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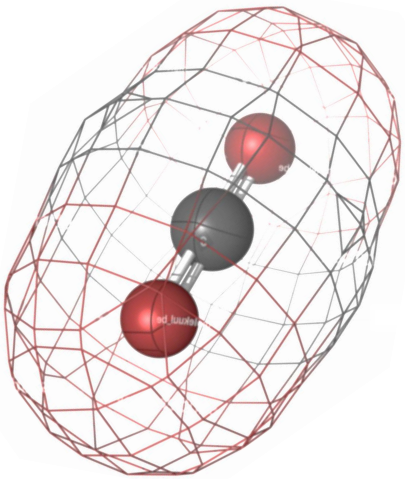

Valorización de aceite de pescado mediante tecnologías de dióxido de carbono supercrítico

R. Melgosa, M.T. Sanz, Ó. Benito-Román, A.E. Illera, S. Beltrán

Dpto. Biotecnología y Ciencia de los Alimentos
Universidad de Burgos

Madrid, 2018

outline

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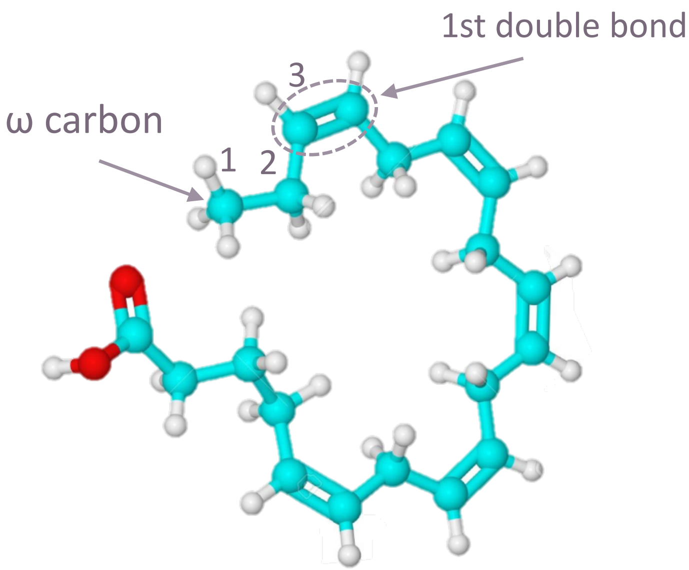
1) Introduction
   1. Omega–3 polyunsaturated fatty acids
   2. Why are omega–3 consumed?
   3. The problem of omega–3 oxidation
   4. Supercritical alternative
2) Materials & methods
3) Results
4) Concluding remarks

omega-3 PUFAs

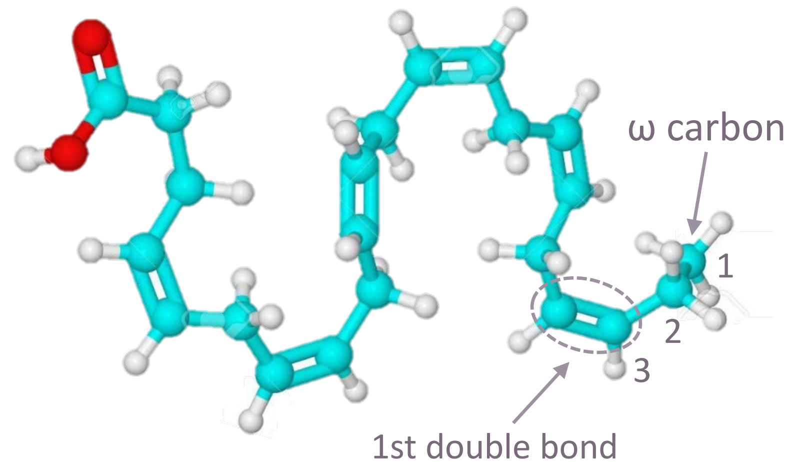
Fatty acid: carboxylic acid with a long aliphatic chain

Unsaturation: double covalent bond between adjacent carbon atoms

Eicosapentaenoic acid (EPA, C20:5 *n*–3)



Docosahexaenoic acid (DHA, C22:6 *n*–3)



why are omega-3 consumed?
healthy effects

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Healthy effects of omega−3 are linked to their structural and regulatory roles

Cardioprotective effects

P.M. Kris-Etherton, *et al.*, Circulation. 106 (2002) 2747–2757.

Neuroprotective

S. Kalmijn *et al.*, Ann. Neurol. 42 (1997) 776–782.
M.C. Morris *et al.*, Arch. Neurol. 60 (2003) 940–946.
F. Calon *et al.*, Neuron. 43 (2004) 633–645.

Antiinflammatory

P.C. Calder, J. Nutr. 142 (2012) 592S–599S.

Immunomodulatory

J.R. Hibbeln *et al.*, Lancet. 369 (2007) 578–585.
S.C. Larsson *et al.*, Am. J. Clin. Nutr. 79 (2004) 935–945.

why are omega-3 consumed?
some data

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NIH National Institutes of Health *Turning Discovery Into Health*

A Joint Effort of the Office of Dietary Supplements and the National Library of Medicine

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Dietary Supplement Label Database

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Home | Search | About | Contact Us | Help Menu

Search e.g. calcium

Ingredient - Omega-3

1,906 product(s) contain the ingredient "Omega-3"

almost 2000 products in USA

The following results include DSLD products that contain any of the following:
"OMEGA-3 FATTY ACIDS"; "OMEGA-3"; "DELETE"; "TOTAL OMEGA-3"; "TOTAL OMEGA-3 FATTY ACIDS"; "OMEGA 3"; "OMEGA 3 FATTY ACIDS FROM FISH OIL"; "OTHER OMEGA-3'S"; "OTHER OMEGA-3 FATTY ACIDS AS TG"; "TOTAL EPA"; "OMEGACHOICE OMEGA-3 ESSENTIAL FATTY ACIDS:"; "PROVIDES 600MG OF TOTAL OMEGA-3 FATTY

Read More [+]

Dietary Supplement Label Database (NIH)

http://www.dsld.nlm.nih.gov/dsld/index.jsp

(accessed june 2018)

Global Market: US$ 1.8 bn/year

Transparency Market Research (2013) Global Fish Oil Market Industry Analysis, Size, Share, Growth, Trends and Forecast, 2012–2018

https://www.transparencymarketresearch.com/fish-oil.html

(accessed june 2018)

the problem of omega-3 oxidation

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Fishing for answers: is oxidation of fish oil supplements a problem?

Cameron-Smith, D., Albert, B.B., Cutfield W.S., J. Nutr. Sci. 4, e36 (2015) 1–2.

[···] in Canada, 50 % [of 171 over-the-counter omega-3 supplements] exceeded voluntary limits for at least one measure of oxidation [···]. In the USA, 27 % of products tested were found to have more than twice the recommended levels of lipid peroxides, while in South Africa and New Zealand more than 80 % of supplements tested exceeded recommended levels.

[···] The levels of oxidation now described in four independent studies since 2012 (analyzing 260 n-3 PUFA products) suggest that the general public is consuming oxidized products [···].

Importantly, the biological effects and health consequences of consuming oxidized fish oil supplements are not yet established. In 2010, the EFSA panel on biological hazards concluded that 'information on the level of oxidation of fish oil (···) and related toxicological effects in humans is lacking'.

the problem of omega-3 oxidation

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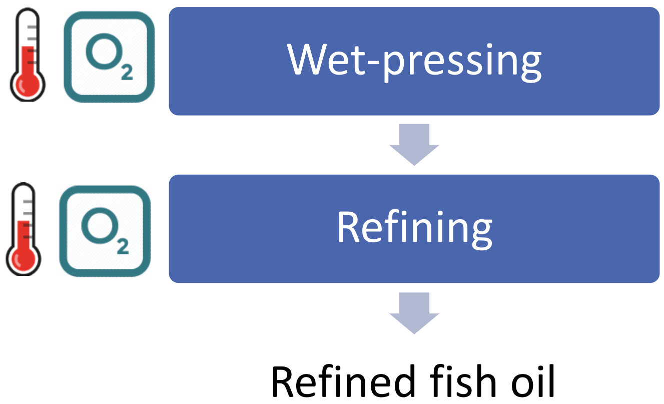

It is not surprising that many retail fish oil products are oxidized, considering the complex process from ocean catch through to the final consumer product.

[…] small pelagic fishes, caught off the coast of Peru and Chile […] transported to shore, processed by fractionation into fish meal and crude fish oil. […] stored in large tanks before being shipped on for further refining, particularly to China […], involving several steps with repeated heating at high temperatures. […]

Cameron-Smith, D., Albert, B.B., Cutfield W.S., J. Nutr. Sci. 4, e36 (2015) 1–2.

the problem of omega-3 oxidation

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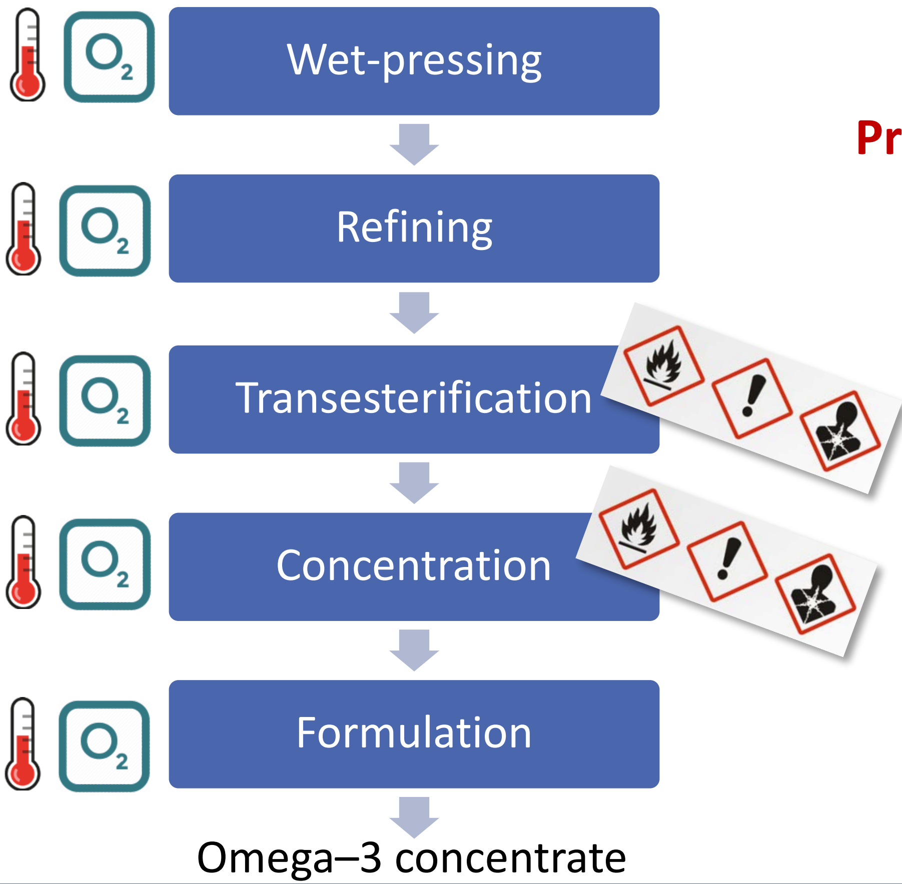

Production of omega–3 concentrates

Further processing may increase oxidation even more

Organic solvents

Toxicity, environmental concerns

More processing steps (more oxidation)

supercritical alternative

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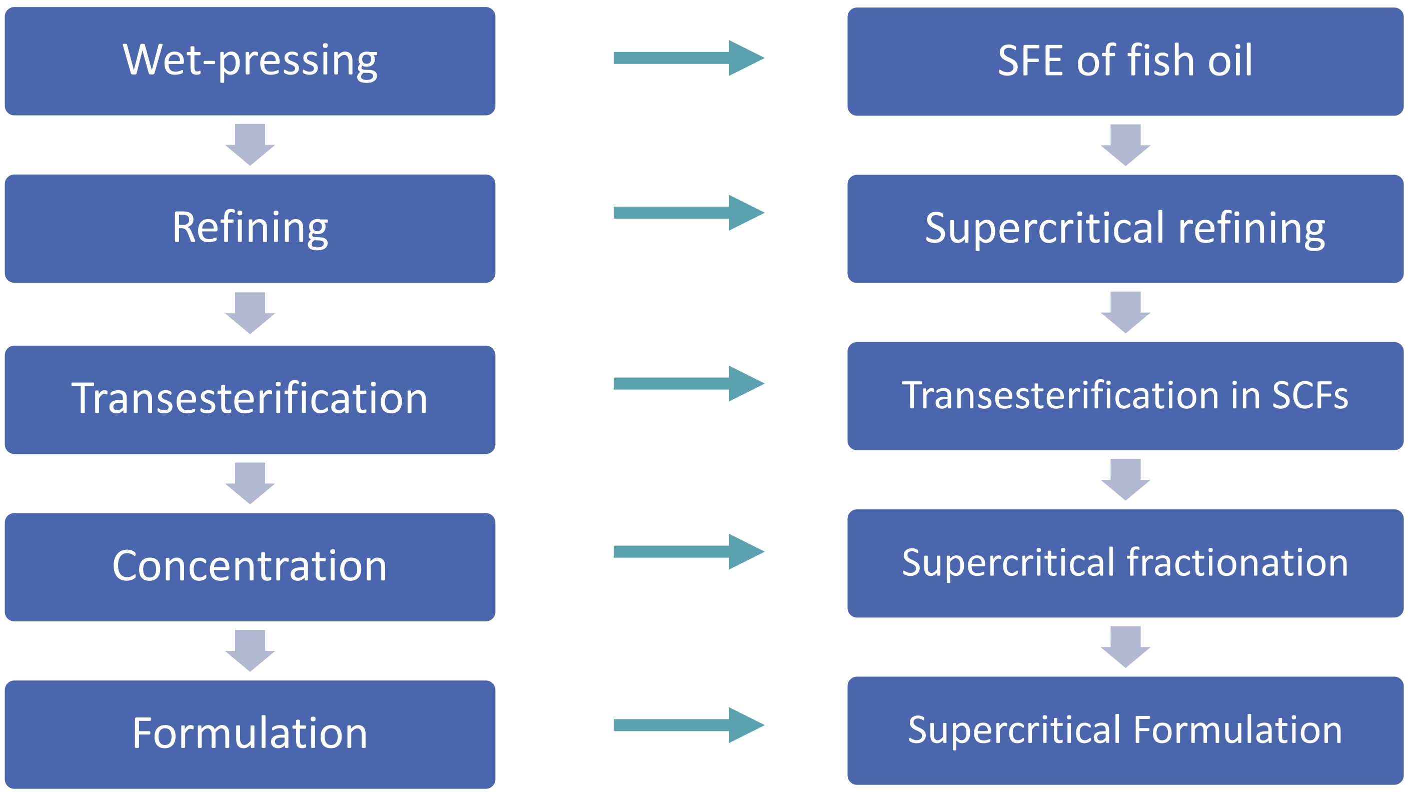

Flow diagram for conventional production of omega–3

Flow diagram for **supercritical** production of omega–3

supercritical alternative

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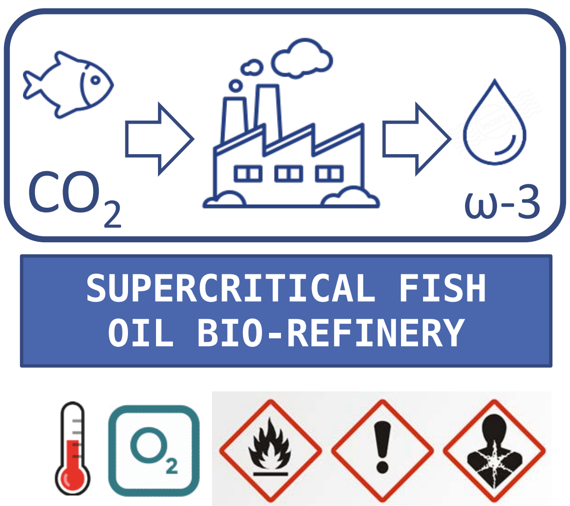

- SFE of fish oil
- Supercritical refining
- Transesterification in SCFs
- Supercritical fractionation
- Supercritical Formulation

Flow diagram for **supercritical** production of omega–3

supercritical alternative

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Study the lipase-catalyzed ethanolysis of fish oil in supercritical CO_2

Focus of this work

- SFE of fish oil
- Supercritical refining
- Transesterification in SCFs
- Supercritical fractionation
- Supercritical Formulation

Flow diagram for **supercritical** production of omega–3

reaction and biocatalyst

3-step transesterification of fish oil TAGs with ethanol as acyl donor

Biocatalyst: Lipozyme RM IM

Rhizomucor miehei

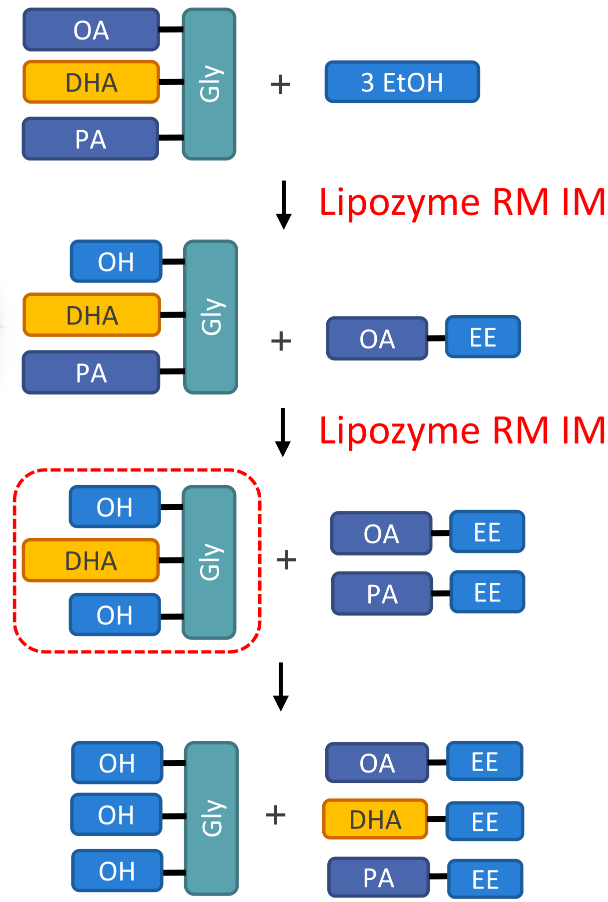
(5 % wt. of substrates)

more resistant to SC-CO_2 than CALB

(Melgosa *et al*., 2015)

immobilized - easy recovery (filtration)

sn-1,3-specific - 2-MAGs can be obtained



Melgosa, R., Sanz, M.T., Solaesa, Á.G., Bucio, S.L., Beltrán, S., J. Supercrit. Fluids 97 (2015) 51-62

residual activity of commercial lipases

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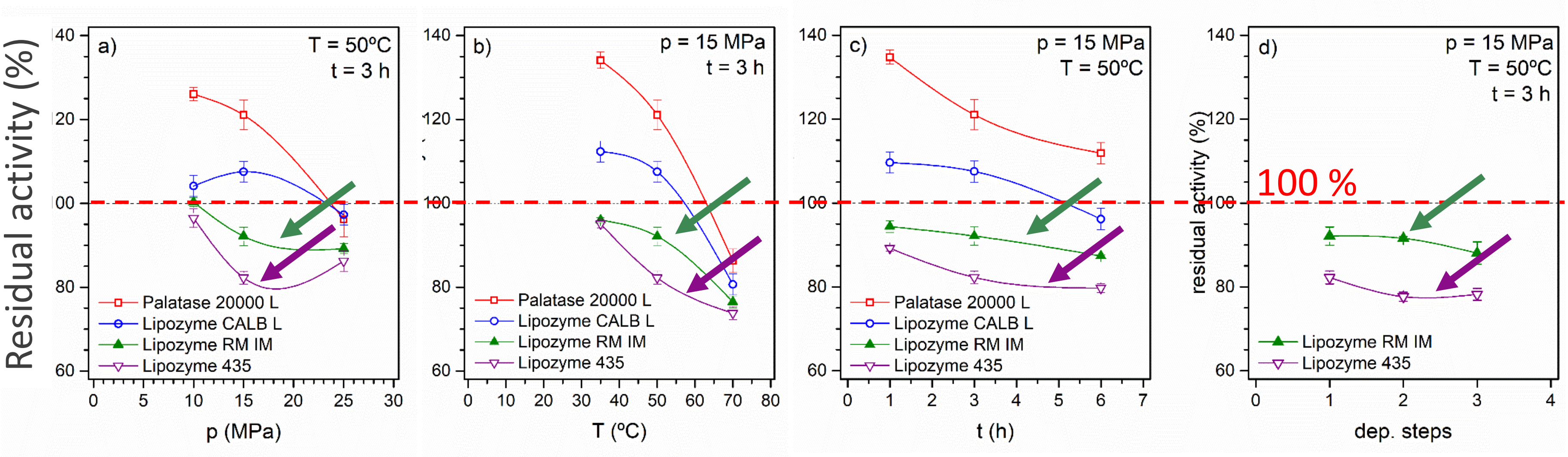

10-25 MPa

35-70 ºC

1-6 h

1-3 cycles

Lipozyme RM IM

Lipozyme 435

fish oil

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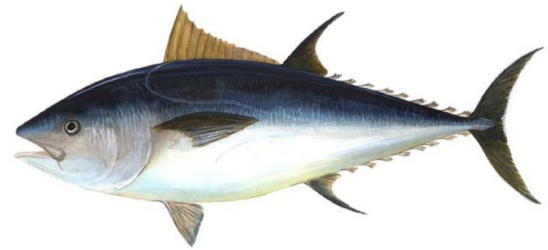
Mixture of tuna and sardine refined oils

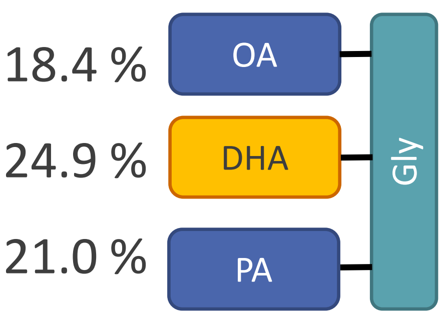
AFAMSA S.A.

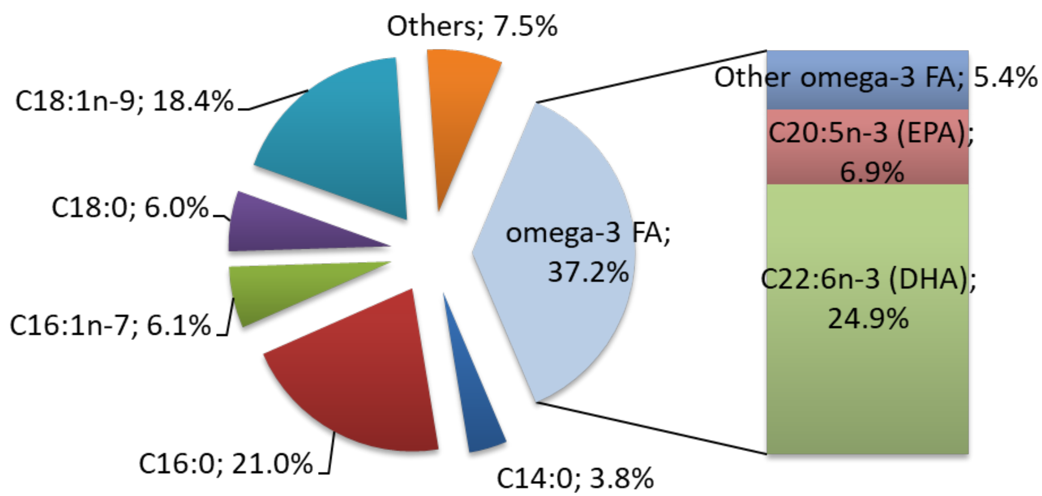
European sardine
Sardina pilchardus



Atlantic bluefin tuna
Thunnus thynnus

Fatty acid profile (AOAC)





apparatus

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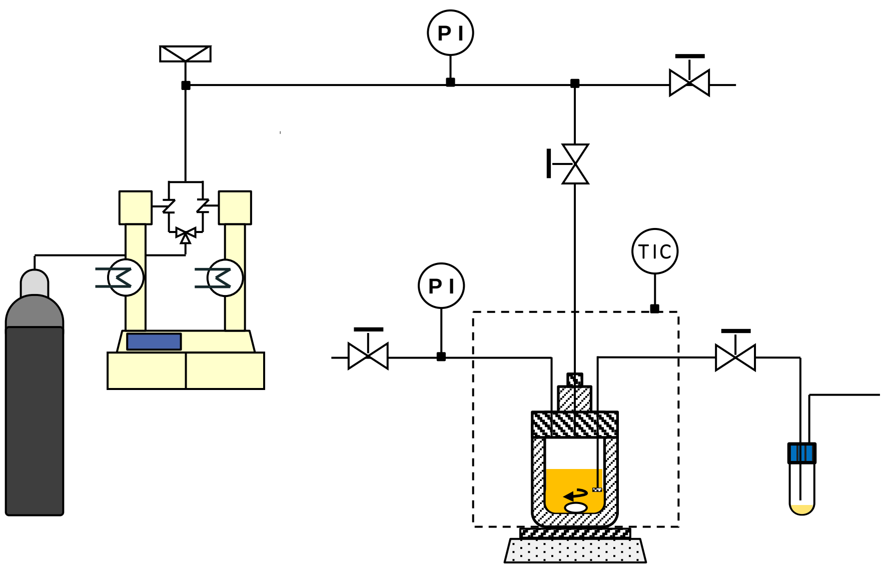
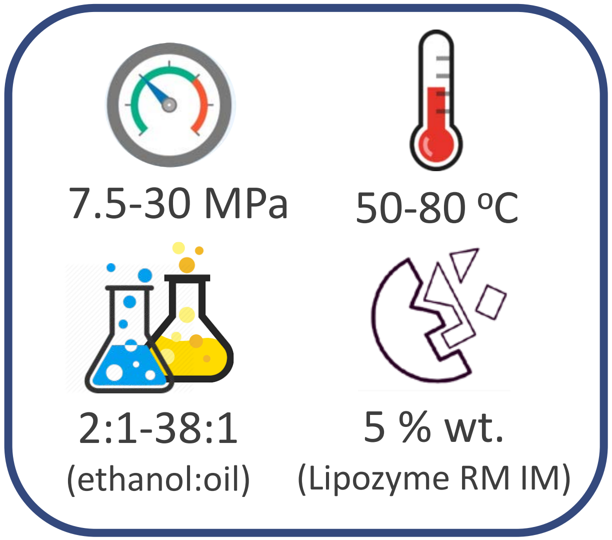


High-pressure batch stirred tank reactor with sampling system design and assembling: BIOIND



Reaction conditions

analysis of the composition

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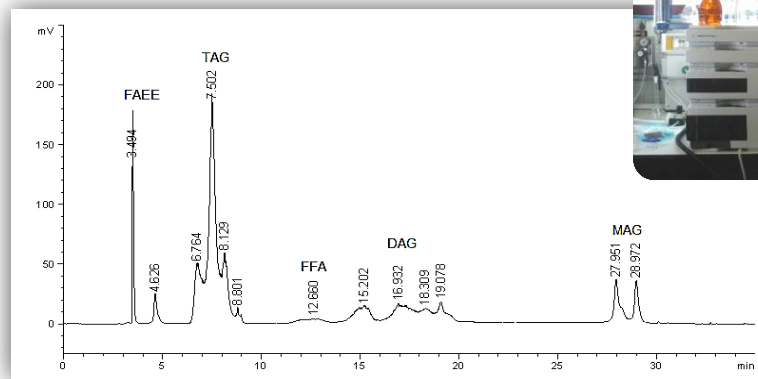
Neutral Lipids profile - TAG, DAG, MAG, FAEE

Normal Phase HPLC ELSD

- Isooctane
- MTBE + AcOH 0.1 %vol
- Calibrated with 13 standards and the original fish oil



HPLC ELSD (Agilent Technologies)

Glycerol and ethanol

Theoretically estimated from the reaction stoichiometry

$$n_{\mathrm{GLY}}^{\mathrm{t}} = \left(n_{\mathrm{FAEE}} - n_{\mathrm{DAG}} - 2\cdot n_{\mathrm{MAG}}\right)/3$$ (Sovová *et al.*, 2008)

$$n_{\mathrm{EtOH}}^{\mathrm{t}} = n_{\mathrm{EtOH}}^{0} - n_{\mathrm{FAEE}}$$

Sovová, H., Zarevúcka, M., P. Bernášek, P., Stamenić, M., Chem. Eng. Res. Design. 86 (2008) 673–681.

reaction kinetics

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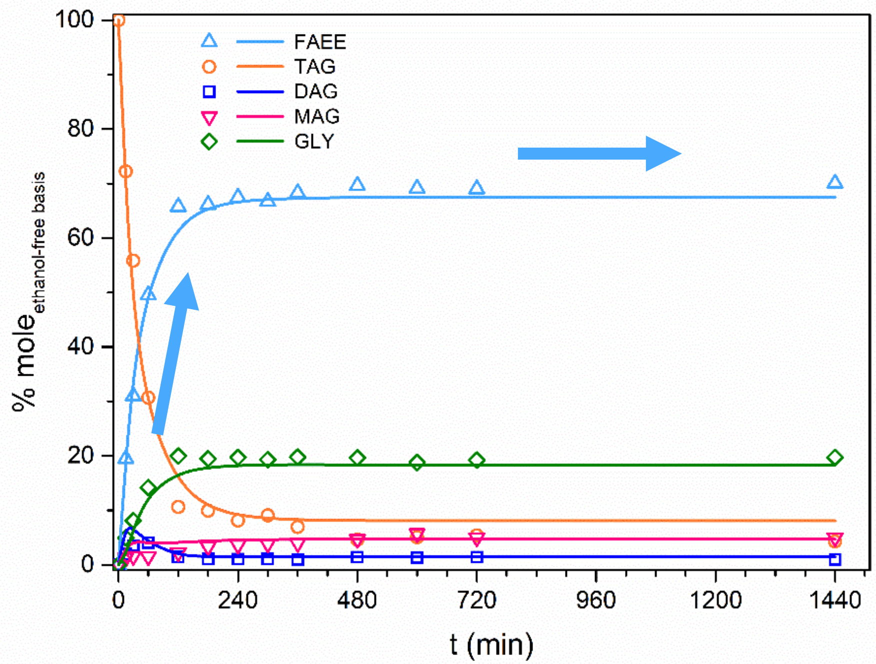
Time course of the reaction

MR = 38:1, T = 50 ºC, p = 10 MPa, enz. = 5 %wt.



Rapid production of FAEE at the beginning

Plateau at eq. conversion

reaction kinetics

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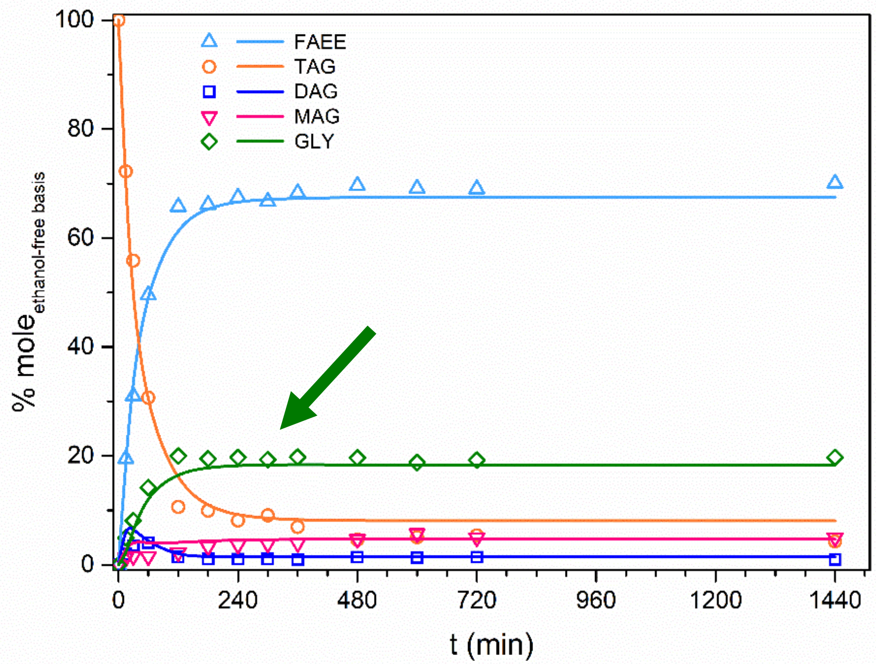
Time course of the reaction

MR = 38:1, T = 50 ºC, p = 10 MPa, enz. = 5 %wt.



Rapid production of FAEE at the beginning

Plateau at eq. conversion

Production of GLY
non *sn*-1,3-specific behaviour

acyl-migration

Support, reaction time, temperature, solvents, water content, etc.
(Xu *et al*., 1998)

X. Xu, A. R. H. Skands, C. E. Høy, H. Mu, S. Balchen and J. Adler-Nissen,, JAOCS 75 (1998) 1179–1186.

effect of initial MR

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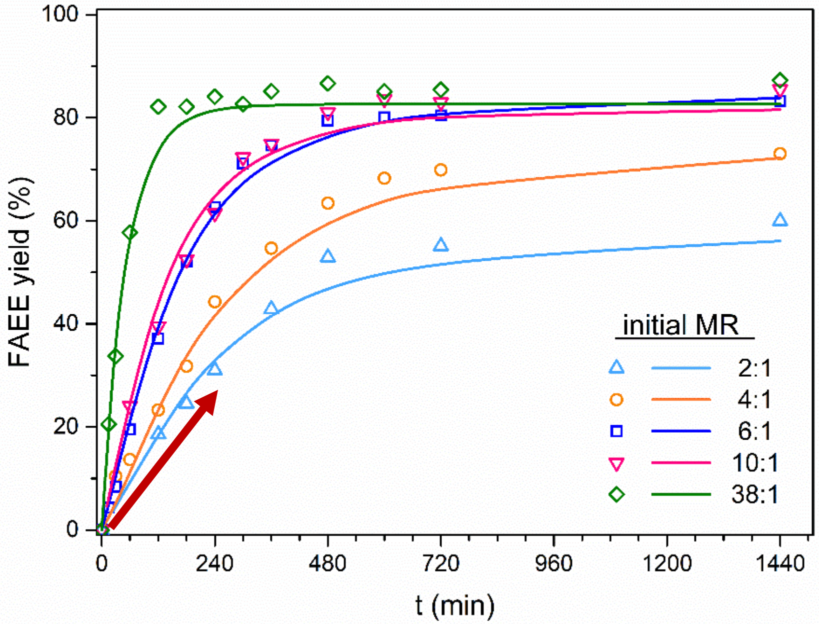
FAEE yield vs. time

T = 50 ºC, p = 10 MPa, enz. = 5 %wt.



MR (ethanol:oil)	r_0 ($\mu mol \cdot g_{enz}^{-1} \cdot min^{-1}$)	Eq. FAEE yield* (%)
2:1	34 ± 2	57 ± 3
4:1	43 ± 5	70 ± 2
6:1	71 ± 5	82 ± 2
10:1	79 ± 3	84 ± 1
38:1	130 ± 10	86 ± 1

: ↓ mass transfer limitations

$$\text{FAEE yield (\%)} = x_{FAEE} / \left(x_{TAG} + x_{DAG} + x_{MAG} + x_{FAEE} \right) \cdot 100$$

effect of initial MR

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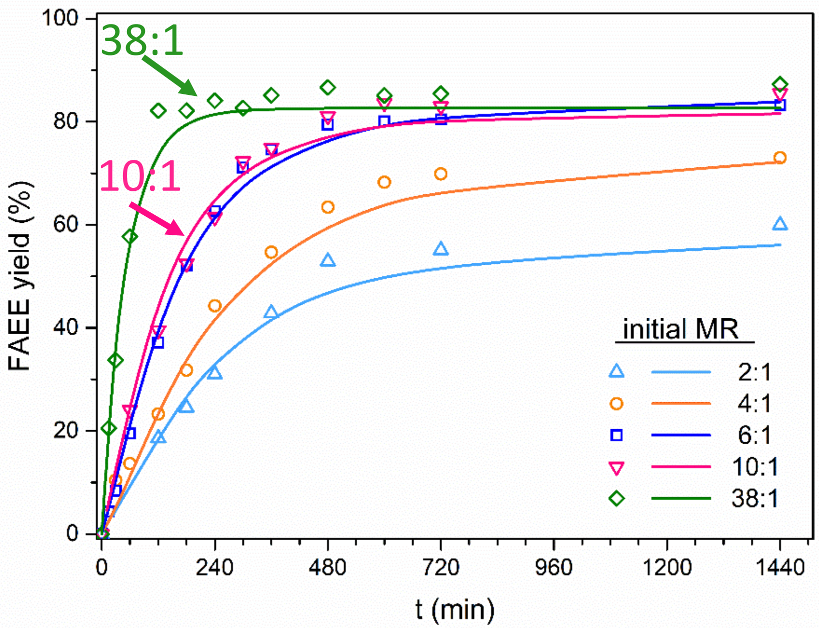
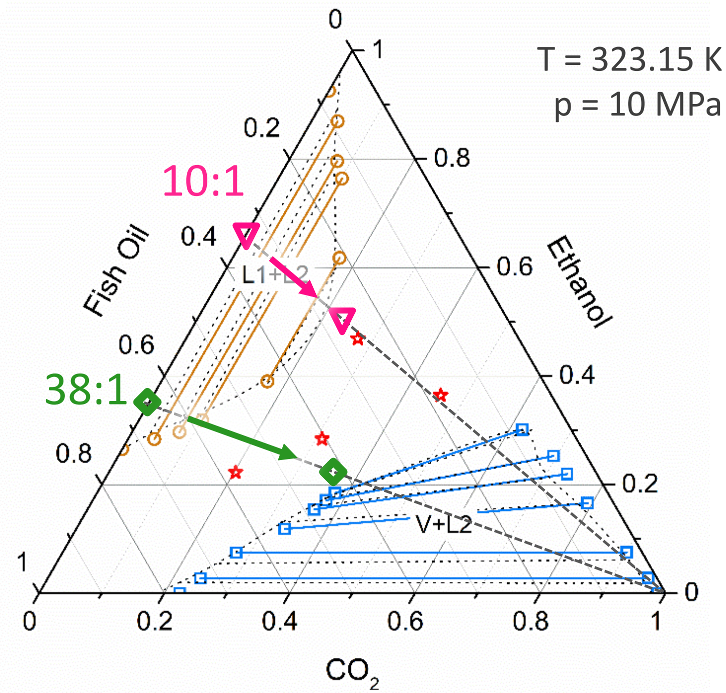
FAEE yield vs. time

T = 50 ^{o}C, p = 10 MPa, enz. = 5 %wt.



$$\text{FAEE yield (\%)} = x_{\text{FAEE}} / \left(x_{\text{TAG}} + x_{\text{DAG}} + x_{\text{MAG}} + x_{\text{FAEE}} \right) \cdot 100$$

10:1 and 38:1 are biphasic without CO_2



SC-CO_2 expands reaction media, which becomes homogeneous

(Melgosa *et al.*, 2017)

Melgosa, R., Sanz, M.T., Solaesa, Á.G., Beltrán, S., J. Chem. Thermodynamics 115 (2017) 106-113

pressure effect

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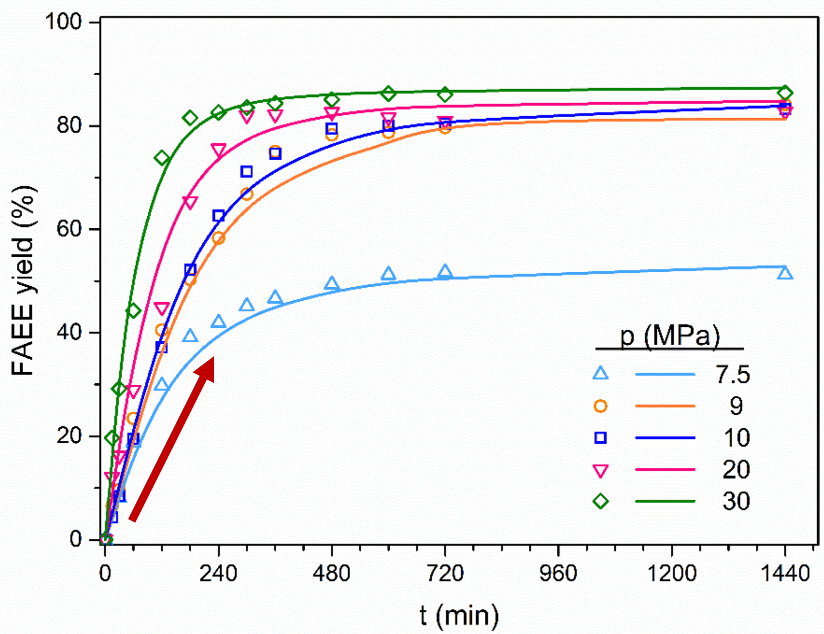
FAEE yield vs. time

MR = 6:1, T = 50 ºC, enz. = 5 %wt.



$$\text{FAEE yield (\%)} = x_{\text{FAEE}} / \left(x_{\text{TAG}} + x_{\text{DAG}} + x_{\text{MAG}} + x_{\text{FAEE}} \right) \cdot 100$$

p (MPa)	r_0 (μmol·g_{enz}^{-1}·min^{-1})	Eq. FAEE yield* (%)
7.5	51 ± 7	51.4 ± 0.3
9	67 ± 6	80 ± 3
10	71 ± 5	82 ± 2
20	110 ± 10	82.1 ± 0.7
30	210 ± 20	86 ± 1

↑  : ↑ r_0

pressure effect

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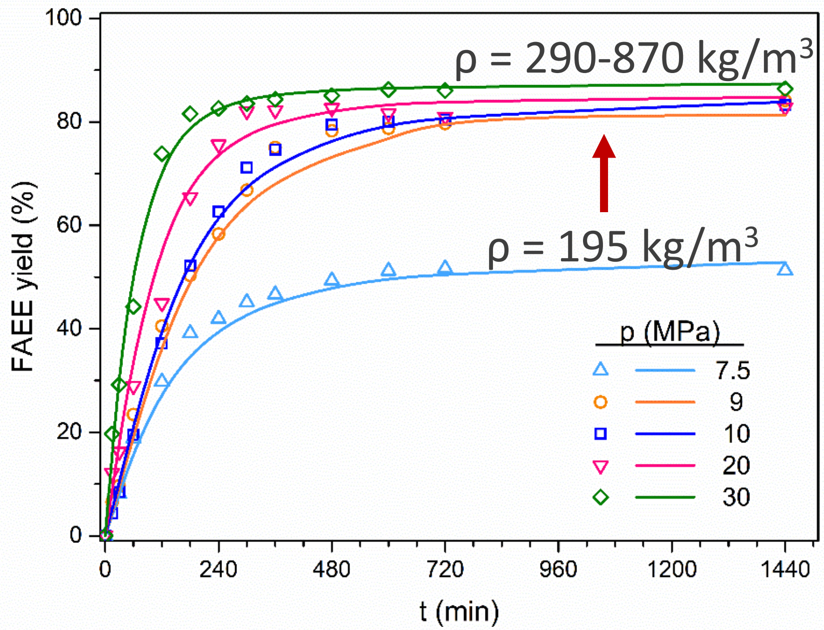
FAEE yield vs. time

MR = 6:1, T = 50 ºC, enz. = 5 %wt.



$$\text{FAEE yield (\%)} = x_{\text{FAEE}} / \left(x_{\text{TAG}} + x_{\text{DAG}} + x_{\text{MAG}} + x_{\text{FAEE}} \right) \cdot 100$$

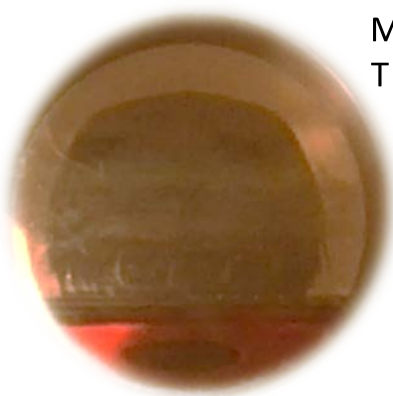
p (MPa)	r_0 ($\mu mol \cdot g_{enz}^{-1} \cdot min^{-1}$)	Eq. FAEE yield* (%)
7.5	51 ± 7	51.4 ± 0.3
9	67 ± 6	80 ± 3
10	71 ± 5	82 ± 2
20	110 ± 10	82.1 ± 0.7
30	210 ± 20	86 ± 1

↑  : ↑ FAEE yield

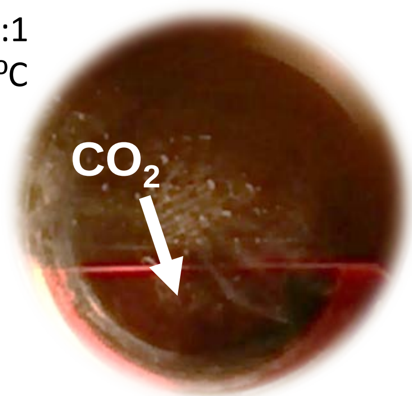
7.5 → 9-30 MPa

pressure effect

MR = 6:1
T = 50 ºC



0.1 MPa



10 MPa

Phase Eq. of the system

expanded liquid + gas

(Melgosa *et al.*, 2017)

↑ pressure: ↑ Dissolved CO_2 ; ↓ mass transfer limitations

No dilution effect

observed in oleic acid + 1-octanol + CO_2
10 → 30 MPa
(Laudani *et al.*, 2007)

Melgosa, R., Sanz, M.T., Solaesa, Á.G., Beltrán, S., J. Chem. Thermodynamics 115 (2017) 106-113
Laudani, C.G., Habulin, M., Knez, Ž., Della Porta, G., Reverchon, E., J. Supercrit. Fluids, 41 (2007) 92–101.

temperature effect

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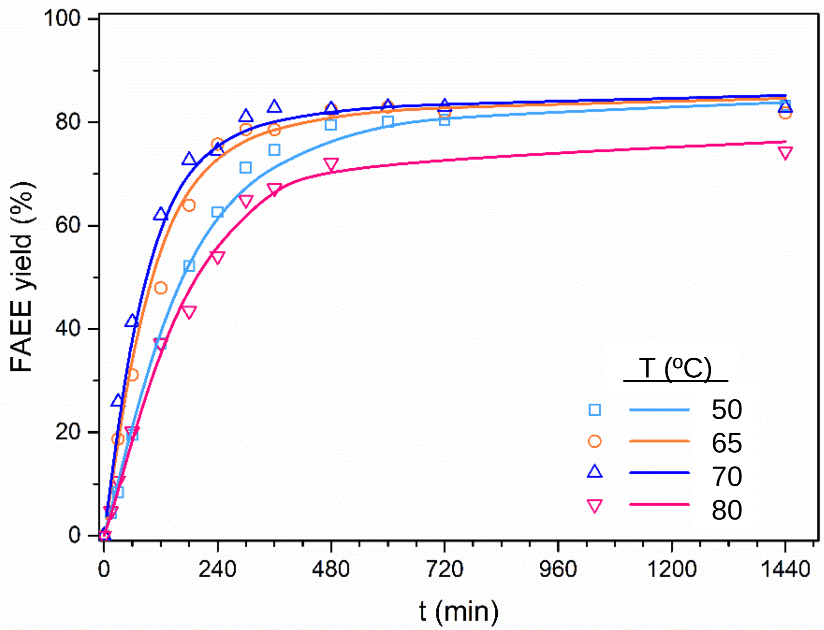
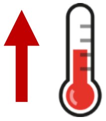
FAEE yield vs. time

MR = 6:1, p = 10 MPa, enz. = 5 %wt.



$$\text{FAEE yield (\%)} = x_{\text{FAEE}} / (x_{\text{TAG}} + x_{\text{DAG}} + x_{\text{MAG}} + x_{\text{FAEE}}) \cdot 100$$

T (ºC)	r_0 (µmol·g_{enz}^{-1}·min^{-1})	Eq. FAEE yield* (%)
50	71 ± 5	82 ± 2
65	110 ± 10	82.3 ± 0.5
70	160 ± 20	82 ± 1
80	64 ± 5	75.0 ± 0.3



50 → 70 ºC :

- ↑ kinetic energy
- ↓ mass transfer limitations

temperature effect

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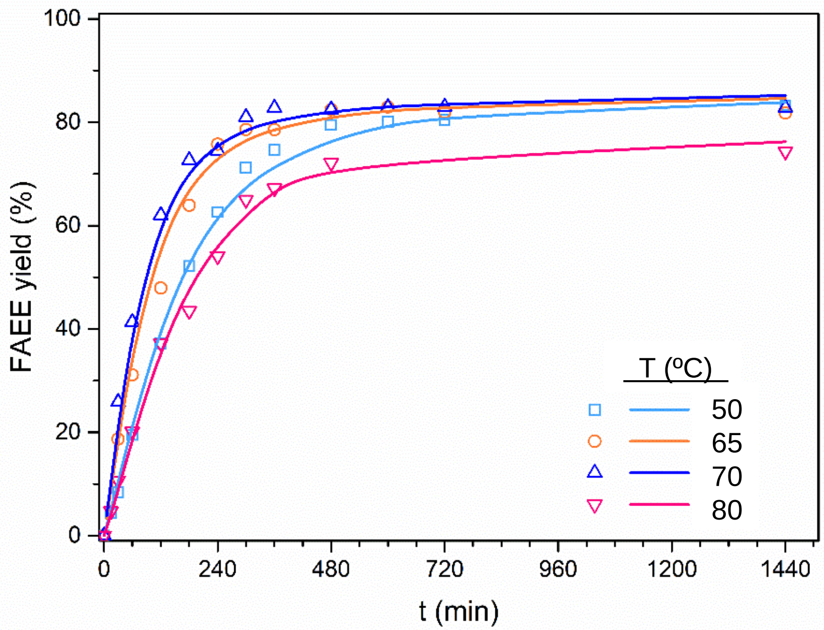
FAEE yield vs. time

MR = 6:1, p = 10 MPa, enz. = 5 %wt.



$$\text{FAEE yield (\%)} = x_{\text{FAEE}} / (x_{\text{TAG}} + x_{\text{DAG}} + x_{\text{MAG}} + x_{\text{FAEE}}) \cdot 100$$

T (ºC)	r_0 ($\mu mol \cdot g_{enz}^{-1} \cdot min^{-1}$)	Eq. FAEE yield* (%)
50	71 ± 5	82 ± 2
65	110 ± 10	82.3 ± 0.5
70	160 ± 20	82 ± 1
80	64 ± 5	75.0 ± 0.3

↑ T: No effect on FAEE yield

low heat of reaction

50 → 70 ºC

Eq. conversion is T-independent

$$\ln\left(\frac{K_2}{K_1}\right) = \frac{\Delta H^o}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right); \quad \Delta H^o \approx 0 \rightarrow K_2 \cong K_1$$

temperature effect

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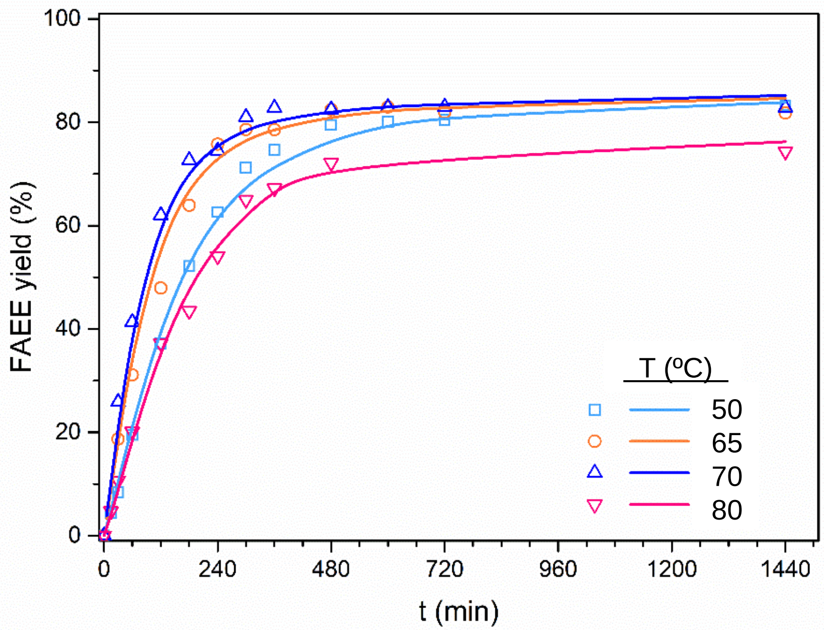
FAEE yield vs. time

MR = 6:1, p = 10 MPa, enz. = 5 %wt.



$$\text{FAEE yield (\%)} = x_{FAEE} / (x_{TAG} + x_{DAG} + x_{MAG} + x_{FAEE}) \cdot 100$$

T (ºC)	r_0 ($\mu mol \cdot g_{enz}^{-1} \cdot min^{-1}$)	Eq. FAEE yield* (%)
50	71 ± 5	82 ± 2
65	110 ± 10	82.3 ± 0.5
70	160 ± 20	82 ± 1
80	64 ± 5	75.0 ± 0.3

80 ºC : Thermal inactivation of the catalyst

T_{opt} = 70 ºC > T_{opt} = 40 ºC n-hexane solvent-free (Oliveira and Oliveira, 2000, Calero *et al.*, 2014)

D. Oliveira, J.V. Oliveira, Ind. Eng. Chem. Res. 39 (2000) 4450–4454.
J. Calero, C. Verdugo, *et al.*, New Biotechnol. 31 (2014) 596–601.

lipid oxidation

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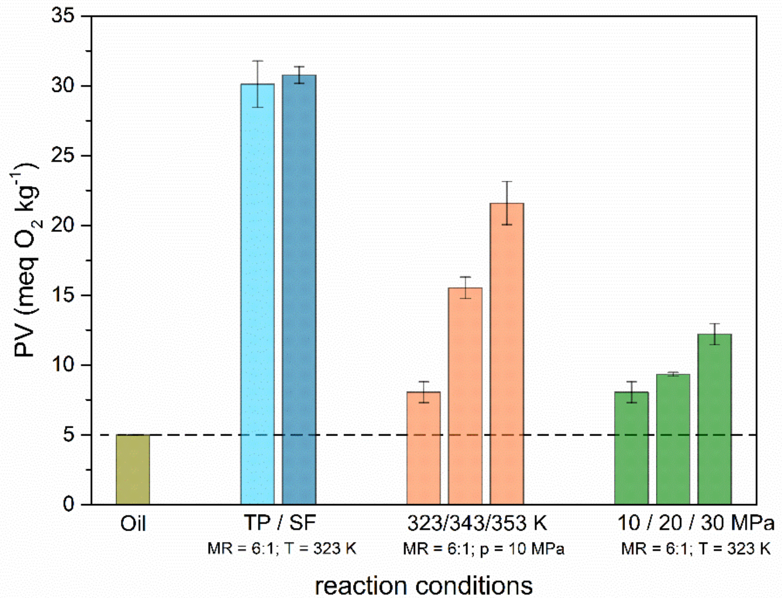

Peroxide value of reaction products

MR = 6:1, different reaction media and p,T conditions
dashed line - *Codex* limit (5 meq O_2/kg oil)

At the same T (50 ºC),
SF and TP show higher PV than SC-CO_2

in SC-CO_2

Pressure has little effect on PV

T negatively affects PV **T_{opt} = 70 ºC ??**

compromise { good reaction performance / low oxidation

Codex Stan 329-2017 - Standard for Fish Oils, Rome, Italy, 2017.

Conclusion

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$SC\text{-}CO_2$ can replace organic solvents in fish oil transesterification, with yields up to 86 % in 2 h (MR = 38:1, 50 ºC, 10 MPa, 5% biocat.)

- Reducing mass transfer limitations
- Protecting the biocatalyst from thermal degradation
- Preventing oxidation of products due to displacement of oxygen

Ongoing work

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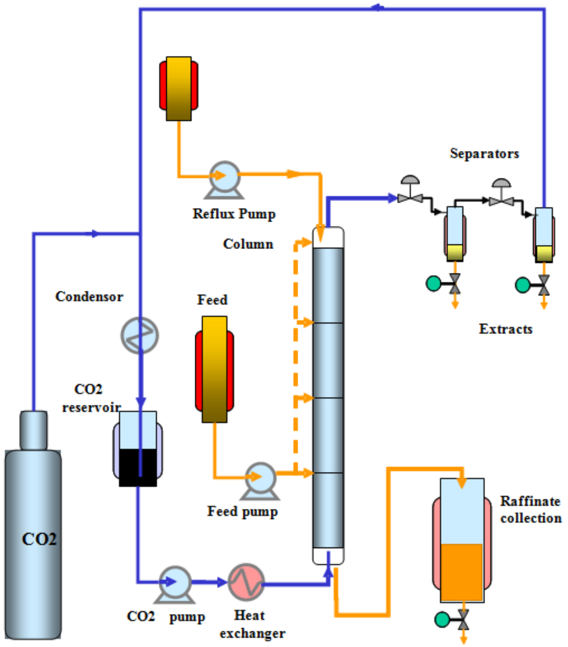
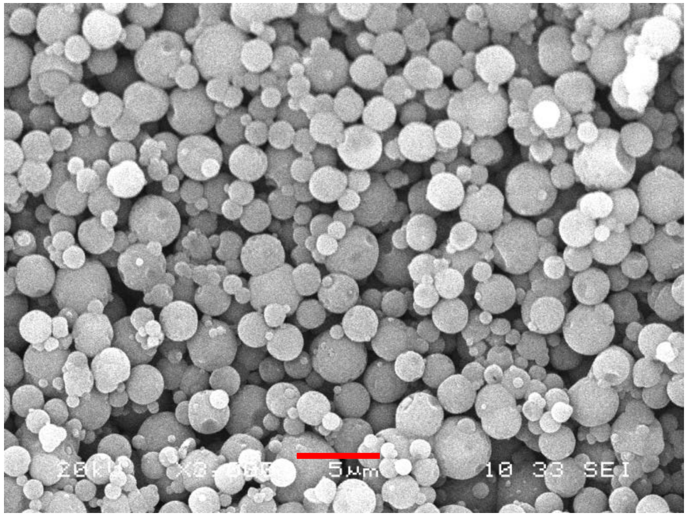


supercritical fractionation process



omega–3 encapsulated in OSA-starch PGSS-dried particles (3000x, 5 µm)

Further studies on supercritical fractionation and formulation of omega–3 will help in the development of a supercritical biorefinery for fish oil production and valorization

Acknowledgements

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This work has been performed within the research group **Industrial and Environmental Biotechnology (BIOIND)**, in the frame of the research projects **CTQ2012-39131-C02-01** and **BU055U16**, financed by **MINECO** and **JCyL**, respectively, and both co-financed by **ERDF**.

www.ubu.es/bioind

I also owe my gratitude to **MINECO** for financing my predoctoral contract (**BES-2013-063937**).

GOBIERNO DE ESPANA MINISTERIO DE ECONOMIA Y COMPETITIVIDAD

Junta de Castilla y León

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND

Thank you for your attention