Supercritical Fluid Extraction and Separation of Biologically Active Components

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Historical notes

• **1822** – *Baron Cagniard de la Tour*
  Critical point of a substance

• **1879 – 1880** – *Hannay and Hogarth*
  KI, KBr, CoCl$_2$ in alcohol ($T_c = 243 \, ^\circ C$, $P_c = 63$ bar)

• **1880 - 1920** - *van der Waals, Amagat, Joule, Thomson, Clausius*
  Pioneer work in research of thermodynamics of one and two component supercritical system
Historical notes

• 1896 – Villard
  Solubilities of camphor, stearic acid, paraffin wax in methane, ethylene, CO₂

• 1943 – Messmore, 1955 - Zhuze
  Deasphalting of petroleum oils with propane/propylene
  \( T = 100 \, ^{°}C, \, P = 100 \, – \, 150 \, \text{bar} \)

• 1964 – Zosel
  The patent includes 68 examples of extractions and separations

• 1970 – 1980 commercial applications
Supercritical Fluid Extraction (SFE) Units ($V > 0.1 m^3$)
SCOPE of presentation

- Physico-chemical properties
- Supercritical Fluid Extraction (SFE)
- SFE of plant constituents
SFE of plant constituents

essential oils
triterpenes and steroids
carotenoids
alkaloids
plant lipids
SCOPE of presentation

- Physico-chemical properties
- Supercritical Fluid Extraction (SFE)
- SFE of plant constituents
- Down stream processing
- Modelling
- Economic issues
- Conclusion
What is a supercritical fluid?

![Supercritical fluid phase diagram]

- $T_c = 31.1^\circ C$
- $p_c = 73.8$ bar
Supercritical fluid

Below the critical parameters, two distinct phases exist
Density of carbon dioxide vs. pressure
Density of carbon dioxide vs. temperature
Density of water vs. temperature
Range over which near-critical extraction have been carried out
Viscosity of carbon dioxide at the near critical region
Viscosity of water
Specific heat capacity of carbon dioxide

- Bruno and Ely, 1991
Thermal conductivity of CO$_2$

Bruno and Ely, 1991
Transport properties of supercritical solvents

![Graph showing properties vs pressure](image)

<table>
<thead>
<tr>
<th>Property</th>
<th>Gas</th>
<th>Fluid</th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho$ (kg/m$^3$)</td>
<td>1</td>
<td>200 – 700</td>
<td>1000</td>
</tr>
<tr>
<td>$\eta$ (Pas)</td>
<td>$10^{-5}$</td>
<td>$10^{-4}$</td>
<td>$10^{-3}$</td>
</tr>
<tr>
<td>$D$ (cm$^2$/s)</td>
<td>$10^{-1}$</td>
<td>$10^{-3} - 10^{-4}$</td>
<td>$10^{-5}$</td>
</tr>
</tbody>
</table>

Schneider et al., 2000
Fluid phase behaviour
Phase diagram of ethanol – CO$_2$

The six basic types of fluid phase behaviour

Van Koynenburg and Scott, 1980
The six basic types of fluid phase behaviour

Van Koynenburg and Scott, 1980
The six basic types of fluid phase behaviour

Van Koynenburg and Scott, 1980
High-pressure phase equilibria

\[ \bar{f}^l_i(T, P, x_i) = \bar{f}^v_i(T, P, y_i) \]

\[ \bar{\phi}^v_i = \frac{\bar{f}^v_i}{P y_i} \quad \bar{\phi}^l_i = \frac{\bar{f}^l_i}{P x_i} \quad \bar{\phi}^v_i y_i = \bar{\phi}^l_i x_i \]

\[ \ln \frac{\bar{f}^v_i(T, P, y_i)}{y_i P} = \ln \bar{\phi}^v_i = \frac{1}{RT} \int_{\infty}^{V} \left[ \frac{RT}{V} - \left( \frac{\partial P}{\partial n_i} \right)_{T,V,n_{j\neq i}} \right] dV - \ln \frac{PV}{RT} \]

\[ P = P(T,V,n_i) \]

The Peng-Robinson equation of state

\[ P = \frac{RT}{V - b} - \frac{a(T)}{V^2 + 2Vb - b^2} \]

Kikic, de Loos, 2001
Solubility of solids in supercritical fluids

\[ \bar{f}_2^{SF} = \bar{f}_2^S = f_2^S = P_{2}^{sub} \phi_2^V \exp \left( \int_{P_{2}^{sub}}^{P} \frac{v_2^{S} dP}{RT} \right) \]

\[ \bar{f}_2^{SF} = y_2 \phi_2^V P \]

\[ y_2 = P_{2}^{sub} \left[ \frac{\phi_2^V \exp \left( \int_{P_{2}^{sub}}^{P} \frac{v_2^{S} dP}{RT} \right)}{\bar{f}_2^{SF} \phi_2^V} \right] \]

Kikic, de Loos, 2001
Solubility of naphthalene in CO$_2$
Solvating power of supercritical fluids
### Critical properties of used solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$T_c$ (°C)</th>
<th>$P_c$ (bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylene (C2H4)</td>
<td>9</td>
<td>50,3</td>
</tr>
<tr>
<td>Ethane (C2H6)</td>
<td>32</td>
<td>48,8</td>
</tr>
<tr>
<td>Propylene (C3H6)</td>
<td>92</td>
<td>46,2</td>
</tr>
<tr>
<td>Propane (C3H8)</td>
<td>97</td>
<td>42,4</td>
</tr>
<tr>
<td>n-pentane (C5H12)</td>
<td>197</td>
<td>33,7</td>
</tr>
<tr>
<td>Benzene (C6H6)</td>
<td>289</td>
<td>48,9</td>
</tr>
<tr>
<td>Toluene (C7H8)</td>
<td>319</td>
<td>41,1</td>
</tr>
</tbody>
</table>
Supercritical fluids in food and pharma industries

- Carbon dioxide ($P_C=73.8$ bar, $T_C=31.1\,^\circ C$)
Carbon dioxide

- GRAS (generally regarded as safe)
- gentle conditions ($P_c=73.8$ bar, $T_c=31.1^\circ C$)
- inert, odourless, tasteless
- easily removed from products
- non-explosive
- readily available, inexpensive
- benign solvent
Supercritical fluids

• Carbon dioxide ($P_C=73.8$ bar, $T_C=31.1$°C)
• Nitrous oxide ($P_C=71.0$ bar, $T_C=36.5$°C)
• Propane ($P_C=42.5$ bar, $T_C=96.7$°C)
Propane

- near-critical liquid \( (T_c=96.7^\circ C) \)
- flammable, explosive mixtures with air
- maximum permitted residue: 50 ppm
- significant solvent loss
- waste solvent
Supercritical fluids

- Carbon dioxide ($P_C=73.8$ bar, $T_C=31.1\, ^\circ C$)
- Propane ($P_C=42.5$ bar, $T_C=96.7\, ^\circ C$)
- Water ($P_C=220.5$ bar, $T_C=374.2\, ^\circ C$)
Supercritical fluids

- Carbon dioxide ($P_C=73.8$ bar, $T_C=31.1^\circ C$)
- Propane ($P_C=42.5$ bar, $T_C=96.7^\circ C$)
- Water ($P_C=220.5$ bar, $T_C=374.2^\circ C$)
- Entrainers (ethanol, ethyl acetate, acetone)
# Modifiers (co-solvent, entrainer)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>( T_c , (^\circ C) )</th>
<th>( P_c , (\text{bar}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-pentane</td>
<td>196,6</td>
<td>33,7</td>
</tr>
<tr>
<td>n-hexane</td>
<td>234,5</td>
<td>30,3</td>
</tr>
<tr>
<td>methanol</td>
<td>239,5</td>
<td>80,8</td>
</tr>
<tr>
<td>ethanol</td>
<td>241</td>
<td>61,4</td>
</tr>
<tr>
<td>n-buthanol</td>
<td>288,9</td>
<td>45</td>
</tr>
<tr>
<td>acetone</td>
<td>235</td>
<td>47</td>
</tr>
<tr>
<td>Dimethyl ether</td>
<td>126,9</td>
<td>54</td>
</tr>
</tbody>
</table>
Phase diagram of ethanol – CO$_2$

### Change of critical parameters by addition of entrainer

<table>
<thead>
<tr>
<th>Concentration</th>
<th>acetone</th>
<th>methanol</th>
<th>ethanol</th>
<th>n-buthanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>mol%</td>
<td>T&lt;sub&gt;c&lt;/sub&gt;(°C)</td>
<td>P&lt;sub&gt;c&lt;/sub&gt;(bar)</td>
<td>T&lt;sub&gt;c&lt;/sub&gt;(°C)</td>
<td>P&lt;sub&gt;c&lt;/sub&gt;(bar)</td>
</tr>
<tr>
<td>1</td>
<td>34,7</td>
<td>77,9</td>
<td>32,7</td>
<td>76,5</td>
</tr>
<tr>
<td>2</td>
<td>36,8</td>
<td>79,7</td>
<td>34,7</td>
<td>78,2</td>
</tr>
<tr>
<td>4</td>
<td>43,7</td>
<td>85,7</td>
<td>37,7</td>
<td>81,7</td>
</tr>
</tbody>
</table>

- Pure CO<sub>2</sub>: T<sub>c</sub>=31,3°C, P<sub>c</sub>=73,8 bar
Developing a Commercial-scale SFE Process

• Analytical/laboratory unit: 1 mL to 1 L
  screening unit
  samples for analytical evaluation

• Pilot plant unit: 2 to 100 L
  processing variables
  samples for customers

• Semi-commercial plant: 100 to 200 L
  production for market tests

• Commercial plant: 200 to 6500 L
Supercritical fluid extraction unit

Component Diagram:
- Extractor
- Heat exchanger
- Valve
- I. separator
- II. separator
- Cooler/condenser
- Pump
- Cooler
- CO₂ storage
Pump process in the T – s diagram

1 liquid
2 undercooler
3 extraction pressure
4 extraction temperature
5 two phase region
5-6 gaseous part
5-7 liquid part
8 gas
Multipurpose extraction unit
(NATEX, Austria)
Preparation of raw materials

- Crushing/grinding
Effect of particle size: SFE of paprika

- Run 32

- Run 33

- Run 34

- Run 35

$d_0 = 0.45 \text{ mm}$

$d_0 = 0.96 \text{ mm}$
Preparation of raw materials

- Crushing/grinding
- Rolling/pelletizing
- Wetting/drying
Effect of moisture content: SFE of olive fruit

Moisture content: 5.12%

Moisture content: 47.67%
Preparation of raw material

- Crushing/grinding
- Rolling/pelletizing
- Wetting/drying
- Chemical/biochemical pretreatment
Extraction conditions

• Pressure
• Temperature
• Solvent composition (co-solvents)
• Extraction time
• Solvent flow rate
• Upflow/downflow
• Packed bed structure (H/D, axial mixing)
• Mixing
Separation conditions

- Pressure
- Temperature
- Multiple separators (fractionation)
- Absorbers
- Adsorbers
- Membranes
Formation of secondary plant products
Process of primary metabolism

\[ \text{CO}_2 \quad \text{H}_2\text{O} \]

Pentose cycle

Saccharides

Glycolysis

Phosphoenol-pyruvic acid

Pyruvate

Acetyl CoA

Krebs cycle

\[ \text{CO}_2 \]
**Products of primary metabolism**

**Process of primary metabolism**

- CO$_2$  
- H$_2$O  

**Pentose cycle**

- Saccharides
- Glycolysis
- Phosphoenol-pyruvic acid
- Pyruvate
- Acetyl CoA

**Krebs cycle**

- CO$_2$

**Oligo and polisaccharides**

- Eritrose-4-PO$_4$
- Shikimic acid
- Aromatic amino acids

**Proteines**

- Aliphatic amino acids

**Malonyl CoA**

- Fatty acids

**IPP biological isopren**

- Squalene
Process of primary metabolism

CO$_2$ $\rightarrow$ H$_2$O

Pentose cycle

↓

Saccharides

↓

Glycolysis

↓

Phosphoenol-pyruvic acid

↓

Pyruvate

↓

Acetyl CoA

↓

Krebs cycle

↓

CO$_2$

Products of primary metabolism

Oligo and polisaccharides

↓

Eritrose-4-PO$_4$

↓

Shikimic acid

↓

Aromatic amino acids

↓

Aliphatic amino acids

↓

Malonyl CoA

Fatty acids

↓

IPP biological isopren

↓

Squalene

Terpenes

Secondary plant metabolism

Glycosides

Mucilages and gums

Phenolic compounds

Lignans

Alkaloids

Alkaloids

Peptides

Fats and waxes

Tetracyclynes

Antraquinones

Terpenes
Terpenoids

Isoprene (C\textsubscript{5}) units

Isoprene does not participate in the biogenesis
Biosynthesis of Limonene and Carvone

Terpenoids

- $C_{10}$ monoterpenes (essential oil)
- $C_{15}$ sesquiterpenes (essential oil)
- $C_{20}$ diterpenes (antioxidants)
- $C_{30}$ triterpenes (phytosterols)
- $C_{40}$ tetraterpenes (carotenes)
- $(C_5)_n$ polyterpenes (caoutchouc)
Solubility

- typical essential oil components

Comparative chemical composition of distilled oils and SFE products

<table>
<thead>
<tr>
<th>Plant</th>
<th>Compound</th>
<th>Steam distillation</th>
<th>SFE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dill</strong> <em>(Anethum graveolens L.)</em></td>
<td>limonene</td>
<td>55.7</td>
<td>42.6</td>
</tr>
<tr>
<td></td>
<td>d-carvone</td>
<td>36.5</td>
<td>48.6</td>
</tr>
<tr>
<td><strong>Coriander</strong> <em>(Coriandrum sativum L.)</em></td>
<td>pinene</td>
<td>15.3</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>linalool</td>
<td>68.5</td>
<td>75.5</td>
</tr>
<tr>
<td><strong>Celery</strong> <em>(Apium graveolens L.)</em></td>
<td>limonene</td>
<td>50.5</td>
<td>33.4</td>
</tr>
<tr>
<td></td>
<td>3-butylphthalide</td>
<td>23.6</td>
<td>40.6</td>
</tr>
<tr>
<td><strong>Parsley</strong> <em>(Petroselinum crispum L.)</em></td>
<td>α-pinene</td>
<td>24.0</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>β-pinene</td>
<td>21.9</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>myristicin</td>
<td>7.4</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>apiole</td>
<td>38.5</td>
<td>84.9</td>
</tr>
</tbody>
</table>
Alteration of essential oil components during distillation

linalyl acetate $\rightarrow$ linalool
lavandin (*Lavandula intermedia* Emeric)
cRARY sage (*Salvia sclarea* L.)
glycosides $\rightarrow$ thymol
thyme (*Thymus vulgaris* L.)
unknown precursor $\rightarrow$ $\alpha$-terpineol
terpinene-4-ol
lavandin (*Lavandula intermedia* Emeric)
pulegone $\rightarrow$ dihydrocarvone
spearmint (*Mentha spicata* L.)
Fractionation of fennel oil
(*Foeniculum vulgare* Mill.)
Fractionation of fennel oil ($P_E=302$ bar, $T_E=38^\circ$C)

1\textsuperscript{st} separator: 75 bar, 23.5 °C

1\textsuperscript{st} separator: 86 bar, 23.5 °C
Effects of the separation parameters on fractionation of fennel oil
## Composition of orange oil

<table>
<thead>
<tr>
<th>Component</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Pinene</td>
<td>0.45</td>
</tr>
<tr>
<td>Myrcene</td>
<td>1.77</td>
</tr>
<tr>
<td>d-Limonene</td>
<td>90.60</td>
</tr>
<tr>
<td>Octanal</td>
<td>0.59</td>
</tr>
<tr>
<td>Decanal</td>
<td>0.52</td>
</tr>
<tr>
<td>Linalool</td>
<td>0.37</td>
</tr>
<tr>
<td>Geranial</td>
<td>0.12</td>
</tr>
</tbody>
</table>
Phase equilibria of orange peel oil
Separation factors of orange peel oil fractions


Distribution coefficient:

\[ K = \frac{y}{x} \]

Separation factor:

\[ \alpha = \frac{K_{Terpenes}}{K_{Aroma \text{fr.}}} \]
Fractionation of citrus oil
## Results of fractionation

<table>
<thead>
<tr>
<th>Pilot plant data on deterpenation of orange oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aromatic content in feed oil</td>
</tr>
<tr>
<td>Aromatic content in concentrated oil</td>
</tr>
<tr>
<td>Recovery of aromatic fraction</td>
</tr>
<tr>
<td>Solvent-to-oil ratio</td>
</tr>
</tbody>
</table>
SFE of sesquiterpene lactones

Chamomile (*Matricaria chamomilla* L.)

- apigenin
- apigenin-7-glucoside
- (-)-α-bisabolol
- (-)-α-bisabololoxide A
- (-)-α-bisabololoxide B
- chamazulene
- cis-en-in-dicycloether
- trans-en-in-dicycloether
- matricine
Fractionated extraction of chamomile

<table>
<thead>
<tr>
<th>P</th>
<th>T</th>
<th>Matricine</th>
<th>α-bisabolol</th>
<th>Spiroether</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>(bar)</td>
<td>(°C)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>80</td>
<td>40</td>
<td>3.5</td>
<td>27</td>
<td>14</td>
<td>0.12</td>
</tr>
<tr>
<td>90</td>
<td>40</td>
<td>17.0</td>
<td>21.0</td>
<td>16.7</td>
<td>0.4</td>
</tr>
<tr>
<td>300</td>
<td>40</td>
<td>7.5</td>
<td>9.2</td>
<td>11.7</td>
<td>1.65</td>
</tr>
</tbody>
</table>

SFE of feverfew (*Chrysanthemum parthenium* Bernh.): effects of pressure and temperature on the yield
Recovery of parthenolide
Diterpenes investigated by SFE

- **baccatin-III**
- **carnosol**
- **rosmanol**
- **sclareol**
- **taxol**
# Isolation of diterpenes from plants

<table>
<thead>
<tr>
<th>Plant</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clary sage</td>
<td>sclareol</td>
</tr>
<tr>
<td>\textit{(Salvia sclarea L.)}</td>
<td></td>
</tr>
<tr>
<td>Yews</td>
<td>paclitaxel</td>
</tr>
<tr>
<td>\textit{(Taxus brevifolia Nutt., Taxus cuspidata Capita)}</td>
<td>baccatin III</td>
</tr>
<tr>
<td>Rosemary</td>
<td>rosmanol</td>
</tr>
<tr>
<td>\textit{(Rosmarinus officinalis L.)}, Sage</td>
<td>7-methyl-epirosmanol</td>
</tr>
<tr>
<td>\textit{(Salvia officinalis L.)}</td>
<td>carnosol</td>
</tr>
<tr>
<td></td>
<td>carnosolic acid</td>
</tr>
</tbody>
</table>
Basic skeleton of triterpenes

\[
\begin{align*}
\text{α-amirin} & \quad \text{β-amirin} \\
\text{faradiol} & \quad \text{β-sitosterol}
\end{align*}
\]
# Isolation of triterpenes

<table>
<thead>
<tr>
<th>Plant</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mulberry</strong></td>
<td><strong>α-amyrin acetate</strong></td>
</tr>
<tr>
<td>(<em>Morus alba</em> L.)</td>
<td></td>
</tr>
<tr>
<td><strong>Neem</strong></td>
<td><strong>nimbin</strong></td>
</tr>
<tr>
<td>(<em>Azadirachta indica</em> A. Juss)</td>
<td><strong>salannin</strong></td>
</tr>
<tr>
<td></td>
<td><strong>azadirachtin</strong></td>
</tr>
<tr>
<td><strong>Marigold</strong></td>
<td><strong>faradiol and its esters</strong></td>
</tr>
<tr>
<td>(<em>Calendula officinalis</em> L.)</td>
<td></td>
</tr>
<tr>
<td><strong>Chaste tree</strong></td>
<td><strong>β-amyrin</strong></td>
</tr>
<tr>
<td>(<em>Vitex agnus castus</em> L.)</td>
<td><strong>β-sitosterol</strong></td>
</tr>
<tr>
<td><strong>Dandelion</strong></td>
<td><strong>β-amyrin</strong></td>
</tr>
<tr>
<td>(<em>Taraxacum officinale</em> Web.)</td>
<td><strong>β-sitosterol</strong></td>
</tr>
</tbody>
</table>
SFE of dandelion leaves: effects of pressure and temperature on the yields
Recovery of sterols from dandelion leaves
Recovery of carotenoids

- bixin (annetto)
- capsanthin (paprika)
- β-carotene (carrot)
- lutein (alfalfa)
- lycopene (tomato)
SFE of pigments from paprika
Extraction of hop
*(Humulus lupulus L.)*

- **Water**
  - Water extract
    - Petrol soluble
      - ‘Soft resin’
        - α acid
    - Petrol insoluble
      - ‘Hard resin’
      - β acid
      - Uncharacterised acid

- **Organic solvent**
  - Total resin

- **Steam distillation**
  - Essential oil
Structure of major components

α acid

iso α acid

β acid
Composition of hop extract versus time

Chaste tree: *Vitex agnus castus* L.

p: 100-275-450 bar

T: 40-50-60 °C
Vitex agnus castus L.

Diterpene

Rotundifurane
Vitex agnus castus L.

**β-sitosterol**

**β-amyrin**

Triterpenes
Vitex agnus castus L.

Flavonoid

Casticin
Vitex agnus castus L.

Minor compounds’ concentration of the extracts [g/kg]:

<table>
<thead>
<tr>
<th></th>
<th>Rotundifurane</th>
<th>β-sitosterol</th>
<th>β-amyrin</th>
<th>Casticin</th>
</tr>
</thead>
<tbody>
<tr>
<td>sc-CO₂ (450 bar, 60°C)</td>
<td>16.2</td>
<td>16.5</td>
<td>9.1</td>
<td>39.3</td>
</tr>
<tr>
<td>ethanol (96 % v/v)</td>
<td>4.7</td>
<td>4.9</td>
<td>3.4</td>
<td>12.4</td>
</tr>
</tbody>
</table>

sc-CO₂/EtOH ~3.5x ~3.5x >2.5x >3x
Alkaloids: decaffeination of coffee

- Solubility isobars of caffeine

Decaffeination of green coffee beans
Nearly continuous SFE of coffee
Extraction of nicotine from tobacco

Tobacco $\rightarrow$ Aroma removal $\rightarrow$ Nicotine removal $\rightarrow$ Adsorbent regeneration $\rightarrow$ Nicotine by-product

$\rightarrow$ CO$_2$

$\rightarrow$ H$_2$O

$\rightarrow$ Drying

Aroma $\rightarrow$ Aroma transfer $\rightarrow$ Aroma distribution $\rightarrow$ Conditioning $\rightarrow$ CO$_2$

$\rightarrow$ Nicotine reduced tobacco
Extraction of alkaloids used in medicine

<table>
<thead>
<tr>
<th>Plant</th>
<th>Alkaloid</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>paprika (Capsicum annuum L.)</td>
<td>capsaicin</td>
<td>good</td>
</tr>
<tr>
<td>pepper (Piper nigrum L.)</td>
<td>piperine</td>
<td>good</td>
</tr>
<tr>
<td>poppy (Papaver somniferum L.)</td>
<td>opium (codein, thebaine, papaverin)</td>
<td>reasonable</td>
</tr>
<tr>
<td>strychnos (Strychnos nux-vomica L.)</td>
<td>strychnine</td>
<td>reasonable</td>
</tr>
<tr>
<td>hemlock fruit (Conium maculatum L.)</td>
<td>coniine</td>
<td>reasonable</td>
</tr>
<tr>
<td>ipecacuanhae radix (Cephyaelis ipecacuanha Will.)</td>
<td>emetin, caphaelin</td>
<td>poor</td>
</tr>
</tbody>
</table>
Fatty oils and waxes

• Solubility isotherms of soybean oil

Effect of alcohol-entrainer on extraction rate

g oil / 100 g dry corn germ

kg CO₂ / kg dry corn germ

Symbols:
- ◇ 0 % alcohol
- △ 2.5 % alcohol
- □ 5 % alcohol
- ● 7.5 % alcohol
- ◊ 10 % alcohol
## List of raw materials for γ-linolenic acid

<table>
<thead>
<tr>
<th>Raw material</th>
<th>Yield CO₂ extraction [%]</th>
<th>C₁₈:₃ in the extract (γ-linolenic acid) [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Oenothera biennis</em> L.</td>
<td>22</td>
<td>8 - 12</td>
</tr>
<tr>
<td><em>Borago officinalis</em> L.</td>
<td>18</td>
<td>18 - 22</td>
</tr>
<tr>
<td><em>Humulus lupulus</em> L.</td>
<td>6</td>
<td>3 - 4</td>
</tr>
<tr>
<td><em>Cannabis sativa</em> L.</td>
<td>35</td>
<td>3 - 6</td>
</tr>
<tr>
<td><em>Ribes rubrum</em> L.</td>
<td>14</td>
<td>4 - 6</td>
</tr>
<tr>
<td><em>Ribes nigrum</em> L.</td>
<td>18</td>
<td>16 - 19</td>
</tr>
<tr>
<td><em>Ribes grossularia</em> L.</td>
<td>15</td>
<td>10 – 12</td>
</tr>
<tr>
<td><em>Rosa canina</em> L.</td>
<td>12</td>
<td>24 - 31</td>
</tr>
<tr>
<td><em>Hippophae rhamnoides</em> L.</td>
<td>12</td>
<td>26 - 30</td>
</tr>
</tbody>
</table>
Partition coefficient and selectivity of fish oil ethyl esters

<table>
<thead>
<tr>
<th>Component</th>
<th>$K=y/x$</th>
<th>$K_{A}/K_{22}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{16}$</td>
<td>0.090</td>
<td>7.5</td>
</tr>
<tr>
<td>$C_{18}$</td>
<td>0.050</td>
<td>4.2</td>
</tr>
<tr>
<td>$C_{20}$</td>
<td>0.027</td>
<td>2.3</td>
</tr>
<tr>
<td>$C_{22}$</td>
<td>0.012</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Two-column process for separation of EPA and DHA

Extraction from lyophilised fermentation broths

<table>
<thead>
<tr>
<th>Compound</th>
<th>Micro-organism</th>
<th>Methanol in CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>¹mevinolin</td>
<td>Aspergillus terreus</td>
<td>3 %</td>
</tr>
<tr>
<td>²chaetoglobosin</td>
<td>Penicillium expansum</td>
<td>20 %</td>
</tr>
<tr>
<td>²mycolutein</td>
<td>unidentified</td>
<td>20 %</td>
</tr>
<tr>
<td>²luteoreticulin</td>
<td>unidentified</td>
<td>20 %</td>
</tr>
<tr>
<td>²C₁₆H₁₂O₇</td>
<td>Aspergillus fumigatus</td>
<td>0 %</td>
</tr>
<tr>
<td>²sydowinin B</td>
<td>Aspergillus fumigatus</td>
<td>20 %</td>
</tr>
<tr>
<td>²elaiophylin</td>
<td>Streptomyces sp.</td>
<td>20 %</td>
</tr>
</tbody>
</table>

¹Larson and King, 1986
²Cocks et al., 1995
## Partition coefficient of different compounds

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$K = y/x$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-alcohols $C_1$ – $C_7$</td>
<td>0.4.....31</td>
</tr>
<tr>
<td>Organic acids (wt basis)</td>
<td></td>
</tr>
<tr>
<td>acetic acid</td>
<td>0.03</td>
</tr>
<tr>
<td>butyric acid</td>
<td>0.08</td>
</tr>
<tr>
<td>Esters</td>
<td></td>
</tr>
<tr>
<td>ethyl formiate</td>
<td>16</td>
</tr>
<tr>
<td>isoamyl acetate</td>
<td>850</td>
</tr>
</tbody>
</table>
Continuous extraction broths

nutrients

fermenter

pressure reduction valve

cells

cell separation unit

CO₂

extractor

product
### Example: Extraction of pleuromutilin

<table>
<thead>
<tr>
<th>Aqueous flow rate ml/min</th>
<th>CO₂ flow g/min</th>
<th>Ratio of CO₂ to aqueous w/v</th>
<th>Average extractor residence time min</th>
<th>Average extraction %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>10/1</td>
<td>130</td>
<td>78.4</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>5/1</td>
<td>65</td>
<td>65.0</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>20/1</td>
<td>130</td>
<td>88.8</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>10/1</td>
<td>65</td>
<td>76.9</td>
</tr>
</tbody>
</table>

Walford et al., 2000
Solubilization of proteins in supercritical fluids

Head (hydrophilic)

Tail (CO₂-philic)

Fluorocarbons

Hydrocarbon polymers
Modelling of SFE: Brunner’s equation

\[
\frac{dx}{dt} = -kx
\]

\[
Y_E = Y_E\infty [1 - \exp(-kt)]
\]

\[
Y_E = Y_{E1\infty} [1 - \exp(-k_1 t)] + Y_{E2\infty} [1 - \exp(-k_2 t)]
\]

• \(x\) concentration of soluble component(s) in solid phase (kg/kg)
• \(t\) time (s)
• \(K\) kinetic parameter (1/s)
• \(Y_E\) extraktion yield (kg/kg)
• \(Y_{E\infty}\) maximal extraction yield (kg/kg)
Fractionation of Greek sage oil

<table>
<thead>
<tr>
<th>Parameters</th>
<th>( Y_{E_\infty} ), %</th>
<th>( k ), 1/min</th>
<th>( R )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P_E = 300 ) bar, ( T_E = 45 ) °C</td>
<td>4,90</td>
<td>0,012</td>
<td>0,999</td>
</tr>
<tr>
<td>( P_{SZ-1} = 90 ) bar, ( T_{SZ-1} = 30-40 ) °C</td>
<td>( Y_{E_{1\infty}} = 2,30 )</td>
<td>( k_1 = 0,010 )</td>
<td>0,999</td>
</tr>
<tr>
<td>( P_{SZ-2} = 20 ) bar, ( T_{SZ-1} = 20 ) °C</td>
<td>( Y_{E_{2\infty}} = 2,59 )</td>
<td>( k_2 = 0,013 )</td>
<td>0,994</td>
</tr>
</tbody>
</table>
Component balances for solid and liquid phases

\[- \rho_s (1 - \varepsilon) \frac{dx}{dt} dV = J(x, y) dV \quad \rho_f \varepsilon \frac{dy}{dt} dV + \rho_f u \frac{dy}{dh} dV = J(x, y) dV\]

- \(x\) concentration of soluble component(s) in solid phase (kg/kg)
- \(y\) concentration of soluble component(s) in supercritical fluid (kg/kg)
- \(J\) specific extraction rate (flux) (kg/(m³s))
- \(h\) length coordinate (at CO₂ inlet \(h = 0\)) (m)
- \(t\) time (s)
- \(u\) fluid velocity (m/s)
- \(\varepsilon\) voidage of packing (m³/m²)
- \(\rho_f\) density of supercritical fluid (kg/m³)
- \(\rho_s\) density of solid matrix (kg/m³)
The Sovová’s model describes the yield in function of the time using 3 equations:

\[
\frac{Y}{x_0} = \vartheta [1 - \exp(-Q)] \quad \vartheta < \frac{q}{Q}
\]

\[
\frac{Y}{x_0} = \vartheta - \frac{q}{Q} \exp[Q(Z_k - 1)] \quad \frac{q}{Q} \leq \vartheta < \vartheta_k
\]

\[
\frac{Y}{x_0} = 1 - \frac{1}{S} \ln \left\{ 1 + [\exp(S) - 1] \exp \left[ S \left( \frac{q}{Q} - \vartheta \right) \right] (1 - q) \right\} \quad \vartheta \geq \vartheta_k
\]

- \( Q, S \) dimensionless model parameters [-]
- \( \tau \) minimal extraction time [s]
- \( q \) soluble material fraction on the surface of the particles [kg/kg]
- \( \vartheta = \frac{t}{\tau} \) dimensionless time [-]
- \( Z_k \) dimensionless length and time where and when the soluble material runs out from the surface [-]

\[ v_k = \frac{q}{Q} + \frac{1}{S} \ln\{1 - q[1 - \exp(S)]\} \]
\[ Z_k = \frac{1}{S} \ln \left[ 1 + \frac{1}{q} \exp \left[ S \left( \vartheta - \frac{q}{Q} \right) \right] - 1 \right] \]

\[ \tau = \frac{m_s x_0}{\dot{m}_f y^*} \]
\[ Q = \frac{m_s k_f a_p \rho_f}{\dot{m}_f (1 - \varepsilon) \rho_s} \]
\[ S = \frac{m_s k_s a_p x_0}{\dot{m}_f (1 - \varepsilon) y^*} \]

- \( y^* \): solubility at extraction pressure and temperature [kg/kg]
- \( x_0 \): initial concentration of soluble material [kg/kg]
- \( \rho_f, \rho_s \): density of the fluid and solid phase [kg/m³]
- \( a_p \): specific surface area of the solid particles [m²/m³]

- \( k_f, k_s \): mass transfer coefficient in the fluid and the solid phase [m/s]
- \( m_s \): amount of the raw material [kg]
- \( \dot{m}_f \): mass flow of the fluid [kg/s]
- \( \varepsilon \): void fraction in bed [m³/m³]
\[ v_k = \frac{q}{Q} + \frac{1}{S} \ln\{1 - q[1 - \exp(S)]\} \quad Z_k = \frac{1}{S} \ln \left[ 1 + \frac{1}{q} \left\{ \exp\left( S \left( \frac{v - q}{Q} \right) \right) - 1 \right\} \right] \]

\[ \tau = \frac{m_s x_0}{\dot{m}_f y^*} \quad Q = \frac{m_s k_f a_p \rho_f}{\dot{m}_f (1 - \varepsilon) \rho_s} \quad S = \frac{m_s k_s a_p x_0}{\dot{m}_f (1 - \varepsilon) y^*} \]

- \( y^* \): solubility at extraction pressure and temperature [kg/kg]
- \( x_0 \): initial concentration of soluble material [kg/kg]
- \( \rho_f, \rho_s \): density of the fluid and solid phase [kg/m³]
- \( a_p \): specific surface area [m²/m³]
- \( k_f, k_s \): mass transfer coefficients [m/s]
- \( m_s \): amount of the raw material [kg]
- \( \dot{m}_f \): mass flow of the fluid [kg/s]
- \( \varepsilon \): void fraction in bed [m³/m³]

**Solubility was estimated from an empirical equation or from the initial slope of the extraction curves**
\[ v_k = \frac{q}{Q} + \frac{1}{S} \ln\{1 - q[1 - \exp(S)]\} \]
\[ Z_k = \frac{1}{S} \ln\left[1 + \frac{1}{q} \left\{\exp\left[S\left(\vartheta - \frac{q}{Q}\right)\right] - 1\right\}\right] \]

\[ \tau = \frac{m_s x_0}{\dot{m}_f y^*} \]
\[ Q = \frac{m_s k_f a_p \rho_f}{\dot{m}_f (1 - \varepsilon) \rho_s} \]
\[ S = \frac{m_s k_s a_p x_0}{\dot{m}_f (1 - \varepsilon) y^*} \]

- \( y^* \) solubility at extraction pressure and temperature \[\text{[kg/kg]}\]
- \( x_o \) initial concentration of soluble material \[\text{[kg/kg]}\]
- \( \rho_f, \rho_s \) density of the fluid and solid phase \[\text{[kg/m}^3]\]
- \( a \) specific surface area of the solid particles \[\text{[m}^2/\text{m}^3]\]
- \( \dot{m}_f \) mass flow of the fluid \[\text{[kg/s]}\]
- \( \varepsilon \) void fraction in bed \[\text{[m}^3/\text{m}^3]\]

- The initial concentration of soluble material was estimated by traditional solvent Soxhlet-extraction using n-hexane as solvent
\[ v_k = \frac{q}{Q} + \frac{1}{S} \ln\{1 - q[1 - \exp(S)]\} \]
\[ Z_k = \frac{1}{S} \ln\left[1 + \frac{1}{q} \left\{\exp\left[S\left(\vartheta - \frac{q}{Q}\right)\right] - 1\right\}\right] \]
\[ \tau = \frac{m_s x_0}{\dot{m}_f y^*} \]
\[ Q = \frac{m_s k_f a_p \rho_f}{\dot{m}_f (1 - \varepsilon) \rho_s} \]
\[ S = \frac{m_s k_s a_p x_0}{\dot{m}_f (1 - \varepsilon) y^*} \]

- \( y^* \): solubility at extraction pressure and temperature [kg/kg]
- \( x_o \): initial concentration of soluble material [kg/kg]
- \( \rho_f \), \( \rho_s \): density of the fluid and solid phase [kg/m^3]
- \( a_p \): specific surface area of the solid particles [m^2/m^3]
- \( k_f \), \( k_s \): mass transfer coefficients [m/s]
- \( m_s \): amount of the raw material [kg]
- \( \dot{m}_f \): mass flow of the fluid [kg/s]
- \( \varepsilon \): void fraction in bed [m^3/m^3]

The density of the carbon dioxide was calculated from the Bender equation of state at the pressure and temperature of the extraction.
Parameters of the Sovová's model

- $y^*$: solubility at extraction pressure and temperature [kg/kg]
- $x_0$: initial concentration of soluble material [kg/kg]
- $\rho_f, \rho_s$: density of the fluid and solid phase [kg/m$^3$]
- $a_p$: specific surface area of the solid particles [m$^2$/m$^3$]
- $k_f, k_s$: mass transfer coefficient in the fluid and the solid phase [m/s]
- $m_s$: amount of the raw material [kg]
- $m_f$: mass flow of the fluid [kg/s]
- $\varepsilon$: void fraction in bed [m$^3$/m$^3$]

The density of the raw material was measured by Hofsäss air pycnometer.
\[ v_k = \frac{q}{Q} + \frac{1}{S} \ln \{1 - q[1 - \exp(S)]\} \quad Z_k = \frac{1}{S} \ln \left[ 1 + \frac{1}{q} \left\{ \exp \left[ S \left( \vartheta - \frac{q}{Q} \right) \right] - 1 \right\} \right] \]

\[ \tau = \frac{m_s x_0}{\dot{m}_f y^*} \quad Q = \frac{m_s k_f a_p \rho_f}{\dot{m}_f (1 - \varepsilon) \rho_s} \quad S = \frac{m_s k_s a_p x_0}{\dot{m}_f (1 - \varepsilon) y^*} \]

- \( y^* \): solubility at extraction pressure and temperature \[ \text{[kg/kg]} \]
- \( x_o \): initial concentration of soluble material \[ \text{[kg/kg]} \]
- \( \rho_f, \rho_s \): density of the fluid and solid phase \[ \text{[kg/m}^3\text{]} \]
- \( a_p \): specific surface area of the solid particles \[ \text{[m}^2\text{/m}^3\text{]} \]
- \( k_f, k_s \):
- \( m_s, \dot{m}_f \):
- \( \varepsilon \): void fraction in bed \[ \text{[m}^3\text{/m}^3\text{]} \]

• The specific surface area was estimated from the surface-volume diameter of the particles, what was determined from sieve experiments using the Rosin–Rammler–Bennett particle size distribution model.
\[ v_k = \frac{q}{Q} + \frac{1}{S} \ln\{1 - q[1 - \exp(S)]\} \]
\[ Z_k = \frac{1}{S} \ln\left[1 + \frac{1}{q}\left\{\exp\left[S\left(\vartheta - \frac{q}{Q}\right)\right] - 1\right]\right] \]

\[ \tau = \frac{m_s x_0}{\dot{m}_f y^*} \]
\[ Q = \frac{m_s k_f a_p \rho_f}{\dot{m}_f (1 - \varepsilon) \rho_s} \]
\[ S = \frac{m_s k_s a_p x_0}{\dot{m}_f (1 - \varepsilon) y^*} \]

- The mass transfer coefficient in the fluid phase was estimated from a Sherwood correlation.

- Parameters of the Sovová's model:
  - \( y^* \): solubility at extraction pressure and temperature \([\text{kg/kg}]\)
  - \( x_o \): initial concentration of soluble material \([\text{kg/kg}]\)
  - \( \rho_f, \rho_s \): density of the fluid and solid phase \([\text{kg/m}^3]\)
  - \( a_p \): specific surface area of the solid particles \([\text{m}^2/\text{m}^3]\)
  - \( k_f, k_s \): mass transfer coefficient in the fluid and the solid phase \([\text{m/s}]\)
  - \( m_s \): amount of the raw material \([\text{kg}]\)
  - \( m_f \): mass flow of the fluid \([\text{kg/s}]\)
  - \( \varepsilon \): void fraction in bed \([\text{m}^3/\text{m}^3]\)
\[ v_k = \frac{q}{Q} + \frac{1}{S} \ln \{1 - q [1 - \exp(S)] \} \]
\[ Z_k = \frac{1}{S} \ln \left[ 1 + \frac{1}{q} \left\{ \exp \left[ S \left( \vartheta - \frac{q}{Q} \right) \right] - 1 \right\} \right] \]

\[ \tau = \frac{m_s x_0}{\dot{m}_f y^*} \quad Q = \frac{m_s k_f a_p \rho_f}{\dot{m}_f (1 - \varepsilon) \rho_s} \quad S = \frac{m_s k_s a_p x_0}{\dot{m}_f (1 - \varepsilon) y^*} \]

*\( y^* \)*: solubility at extraction pressure and temperature [kg/kg]

*\( x_0 \)*: initial concentration of soluble material [kg/kg]

*\( \rho_f, \rho_s \)*: density of the fluid and solid phase [kg/m³]

*\( a_p \)*: specific surface

*\( k_f, k_s \)*: mass transfer coefficients

*\( m_s \)*: amount of the raw material [kg]

*\( \dot{m}_f \)*: mass flow of the fluid [kg/s]

*\( \varepsilon \)*: void fraction in bed [m³/m³]

**The void fraction in bed was calculated from the density of the raw material and the bulk density.**
• Extraction of marigold: $p_E=350$ bar, $T_E=40^\circ$C

![Graph showing experimental yield points](image)
• Extraction of marigold: $p_E=350$ bar, $T_E=40^\circ$C
Extraction of marigold: $p_E = 350\, \text{bar}$, $T_E = 40\, ^\circ\text{C}$.
Effect of the solvent flow rate on the extraction of corn germ, Sovová model, $P_E = 450$ bar, $T_E = 40°C$. 

- 11 kg/h, experimental
- 7 kg/h, experimental
- 4 kg/h, experimental
- 11 kg/h, calculated
- 7 kg/h, calculated
- 4 kg/h, calculated
Investment costs of high pressure units


\[ I = A(V_T W)^{0.25} \]
Production costs of SFE
(NATEX, Austria)
<table>
<thead>
<tr>
<th>Plant capacity (t/year)</th>
<th>Production cost (EUR/kg raw material)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>300-400</td>
<td>SE 3-8  SFE 4-10</td>
<td>medicinal plants, cosmetics, spices</td>
</tr>
<tr>
<td>1000-1200</td>
<td>1-3.5 2-5</td>
<td>food additives, specific vegetable oils</td>
</tr>
<tr>
<td>10000-12000</td>
<td>0.5-1.2 0.75-1.2</td>
<td>coffee, hop</td>
</tr>
<tr>
<td>1000000-120000</td>
<td>0.2-0.4 ?</td>
<td>oilseeds</td>
</tr>
</tbody>
</table>
CONCLUSIONS

• There is a growing interest in new natural products that act very specifically without any side-effects.

• Supercritical fluid extraction is ideal for preparation of medicinal plant extracts.

• Carbon dioxide extracts are different from the traditional preparations not only in chemical composition, but in phytotherapeutical activity as well.

• There is an urgent need for more detailed phytochemical analysis of extracts.
CONCLUSIONS

- Commercial supercritical fluid processes have been still limited to few areas.
- Large capacity plants, with optimised design and operation lead to prices that are very often of the same order of magnitude as those related to classical processes.
- Moreover, there are also cases were supercritical fluids permit to make products or operations, that cannot be realised by any other methods.
Thank you for your kind attention!