Microwave, ultrasound, thermal treatment, and mechanochemistry for extraction of lipids from microorganisms.

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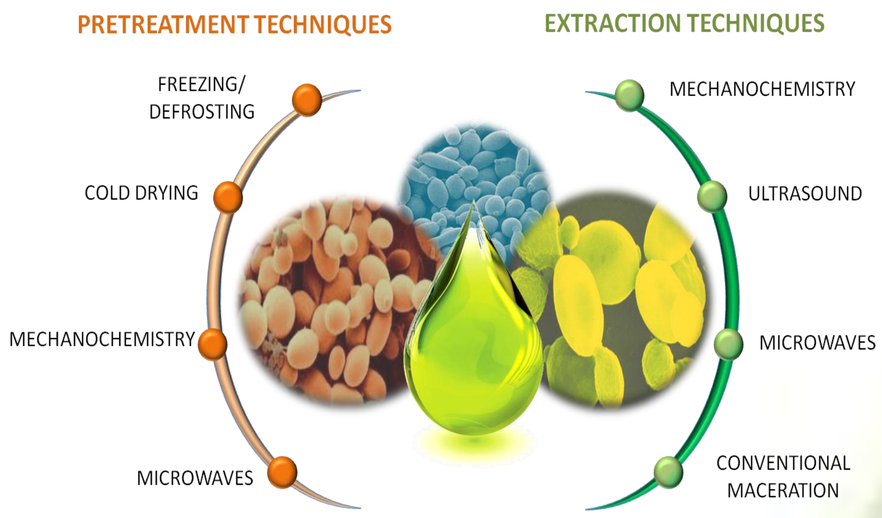
INTRODUCTION & OBJECTIVES



Microbial oil exhibits a significant proportion of various fatty acids, and thus offers a good potential as possible sources of bio fuel. Usually, solvent mixture of Bligh & Dyer method such as methanol and chloroform are currently employed for conventional lipid extraction from microorganisms [1].

Mainly microorganisms such as *Yarrowia lipolityca* yeast strain have a rigid cell wall (composed of polysaccharides such as chitin) [2]. In consequence it is difficult to obtain a complete extraction process with a simple B&D maceration, few studies has shown the efficiency of US and MW techniques for lipid recovery from microorganisms [3].

For this study, our objectives are presented bellow:

- Compare various intensified techniques for lipid recovery such as bead mills, microwaves, and ultrasound and various pretreatments such as freezing/defrosting, cold drying, ultrasound, microwaves and bead mills;
- Obtain a complete extraction of intracellular lipids from *Yarrowia lipolityca*;
- Optimize the best method of extraction;
- Comprehension of mechanism.

GLOBAL PROTOCOL

Bligh and Dyer method (1959) [4] is constituted of a mixture of chloroform and methanol (ratio of 2:1), forming a monophasic solvent system, to extract and dissolve lipids. A biphasic system is then produced by the addition of water or saline solution leading to the migration of polar compounds along with the methanol into an upper water phase and leaving the lipids in the lower chloroform phase.

Various intensified extraction processes (bead mills, microwaves, and ultrasound) and pretreatments (freezing/defrosting, cold drying, US, MW and bead mills) were compared to conventional maceration. All these extractions were performed with the standard solvent mixture of chloroform and methanol (2:1, v/v).

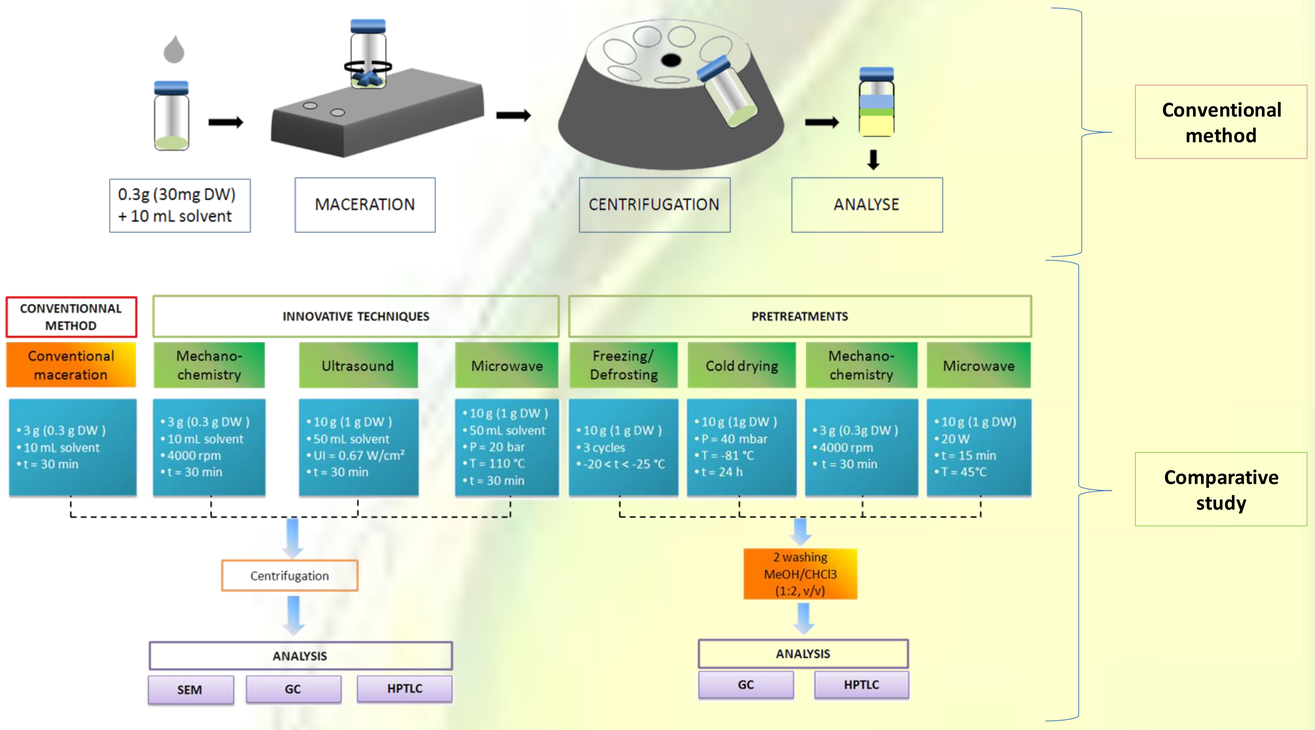


Figure 1. Global protocol of experiments

WHY MECHANOCHEMISTRY ARE MORE EFFICIENT THAN CONVENTIONAL MACERATION?

In order to understand the impact of mechanochemistry by bead mills on lipids extraction a kinetic (from equation 1) comparative study was performed between mechanochemistry and conventional maceration in the same conditions (except bead mills).

- Mechanochemistry improves clearly the kinetic of extraction (fig. 8).
- $k_{1MAC} << k_{1MEC}$ ➔ limitation of time and diffusion by conventional maceration due to the rigid cell walls of yeast.
- Beads in movement (4000 rpm) permits to disrupt and broke the cell walls of yeast (fig. 9), thus lipid recovery are maximize.

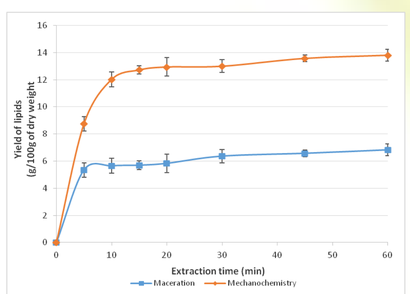


Figure 7. Kinetics of lipids extracted by mechano-chemistry compared with conventional maceration

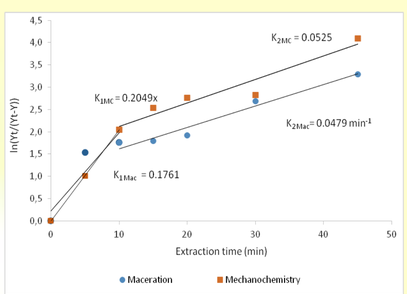


Figure 8. Modelization of extraction kinetics of lipids by mechanochemistry compared with conventional maceration

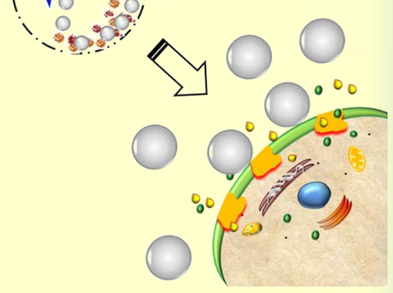


Figure 9. Mechanism of bead mills extraction on cell walls of yeast

$$\left(ln\frac{C_\infty}{C_\infty - C(t)}\right) = k_i + a$$

Equation 1. First-order rate equation derived from a quasi-steady model of extraction [5]

EXPERIMENTAL RESULTS

❖Comparison of various techniques in terms of lipids extraction yield:

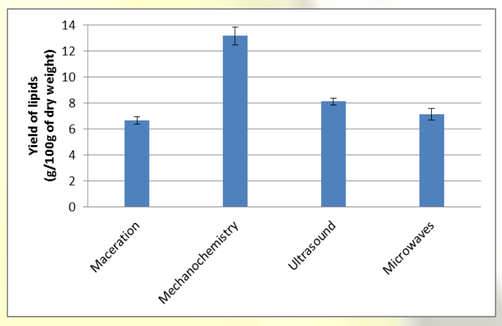


Figure 2. Effect of extraction processes on lipids extraction yield

- *Yarrowia l. IFP29* strain has a rigid cell wall.
- Difficult to obtain a complete extraction process with a simple B&D (6.23 ± 0.51 g/100 g of dw).
- Mechanochemistry using bead mills is the most efficient technique (13.16 ± 0.68 g/100 g of dw).
- Ultrasound and Microwaves are less effective than Mechanochemistry (bead mills).

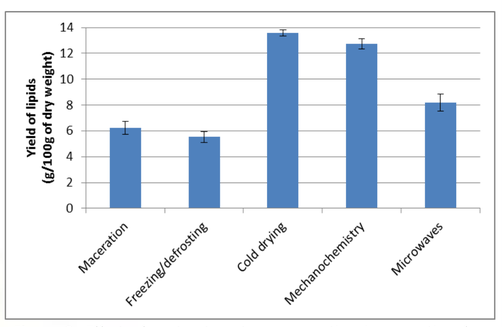


Figure 3. Effect of pretreatment on conventional maceration for lipids extraction

- Cycles of freezing/defrosting are not efficient.
- A cold drying under pressure has disrupted the cells walls and permits to reach the maximum lipids yield .
- A pretreatment by bead mills appears to be efficient (12.73 ± 0.41 g/100 g of dw).
- Microwaves are less effective than other pretreatments.

❖Process impact on lipids profiles:

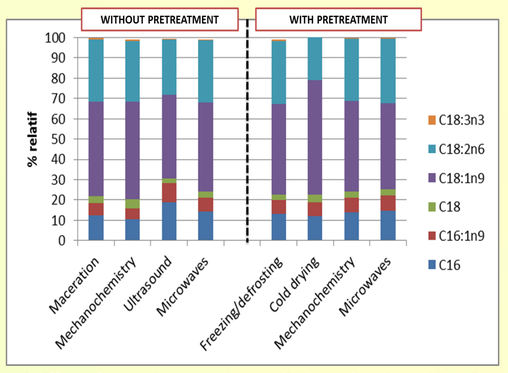


Figure 4. Effect of extraction processes on lipids profile (Analysis by Gas Chromatography)

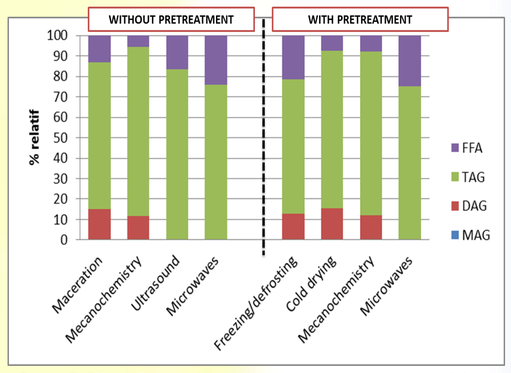


Figure 5. Effect of extraction processes and pretreatment on lipids classes (Analysis by High Performance Thin Layer Chromatography)

- Mainly fatty acids :
  - Linoleic acid (48%)
  - Oleic acid (30%)
  - Palmitic acid (10%)
  - Palmitoleic (5%)
- TAGs mainly present (80%).
- For microwaves and ultrasounds techniques => Degradation of DAG in FFA
- Similar profil between all the techniques, no selectivity.

❖Process impact on microstructure of yeast:

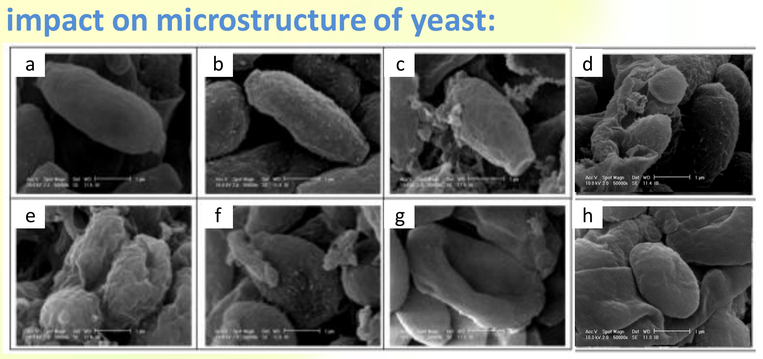


Figure 6. Effect of extraction processes and pretreatments on *Yarrowia l.* structure. Analysis by Scanning Electron Microscopy.

Effect of different treatments on microstructure of *Yarrowia l.* cells:

a) Control: cells are oval and regular (without any treatment).
b) Conventional maceration: some insignificant cellular debris on the cell.
c) Beads mill : many cellular debris, deformation of the cell.
d) Cold drying under pressure: large destruction of the cell and release of lipids into the medium.
e) Ultrasound : large deformation of the cell, some cell debris are visible.
f) g) Microwaves : large cellular debris are present into the medium.
h) Freezing/ Defrosting : deformation of the cell, but no debris.

CONCLUSION

- Ultrasound and Mechanochemistry are the most efficient techniques for maximize lipid recovery;
- No major modification of lipids profiles for all the intensified techniques used;
- Mechanochemistry enhances the transfer of diffusion and movement quantity;
- Intensified processes permit to save time and energy.

References

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