Synthesis of structured triacylglycerides starting from commercial salmon oil by lipase catalysis under supercritical conditions

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Summary

The consumption of long-chain polyunsaturated fatty acids (LC-PUFA) belonging to the omega-3 series (namely, eicosapentaenoic and docosahexaenoic acids; EPA and DHA, respectively) has been reported to lead to inhibition of the development of different kinds of illnesses such as cardiac, circulatory and inflammatory. Bioavailability of such fatty acids has shown to depend on their location in triacylglycerides (TAG) molecules, so that location at the *sn*-2 position has shown to be the most profitable. Consequently, a great attention has been accorded to the synthesis of structured TAG (STAG), which include LC-PUFA at such location. For this objective, lipases application has been found very convenient, especially if employed under CO_2-supercritical conditions, so that a solubility increase of lipid/hydrophobic compounds in non-polar media is provoked, tis leading to an enhancement of synthesis processes such as esterification and transesterification.

In this study, STAG were prepared starting from refined commercial salmon oil. For it, lipase NovozymeR 435 under CO_2-supercritical condition was applied. According to the reaction time employed, four different fractions were obtained. Location of EPA and DHA in the resulting glycerol backbone was detected by mass spectrometry (MALDI-TOF) analysis. In all fractions obtained, a marked reduction of the starting TAG was observed; additionally, a marked decrease of EPA content at the *sn*-2 location was observed, while a substantial increase of the DHA content at such position was implied. Interestingly, the fraction obtained after the longest reaction time period (i.e., 2 hours) led to the highest yield of DHA in the resulting STAG molecule.

Physiological advantages of EPA+DHA:
- Arterial pressure
- TAG level in blood
- Inflammation, endothelial function and cardiac diastolic function

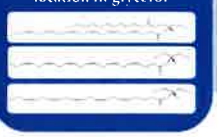
Consumption of 0.250 g/day of EPA+DHA leads to a reduced coronary illnesses risk and sudden cardiac death. DHA consumption leads to positive development of brain and retina during the foetal period and the two first years.

TAG showing omega-3 LC-PUFA in the *sn*-2 location show nutritional and physiological activity, being easily absorbed. This fact can be explained on the basis that pancreatic lipase hydrolyses the two extreme positions of TAG, being employed as energy source; furthermore, fatty acids located at the *sn*-2 position are absorbed easily and quickly at the enterocyte (Dominiczak *et al.*, 2014).

The employment of supercritical (CO_2SC) conditions allows obtaining fractions of a mixture products designed in agreement to diet needs (King, 2004).

The most important advantages of CO_2SC versus other liquid solvents are its high diffusivity, low viscosity and surface tension, which allow a fast mass transfer in enzymatic reactions (Rubio-Rodríguez *et al.*, 2010).

Manufacture structured lipids by enzymatic treatment under CO_2SC conditions leading to changes in the EPA/DHA location in glycerol



Identification of location changes by mass spectrometry (MALDI-TOF)

Materials and methods

Refined and commercial salmon oil (RCSO)

GLC analysis of total fatty acids (TFA)

Enzymatic partitioning in CO_2SC (140 bar, 40 °C, 20% lipasa Novozyme 435)

Four extractions
Extractions 1, 2, 3 and 4 (30, 60, 90 and 120 min, respectively)

Preparation of the sample for the mass spectrometry analysis

Sample weighing in a 250-mL flask

Addition of warm ethanol (95%) and phenolphthalein. Titration with NaOH

Washing with hexane/distilled water (three times) and recovery of hexane phases

Washing of hexane phases till complete soaps elimination. Distillation under pressure (37 °C)

Hexane sample is diluted to 1/1 with an isopropanol/chloroform mixture

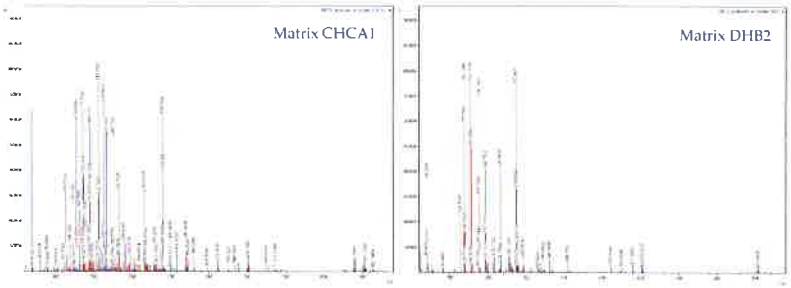
MALDI-TOF analysis

Fatty acid composition

Fatty acid	g/100g TFA
C12:0	0.06 ± 0.00
C14:0	2.90 ± 0.01
C16:0	12.76 ± 0.03
C16:1 9t	0.07 ± 0.00
C16:1 9c	3.74 ± 0.01
C17:0	0.22 ± 0.00
C 17:1 10c	0.13 ± 0.00
C18:0	3.64 ± 0.01
C18:1n9	36.95 ± 0.08
C18:1n7c	3.32 ± 0.00
C18:2 9t 12t	0.06 ± 0.00
C18:2 9c 12c	15.7 ± 70.07
C20:0	0.32 ± 0.00
C18:3 6c 9c 12c	0.22 ± 0.00
C 20:1 8c	0.44 ± 0.01
C 20:1 11c	1.84 ± 0.04
C 18:3 9c 12c 15c	4.91 ± 0.00
C 20:2 11c 14c	1.34 ± 0.01
C 22:0	0.36 ± 0.00
C 20:3	0.33 ± 0.00
C 22:1	0.29 ± 0.01
C 20:4	0.35 ± 0.04
C 22:2	0.12 ± 0.01
C 20:5 (EPA)	3.92 ± 0.04
C 24:1	0.27 ± 0.03
C 22:4	0.15 ± 0.03
C 22:5	1.68 ± 0.05
C 22:6 (DHA)	3.83 ± 0.04
EPA+DHA	7.75 ± 0.04

Results and discussion

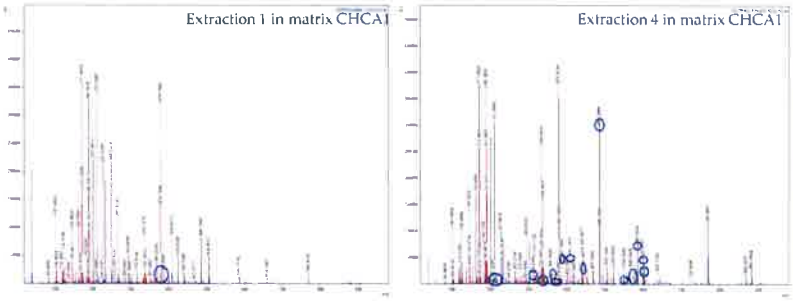
Mass spectra showing the presence of EPA and DHA at the *sn*-2 location in the RCSO



Summary of the number of presences of EPA and DHA identified in different matrices of RCSO

Matrix	EPA in *sn*-2	DHA in *sn*-2	DHA in *sn*-3	EPA (*sn*-2) DHA (*sn*-3)
CHCA1: 10 mg/mL in 70/30 (v/v) acetonitrile/0.1% trifluoroacetic acid	52	2	12	4
CMBT1: 10 mg/mL in methanol	0	0	0	0
DHB1: 77 mg/mL in methanol	0	0	0	0
DHB2: 77 mg/mL in 0.1% (p/v) trifluoroacetic acid/methanol	12	0	12	0

Mass spectra showing the presence of EPA and DHA at *sn*-2 location in RCSO



- The final composition of EPA+DHA (g/100g TFA) in extractions 3 and 4 was 6.05 and 6.14, respectively.
- Most abundant TAG in RCSO were: **12:0/20:5/22:5, 18:0/20:5/20:5, 16:0/20:5/22:5, 18:3/20:5/20:5 and 16:1/20:5/22:4.**
- Monoglycerides and diglycerides were also observed in all extractions; most abundant were: **14:1/20:5/–, 12:0/22:6/–, –/20:5/ – and –/22:6/–.**

Conclusions

- Evaluation of the RCSO by GLC showed a fatty acid composition corresponding to a marine oil and confirmed the presence of TAG in the initial mass spectra.
- The RCSO possesses EPA and DHA at the *sn*-2 location according to the mass spectrometry analyses. Matrix CHCA1 was chosen as the most convenient.
- Fractionation in CO_2SC leads to changes in the fatty acid composition of fractionated samples obtained when compared to the initial oil when the reaction is catalysed by the B-lipase enzyme (i.e., *Candida antarctica*), a significant decrease of the EPA and DHA content being evident in the latest extractions carried out (90 and 120 minutes).
- Fractionated samples obtained under CO_2SC conditions led to changes in the position of EPA and DHA when analysed by mass spectrometry (MALDI-TOF); thus, monoglycerides including EPA and DHA at *sn*-2 location were obtained in extraction 4 at 120 min.
- Mass spectrometry allowed the identification of possible positional changes of EPA and DHA in glycerol of the STAG, this showing a reduction of the number of TAG in all extractions when compared to the non-fractionated oil. EPA content in the *sn*-2 location decreased in the different extractions but a DHA content increase at the *sn*-2 location was evident in extraction 4 when compared to the initial oil.

References: **Dominiczak MH *et al.* (2014).** "Digestion and absorption of nutrients: The gastrointestinal tract" In: Baynes JW, Dominniczak MH. Medical Biochemistry. 4th ed. London: Saunders Elsevier. **King JW. (2004)** "Critical fluid technology for the processing of lipid-related natural products" Comptes Rendus Chimie 7:647-659. **Rubio-Rodríguez N et al. (2010).** "Production of omega-3 polyunsaturated fatty acid concentrates: A review". Innovative Food Science & Emerging Technologies, 11:1-12.