adebiotech

23 & 24 novembre 2015

LES LIPIDES DU FUTUR :

les lipases au cœur des développements scientifiques et industriels

LIPINOV

BIOCITECH, CITÉ DES ENTREPRISES DE SANTÉ ET DE BIOTECHNOLOGIES, ROMAINVILLE

# SUSTAINABLE EXTRACTION OF PLANT LIPIDS BY ENZYMATIC AQUEOUS EXTRACTION (E.A.E.)

**Lionel MUNIGLIA, PhD**
**Scientific Director**

UNIVERSITÉ DE LORRAINE

ensaia

LIBio
Laboratoire d'Ingénierie des Biomolécules

BIOLIE
L'extraction au sens propre

- **Associate professor**

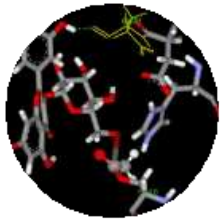
  **French National superior school of agronomy and food industries**

  **Food chemistry – Biochemistry**

  LIBio
  Laboratoire d'Ingénierie des Biomolécules

  **Laboratory of Biomolecules Engineering**

  **Enzymatic catalysis – Green Chemistry**



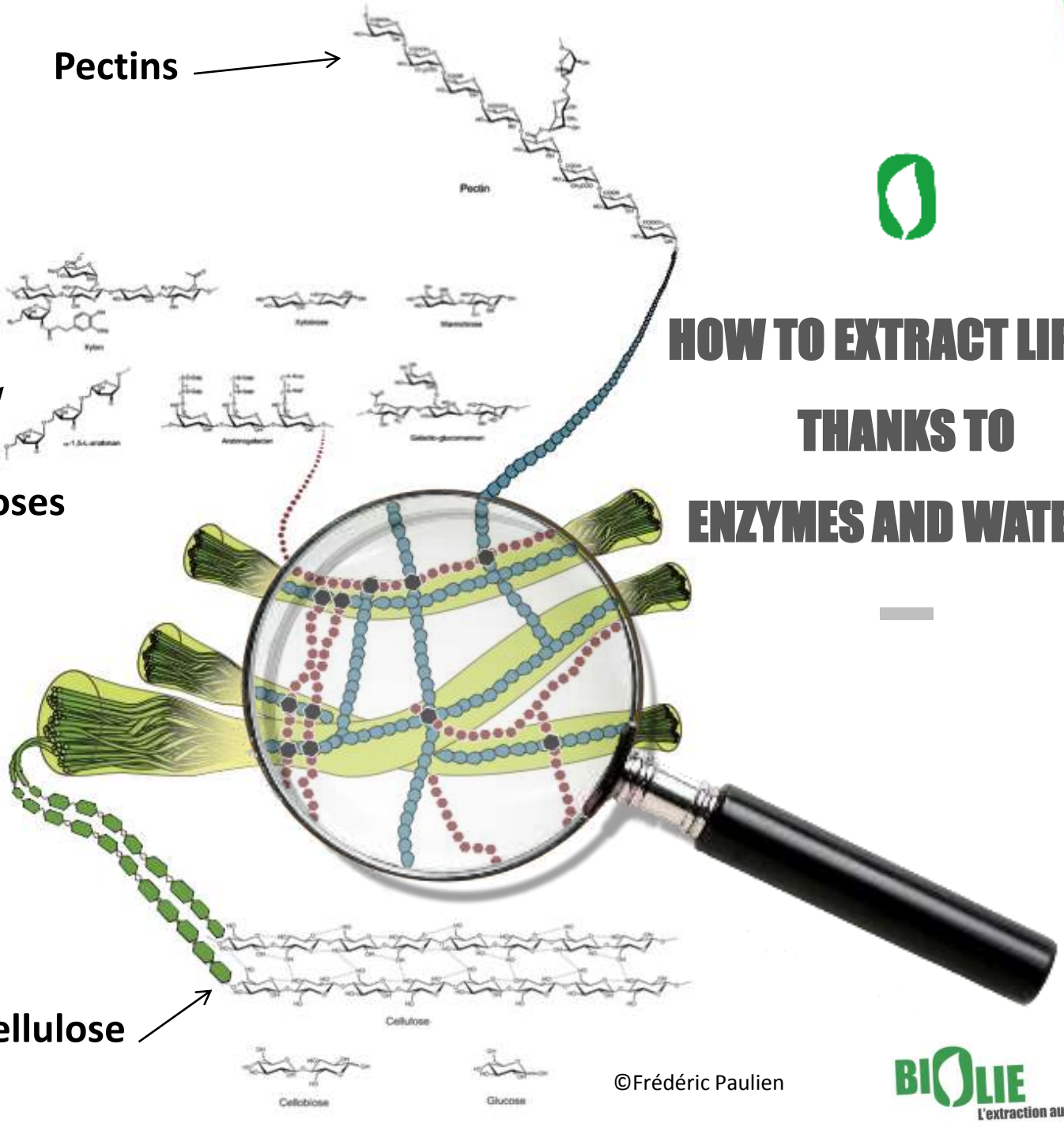
- **Scientific director of a spin-off company**

  BIOLIE
  L'extraction au sens propre

Pectins

Hemicelluloses

Cellulose




# HOW TO EXTRACT LIPIDS THANKS TO ENZYMES AND WATER?

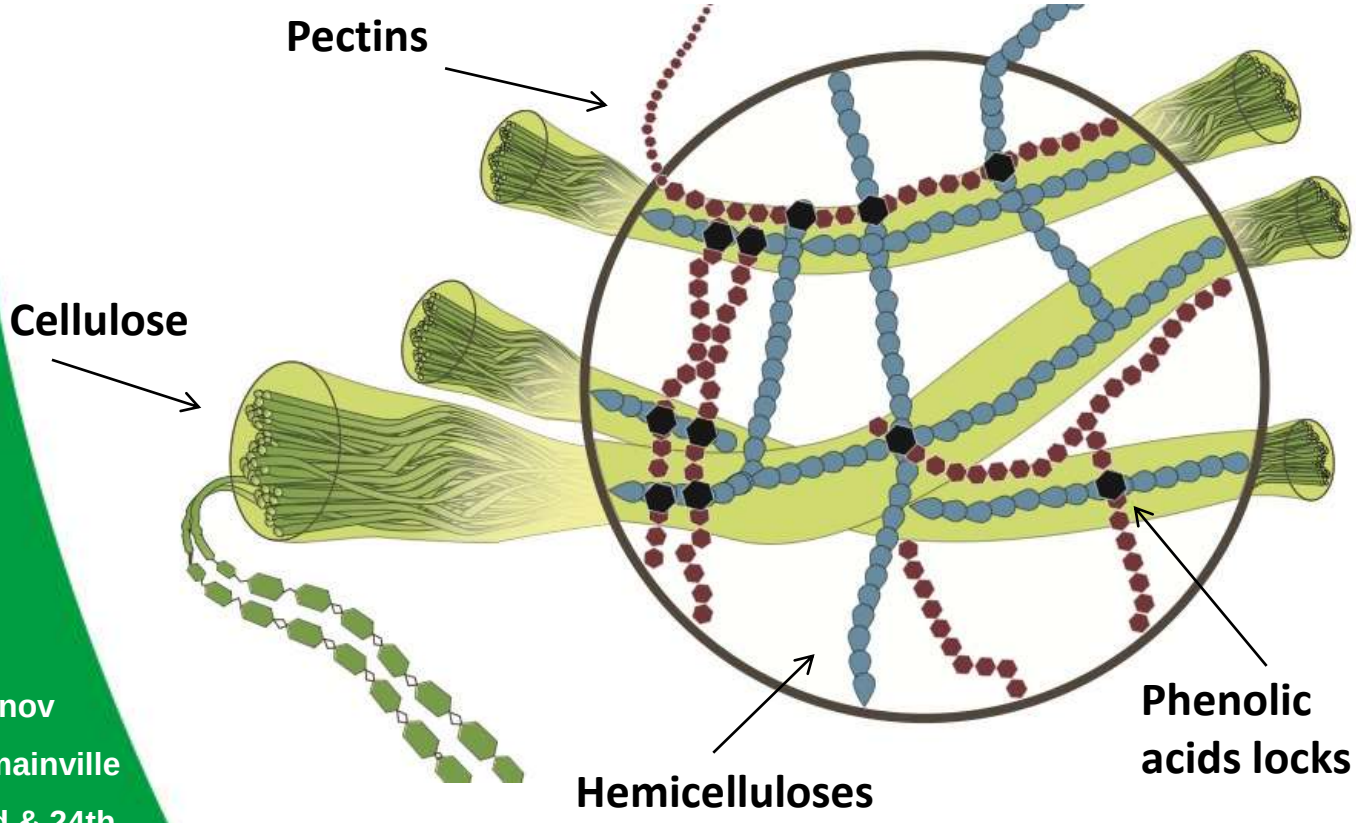




BIOLIE L'extraction au sens propre

# ENZYMES: A SOLUTION TO IMPROVE MOLECULES EXTRACTION

…thanks to the hydrolysis of cellular walls of the raw material

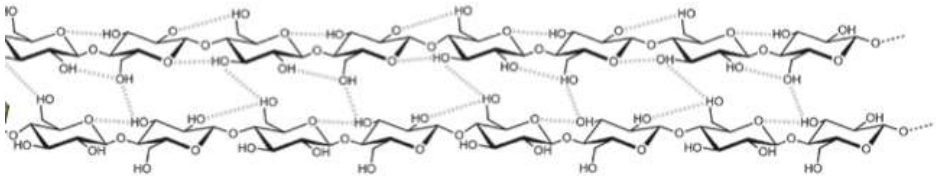



**Composition of cellular wall of raw material?**

Enzymes for selective hydrolysis and synergy?

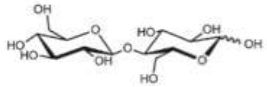
Ricochon and Muniglia (2010). OCL, 17 (6) 356-359.

# ENZYMES: A TAILORED CHOICE

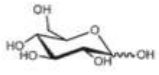


Cellulose

## Cellulose

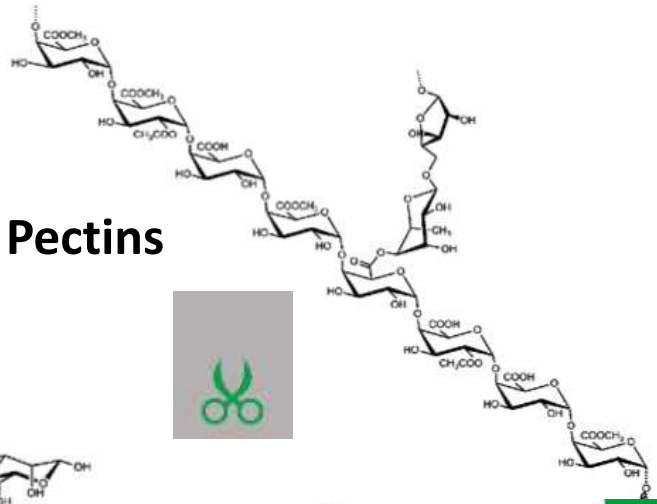


Cellobiose

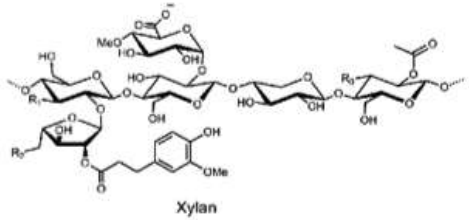


Glucose

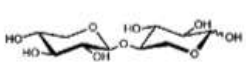
## Pectins



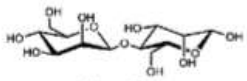




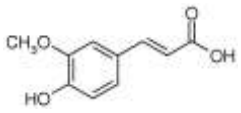
Xylan



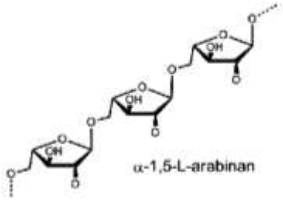
Xylobiose



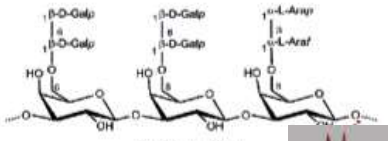
Mannobiose



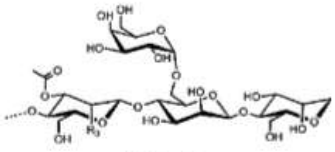
## Phenolic acids locks



α-1,5-L-arabinan



Arabinogalactan



Galacto-glucomannan

## Hemicelluloses

## ~~Proteases~~



Ricochon et al. (2011) Bioresource Technology 102, 9599–9604

# ENZYMES: A TAILORED CHOICE

| Activities | Cellulases | | | Hemicellulases | | | Pectinases | | Proteases |
|---|---|---|---|---|---|---|---|---|---|
| Cocktails | C1 | C2 | C3 | H1 | H2 | H3 | PG | PME | |
| E1, batch A | 5 | 4 | 0 | 0 | 2050 | 1720 | 8 | 0 | / |
| E1, batch B | 4 | 5 | 0 | 0 | 1950 | 1680 | 7 | 4 | / |
| E2 | 10 | 70 | 31 | 1660 | 30 | 2160 | 144 | 1 | / |
| E3 | 35 | 11 | 0 | 0 | 0 | 0 | 0 | 115 | 1 |
| E4 | 10 | 0 | 0 | 3120 | 110 | 1330 | 59 | 3 | 536 |
| E5 | 8 | 14 | 2 | 10 | 0 | 450 | 58 | 4 | / |
| E6 | 1 | 6 | 1 | 330 | 170 | 2950 | 7 | 0 | / |
| E7 | 2 | 0 | 1 | 530 | 0 | 460 | 0 | 0 | / |
| E8 | 2 | 0 | 0 | 0 | 2070 | 0 | 0 | 0 | / |
| … | … | … | … | … | … | … | … | … | … |

PG: PolyGalacturonase

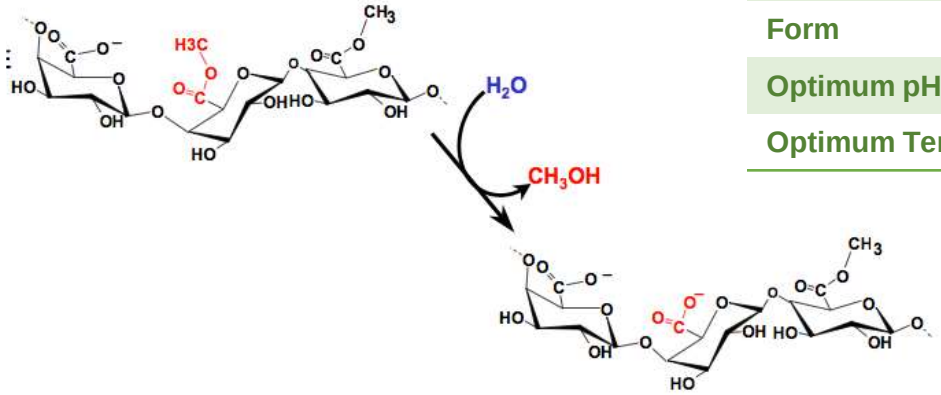
PME: Pectin Methyl Esterase

**Need for specific formulation of enzymatic mixtures**

# ENZYMES: A TAILORED CHOICE

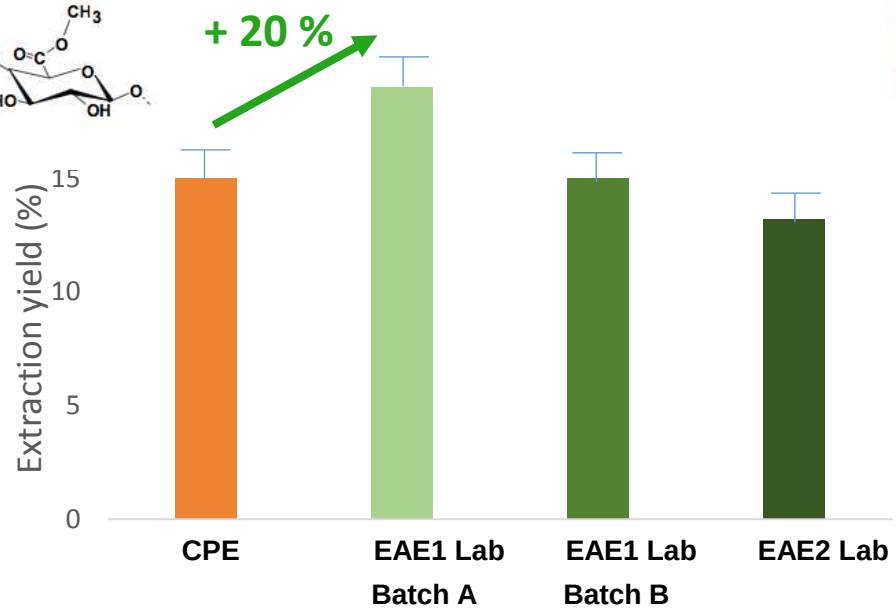
**Ex.: Hydrolysis of pectins**

**(polygalacturonic acid)**




| Activity | Cellulase 1800 u/g |
|---|---|
| Biological Source | *Trichoderma sp* |
| Form | Liquid |
| Optimum pH range | 3.5 – 6.5 |
| Optimum Temperature range | 45 – 65°C |

- Commercial cocktail known for its polyGalacturonase (PG) activity
- Pectin Methyl Esterase (PME) is a secondary activity not standardized in the used cocktail




(EAE for Enzymatic Aqueous Extraction process and CPE for Cold-Pressed Extraction)

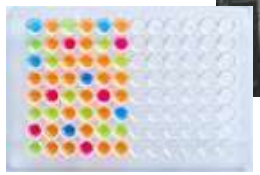
Ricochon et al. (2011)

Bioresource Technology 102, 9599–9604

# ENZYMES: A TAILORED CHOICE

**New platform for high-throughput screening of enzyme activities**

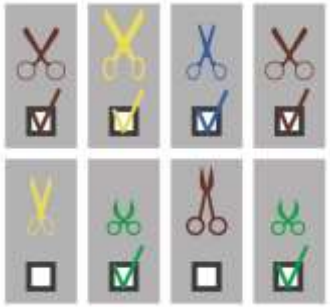
- **Full characterization of the activities in a commercial mixture**
- **Optimization of the formulation of owner cocktails: formulation tailored to the raw material and the molecules to be extracted**
- **More precise formulation of the composition of mixtures**
- **Time and costs saving**
- **Better efficiency**

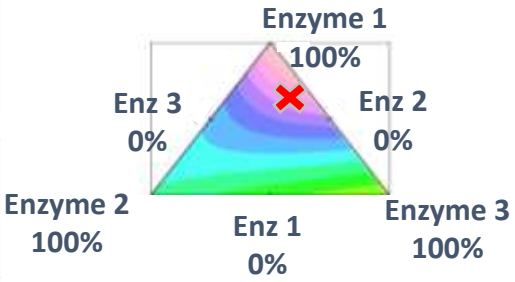


# ADAPTATION TO SPECIFIC RAW MATERIAL

## 1 Structural Analysis of plant cell walls

**Study of the plant components (polysaccharides, phenols, proteins, oil…)**






## 2 Design of a customized enzymatic cocktail – Screening of enzymes and formulation

**(commercially available enzymes)**

## 3 Enzymatic Aqueous Extraction

**Optimization of physico-chemical parameters**

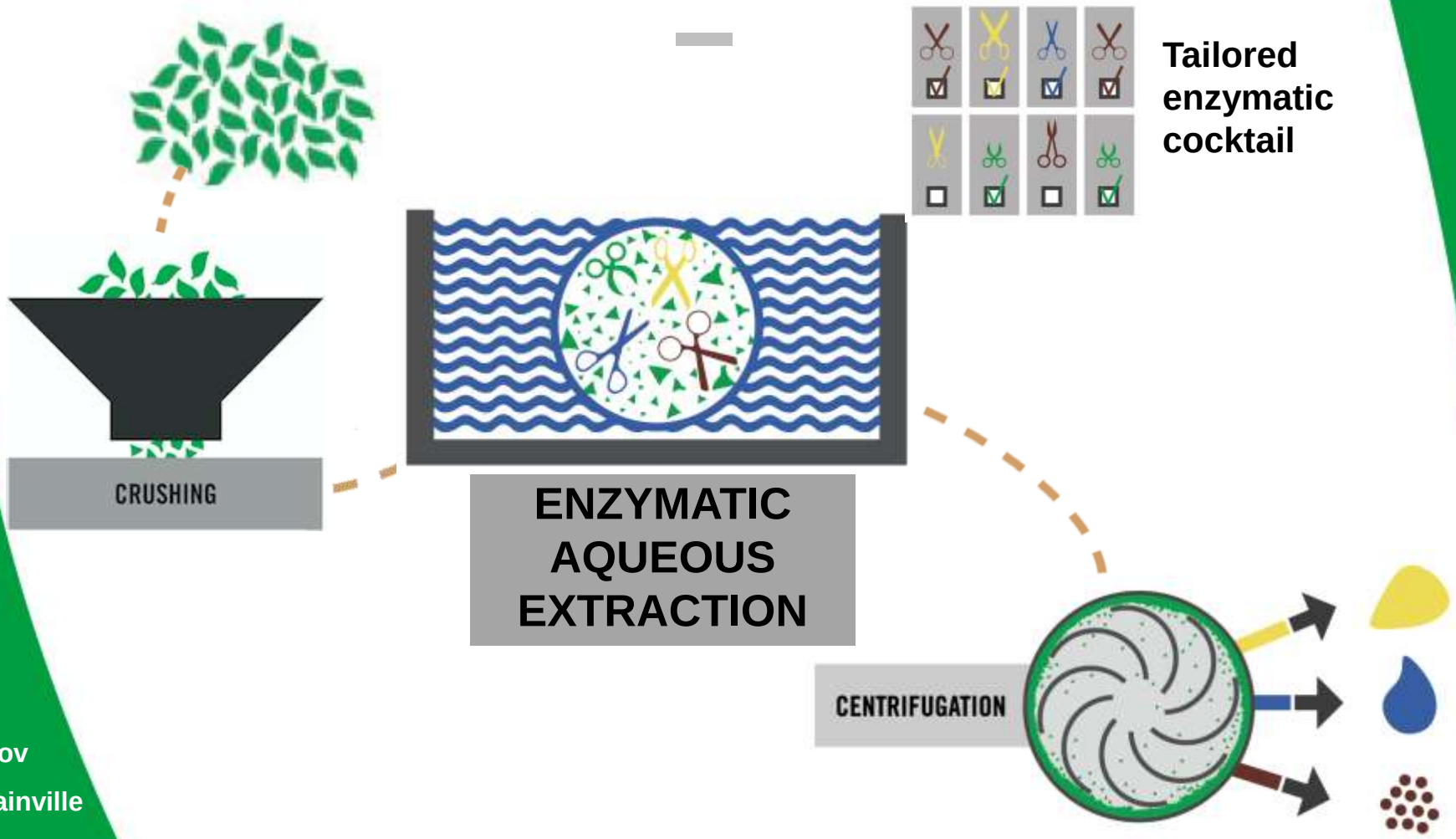
4 hours
50° C
Atm. pressure
In water
No pH control



Ricochon et al. (2011).
Journal of Chromatography B, 879 (2011) 1529–1536

BIOLIE L'extraction au sens propre

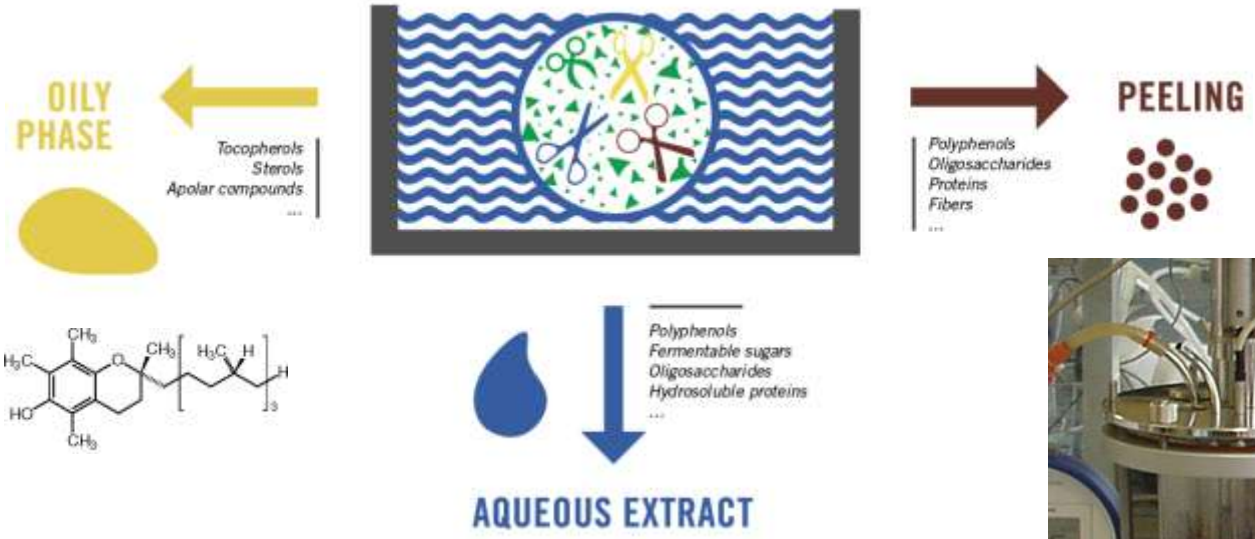
# A PROCESS IN 3 MAIN STEPS






PATENT: WO21011045387

# PROCESS PRINCIPLES: 1 step, 3 valuable fractions






**L. Muniglia, N. Claisse, P.H. Baudelet, G. Ricochon (2014).**

**Enzymatic Aqueous Extraction (EAE),**

**Alternative Solvents for Natural Products Extraction, Green Chemistry and Sustainable Technology, Chap. 8, 167-204, F. Chemat and M. Abert Vian (eds.) Springer-Verlag Berlin Heidelberg**

# FROM LAB TO INDUSTRIAL SCALE


0,1 liter → 2-7 liters → 70 liters → 3000 liters

2002 — 2005 — 2009 — 2014

# LIPID PRODUCTION
# THANKS TO
# ENZYMATIC AQUEOUS EXTRACTION

# EXAMPLES OF ENZYMATIC OILS

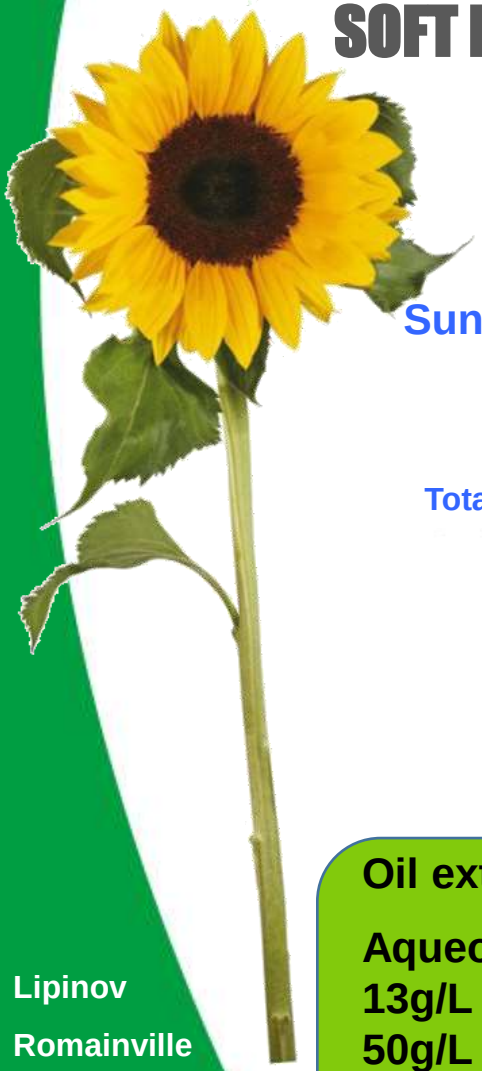
**Sunflower seeds**

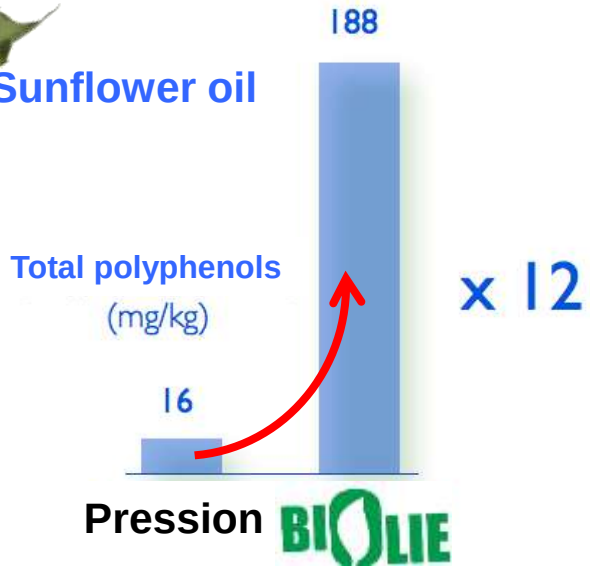
**Apricot kernel**

**Elderberry seeds**

**Fir seeds**

# SOFT PROCESS FOR SUNFLOWER OIL EXTRACTION





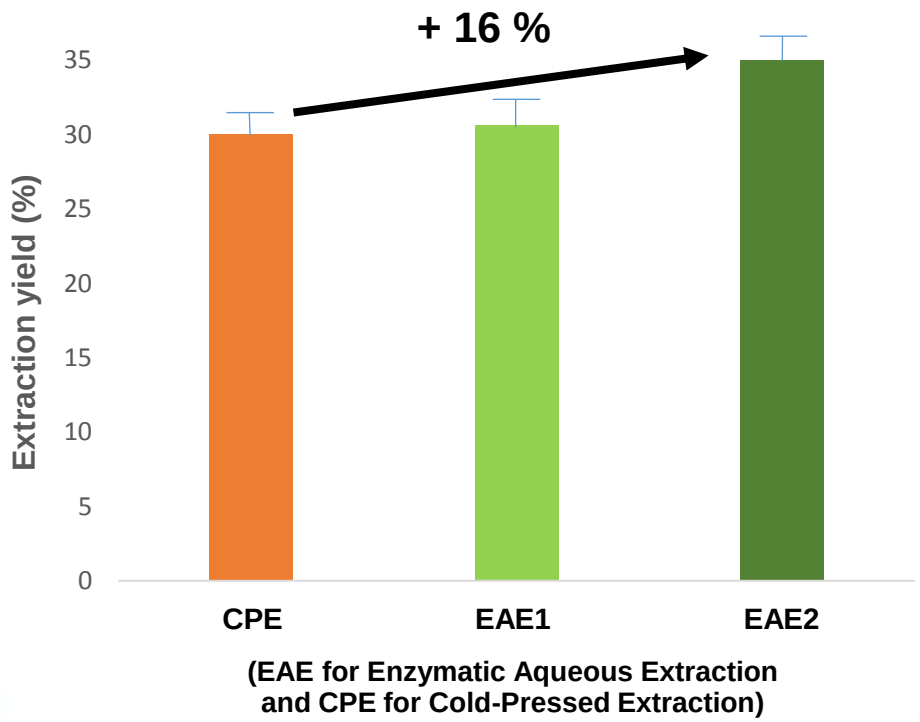

| | Aqueous extraction | Hexan extraction |
|---|---|---|
| α-tocopherol (mg/kg) | 612 | 474 |
| β-tocopherol (mg/kg) | 46 | 20 |
| γ-tocopherol (mg/kg) | 10 | 4.5 |
| δ-tocopherol (mg/kg) | <2 | <2 |
| α-tocotrienol (mg/kg) | <2 | <2 |
| β-tocotrienol (mg/kg) | <2 | <2 |
| γ-tocotrienol (mg/kg) | <2 | <2 |
| δ-tocotrienol (mg/kg) | <2 | <2 |
| Total content (mg/kg) | 668 | 499 |
| Vitamin activity E (mg αTE/100g oil) | 63.6 | 48.5 |

**Oil extraction Yields up to 95%**

**Aqueous extract containing:**
**13g/L polyphenols**
**50g/L reducing sugars**
**90g/L water soluble proteins**
**16% dry matter**

# A SUCCESSFUL SYNERGY: APRICOT KERNEL OIL

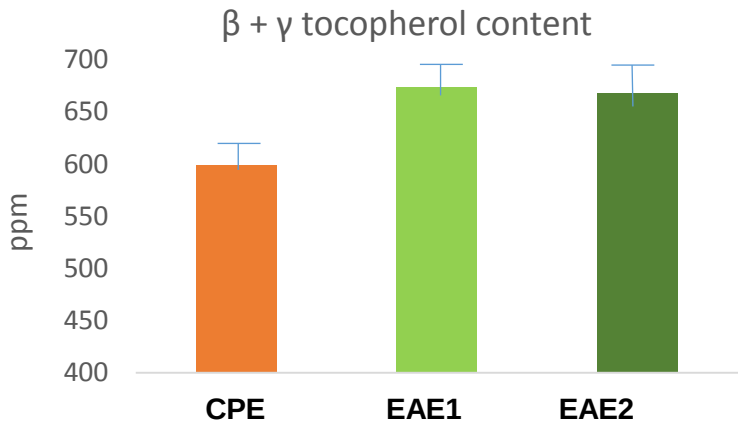
- **Apricot oil selected as a well known model**
- **Test of two very different enzymatic cocktails (EAE1 & EAE2)**



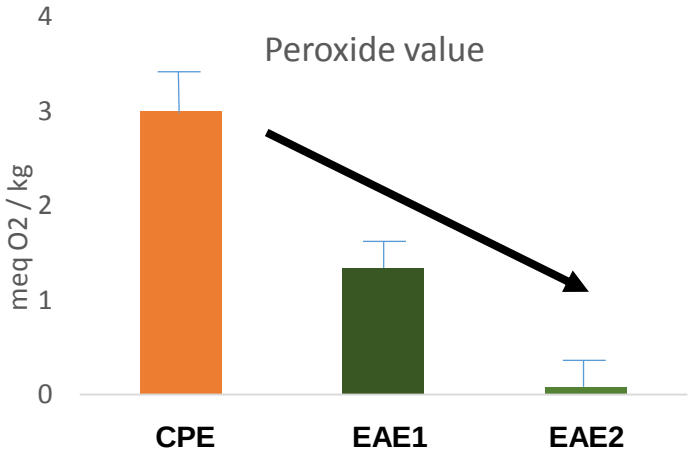

(EAE for Enzymatic Aqueous Extraction and CPE for Cold-Pressed Extraction)

# A SUCCESSFUL SYNERGY: APRICOT KERNEL OIL

## β- and γ-tocopherols contents increased



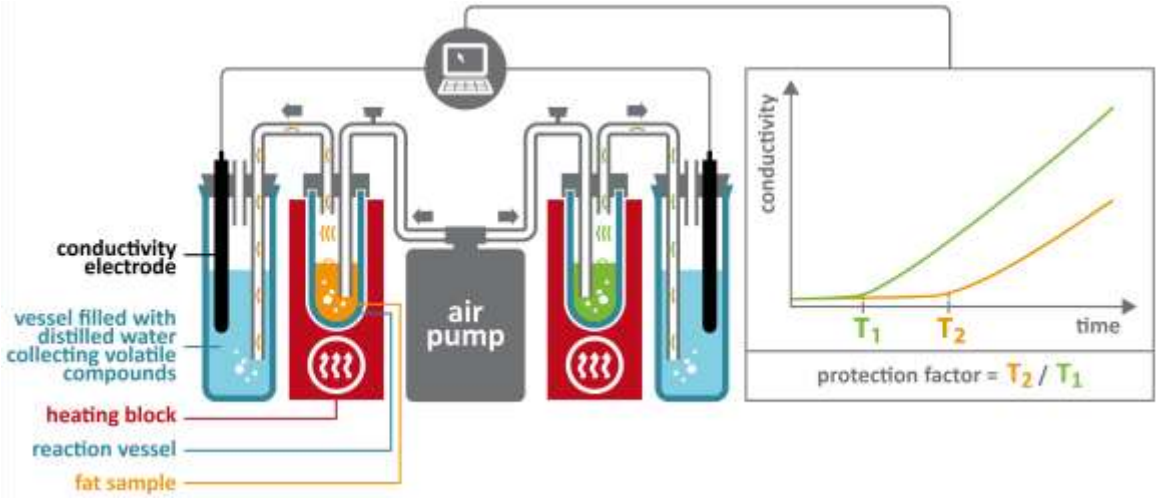

## Lower peroxide values for EAE oils

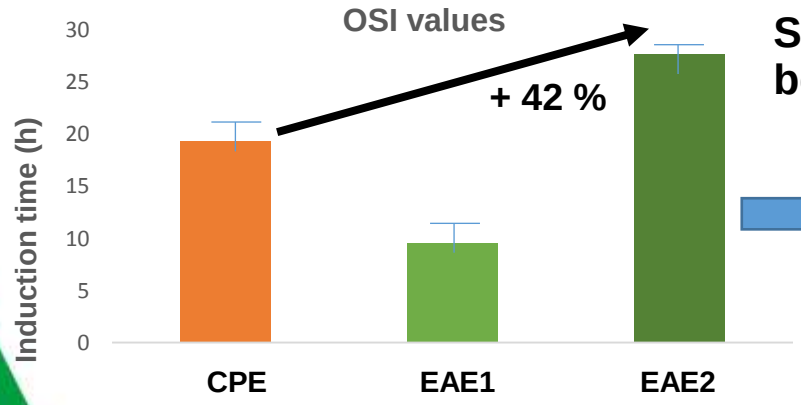



**High level in Benzaldehyde (CPE X20)**

# A SUCCESSFUL SYNERGY: APRICOT KERNEL OIL

**Rancimat stability measured at 110 °C & 10 L/h improved only for EAE2**



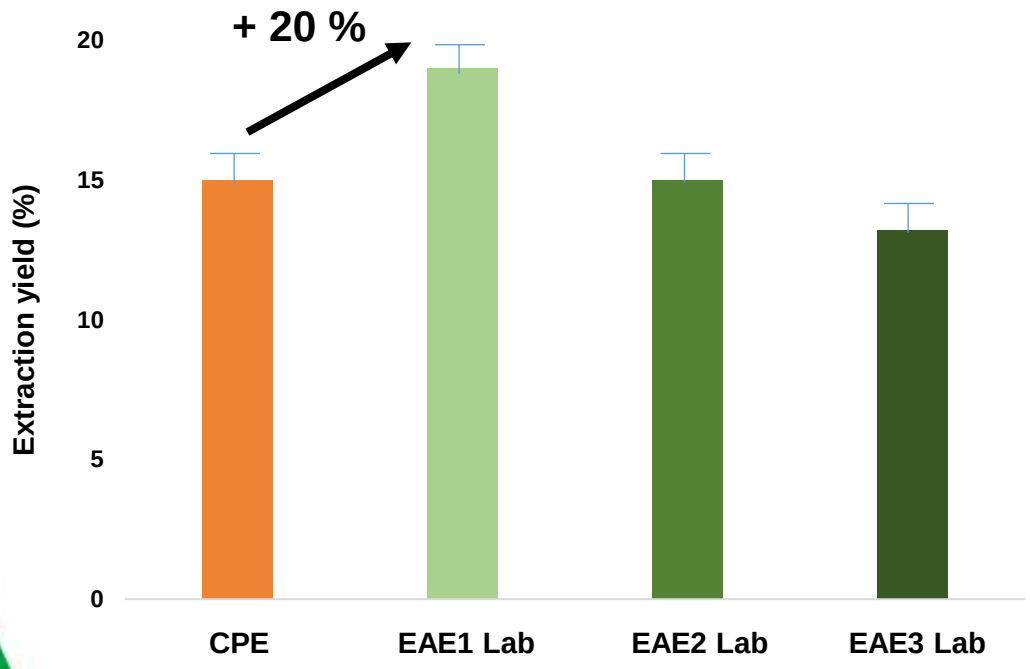




**Significant differences were found between both EAE oils**

**Improved yield, tocopherols content and stability for EAE2**

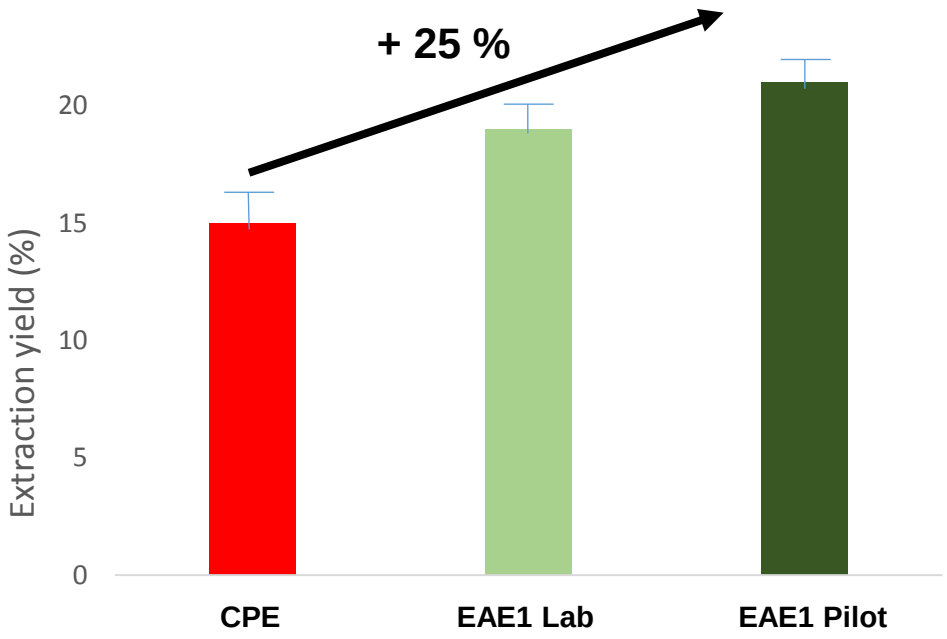
# ENZYMATIC EXTRACTION OF A SENSITIVE ELDERBERRY OIL

- **Lab-screening of enzymatic cocktails shows differences in terms of yield**




# ENZYMATIC EXTRACTION OF A SENSITIVE ELDERBERRY OIL

- **Up-scaling resulted in improved yield**




# BIOREFINERY OF ELDERBERRY SEEDS

## Valorization of all fractions

Elderberry seeds

100 kg

Enzymatic extraction

Aqueous extract

23 kg
on dry basis

Skin & Hair care
Nutraceuticals

Pomace

56 kg

Exfoliation, feed or new extraction

Plant Oil

21 kg

Skin & Hair care

Patented extracts

# BIOREFINERY OF FIR SEEDS *Abies Alba* (VOSGES)

→ **All fractions are valuable**

Vosges' Fir Seeds — **100 kg**

Enzymatic extraction

**Cosmetic applications**

Aqueous extract — **35 L**

**Anti-aging**
**Soothing**
**Depigmenting**

Plant oil — **21 L**

**Anti-aging**
**Antioxidant**
**Radical scavenging**
**Moisturizer**
**Nutraceutical**

Pomace — **57 kg**

**Exfoliation, feed, new extraction, methanation…**

Patented extracts

# BIOREFINERY OF FIR SEEDS *Abies Alba* (VOSGES)

## Huile

| | |
|---|---|
| I s | 137 |
| I i | 183 |
| I a | 32.66 |
| I p | 3.41 |
| n | 1.49 |
| d | 0.93 |
| DPPH ($IC_{50}$) | 0.68% V/V |
| Polyphenols | 388 mg/kg |
| Sterols | 3315 mg/kg MG |
| Tocopherols | 283 mg/kg MG |
| Activity Vit E | 15.7mg α TE/100g MG |
| FA sat. | 6.8% |
| FA monoinsat. | 26.3% |
| FA polyinsat. | 64.9% |

**High level in PUFA Delta5 (>20%)**

| | |
|---|---|
| C16:0 (palmitic acid) | 3.2 |
| C16:1 (palmitoleic acid) | <0.1 |
| C17:0 (margaric acid) | 0.3 |
| C18:0 (stearic acid) | 1.8 |
| C18:1 (oleic acid) | 25.6 |
| C18:2 Δ5 (taxoleic acid) | 6.7 |
| C18:2 (linoleic acid) | 42.4 |
| C18:3 Δ5 (pinolenic acid) | 12.4 |
| C18:3 (linolenic acid) | 0.4 |
| C20:0 (arachidic acid) | 0.5 |
| C20:1 (gondoic acid) | 0.7 |
| C20:2 (eicosadienoic acid) | 0.3 |
| C20:2 Δ5 (keteleronic acid) | 0.5 |
| C20:3 Δ5 (sciadonic acid) | 2.2 |
| C22:0 (behenic acid) | 0.5 |
| C24:0 (lignoceric acid) | 0.5 |
| Other | 2.0 |

# MESSAGE TO BRING BACK HOME

**« Safe » products**

no solvent,
nor chemicals
**No toxicity**

**Biorefinery**
No waste
Renewable raw materials
**Mild extraction conditions**

**Sustainable technology**

**Competitive yields**

Easy industrial scale
Low production costs
**Economical balance**

**New products**
Polyphenols, sterols, CoQ 9 and 10, vitamin A and E
**High added values**

**High contents in actives**

Ma démarche environnementale est reconnue ENVOL
*Engagement volontaire de l'entreprise pour l'environnement*
*par AFNOR Certification*

# THANK YOU
## FOR YOUR ATTENTION


Lionel MUNIGLIA, Scientific Director
lionel.muniglia@biolie.fr

Nicolas Attenot, President
nicolas.attenot@biolie.fr

BIOLIE
24-30 rue Lionnois
BP 60120
54003 NANCY Cedex
✆+33 (0) 83 59 64 98
🖷 +33 (0)3 83 68 52 71
www.biolie.fr