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

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Brew2Bio

European Union
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MANAGING AUTHORITY OF EPAnEK

EPAnEK 2014–2020
OPERATIONAL PROGRAMME
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ENTREPRENEURSHIP
INNOVATION

ΕΣΠΑ 2014-2020
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Co-financed by Greece and the European Union

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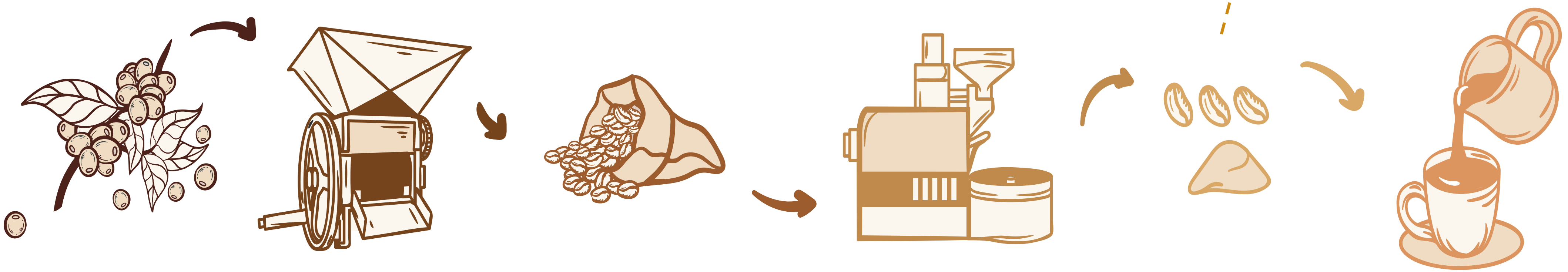
Objectives

- 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

More than 330,000 t of SCGs are generated from coffee catering services in the EU



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

Pretreatment stage

Residual SCGs

Alkaline pretreatment
Solid to liquid ratio: 1:10 (w/v)
Pretreament duration: 1h
X1: NaOH: 0-2% (w/v)
X2: Temperature: 70-140^{o}C

Enzymatic hydrolysis
Commercial enzymes
Temperature: 50 ^{o}C

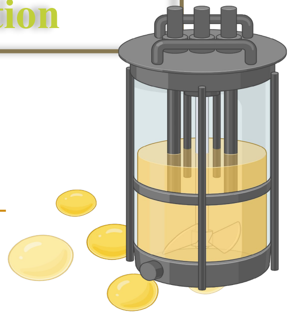
Sugar-rich hydrolysate

Central composite design

Run	X1	X2	X1	X2
1	1	1	1.7	130
2	1	-1	1.7	80
3	-1	1	0.3	130
4	-1	-1	0.3	80
5	1.414	0	2	105
6	-1.414	0	0	105
7	0	1.414	1	140
8	0	-1.414	1	70
9	0	0	1	105
10	0	0	1	105
11	0	0	1	105
12	0	0	1	105

Bioprocess development for microbial oil production

Fermentation → **Microbial oil production**



Compositional analysis of SCGs

Composition (% dry basis)	This study	Literature
Ash	1.8	0.4 - 2.2
Protein	14.8	6.7 - 13.7
Oil	12.2	10.0 - 15.0
Phenolics	0.92	
Glucan	10.6	8.6 - 15.3
Hemicellulose	28.9	30.0 - 39.0
Arabinan	1.9	1.7
Mannan	17.2	21.2
Galactan	8.9	13.8
Xylan	1.0	
Lignin	28.1	23.9 - 33.6

Recovery of value-added components

Extraction of coffee oil

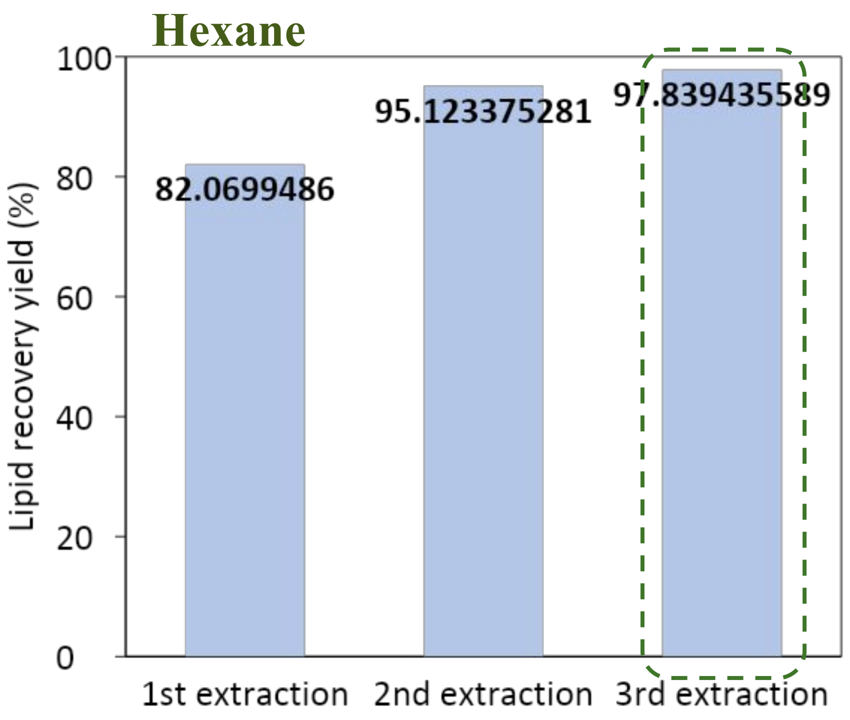
Extraction conditions:

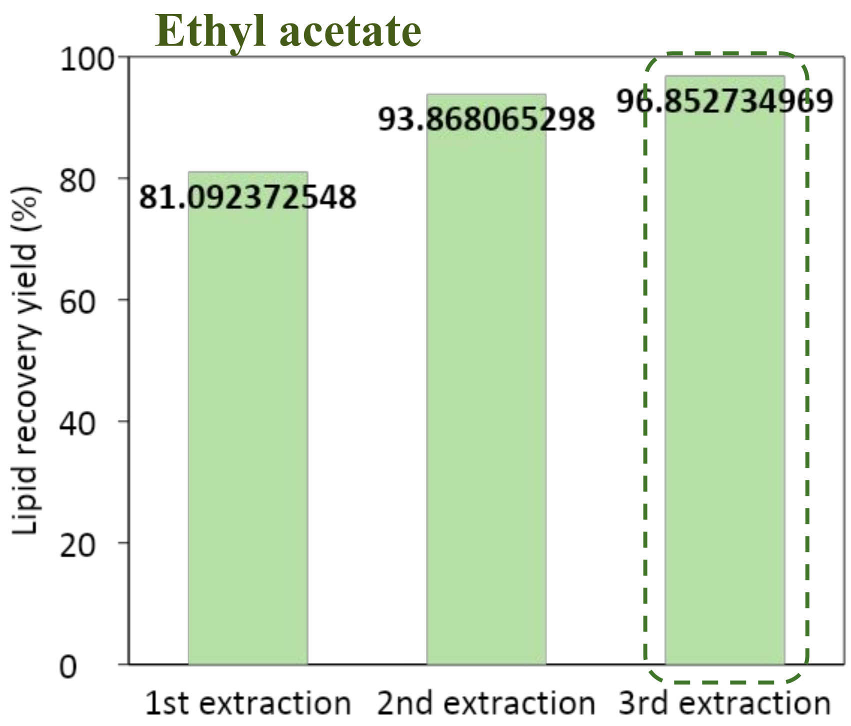
Solid to liquid ratio: 1:10 (w/v)

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

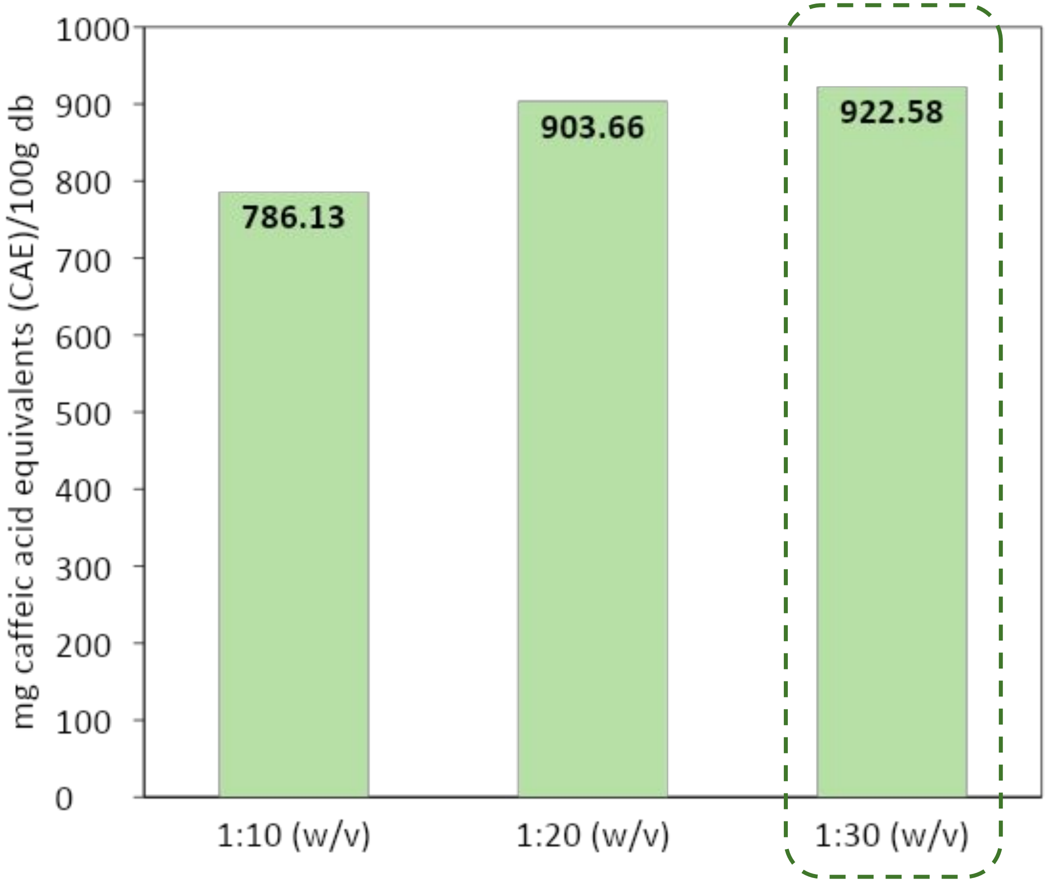
Extraction conditions:

Extraction solvent: 70% EtOH

Ultrasound 20 min, 3 times

Different solid to liquid ratio:

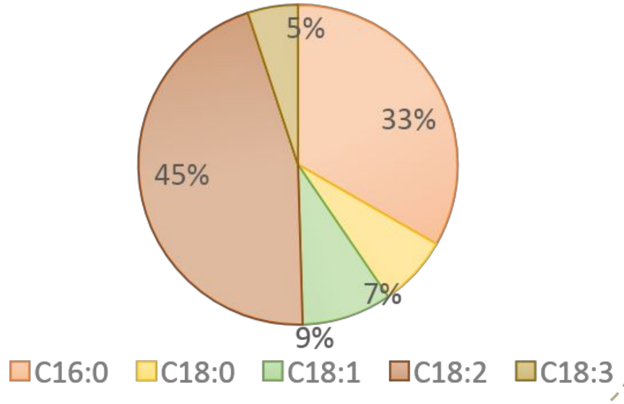
- 1:10 (w/v)
- 1:20 (w/v)
- 1:30 (w/v)



Biorefinery development of SCGs

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

Composition of coffee oil



Initial solid 1000 g (dry basis)

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

Ethyl acetate extraction

Oil 118.50 g

Recovery yield: 97%

Residual solids 881.5 g

Extraction conditions
70% EtOH, 1:10 (w/v)
Ultrasound 20 min, x 3 times

Extraction of phenolic compounds

Crude extract of phenolic compounds 54.2 g

7.87 g CAE
85% recovery yield

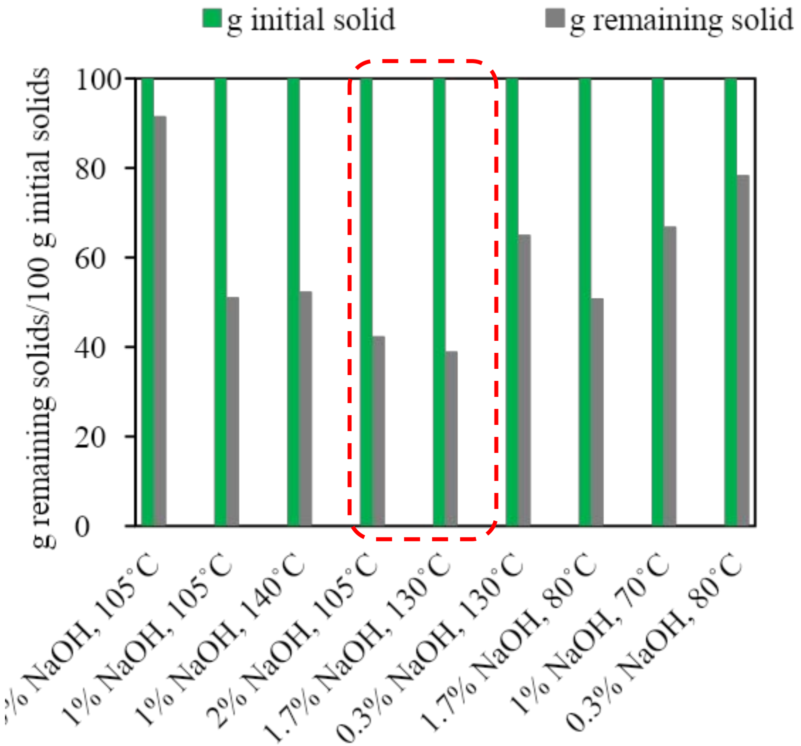
Residual solids 827.3 g

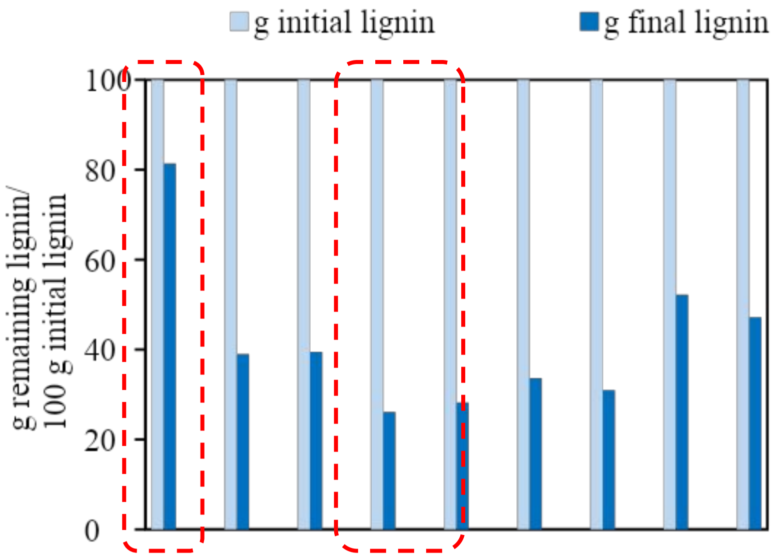
Glucan: 12.1%
Hemicellulose: 33.5%
Lignin: 31.8%
Protein: 15.2%

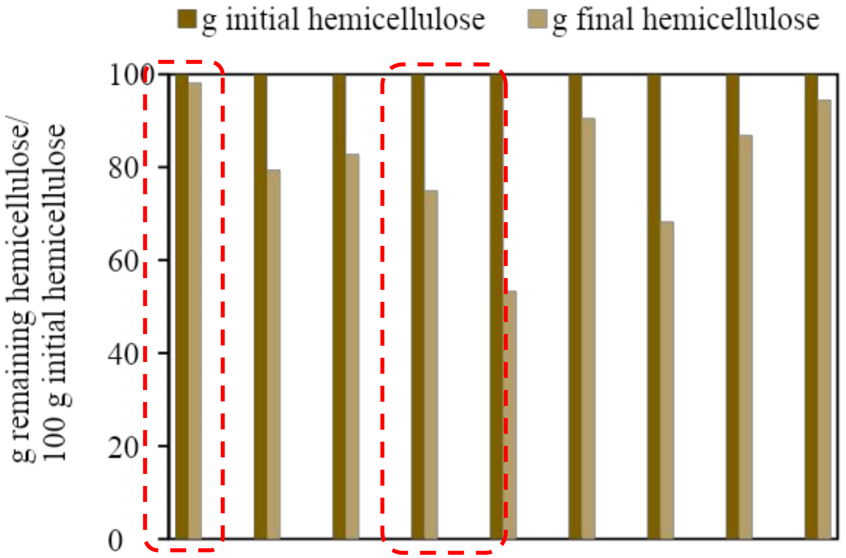
Alkaline pre-treatment → **Enzymatic hydrolysis**

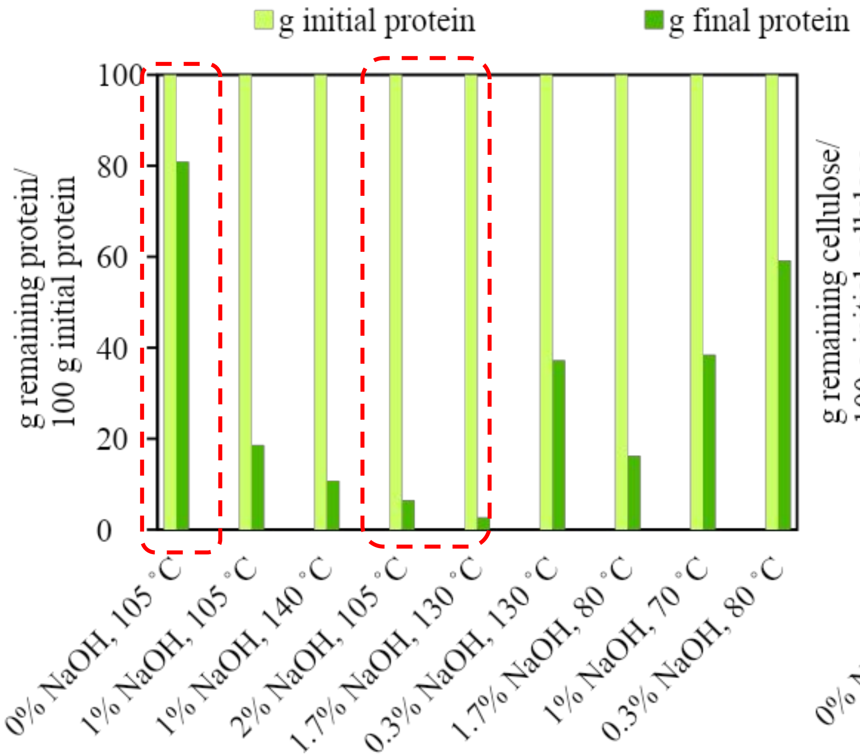
Microbial oil production

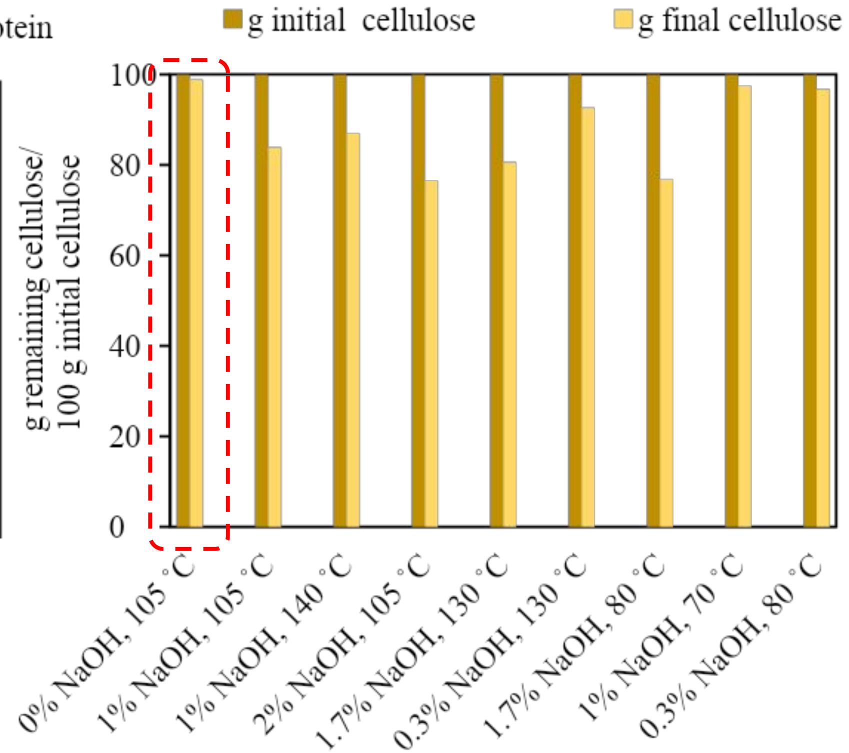
Alkaline pretreatment of residual SCGs



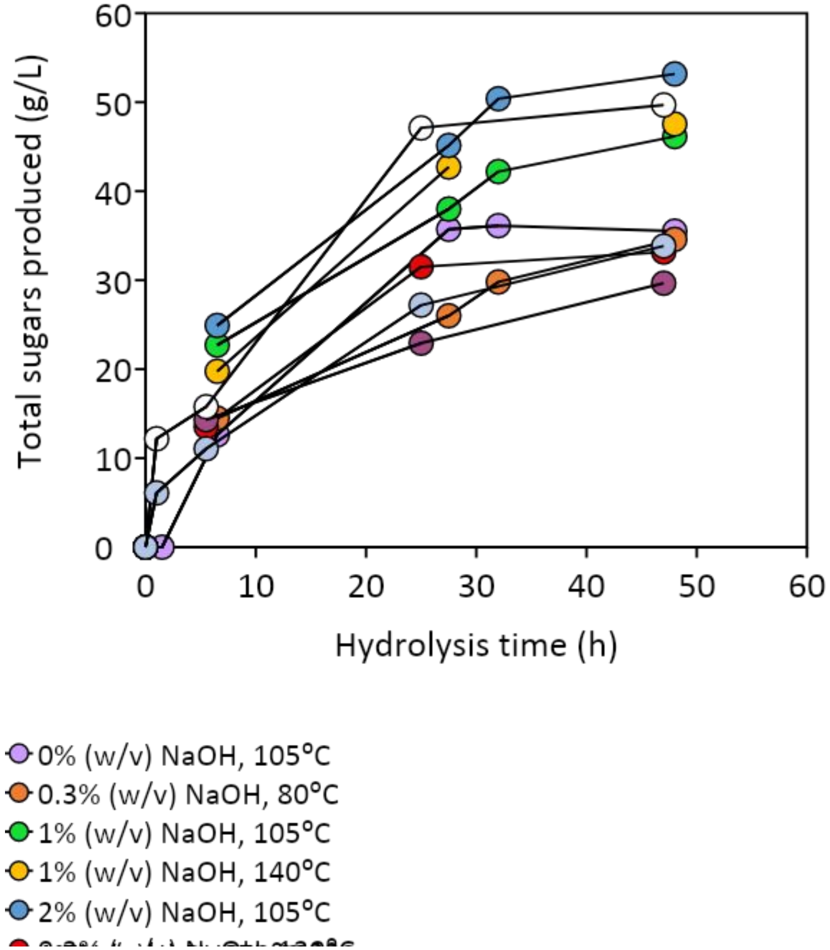


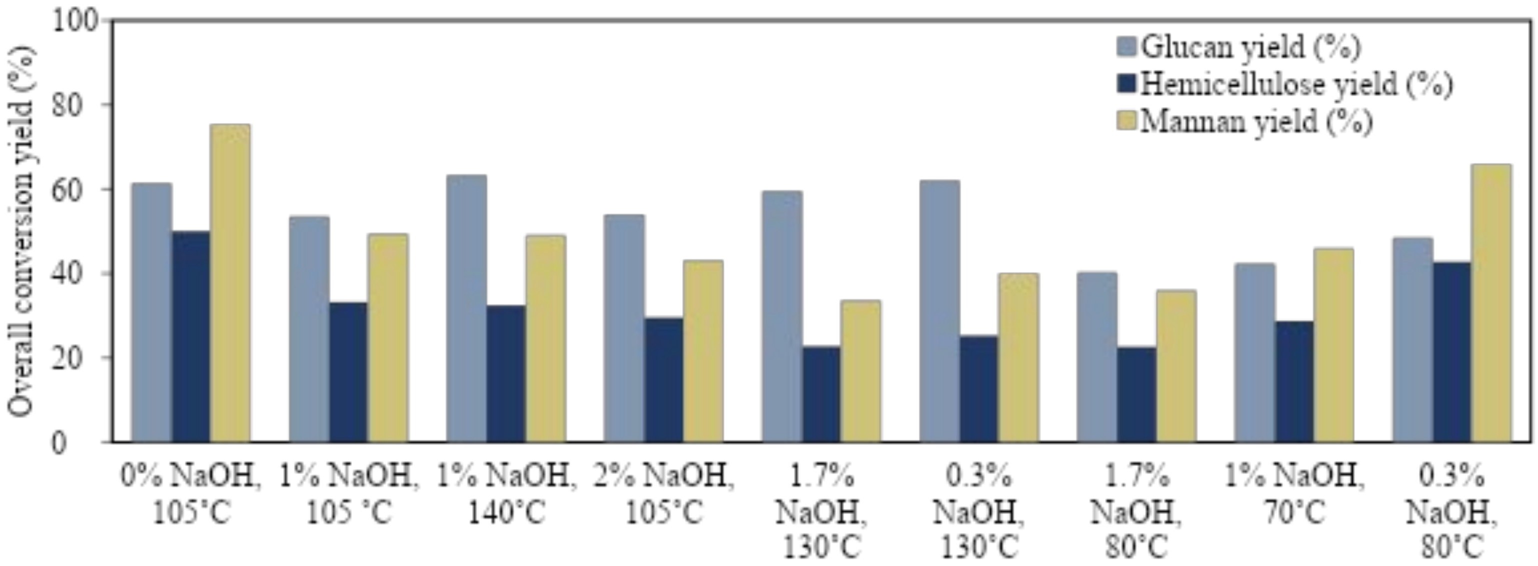


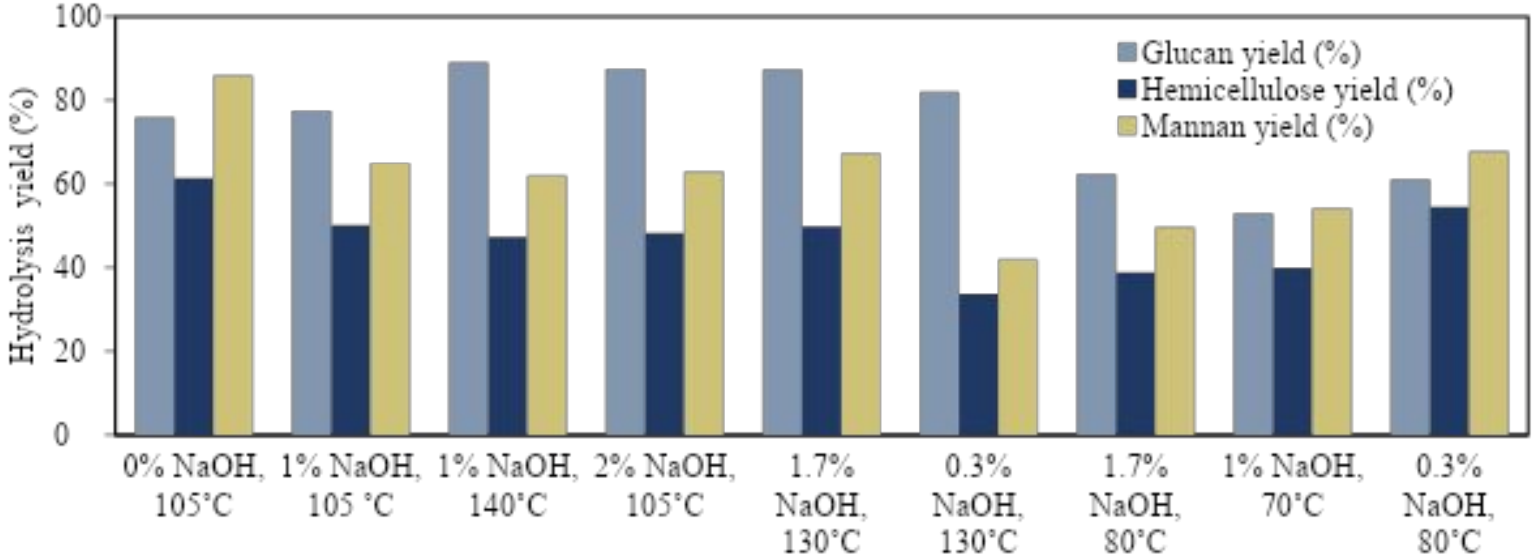




Alkaline treatment and subsequent enzymatic hydrolysis of residual SCGs







Validation of experimental design

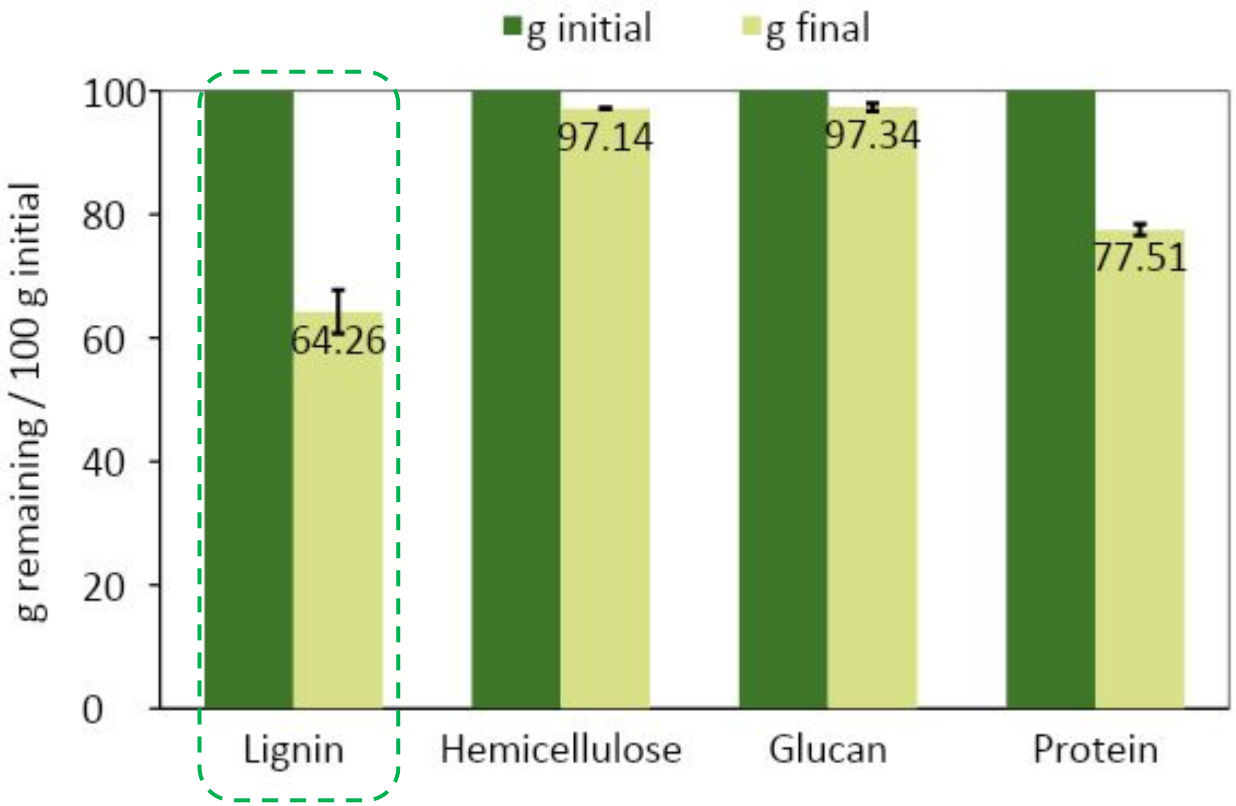
Optimisation approach Lignin removal (%)

NaOH (%, w/v) (coded)	Temperature (^oC) (coded)	NaOH (%, w/v) (real)	Temperature (^oC) (real)
-1.3234	-0.2236	0.06	99.47
Constrains			
Glucan removal (%)		Hemicellulose removal (%)	
< 2.5		< 2.5	

Optimum response:

Lignin removal: 36%

Validation of experimental design



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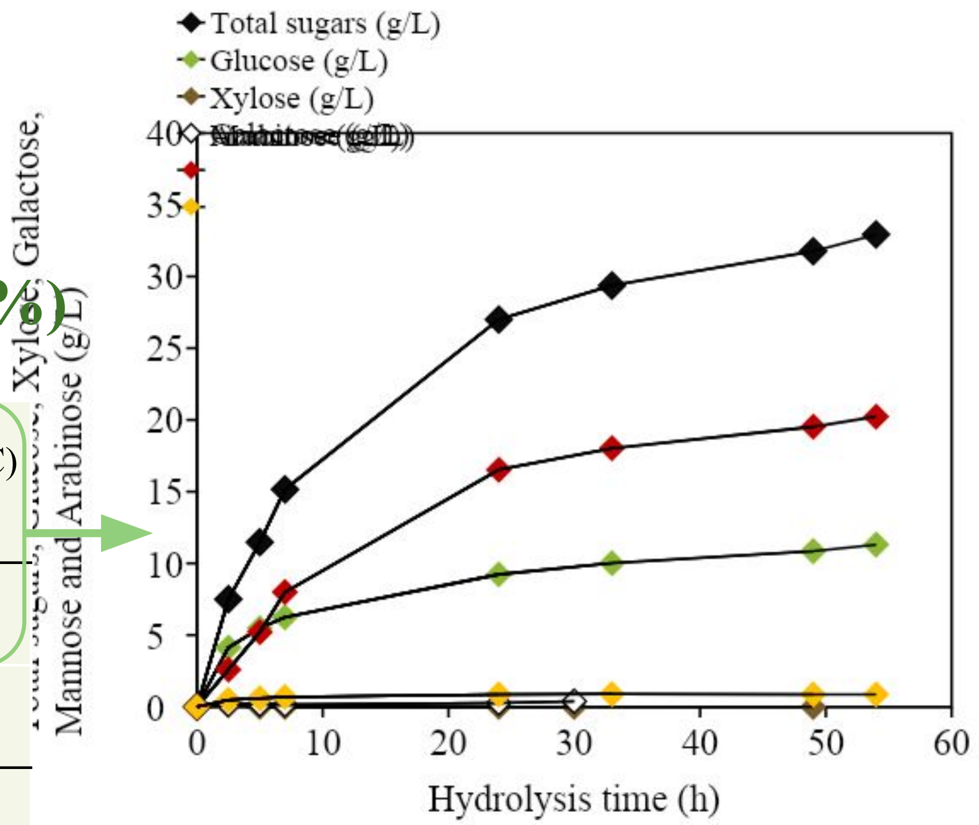
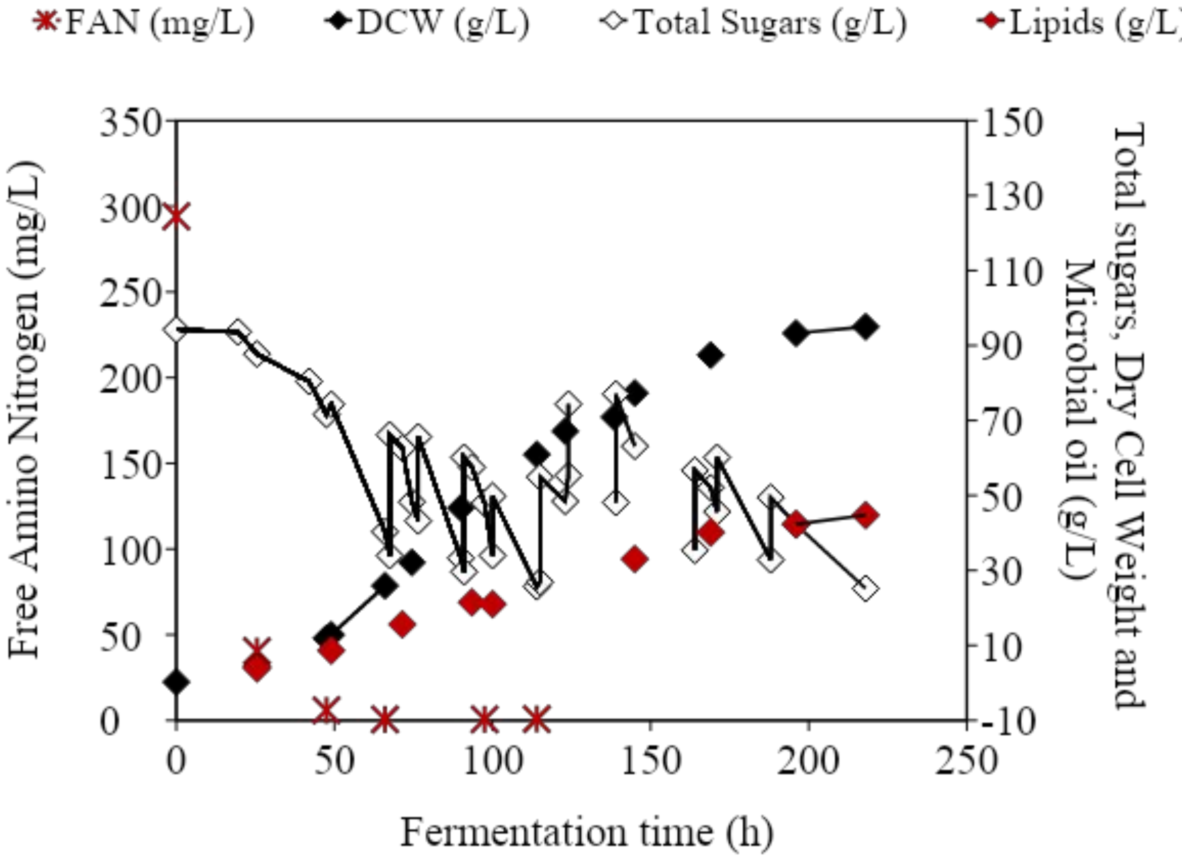
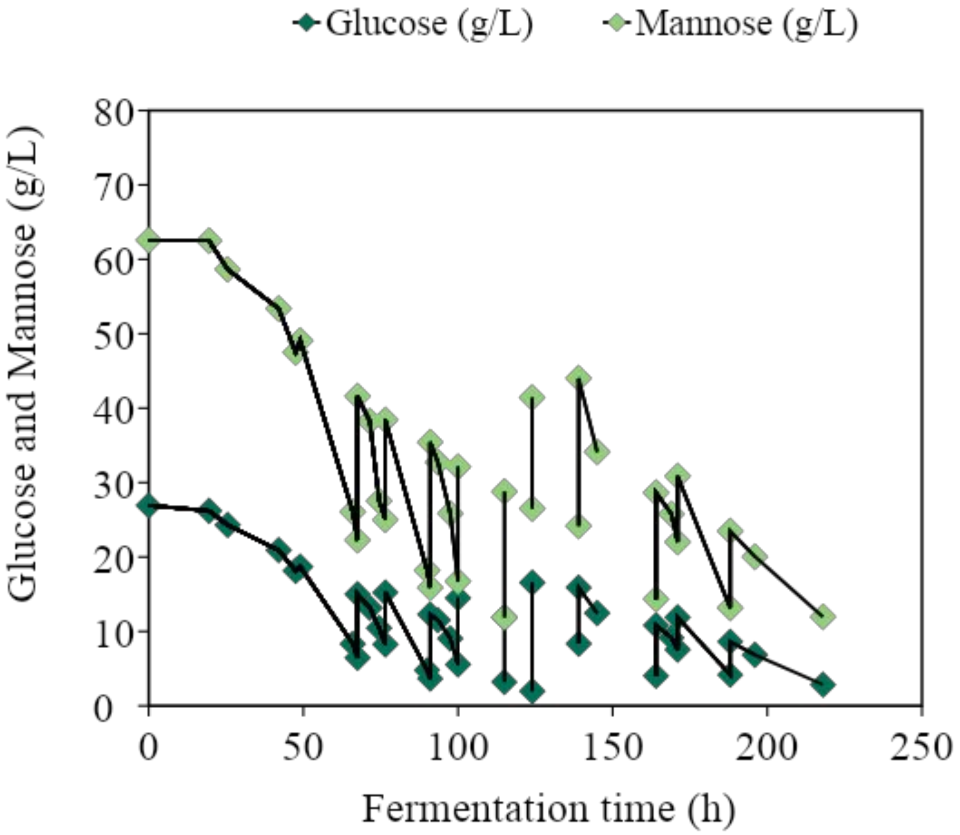


Overall glucan conversion yield:
63.4%
Overall hemicellulose conversion yield:
44.4%

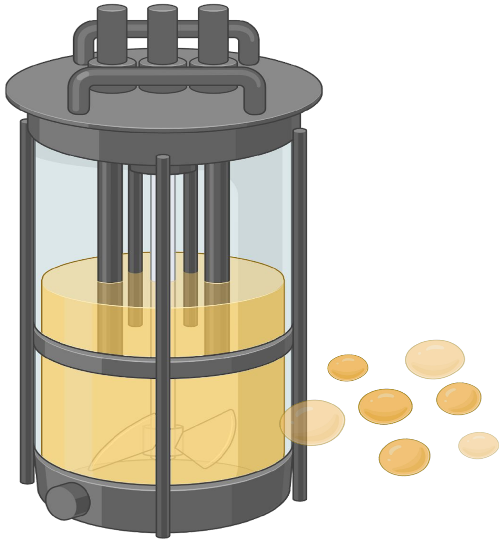
Fermentation with *Lipomyces starkeyi* for microbial oil production

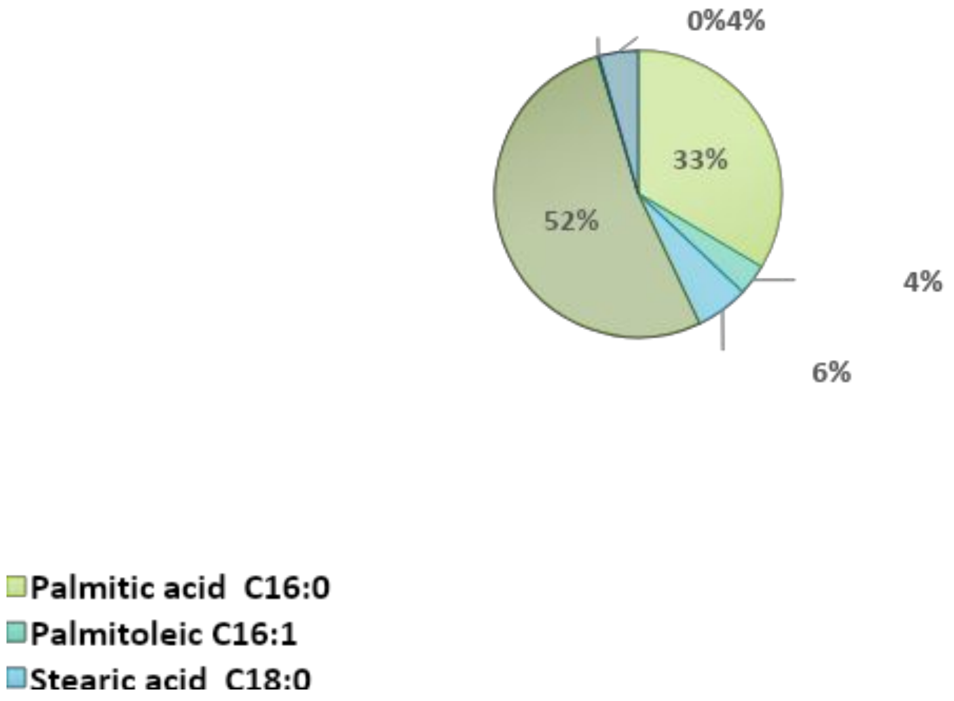




Fermentation time (h)	DCW (g/L)	Microbial oil content (%)	Microbial oil (g/L)	Yield (g/g)	Productivity (g/(L·h))
170	87.5	49.0	40.2	0.16	0.24

Fatty acid methyl esters profile





Fermentation time (h)	Palmitic acid C16:0	Palmitoleic C16:1	Stearic acid C18:0	Oleic acid C18:1	Linoleic acid C18:2	Others
25	36.4	3.1	9.9	43.8	0.5	6.4
94	33.5	3.5	6.0	52.4	0.2	4.4
218	34.3	0	6.8	56.1	1.6	1.2

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^{o}C without NaOH addition
- Optimum conditions for delignification of residual SCGs obtained were 0.06% (w/v) NaOH at 99.5^{o}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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