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# Modern Extraction and Isolation Methods

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# Introduction to Extraction and Isolation

# Why to extract?

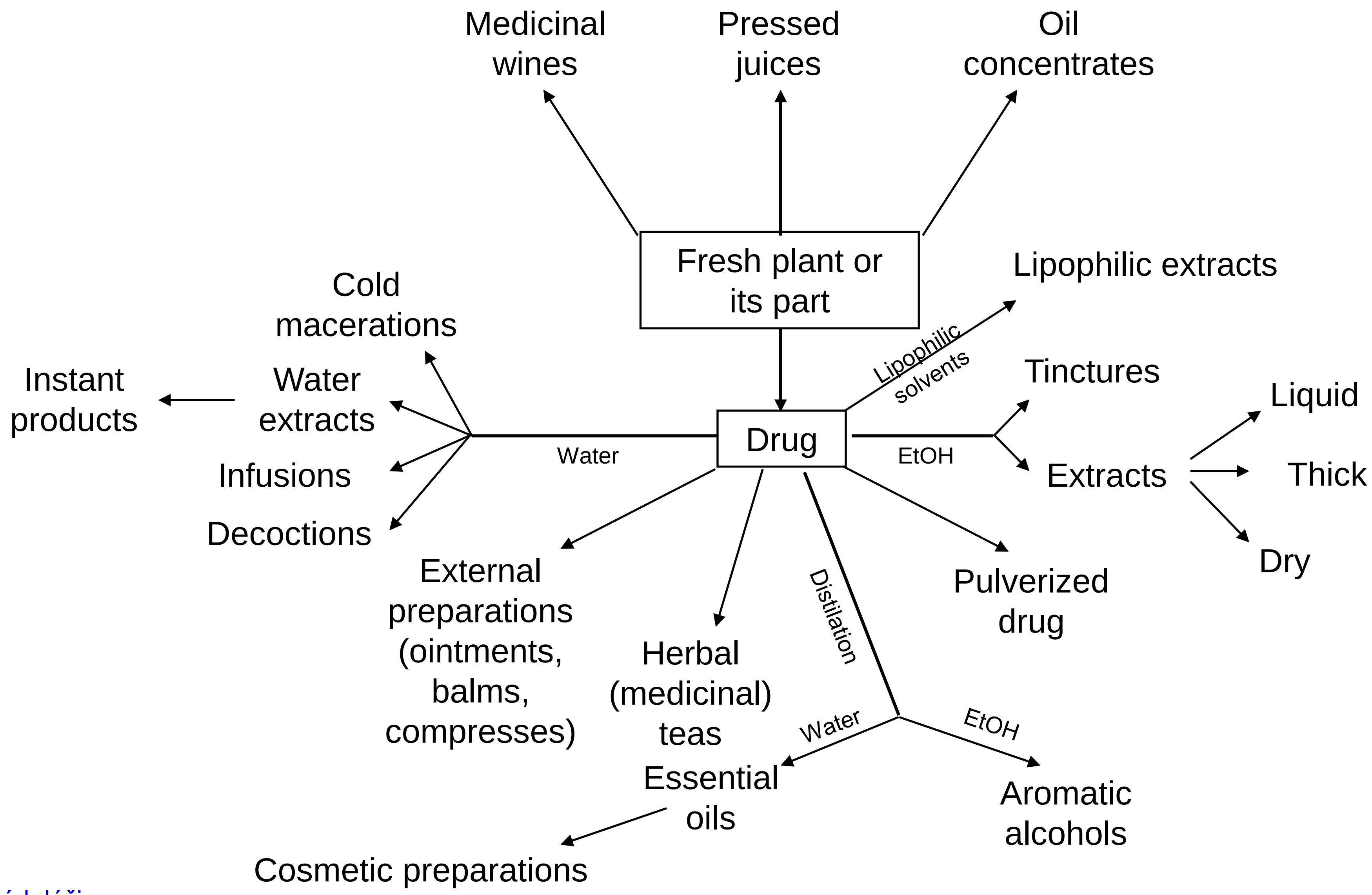
- Content of active compounds in drug low
- Content of active compounds variable
- Presence of unwanted compounds
- Drug not acceptable because of bad organoleptic properties
- The amount for direct preparation of application form/administration too big
- Better possibility of dosage
- Better compliance of patient

# Content compounds

- Main active compounds
- Supporting content compounds
- Ballast compounds

- The aim of extraction:
  - **Remove ballast substances, to maintain main and supporting content compounds, the obtain extract rich in target substance**

# Traditional processing of medicinal plants, their parts and drugs



# Methods of standardization

## Analytical techniques for determination of markers and standardization

### *Determination of class of compounds*

Total phenolics, total flavonoids, total alkaloids…
Amount of volatile substances (essential oil quantification)
Common utilization of color reactions
- Fast, simple, cheap
- Possible false-positive results, possible falsification

**Requirements for marker:**

1) Bioactivity
2) Sufficient content
3) Physico-chemical stability

**Combination of analytical technique and biological activity**

### *Single compounds as markers*

- HPLC-DAD, HPLC-MS, GC-MS
- Quantification of one or more compounds
- Precise, more expensive, low chance of falsifying

# Extraction - Fick diffusion law

$$\Delta n / \Delta t = -(DA/h) \times (c_0 - c)$$

- **Δn/Δt** – velocity of diffusion
- **D** – diffusion coefficient based on temperature and diameter of diffunding particles
- **A** – diffusion space (surface)
- **h** – diffusion layer
- **($c_0$-c)** – concentration gradient

# Improved effectiveness of extraction

- **Maceration** **(one batch method – periodic)**
  - Decantation, centrifugation, filtration

**Extraction - Fick diffusion law**

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***Matrix effects***

Desintegration of material
Meating
Stirring
Repetition of process
Solvent selection
Sonication

## Improved methods

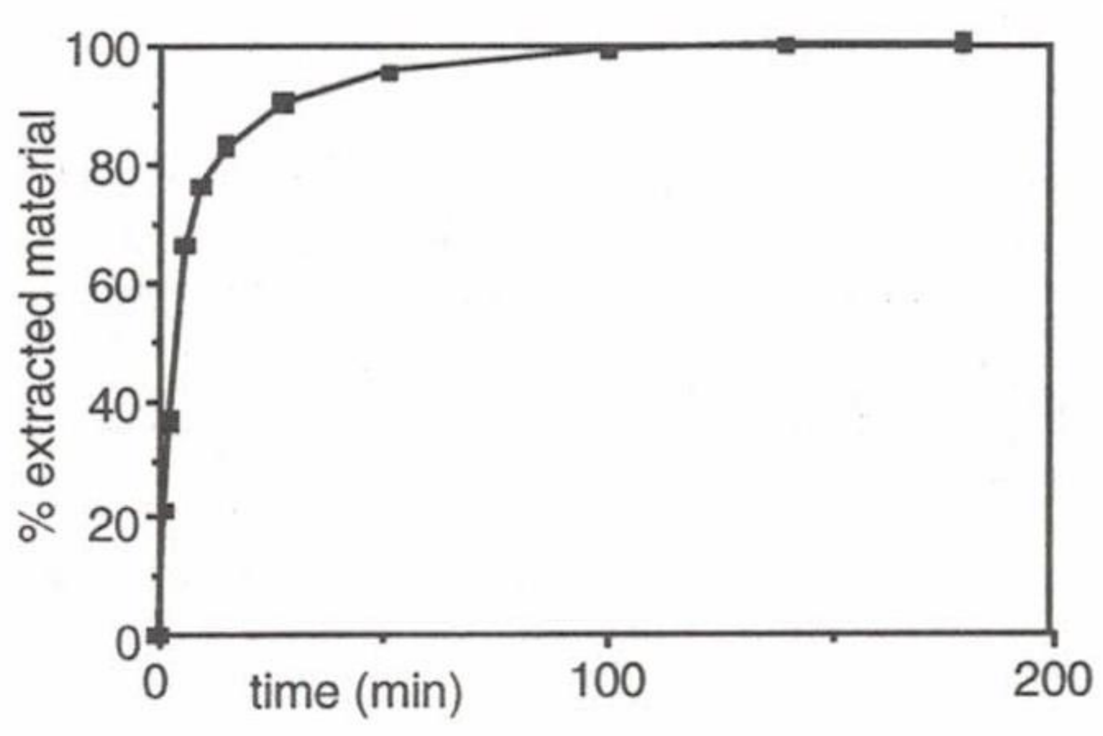
**(semicontinual)**

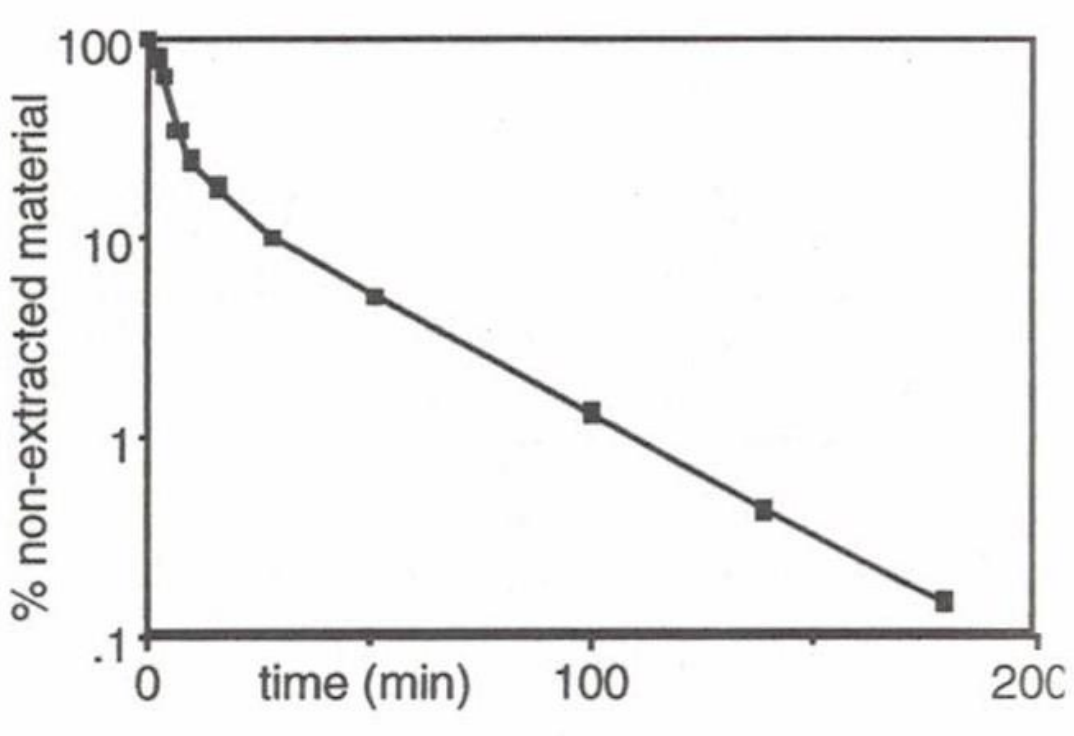
- Percolation
- Digestion
- Variations on Soxhlet extractor

# Solid-liquid extraction techniques

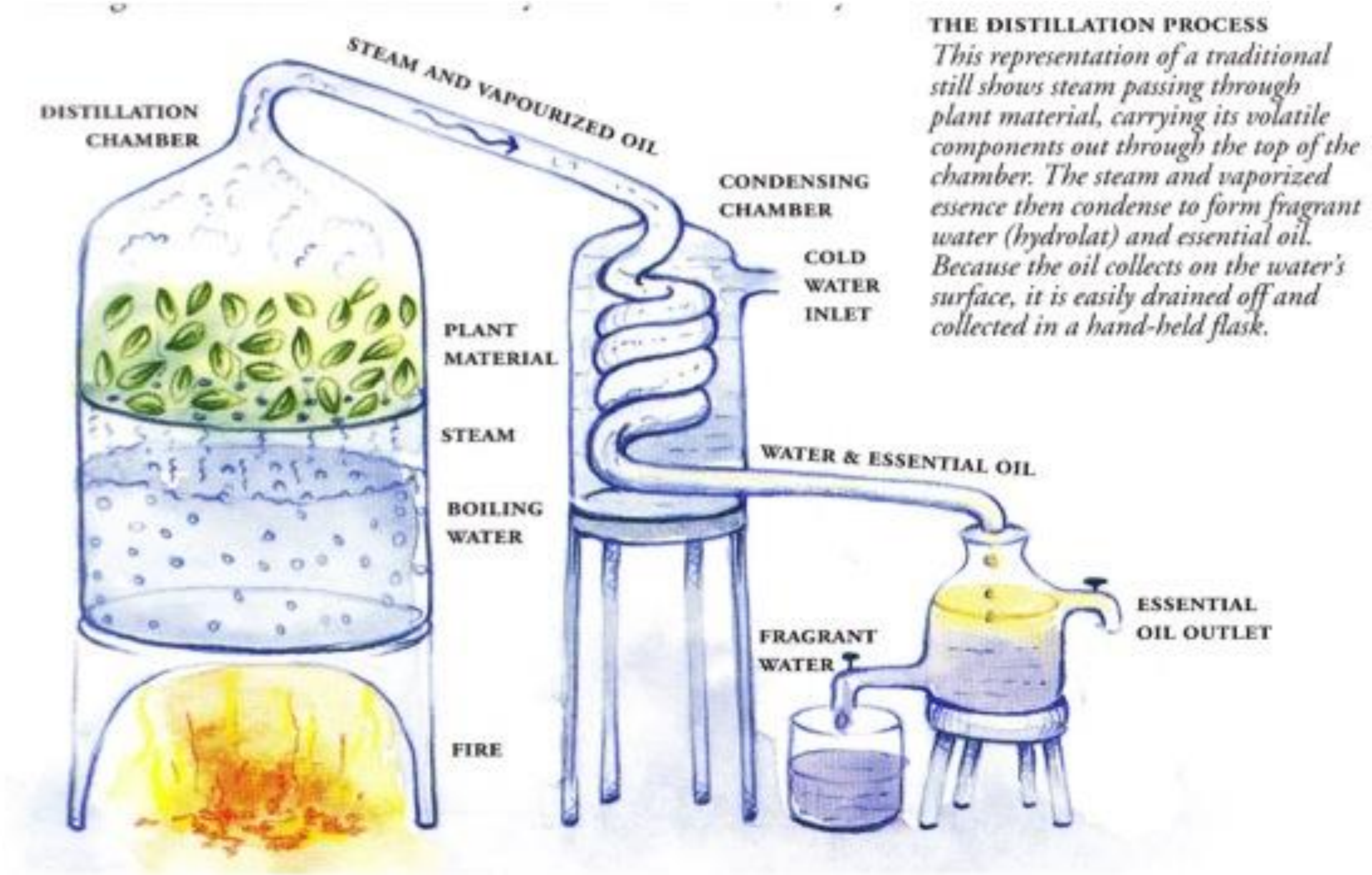
- Conventional extraction techniques
  - maceration, percolation, squeezing, counter-current extraction, extraction through Soxhlet, distillation, etc.
  - high quantities of expensive and pure solvents, a low selectivity of extraction; a high solvent evaporation rate during the process; long extraction times, thermal decomposition of thermolabile compounds

- Unconventional (innovative) extraction techniques
  - ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), extraction with accelerated solvent, solid phase microextraction, enzyme-assisted extraction, and rapid solid-liquid extraction dynamic (RSLDE) via the Naviglio extractor

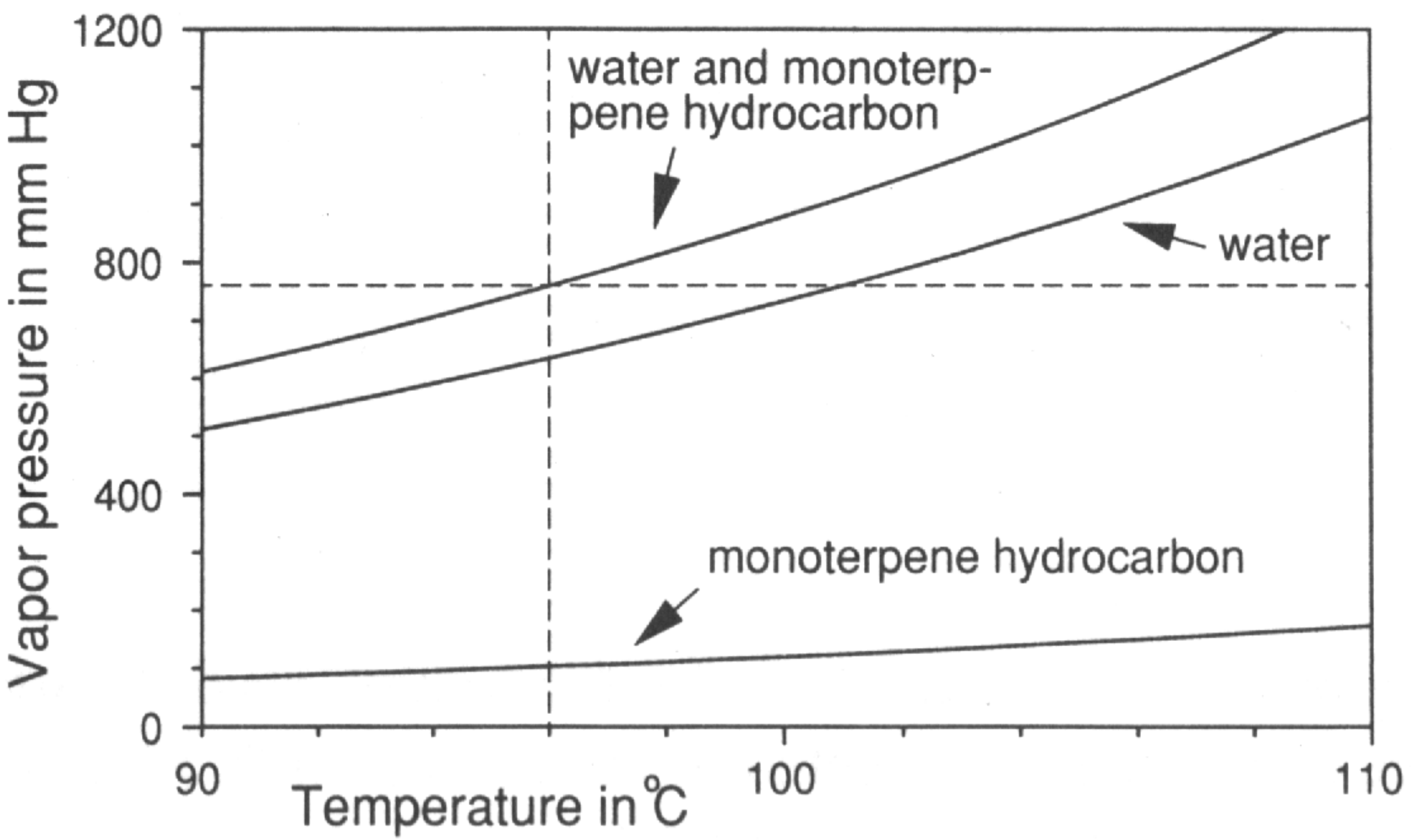

% extracted material
100
80
60
40
20
0
0
time (min)
100
200


% non-extracted material
100
10
1
.1
0
time (min)
100

# Water steam destillation



# Dalton´s law

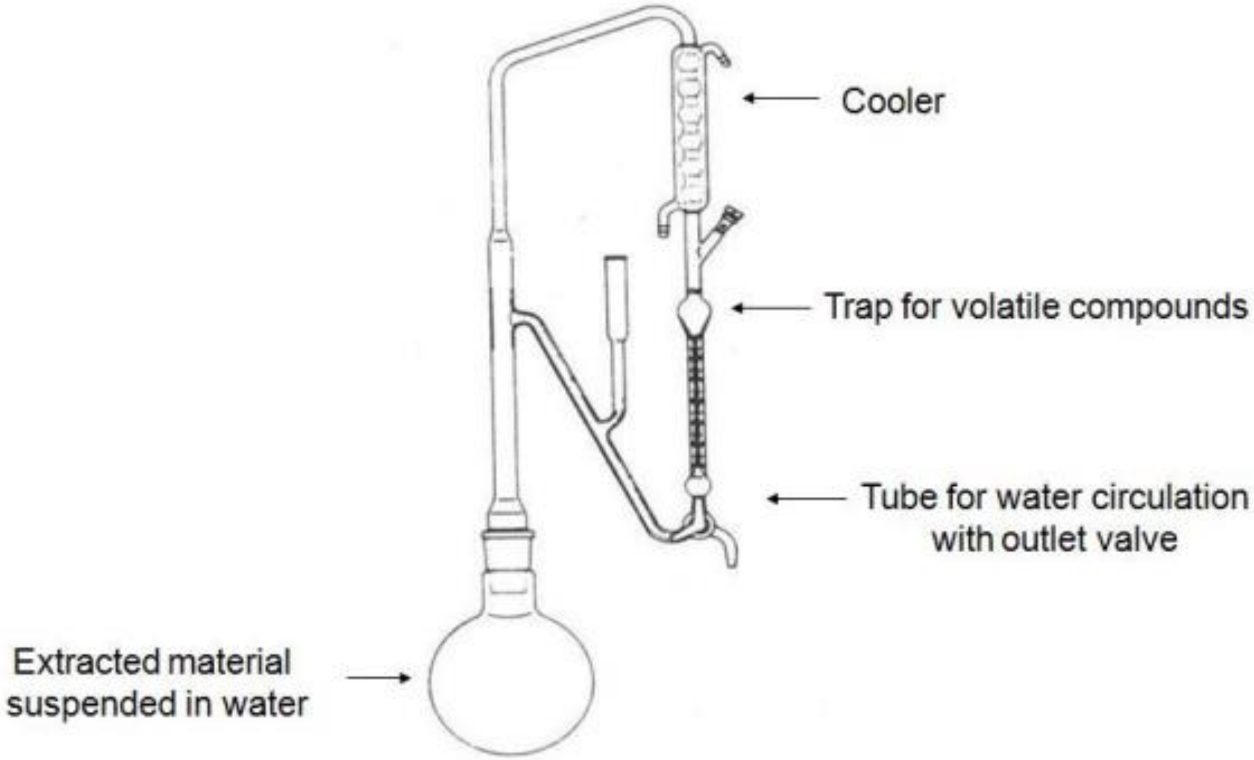


1200
800
400
0
Vapor pressure in mm Hg
water and monoterp-
pene hydrocarbon
water
monoterpene hydrocarbon
90
100
110
Temperature in °C

# Steam distillation

Suitable for water-insoluble substances, only volatile compounds

Selective
Simple
Cheap



# Products of steam distillation

- Essential oils
  - Eucalyptus, tea-tree oil, camphora
- Hydrolates
  - Rose water
  - Orange blossom water
- Aromatic spirits

**Table 1: Table of analytes suitable for steam-distillation extraction.**

| Analyte | Matrix | Quantification Method | Related Norms |
|---|---|---|---|
| Protein (nitrogen), TKN, TVBN | Food, beverages, pharmaceuticals, feed, waste water | Potentiometric / Colorimetric Titration | AOAC 2001.11 AOAC 920.87 ISO 937 ISO 3188 |
| Ammonium, nitrite, nitrate (Devarda), urea | Fertilizer, soil, cosmetics, hair dye | Potentiometric / Colorimetric Titration | AOAC 892.01 AOAC 955.04 83/514/EEC |
| Alcohol | Wine, beer, spirits | Densitymeter | EC 2870/2000 |
| Volatile acids | Wine, juice | Potentiometric Titration | OIV-MA-AS313-02 |
| Sulfite, Sulfur dioxide | Wine, beer, dried fruits, seafood | Potentiometric Titration | AOAC 962.16 |
| Cyanide, Amygdalin | Food, feed, waste water | Complexometric Titration | ISO 2164-1975, AOAC 915.03 |
| VDKs | Beer | UV-Vis Spectrometry | |
| Phenol | Soil, waste water | UV-Vis Spectrometry | ISO 6439:1990 DIN 38409-H16-3 |
| Formaldehyde | Textiles, maple sirup | UV-Vis Spectrometry | ISO 14184-1 AOAC 964.21 |
| Limonene (essential oils) | Juice, fragrances, hops | Redox Titration | |

# Equipment

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# Equipment



lavender


The Front
The Side

# Superkritical fluid extraction

- **Extraction with utilization of supercritical fluids**
- **Supercritical fluid**

  Pressure and temperature over critical values
  Physical properties form a transition between the properties of gases and liquids

  Density close to liquids → good dissolving ability
  Diffusion constant close to gases → rapid mass transfer
  Viscosity lower than liquid → advantage of better flow properties
  Low surface tension → easy material penetration


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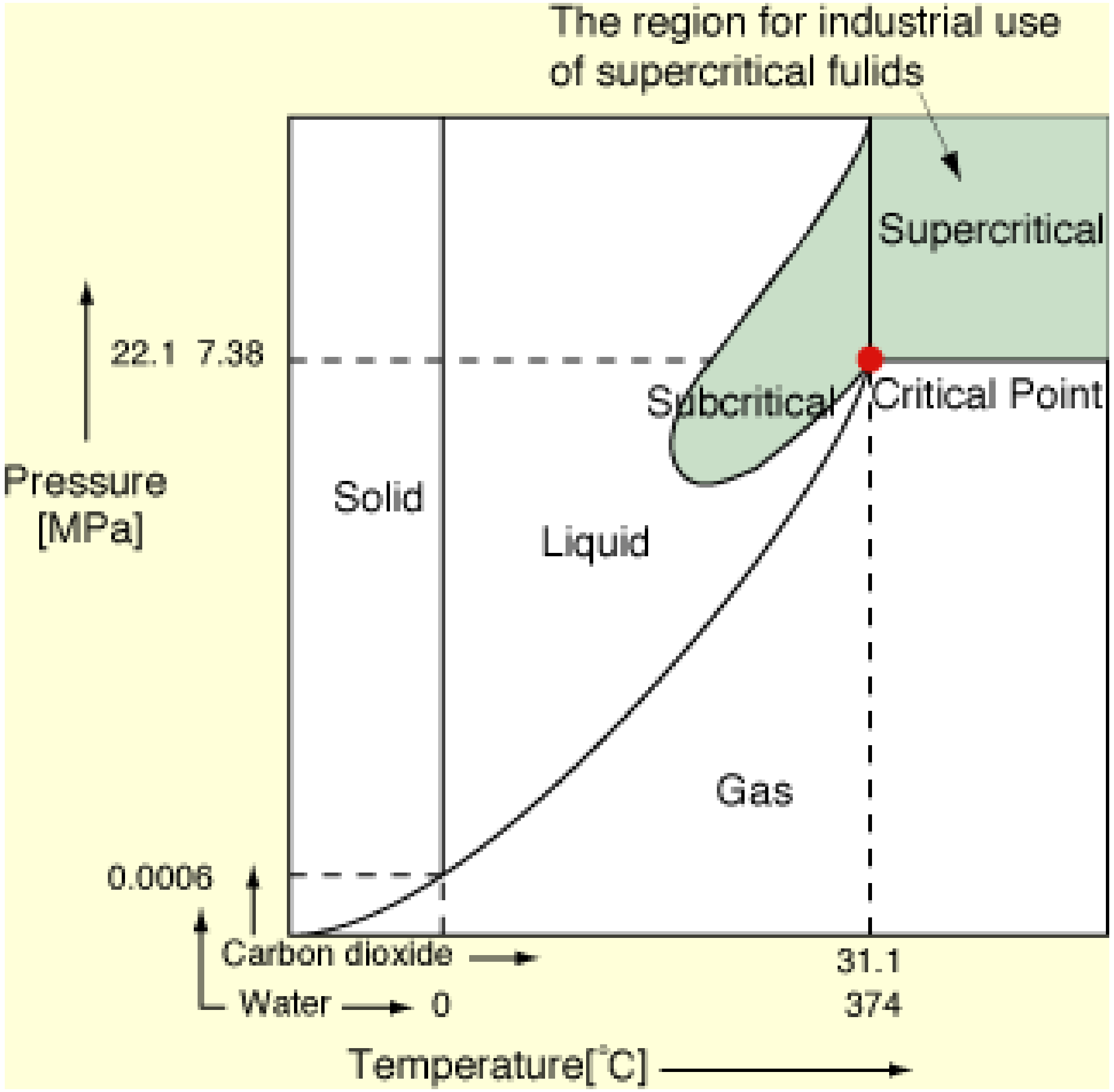
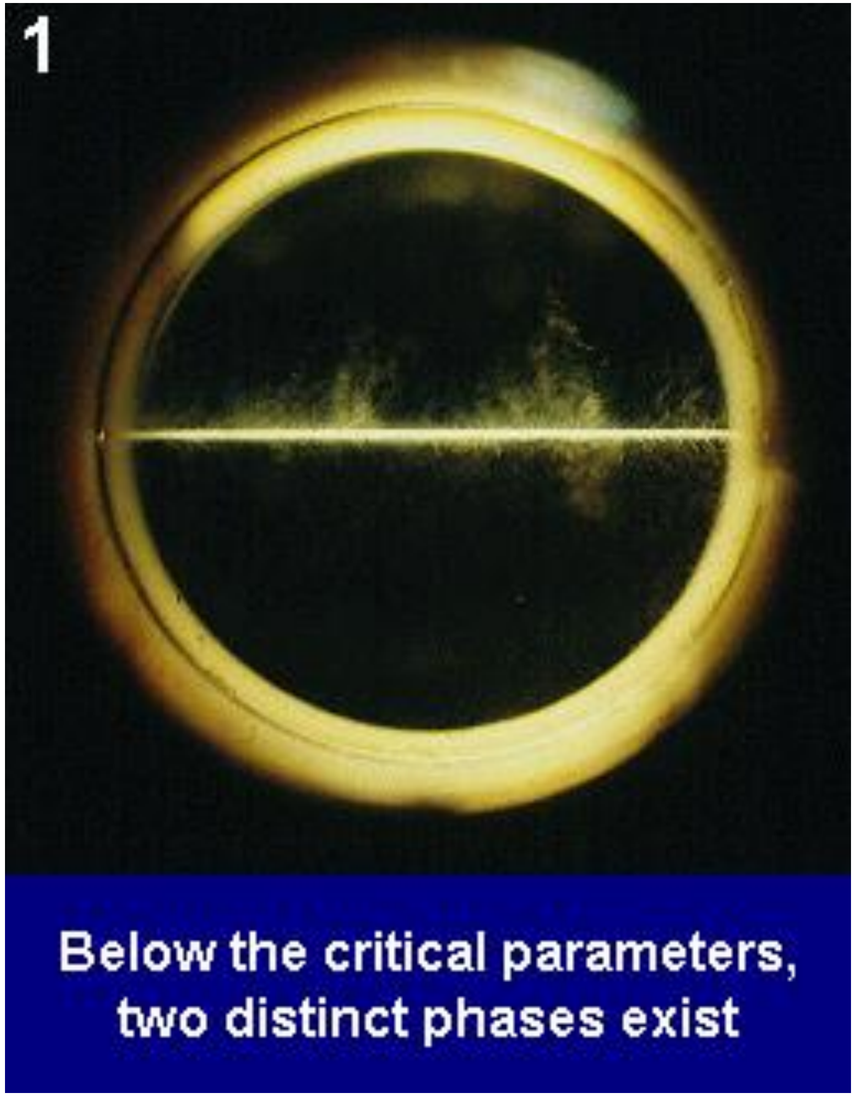
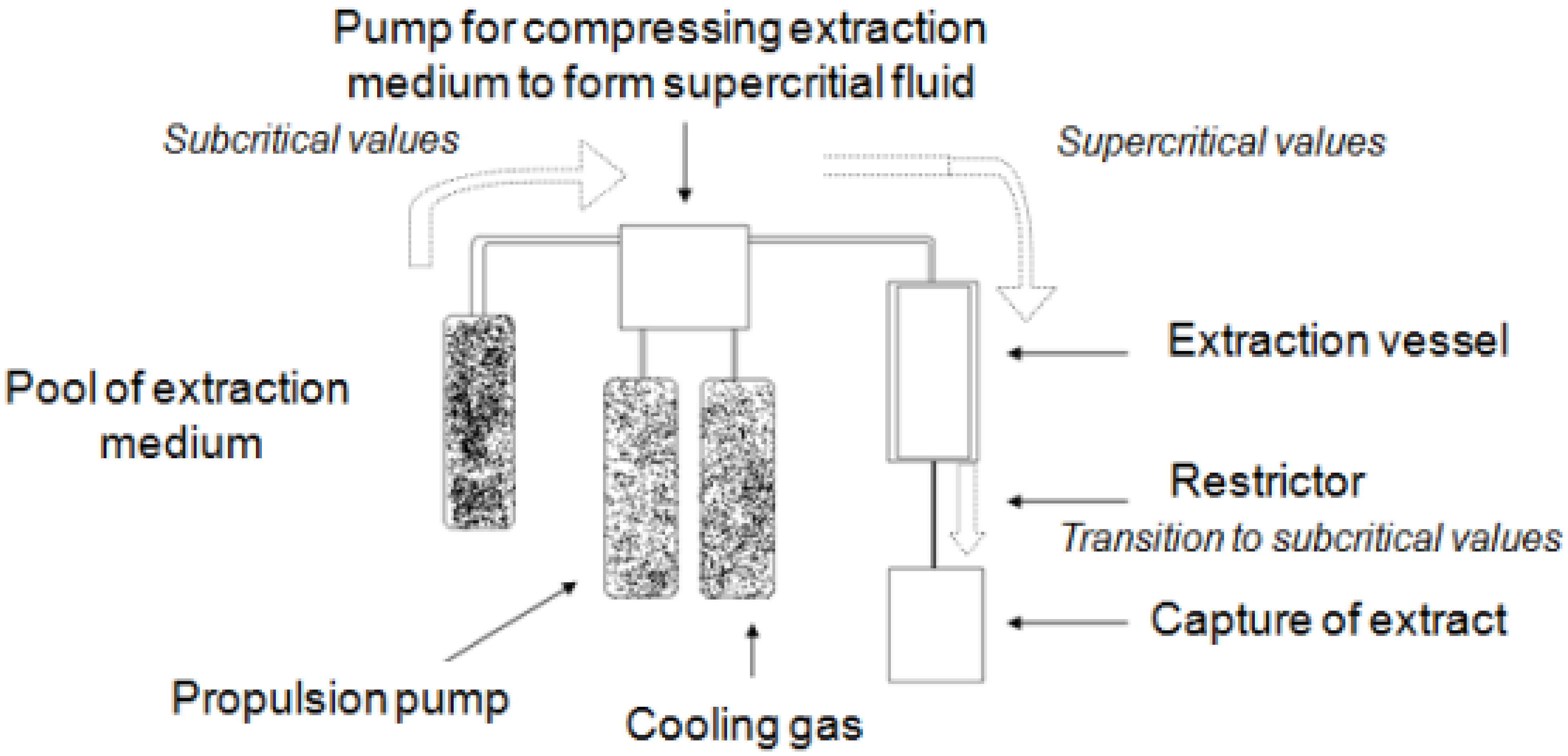

The region for industrial use
of supercritical fulids
Supercritical
22.1 7.38
Subcritical
Critical Point
Pressure
[MPa]
Solid
Liquid
Gas
0.0006
Carbon dioxide
Water
0
31.1
374
Temperature[˚C]


1
Below the critical parameters,
two distinct phases exist

# Supercritical fluid extraction



# Advantages of SFE:

Gentle technique.
Ideally, no organic solvents are needed.
Ecologically harmless.
Cheap.
Fast.
Possibility of automation.
Changes of solvation strength by changes of pressure.

# Disadvantages of SFE:

Less suitable for polar compounds.
More demanding instrumentation.
Requires the use of high pressures.
Less suitable for leaf extraction.
Extraction tuning issues.
Difficult extraction of fresh material (water content).

**$CO_2$ –** non-flammable, non-explosive, easily available, cheap, environmentally friendly, advantageous supercritical region (T=31.1 °C; P=7.28 MPa), suitable for the extraction of low polar substances (essential oils, oils, waxes, carotenoids)

- **Utilization:**

  Hop extraction. Decaffeination of coffee. Extraction of taxol from *Taxus brevifolia*. Extraction of essential oils and spices. Non-pharmaceutical purposes

# SFE

https://www.youtube.com/watch?v=DunzmKCDk0c



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# Accelerated solvent extraction (ASE)

- Increased extraction yields and reduced time
- An increased diffusion
- Liquids operating above their boiling temperature while being maintained in a liquid state by the increase in pressure
- A cylindrical steel container, the extracting solvent is introduced
- The temperature of the system is raised above the boiling point of the solvent, which is maintained in the liquid state thanks to a simultaneous increase in pressure (the vial is sealed to resist high pressure values: 100–200 bar)
- Not suitable for thermally labile substances

# Accelerated solvent extraction (ASE)

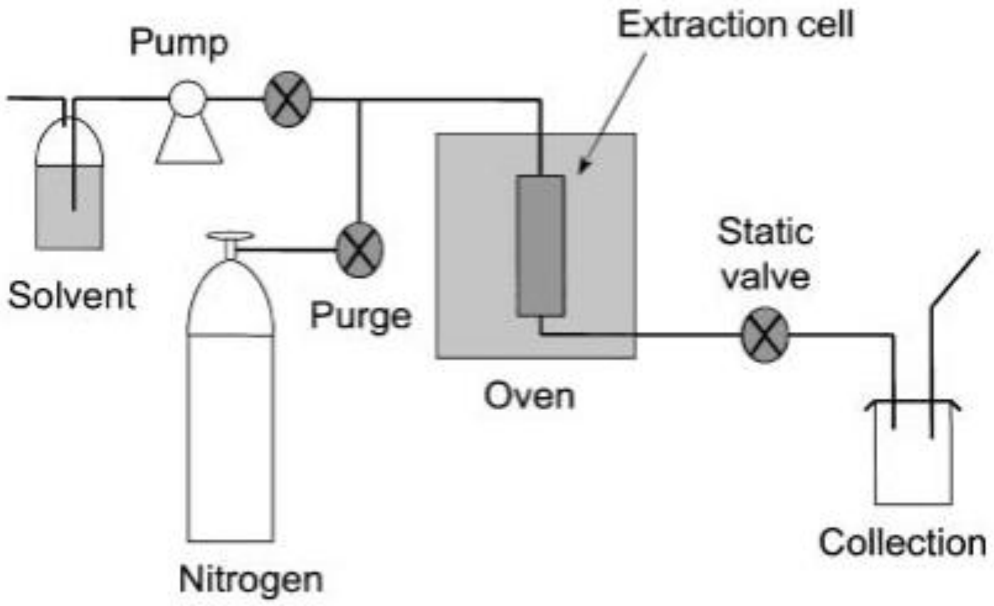
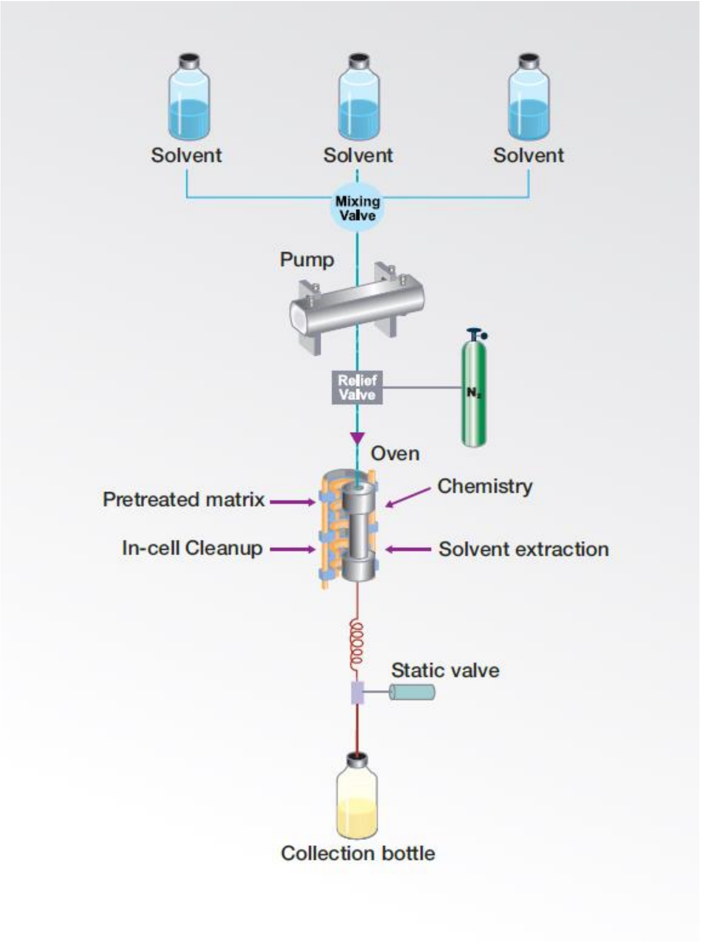


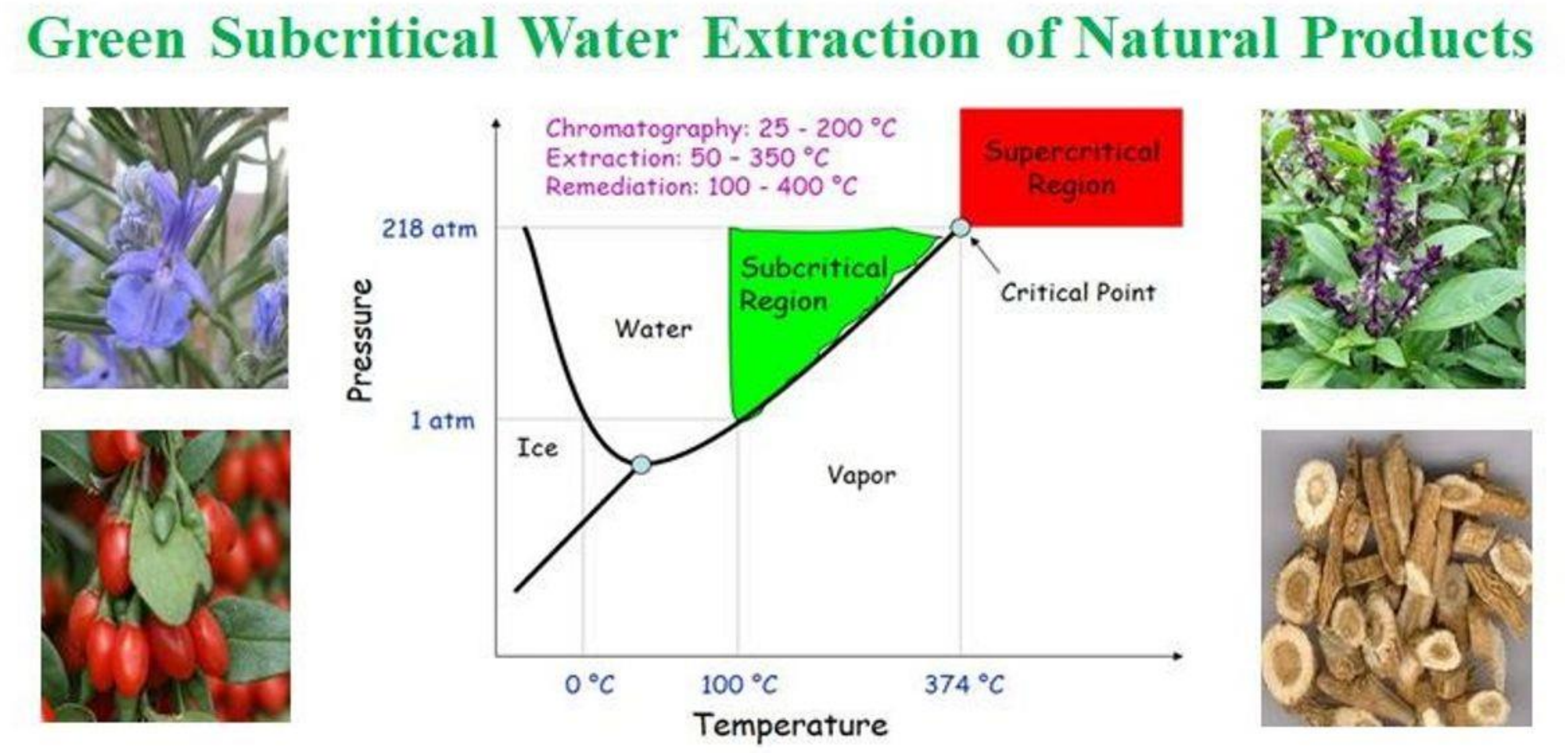

**Figure 5.** Scheme of an accelerated solvent extraction (ASE) system (from Richter *et al.*, 1996).




https://www.thermofisher.com/cz/en/home/industrial/chromatography/chromatography-sample-preparation/automated-sample-preparation/accelerated-solvent-extraction-ase.html

https://www.thermofisher.com/order/catalog/product/083114

# Subcritical water extraction (SBWE)



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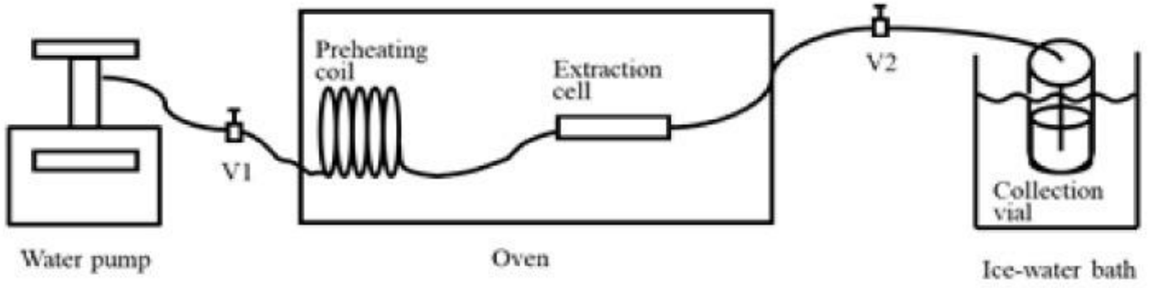
# Subcritical water extraction (SBWE)

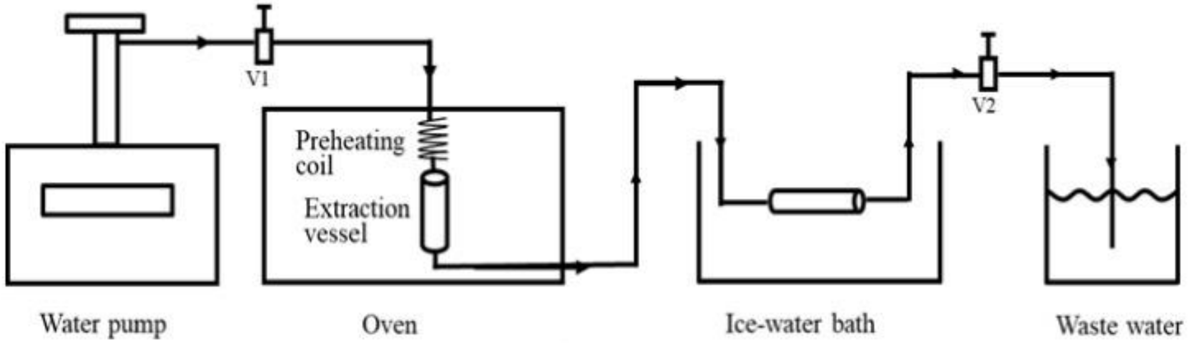
- 1) reversed-phase liquid chromatography using subcritical water as the sole mobile phase-subcritical water chromatography
- 2) extraction of environmental samples
- 3) hydrolysis, degradation, polymerization, and synthesis reactions using subcritical water as both a solvent or a reactant
- 4) environmental remediation such as cleaning contaminated sewages and soils, decomposing pollutants (pesticides, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls) and explosives
- 5) extraction of active ingredients from medicinal and seasoning herbs, vegetables, fruits, and other plant related matrices

# Subcritical water extraction (SBWE)

- High-temperature and high-pressure water
  - Temperature and pressure below its critical point (Tc = 374.15 °C, Pc = 22.1 MPa).
- Its polarity can be dramatically decreased with increasing temperature
  - Dielectric constant, viscosity, and surface tension all decrease steadily
  - Its diffusion coefficient is improved with increasing water temperature
- SW can behave similar to methanol or ethanol
- Co-solvents include ethanol, methanol, salts, and ionic liquids
- Support by microwave and sonication

*Static model*




*Static × dynamic*



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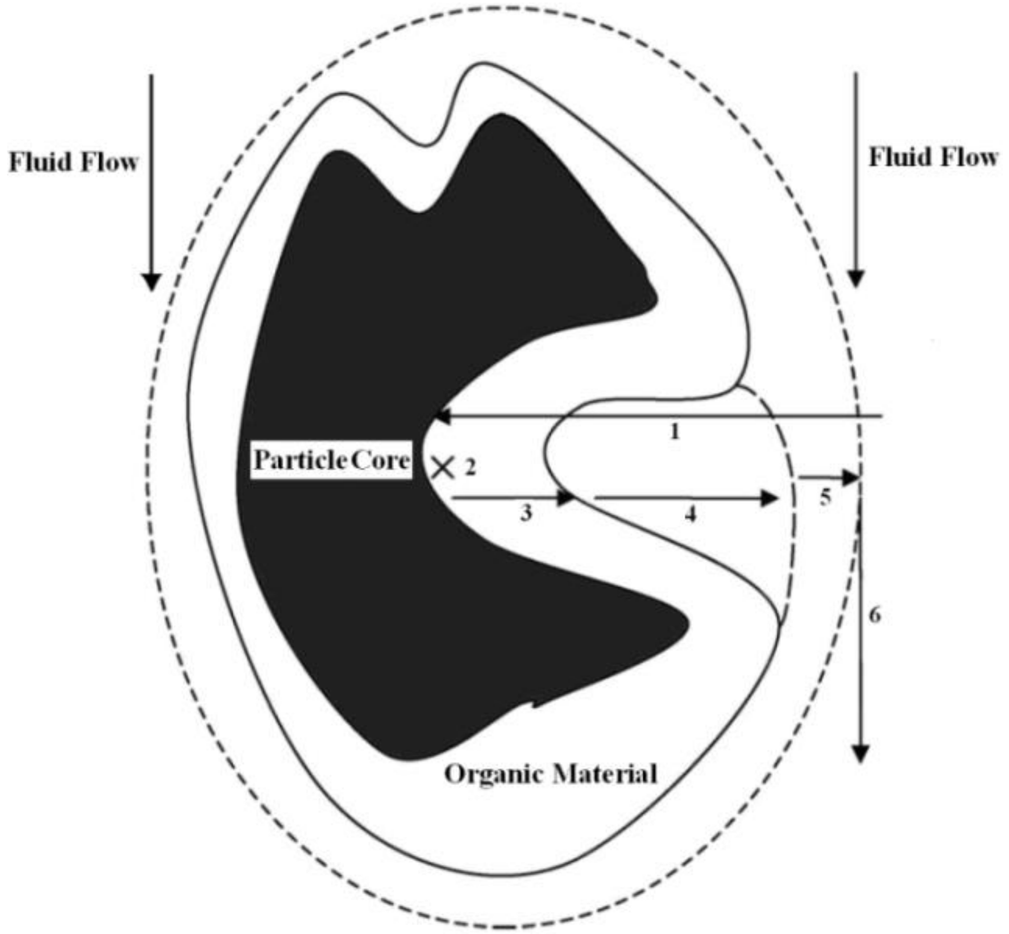
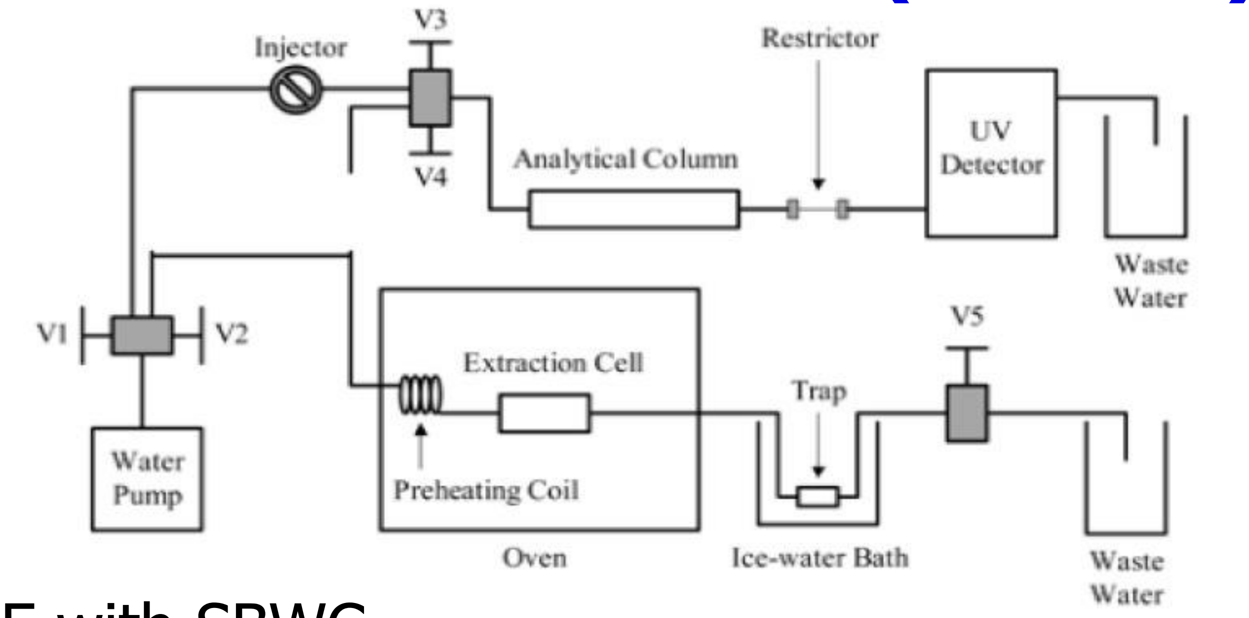
# Subcritical water extraction (SBWE)

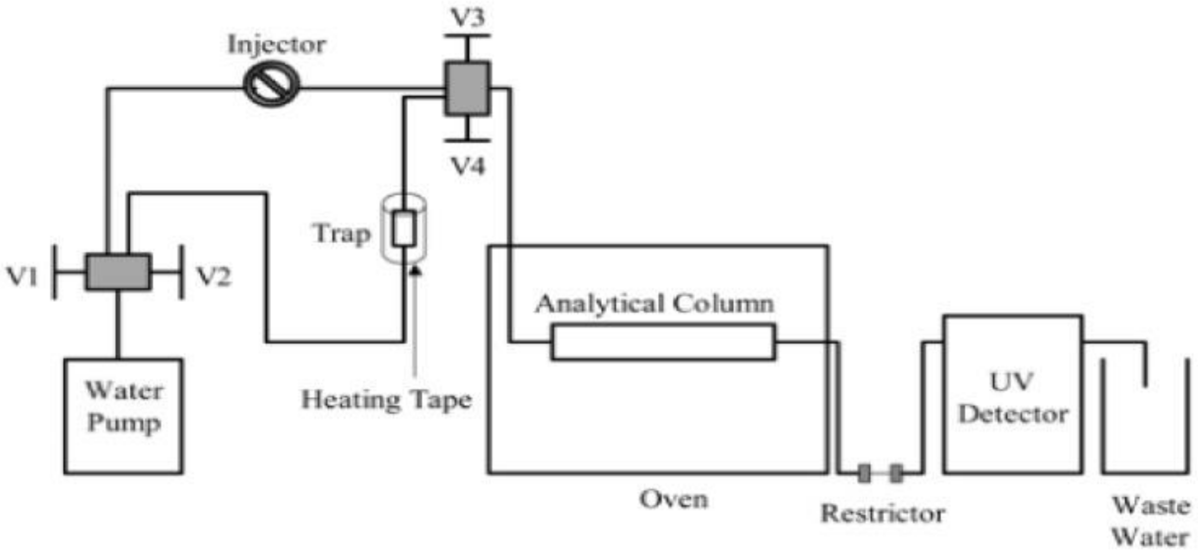
The SWE process can be proposed to have six sequential steps: (1) rapid fluid entry; (2) desorption of solutes from matrix active sites; (3) diffusion of solutes through organic materials; (4) diffusion of solutes through static fluid in porous materials; (5) diffusion of solutes through layer of stagnant fluid outside particles; and (6) elution of solutes by the flowing bulk of fluid



# Subcritical water extraction (SBWE)




Offline coupling of SBWE with SBWC



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# Subcritical water extraction (SBWE)




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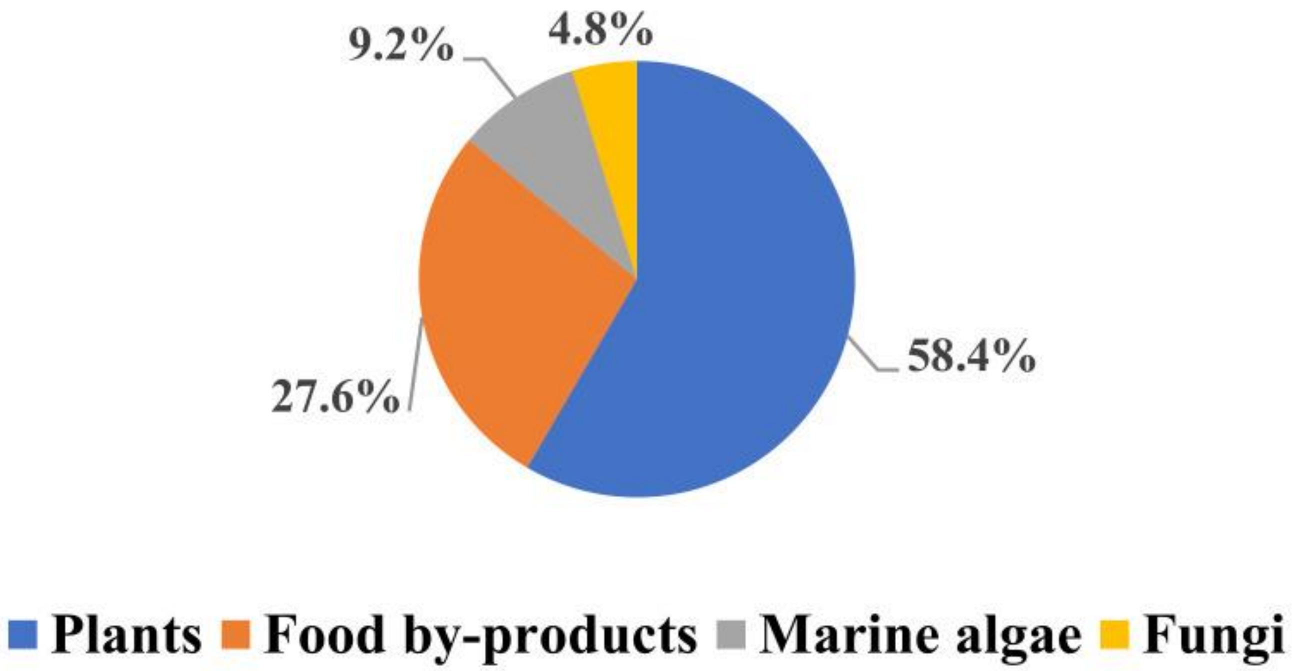
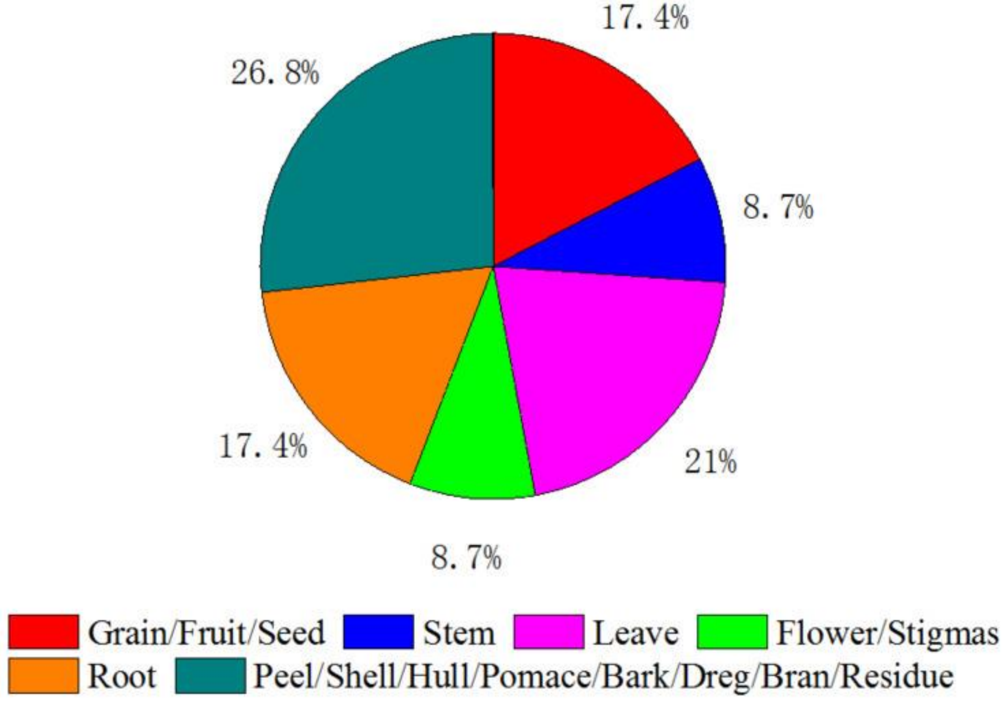
# Subcritical water extraction (SBWE)

- The major advantage of SBWE
  - Water is nontoxic → suitable for the extraction of herbs, vegetables, and fruits
  - No liquid waste disposal
  - Ecology, economy, and safety
  - Density, ion product, and dielectric constant can be adjusted by temperature
- Optimal temperature 130 to 240 °C
- Flavonoids, organic acids, glycosides, carbohydrates, polyphenols, essential oils, alkaloids, lignans, steroids

# Subcritical water extraction (SBWE)



# Ultrasound assisted extraction

- An innovative technique, used in different settings
- “Clean technology”
- Use of low solvent volumes
- Short Ets
- Few instrumental requirements
- Low economic and environmental impact
- Technique employs ultrasonic waves frequencies between 20 kHz and 10 MHz
  - power ultrasound (20–100 kHz), characterized by a high intensity, used for extraction and processing applications
  - signal or diagnostic ultrasound (100 kHz–10 MHz), employed as a clinical diagnostic technique, and for control and quality assessment
- Acoustic cavitation (AC)

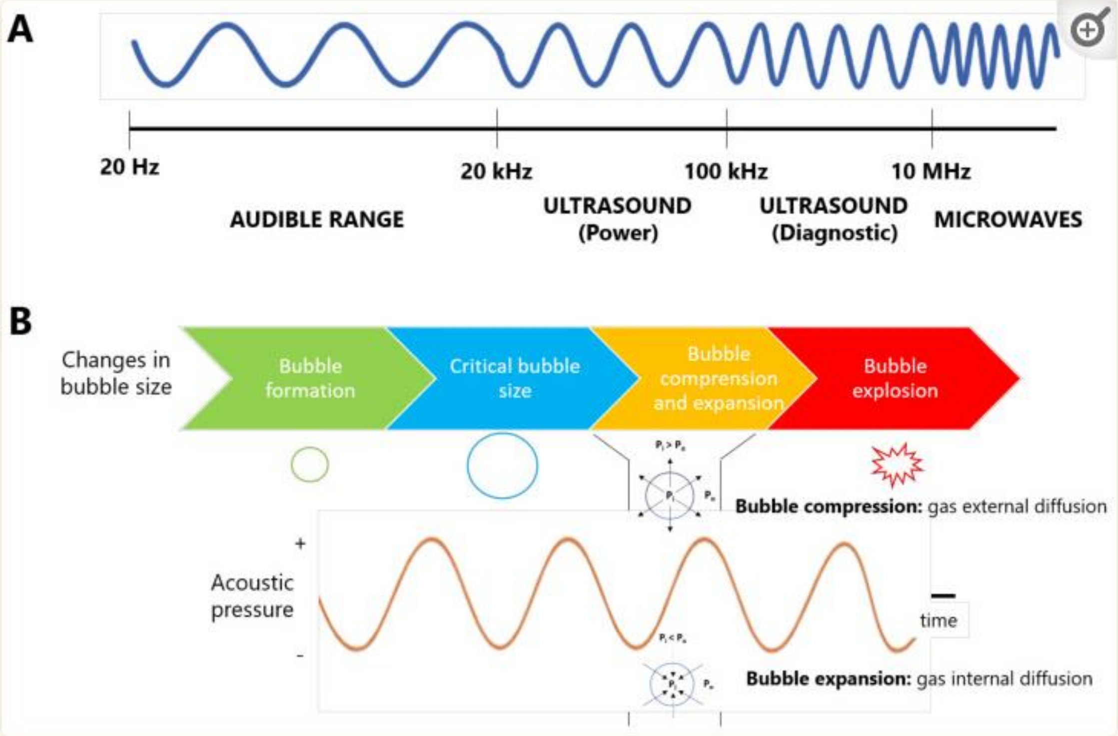



Figure 1

(A) The sound spectrum: audible range (20 Hz–20 kHz), ultrasound range (20 kHz–10 MHz) and microwave range (>10 MHz). (B) Bubble growth cycle during acoustic cavitation. Rarefaction and compression. A cycle of ultrasonic wave holds an expansion (rarefaction) and compression phases. In rarefaction phase, gas diffused into a bubble because of external pressure ($P_o$) is higher than internal pressure ($P_i$). However, gas diffused out of bubble during compression phase due to internal pressure is higher than external pressure.

doi: 10.3390/ijerph18179153

# Ultrasound assisted extraction (UAE)

## – UAE-Associated Mechanisms

- Fragmentation → the reduction of matrix particle size guided by the ultrasonic action → increases the solid surface area to develop mass transfer, driving to better extraction yields
- Erosion → the release of solid structures from the matrix into the extractive solvent, caused by the collapse of cavitation bubbles
- Sonocapillary → an enhanced penetration of solvent into the canals and pores of the matrix
- Detexturation → the solid matrix destruction
- Sonoporation → an increase in cell membranes permeability, forming of membrane pores
- Local shear stress → generation of shear forces onto the matrix surface, causing the later rupture of its structures and the extraction of inner compounds in the solvent

## – Relevant Parameters Associated with UAE

- Physical parameters - power, frequency, and ultrasound intensity, related with the ultrasound equipment – ET, shape and size of ultrasonic reactors
- Medium parameters - the solvent nature and its properties (polarity,, viscosity, surface tension, solvent vapor pressure), extraction time, the presence of gases, new green solvents for lipophilic extraction → ionic liquids and deep eutectic solvents
- Matrix parameters
  - type of matrix, structure, pre-treatment, particle size, or solid-liquid ratio

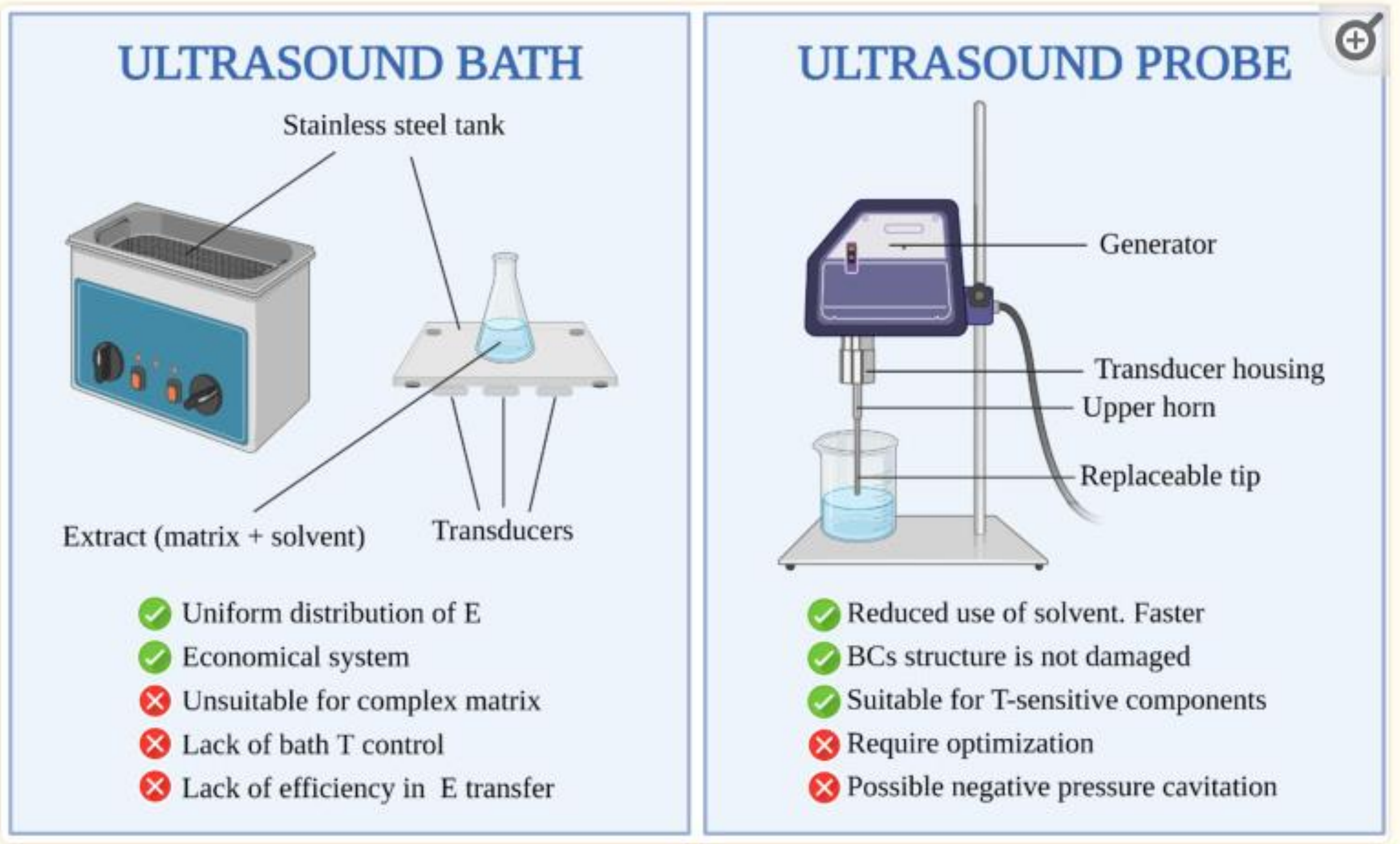
# Ultrasound assisted extraction

**Advantages**

- Similar results as extraction by pressing (squeezing)
- High speed
- Economic advantage
- Relatively low-cost technology involved

**Disadvantages**

- The system heats up due to the prolonged treatment
- The solid matrix is completely crushed → difficult to separate the mass from extract
- The use of ultrasound energy of more than 20 kHz may influence the active phytochemicals through the formation of free radicals.



# Microwave assisted extraction

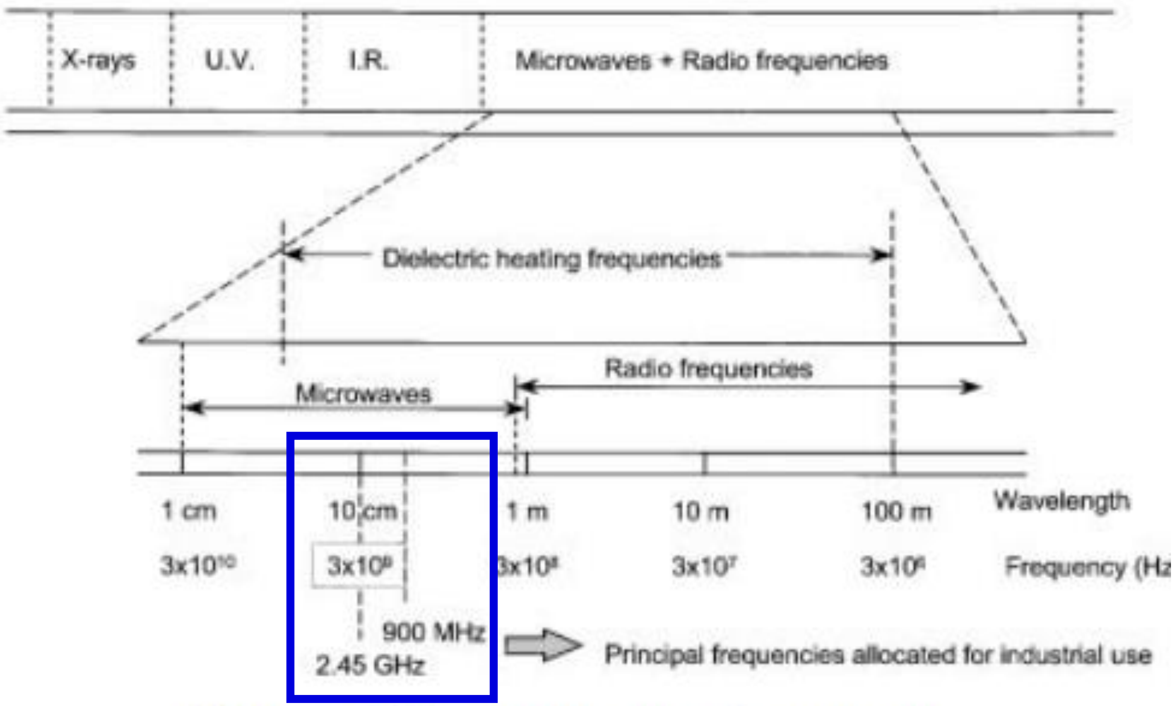



**Figure 1.** The electromagnetic spectrum.

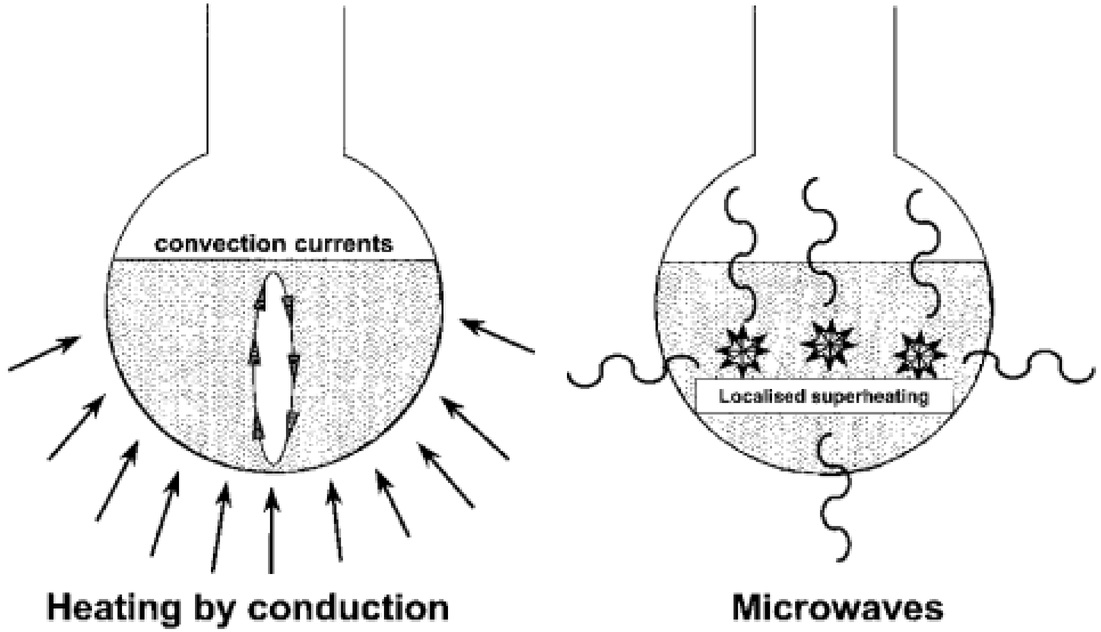



**Figure 2.** Scheme of the heating principle by conduction in the classical method of extraction and by microwave irradiation in microwave-assisted extraction.

# Microwave assisted extraction

- Microwaves at 2.45 GHz
- Electric field causes heating
  - Dipolar rotation
    - Molecules with dipol moment (permanent or induced), both in solvent and solid
    - Oscillation caused collisions and interactions with surrounding molecules → deliberation of thermal energy
    - Larger dielectric constant of solvent – greater heating
  - Ionic conduction
    - Ion currents formation – resistence induces heat

**Table 1. Dielectric constants and dipole moment values of some commonly used solvents**

| Solvent | Dielectric constant (20°C) | Dipole moment (25°C) (Debye) |
|---|---|---|
| Hexane | 1.89 | <0.1 |
| Toluene | 2.4 | 0.36 |
| Dichloromethane | 8.9 | 1.14 |
| Acetone | 20.7 | 2.69 |
| Ethanol | 24.3 | 1.69 |
| Methanol | 32.6 | 2.87 |
| Water | 78.5 | 1.87 |

- Extraction
  - Disruption of weak hydrogen bonding
  - Viscosity decreases effect
  - Migration of ions increases solvent penetration into matrix
  - In some cases, the matrix itself interacts with microwaves while the surrounding solvent possesses a low dielectric constant and thus remains cold
    - Advantageous in the case of thermosensitive compounds - the extraction of essential oils, microwaves interact selectively with the polar molecules present in glands, trichomes or vascular tissues. Localized heating leads to the expansion and rupture of cell walls and is followed by the liberation of essential oils into the solvent

# Microwave assisted extraction

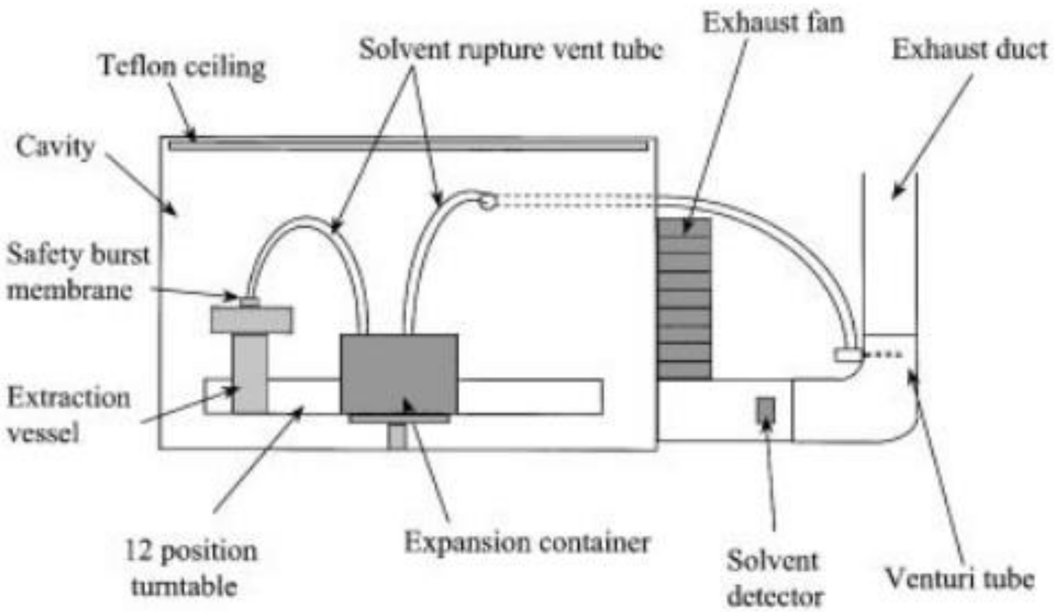



**Figure 3.** Schematic diagram of a closed-vessel microwave system for extraction.

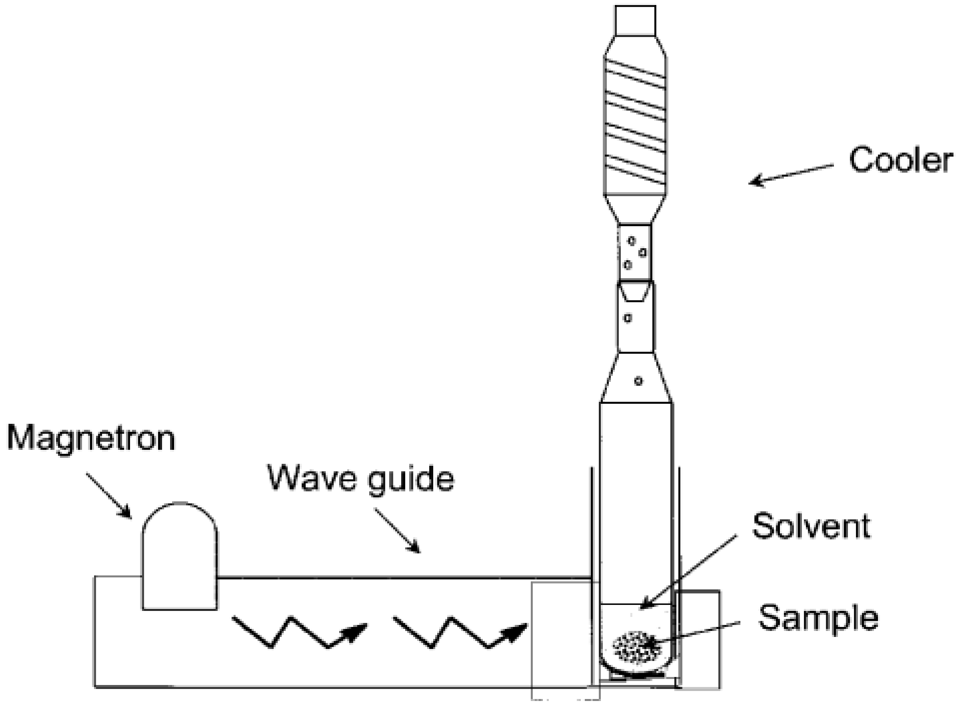



**Figure 4.** Schematic diagram of an open focused-microwave system for extraction.

– Closed and open vessels

# Microwave assisted extraction

– Combination of MAE with different methods
  – Maceration
  – Percolation
  – Distillation

**Table 2. Application of MAE to natural product extraction**

| Compounds | Matrix | System | Extraction conditions | Reference |
|---|---|---|---|---|
| Vicine, convicine (pyrimidine glycosides) | Faba beans (*Vicia faba*) | Domestic oven | Methanol:water (1:1); two successive irradiations (30 s) with an intermediate cooling step | Ganzler *et al.* (1986a, 1986b); Ganzler and Salgò (1987) |
| Gossypol | Cotton seeds | Domestic oven | Three cycles of irradiation (30 s) with cooling steps in between | Ganzler *et al.* (1986a); Ganzler and Salgò (1987) |
| Sparteine (alkaloid) | Lupine seeds | Domestic oven | Four cycles (30 s) with cooling steps in between | Ganzler *et al.* (1986b, 1990) |
| Terpenes (linalool, terpineol, citronellol, nerol and geraniol) | Must (*Vitis vinifera*) | Closed vessels | 10 mL dichloromethane; 475 W; 10 min; 90°C | Carro *et al.* (1997) |
| Essential oils | *Monarda fistulosa, Allium* sp. | Modified domestic oven | Hexane | Paré (1990) |
| Volatile oils | *Mentha piperita, Thuja occidentalis* | Modified domestic oven | Hexane, alkanes (transparent solvents) | Paré (1994) |
| Essential oils | Rosemary and peppermint leaves | Domestic oven | Hexane, carbon tetrachloride, toluene; 750 W; <60 s | Chen and Spiro (1994) |
| Essential oils | Plant leaves | Domestic oven | Hexane; <60 s | Collin *et al.* (1991) |
| Essential oils | Fresh leaves of *Lippia sidoides* | Domestic oven | "High cooking level"; 5 min | Craveiro *et al.* (1989) |
| Carotenoids | Paprika powder | Closed vessels | 50 W; 120 s; <60°C | Csiktunadi Kiss *et al.* (2000) |
| Taxanes (paclitaxel) | Needles of *Taxus* sp. | Closed vessels | 5 g fresh needles pre-soaked with 5 mL water prior to extraction with 10 mL of 95% ethanol; 100% power; 54 s; 85°C | Incorvia Mattina *et al.* (1997) |
| Ergosterol | Fungal contaminations | Domestic oven | 375 W; 35 s | Young (1995) |
| Withanolides | *Iochroma gesnerioides* leaves | Open cell, focused | 100 mg material pre-soaked with 0.6 mL water prior to extraction with 5 mL methanol; 25 W; 40 s | Kaufmann *et al.* (2001a) |
| Cocaine and benzoylecgonine | *Erythroxylum coca* leaves | Open cell, focused | Methanol; 125 W; 30 s | Brachet *et al.* (2002) |
| Alkaloids | *Senecio* sp. | Closed vessels | 65–100°C | Bicchi *et al.* (1992) |

# Rapid Solid-Liquid Dynamic Extraction (RSLDE)

- a negative gradient of pressure between the inner material and the outside of the solid matrix (high pressure inside and low pressure outside; Naviglio's principle).
- when the gradient of pressure is removed, the liquid flows out of the solid in a very fast manner and carries out all substances not chemically bonded to the main structure of the solid.


https://doi.org/10.3390/foods8070245

an "active" process → the gradient of pressure forces out the molecules

techniques based on diffusion and osmosis → "passive" processes, molecules are not forced out of the matrix

# Rapid Solid-Liquid Dynamic Extraction (RSLDE)

- An extractive cycle consists of both static and dynamic phases
- The static phase
  - → the liquid is maintained under pressure at about 10 bar on the solid to be extracted
  - left long enough to let the liquid penetrate inside the solid and to balance the pressure between the inside and the outside of the solid (about 1–3 min)
- The dynamic phase
  - the pressure immediately drops to atmospheric pressure
  - a rapid flowing of liquid from the inside to the outlet of the solid matrix
  - a suction effect of the liquid from the inside towards the outside of the solid.
  - Extractable material transported by solvent
- The cycles can be repeated until the solid runs out, about 30 extractive cycles (two-minute static phase; two-minute dynamic phase)

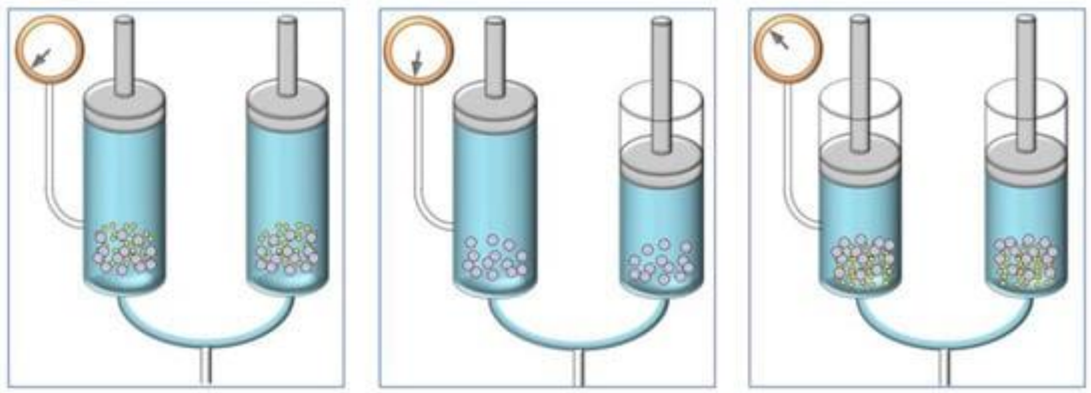
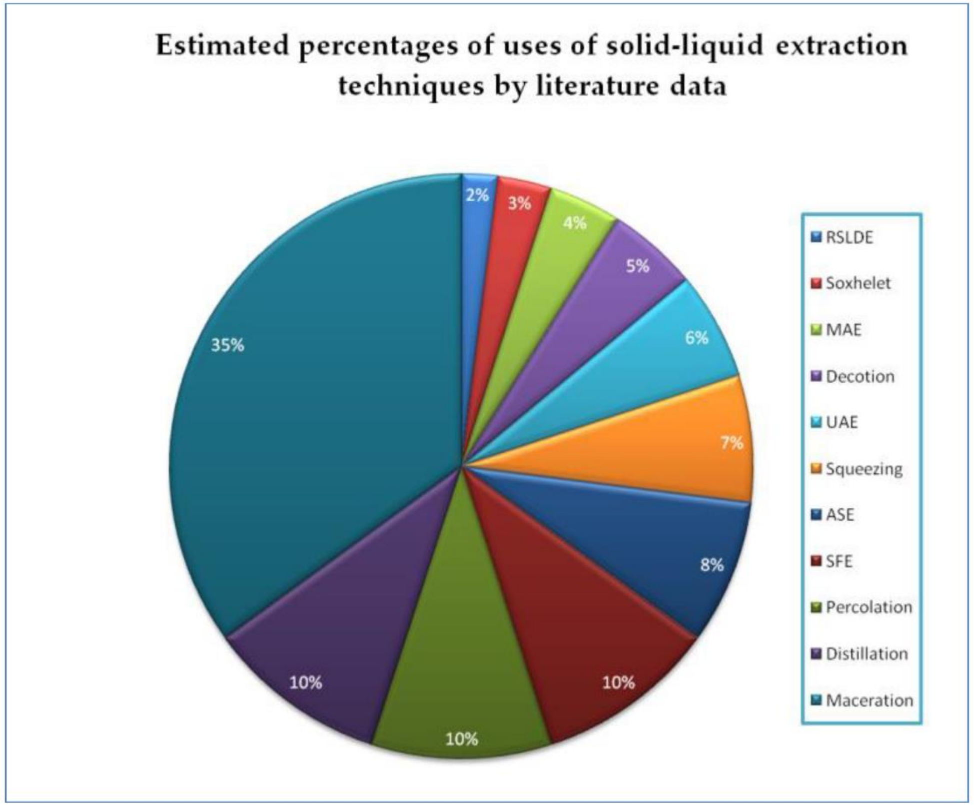


**Figure 1.** Schematic representation of the Naviglio extractor consisting of two extraction chambers connected via a conduit: the first two images show the dynamic phase, while the third image the static phase.

# Comparison of different extraction techniques

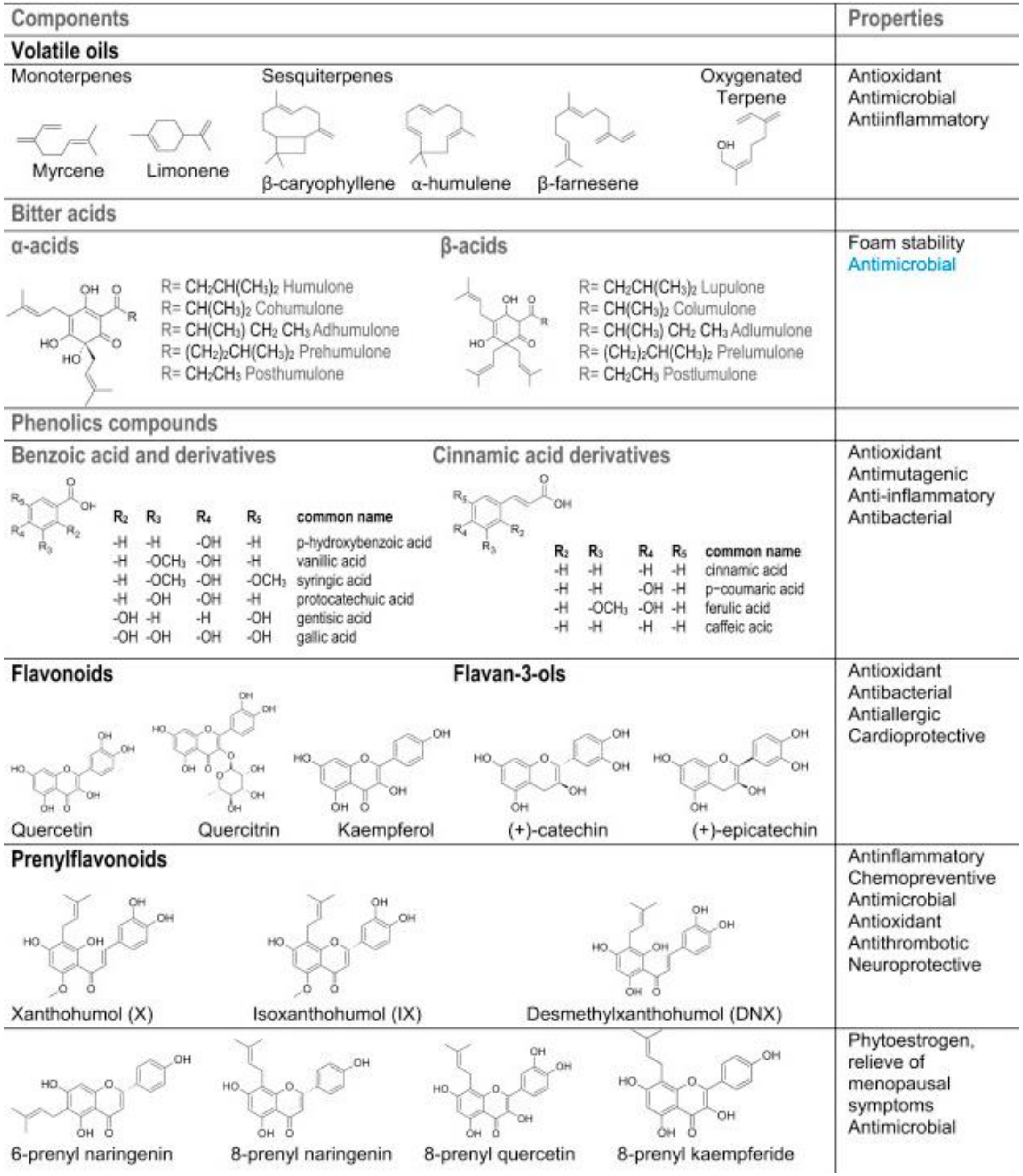



| Extraction Technique | Solvent | Granulometry | Time | Yield | Quality Extracted | Extract Stability |
|---|---|---|---|---|---|---|
| Squeezing | Indifferent | Not important | Minimum | Exhaustive | Poor | Poor |
| Maceration | Fundamental | Important | Long | Exhaustive | Great | Great |
| Decotion | Fundamental | Important | Long | Exhaustive | Great | Great |
| Percolation | Fundamental | Important | Middle | Partial | Good | Good |
| Soxhlet | Fundamental | Important | Long | Exhaustive | Poor | Poor |
| SCD | Indifferent | Not important | Middle | Partial | Poor | Poor |
| MAE | Fundamental | Not important | Middle | Partial | Poor | Poor |
| UAE | Fundamental | Not important | Middle | Partial | Great | Great |
| SFE | Indifferent | Not Important | Middle | Exhaustive | Poor | Poor |
| ASE | Fundamental | Not important | Minimum | Exhaustive | Poor | Poor |
| RSLDE | Indifferent | Not important | Minimum | Exhaustive | Great | Great |

# *LUPULI FLOS* – Hop flowers (ČL 2017)

## *Humulus lupulus* L. – Hop, Chmel otáčivý (Cannabaceae)

- a dioecious, right-handed winding climbing plant
- cultivated in US, Europe and northern Asia
- only female plants are grown, they reproduce vegetatively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# Hop processing

**Hop material**
***(raw, granulate, pelets)***

*Hot water extraction* → **Phenolics**

*Steam distillation* → **Essential oils**

*Methanol, ethanol, diethylether extraction* → **Total resins**

*Hexane extraction* →

**Soft resins** (hexan soluble)
***α-bitter acids***
***β-bitter acids***
***unspecified soft resins***
*(resupones)*

**Hard resins** (hexan unsoluble)
***γ-hard resins***
***δ-hard resins***

# Hop products and extracts

**Products**

- Mechanical products – compressed hop, granulates

**Extracts**

- Ethanol (MW assisted, Rapid Solid-Liquid Dynamic Extraction (RSLDE) – Naviglio´s principles)
  - Extract containg besides α-acids, β-acids and hop essential oils also phenolics and tannins
  - 90% ethanol, possible fractionation to get hop tannins (water soluble)
- $CO_2$
  - Supercritical or subcritical – effectivity/yield/conditions
  - pure hop resin extract containing α-acids, β-acids and hop essential oils.
- Steam distillation (MW assisted)
  - pure extract hop essential oils
- Vaccum distillation and chromatographic purification of $CO_2$ extracts



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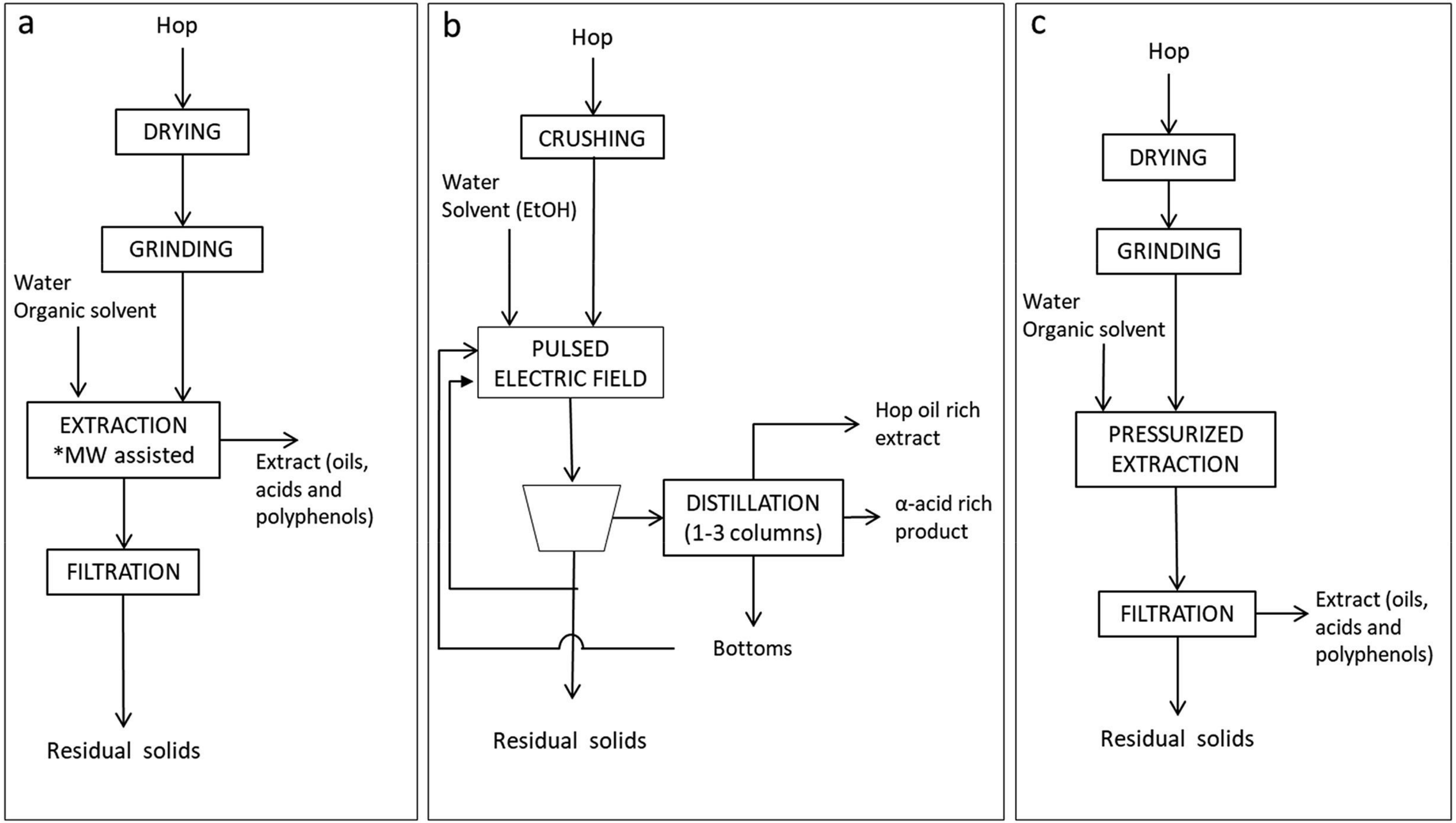


Extraction of hop components conventionally or assisted by intensified technologies, **a) solvent extraction** optionally assisted by microwave (Jeliazkova et al., 2018; Tyśkiewicz et al., 2018), **b) extraction assisted by pulsed electric field** (Held & Stanis, 2018), and **c) pressurized extraction**

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Table 2. Examples of the influence of the operational conditions with dense $CO_2$ on the extraction of hop components.

| Operational conditions | Composition of the extract | Reference |
|---|---|---|
| **Liquid $CO_2$** | | |
| 5–6.1 MPa, 16–25 °C | Oils, α-acids (>40%), β-acids | Laws et al., 1977 |
| **One stage supercritical $CO_2$** | | |
| 8–11 MPa, 40–60 °C, pure $CO_2$ | Essential oil | Van Opstaele et al., 2012a; Katono et al., 2018 |
| | Essential oil, α acids, β acids | Del Valle et al., 2003; Zanoli & Zavatti, 2008; Formato et al., 2013; Kupski et al., 2017 |
| **Two stages supercritical $CO_2$** | | |
| 1 8–9 MPa, 50 °C<br>2 11 MPa, 50 °C | Essential oil (floral)<br>Essential oils (spicy, sesquiterpenoids) | Goiris et al., 2002; Van Opstaele et al., 2012b |
| 1 15 MPa, 40 °C. Sep: 1.5 MPa, 20 °C<br>2 25–30 MPa, 40–48 °C. Sep. 1.5 MPa, 20 °C | Essential oil<br>Acids, α-acids (41%) | Zeković et al., 2007; Byelashov et al., 2018 |
| 1 28–30 MPa, 50 °C<br>2 85–90 MPa, 75–90 °C, pure $CO_2$ or EtOH-mod. (20 wt%). Sep.: 6–7 MPa, 40–60 °C | Essential oils and bitter acids<br>Xanthohumol | Forster et al., 2003; Jakowski et al., 2015 |
| 1 20 MPa, 40 °C<br>2 25 MPa, 50 °C, EtOH-mod. | Essential oils and bitter acids<br>Flavonoids | He et al., 2005 |
| 1 alcohol, acetone or mixtures, soaking in $CO_2$ (2.5–6 MPa)<br>2 sc-$CO_2$ (25–55 MPa, 40–85 °C). Sep.: 5–7 Mpa, 17–40 °C | Xanthohumol | Rój and Kozlowski, 2016 |

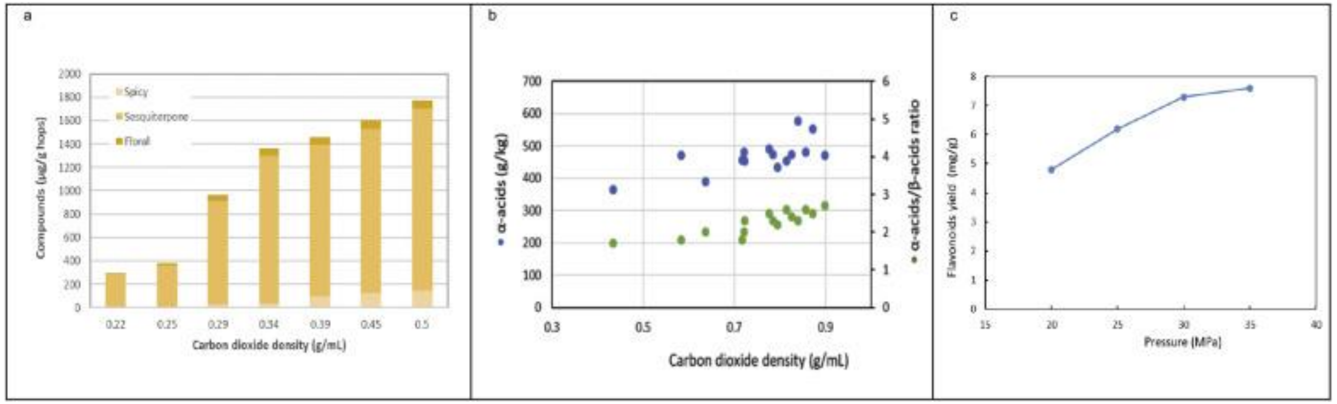


Fig. 2. Effect of solvent density during supercritical $CO_2$ extraction of a) essential oils (Van Opstaele et al., 2012a), b) bittering compounds (Del Valle et al., 2003) from hop pellets and c) effect of pressure during sc-$CO_2$ extraction using ethanol (70%) as cosolvent, on the phenolic yield from waste hops (He et al., 2005).