Sustainable Processes for Bioactives Extraction: Present and Future

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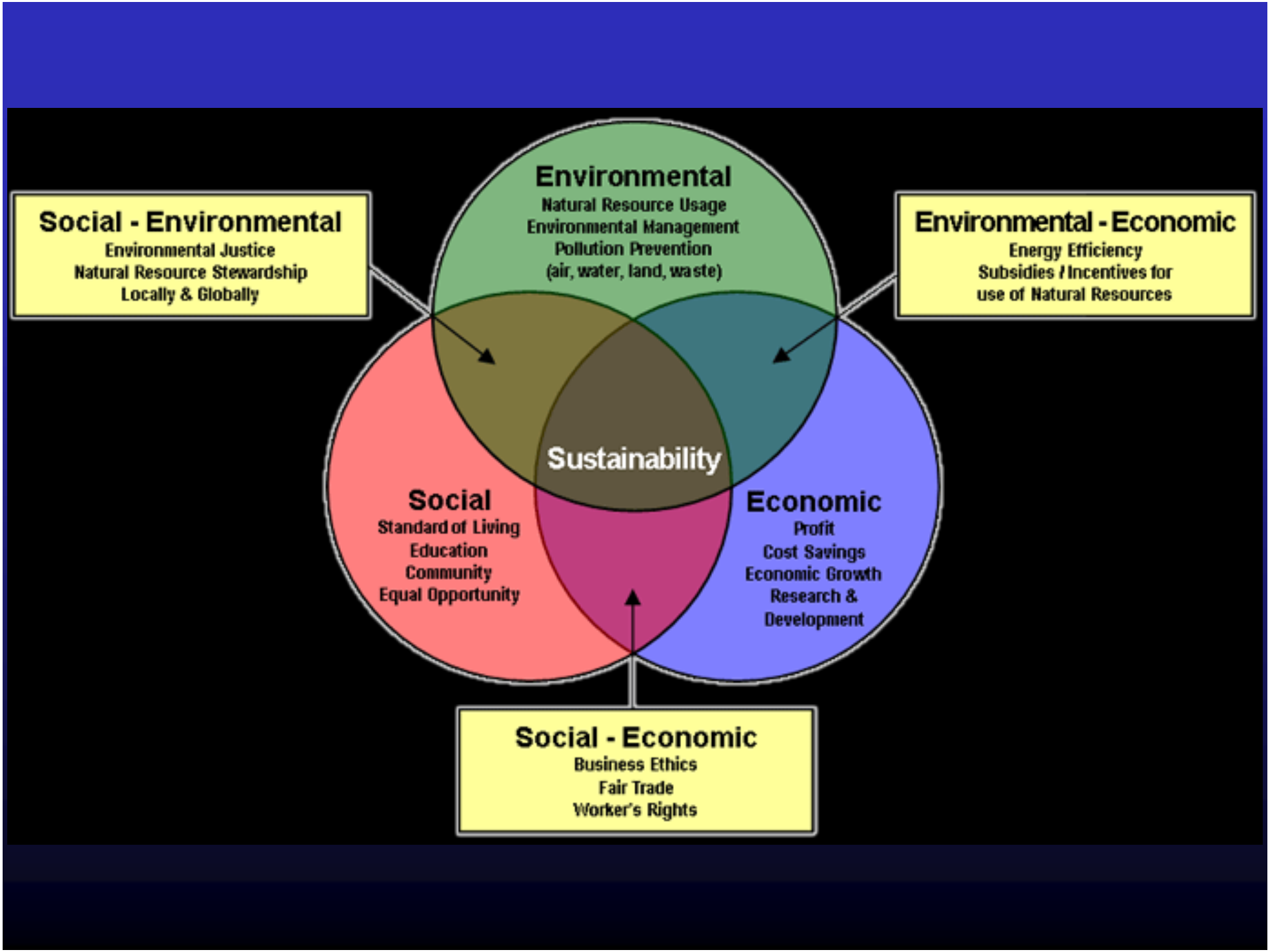
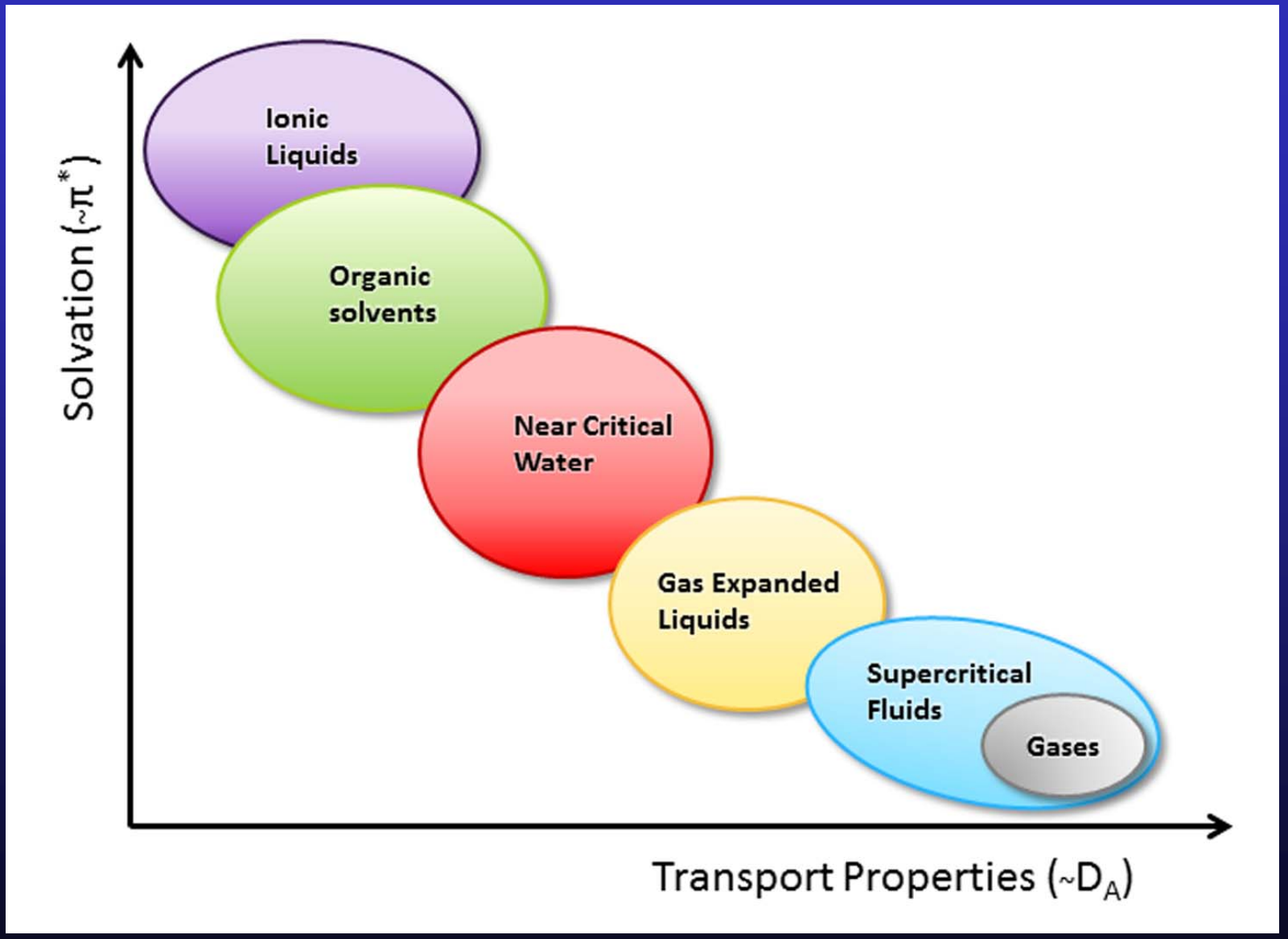
http://www.cial.uam-csic.es/pagperso/foodomics


Environmental
Natural Resource Usage
Environmental Management
Pollution Prevention
(air, water, land, waste)
Social - Environmental
Environmental Justice
Natural Resource Stewardship
Locally & Globally
Environmental - Economic
Energy Efficiency
Subsidies / Incentives for
use of Natural Resources
Sustainability
Social
Standard of Living
Education
Community
Equal Opportunity
Economic
Profit
Cost Savings
Economic Growth
Research &
Development
Social - Economic
Business Ethics
Fair Trade
Worker's Rights


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

SOLVENTS FOR SUSTAINABLE CHEMICAL PROCESSES



Green Chem, 2014, 16, 1034

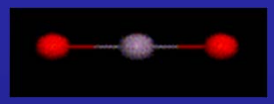
CHALLENGES IN THE DEVELOPMENT OF GREEN EXTRACTION PROCESSES

FAST

SELECTIVE

GREEN

COST-EFFECTIVE

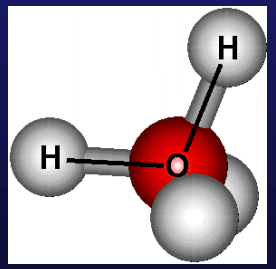


HIGH YIELDS

SUSTAINABLE

EFFICIENT

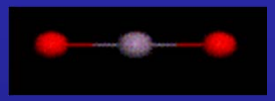
SUSTAINABLE PROCESSES



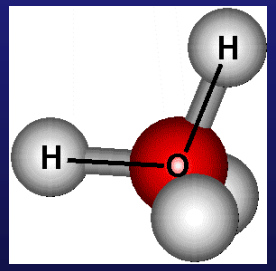
Without organic solvents

CHALLENGE

EXTRACTION BIOACTIVE COMPOUNDS FROM NATURAL SOURCES THROUGH GREEN PROCESSES

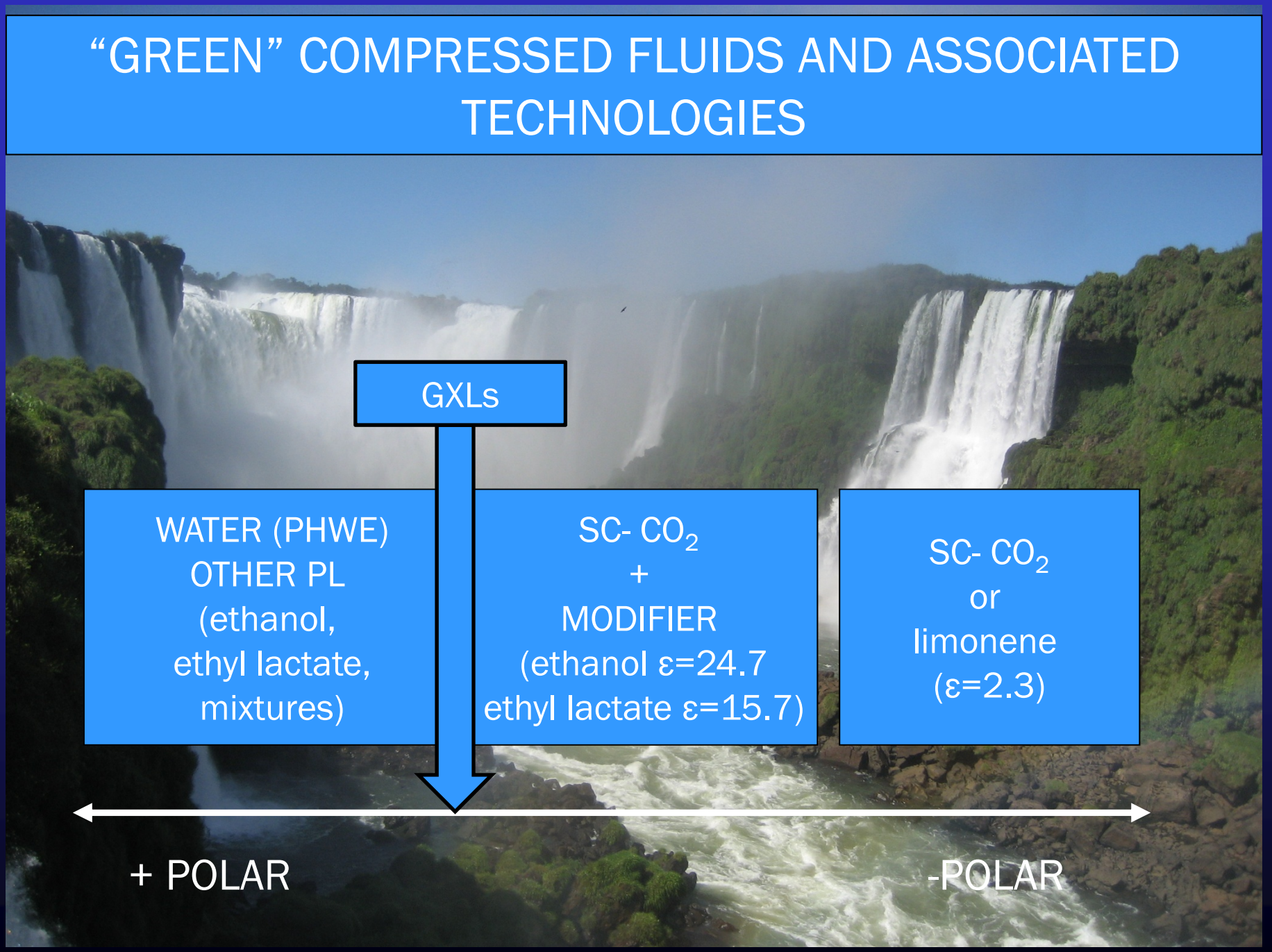


INTENSIFICATION OF PROCESSES



PRESSURE
TEMPERATURE
MICROWAVES
ENZYMES

“GREEN” COMPRESSED FLUIDS AND ASSOCIATED TECHNOLOGIES



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

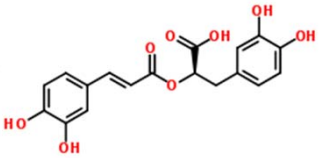
A. P. Sánchez-Camargo, J. A. Mendiola, A. Valdés, M. Castro-Puyana, V. García-Cañas, A. Cifuentes, M. Herrero, E. Ibáñez

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

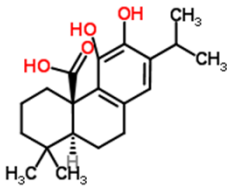
INTRODUCTION

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

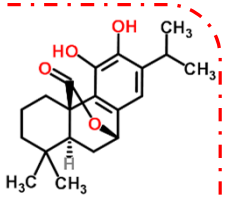
Rosmarinus officinalis



Rosmarinic Acid (RA)

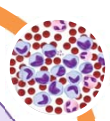


Carnosic Acid (CA)



Carnosol (CS)

Chemoprentive Activities Cancer cells

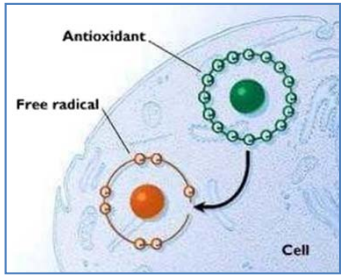


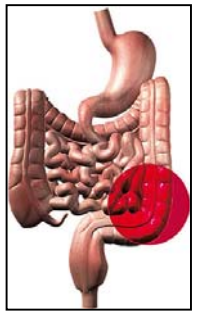
Leukemia

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

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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

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. Cifuentes[b], E. Ibáñez[b,*]

PLE

EtOH/H2O

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]
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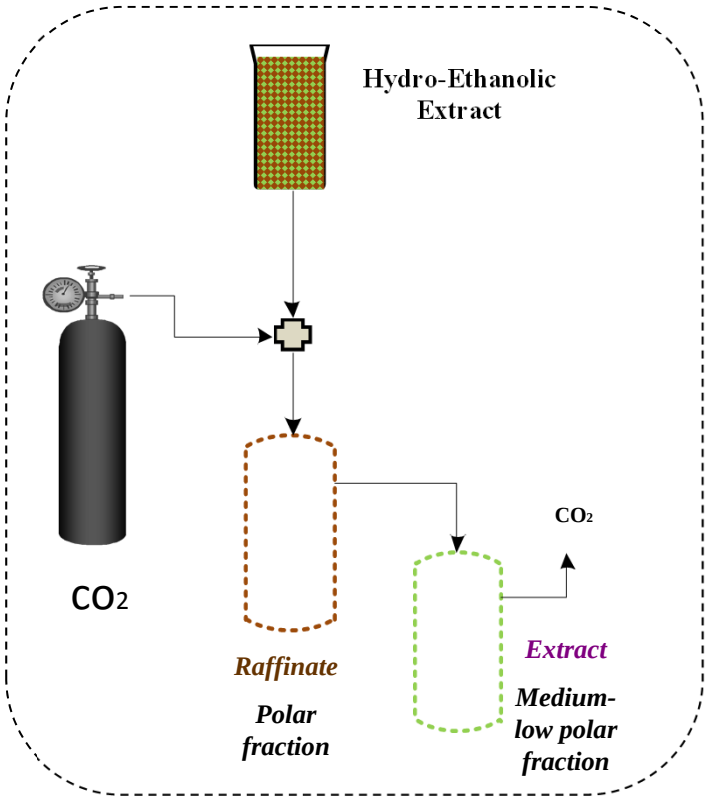
Lourdes Rocamora-Reverte[2]
José Antonio Ferragut[2]
Virginia García-Cañas[1]
Alejandro Cifuentes[1]

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

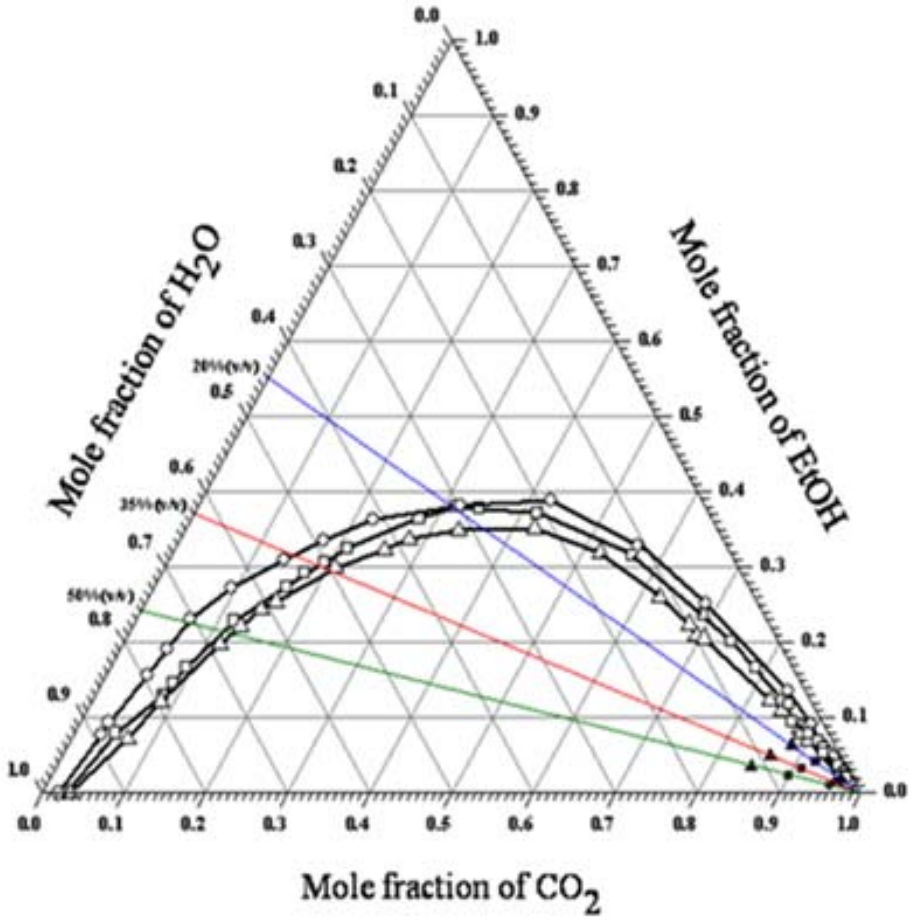
Phase equilibrium for CO_2-Ethanol-Water

Solvent selectivity & ethanol partition coefficients

Main influencing factors involved:

Feed/SC-CO2 → Pressure → %H2O feed

Optimization according the compounds' nature



OBJECTIVE

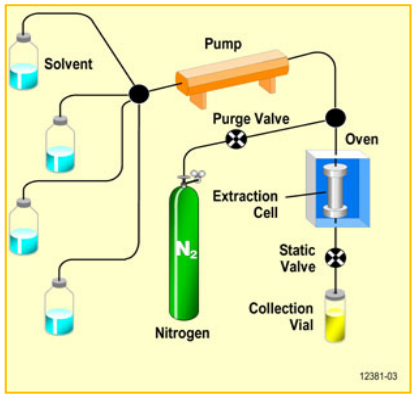
To optimize the single-step supercritical antisolvent fractionation (SAF) of a rosemary PLE extract (obtained using a mixture of ethanol and water as extracting solvent), in order to produce a CA+CS-enriched fraction which is expected to be more active against human colon cancer cells (HT-29)

Workflow

Rosemary leaves

Dryed by sunlight + Grinded (500-999µm)

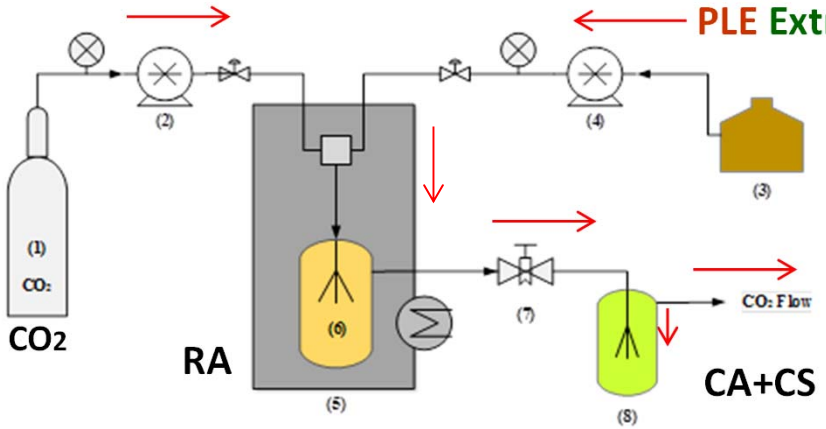
Pressurized Liquid Extraction (PLE)



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	%

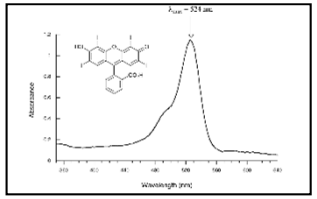
Multiple Response Optimization

Methodology

PLE Hydro-alcoholic extract – raffinate - extract

Total phenols content

Folin-Ciocalteu



Antioxidant Activity

***In vitro* assays**

TEAC Assay

DPPH radical EC50

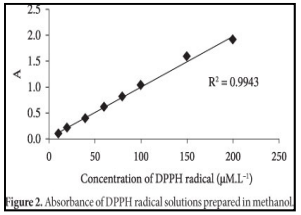
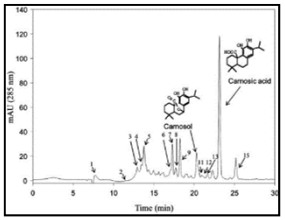


Figure 2. Absorbance of DPPH radical solutions prepared in methanol

UHPLC-MS

Quantification of phenolic compounds



Rosmarinic Acid
Carnosic Acid
Carnosol

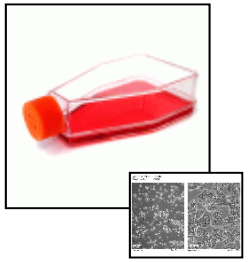
Responses

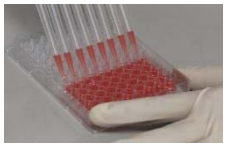
❖ **Anti-proliferative activity against human colon cancer cells.**

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

30 µg/mL

Colon adenocarcinoma HT-29 cells

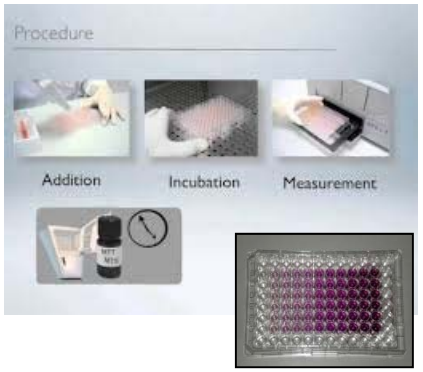




CELL CULTURE
10.000 cells/cm^2
96-well plate

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

PLE

rosemary extraction:

upstream process

+

SAF

Supercritical Antisolvent Fractionation process:

downstream process

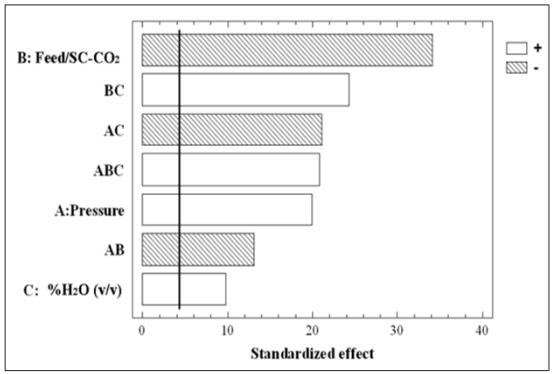
Optimization of the Supercritical Antisolvent Fractionation process:
downstream process

RAFFINATE

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 (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

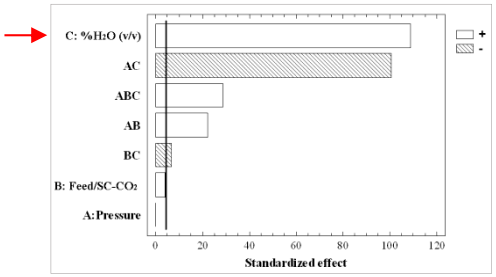
Improved Anti-proliferative activity :
SCFE Single-Step: 64.5% (33% CA+ CS)
Two-sequential steps: 38.7% (40% CA+CA)
Supercritical Antisolvent Fractionation: 16.9%

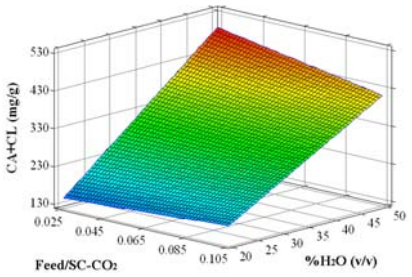
Optimization of the Supercritical Antisolvent Fractionation process:

downstream process

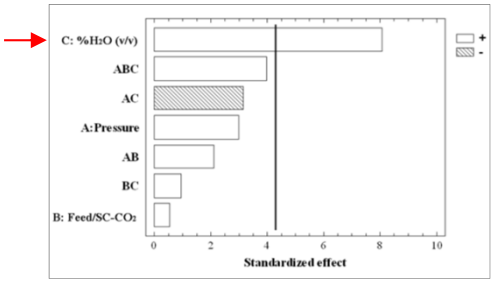
EXTRACT

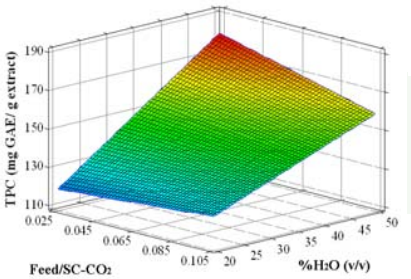
CA+CS relative amount



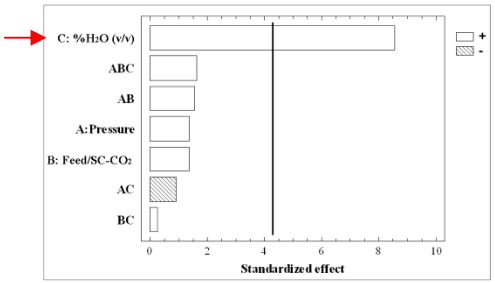


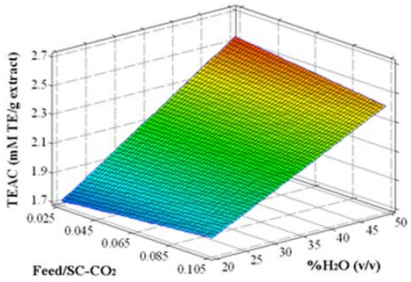
Total Phenolic Content (TPC)



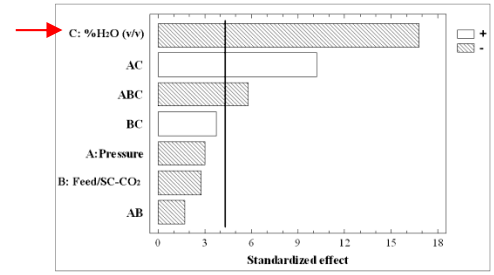


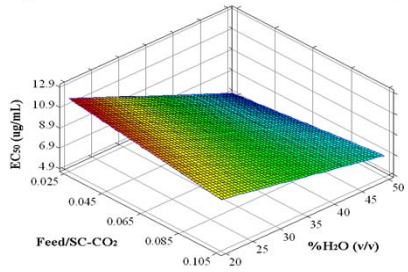
Antioxidant Activity TEAC



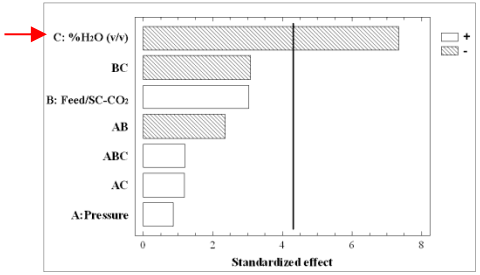


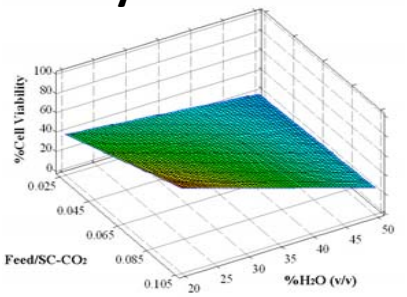
Antioxidant activity DPPH





%Cell Viability





Optimal conditions

P= 100 bar

Feed /SC-CO_2 =0.025

%H_2O in feed (v/v) =50

CONCLUSIONS

- Integrated Process: PLE + SAF → two different polarity fractions.
- RA was concentrated 2.7-fold in the raffinate, compared to the feed.
- Enriched fractions with up to 48% CA + CS were obtained in the extract.
- Response surface methodology was used to optimize fractionation conditions:

  P=100 bar

  Feed/SC-CO_2 =0,025

  %Water in feed=50% (v/v)

- Anti-proliferative activity with 17% of cell survival after 24 h of treatment

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

DEVELOPMENT OF NEW STRATEGIES OF INTEGRATED GREEN PROCESSES FOR OBTAINING PHLOROTANNIN-ENRICHED EXTRACTS FROM BROWN ALGAE *Cystoseira abies-marina*.

A. P. Sánchez-Camargo, L. Montero, A. Cifuentes, M. Herrero, 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:

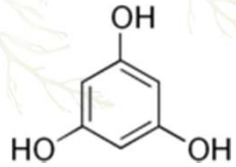
- **Most available species living on Mediterranean Sea and Atlantic Ocean ecosystems**
- **Produced to face biotic and abiotic stress conditions factors**

DEFENSIVE METABOLITES

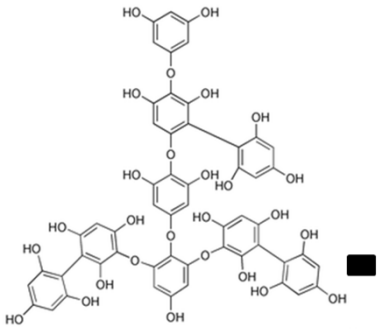
PHLOROTANNINS

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

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



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

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

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

- **Enzymes**: Proteases & Carbohydrases
- **Pressurized Liquid Extraction (PLE)**

2. CHARACTERIZATION

1644

Electrophoresis 2014, *35*, 1644–1651

Lidia Montero
Miguel Herrero
Elena Ibáñez
Alejandro Cifuentes

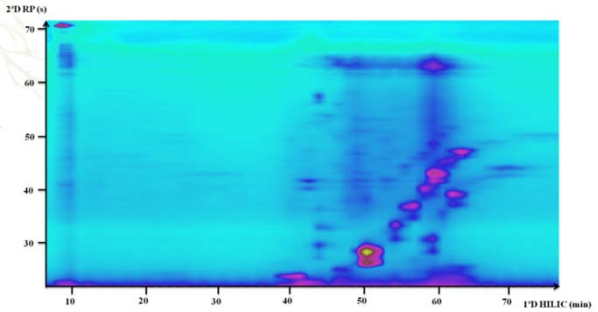
Laboratory of Foodomics, Institute of Food Science Research (CIAL, CSIC), Campus de Cantoblanco, Madrid, Spain

Research Article

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

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

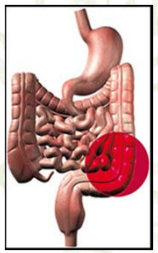
Contents lists available at ScienceDirect

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]



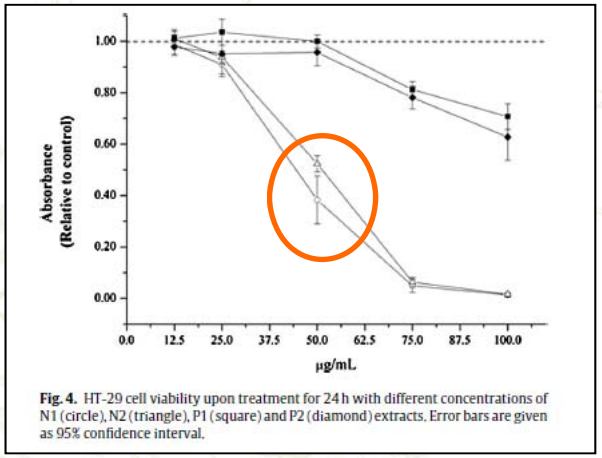


Fig. 4. HT-29 cell viability upon treatment for 24 h with different concentrations of N1 (circle), N2 (triangle), P1 (square) and P2 (diamond) extracts. Error bars are given as 95% confidence interval.

4. PHLOROTANNINS PURIFICATION

Seaweed extracts

- Lipids
- Ash
- Proteins
- Carbohydrates

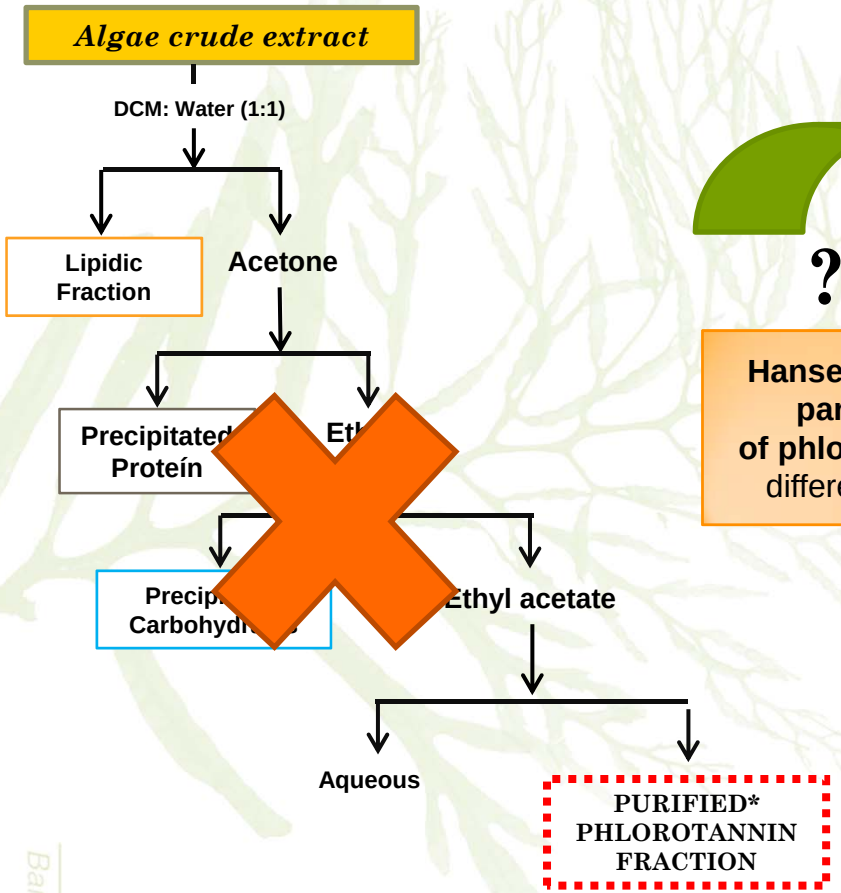
LIQUID / LIQUID PURIFICATION

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

SFE

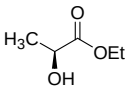
PLE

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"

$$E/V = E_D/V + E_P/V + E_{HB}/V$$

D - Dispersion or non-polar (Van der Waals)
P - Polar (Dipole moment)
H - Hydrogen Bonds (Electron Interchange)
V - Molar Volume

$$\delta^2 = \delta^2_D + \delta^2_P + \delta^2_{HB} \quad (1)$$

INTRODUCTION

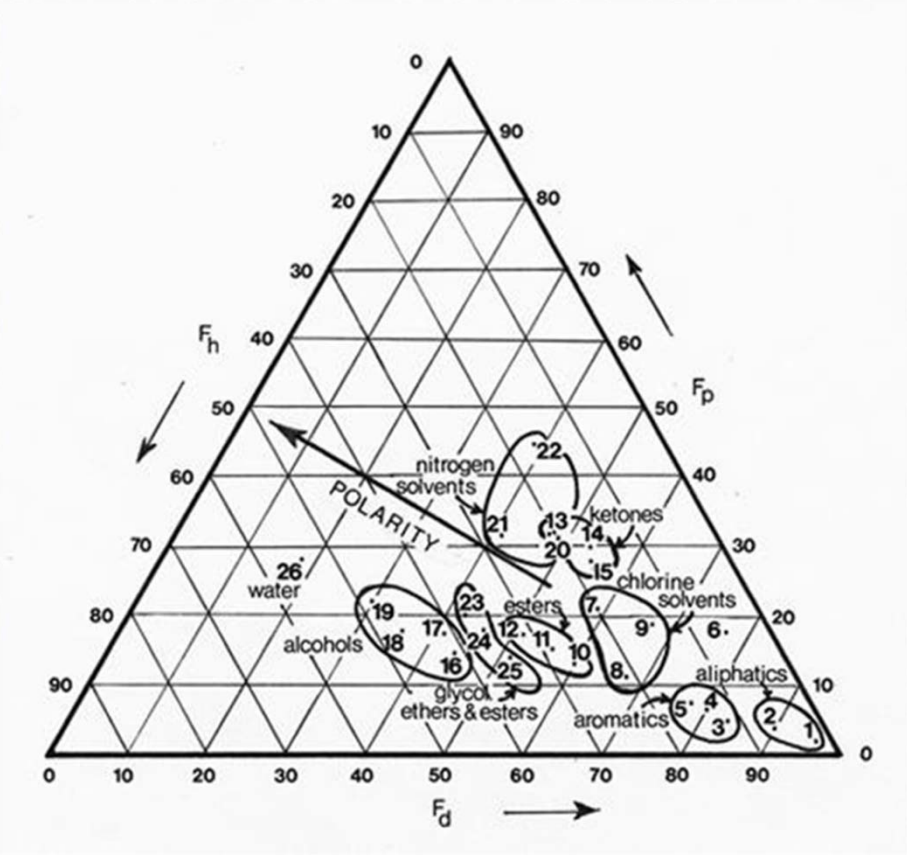
Teas (1968) : Triangular Plot for Solvent Selection

MODIFICATION OF HANSEN PARAMETERS

$$F_d = \delta_D/(\delta_D + \delta_P + \delta_{HB}) \quad (2)$$

$$F_p = \delta_P/(\delta_D + \delta_P + \delta_{HB}) \quad (3)$$

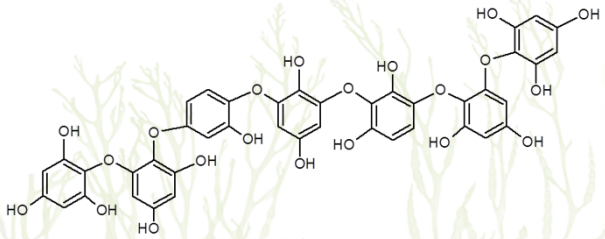
$$F_h = \delta_H/(\delta_D + \delta_P + \delta_{HB}) \quad (4)$$



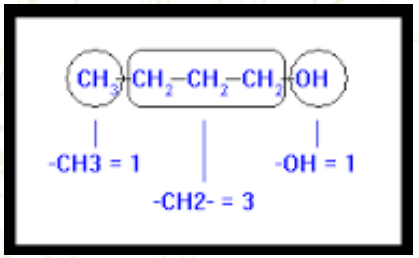
INTRODUCTION

No experimental data

δ_D, δ_P, δ_H ???



Additive rules



GROUP CONTRIBUTION METHODS

Thermodynamic properties
Tc, Pc, Vm

Rackkett method (1970)
Fedors method (1974)
Joback method (1987)

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

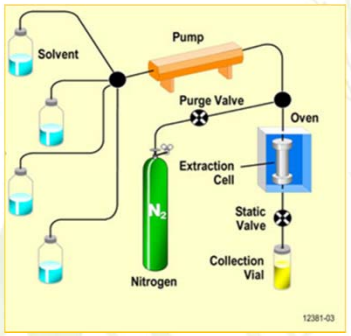
OBJECTIVE

To develop a new extraction strategy using the estimation of Hansen solubility parameters of phlorotannins in green solvents to obtain enriched extracts from brown algae *Cystoseira abies-marina*

Workflow

Dried *cystoseira abies-marina*

Pressurized Liquid Extraction (PLE)



T = 100°C, 160°C
P = 100 bar
t= 20 min

Solvents:

EtOH:Water (95:5)
Acetone:Water (70:30)

Cystoseira crude extract → Phlorotannin Content DMBA Analysis (mg PGE/g extract)

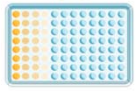
DCM: Water (1:1)

- Lipidic Fraction
- Aqueous
  - Acetone (3:1)
    - Precipitated Protein
    - Acetonic fraction (Evaporation and suspension in water)
      - EtOH (3:1)
        - Precipitated Carbohydrates
        - Ethanolic fraction (Evaporation and suspension in water)
          - Ethyl acetate (1:1)
            - Aqueous
            - **PURIFIED* PHLOROTANNIN FRACTION** (Evaporation and suspension in water)

PURIFIED* PHLOROTANNIN FRACTION

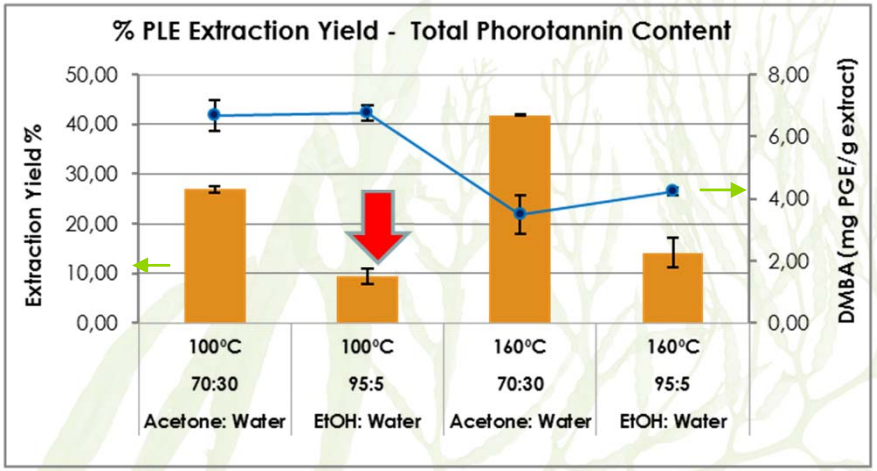
Characterization

- **Phlorotannin Content DMBA Analysis (mg Phloroglucinol Equivalent/g extract (515 mn)**
- **Comprenhensive two-dimensional liquid chromatography LCxLC-MS (HILIC x RP)**

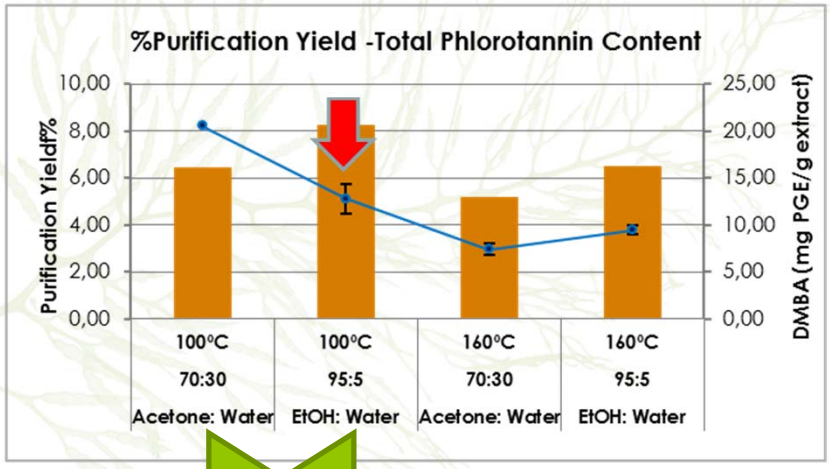


RESULTS : PLE Extraction

Before purification



After purification

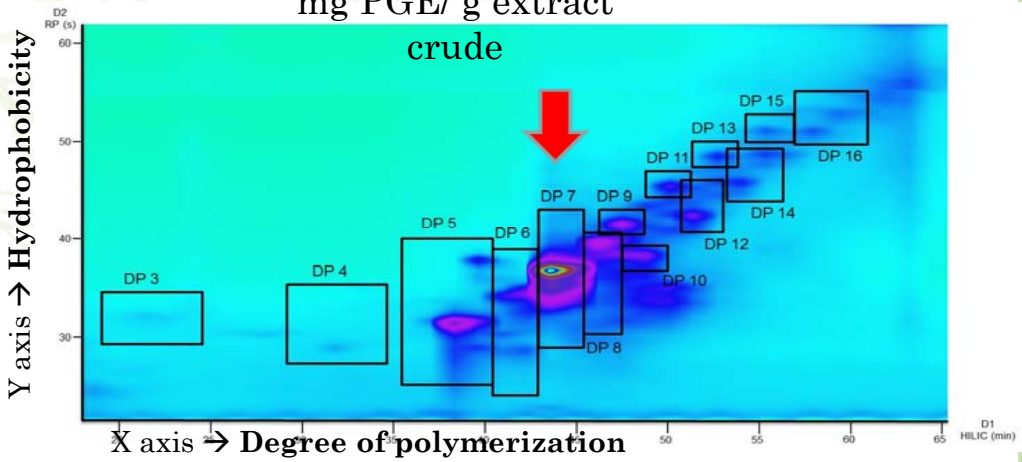


EtOH: Water 95:5 (100ºC) sample

132 vs. 106
mg PGE/ g extract
crude

LCxLC-MS/MS

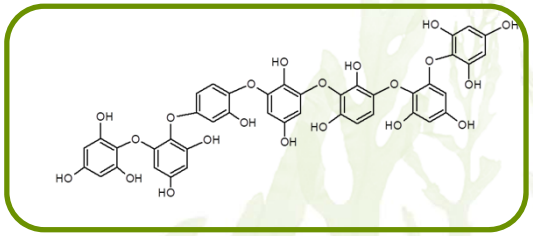
Phloretols
3-16 units
Polimerization Degree



Solubility parameters estimation

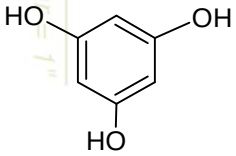
GROUP CONTRIBUTION METHODS: Solute

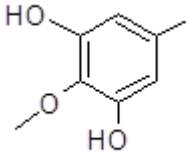
Heptaphloretol



Atomic group contributions

Atom or group	No.
-CH=	15
>C=	27
-O-	6
-OH	15
Ring	7





Critical property data → Joback method (1987)

$$T_c[K] = T_b\left[0.584 + 0.965\sum T_{c,i} - \left(\sum T_{c,i}\right)^2\right]^{-1} \quad \textbf{(5)}$$

Molar Volume → Fedors method (1987)

$$V = \sum V_i n_i \quad \textbf{(6)}$$

Solubility parameters → Hansen (1966)

$$\frac{E}{V} = \frac{E_d}{V} + \frac{E_p}{V} + \frac{E_{hb}}{V} \qquad \delta_T = \sqrt{\delta_d + \delta_p + \delta_{hb}} \quad \textbf{(7)}$$

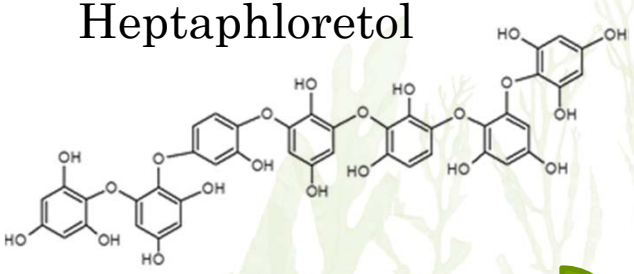
Temperature dependence of HSP → Jayasri and Yaseen (1980)

$$\frac{\delta_2}{\delta_1} = \left(\frac{1-T_{r2}}{1-T_{r1}}\right)^{0.34} \qquad T_r = \frac{T}{T_C} \quad \textbf{(8)}$$

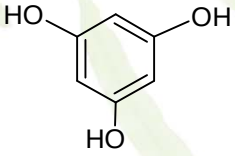
RESULTS : Hansen Solubility parameters

Solute

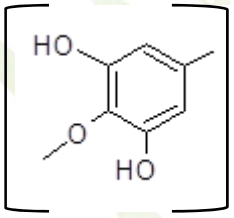
Heptaphloretol



T (°C)	δ_D	δ_P	δ_{HB}	δ_T
25	32,3	15,5	33,8	49,3
100	32,1	15,5	33,6	49,0
200	31,8	15,3	33,4	48,6

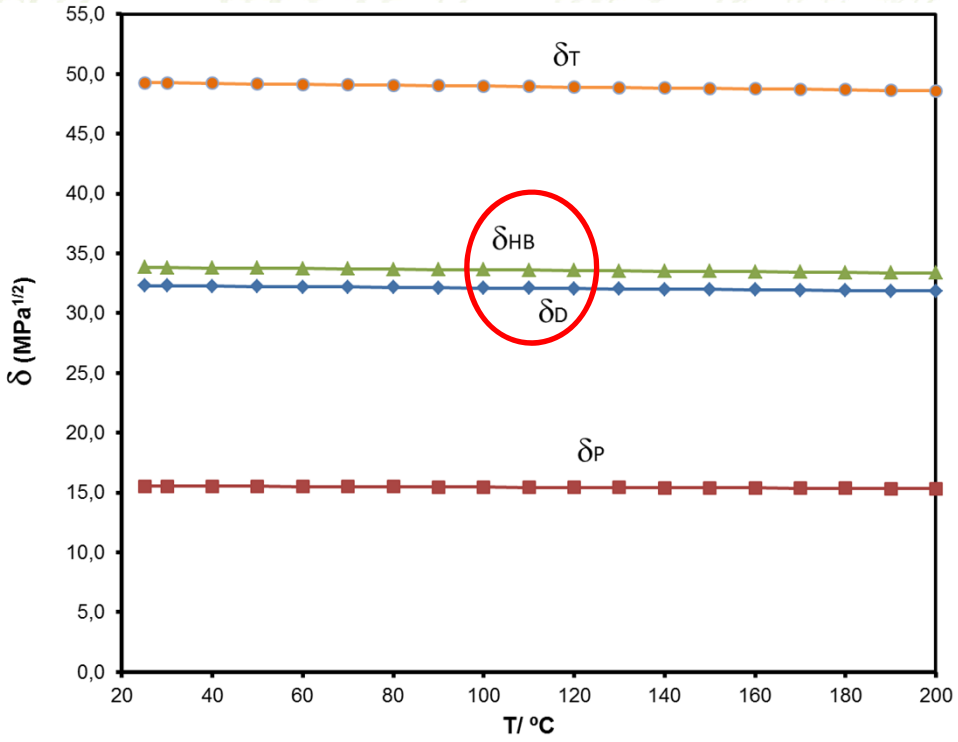


Phloroglucinol



Monomeric unit

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



No dependence of f(T)

Solubility parameters estimation

GROUP CONTRIBUTION METHODS: Solvents

Solvents

- **Ethanol**
- **Water**
- **Ethyl Lactate**
- **CO_2**
- **CO_2+ETOH**

Physical properties:

Rackett equation & Gunn–Yamada met

$$V(T) = \frac{f(T)}{f(T^R)} V^R \qquad f(T) = H_1(1 - \omega H_2) \qquad (8)$$

Solubility parameters → Hansen (1966)

$$\delta_T = \sqrt{\delta_d + \delta_p + \delta_h} \qquad \delta_{i,mix} = x_1 \cdot \delta_{i,1} + x_2 \cdot \delta_{i,2} \quad i=d,p,h \qquad (9)$$

Solubility parameters for <u>subcritical & supercritical conditions</u> → Williams et al., 2004

$$\frac{\delta_{D,ref}}{\delta_D} = \left(\frac{V_{ref}}{V}\right)^{-1.25} \qquad \frac{\delta_{P,ref}}{\delta_P} = \left(\frac{V_{ref}}{V}\right)^{-0.5} \qquad (10)$$

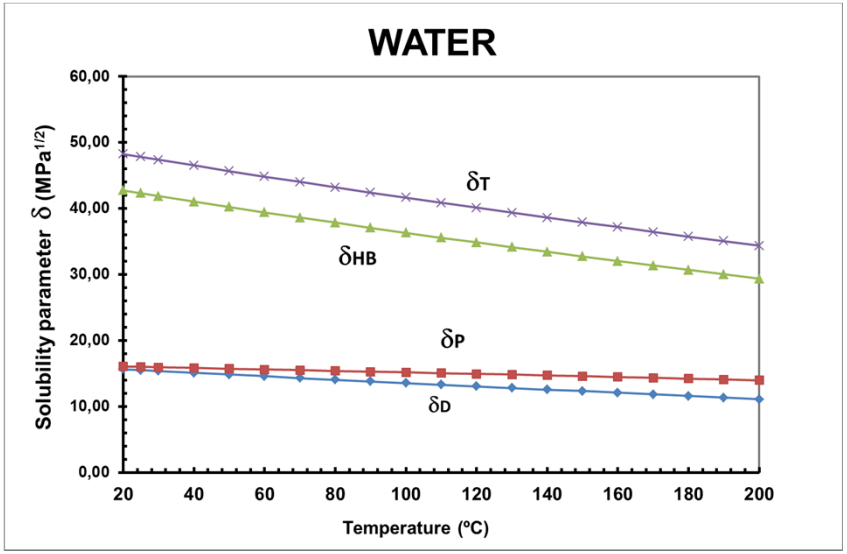
$$\frac{\delta_{D,ref}}{\delta_D} = \exp\left[-1{,}32 \cdot 10^{-3}\left(T_{ref} - T\right) - ln\left(\frac{V_{ref}}{V}\right)^{0.5}\right]$$

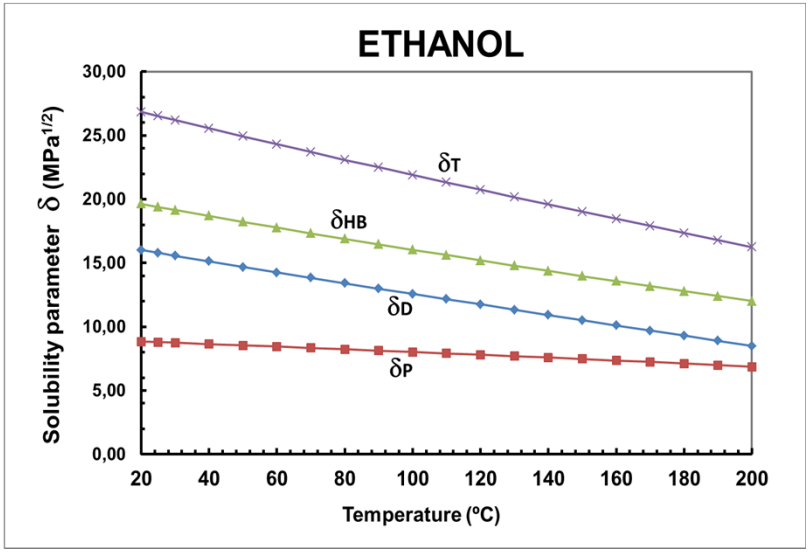
RESULTS: Hansen Solubility parameters

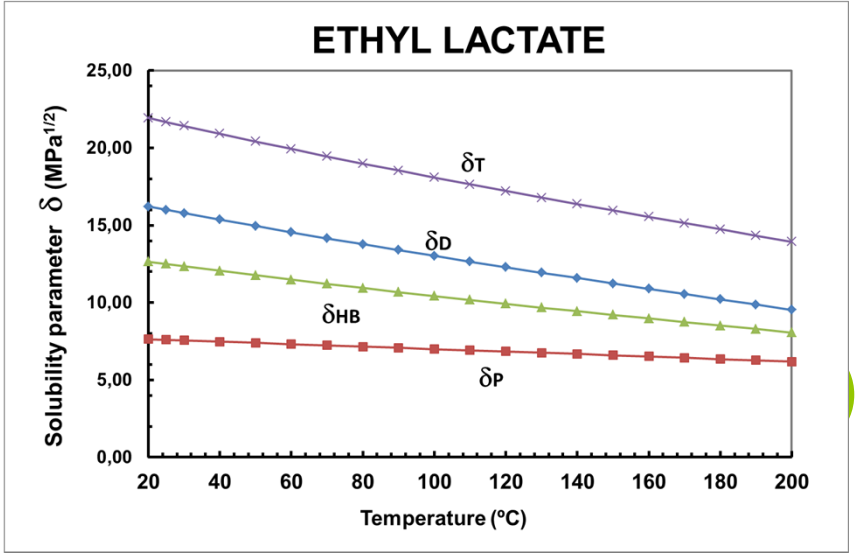
Solvents

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

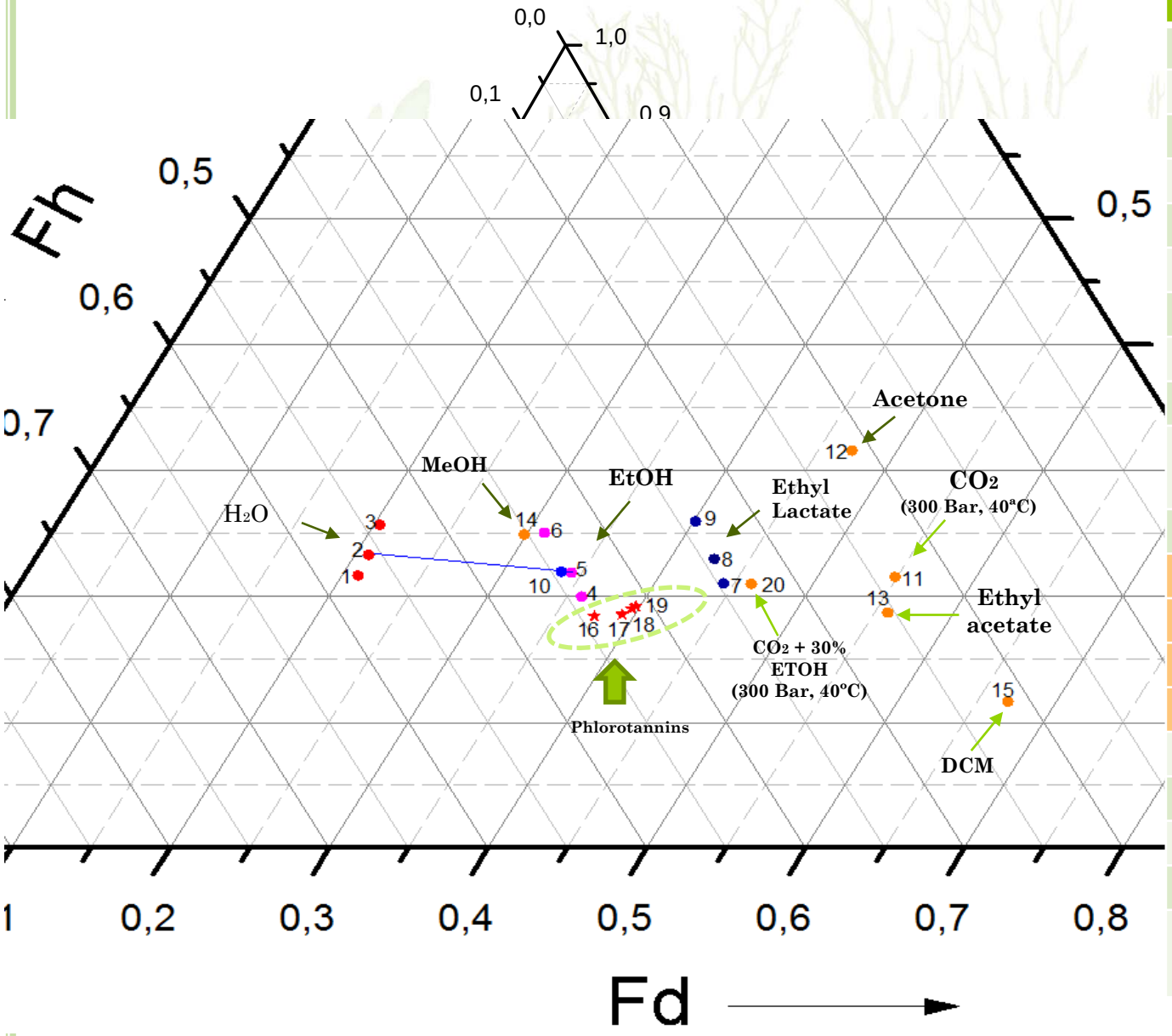
By manipuling the subcritical fluids' **T** there is a change in the contribution of various intermolecular forces







RESULTS : Teas Graph (Fd, Fp, Fh)



No.	Compound	T (°C)
1	Water	25
2	Water	100
3	Water	200
4	Ethanol	25
5	Ethanol	100
6	Ethanol	200
7	Ethyl Lactate	25
8	Ethyl Lactate	100
9	Ethyl lactate	200
10	Ethanol : Water 95:5	100
11	CO2 (300Bar)	40
12	Acetona	25
13	Ethyl Acetate	25
14	Methanol	25
15	Dicloromethane	25
16	Phloroglucinol	25
17	Phloretholes PD3	25
18	Phloretholes PD7	25
19	Monomeric unit	25
20	CO2 + 30% ETOH (300Bar)	40

CONCLUSIONS

- Ethanol/Water (95:5 v/v) mixture at 100ºC showed the highest selectivity to extract phlorotannins from *Cystoseira abies-marina*

- The main type of phlorotannins found in the purified samples were phloroethols with a degree of polymerization from 3-16 units

- Temperature does not have an strong effect on the solubility parameters of the phlorotannins studied.

- Hansen solubility parameters is an interesting predicting method to estimate the solubility of structurally complex, biological solutes in sub-and/or supercritical fluid, optimizing conditions for their extraction from natural matrices.

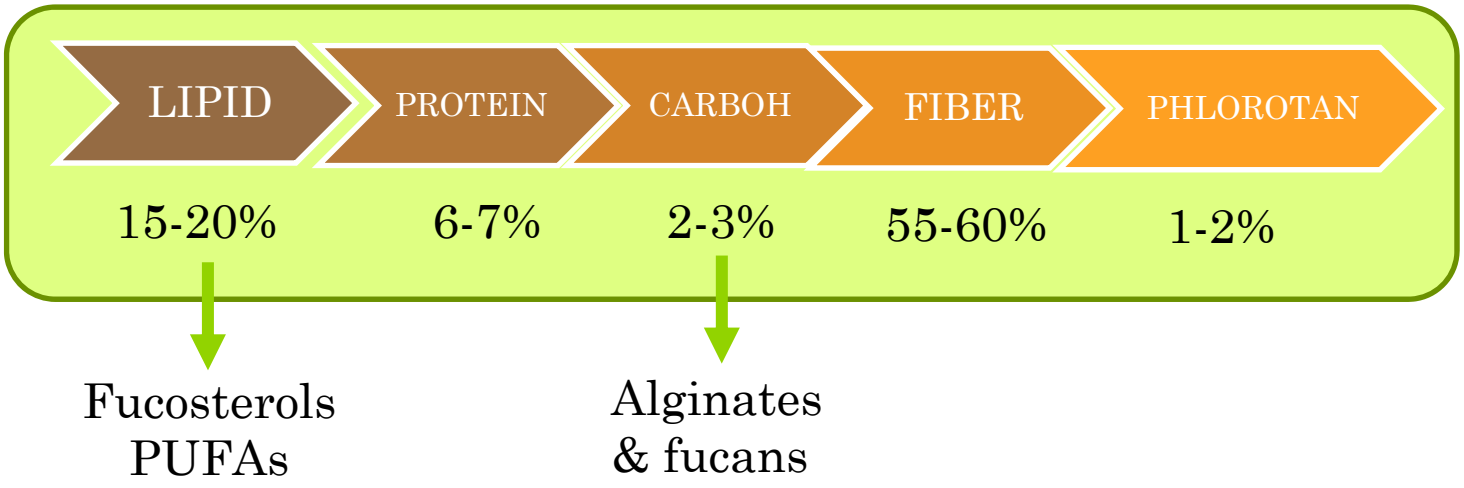
- Solvents such as ethanol, ethyl lactate and CO2 + 30% ethanol can be suitable to design a new Green Purification method for phlorotannins extraction.

FUTURE CHALLENGES

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

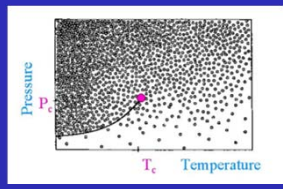
Multi-step process

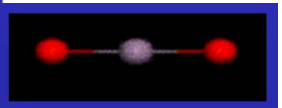
Biorefinery concept



Green Solvents, Absorbents, Physical Separations

STEP TO THE FUTURE





GREEN PROCESSING PLATFORM

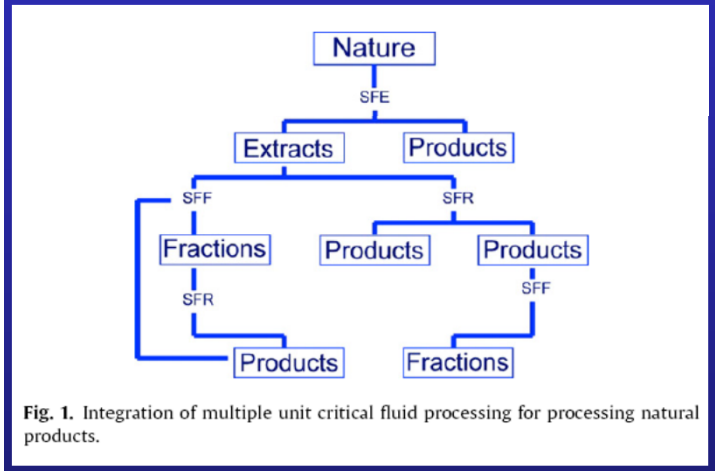
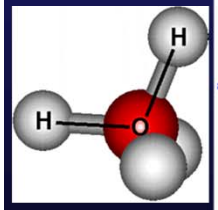
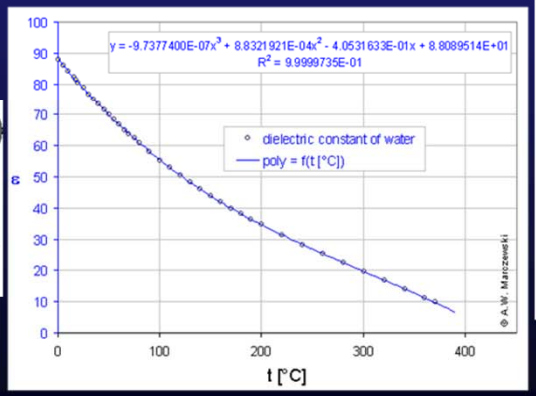
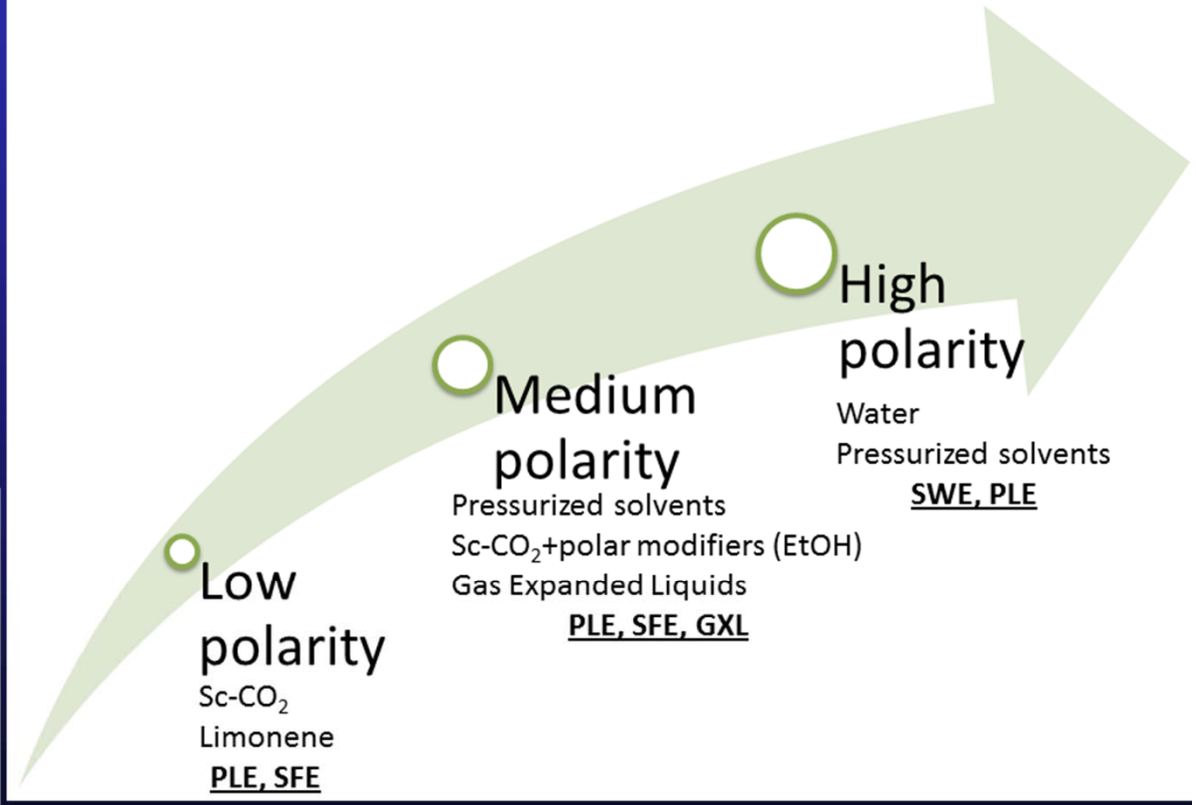


Fig. 1. Integration of multiple unit critical fluid processing for processing natural products.

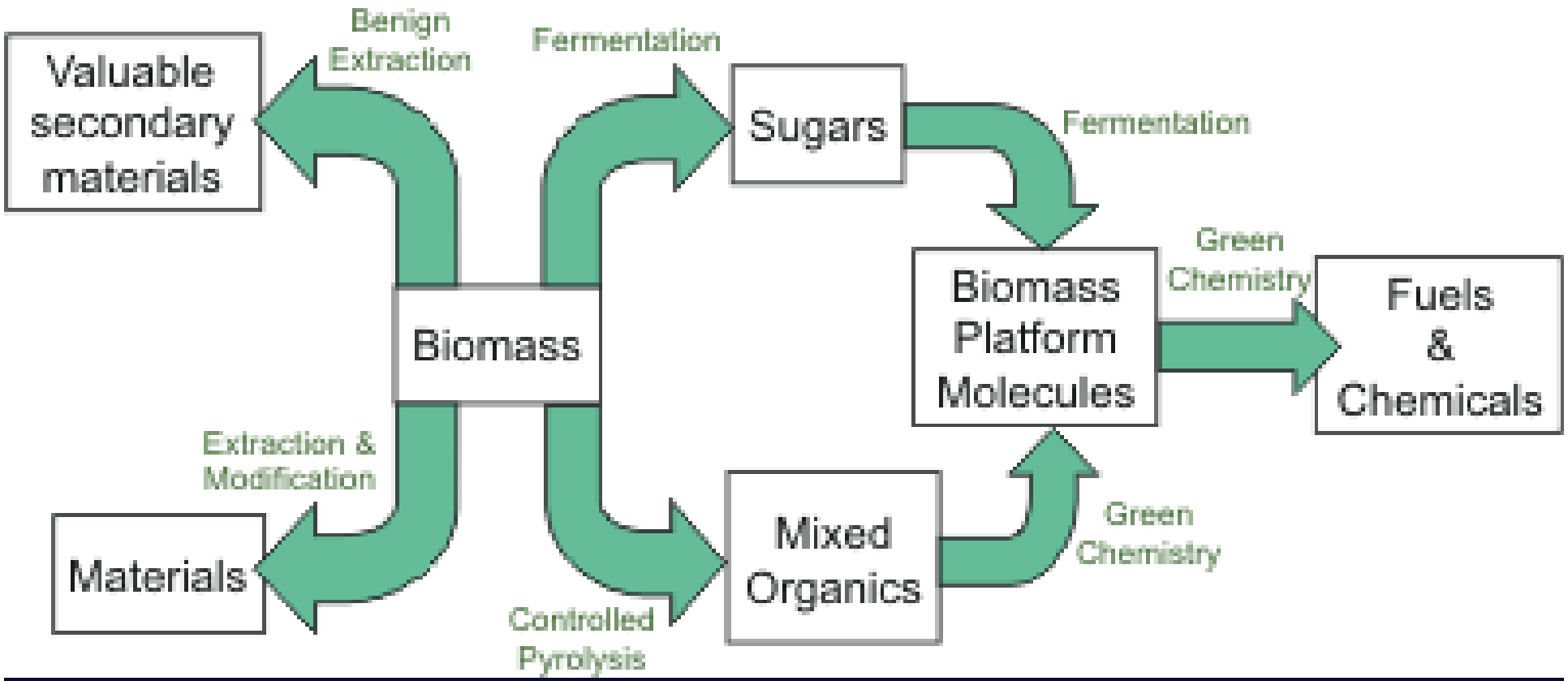
JerryW. King, Keerthi Srinivas, J. of Supercritical Fluids 47 (2009) 598–610

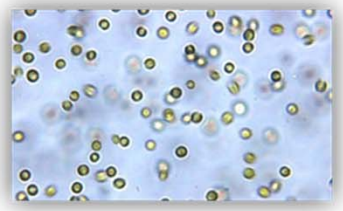






Green Chemistry and the Biorefinery





1 $scCO_2$ 300 bar, 50 °C

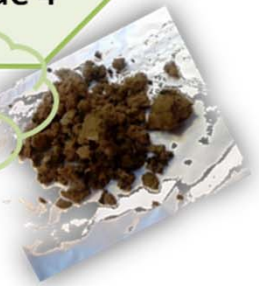
2 CXE $scCO_2$, 70 bar, 50 °C/ 15-45-75% EtOH

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

- Carotenoids
- Non-polar lipids

Extract 2

- Carotenoids
- Chlorophylls
- Mid and high-polar lipids

Extract 3

- Antioxidant activity
- Protein
- Sugars

Extract 4

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

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