GREEN PROCESS FOR ENRICHMENT OF EPA/DHA FROM MARINE SOURCES BY BIOTECHNOLGY AND SUPERCRITICAL FLUIDS

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ABSTRACT

EPA and DHA (eicosapentaenoic acid and docosahexaenoic acid) from marine sources are concentrated from 30-40% till 70% in three green steps: transesterification with enzyme, concentration by supercritical CO_2 and transesterification with enzyme.

INTRODUCTION

EPA (eicosapentaenoic acid – fig. 1) and DHA (docosahexaenoic acid – fig. 2) are essential fatty acids found in marine sources like fish, algae and micro algae. According to European Commission authorized health claims, EPA/DHA contribute to normal function of heart and brain. In nutraceutical applications, EPA/DHA must be enriched because of technical requirement. Enrichment can be done by chemical process using catalyst and/or organic solvent or by a green process that involves **biotechnology** and **supercritical fluids**.

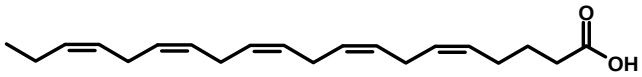

Figure 1: EPA

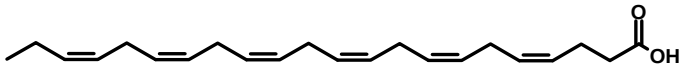

Figure 2: DHA

Usually, EPA/DHA are found in fish oil and micro algae oil as triglycerides (fig. 3) with a 30-40% concentration based on total fatty acids.
The green process is divided in three steps:

1. transesterification of fatty acids with enzymes and ethanol to produce FAEE (fatty acid ethyl ester)
2. concentration of EPA/DHA ethyl ester by supercritical fluids
3. transesterification of fatty acids ethyl ester with enzymes and glycerol to produce triglycerides with a higher content of EPA/DHA.

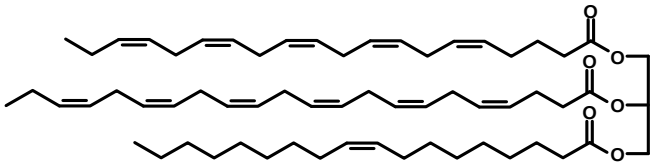

Figure 3: triglyceride

In the 1st and 3rd steps, different enzymes are compared to evaluate their transesterification efficiency and recovery.
The second step, which is enrichment of EPA/DHA, is done in a supercritical liquid-liquid column. Different parameters are tested (pressure, temperature, flow rate, reflux) to reach the best balance between concentration and price. Others trials are in progress with supercritical chromatography to reach a highly purified EPA/DHA for pharmaceutical applications.

MATERIALS AND METHODS

Transesterification are done in a 1L double jacket reactor with mechanical stirrer.

In the first step, enzymes, ethanol, water and fish oil are mixed and stirred. After reaction, water is added to separate enzymes and glycerol from FAEE. Different parameters are tested: type and quantity of enzyme, type and quantity of ethanol (water content), reaction time.

In the third step, enzymes, FAEE and glycerol are mixed and stirred. After reaction, water is added to remove ethanol. Different parameters are tested: type and quantity of enzyme and reaction time.

Fish oil is a commercial fish oil containing 35% of EPA/DHA (Croda). Ethanol is absolute ethanol from agricultural origin (Sigma-Aldrich). Enzymes come from Lyven: Lipolyve CC (*Candida rugosa*) and Lipolyve R (*Rhizopus arrhizus*).

CO2 is a green solvent used in food, perfumery, cosmetic, biomedical, dyeing…CO2 is mainly used for plant extraction in supercritical state: above 73bar and 31°C (fig. 4).

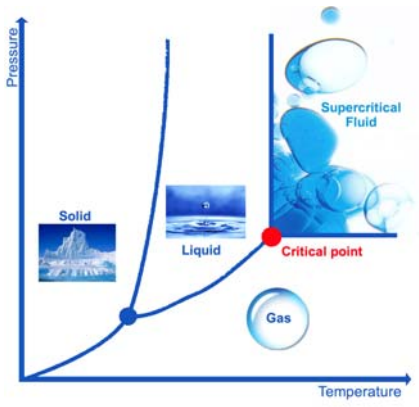

Figure 4: carbon dioxide state diagram

CO_2 trials are done on a Waters pilot unit equipped with a liquid-liquid column (fig. 5 & 6).

Figures 5 and 6: Waters supercritical CO2 pilot and counter current column

In the second step, the aim is to separate as much as possible EPA/DHA ethyl ester from other FAEE (oleic acid ethyl ester for example). Different parameters are tested to reach the best balance between concentration of EPA/DHA and price:

- pressure : 74 to 120bar
- temperature: 30 to 45°C
- with or without reflux
- CO_2 flow rate
- FAEE flow rate

RESULTS

In the first step, for transesterification, the best results are obtained with:

- Lipolyve R at 0.1%
- Absolute ethanol without water. 10 parts of alcohol for 1 part of fish oil
- Reaction temperature 52°C
- Reaction time: 3h.

With these parameters, 85% of triglyceride are converted to FAEE.

In the second step, highest selectivity between EPA/DHA EE and the others FAEE is around 3.5 at 76bar (fig. 7) at 35°C with a reflux at the top of the column obtained with a 65°C temperature.

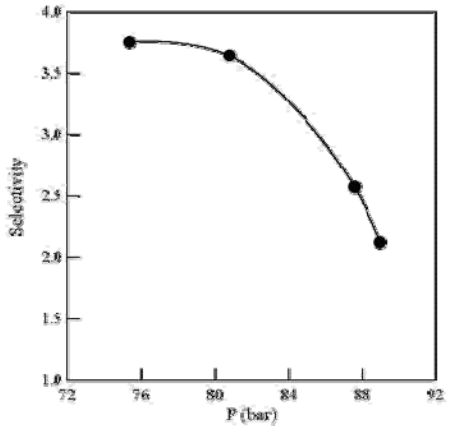

Fig 7: selectivity between EPA/DHA EE and C18 FAEE in CO_2

With these parameters, EPA/DHA EE concentration reaches up to 70% with a 80% EPA/DHA recovery.

In the last step, for transesterification, the best results are obtained with:

- Lipolyve R at 0.2%
- Reaction temperature 50°C
- Reaction time: 3h.

With these parameters, 95% of FAEE are converted to triglyceride.

CONCLUSION

With this process, the enriched EPA/DHA oil contains up to 70% of ω-3.

These 3 steps process has many advantages:

- ↳ green process (no organic solvent or chemical reaction)
- ↳ higher recovery of EPA/DHA
- ↳ low temperature
- ↳ no oxidation/degradation of EPA/DHA

Highest concentration can be obtained using UPC² from Waters (Ultra Performance Convergence Chromatography) for pharmaceutical applications.