



Bioactives in fruit and vegetables and their extraction: state of the art and perspectives

Catherine M.G.C. Renard

UMR408 Sécurité et Qualité des Produits d'Origine Végétale,
INRA, Université d'Avignon et des Pays du Vaucluse



Outline

- ↗ Introduction
- ↗ Bioactive compounds in fruit and vegetables
- ↗ Fruit and vegetable co-products and their stabilisation
- ↗ Extraction technologies
 - Traditional methods
 - New extraction methods
 - Alternative solvents
- ↗ Research ... and organisation needs



Why a renewed interest in bioactives from plants?

↳ **Socio-cultural factors**

- Consumers' attraction for « naturality »
- Good image of plant products

↳ **But also a long history**

- Many colorants and hydrocolloids
- Antioxydants
- Presumed health effects - nutraceuticals

↳ **Recent innovations in extraction**

- Process intensification : « XX-assisted extraction »
- REACH regulation (2006)



« Bioactive compounds »

↳ **They are**

- Secondary metabolites - but not all secondary metabolites
- Present among others in fruit and vegetables
- Demonstrated or supposed preventive impact on chronic degenerative pathologies
- OR with interesting functionalities: color, antioxidant...

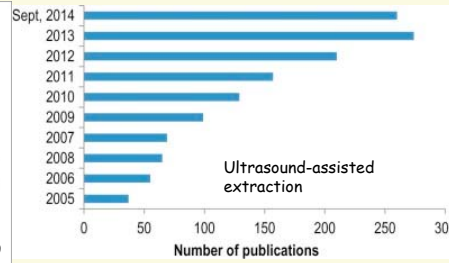
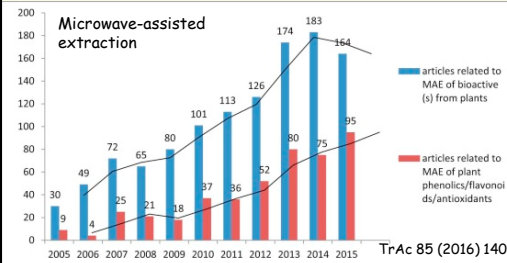
↳ **They are not**

- Nutrients sensu stricto (vitamins, minerals)
- With pharmacological (or toxic) activities
- Used in traditional medicine





Abundant recent scientific publications



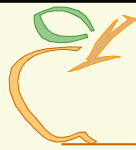
- Two most studied technologies:
 - Microwaves and ultrasounds
 - For extraction or for analytical purposes
- Most of the literature is from Eastern countries
 - Polyphenols: China >> India - Spain
 - Essential oils: China > Iran >> France



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Main classes of bioactives in F&V

Terpenes

- Tetraterpenes: carotenoids
- Monoterpenes (essential oils)
- Saponins, phytosterols...

Phenolic compounds

- Non flavonoids: phenolic acids, hydrolysable tannins, lignans, stilbenes (resveratrol), coumarins...
- Flavonoids: anthocyanins, condensed tannins, flavonols, flavanones...

Organosulfur compounds

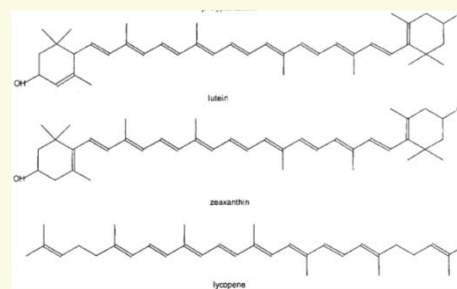
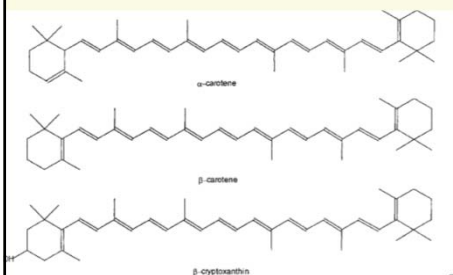
- Thiosulfinates and glucosinolates

Nitrogen-containing compounds

- Capsaicinoids
- Betalains



Carotenoids



Red to yellow pigments - hydrophobic

Include provitamins A, lutein and lycopene

Two subclasses: carotens (hydrocarbons) and xanthophylls

Colorants and Antioxydants



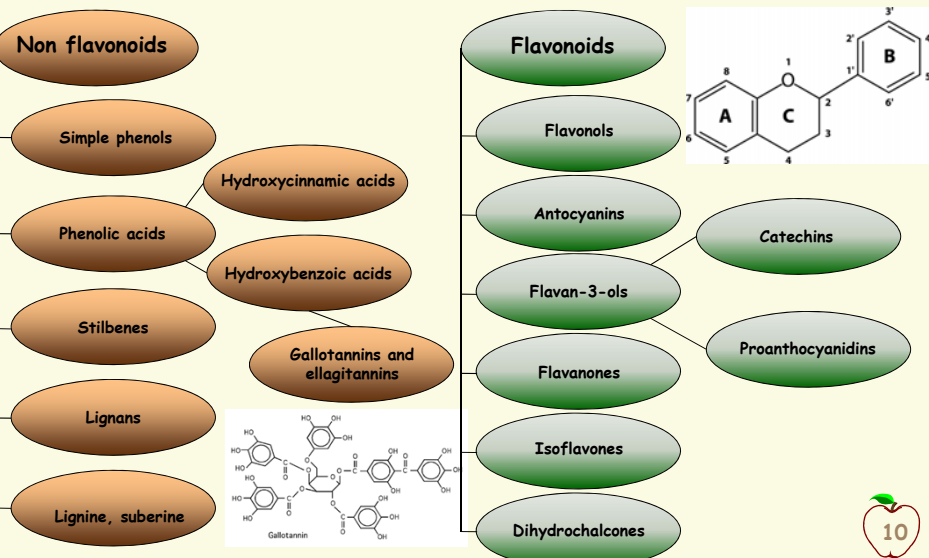


Polyphenols

- ↳ **Ubiquitous**
 - Presents in F&V but mostly in tea, coffee, cocoa, wine, cider...
- ↳ **Many have interesting properties**
 - Color: anthocyanins and flavonols
 - Antioxydants
 - Nutraceutical effects, often mediated by microbiota
- ↳ **But also a number of drawbacks**
 - Bitter and/or astringent
 - Brown color after oxidation (enzymic browning)
 - Enzyme inhibition (technological but also digestive enzymes - antinutrients)



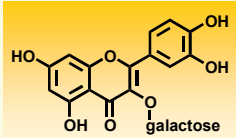
Classification and a few formulae



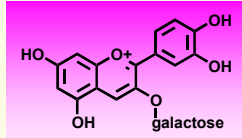


A few common flavonoids

Flavonols

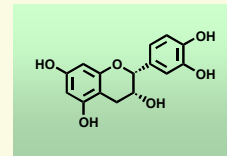


Anthocyanins

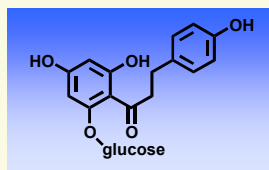


Flavan-3-ols

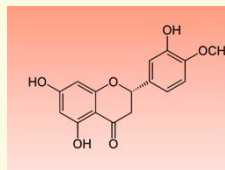
Catechins



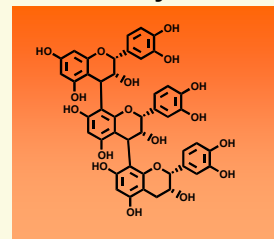
Dihydrochalcones



Flavanones



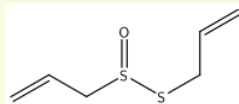
Proanthocyanidins



Organo-sulfur compounds

Thiosulfinates

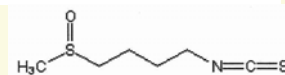
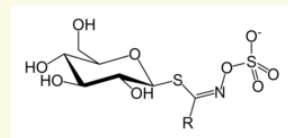
- Alliin - alliin
- In Alliaceae
- Degradation by alliinase to give typical odor



Aliphatique
Aromatique
Indolique

Glucosinolates

- R: aliphatic, aromatic or indolic
- Degradation by myrosinase to active thiocyanates, isothiocyanates...
- In Brassicaceae
 - Odor and pungency...



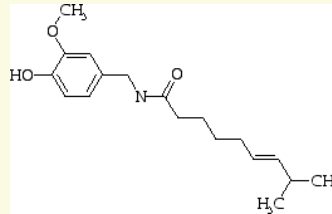
sulforaphane



Nitrogen-containing compounds

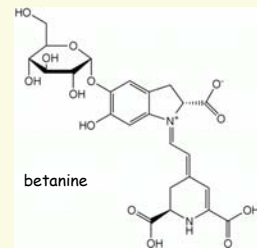
↳ Capsaicinoids

- capsaicine, dihydrocapsaicine
- Alkaloids present in Capsicum
- Responsible for their « burning » sensation
- Pain relief



↳ Betalaines

- Betacyanines (red to purple) or betaxanthines (yellow to orange)
- In Caryophyllaceae
 - Beet, prickly pear, pitaya, ...



Different compounds distribution in different species



Flavan-3-ols (incl. procyanidins), hydroxycinnamic acids, flavonols & dihydrochalcones



Anthocyanins, flavonols, phenolic acids, flavan-3-ols (procyanidins)



Flavan-3-ols (incl. procyanidins), flavonols, hydroxycinnamic acids & anthocyanins

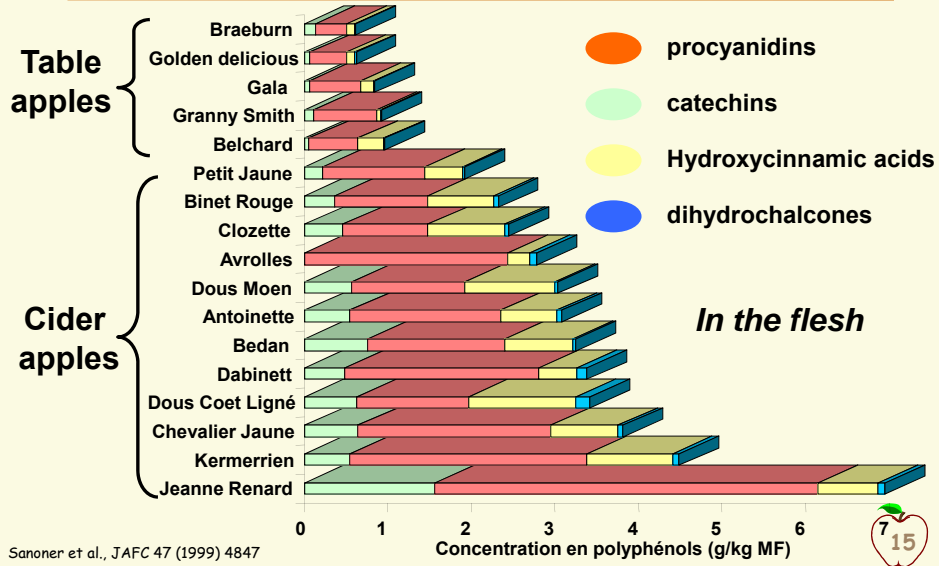


Flavan-3-ols (incl. procyanidins), anthocyanins, flavonols & hydroxycinnamic acids





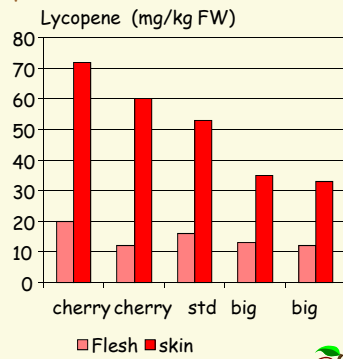
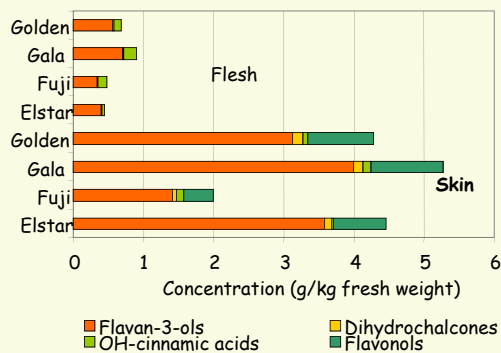
Contents are also very variable



They can be concentrated in some fractions of the F&V

Often in higher concentrations in tough, outer parts

- So that they are more concentrated in co-products
- Epidermis: anthocyanins, flavonols
- Sometimes higher concentrations in pips or stones



Markovic et al., 2010





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What are the potential sources ?

- ↳ **Co-products ... if there are products**
 - Grape, apple, red fruit pomaces
 - Citrus peels
 - Mango and other tropical fruit peels
 - Tomato by-products
 - Olives processing water
 - Onion peels or salad outer leaves
 - Sugar-beet pulp, potato pulp
- | |
|-----------------|
| Juices, wine |
| Tomato products |
| Oil |
| Fresh cut |
| Sugar, starch |
- ↳ **The co-products must be stabilised**
 - Availability limited in time plus highly fermentescible
 - Generally by drying, which can impact bioactives
 - Sometimes pasteurization (storage)





Bioactive compounds are a minor part of the co-products

- ▮ **Fibers represent > 50% of F&V co-products**
 - Dietary fibers: hydration, texturants
 - Apple and citrus: production of pectins - 10 - 25%
 - A major residue after extraction of bioactives
- ▮ **Seeds & pips can be rich in oils and proteins**

Some typical concentrations

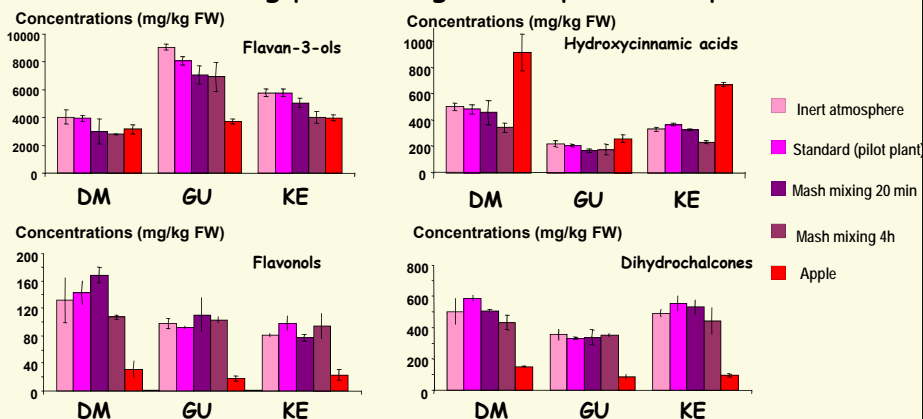
lycopene in tomato co-product: 0,1% - 1 g/kg ms Kalogeropoulos et al., 2012
carotenoids in mango peel: 0,3% - 3 g/kg ms Ajila et al., 2010
carotenoids in carrot pomace: 0,015% - 150 mg/kg ms Pieszka et al., 2015

polyphenols in apple pomace: 0,5-3 % 5-30 g/kg ms
polyphenols in blackcurrant pomace: 1,2% 12 g/kg ms Juskiewicz et al., 2015
procyanidins in perry pear pomace: 1 % 10 g/kg mf Brahem et al., 2016



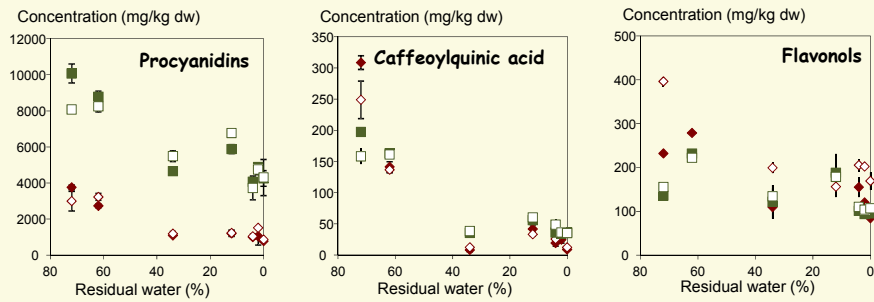
Retention in pomace is selective

And oxidation during processing also impacts compositions





Drying can modify the molecules and their extractability



Outline

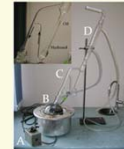
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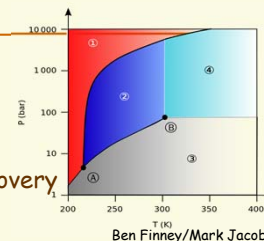
The « traditional » extraction methods

- ↳ **By simple contact between plant matter and its solvent**
 - Maceration - followed by filtration - with ethanol
 - Decoction - cold water or infusion - hot (boiling) water
 - Percolation - renewed solvent
- ↳ **Expression**
 - Mechanical effect, liquid/solid separation
 - Citrus essential oils, oils
- ↳ **Steam distillation**
 - Essential oils plus hydrosols
- ↳ **Enfleurage**
 - Extraction by contact with odorless fats
- ↳ **Soxhlet**
 - Constant renewal of solvent plus heat
- ↳ **Grinding or micronization**
 - Traditional pretreatments to accelerate extraction
 - Solid/liquid separation may become more difficult



Supercritical fluid extraction

- ↳ **Pressure and temperature**
 - CO_2 : $> 31.1^\circ\text{C}$, 7.39 MPa
 - But also ethane, propane...
 - Advantages: low temperature, easy extract recovery
 - CO_2 : GRAS, low cost, non explosive
 - Low polarity but it can be modulated by co-solvents
 - Ethanol
- ↳ **Large scale industrial uses**
 - Decaf, hop extract
 - Essential oils and pharmaceuticals
 - Carotenoids





New extraction methods

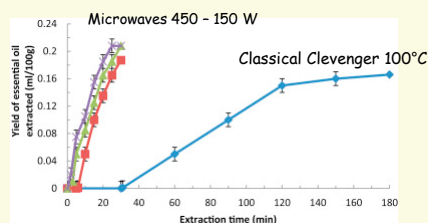
- ↳ **Intensification: increase yield, decrease solvent use and extraction duration**
 - Extraction itself « one pot »
 - Pretreatment: degradation of plant tissues followed by extraction
- ↳ **Various physical principles have been proposed**
 - Most often « athermal » - except microwaves
 - Increased tissue destructuration and facilitated transfer
 - Grinding or micronization
 - Pressure / vacuum
 - Directly or using ultrasound
 - Electrotechnologies
 - Mechanical, enzymic...



Microwaves-assisted extraction

Electromagnetic waves (2,45 GHz) which generate heat by interacting with polar molecules (such as water)

High temperature: hydrodistillation « without added water »



Extraction of pomelo essential oil

Chen et al., 2016

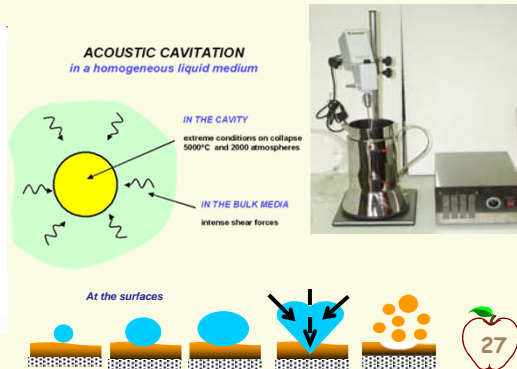
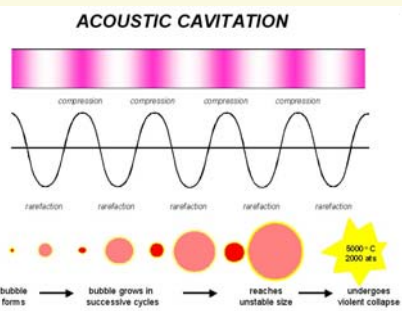




Ultrasound-assisted extraction

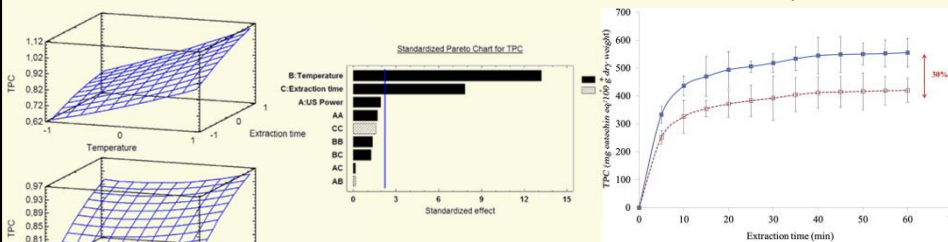
The underlying mechanism is cavitation created by ultrasound waves in the solvent

Explosion of cavitation bubbles erodes the surface of the plant tissues and desaggregates particles while facilitating homogenisation



Application to extraction of polyphenols from apple pomace

Experimental planning to optimize yield as function of temperature, ultrasound, extraction duration, S/L ratio, solvent composition



The (statistical) optimum corresponds to max conditions

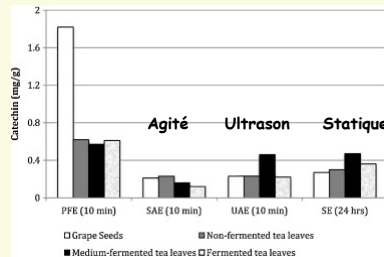
Generally a very limited effect of ultrasound power

Pilot to industrial-scale equipments



Pressurized solvent extraction

PSE or ASE™: solvents under pressure and high temperature
Subcritical conditions but solvent polarity (hence specificity) is modified
Facilitated penetration in the pores of the material
Mostly analytical use



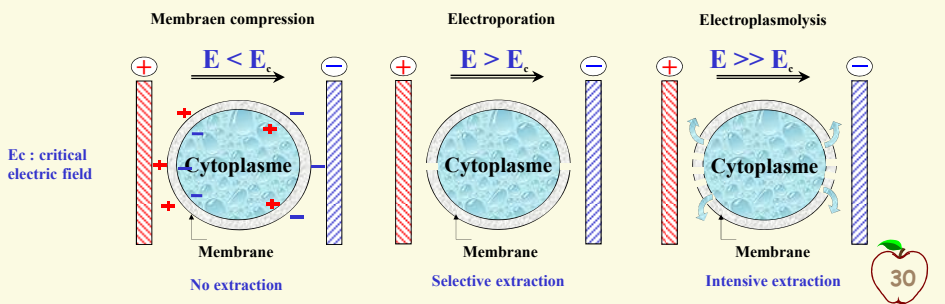
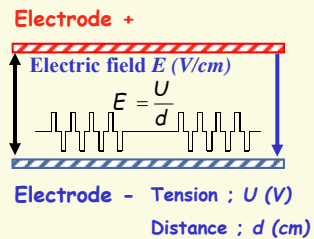
Pinero et al., 2004



Pulsed electric fields

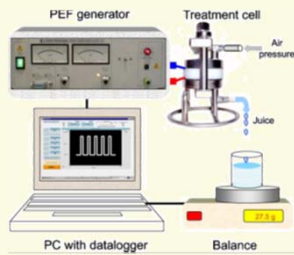
Electroporation i.e. opening of pores in the cell membranes by an electric field

Used to facilitate extraction from intracellular liquids - juices rather than extraction of bioactives from co-products

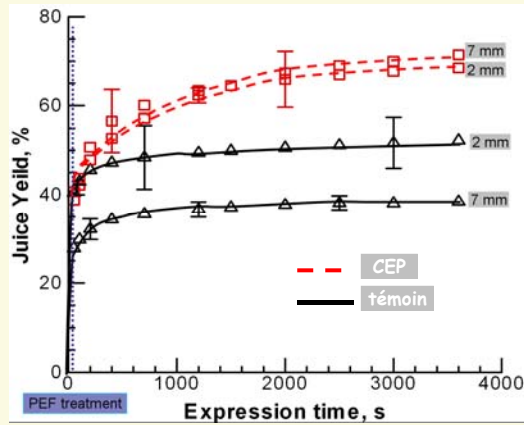




Apple juice extraction

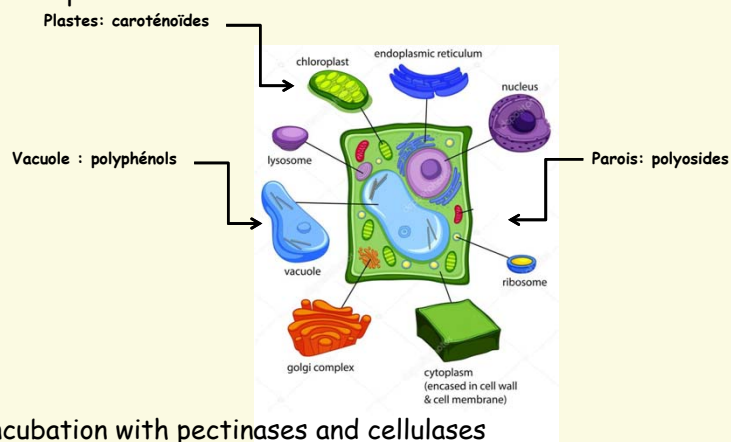


Pilot-scale extraction on a belt press, with different apple fragmentation sizes



Enzymes that degrade the plant cell walls

To facilitate diffusion by tissue degradation and decrease of adsorption

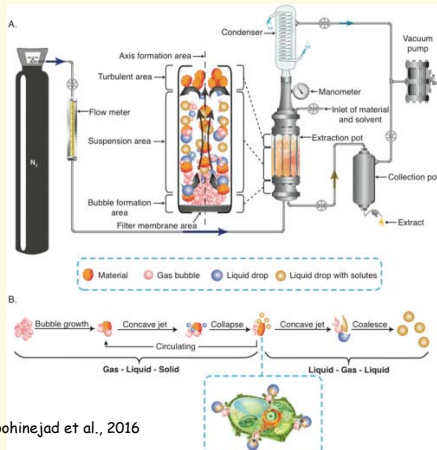


Incubation with pectinases and cellulases



Negative pressure cavitation

Cavitation by depression of a gas-saturated liquid to generate intense erosion of solid particles and increase turbulence and mass transfer from the solid matrix to the solvent



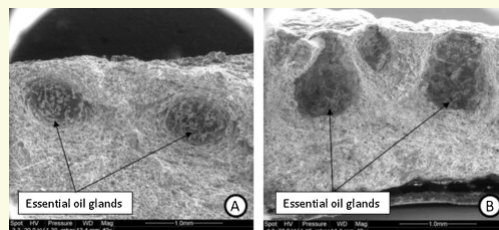
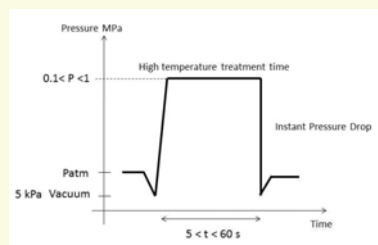
Cavitation is due to gas expansion, and the bubbles also contribute to a turbulent flow and intense mass transfer



Détente instantanée contrôlée

In instant pressure drop the sample is treated in a stream of saturated steam then the water is vaporized by sudden depression, resulting in cell wall expansion

Essential oils can be recovered with the cooled vapors while other compounds are easily extracted from the fine solid powder



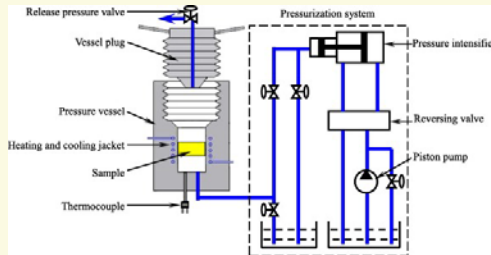
Optnein of orange essential oil glands by DIC treatment



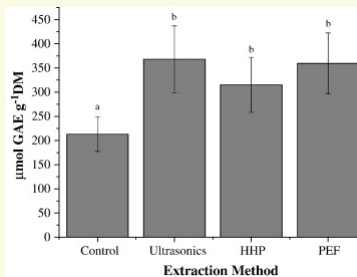


High pressure homogenization

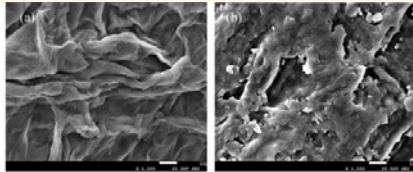
The (wet) plant particles are disintegrated by high intensity mechanical stresses as the liquid flows through an homogenization chamber



Extraction of phenolics from grape by-products



Corrales et al., 2008



Alternative solvents

The incentive is the need to replace petrochemical solvents with negative evaluation according to REACH legislation

In choosing a solvent its solution properties as well as recovery / elimination have to be assessed

Different options have been proposed:

- « classical », GRAS solvents such as water (removal...) or ethanol
- Natural « green » solvents: terpenes (limonene, alpha-pinene...)
- Liquefied but not supercritical gases (n-propane, n-butane)
- Ionic liquids notably natural deep eutectic solvents

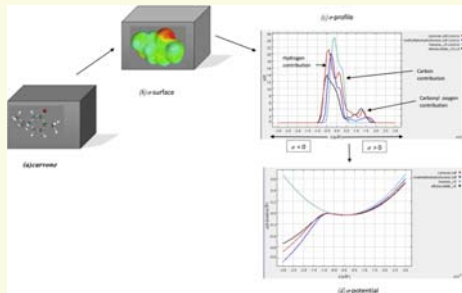




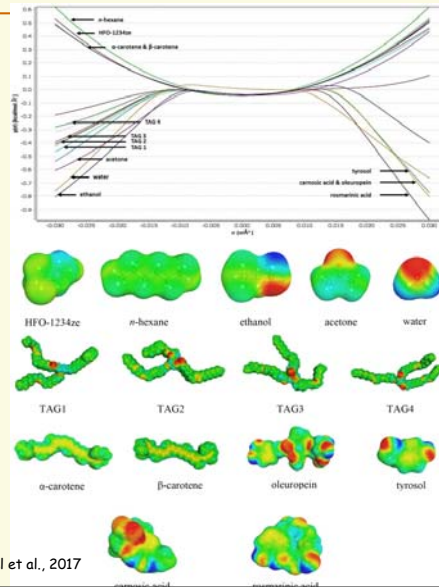
Computer-aided solvent choice

Solvent and solutes compatibility using Cosmo-RS

3D distribution of the polarization charges on the molecule surface



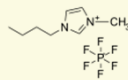
Rapinel et al., 2017



Ionic liquids and NADES

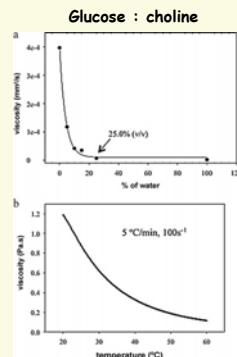
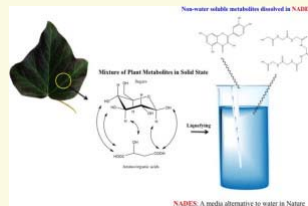
Ionic liquids:

- salts in the liquid state



Natural deep eutectic solvents:

- Approximate the medium in which the compounds were dissolved in the cell
- Mixtures of sugars and amino-acids - and water



Dai et al., 2013



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Needs in research... And organisation

- ↳ **Extraction itself is the easy part...**
 - Extraction intensification has been the focus of recent research
 - Validated methods, some available at pilot / industrial scale
- ↳ **Research perspectives**
 - Go beyond optimization
 - Improve pre-extraction (drying, stabilisation) and post-extraction treatments (purification, stabilisation...)





A need of organisation of value chains

- ↪ **Extraction of WHAT from WHICH material: co-product or dedicated production?**
 - Choice / cost / concentration / « food first »
 - Can we reconcile those two very different questions:
 - I have a co-product, what can I do with it?
 - I want this molecule/property, how can I get it?
- ↪ **Which properties are relevant?**
 - Nutraceutical niche markets?
 - Replacement of synthetic colorants or antioxidants?
 - Legislation?
- ↪ **Stabilisation**
 - Extraction during a large part of the year
 - Versatility to adapt to different raw materials/co-products or stabilization to expand period of use?
 - What about the residues? Solvent recovery?
 - What about contamination by pesticides / mycotoxins?



Thank you for your attention



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