 °C
4
Water
100 bar, 80 °C
Isochrysis galbana
Residue 1
Residue 2
Residue 3
Residue 4
Exhausted biomass
Extract 1
Extract 2
Extract 3
Extract 4
• Carotenoids
• Non-polar lipids
• Carotenoids
• Chlorophylls
• Mid and high-polar lipids
• Antioxidant activity
• Protein
• Sugars

Green Chemistry

PAPER

Cite this: *Green Chem.*, 2015, **17**, 4599

Downstream processing of *Isochrysis galbana*: a step towards microalgal biorefinery†

Bienvenida Gilbert-López,[a] José A. Mendiola,[a] Javier Fontecha,[b] Lambertus A. M. van den Broek,[c] Lolke Sijtsma,[c] Alejandro Cifuentes,[a] Miguel Herrero[a] and Elena Ibáñez*[a]

MIRACLES

SPECIALTIES FROM ALGAE

ACKNOWLEDGEMENTS:

Finantial support:

- Spanish Ministry of Science and Innovation (now Ministry of Economy and Competitiveness)- (Project AGL2011-29857-C03-01)
- EU 613588- MIRACLES, "Multi-product Integrated bioRefinery of Algae: from Carbon dioxide and Light Energy to high-value Specialties"