Development of a citrus processing waste-based biorefinery for production of high-added value commodities

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²KEAN Soft Drinks, Ltd, Promachon Eleftherias, 4103, Agios Athanasios, Limassol, Cyprus
- Worldwide citrus fruits production: $143 \times 10^6$ t per year
- Industrial generation of citrus peel waste (CPW): $24 \times 10^6$ t per year
- 50% of the total mass is considered as CPW
  - Peel
  - Membranes
  - Seeds
  - Pulp
- 1 – 17 m$^3$ citrus processing wastewater (CPWW) per t processed fruit
  - Factory cleaning
  - Juice concentration
  - Essential oils extraction
- Up to 17 m³ citrus processing wastewater (CPWW) per t processed fruit
  - Factory cleaning
  - Juice concentration
  - Essential oils extraction
Previous CPWW Exploitation Studies

- All studies aimed to reduce the COD content
**Essential Oils**
- Antioxidants
- Preservative
- Flavoring
- Anti-microbial properties

**Polyphenols**
- Antioxidant Characteristics:
  - Anticancer
  - Antiviral
  - Anti-inflammatory properties

**Carotenoids**
- Antioxidants
- Pro-vitamin A activity
  - (β-carotene)

**Bacterial Cellulose**
- High crystallinity
- Biodegradability
- High degree of polymerization
- High water-holding capacity
- Enhanced Mechanical strength
- High Purity

**Applications**
- Antimicrobial agents
- Foods
- Medicine
- Green solvent
- Platform chemical

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Bacterial Cellulose

Properties
- High crystallinity
- Biodegradability
- High degree of polymerization
- High water-holding capacity
- Enhanced Mechanical strength
- High Purity

Drawbacks
- High production cost
- Low productivity
- Extensive cultivation time
Aim and Objectives

Biorefinery development for manufacture of value-added extractable products and bacterial cellulose using citrus processing wastewater

- Recovery of essential oils
- Assessment of different organic solvents for carotenoids extraction
- Assessment of different adsorption materials for polyphenols recovery
- Production of bacterial cellulose
CPWW Composition

Three streams:
- Heating/Cooling
- Juice vacuum concentration
- Essential Oils Extraction

COD content < 0.6 g L\(^{-1}\)

Sugars and bioactive compounds **were not detected**

<table>
<thead>
<tr>
<th></th>
<th>Valencia, Mandora, Lemon, Grapefruit wastewater</th>
<th>Current Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD [g L(^{-1})]</td>
<td>87.2 – 104.6</td>
<td>104.6</td>
</tr>
<tr>
<td>Reducing sugars [g L(^{-1})]</td>
<td>33.0 – 92.7</td>
<td>48.0</td>
</tr>
<tr>
<td>Free Amino Nitrogen [mg(_{\text{glycine}}) L(^{-1})]</td>
<td>59.7 – 108.9</td>
<td>95.0</td>
</tr>
<tr>
<td>Total Phenolic Content [g(_{\text{GA eq.}}) L(^{-1})]</td>
<td>1.1 – 2.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Essential Oils [mg L(^{-1})]</td>
<td>196.4 – 637.8</td>
<td>450.1</td>
</tr>
<tr>
<td>Total Solids [%]</td>
<td>5.6 – 6.7</td>
<td>5.5</td>
</tr>
<tr>
<td>pH-value</td>
<td>3.6 – 4.1</td>
<td>3.9</td>
</tr>
</tbody>
</table>
Proposed Biorefinery Process

- **Organic Solvent**
- **Centrifugation**
- **Solid**
- **Solvent Extraction**
- **Carotenoids**
- **Essential Oils**
- **K. sucrofermentans**
- **Water**
- **Ethanol**
- **Adsorption**
- **Sugars**
- **Fermentation**
- **Bacterial Cellulose**
- **Sugars**
- **Polyphenols**
Essential Oils Extraction

Experimental Conditions:
- 3500 rpm
- 25 °C
- 30 min
- 5:1 CPWW-to-solvent

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$n$-Pentane
$n$-Heptane
$n$-Hexane

![Graph showing essential oils extraction](image)

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Carotenoids Extraction

Experimental Conditions:
- 100 rpm
- 35 °C
- 35 min
- 2% solid loading

![Bar chart showing total carotenoids yield for different solvent combinations.

- Ethanol: 1.91 mg g⁻¹
- Acetic Acid: 1.97 mg g⁻¹
- Acetone: 1.69 mg g⁻¹
- Hexane: 1.00 mg g⁻¹
- Limonene: 1.00 mg g⁻¹
- Isopropanol: 1.00 mg g⁻¹

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<table>
<thead>
<tr>
<th>Feedstock</th>
<th>Extraction method</th>
<th>Solvent</th>
<th>Production</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange peel</td>
<td>Ultrasound-assisted extraction</td>
<td>50%, v/v Ethanol</td>
<td>0.63 mg β-carotene/100 g dry basis</td>
<td>Montero-Calderon et al., 2019</td>
</tr>
<tr>
<td>Kinnow mandarin peel</td>
<td>Ultrasound-assisted extraction</td>
<td>Ethanol</td>
<td>2.97 mg lutein/100 g dry basis</td>
<td>Saini et al., 2020</td>
</tr>
<tr>
<td>Mandarin peel</td>
<td>Solid-liquid extraction</td>
<td>Ethanol/Acetone/Hexane (25/25/50 v/v)</td>
<td>27 mg/100 mg dry basis</td>
<td>Barman et al., 2020</td>
</tr>
<tr>
<td>Mandarin peel</td>
<td>Supercritical fluid extraction</td>
<td>CO₂-acetone (7%)</td>
<td>0.39 mg β-carotene/100 g dry basis</td>
<td>Tsitsagi et al., 2018</td>
</tr>
<tr>
<td>Mandarin peel</td>
<td>High Voltage electric discharges</td>
<td>N/A</td>
<td>0.369 mg β-carotene/100 ml of extract</td>
<td>Buniowska et al., 2015</td>
</tr>
<tr>
<td>Orange Peel</td>
<td>Ultrasound-assisted extraction</td>
<td>Limonene</td>
<td>11.25 mg β-carotene/100 g dry basis</td>
<td>Boukroufa et al., 2017</td>
</tr>
<tr>
<td>CPWW solid</td>
<td>Solvent extraction</td>
<td>Ethanol/Acetone/Limonene (25/25/50 v/v)</td>
<td>191 mg β-carotene/100 g dry basis</td>
<td>Current study</td>
</tr>
</tbody>
</table>
## Polyphenols Recovery

Assessment of different adsorption materials and different concentrations

<table>
<thead>
<tr>
<th>Adsorption material</th>
<th>Polarity</th>
<th>Particle size diameter [mm]</th>
<th>Surface area [m² g⁻¹]</th>
<th>Pore size [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amberlite XAD4</td>
<td>non-polar</td>
<td>0.56 - 0.71</td>
<td>750</td>
<td>10</td>
</tr>
<tr>
<td>Amberlite XAD16N</td>
<td>non-polar</td>
<td>0.56 - 0.71</td>
<td>800</td>
<td>20</td>
</tr>
<tr>
<td>Amberlite XAD7HP</td>
<td>moderate polar</td>
<td>0.56 - 0.71</td>
<td>380</td>
<td>30-40</td>
</tr>
<tr>
<td>PuroSorb PAD900</td>
<td>non-polar</td>
<td>0.35 – 1.20</td>
<td>850</td>
<td>15-30</td>
</tr>
<tr>
<td>Biochar*</td>
<td>non-polar</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Activated Biochar**</td>
<td>non-polar</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

*Pistachio shells pyrolyzed at 500 °C for 3 min (Kyriakou et al., 2020)

**Pistachio Biochar Activation performed using 1 M KOH for 24 h at 25 °C
Polyphenols Adsorption

Batch Experiments, 25 °C, 100 rpm, 1 h, conical flasks 50 ml CPWW

(a) Polyphenols (b) Sugars
Polyphenols Desorption

Desorption Experiments: 10 g of each adsorption material in columns

1 resin volume [rv] = 10 ml

Polyphenols Desorption [% w/v]

(a) 5 rv h\(^{-1}\) (b) 10 rv h\(^{-1}\) (c) 15 rv h\(^{-1}\)

- XAD7HP
- XAD16N
- XAD4
- PAD900
- Activated Biochar

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Polyphenols Desorption

Desorption Experiments: 10 g of each adsorption material in columns

Chosen resin: Pursorb PAD900

1 resin volume [rv] = 10 ml

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Polyphenols Desorption

Desorption Experiments: 10 g of each adsorption material in columns

100% Polyphenols
100% Sugars

7 rv CPWW
10 rv h⁻¹

1 rv
82.9% Polyphenols
27.5% Sugars

2 rv H₂O
10 rv h⁻¹

1 rv
73.6% Polyphenols
13.6% Sugars

5 rv Ethanol
10 rv h⁻¹

1 rv
16.7% Polyphenols
13.0% Sugars

56.9% Polyphenols
0.62% Sugars

~80% Desorption!

1 resin volume [rv] = 10 ml
Polyphenols Desorption

Desorption Experiments: 10 g of each adsorption material in columns

![Graph showing polyphenols recovery for different solvents.]

1 resin volume [rv] = 10 ml

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Bacterial Cellulose Production

Experimental Conditions:

- *K. sucrofermentans* DSM 15973
- T = 30 °C, 150 rpm (for 2 d then static)
- 15 d fermentation duration
- Initial sugars conc.: 22.5 g L\(^{-1}\)

✓ Final BC conc.: 3.9 g L\(^{-1}\)
✓ Yield: 0.19 g\(_{BC}\) g\(_{sugar}\)\(^{-1}\)
✓ Productivity: 0.39 g L\(^{-1}\) d\(^{-1}\)
<table>
<thead>
<tr>
<th>Industrial Waste</th>
<th>Additional Nutrient</th>
<th>Culture</th>
<th>BC Conc. [g L⁻¹]</th>
<th>BC Yield [gbc g⁻¹]</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus peel fluid</td>
<td>Acetate buffer</td>
<td><em>G. xylinus</em></td>
<td>2.3</td>
<td>0.12</td>
<td>Kuo et al., 2019</td>
</tr>
<tr>
<td>Sugar beet molasses</td>
<td>Null</td>
<td><em>G. xylinus</em>  PTCC 1734</td>
<td>4.6</td>
<td>0.25</td>
<td>Salari et al., 2019</td>
</tr>
<tr>
<td>Cheese whey</td>
<td>Null</td>
<td><em>G. xylinus</em>  PTCC 1734</td>
<td>3.6</td>
<td>0.14</td>
<td></td>
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<tr>
<td>Sweet lime pulp</td>
<td>Null</td>
<td><em>K. europaeus</em> SGP37</td>
<td>6.3</td>
<td>0.19</td>
<td>Dubey et al., 2018</td>
</tr>
<tr>
<td>Sugarcane molasse</td>
<td>Null</td>
<td><em>K. rhaeticus</em></td>
<td>1.9</td>
<td>0.04</td>
<td>Machado et al., 2018</td>
</tr>
<tr>
<td>Ripe dates</td>
<td>Yeast extract, peptone, Na₂HPO₄, citric acid</td>
<td><em>A. xylinum</em> 0416</td>
<td>5.8</td>
<td>0.19</td>
<td>Lotfiman et al., 2018</td>
</tr>
<tr>
<td>Pecan nutshell</td>
<td>Yeast extract, peptone, ethanol</td>
<td><em>G. entanii</em></td>
<td>2.8</td>
<td>0.07</td>
<td>Dorame-Miranda et al., 2019</td>
</tr>
<tr>
<td>CPWW</td>
<td>Yeast extract, peptone, Na₂HPO₄, citric acid</td>
<td><em>K. saccharofetans</em> DSM 15973</td>
<td>3.9</td>
<td>0.19</td>
<td>Current Study</td>
</tr>
</tbody>
</table>
In Summary …

1 m³

Centrifugation

Essential Oils

0.45 kg

Organic Solvent

Solvent Extraction

Solid

Carotenoids

10.3 g

K. sucrofermentans

Fermentation

Sugars

42 kg

Organic Solvent

Water

Ethanol

Adsorption

Sugars

Polyphenols

0.5 kg

Bacterial Cellulose

9.5 kg

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Conclusions

✓ First study in our knowledge used CPWW for polyphenols recovery through adsorption yielding up to 65%.

✓ 0.45 kg m\(^{-3}\) of essential oils can be recovered from CPWW using \(n\)-heptane.

✓ 1.91 mg g\(_{db}\)^{-1} of carotenoids can be recovered employing ethanol.

✓ Production of 3.9 g L\(^{-1}\) of bacterial cellulose employing \(K.\ sucrofermentans\) DSM 15793.
Future Work

- Study the effect of air-supplement
- Study fed-batch and continuous fermentation modes
- Upscale in an airlift bioreactor
Thank you for your attention!