Alkaline pretreatment of spent coffee grounds for microbial oil production using the oleaginous yeast strain *Lipomyces starkeyi*

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Objectives

- Valorisation of spent coffee grounds (SCGs) from catering services
- Biorefinery development for the production of value-added products
- Experimental design for the alkaline pretreatment of residual SCGs
- Valorisation of SCGs hydrolysate via bioprocess development for microbial oil production
Spent coffee grounds (SCGs)

- In 2019, over 1.8 million t of coffee were processed in the European Union
- European coffee consumption in 2018/2019 generated an estimated 6.5 million t of SCGs
- For every kg of coffee beverage, 2 kg of solid waste are produced as SCGs
- SCGs management is an important issue in the EU
- Nowadays, the majority of SCGs is disposed via landfilling
Biorefinery development of SCGs

- Carbohydrates
- Lipids
- Phenolic compounds
- Protein
- Minerals

Conventional and prospective applications

- Feed additive
- Fertilizer
- Cosmetics industry
- Pharmaceutical industry
- Biofuel production
- Microbial fermentation
Experimental design

Spent Coffee Grounds (SCGs)

Recovery of value-added components
- Coffee oil
- Phenolic compounds

Residual SCGs

Alkaline pretreatment
Solid to liquid ratio: 1:10 (w/v)
Pretreatment duration: 1h
X1: NaOH: 0-2% (w/v)
X2: Temperature: 70-140°C

Enzymatic hydrolysis
Commercial enzymes
Temperature: 50°C

Sugar-rich hydrolysate

Central composite design

<table>
<thead>
<tr>
<th>Run</th>
<th>X1</th>
<th>X2</th>
<th>X1</th>
<th>X2</th>
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<td>130</td>
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<td>1</td>
<td>-1</td>
<td>1.7</td>
<td>80</td>
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<td>80</td>
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<td>0</td>
<td>0</td>
<td>105</td>
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<td>140</td>
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<td>105</td>
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<td>0</td>
<td>0</td>
<td>1</td>
<td>105</td>
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<td>0</td>
<td>1</td>
<td>105</td>
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<td>12</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>105</td>
</tr>
</tbody>
</table>

Bioprocess development for microbial oil production

Fermentation

Microbial oil production
### Compositional analysis of SCGs

<table>
<thead>
<tr>
<th>Composition (% dry basis)</th>
<th>This study</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>1.8</td>
<td>0.4 - 2.2</td>
</tr>
<tr>
<td>Protein</td>
<td>14.8</td>
<td>6.7 - 13.7</td>
</tr>
<tr>
<td>Oil</td>
<td>12.2</td>
<td>10.0 - 15.0</td>
</tr>
<tr>
<td>Phenolics</td>
<td>0.92</td>
<td></td>
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<tr>
<td>Glucan</td>
<td>10.6</td>
<td>8.6 - 15.3</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>28.9</td>
<td>30.0 - 39.0</td>
</tr>
<tr>
<td>Arabinan</td>
<td>1.9</td>
<td>1.7</td>
</tr>
<tr>
<td>Mannan</td>
<td>17.2</td>
<td>21.2</td>
</tr>
<tr>
<td>Galactan</td>
<td>8.9</td>
<td>13.8</td>
</tr>
<tr>
<td>Xylan</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Lignin</td>
<td>28.1</td>
<td>23.9 - 33.6</td>
</tr>
</tbody>
</table>
Recovery of value-added components

Extraction of coffee oil

Extraction conditions:
- Solid to liquid ratio: 1:10 (w/v)
- Ultrasound 20 min, 3 times

Different extraction solvents:
- Hexane
- Ethyl acetate

- Hexane resulted to oil recovery of 97.8%
- Ethyl acetate, as an alternative green solvent, led to oil recovery of 96.9%
Recovery of value-added components

Extraction of phenolic compounds

Extraction conditions:
Extraction solvent: 70% EtOH
Ultrasound 20 min, 3 times
Different solid to liquid ratio:
  • 1:10 (w/v)
  • 1:20 (w/v)
  • 1:30 (w/v)
Biorefinery development of SCGs

**Initial solid 1000 g** (dry basis)

**Ethyl acetate extraction**
- Solid to liquid ratio 1:10 (w/v)
- Ultrasound 20 min, x 3 times

**Residual solids 881.5 g**

**Extraction of phenolic compounds**
- Solid to liquid ratio 1:10 (w/v)
- Ultrasound 20 min, x 3 times

**Residual solids 827.3 g**

**Alkaline pre-treatment**

**Enzymatic hydrolysis**

**Composition of coffee oil**
- 45% C16:0
- 33% C18:0
- 9% C18:1
- 7% C18:2
- 5% C18:3

**Initial solid 1000 g**

**Oil 118.50 g**

**Recovery yield:** 97%

**Crude extract of phenolic compounds 54.2 g**

**Glucan:** 10.6%
**Hemicellulose:** 28.9%
**Lignin:** 28.1%
**Oil:** 12.2%
**Protein:** 14.8%

**Initial solid 1000 g**

**Oil 118.50 g**

**Residual solids 827.3 g**

**Crude extract of phenolic compounds 54.2 g**

**Glucan:** 12.1%
**Hemicellulose:** 33.5%
**Lignin:** 31.8%
**Protein:** 15.2%

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**Oil 118.50 g**

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**Lignin:** 28.1%
**Oil:** 12.2%
**Protein:** 14.8%
Alkaline pretreatment of residual SCGs
Alkaline treatment and subsequent enzymatic hydrolysis of residual SCGs
Validation of experimental design

### Optimisation approach □ Lignin removal (%)

<table>
<thead>
<tr>
<th>NaOH (%, w/v) (coded)</th>
<th>Temperature (°C) (coded)</th>
<th>NaOH (%, w/v) (real)</th>
<th>Temperature (°C) (real)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1.3234</td>
<td>-0.2236</td>
<td>0.06</td>
<td>99.47</td>
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</tbody>
</table>

Constrains

- Glucan removal (%): < 2.5
- Hemicellulose removal (%): < 2.5

**Optimum response:**
Lignin removal: 36%
Validation of experimental design

Optimum response:
- Lignin removal: 36%

Overall glucan conversion yield: 63.4%
Overall hemicellulose conversion yield: 44.4%
### Fermentation with *Lipomyces starkeyi* for microbial oil production

<table>
<thead>
<tr>
<th>Fermentation time (h)</th>
<th>DCW (g/L)</th>
<th>Microbial oil content (%)</th>
<th>Microbial oil (g/L)</th>
<th>Yield (g/g)</th>
<th>Productivity (g/(L·h))</th>
</tr>
</thead>
<tbody>
<tr>
<td>170</td>
<td>87.5</td>
<td>49.0</td>
<td>40.2</td>
<td>0.16</td>
<td>0.24</td>
</tr>
</tbody>
</table>

**Graphs:**
- Free Amino Nitrogen (mg/L)
- DCW (g/L)
- Total Sugars (g/L)
- Lipids (g/L)
- Glucose (g/L)
- Mannose (g/L)

**Table:**
- Fermentation time (h)
- DCW (g/L)
- Microbial oil content (%)
- Microbial oil (g/L)
- Yield (g/g)
- Productivity (g/(L·h))
### Fatty acid methyl esters profile

<table>
<thead>
<tr>
<th>Fermentation time (h)</th>
<th>Palmitic acid C16:0</th>
<th>Palmitoleic C16:1</th>
<th>Stearic acid C18:0</th>
<th>Oleic acid C18:1</th>
<th>Linoleic acid C18:2</th>
<th>Others</th>
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<tbody>
<tr>
<td>25</td>
<td>36.4</td>
<td>3.1</td>
<td>9.9</td>
<td>43.8</td>
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<td>94</td>
<td>33.5</td>
<td>3.5</td>
<td>6.0</td>
<td>52.4</td>
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<tr>
<td>218</td>
<td>34.3</td>
<td>0</td>
<td>6.8</td>
<td>56.1</td>
<td>1.6</td>
<td>1.2</td>
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</table>
Concluding remarks

- Development of a novel biorefinery is a promising way to ensure sustainable SCGs, with the recovery of value-added products
- Ethyl acetate could efficiently replace hexane as an alternative green solvent for the extraction of coffee oil
- The lowest removal of all components was obtained when the pretreatment was carried out at 105°C without NaOH addition
- Optimum conditions for delignification of residual SCGs obtained were 0.06% (w/v) NaOH at 99.5°C leading to lignin removal of 36%
- Fermentation of SCGs hydrolysate with *Lipomyces starkeyi* resulted in 87.5 g/L of DCW with 49% oil content
Thank you for your attention!

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