Treatment of two-phase olive mill wastes and recovery of phenolic compounds

Isolation of phenolic compounds from agroindustrial Byproducts


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Recovery of phenolic compounds from:
Olive Mill Wastewater + Olive Mill solid wastes - Olive leaves

- Olive oil is a major agro-industrial product for all Mediterranean countries.
- Unfortunately large quantities of olive mill wastewater (OMW) or semi-solid wastes are produced.
- Its treatment is difficult and expensive due to its high organic load and phenolic content.
Treatment of Wastewaters and Solid Wastes - Isolation, Recovery and Purification of phenolic compounds from agricultural by–products (wastes)

• Purification of olive mill wastewater phenols
• Purification of olive leaf phenols
• Purification of grape marc phenols
• Purification of olive mill solid waste phenols
• Purification of coffee beans phenols
Scope

• Large amounts of agricultural byproducts are produced every year, some of them rich in phenolic compounds.

• Phenols are antioxidants with high-added value and positive effects to the human health.

• Their separation for the production of cosmetic products, food supplements etc., is of great interest.

• For this purpose, a combination of solid-liquid extraction, membrane filtration and resin adsorption/desorption following by evaporation is proposed, for the production of phenolic concentrates.

• The final products of the proposed process contain a large percentage of the byproducts’ phenolic content, in a small fraction of the initial volume.

• This technique, after modification, can be applied to a variety of phenol-rich byproducts, allowing the operation of phenol separation plant adjustable to local agricultural activities.
### Physicochemical Separation Techniques

- **Solid-liquid extraction** is the separation of target compounds from a solid matrix through the use of the appropriate solvent.

**Solvents:** WATER- ETHANOL (accepted in food industry)

#### Important parameters:
- **Physical characteristics of the solid**
- **Solvent**
- **Temperature**
- **Agitation**

<table>
<thead>
<tr>
<th>Type of extraction</th>
<th>Solutes</th>
<th>Free solids</th>
<th>Solvent/product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffusional</td>
<td>Soluble coffee</td>
<td>Coffee beans</td>
<td>water</td>
</tr>
<tr>
<td></td>
<td>Sugar</td>
<td>Sugar beets</td>
<td>Water, to produce sugar</td>
</tr>
<tr>
<td>Washing extraction</td>
<td>Vegetable oils</td>
<td>Oilseeds</td>
<td>Hexane, hydrocarbons</td>
</tr>
<tr>
<td></td>
<td>Flavors/Odors</td>
<td>Flowers</td>
<td>Ethanol</td>
</tr>
<tr>
<td></td>
<td>Sugar</td>
<td>Sugarcane</td>
<td>Water</td>
</tr>
<tr>
<td>Leaching</td>
<td>Phosphoric acid</td>
<td>Phosphate rock</td>
<td>Sulfuric acid</td>
</tr>
<tr>
<td>Gold</td>
<td>Gold ore</td>
<td>Sodium cyanide</td>
<td></td>
</tr>
<tr>
<td>Chemical reaction</td>
<td>Gelatin</td>
<td>Bones and skins</td>
<td>Aqueous solution (pH 3 to 4)</td>
</tr>
<tr>
<td>Lignins</td>
<td>Wood chips</td>
<td>NaOH solution, sulfide/sulfite</td>
<td></td>
</tr>
</tbody>
</table>
Physicochemical Separation Techniques

- **Membrane filtration** is a separation technique that has many applications in chemical process industries.

The most important attributes of a membrane material are:

- Good permeability
- High selectivity
- Chemical stability
- Resistance to fouling

http://www.watertechonline.com/sustainable-membranes-for-wastewater-applications/
Analytical Techniques

- **Total Phenols**: Folin-Ciocalteu reagent, gallic acid as standard, 720 nm.
- **Total Carbohydrates**: L-Tryptophane reagent, glucose as standard, 525 nm.
- **COD**: 5220 D, Standard Methods.
- **TS**: 2540 B, Standard Methods.
- **TSS**: 2540 D, Standard Methods.
- **Simple Phenols**: HPLC analysis, gradient elution, DAD detector.
Olive Mill Wastewater Phenolic Compounds

• Olive mill wastewater (OMW) is a byproduct of the THREE-phase extraction systems during the production of olive oil.

• Olive mill semi-solid (alperujo or pomace) is a byproduct of the TWO-phase extraction systems during the production of olive oil.

• Because of their partition coefficient, most phenolic compounds of olive fruits end up in the wastewater produced and not in olive oil.

• Oleuropein is the most common phenolic compound of unripe olive fruits, but during maturity it is hydrolyzed to several simpler phenolic compounds like hydroxytyrosol and tyrosol.
Membrane Filtration of OMW (three phase decanter)

<table>
<thead>
<tr>
<th>Process</th>
<th>COD (g/L)</th>
<th>TS (g/L)</th>
<th>TSS (g/L)</th>
<th>Ch (g/L)</th>
<th>Ph (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial OMW</td>
<td>107.23</td>
<td>63.4</td>
<td>44</td>
<td>12.34</td>
<td>2.64</td>
</tr>
<tr>
<td>&lt;0.125 mm</td>
<td>99.08</td>
<td>58.8</td>
<td>33</td>
<td>13.19</td>
<td>2.65</td>
</tr>
<tr>
<td>UF Feed</td>
<td>257.73</td>
<td>121.36</td>
<td>141</td>
<td>19.37</td>
<td>6.59</td>
</tr>
<tr>
<td>UF Conc.</td>
<td>51.10</td>
<td>37.35</td>
<td>1.33</td>
<td>10.93</td>
<td>2.17</td>
</tr>
<tr>
<td>NF Feed</td>
<td>61.03</td>
<td>43.82</td>
<td>1.77</td>
<td>11.97</td>
<td>2.64</td>
</tr>
<tr>
<td>NF Conc.</td>
<td>32.72</td>
<td>22.15</td>
<td>0.95</td>
<td>5.09</td>
<td>0.86</td>
</tr>
<tr>
<td>NF Filtr./RO Feed</td>
<td>65.48</td>
<td>60.44</td>
<td>1.67</td>
<td>14.96</td>
<td>2.09</td>
</tr>
<tr>
<td>RO Conc.</td>
<td>6.47</td>
<td>1.48</td>
<td>0.08</td>
<td>0.21</td>
<td>0.04</td>
</tr>
<tr>
<td>RO Filtr.</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>
Resin Adsorption/Desorption of OMW RO$_C$

- XAD$_4$ and XAD$_{16}$N yielded the best results. Even though the sample contained more carbohydrates than phenols, resins adsorbed the dissolved phenols at a higher percentage.
- When water was used as a desorption solvent, the small amount of carbohydrates that was adsorbed on the resin was desorbed at a high percentage (60%). Ethanol, on the other hand, almost selectively removed the adsorbed phenols, while acetone removed both, carbohydrates and phenols.
- Kinetic experiments allowed the optimization of flow rates and total volume of treated sample before the resin surface was saturated.
Final Concentrate of OMW Phenolic Compounds

- After carbohydrates removal via the proposed resin process, the distillation under vacuum (-0.95 bar, 55 °C) of the resin ethanolic effluent resulted to a final phenol concentration of 378 g/L in gallic acid equivalents in the distillation residue.

<table>
<thead>
<tr>
<th></th>
<th>Initial OMW</th>
<th>RO concentrate</th>
<th>Ethanolic resin effluent</th>
<th>Distillation residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume, mL</td>
<td>16700</td>
<td>2000</td>
<td>1500</td>
<td>9</td>
</tr>
<tr>
<td>Phenols, g/L</td>
<td>2.64 ±0.04</td>
<td>2.09 ±0.02</td>
<td>2.36 ±0.01</td>
<td>377.50 ±8.34</td>
</tr>
<tr>
<td>Carbohydrates, g/L</td>
<td>12.34 ±0.49</td>
<td>14.96 ±0.03</td>
<td>3.84 ±0.01</td>
<td>293.92 ±1.28</td>
</tr>
</tbody>
</table>
Olive Leaf Phenolic Compounds

• Olive leaves are a byproduct of olive fruit harvesting and initial stages of olive oil extraction, during their separation from olive fruits.

• Olive leaf extracts have been proven to be rich in phenolic compounds, with the most prominent one being oleuropein, which, unlike in the olive fruit, it is not hydrolyzed to simpler phenols.

• Oleuropein can be either bound to a sugar molecule (Oleuropein glycoside) or be present in its free form (Oleuropein aglycon).
Extraction of Olive Leaf Phenols

(a) Effect of Ethanol (% v/v) on Extraction
(b) Effect of Solids (g/L) on Extraction
(c) Effect of Time (min) on Extraction

Optimum extraction conditions:
- Ethanol %: 0
- Duration: 120 min
- Solids/Solvent: 250 g/L
Membrane Filtration of Olive Leaf Extract

<table>
<thead>
<tr>
<th>Volume L</th>
<th>Initial UF conc.</th>
<th>UF filtr.</th>
<th>NF conc.</th>
<th>NF filtr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>75</td>
<td>89</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>Total Ph mg/L</td>
<td>468 ±15</td>
<td>774 ±3</td>
<td>325 ±7</td>
<td>988 ±25</td>
</tr>
<tr>
<td>Total Ch mg/L</td>
<td>2801 ±30</td>
<td>3458 ±27</td>
<td>2140 ±179</td>
<td>5410 ±37</td>
</tr>
</tbody>
</table>

Diagram showing the process of membrane filtration with initial UF concentration, UF filtrate, NF concentration, and NF filtrate.
• 1.44 L of NF concentrate were treated with the proposed resin process, leading to the production of 0.72 L of ethanolic effluent that was evaporated under vacuum (0.05 bar, 50 °C). The final concentrate had a volume of 10 mL.

<table>
<thead>
<tr>
<th></th>
<th>Volume mL</th>
<th>Total Phenols mg/L</th>
<th>Total Carbohydrates mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFc</td>
<td>1440</td>
<td>988 ±25</td>
<td>5410 ±37</td>
</tr>
<tr>
<td>Desorbed</td>
<td>720</td>
<td>1480 ±1</td>
<td>5260 ±35</td>
</tr>
<tr>
<td>Final concentrate</td>
<td>10</td>
<td>97890 ±1230</td>
<td>322333 ±3933</td>
</tr>
</tbody>
</table>
Olive oil semi-solid wastes (pomace or alperujo)
Residual from the 2-phase olive oil extraction process
60-70% moisture
Extraction of phenols from olive oil semi-solid wastes: A parametric study

![Graph 1: Solvent Concentration vs TPC and Carbohydrates](image1)

![Graph 2: Mass of Solid Waste vs Concentration](image2)
Extraction of phenols from olive oil semi-solid wastes: A parametric study

![Graphs showing the extraction of phenols and carbohydrates under different conditions.](image)
Extraction of phenols from olive oil semi-solid wastes: A parametric study

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**Extraction repeatability**

- Phenols (50% ethanol)
- Phenols (100% water)
- Carbohydrates (50% ethanol)
- Carbohydrates (100% water)

**Concentration (mg/L)**

- 0
- 200
- 400
- 600
- 800
- 1000
- 1200
- 1400
- 1600
- 1800

**Extraction rate, rpm**

- Phenols (50% ethanol)
- Phenols (100% water)
- Carbohydrates (50% ethanol)
- Carbohydrates (100% water)

**Concentration (mg/L)**

- 0
- 1000
- 1200
- 1400
- 1600
- 1800
- 2000
- 2200

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Extraction of phenols from olive oil semi-solid wastes: A parametric study

![Graph showing extraction time vs concentration for phenols and carbohydrates in 50% ethanol and 100% water](image)

<table>
<thead>
<tr>
<th>Solvent Type</th>
<th>Conditions</th>
<th>Maximum TPC Concentration (mg/L)</th>
<th>Maximum Carbohydrate Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% H₂O</td>
<td>H₂O</td>
<td>471.0</td>
<td>1020.3</td>
</tr>
<tr>
<td>50% H₂O</td>
<td>(95%), 50%</td>
<td>1020.3</td>
<td>2060.0</td>
</tr>
<tr>
<td>100% H₂O</td>
<td>H₂O</td>
<td>1516.7</td>
<td>1373.3</td>
</tr>
<tr>
<td>50% H₂O</td>
<td>(95%), 50%</td>
<td>1516.7</td>
<td>1603.3</td>
</tr>
<tr>
<td>Temperature, 20°C</td>
<td></td>
<td>735.5</td>
<td>956.3</td>
</tr>
<tr>
<td>Rate, 100 rpm</td>
<td></td>
<td>735.5</td>
<td>1516.7</td>
</tr>
<tr>
<td>Duration, 60 min</td>
<td></td>
<td>735.5</td>
<td>1708.3</td>
</tr>
<tr>
<td>HCl, 0 mL</td>
<td></td>
<td>678.0</td>
<td>892.5</td>
</tr>
<tr>
<td>Mean Concentration</td>
<td></td>
<td>655.0</td>
<td>969.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1632.3</td>
<td>1572.6</td>
</tr>
</tbody>
</table>
Extraction of phenols from olive oil semi-solid wastes: A pilot study

Extracted solution

UF

Permeate

Concentrate, 20 L

100 L

NF

Permeate

Concentrate 10 L

90 L

RO

Permeate

Concentrate 11 L

79 L
Extraction of phenols from olive oil semi-solid wastes: A pilot study

UF- Feed- intermediate permeates- concentrates

NF- Feed- intermediate permeates- concentrates

RO Feed- intermediate permeates- concentrates
Extraction of phenols from olive oil semi-solid wastes: A pilot study

TPC concentration at the raw, feed, permeate and concentrate streams at the UF, NF, RO membranes.
Extraction of phenols from olive oil semi-solid wastes: A pilot study

Carbohydrates concentration at the raw, feed, permeate and concentrate streams at the UF, NF, RO membranes
Extraction of phenols from olive oil semi-solid wastes: A pilot study

COD at the raw, feed, permeate and concentrate streams at the UF, NF, RO membranes.
Conclusions: Extraction of phenols from olive oil semi-solid wastes

*Optimal conditions*
Solvent: Water 50% - ethanol 50%
Solid/solvent ratio: 20 gr/100 ml
Temperature: 30-40 °C
Stirring rate: 100 rpm
Duration: 1 hr

*Pilot study*
Fat and lipids and polyphenols are concentrated in UF retentate stream
Simple phenolics are included in NF and RO concentrates
Unfortunately carbohydrates are everywhere...
Adsorption/desorption on specific resins / cooling crystallization/ melting crystallization/ freeze drying → better purification of phenols... current work
Acknowledgements

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Thank you for your attention 😊