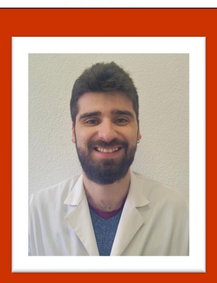
Enzymatic synthesis and purification of structured lysophospholipids from DHA-rich algal oil



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KEYWORDS

Lysophosphatidylcholine (LPC), free fatty acid (FFA) docosahexaenoic acid (DHA), algal oil, lipases.

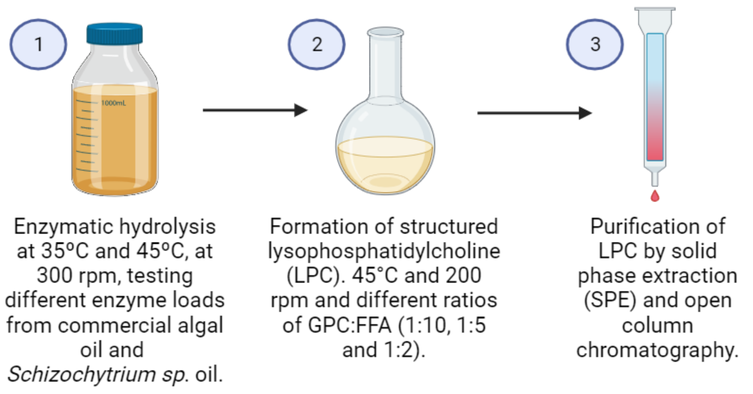
1. INTRODUCTION

- Deficiency of docosahexaenoic acid (C22:6 n-3, DHA) in the brain has been associated with certain neurodegenerative diseases, such as Alzheimer's and Parkinson's disease.
- Phospholipids are currently of great interest as they are more effective than triacylglycerides (TAGs) in incorporating various fatty acids into the cell membrane. (1,2)

2. AIM

The goal of this study is to obtain structured lysophospholipids enriched in DHA ω-3 fatty acid from commercial algal oil and to purify them by different techniques.

3. METHODS



The three steps were monitored by high-performance liquid chromatography (HPLC).

Figure 1. Summary of equipment used, and methodology applied.

❖ Commercial algal oil (DHA-rich algal oil 40%S-IF Type 400, ProgressBiotech) was used for this study, containing 45.64% ± 0.01% DHA, and also, oil extracted from *Schizochytrium sp*. with a very similar fatty acid composition was used to optimize hydrolysis.

1) Commercial RML was used for hydrolysis. It was scaled 20 times to have enough for the following steps.
2) Commercial RML was also used to produce structured lysophosphatidylcholine under vacuum conditions, first optimizing the reaction with linolenic acid. These conditions were then tested with FFA of commercial algal oil and the process was scaled 10 times.
3) Silica (500 mg), amino (500 mg), and modified graphene cartridges were tested for SPE purification. Using tetrahydrofuran (THF) for cartridge conditioning, hexane:ethyl acetate (70:30) and chloroform:methanol (50:50).

For the open column, silica conditioned with t-butanol was used, and ter-butanol, t-butanol:methanol (50:50), methanol, and methanol:water (95:5) were eluted.

4. RESULTS AND DISCUSSION

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- At 35 ºC the results are not as expected, since after 48 hours we found 45.64% ± 4.26% FFA and we still had 45.82%± 3.44% TAG. Therefore, an attempt was made to optimize the reaction by increasing the temperature to 45 ºC and doubling the enzyme load.
- As can be seen in **Figure 2**, with the increase in enzyme load and temperature, the reaction rate increases, obtaining 98.47%± 0.21% FFA at 24 hours, whereas the previous reaction at the same time obtained 25.69% ± 2.14%.
- In the x20 scale-up using commercial algal oil, 100% FFA is also achieved at 48h. The use of this other source of DHA is due to the fact that the amount of oil extracted is limited.

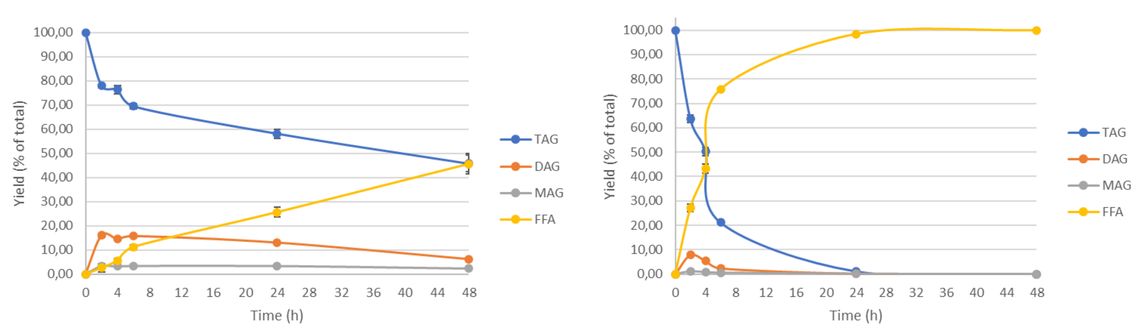

Figure 2. On the left, kinetics of enzymatic hydrolysis of RML at 35 ºC and 300rpm. On the right, kinetics of enzymatic hydrolysis with double load of RML at 45 ºC and 300rpm from *Schizochytrium sp. oil*.

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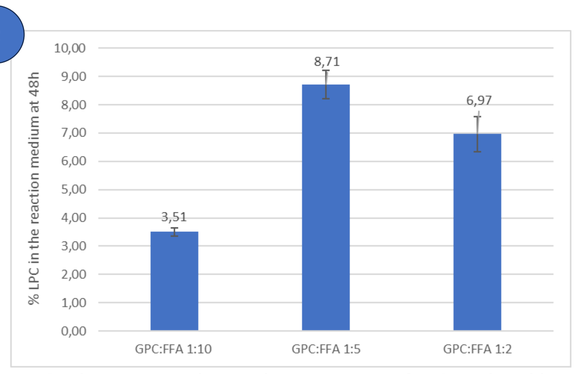

Figure 3. Plot corresponding to the percentage of LPC produced by RML from linolenic acid at 45 ºC and 200 rpm under vacuum at different GPC:FFA ratios.

As shown in **Figure 3**, using the ratio GPC:FFA 1:5 gives the highest proportion of LPC in the reaction, possibly more LPC is achieved than with the 1:2 ratio as the viscosity in this reaction is very high and being in a solvent-free system, their diffusion in the medium is not the best.

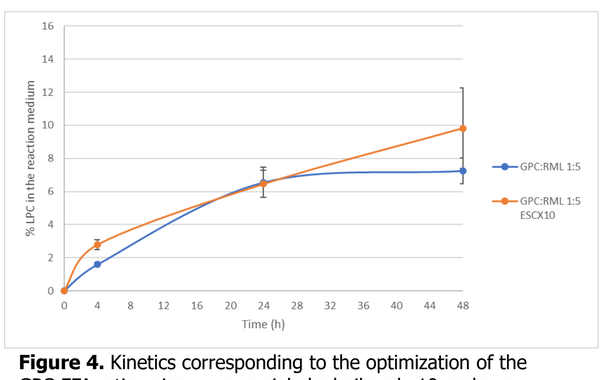

Figure 4. Kinetics corresponding to the optimization of the GPC:FFA ratio using commercial algal oil and x10 scale-up.

When using FFA from commercial algal oil, 7.25% ± 0.78% LPC is obtained, which is lower than when using linolenic acid, but when the process is scaled up, 9.83% ± 2.43% is obtained, although with a high SD compared to the value obtained.

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Figure 5. Modified graphene cartridge.

Purification of LPC was achieved with the 3 materials used:

- Adding 6 mL of hexane:ethyl acetate (70:30) to elute the FFA and 3 mL of chloroform:methanol to elute the LPC in the case of the **amino** cartridge.
- Adding 6 mL of hexane:ethyl acetate (70:30) to elute the FFA and 3 mL of chloroform:methanol and another 3 mL of methanol to elute the LPC in the case of the **silica** cartridge.
- Adding 4 mL hexane:ethyl acetate (70:30) to elute FFA and 2 mL chloroform:methanol to elute LPC in the case of the **modified graphene** cartridge.

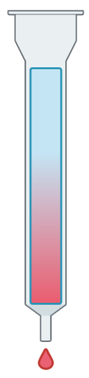

Figure 6. Example of an open column.

In the open column, the LPC elutes when methanol:water (95:5) is added, the advantage of this technique is the possibility to add more of the mixture. In the SPE between 50 and 30 mg are added, in the open column 300 mg are added and there is the possibility to scale up the process.

5. CONCLUSIONS

- In the enzymatic hydrolysis, 100% FFA was obtained, so the reaction was optimized, and scaling up was also achieved to have more FFA for further experiments.
- GPC:FFA ratios were optimized and LPC was achieved using FFA from commercial DHA-rich algal oil.
- Upon purification of the structured DHA-rich phospholipid, bioactivity tests would need to be performed to demonstrate evidence from the literature.

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