FLOW LIPASE-MEDIATED SYNTHESIS OF *N*-PALMITOYLETHANOLAMINE AND ANANDAMIDE ANALOGUES

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Introduction & aim

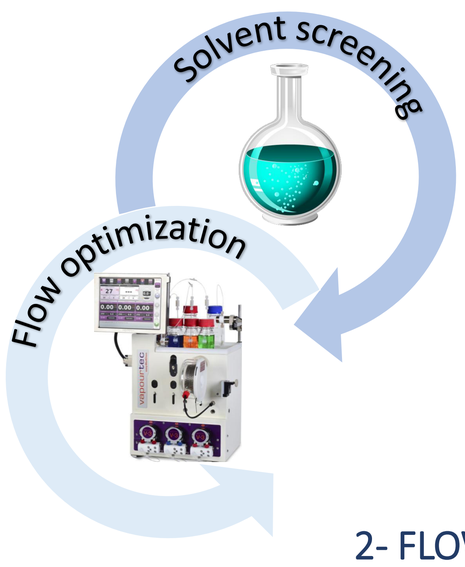
Fatty amides are naturally occurring compounds formed by the condensation of fatty acids with alkanolamines. Among them, **palmitoylethanolamide (PEA)** is an endogenous *N*-acylethanolamine (NAE) signalling molecule with anti-inflammatory and neuroprotective properties.[1] Structurally related PEA analogues, such as **oleoyl ethanolamide (OEA)** and **arachidonoyl ethanolamide (AEA, anandamide)**, act as endogenous lipid mediators, modulating various physiological processes, including metabolism, immune response, and neuronal signalling. OEA is FDA-approved as a dietary supplement for weight loss, highlighting its pharmacological relevance.[3]

However, a key challenge in their therapeutic application is **poor metabolic stability**, as they are rapidly degraded by fatty acid amide hydrolase (FAAH). To address this, the development of stable PEA and AEA analogues resistant to enzymatic hydrolysis offers a promising strategy for enhancing their bioavailability and therapeutic potential.[1,2]

To date, both chemical and biocatalytic approaches have been employed for fatty amides synthesis. Whereas, the first ones involve drastic conditions and toxic reagents, biocatalytic methods rely on chemo-selective enzymatic catalysts and milder reaction conditions.[1] *Candida antarctica* lipase B (CaL B) demonstrated to be the most suitable biocatalyst to perform the esterification and the aminolysis steps on long and (poly-)unsaturated fatty acids, such as oleic, linoleic, linolenic and arachidonic acids, with various organic solvents and solvent-free conditions.[3]

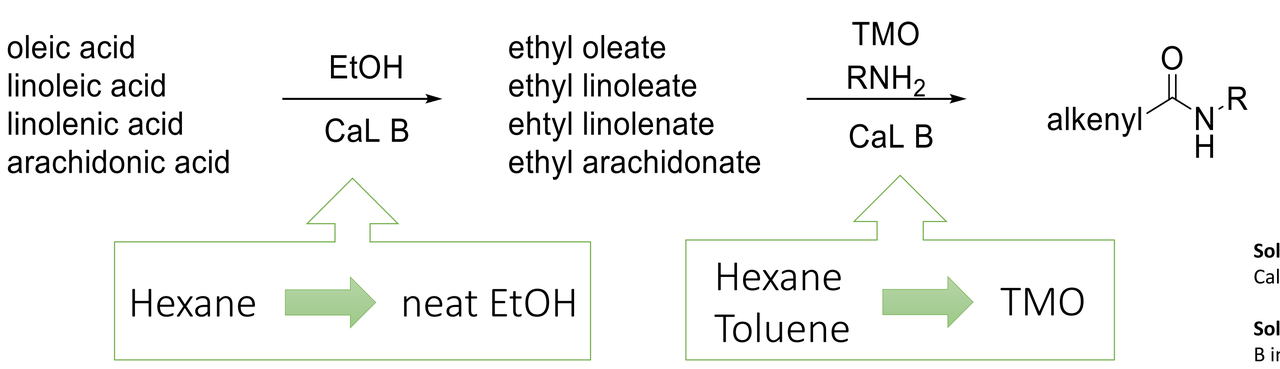
This project focuses on the implementation of a sustainable lipase-mediated synthesis of the PEA analogues exploiting the technology of **flow** and **rotating bed reactors**.

Flow synthesis optimization



1- SOLVENT SCREENING in BATCH MODE

Firstly, different solvents in batch mode for both esterification and aminolysis steps have been investigated employing linoleic acid, ethanol (EtOH) and ethanolamine (ETA) as model reactants.



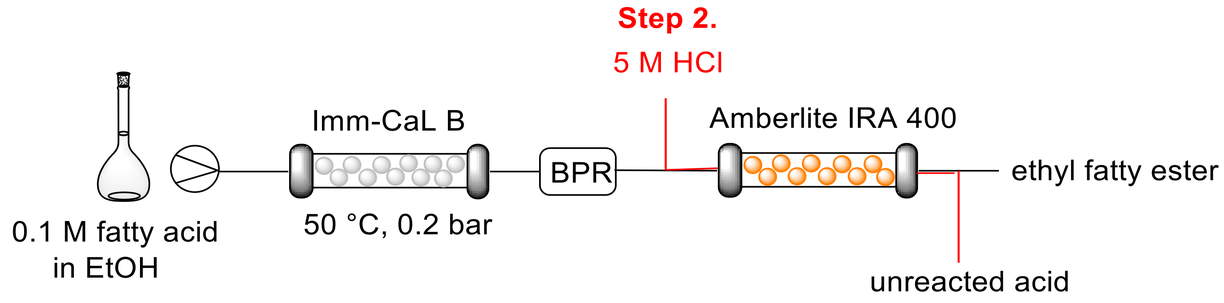
Solvent screening for esterification reaction. Reaction conditions: 40 mg/mL Cal B immobilized on acrylic resin, 24 h, 50 °C.

Solvent screening for amynolysis reaction. Reaction conditions: 60 mg/mL Cal B immobilized on acrylic resin, 24 h, 50 °C. TMO = 2,2,5,5-tetramethyloxolane.

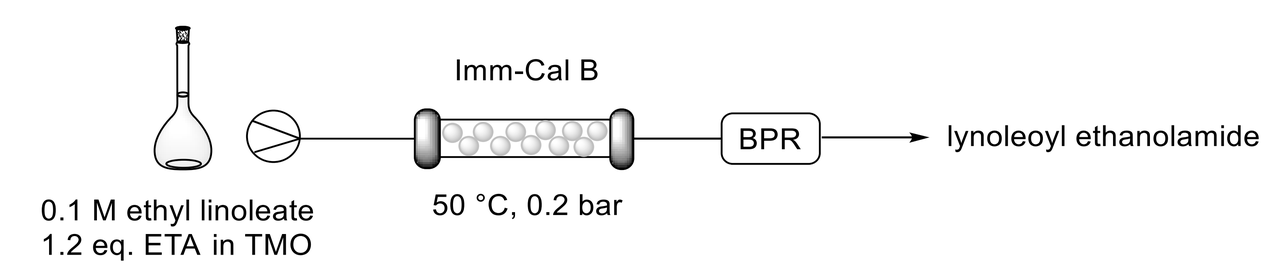
2- FLOW REACTOR OPTIMIZATION: R_t (min) and T (°C)

The neat EtOH condition for the esterification of fatty acids has been optimized under continuous conditions. Best reaction conditions: **Rt = 30 min, temperature 50°C** resulting in **yields of 85 - 90%.**

Flow optimization of the aminolysis reaction for the preparation *N*-fatty ethanolamides employing the green solvent TMO resulted in **yields** ranging **from 20 to 90%** at **Rt = 60 min** and **50°C** as best conditions.



Scheme 1 Flow esterification reaction. Reaction conditions: 0.1 M of fatty acid in EtOH at 50 °C. P= 0.2 bar. Flow rate: 0.1 mL/min.



Scheme 2 Flow aminolysis reaction. Reaction conditions: 0.1 M ethyl fatty ester, 1.2 eq ETA in TMO at 50 °C. P= 0.2 bar. Flow rate: 0.1 mL/min.

- ✓ Petroleum-based solvents replaced with green solvents or neat solvent system.
- ✓ In-line purification enhancing the automation of the system.
- ✓ Improved reaction time, from 48 h in batch mode to 90 min in continuous conditions.

Cytotoxicity and neuroprotective activity test:

The synthesized NAEs demonstrated cytocompatibility in SH-SY5Y and BV2 cells, supporting their potential neuroprotective applications.

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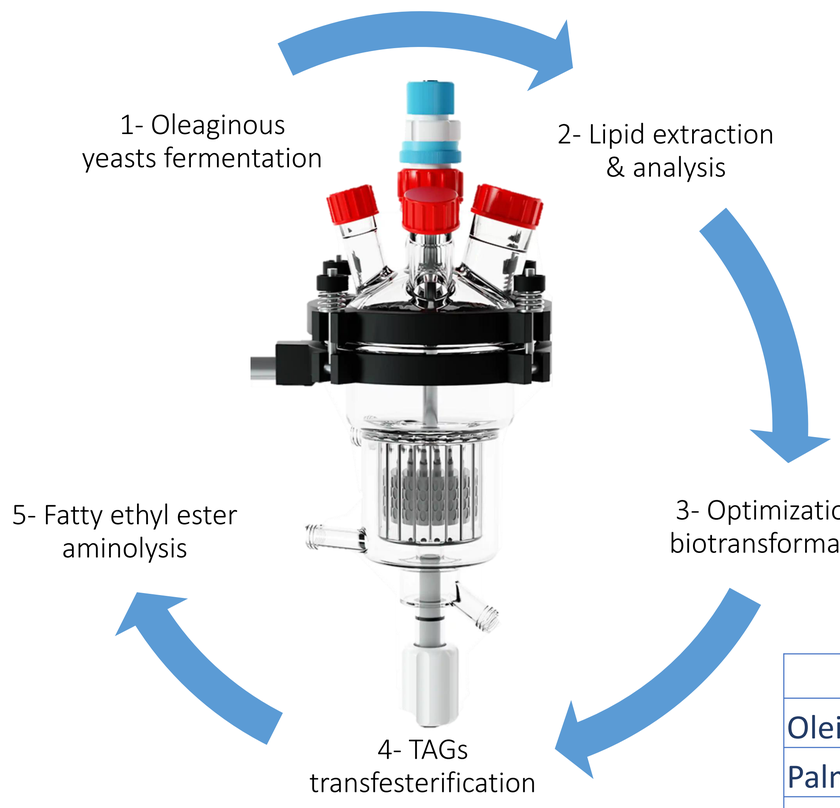
3- SCALABLE AND ECO-FRIENDLY ROUTE TO BIOACTIVE LIPID-BASED THERAPEUTICS.



To align with circular economy principles, a sustainable biocatalytic process was optimized to produce a bioactive fatty amide mixture:

1. *Cutaneotrichosporon oleaginosus* ATCC 20509 accumulating 40 g/L of TAGs when cultivated on whey permeate and candied fruit syrup in a 2L bioreactor for 90h, resulting in fatty acids composition as reported in Table 1.

2. Lipids were extracted from lyophilized biomass by ultrasound-assisted treatment in *n*-heptane/i-PrOH (3:2) and sonicated for 15 min (lipids yield 39%).[4]

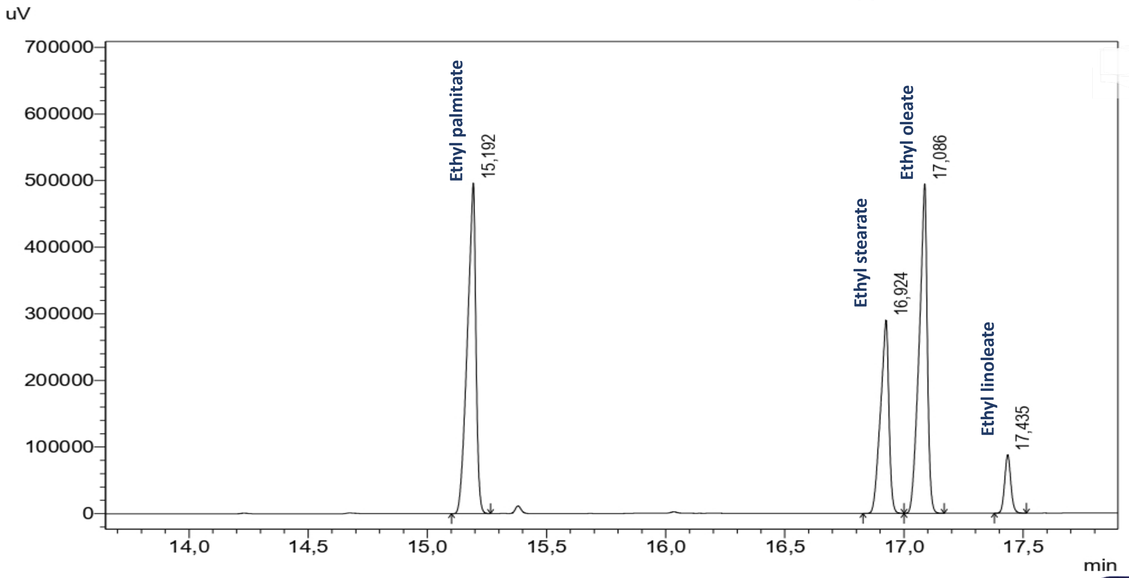
3. Processes optimization: direct transesterification of TAGs in neat EtOH and aminolysis of ethyl fatty acids in different green solvents.

4. **Enzymatic transesterification** (Novozym 435®) of extracted TAGs into ethyl fatty esters was performed in neat EtOH for 4h, yield: 99% (Scheme 3).

5. **Aminolysis** of fatty esters to produce a mixture of bioactive fatty amides with ETA in **eucalyptol** yields to ≥99% in 6h.

FA	%
Oleic acid (C18:1)	45%
Palmitic acid (C16:0)	30%
Stearic acid (C18:0)	19%
Linoleic acid (C18:2)	6%

Table 1 Lipids profile analysis .



Scheme 3 GC chromatogram of TGA transesterification .

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