



**GROUPE DE RECHERCHE
EN ECO-EXTRACTION
DE PRODUITS NATURELS**

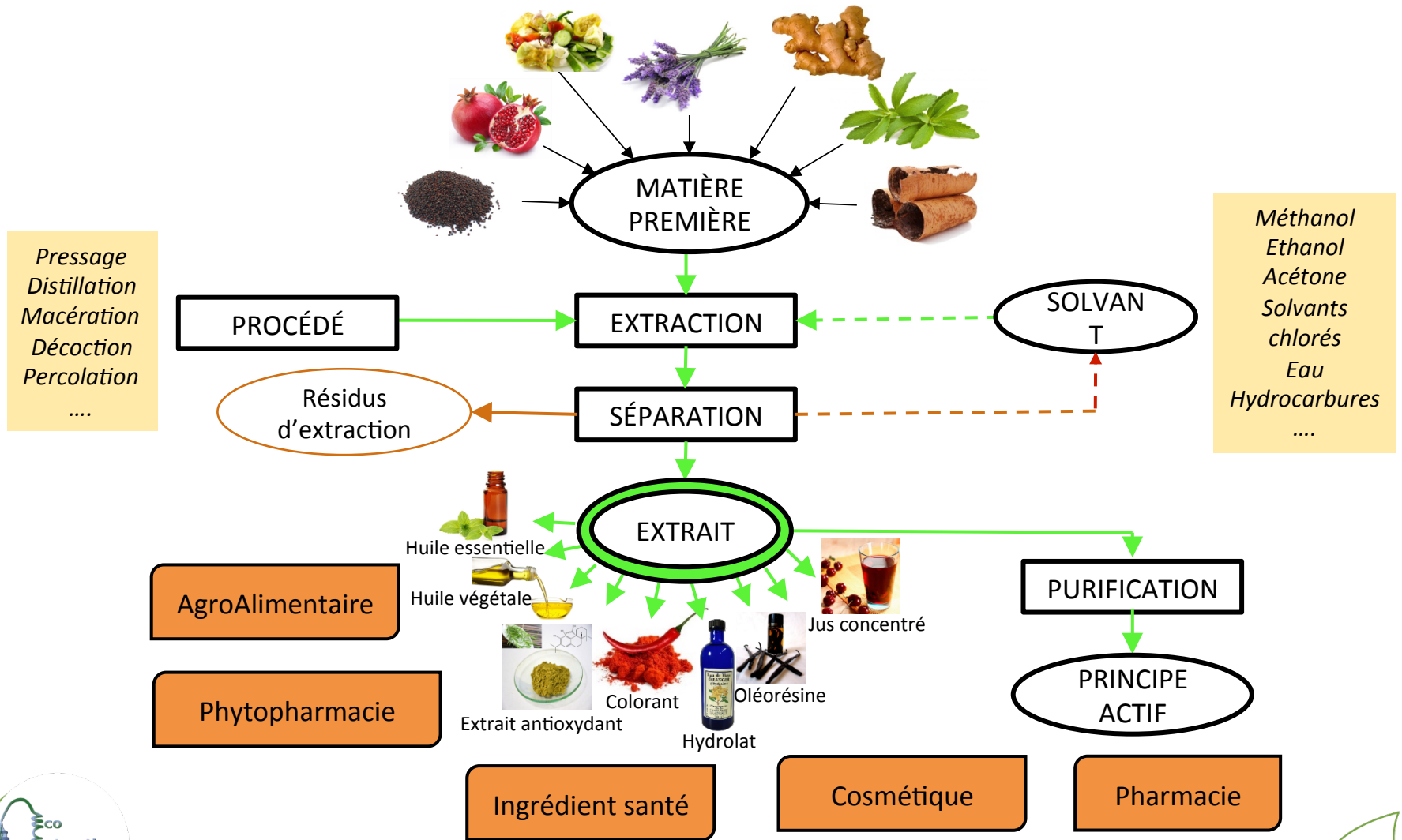
Complémentarité de l'HPTLC et des techniques d'éco-extraction

Avignon, 13 Avril 2017

Maryline VIAN, MC HDR

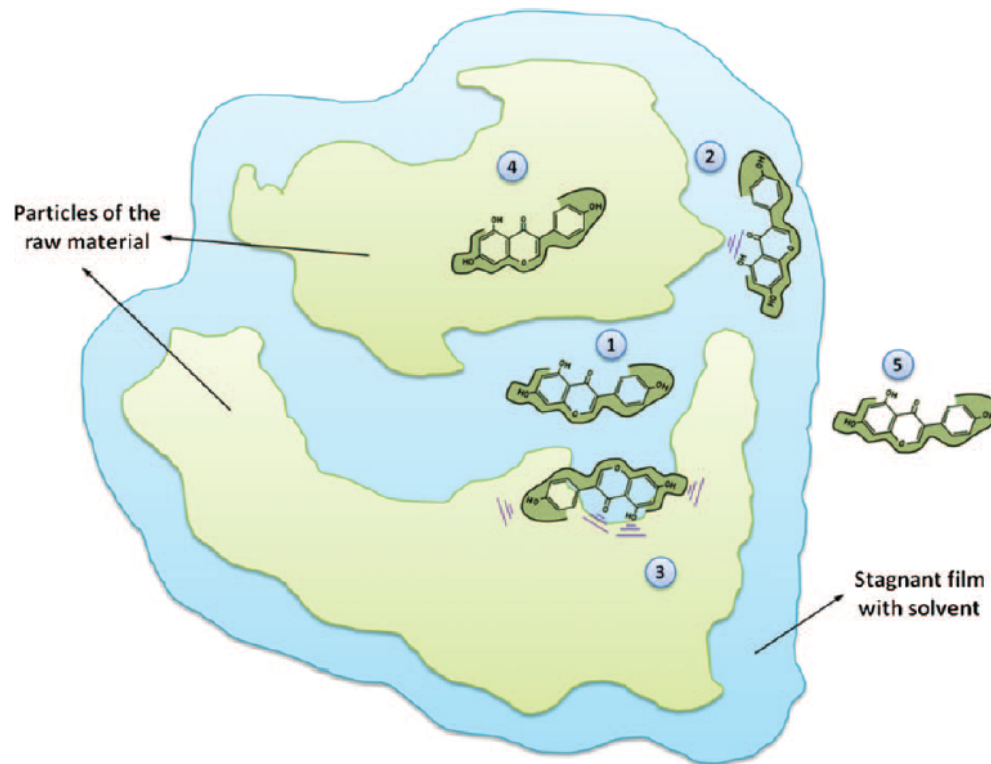


Qu'est ce que l'extraction végétale?



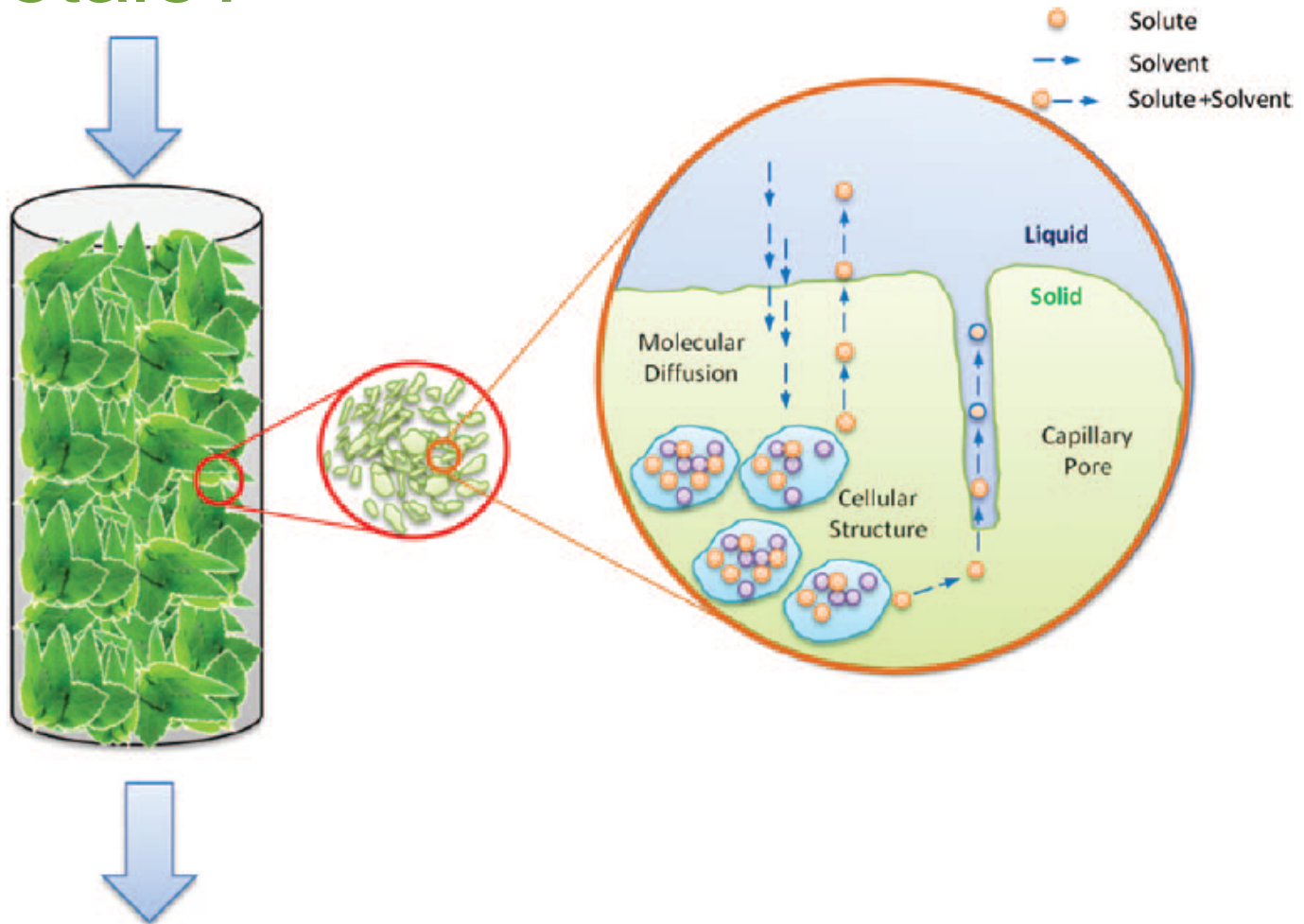
Qu'est ce que l'extraction végétale?

- Où sont localisées les molécules d'intérêt dans le végétal?



D'après *Natural Product Extraction. Principles and Applications*. RCS Publishing, 2013.

Le mécanisme de l'extraction végétale?



D'après *Natural Product Extraction. Principles and Applications*. RCS Publishing, 2013.

Les paramètres de l'extraction végétale?

Matière Première

- Origine/nature
- Granulométrie
 - Humidité
- Degré de « processing »

Procédé

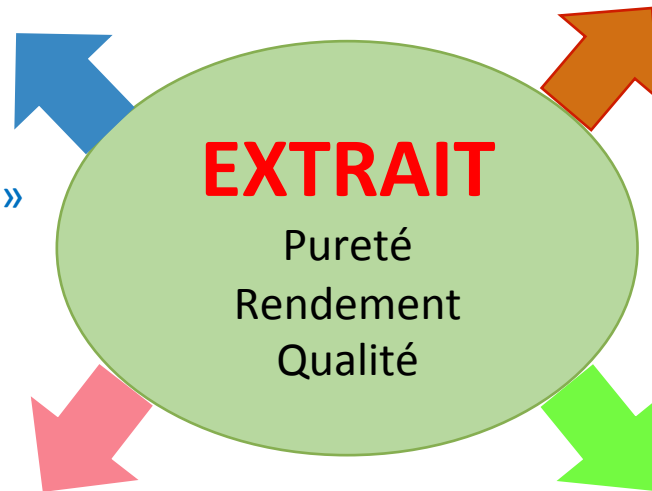
- Design
- Température
- Temps
- Pression

Solvant

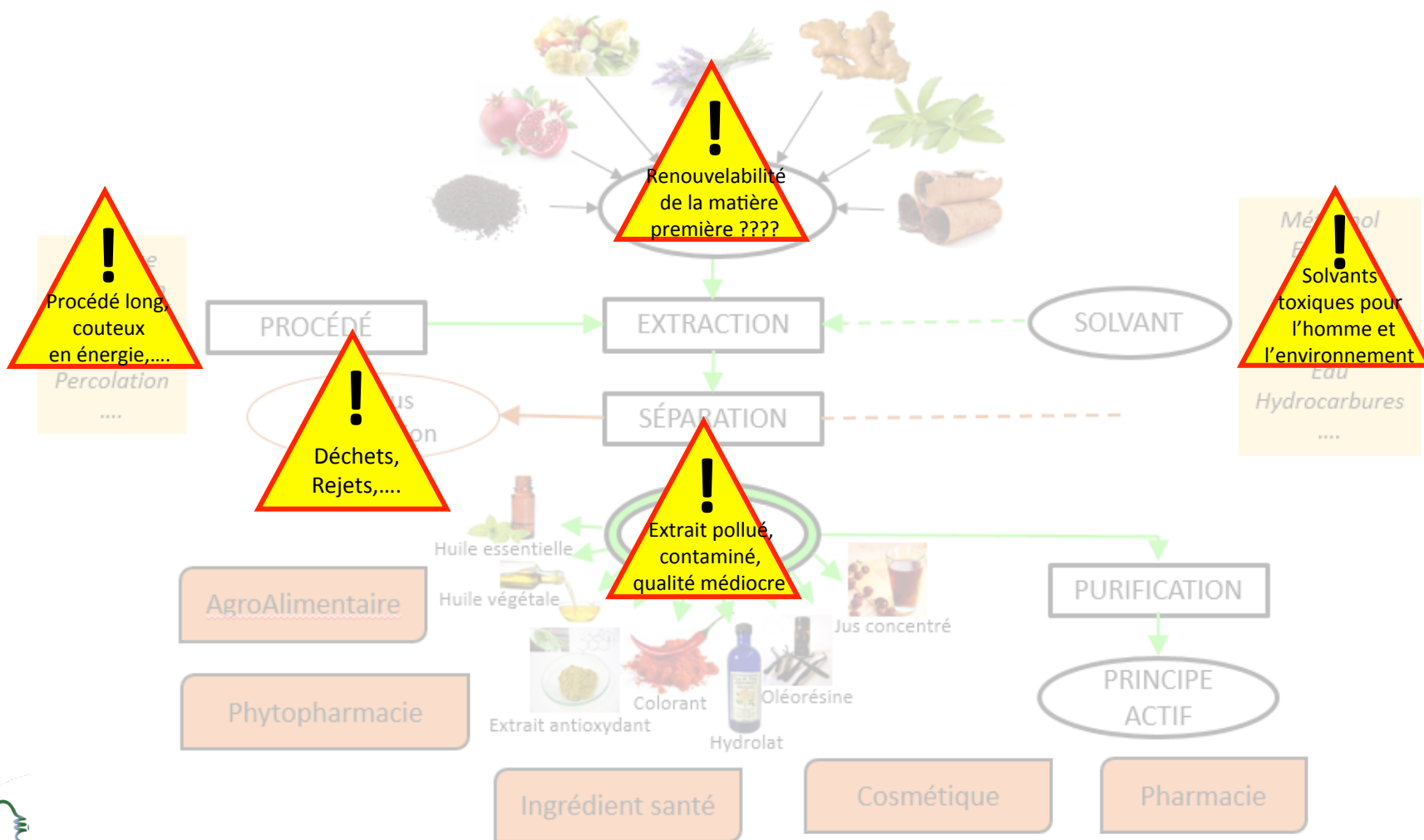
- Nature chimique
- Sélectivité, P.E.
- ratio solide/liquide
- Toxicité, Inflammabilité

Equipement

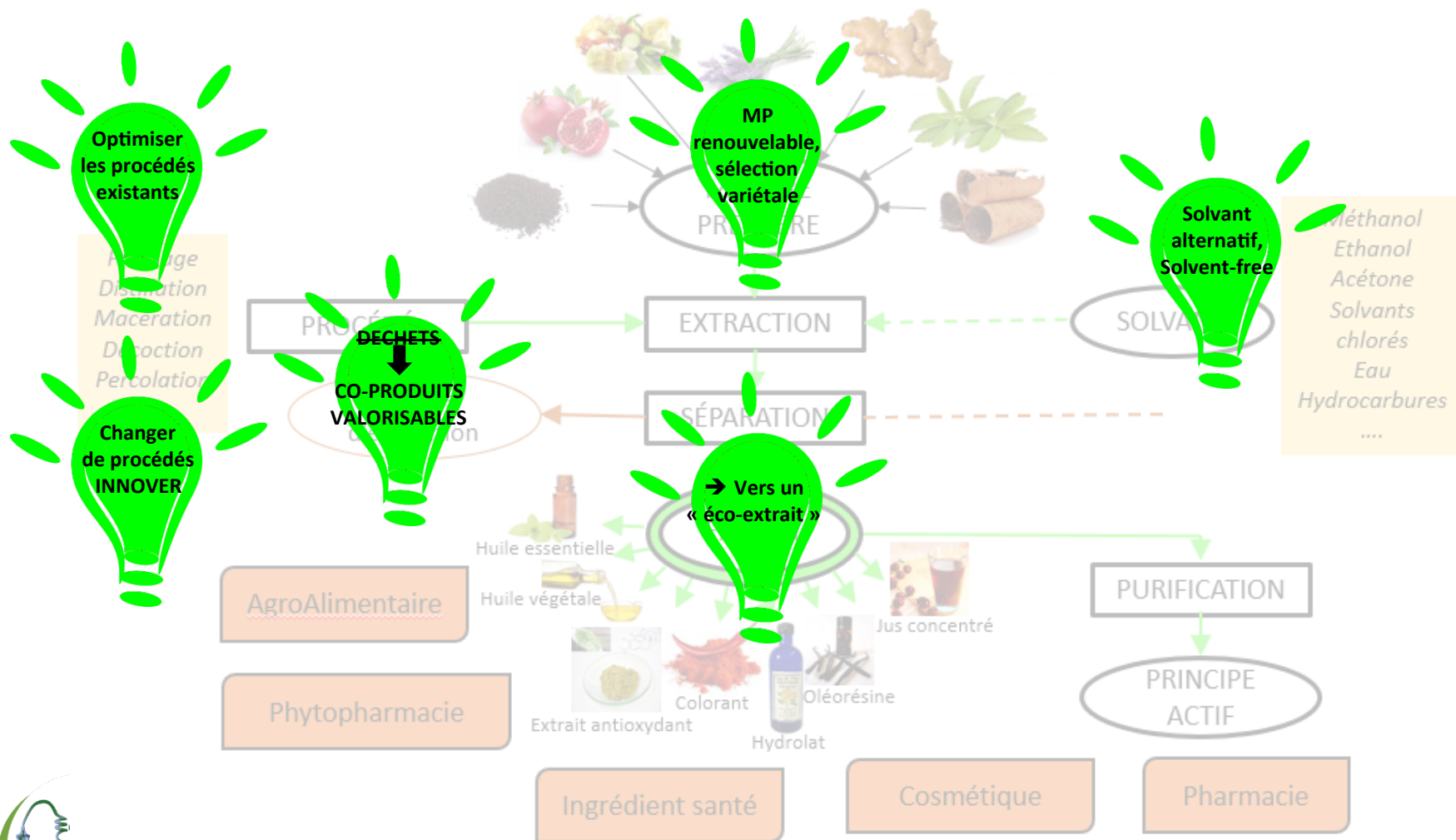
- Scale-up
- Capacité
- Batch/continu
- Agitation



Les problématiques rencontrées?



Vers l'éco-extraction végétale....



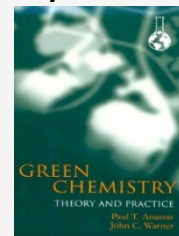
Définition de l'éco-extraction

- Emergence du concept en 2010
- Initié par GREEN en association avec pôles de compétitivité et industriels
- En s'inspirant des 12 principes de la chimie verte, définition de 6 principes de l'éco-extraction

1. Prevent Waste
2. Maximize Atom Economy
3. Design less Hazardous Chemical Syntheses
4. Design safer Chemicals and Products
5. Use safer Solvents and Reaction conditions
6. Increase Energy Efficiency
7. Use Renewable Feedstocks
8. Avoid Chemical Derivatives
9. Use Catalysts, not Stoichiometric Reagents
10. Design Chemicals and Products that Degrade

11. Analyze in Real time to Prevent Pollution
 12. Minimize the Potential for Accidents
- Anastas, Paul T.; Warner, John C. *Green Chemistry Theory and Practice*; Oxford University Press: New York, 1998**

Les 12 Principes de la Chimie Verte

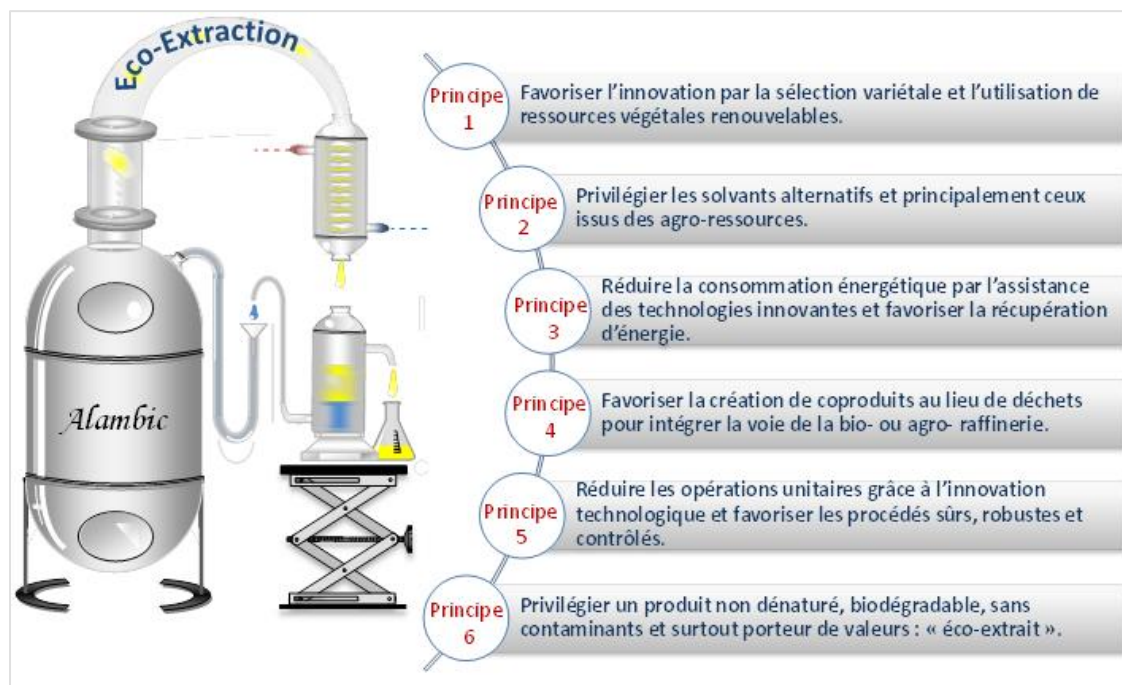


Définition de l'éco-extraction



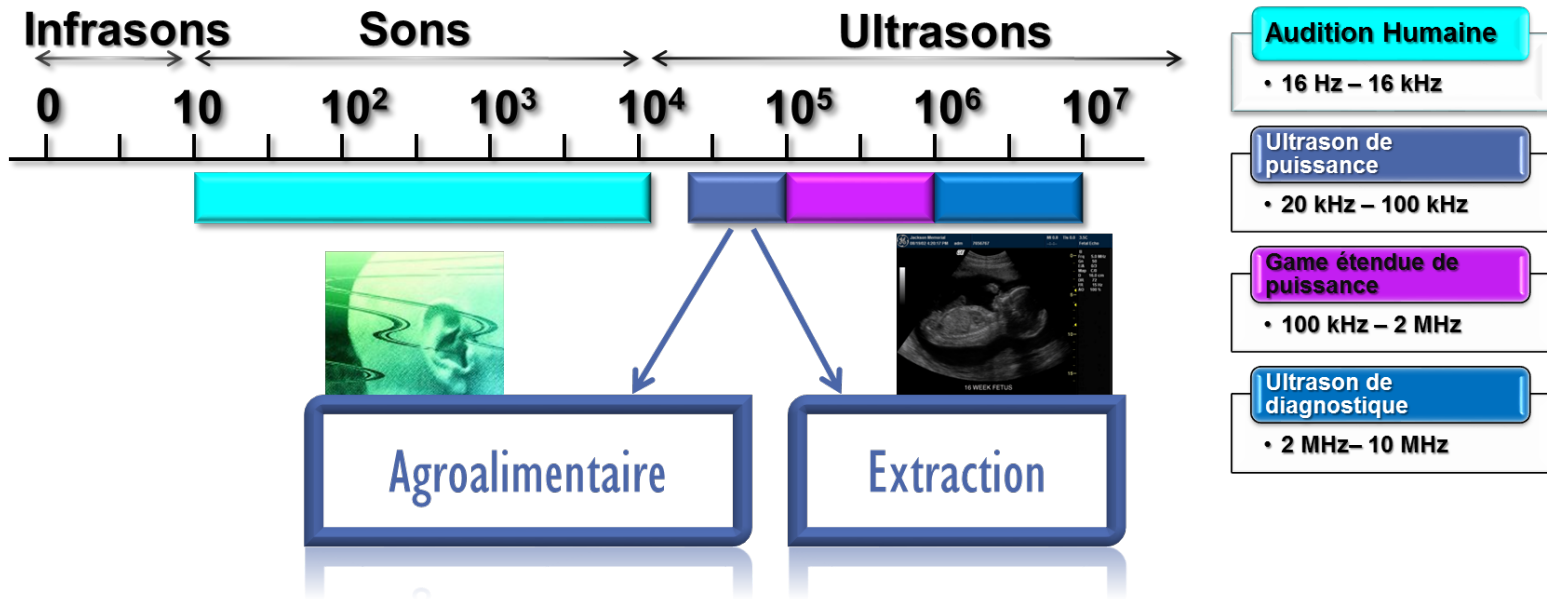
1 • ÉCO-EXTRACTION : CONTEXTE ET INNOVATION

MARYLINE ABERT VIAN, FARID CHEMAT,
LAURENT PRAT ET CHRISTOPHE GOURDON



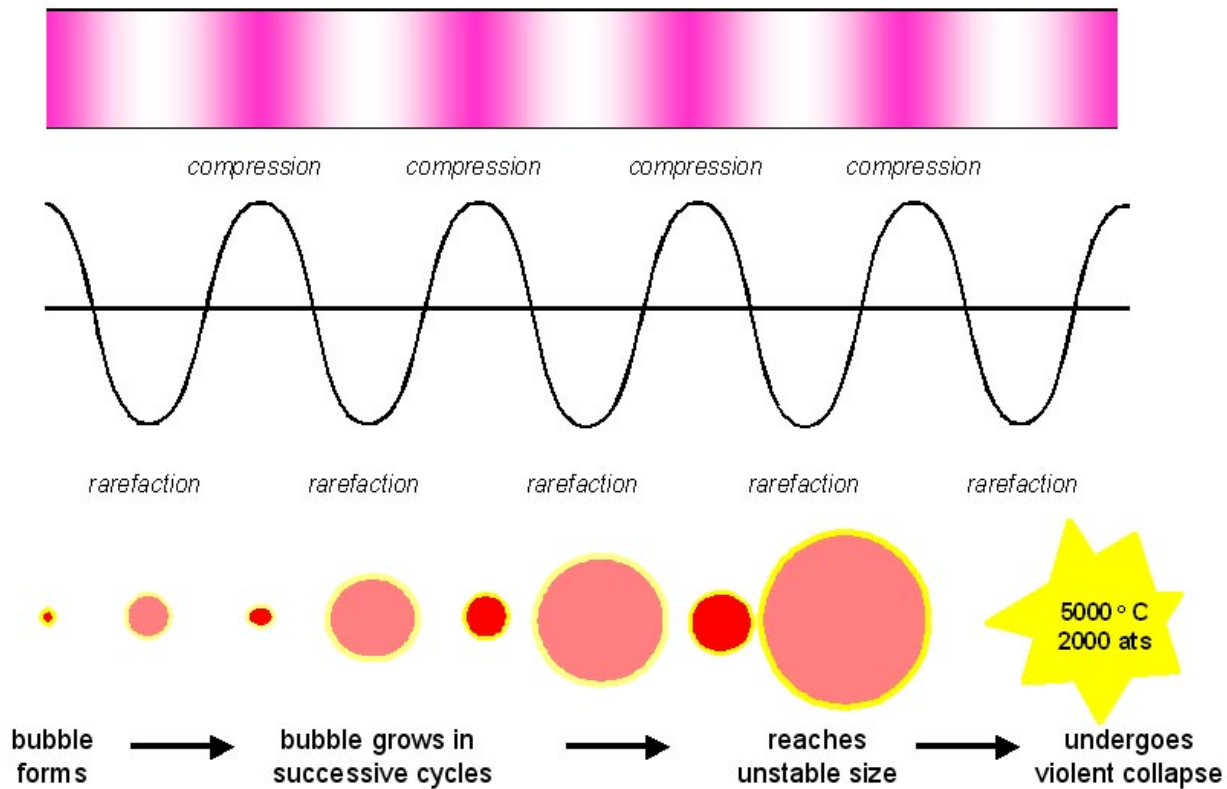
D'après *L'éco-extraction du végétal*, Dunod

Les ultrasons

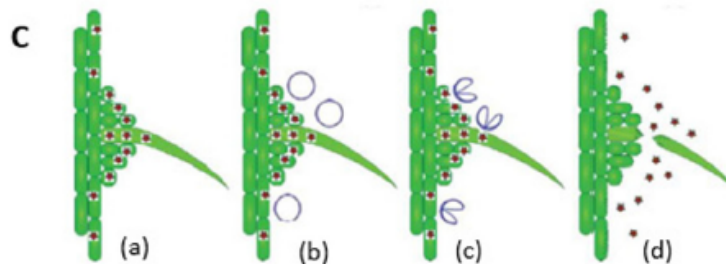
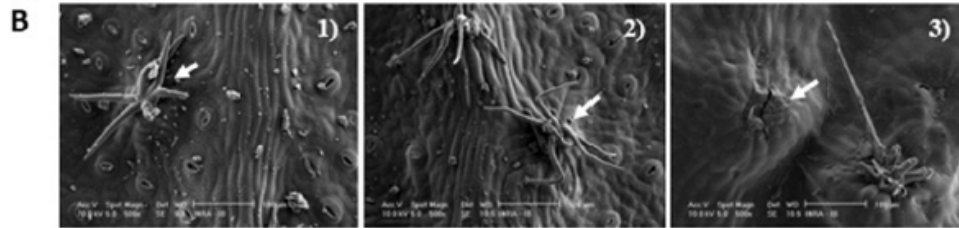
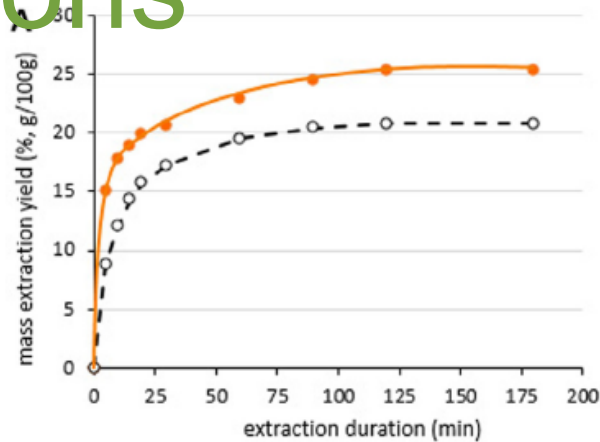


Les ultrasons

ACOUSTIC CAVITATION



L'extraction assistée par ultrasons



D'après L. Petigny, S. Périno-Issartier, J. Wajsman, F. Chemat. *Batch and Continuous Ultrasound Assisted Extraction of Boldo Leaves (Peumus boldus Mol.)*. *Int. J. Mol. Sci.* 2013, 14, 5750-5764.



L'extraction assistée par ultrasons

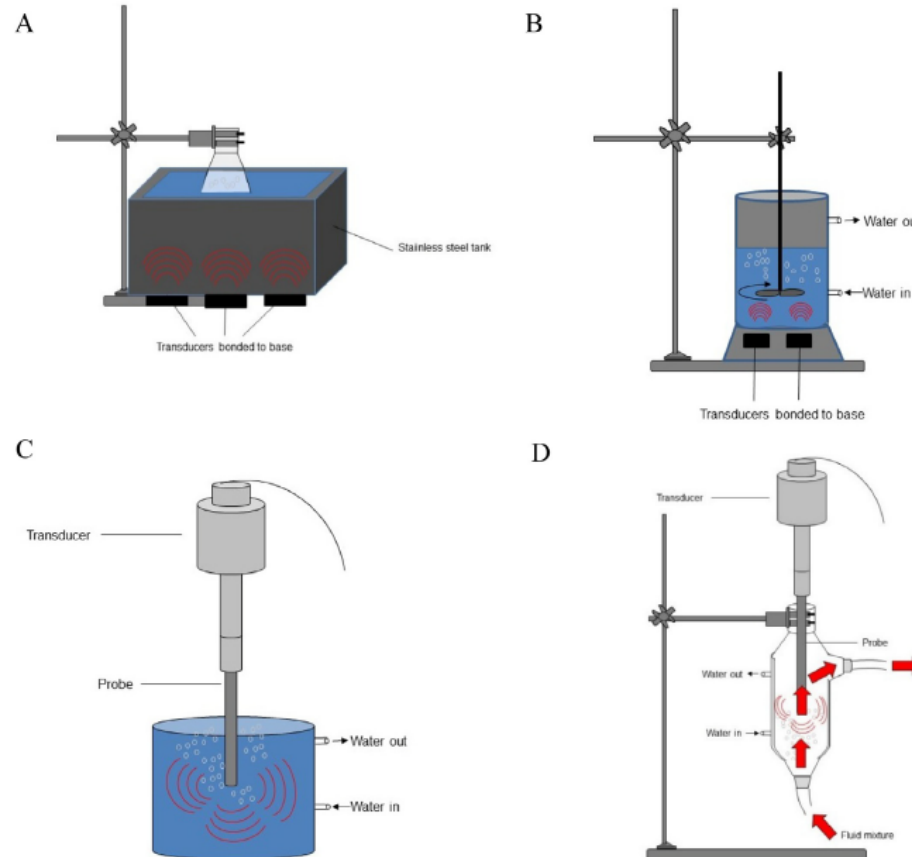


Fig 3. Commonly used ultrasonic systems (A: Ultrasound bath, B: Ultrasound reactor with stirring, C: Ultra sound probe, D: Continuous sonication with ultra sound probe).

D'après F. Chemat, N. Rombaut, A.G. Sicaire, A. Meullemiestre, A.S. Fabiano-Tixier, M. Abert-Vian. *Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. Ultrasonics Sonochemistry* 34 (2017) 540–560



L'extraction assistée par ultrasons



50 L



1000 L



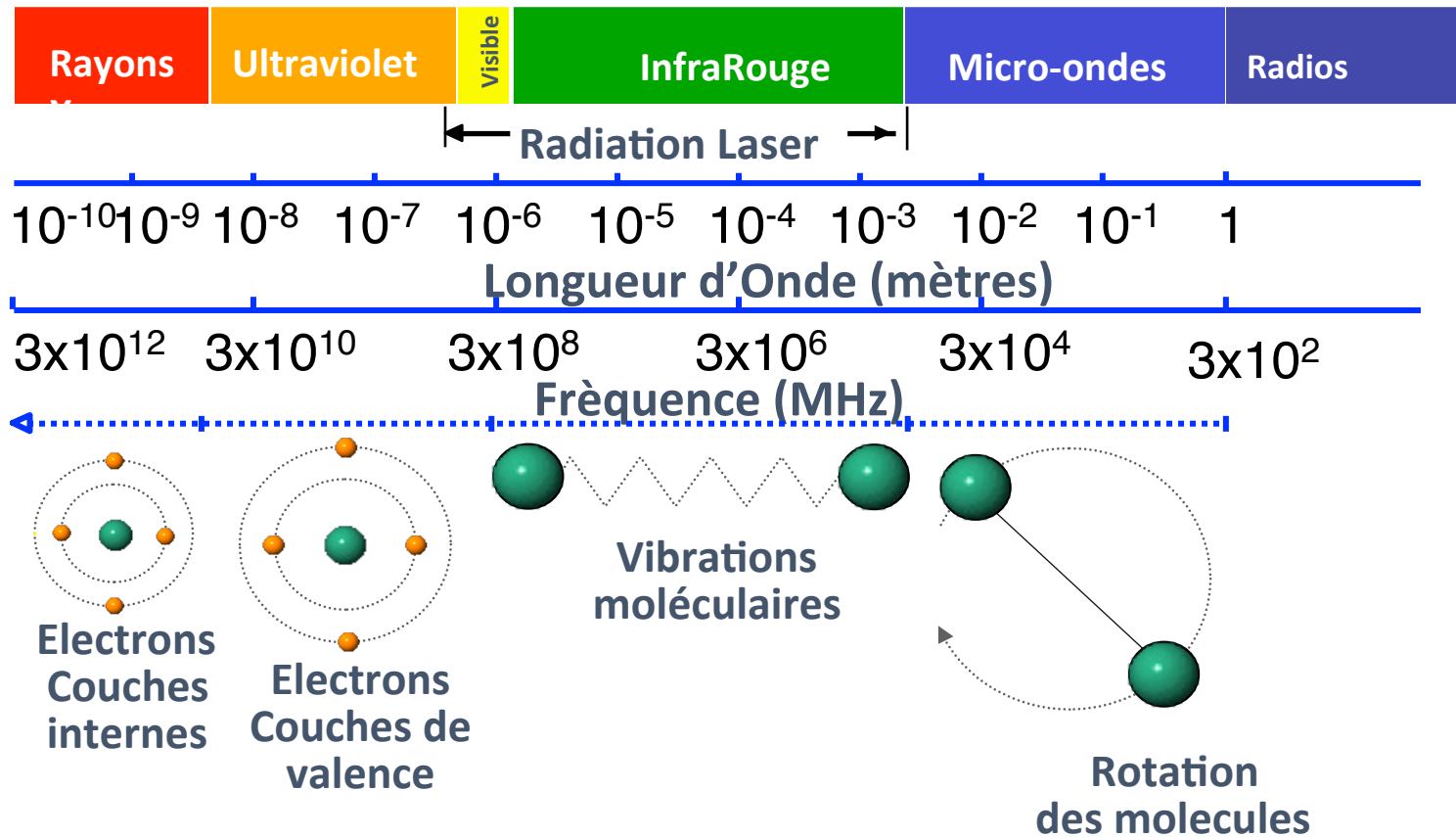
Fig. 11. Industrial ultrasonic continuous equipments (Hielscher - www.hielscher.com).

Fig. 12. Industrial ultrasonic batch equipments: 50, 500 and 1000 L (Reus - www.etsreus.com).

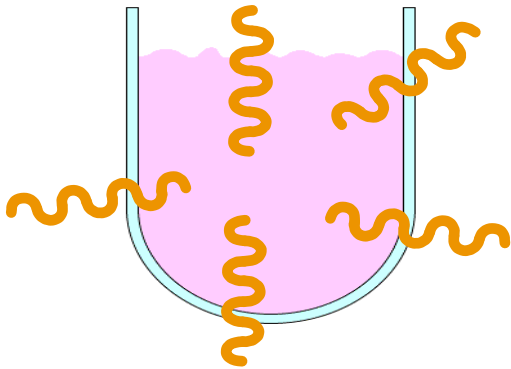


D'après F. Chemat, N. Rombaut, A.G. Sicaire, A. Meullemiestre, A.S. Fabiano-Tixier, M. Abert-Vian. *Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. Ultrasonics Sonochemistry* 34 (2017) 540–560

Les micro-ondes

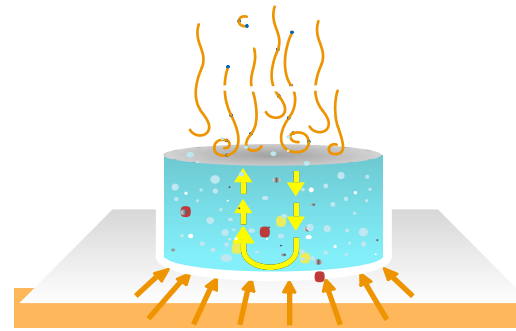


Le chauffage par micro-ondes



Chauffage par micro-ondes

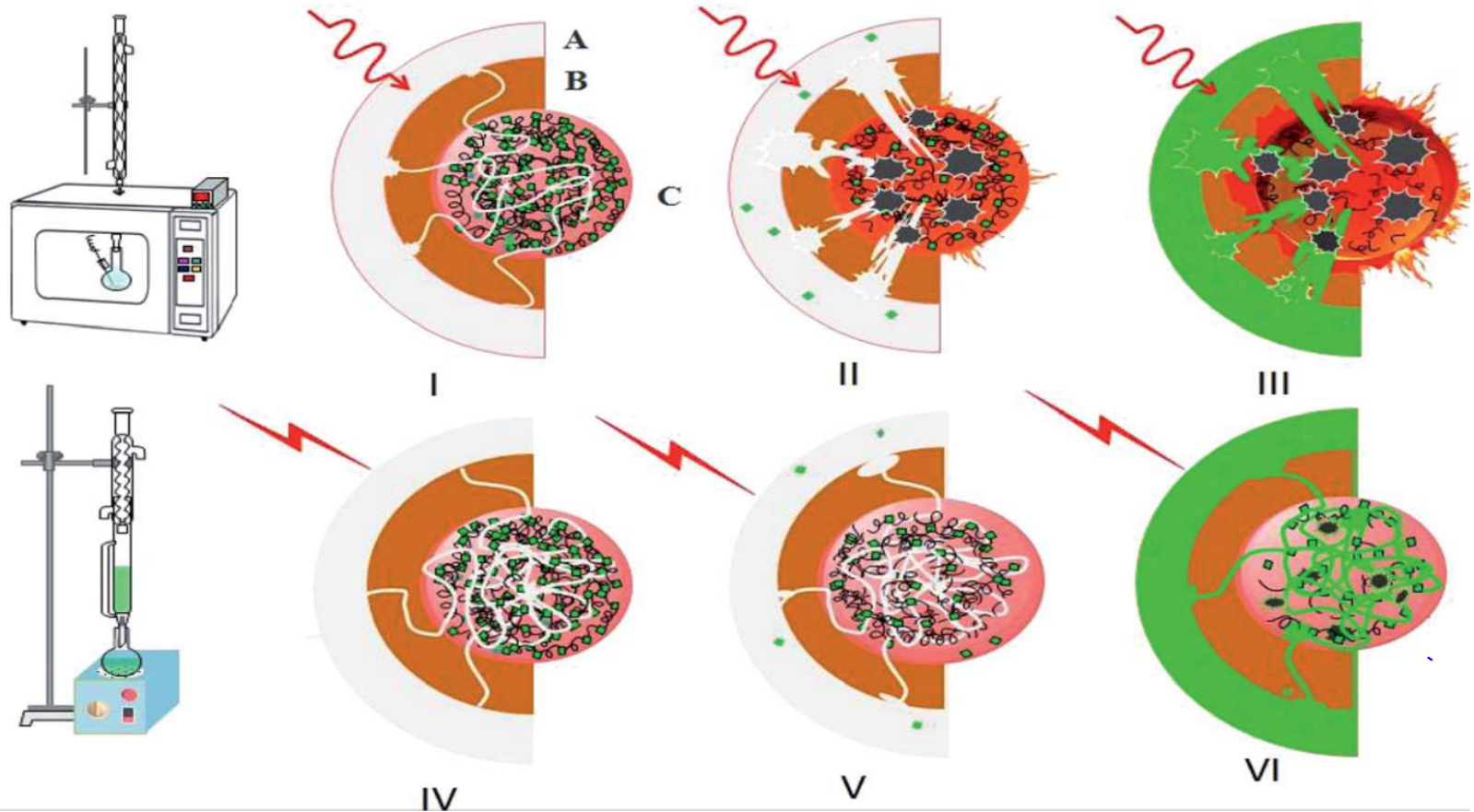
- Chauffage de l'intérieur vers l'extérieur
- Pénétration immédiate et instantanée
- Chauffage sélectif des substances polaires
- Grande rapidité d'élévation thermique



Chauffage conventionnel

- La température des parois extérieures est supérieure à la température d'ébullition du liquide
- Processus lent et non sélectif

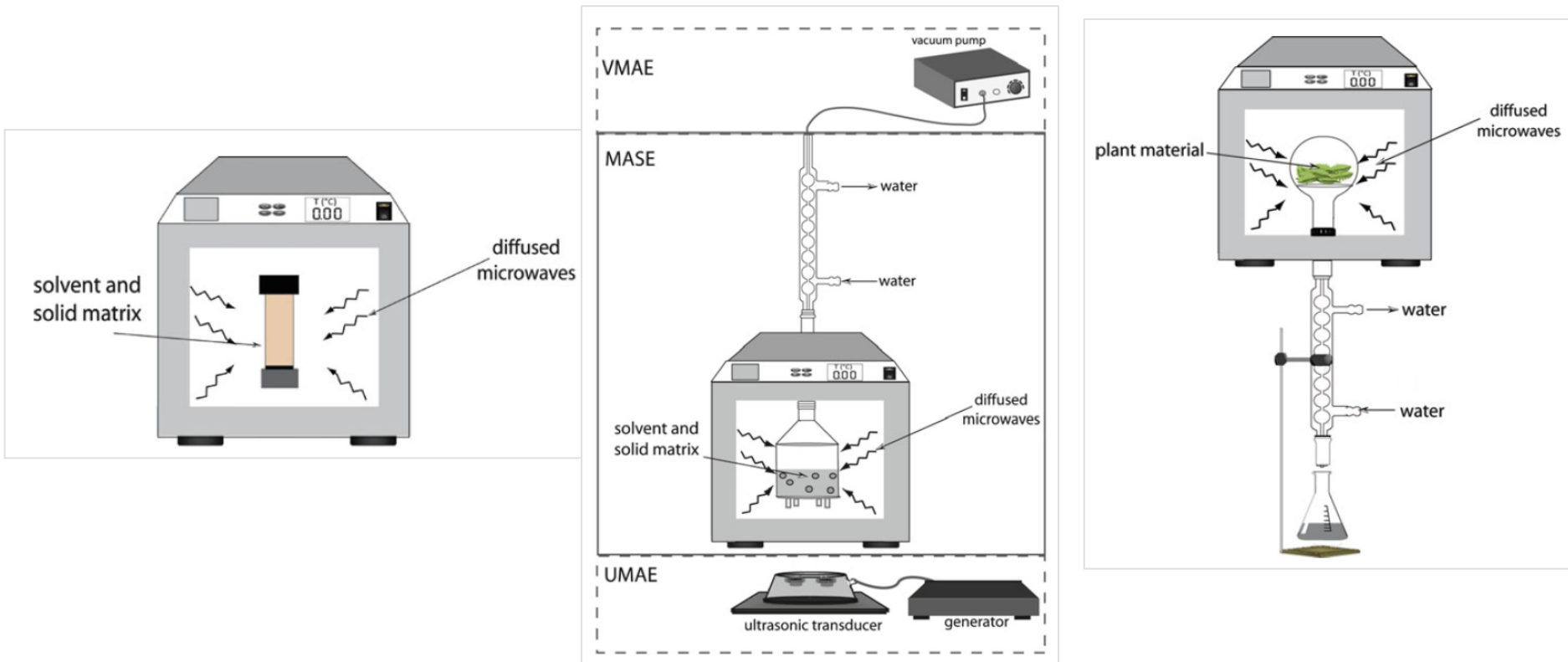
Mécanisme de l'extraction assistée par micro-ondes



D'après H.K. Kala, R. Mehta, K.K. Sen, R. Tandey, V. Mandal. Strategizing method optimization of microwaves assisted extraction of plant phenolics by developing standard working principles for universal robust optimization. *Anal. Methods*, 2017, 9, 2089

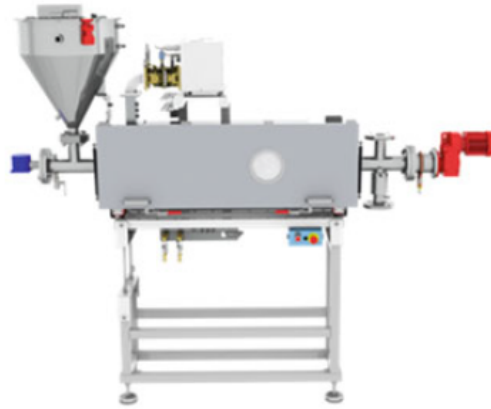


L'extraction assistée par micro-ondes



D'après *Microwave-assisted extraction for bioactive compounds. Theory and Practise. Springer 2013. Chemat & Cravotto Editors*

L'extraction assistée par micro-ondes



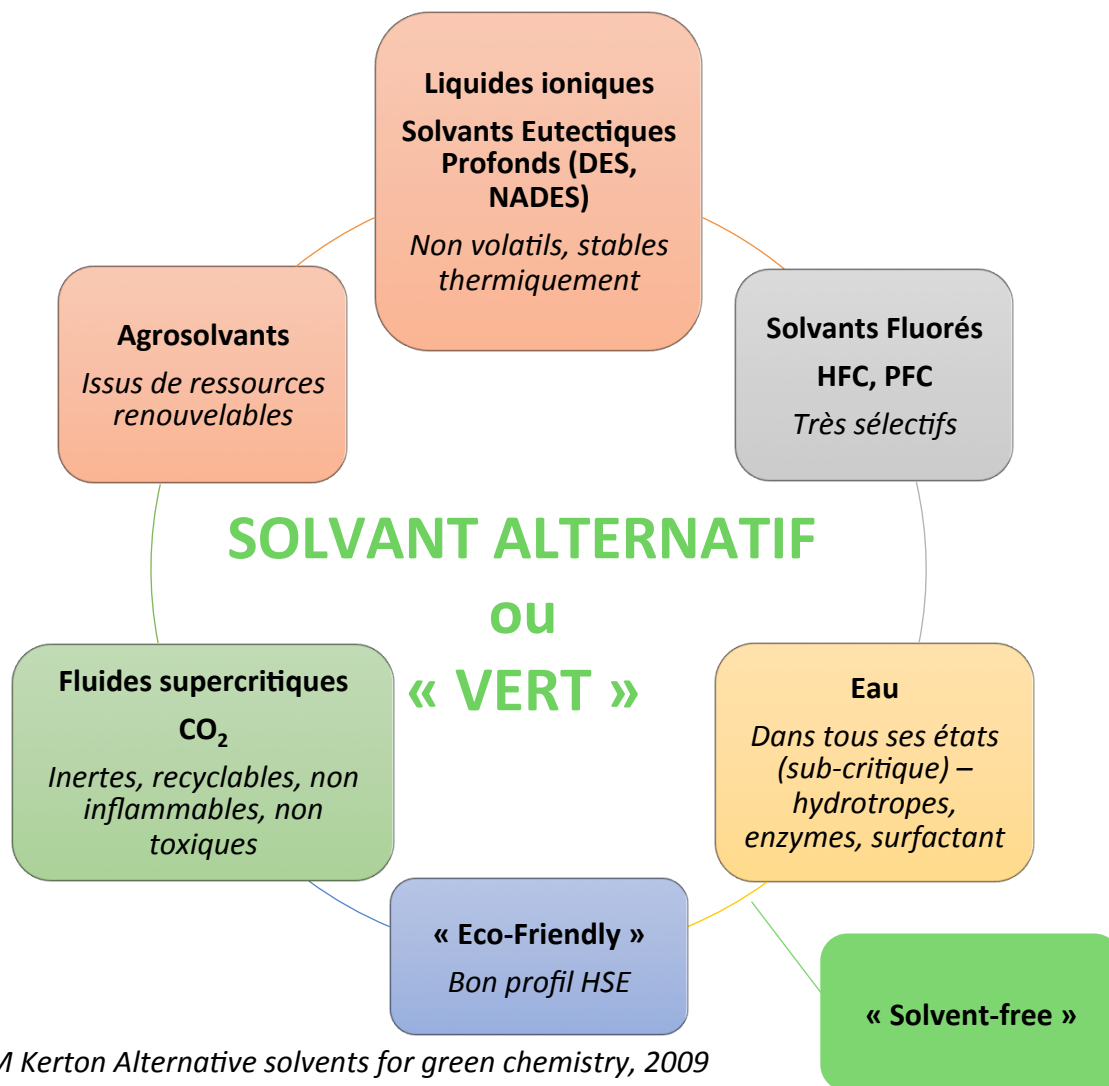
Schematic of continuous flow reactor



Photo of the continuous flow reactor



Les solvants alternatifs

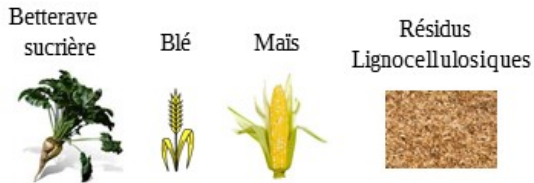


D'après FM Kerton *Alternative solvents for green chemistry*, 2009



Les agrosolvants

Filière céréalière/sucrière



Filière bois



Filière oléo-protéagineuse



TRANSFORMATIONS MECANIQUES, BIOCHIMIQUES ET CHIMIQUES

Saccharose

Amidon

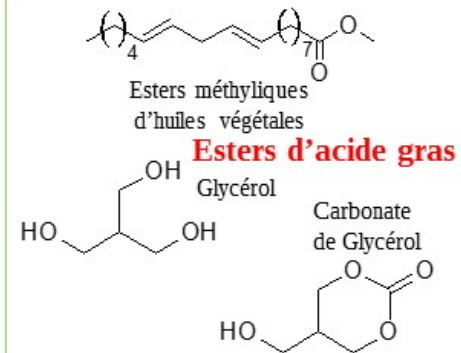
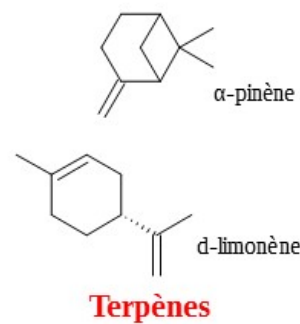
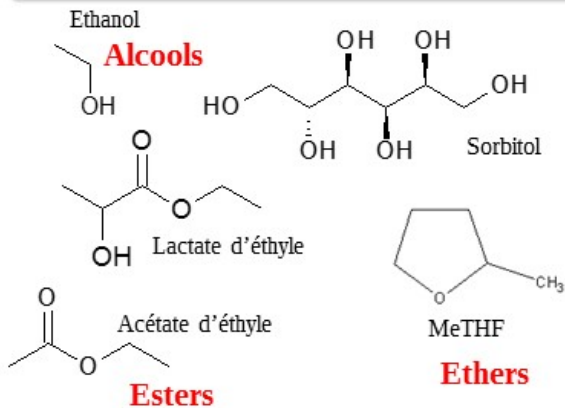
Lignine

Cellulose

Huiles essentielles

Huiles végétales

TRANSFORMATIONS BIOCHIMIQUES ET CHIMIQUES



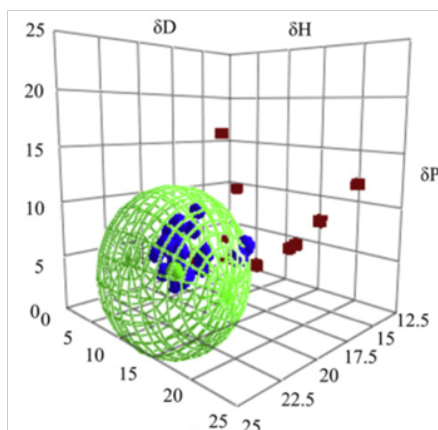
Outils de prédiction des interactions soluté/solvant

Approche Classique

Para mètres de solubilité de Hansen

Calcul de δ_d , δ_p , δ_h

« Le semblable dissout le semblable »

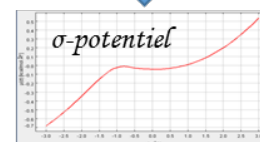
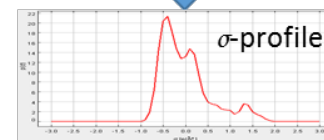
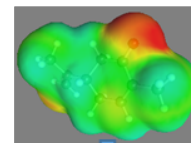


Approche Récente

COSMO-RS

Basé sur la Chimie quantique

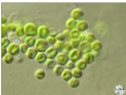





Calcul de distribution des charges sur une molécule individuelle

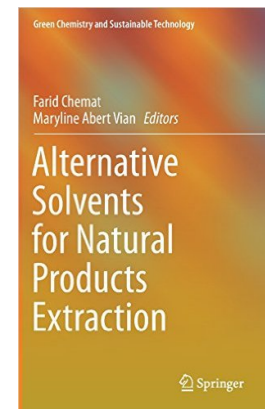


Prédictions des propriétés physico-chimiques



Les solvants alternatifs pour l'extraction végétale

Biomasse	Analyte ciblé	Objectif Application
 Micro-algues	Huile	Emploi d'un solvant vert Bioénergie - Biocarburant
 Micro-algues	Caroténoïdes	Emploi d'un solvant vert Cosmétique
 Tourteau de colza	Huile	Substitution du n-hexane dans le procédé de trituration Agro-alimentaire
 Bourgeons de Cassis	Arômes	Substitution du n-hexane dans la fabrication des concrètes Parfums
 Carvi	Arômes	Substitution du n-hexane dans la fabrication extraits aromatiques Agro-alimentaire
 Mangoustanier	Antioxydants	Substitution de solvants chlorés dans la fabrication extraits actifs Cosmétique – Pharmacie



L'HPTLC, outil d'analyse des « éco-extraits »



ATS 4

Dépôt automatisé des extraits sur la plaque de silice



ADC 2

Développement automatisé de la plaque



Scanner

E v a l u a t i o n
densitométrique



Visualizer




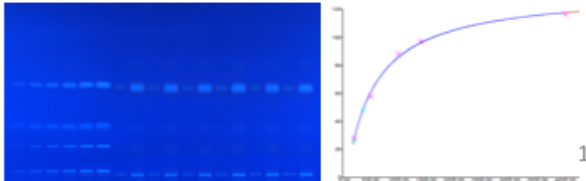
Documentation par image

➔ Acquisition récente de l'AMD 2



L'HPTLC pour l'analyse des classes de lipides

- Méthode développée par C. Dejoye-Tanzi puis optimisée par C. Breil

<p>Conditionnement de la plaque</p>	<ul style="list-style-type: none">❖ Solvant: <u>MeOH/CHCl₃</u>❖ Séchage 1h, 110 °C
	<ul style="list-style-type: none">❖ Standards Courbe étalon à 7 points❖ Echantillons <u>Spotter</u> minimum 2 fois à différents volumes❖ 6 échantillons / plaque
	<ul style="list-style-type: none">❖ Solvants <p>Migration jusqu'à 70 mm: n-hexane / <u>diéthyléther</u> / acide acétique 70/30/2 (v/v/v)</p>
<p>Révélation</p>	<ul style="list-style-type: none">❖ Solvants : <u>Primuline</u>, H₂O, acétone , séchage 5 min
	<ul style="list-style-type: none">❖ Lampe: Hg❖ Longueur d'onde : 366 nm 

L'HPTLC pour l'analyse des classes de lipides

Lipides de micro-algues

Nannochloropsis oculata and *Dunaliella salina*

⇒ procédure d'extraction au moyen d'agrosolvants de lipides de micro-algues fraîches



Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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



New procedure for extraction of algal lipids from wet biomass: A green clean and scalable process

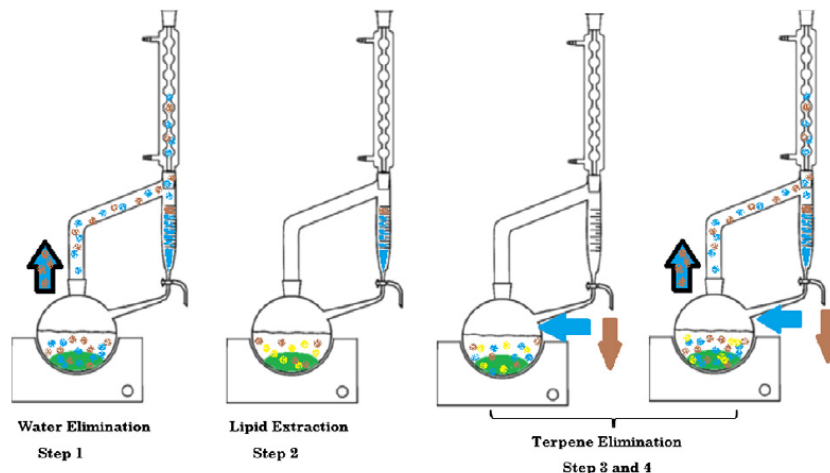


Celine Dejoye Tanzi, Maryline Abert Vian*, Farid Chemat

University of Avignon, INRA, UMR408, 84000 Avignon, France

HIGHLIGHTS

- ▶ A new extraction procedure of lipids from wet microalgae.
- ▶ *Nannochloropsis oculata* and *Dunaliella salina* were chosen as a microalgae.
- ▶ Influence of operating parameters on the extraction yields will be studied.
- ▶ Utilization of terpenes bio-solvents recognized as environmentally safer.



	<i>N. oculata</i>				<i>D. salina</i>					
	B&D	SDEP	Sox		B&D	SDEP	Sox			
		d-Limonene	α -Pinene	p-Cymene		d-Limonene	α -Pinene	p-Cymene		
Lipids Yield (%)	23.78 ± 2.13	18.73 ± 6.58	18.75 ± 3.01	21.45 ± 2.64	8.31 ± 1.05	4.03 ± 0.01	2.94 ± 0.02	3.29 ± 0.05	2.99 ± 0.01	1.90 ± 0.03
Lipid class composition										
FA: free fatty acid	20.21 ± 0.03	27.12 ± 0.12	23.42 ± 0.27	25.84 ± 0.32	16.92 ± 0.13	40.64 ± 0.36	49.77 ± 0.08	47.55 ± 0.32	42.95 ± 0.01	45.25 ± 0.09
TAG: triacylglycerol	69.94 ± 0.16	61.18 ± 0.05	57.67 ± 0.65	62.02 ± 0.82	67.30 ± 2.68	59.36 ± 0.02	50.23 ± 0.20	52.45 ± 0.05	57.05 ± 0.24	54.75 ± 0.04
DAG: diacylglycerol	9.85 ± 0.06	11.71 ± 0.06	18.91 ± 0.09	12.15 ± 0.15	15.78 ± 0.12	-	-	-	-	-



L'HPTLC pour l'analyse des classes de lipides

Lipides d'oléagineux

Graines de Colza

⇒ Substitution du n-hexane par un solvant alternatif, le MeTHF, pour l'extraction de l'huile de colza

Int. J. Mol. Sci. 2015, 16, 8430-8453; doi:10.3390/ijms16048430

OPEN ACCESS
International Journal of
Molecular Sciences
ISSN 1422-0067
www.mdpi.com/journal/ijms

Article

Alternative Bio-Based Solvents for Extraction of Fat and Oils: Solubility Prediction, Global Yield, Extraction Kinetics, Chemical Composition and Cost of Manufacturing

Anne-Gaëlle Sicaire ¹, Maryline Vian ^{1,*}, Frédéric Fine ², Florent Joffre ³, Patrick Carré ⁴, Sylvain Tostain ⁵ and Farid Chemat ¹

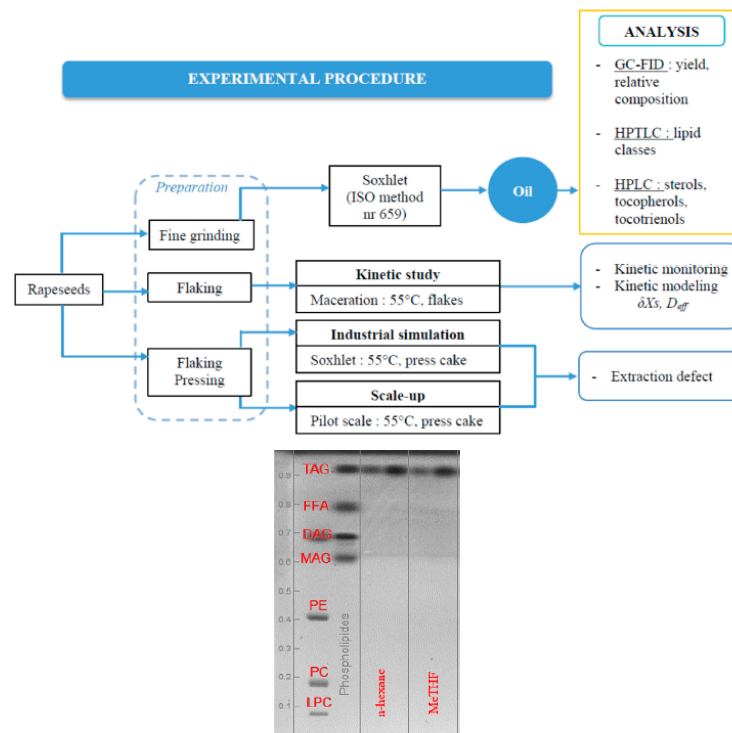


Figure 3. HP-TLC plate: lipid classes in rapeseed oil extracted with *n*-hexane and MeTHF (TAG: triglyceride; FFA: free fatty acids; DAG: diglyceride; MAG: monoglyceride; PE: phosphatidylethanolamine; PC: phosphatidylcholine; LPC: lysophosphatidylcholine).



L'HPTLC pour l'analyse des classes de lipides

Lipides de levures *Yarrowia lipolytica*

⇒ Etude de différentes techniques pour l'extraction de lipides microbiens



Contents lists available at ScienceDirect

Bioresource Technology

Journal homepage: www.elsevier.com/locate/biortech



Microwave, ultrasound, thermal treatments, and bead milling as intensification techniques for extraction of lipids from oleaginous *Yarrowia lipolytica* yeast for a biojetfuel application

Alice Meullemiestre, Cassandra Breil, Maryline Abert-Vian*, Farid Chemat

Université d'Avignon et des Pays du Vaucluse, INRA, UMR 408, GREEN Extraction Team, F-84000 Avignon, France



HIGHLIGHTS

- Maximize the lipids recovery by various extraction processes and pretreatment.
- Microbial oils extracted were quantified and qualified using GC/MS and HPTLC.
- Extraction by bead milling is the most efficient extraction method.
- The best pretreatment in term of yield is the cold drying under pressure.
- Bead milling extraction is the lower energy-consuming and eco-friendly technique.

GRAPHICAL ABSTRACT

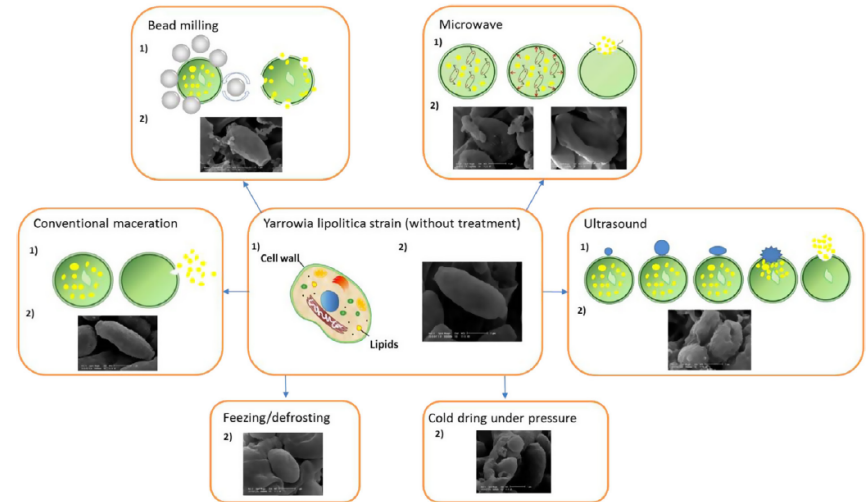
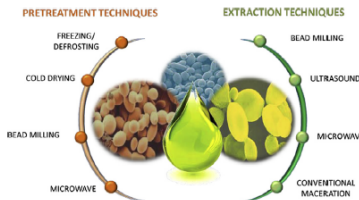


Fig. 6. Effect of extraction processes and pretreatment on *Y. lipolytica* structure.

Table 1

Crude lipids, distribution of lipid class and fatty acid composition of extracts obtained by different methods. (PUFAs polyunsaturated fatty acids, MUFAs monounsaturated fatty acids, SFAs saturated fatty acids).

	Conventional maceration	Bead milling	Ultrasound	Microwave	Freezing/defrosting	Cold drying	Bead milling	Microwave
Lipid yield (%)	6.23 ± 0.51	13.16 ± 0.68	8.10 ± 0.24	7.13 ± 0.45	5.53 ± 0.43	13.56 ± 0.24	12.73 ± 0.41	8.18 ± 0.67
Lipid classes composition								
DAG	15.16 ± 0.01	11.61 ± 0.06	–	–	12.79	15.43	11.96	–
TAG	71.60 ± 0.18	82.69 ± 0.15	83.28 ± 0.18	75.76 ± 0.13	65.65 ± 0.08	76.97 ± 0.11	80.22 ± 0.14	75.11 ± 0.07
FFA	13.24 ± 0.05	5.70 ± 0.02	16.72 ± 0.07	24.24 ± 0.05	21.56 ± 0.03	7.60 ± 0.02	7.82 ± 0.01	24.84 ± 0.03



L'HPTLC pour l'analyse des classes de lipides

Lipides de levures

Yarrowia lipolytica



Article

Bio-Based Solvents for Green Extraction of Lipids from Oleaginous Yeast Biomass for Sustainable Aviation Biofuel

Cassandra Breil †, Alice Meullemiestre †, Maryline Vian *† and Farid Chemat †

⇒ Etude de solvants biosourcés pour l'extraction de lipides microbiens (théorie vs expérimental)

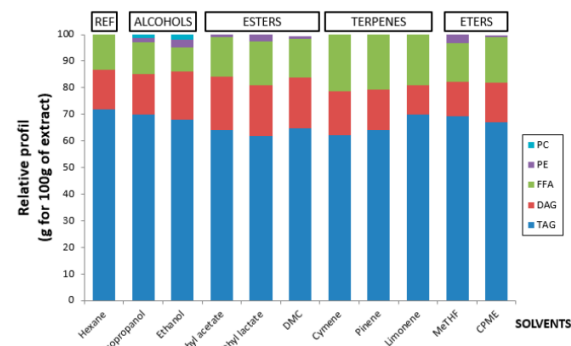
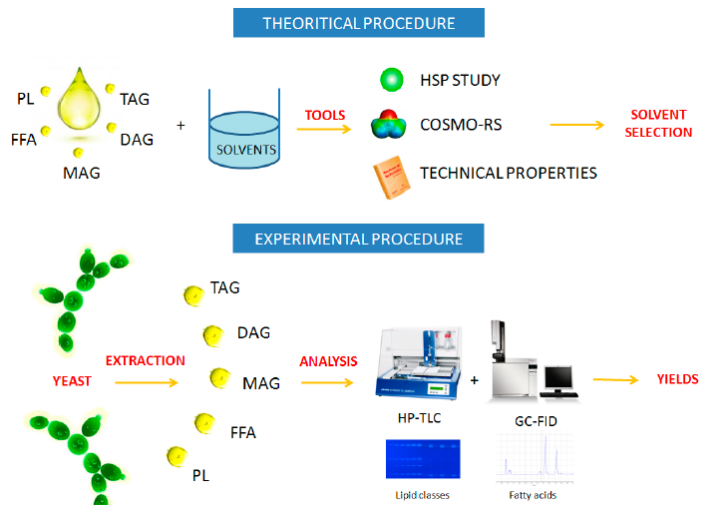


Figure 4. Comparison of lipid classes (TAG, DAG, MAG, FFA and PL) by HPTLC.

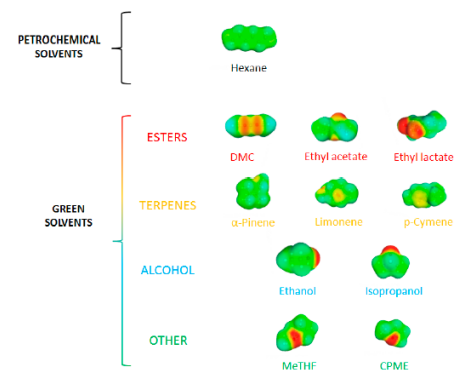


Figure 9. Modelization of solvents by COSMO-RS show the polarity in the surface of solvents.



L'HPTLC pour l'analyse des classes de lipides

Lipides de levures *Yarrowia lipolytica*

⇒ Développement d'un « green Bligh & Dyer »



International Journal of
Molecular Sciences

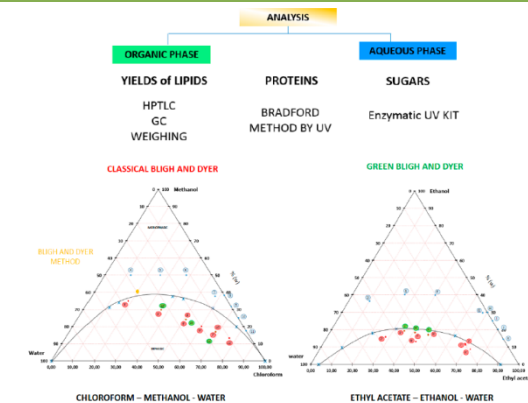
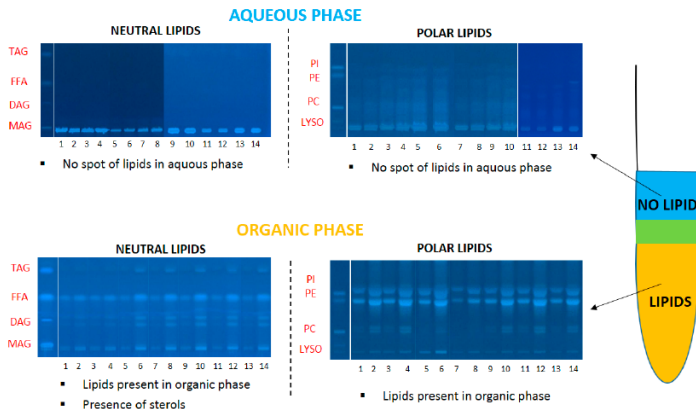


Article

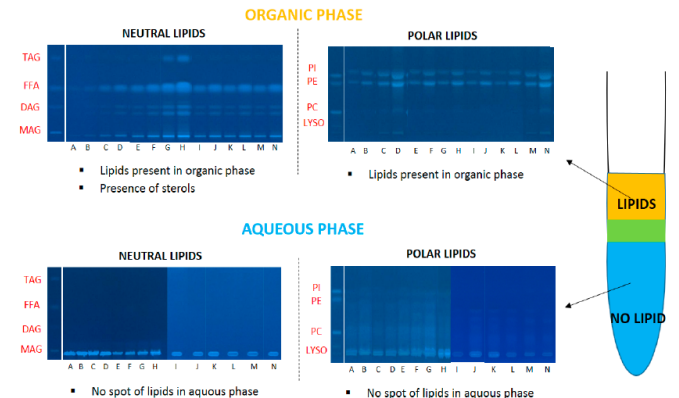
“Bligh and Dyer” and Folch Methods for Solid-Liquid-Liquid Extraction of Lipids from Microorganisms. Comprehension of Solvation Mechanisms and towards Substitution with Alternative Solvents

Cassandra Breil¹, Maryline Abert Vian¹, Thomas Zemb², Werner Kunz³ and Farid Chemat^{1,*}

CLASSICAL BLIGH & DYER



GREEN BLIGH & DYER



L'HPTLC pour l'analyse des substances naturelles

Alcaloïde contenu dans les feuilles de Boldo (*Peumus boldus Mol.*)

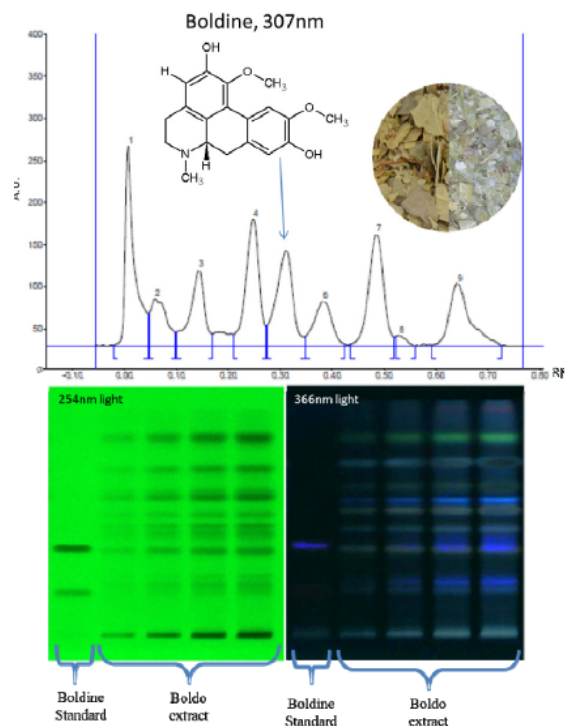
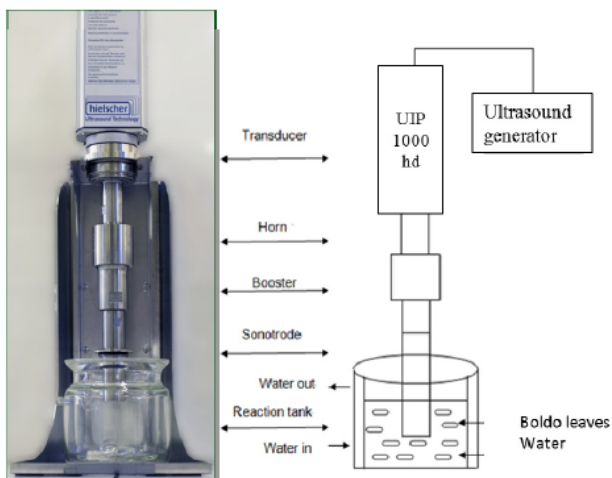
⇒ Extraction aqueuse par ultrasons.

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Article

Batch and Continuous Ultrasound Assisted Extraction of Boldo Leaves (*Peumus boldus Mol.*)

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L'HPTLC pour l'analyse des substances naturelles

- Caroténoïdes de micro-algues
- Test antioxydant DPPH
- Anthocyanes de myrtilles
- Steviosides de la stevia



