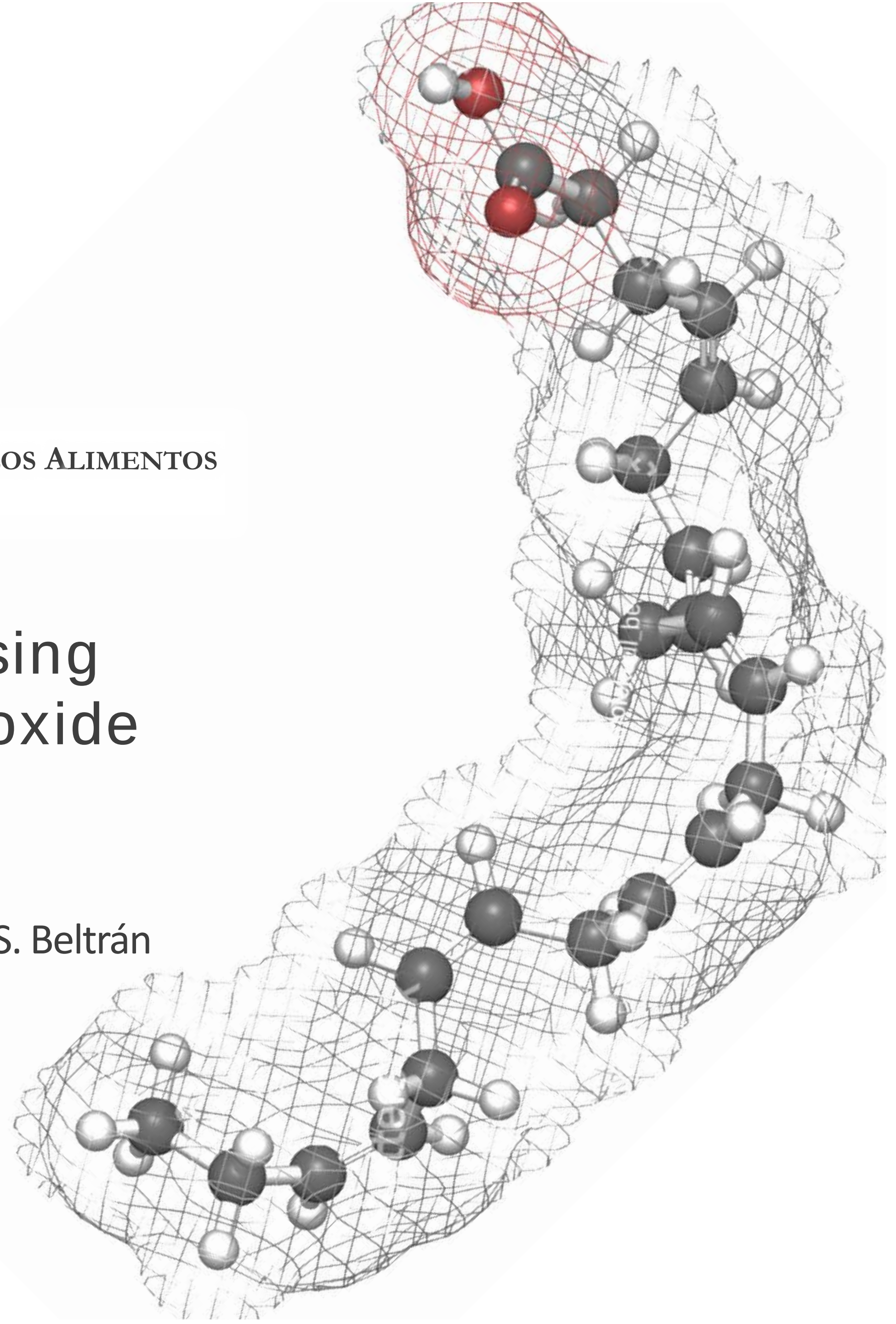
17th European Meeting on SUPERCRITICAL FLUIDS

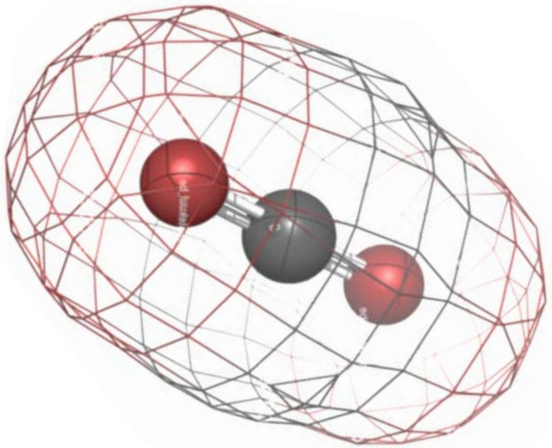
7th European Meeting on HIGH PRESSURE TECHNOLOGY

UNIVERSIDAD DE BURGOS

DEPARTAMENTO DE BIOTECNOLOGÍA Y CIENCIA DE LOS ALIMENTOS



Fish Oil Valorization using Supercritical Carbon Dioxide



R. Melgosa*, M.T. Sanz, Ó. Benito-Román, S. Beltrán

Ciudad Real, 2019

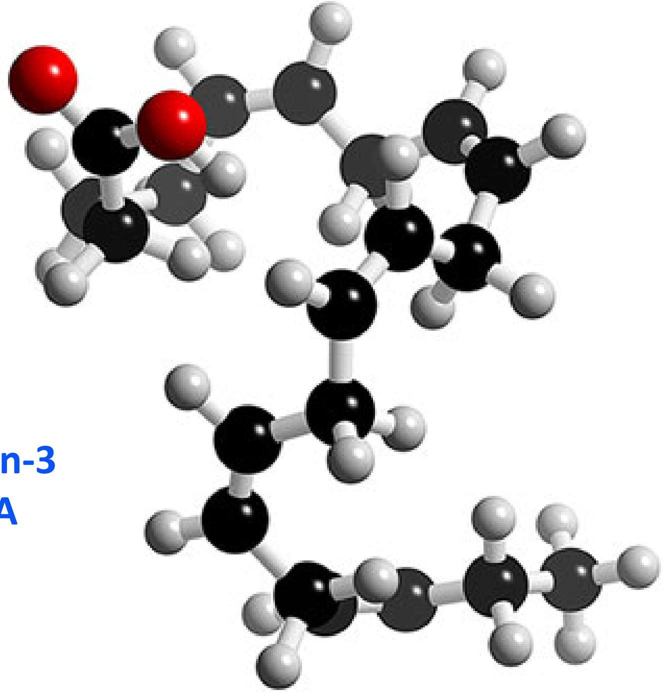
Fish Oil Valorization – **Why?**

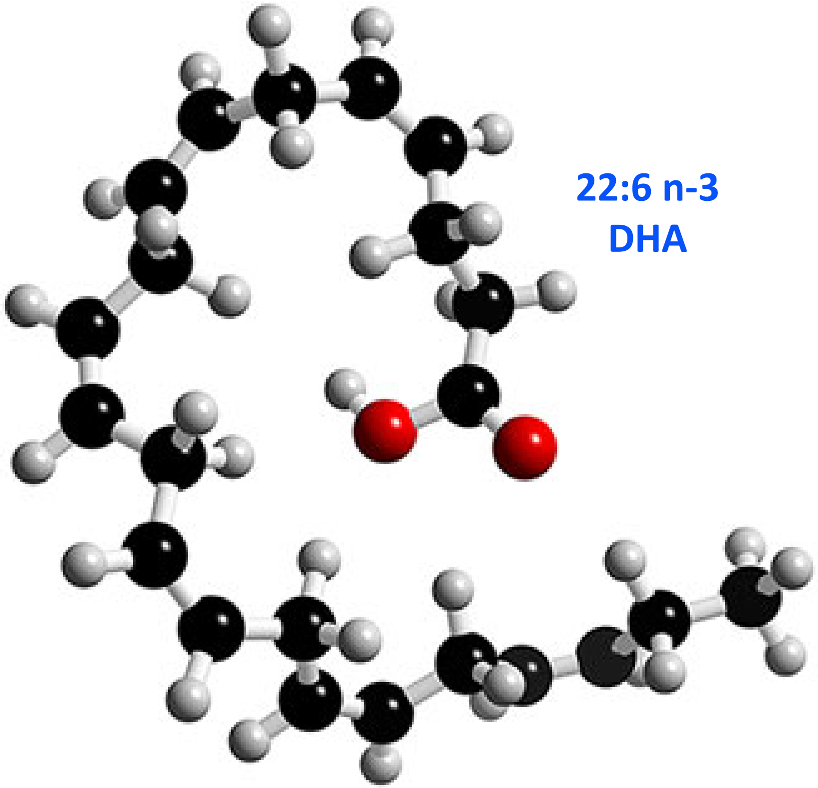
Because **fish oil** is the main source of omega-3 polyunsaturated fatty acids (PUFAs)

Eicosapentaenoic acid (EPA, C20:5 n-3)

Docosahexaenoic acid (DHA, C22:6 n-3)

20:5 n-3
EPA





22:6 n-3
DHA

Fish Oil Valorization – **Why?**

Because **fish oil** is the main source of omega-3 polyunsaturated fatty acids (PUFAs)

Eicosapentaenoic acid (EPA, C20:5 n-3)

Docosahexaenoic acid (DHA, C22:6 n-3)

Because **omega-3 PUFAs** exert important healthy effects on the human body

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

Fish Oil Valorization – **Why?**

Because **fish oil** is the main source of omega-3 polyunsaturated fatty acids (PUFAs)

- Eicosapentaenoic acid (EPA, C20:5 n-3)
- Docosahexaenoic acid (DHA, C22:6 n-3)

Because **omega-3 PUFAs** exert important healthy effects on the human body

Because **fish waste** is so far used as animal feeding or disposed of at high economic and environmental costs

35 % of fish and seafood is wasted through the food chain (FAO, Save Food Initiative)

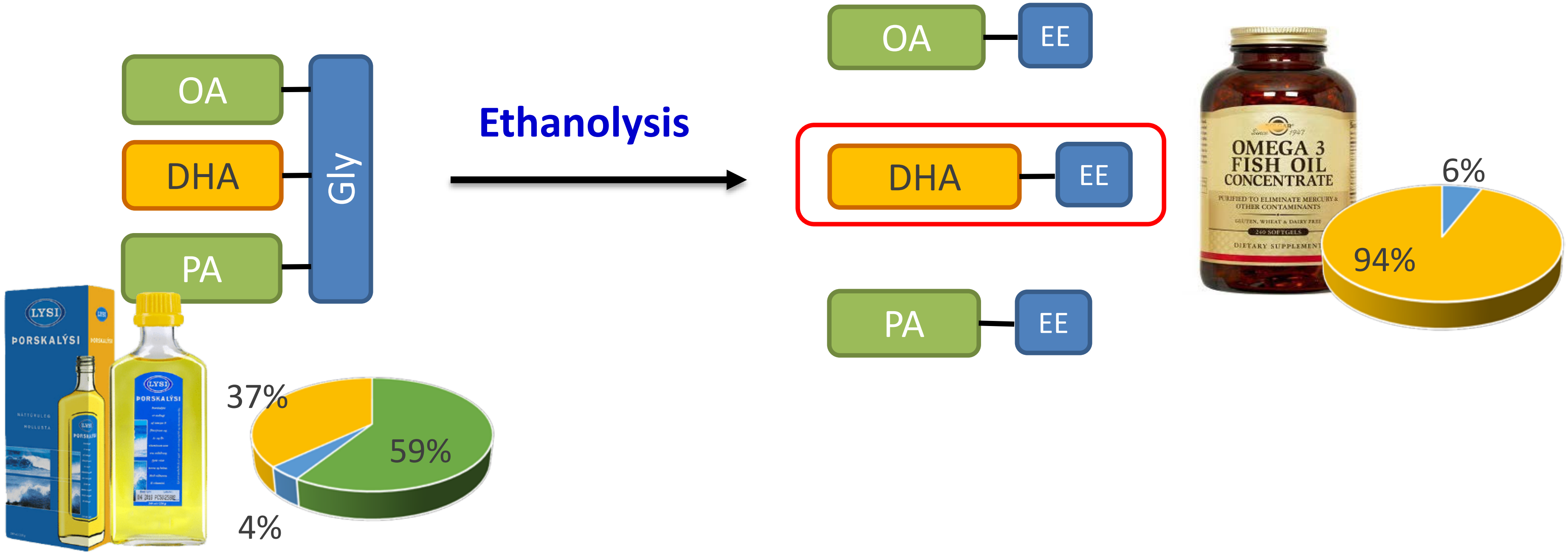
Hand feeding in a salmon hatchery (New Zealand). Photo: NZ King Salmon

Bycatch in a fishing boat. Photo: Johns Hopkins University

Omega-3 concentrates

Increase efficiency of omega-3 and flexibility in formulation of functional foods

Reaction and subsequent **separation of products** (concentration) are required



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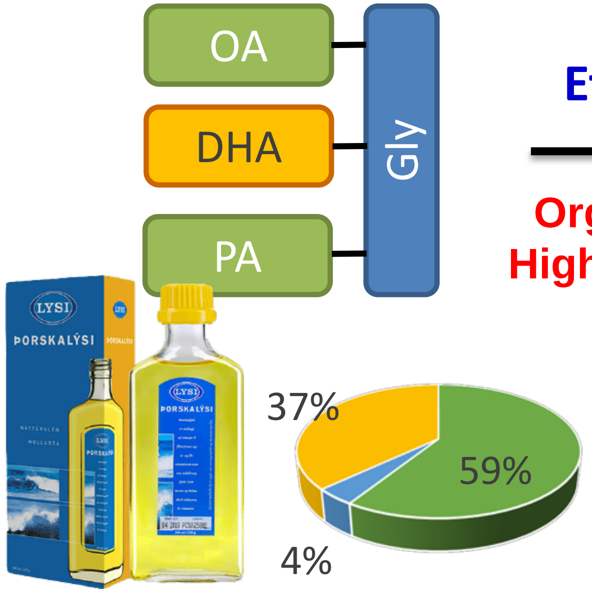


- **Solvent residues** must be removed in downstream steps
- **High temperatures** promote **oxidation**

Omega-3 concentrates – **Oxidation**

INVITED COMMENTARY

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

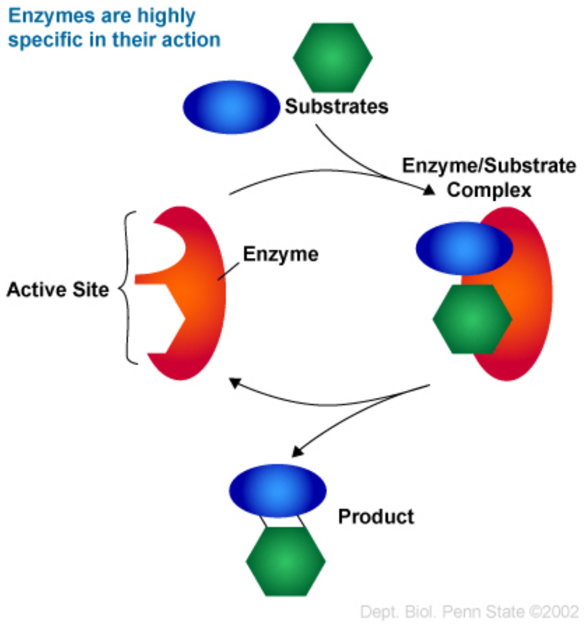
The end result is that consumers are at risk of purchasing an oxidised supplement, for which there is little tangible information on the packaging to provide details of the oil's original source, age and levels of refinement. The levels of oxidation now described in four independent studies since 2012 (analysing 260 *n*-3 PUFA products) suggest that the general public is consuming oxidised products exceeding voluntary industry-standard levels. Importantly, the biological effects and health consequences of consuming oxidised fish oil supplements are not yet established. In 2010, the European Food Standards Authority (EFSA) panel on biological hazards presented a scientific opinion on fish oil for human consumption[(15)], concluding that 'information on the level of oxidation of fish oil (as measured by peroxide and anisidine values) and related toxicological effects in humans is lacking'.

D. Cameron-Smith *et al.*, J. Nutr. Sci. 4, e36 (2015) 1–2

Objective

Valorization of fish oil to obtain omega-3 concentrates through bio-catalysis in supercritical CO_2 media

- Enzymatic reactions of oils in SC-CO_2 combine the advantages of **enzyme specificity** and **high mass transfer in SCFs**
- **Mild temperatures and inert atmosphere avoid oxidation** of omega-3
- Moreover, **the solvent is easily separated from the reaction products** by simple pressure decrease



Properties of SCFs	
Gas-like	**Liquid-like**
High diffusivity (good mass transfer in complex matrices) **Low viscosity** (favorable flow characteristics)	**High solvating power** (depending on density)

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Specific

- Fundamental studies
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    R. Melgosa *et al.*, J. Supercrit. Fluids 97 (2015) 51–62.
  - ✓ Fluid phase equilibria of the reaction system
    R. Melgosa et al., J. Chem. Thermodyn. 115 (2017) 106–113.
  - ✓ Reaction kinetics
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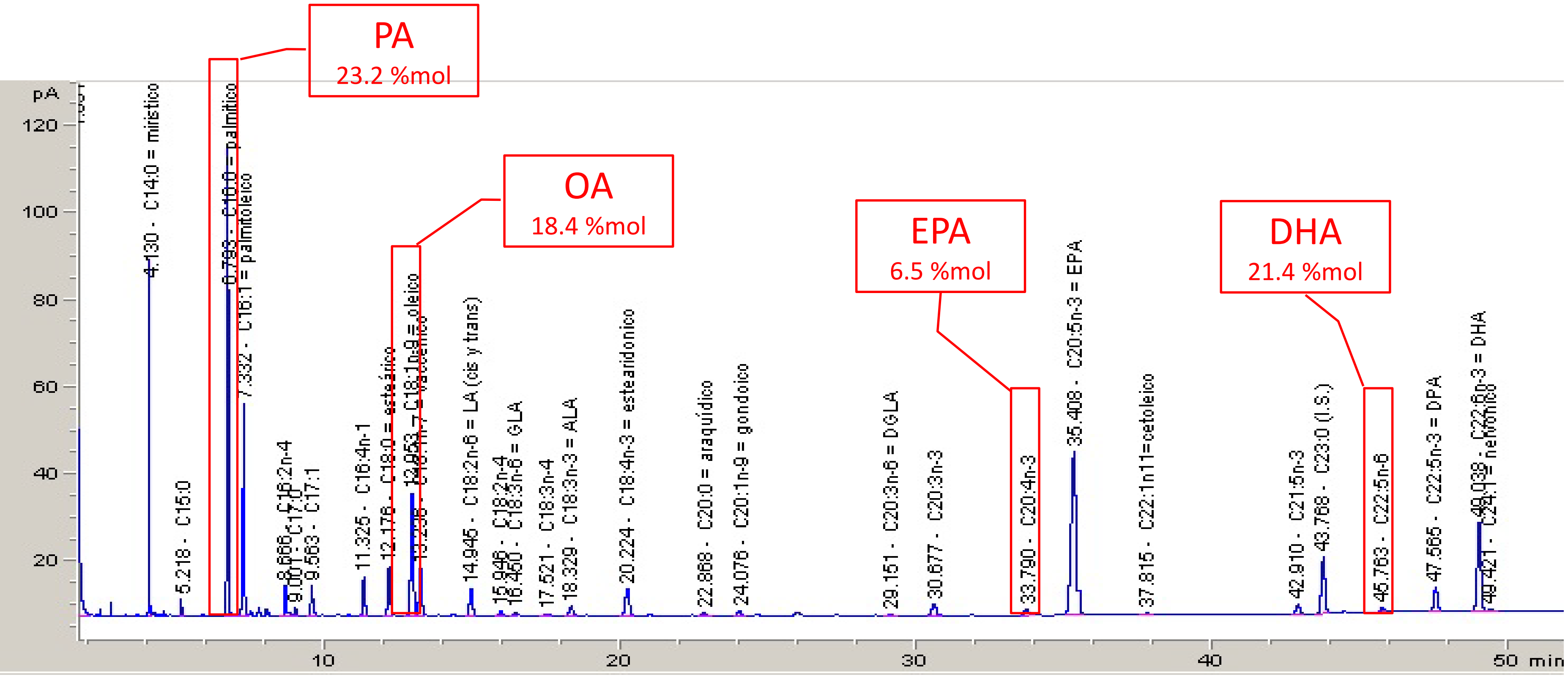
    R. Melgosa et al., J. CO_2 Util. 31 (2019) 65–74.
- Applied studies
  - ✓ Product separation
    R. Melgosa et al., J. CO_2 Util. 31 (2019) 65–74.
  - ✓ Formulation
    R. Melgosa et al., Food Chem. 270 (2019) 138–148.
  - ✓ Stability against oxidation
  - ✓ Continuous process

Refined fish oil (AFAMSA S.A.)

Mixture of tuna (*Thunnus* sp.) and sardine (*Sardina pilchardus*) oil

Fatty Acid profile (AOAC Method)

(Solaesa *et al.*, J. Oleo Sci. 63 (2014) 449–460.)



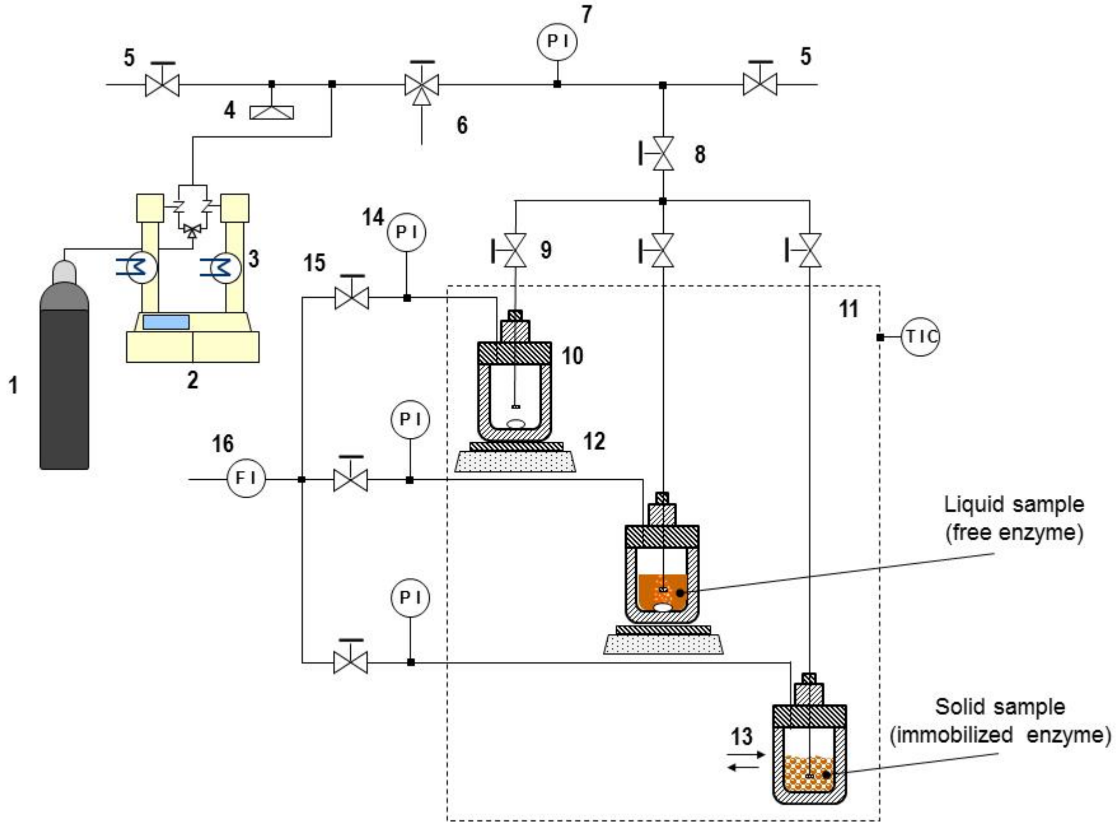
Enzyme stability in scCO_2

Lipase from *Rhizomucor miehei*

- Palatase 20000 L (free, aq. solution)
- Lipozyme RM IM (immobilized)

Lipase B from *Candida antartica*

- Lipozyme CALB L (free, aq. solution)
- Lipozyme 435 (immobilized)

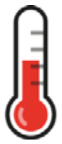


1: CO_2 cylinder
2: syringe pump
3: cryostat
4: rupture disk
5: vent valve
6: process valve
7: general pressure gauge
8: general inlet valve
9: individual inlet valve
10: high pressure cell
11: thermostatic water bath
12: magnetic stirrer
13: mechanical agitation
14: pressure gauge
15: depressurization valve
16: total flow-meter

R. Melgosa *et al.*, J. Supercrit. Fluids 97 (2015) 51–62.

Enzyme stability in $scCO_2$

Pressure (10-25 MPa)

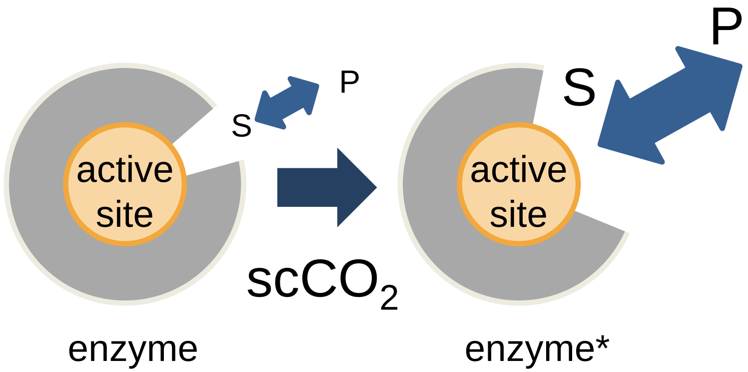


Temperature (35-70 °C)

Exposure time (1-3 h)

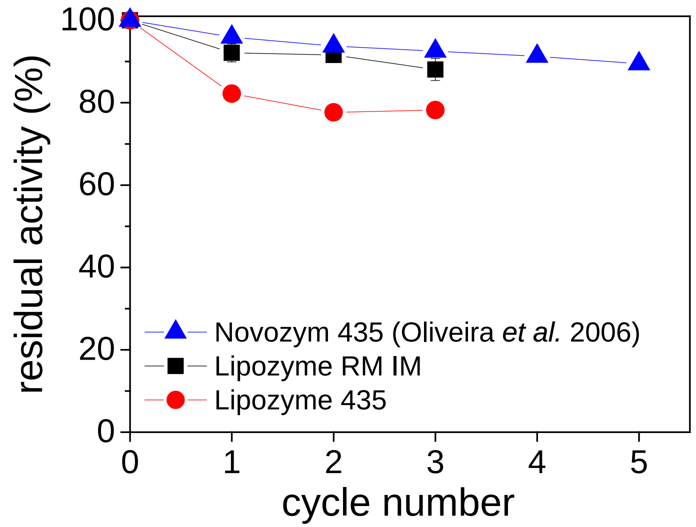
Treatment cycles (1-3)

Activity of free lipases was enhanced



$scCO_2$ opened the lipase conformation

Immobilized enzymes were reused several times with no significant activity loss



(up to 90 % RA after 5 cycles)

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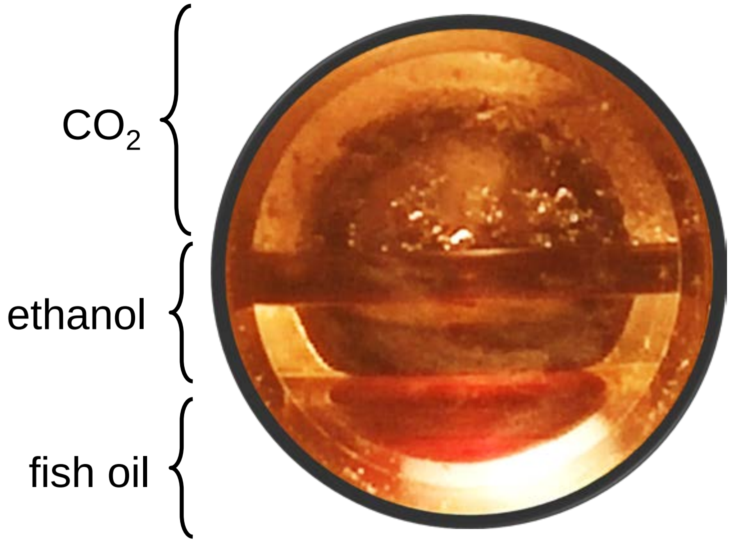
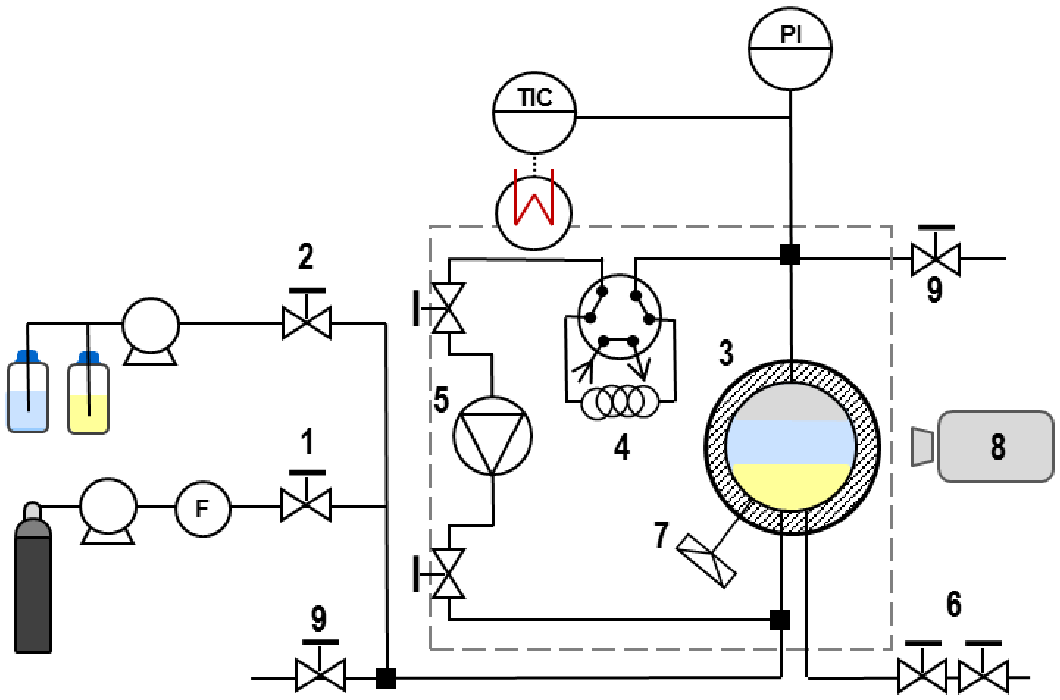


Visual observation of the reaction mixture
(unmixed components)

Fluid phase equilibria

An T VCir method

Analytical, iso**T**hermal, with re**Cir**culation of the **V**apor phase



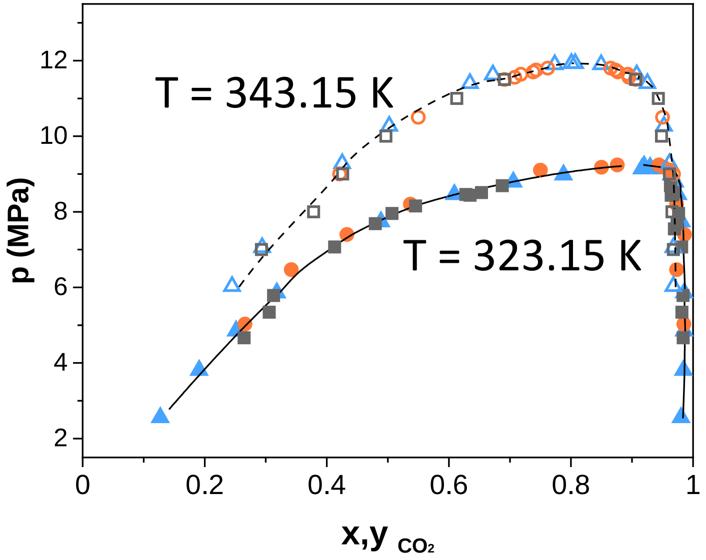
High Pressure View Cell HPVC500
Design and construction: Eurotechnika GmBH
Modified by BIOIND

1. CO_2 inlet
2. Fish oil and ethanol inlet
3. High-pressure, variable volume, equilibrium view cell
4. 6-way valve
5. Gear pump
6. Micro-mettering valves
7. Rupture disk
8. Camera endoscope and video recorder
9. Vent valves

F. Coriolis mass flow meter

R. Melgosa *et al.*, J. Chem. Thermodyn. 115 (2017) 106–113.

Fluid phase equilibria



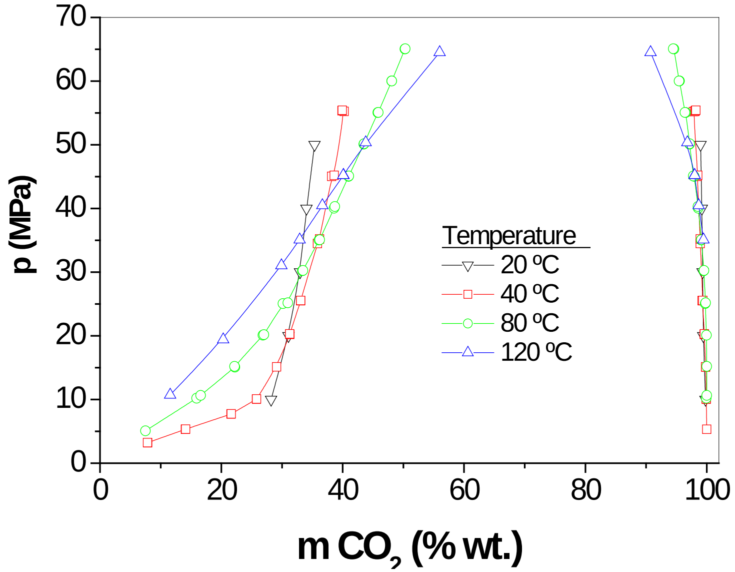
CO_2 + ethanol

full symbols: T = 323.15 K
hollow symbols: T = 343.15 K

□, ■: this work
R. Melgosa et al., J. Chem. Thermodyn. 115 (2017) 106–113

●, ○: data from Lim *et al.*,
Lim et *al.*, J. Supercrit. Fluids, 7 (1994) 219–230.

▲, Δ: data from Joung *et al.*
Joung *et al*. Fluid Phase Eq. 185 (2001) 219–230.



CO_2 + fish oil

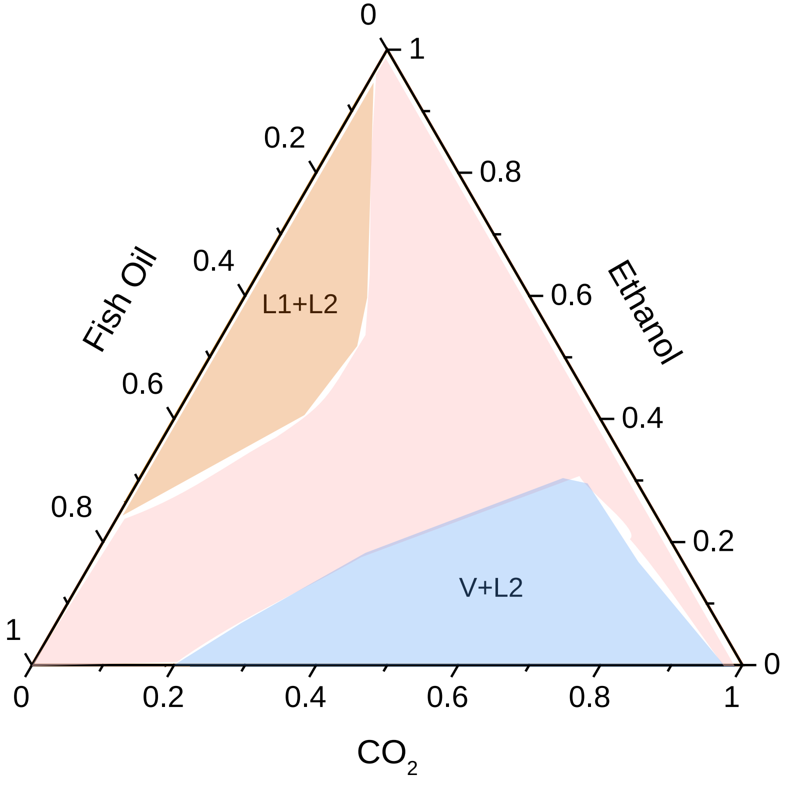
Data from Borch-Jensen & Mollerup
Borch-Jensen & Mollerup,
Fluid Phase Equilib. 138 (1997) 179–211.

Fluid phase equilibria

L1 + L2
V + L2
1-phase

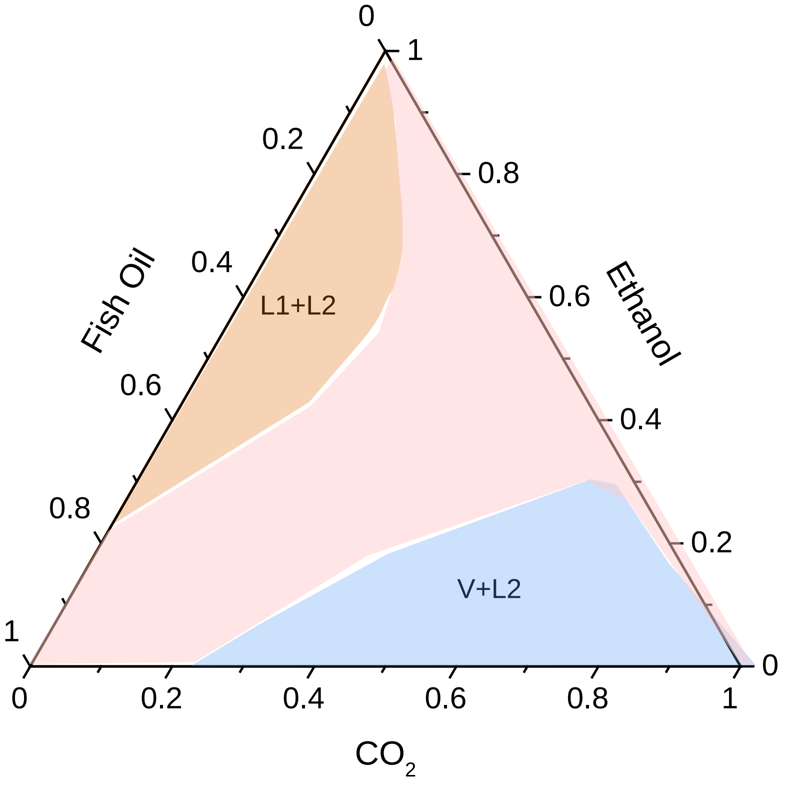
-○-: L1+L2 tie-lines
-□-: V+L2 tie-lines
☆: monophasic mixtures
······ : PR EoS vdW2 model

CO_2 (V) + etanol (L1) + fish oil (L2)



10 MPa, 50 ºC

30 MPa, 50 ºC



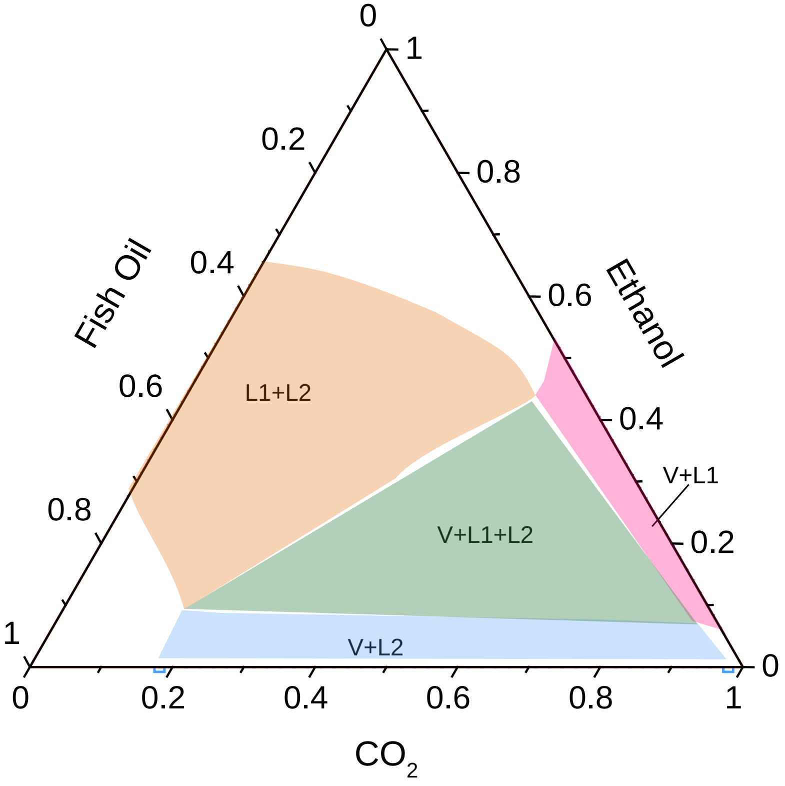
R. Melgosa *et al.*, J. Chem. Thermodyn. 115 (2017) 106–113.

Fluid phase equilibria

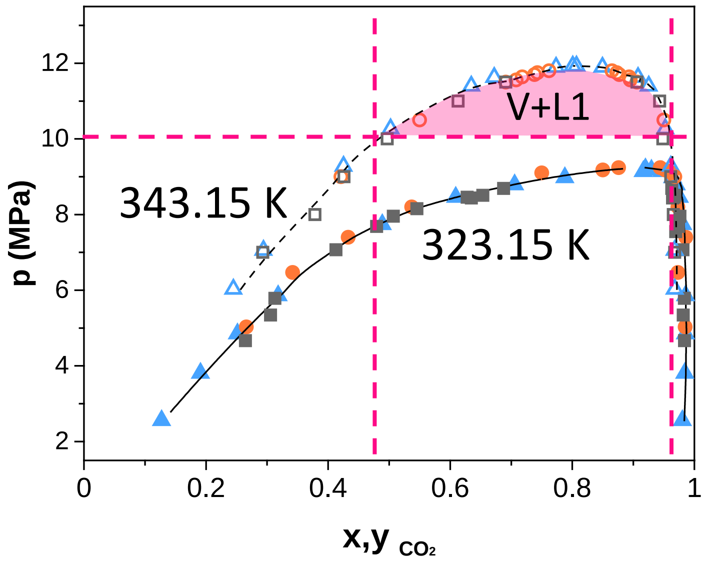
CO_2 (V) + etanol (L1) + fish oil (L2)

V + L1
L1 + L2
V + L2
V + L1 + L2

-△- V+L1 tie-lines
-○-: L1+L2 tie-lines
-□-: V+L2 tie-lines
······ : PR EoS vdW2 model



10 MPa, 70 ºC



CO_2 + ethanol system
is partially miscible at 10 MPa and 70 °C

R. Melgosa *et al.*, J. Chem. Thermodyn. 115 (2017) 106–113.

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Ethanolysis of fish oil

3-step transesterification

$$\mathrm{TAG} + \mathrm{EtOH} \rightleftharpoons \mathrm{DAG} + \mathrm{FAEE}$$

$$\mathrm{DAG} + \mathrm{EtOH} \rightleftharpoons \mathrm{MAG} + \mathrm{FAEE}$$

$$\mathrm{MAG} + \mathrm{EtOH} \rightleftharpoons \mathrm{GLY} + \mathrm{FAEE}$$

TAG: Triacylglyceride; EtOH: Ethanol;

FAEE: Fatty Acid Ethyl Ester; DAG: Diacylglyceride;

MAG: Monoacylglyceride; GLY: Glycerol

Reaction kinetics

Ethanolysis of fish oil from tuna and sardine in $scCO_2$ catalysed by immobilized lipases

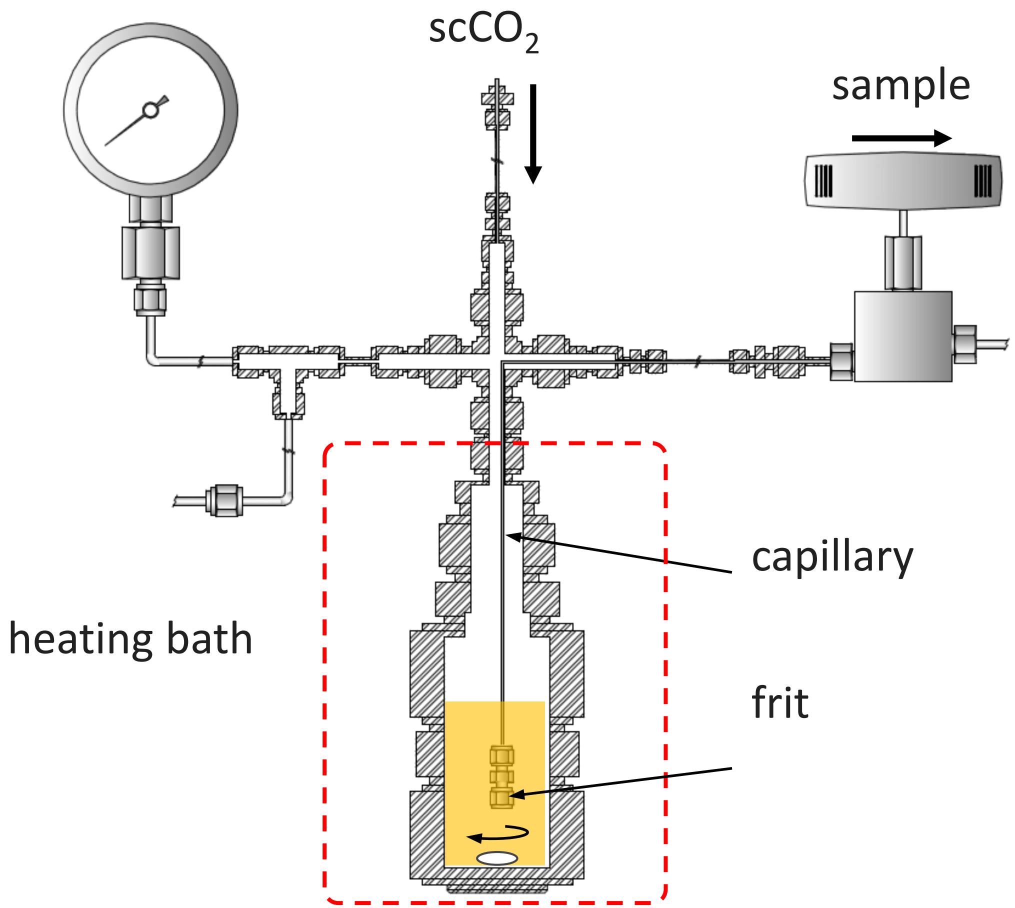
- Lipozyme RM IM from *R. miehei*
- Lipozyme 435 from *C. Antarctica*

Schematic diagram

HP-BSTR

V = 100 mL

MAWP = 326 bar



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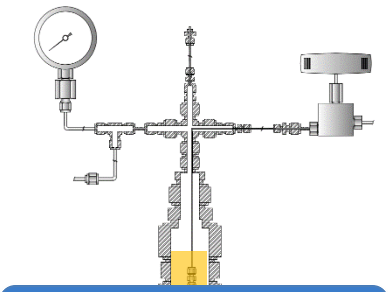
Reaction kinetics

Ethanolysis of fish oil
3-step transesterification

$$TAG + EtOH \rightleftharpoons DAG + FAEE$$

$$DAG + EtOH \rightleftharpoons MAG + FAEE$$

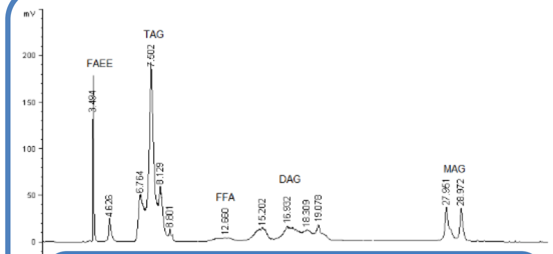
$$MAG + EtOH \rightleftharpoons GLY + FAEE$$



Ethanolysis reaction

Sampling

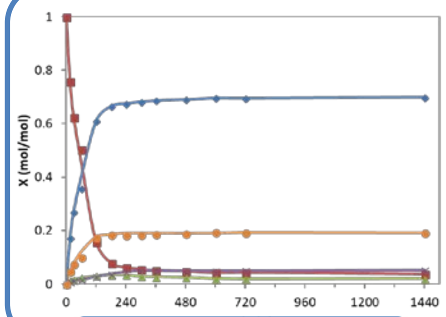
Periodically during 8 h



Analysis

Neutral ipid Profile (HPLC)
(Solaesa *et al.* 2016)

Glycerol and ethanol (HTGC)
(Bucio *et al.* 2013)



Time course of the reaction

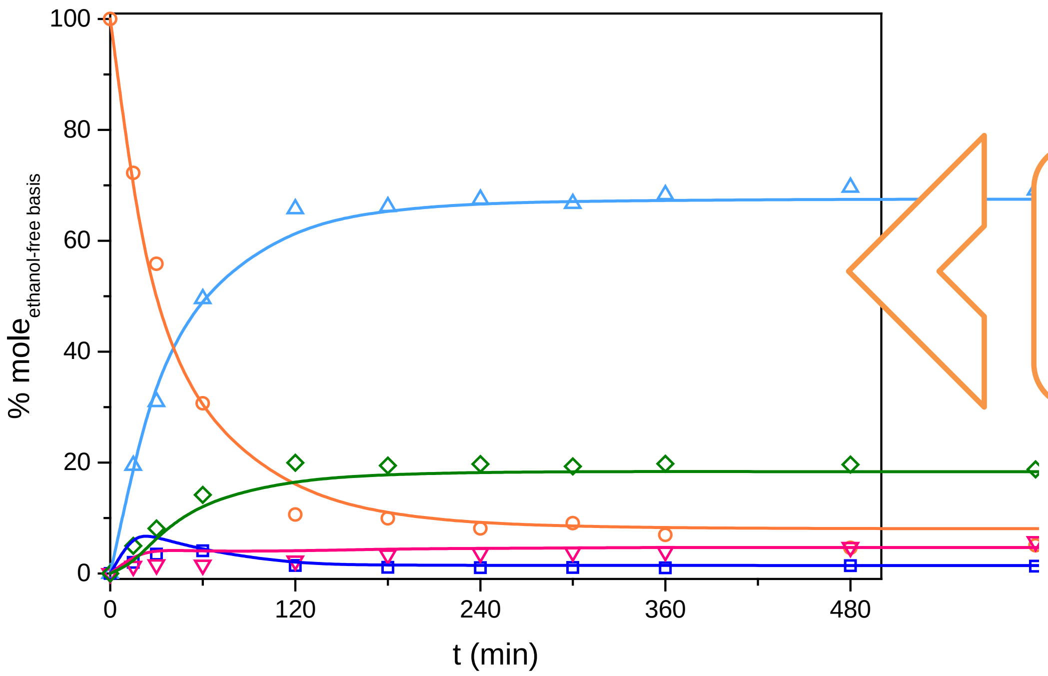
Experimental conditions:

- Lipozyme RM IM (5 %wt.) and Lipozyme 435 (10 %wt.)
- SMR (2:1 - 76:1)
- p (7.5-30 MPa)
- T (50-80 °C)

R. Melgosa *et al.*, J. CO_2 Util. 17 (2017) 117–119.
R. Melgosa *et al.*, J. CO_2 Util. 31 (2019) 65–74.

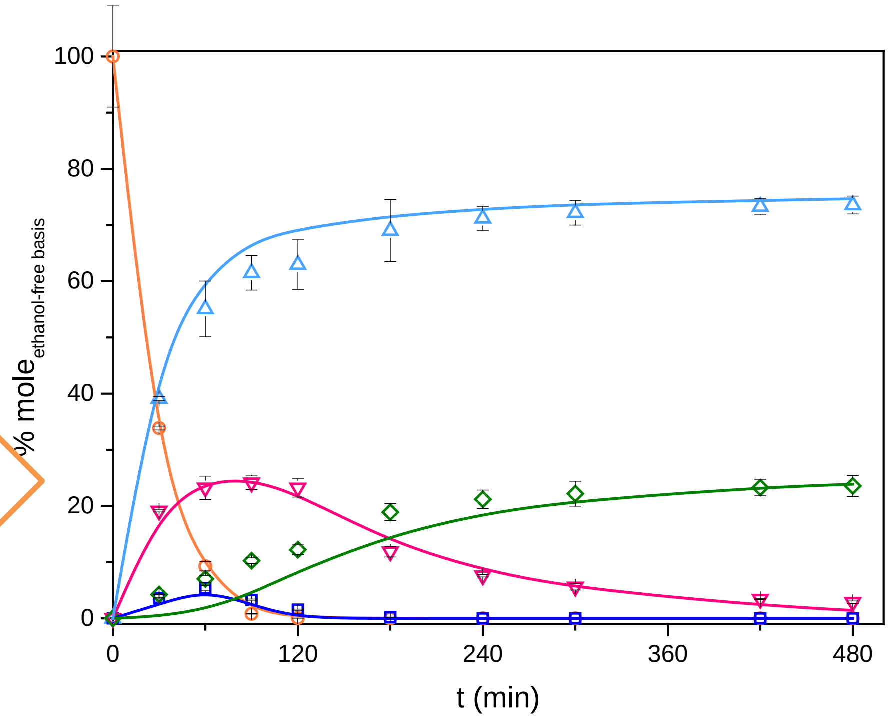
Reaction kinetics

-△- FAEE; -○- TAG; -□- DAG; -▽- MAG; -◇- GLY



Lipozyme RM IM (5 %wt.)
SMR = 38:1
T = 50 °C
P = 10 MPa

Lipozyme 435 (10 %wt.)
SMR = 76:1
T = 50 °C
P = 10 MPa



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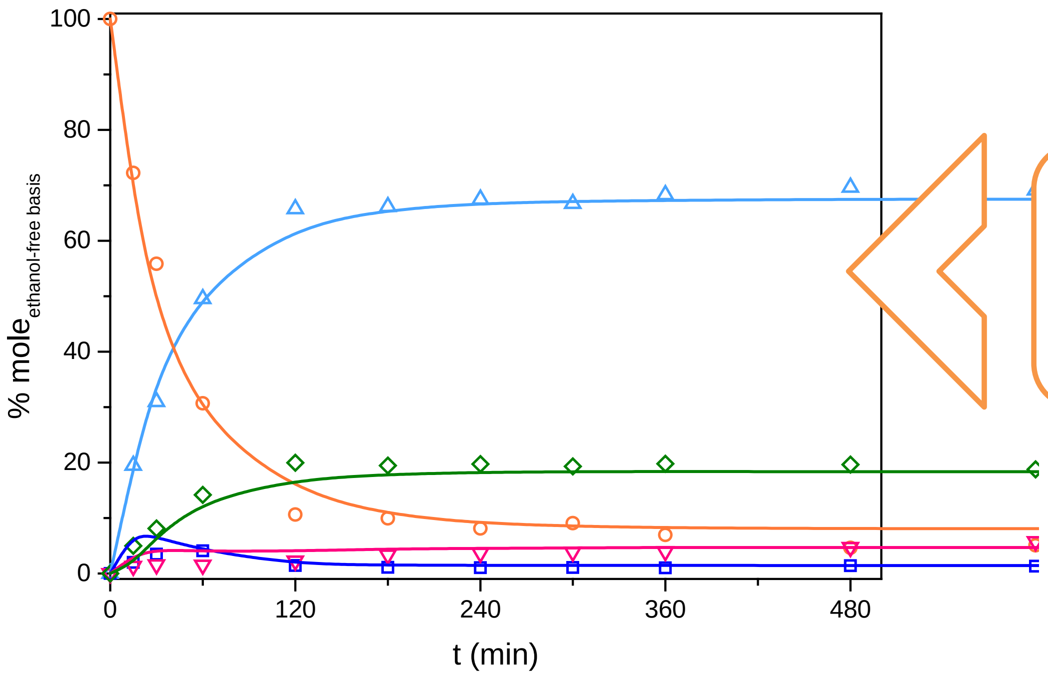

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SMR = 38:1
T = 50 °C
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Lipozyme 435 (10 %wt.)
SMR = 76:1
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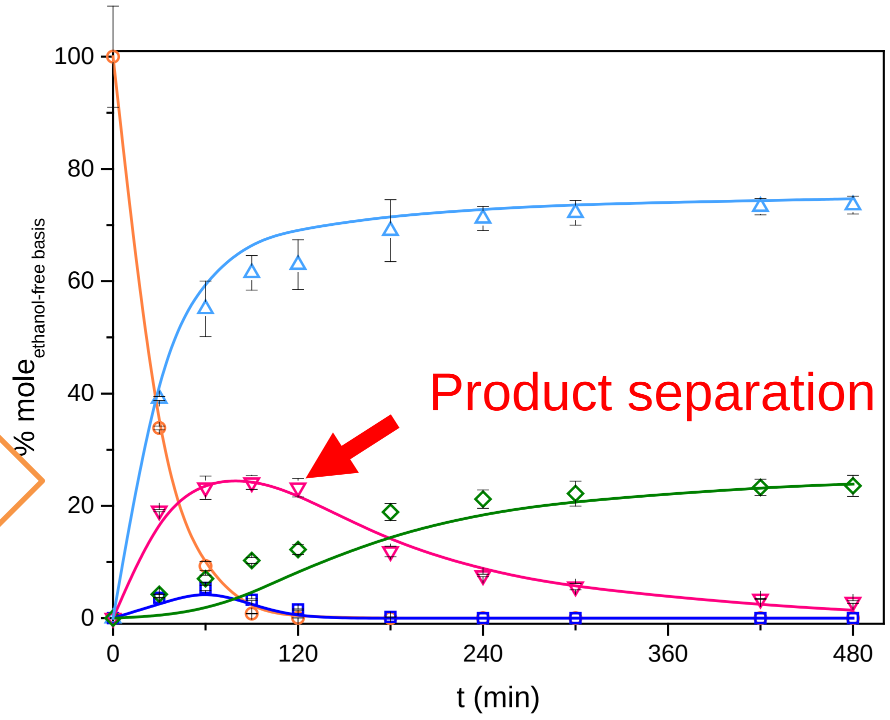
P = 10 MPa



R. Melgosa *et al.*, J. CO_2 Util. 17 (2017) 117–119.
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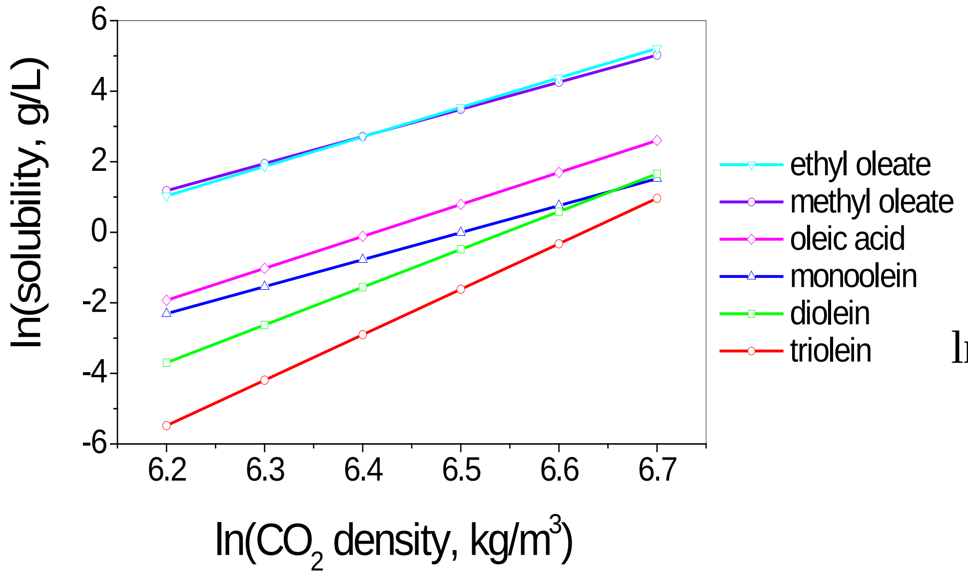
Product separation



Güçlü-Üstündağ &Temelli.
Ind. Eng. Chem. Res. 39(2000) 4756-66.

$$\ln\left[\rho_{CO_2}(25\,\text{MPa}, 40\,^{o}\text{C})\right] = 6.78$$

One-step extraction

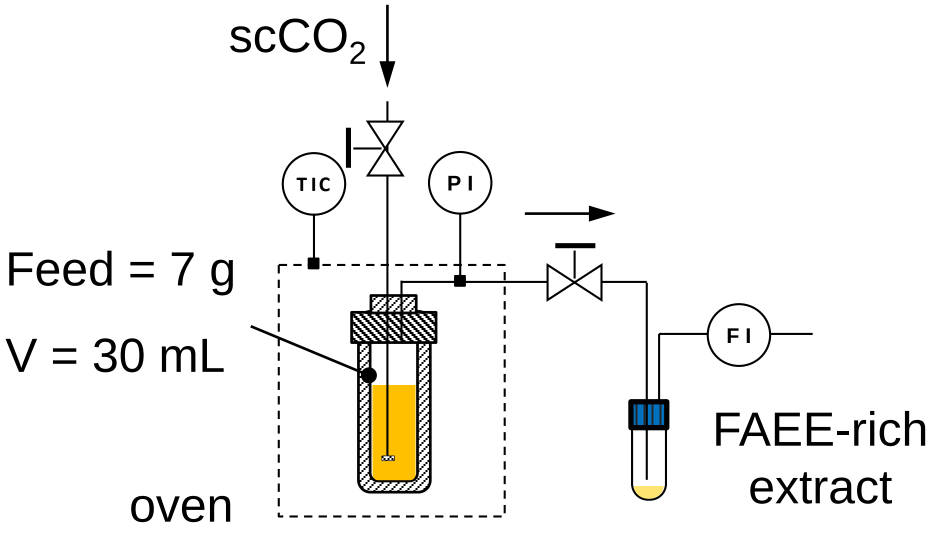
Experimental conditions

10-30 MPa

40-60 ºC

ṁ = 1-3 g/min

30 min extraction



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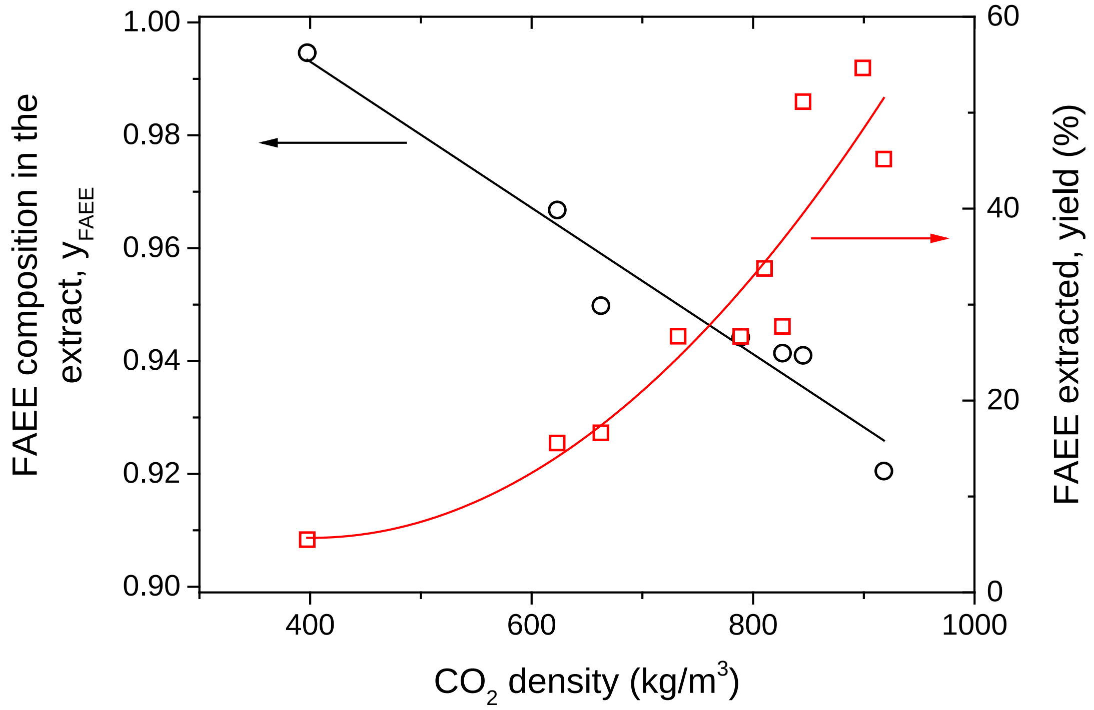
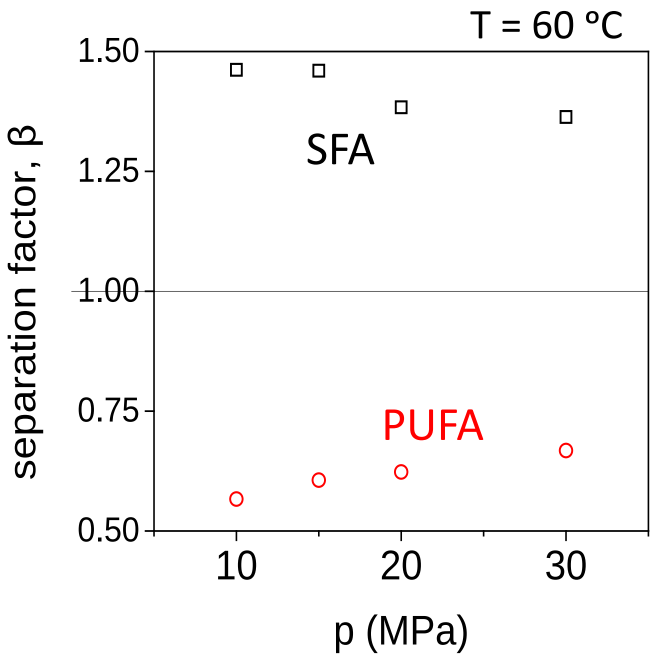


Recovery of FAEE derivatives in the extract increases with CO_2 density, however, its purity decreased since there is co-extraction of MAG

R. Melgosa *et al.*, J. CO_2 Util. 31 (2019) 65–74.

Product separation



$$\beta = \frac{\text{composition in the extract, } y}{\text{composition in the raffinate, } x}$$

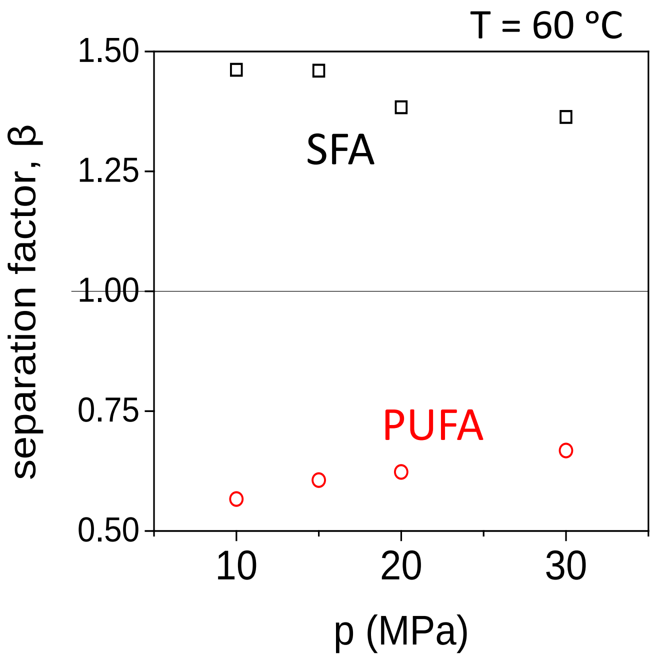
Fatty acid profile analysis
(AOAC Method)

MAG are therefore recovered in the raffinate, with higher omega-3 PUFA concentration than the feed

Raffinate contained
up to **80 %wt. MAG,** and a
EPA+DHA concentration of 37.6 %wt.
(20 % more than initial feed)

R. Melgosa *et al.*, J. CO_2 Util. 31 (2019) 65–74.

Product separation



$$\beta = \frac{\text{composition in the extract, } y}{\text{composition in the raffinate, } x}$$

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(AOAC Method)

Future work >> CC-SFF

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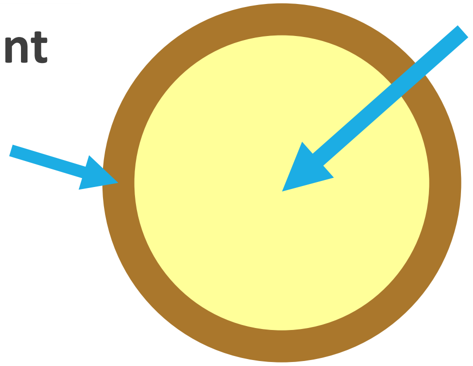


Formulation

Particles from Gas-Saturated Solutions (PGSS)-Drying

Features

high atomization
(bubble formation and gas expansion)

intense cooling
(Joule-Thomson effect)

inert atmosphere
(oxygen displacement)

Experimental conditions

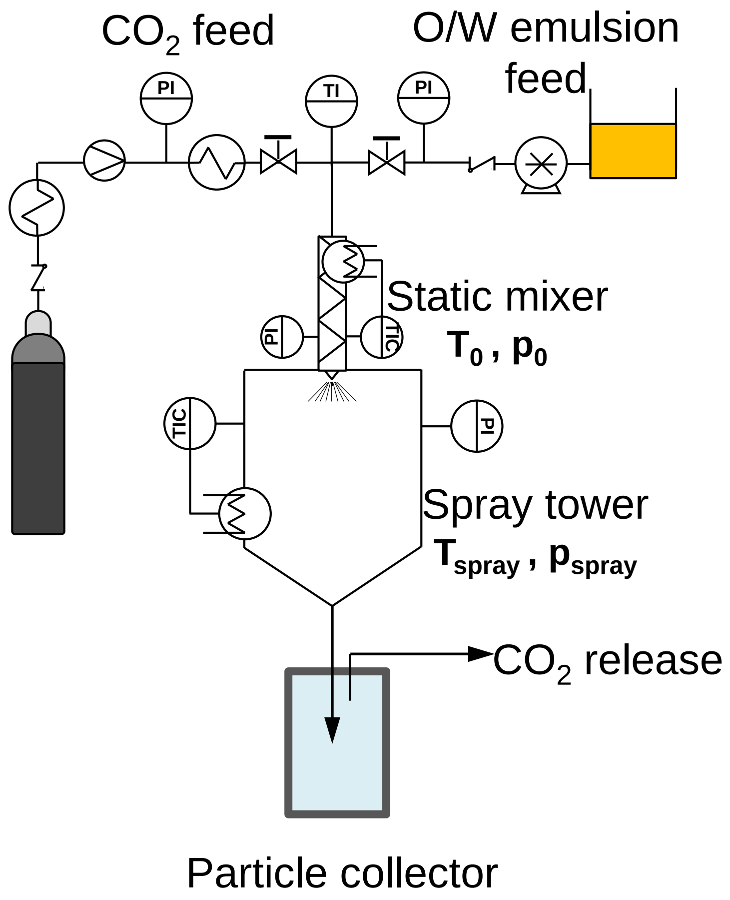
T_0 = 110 ºC, p_0 = 10 MPa

T_{spray} = 55 ºC, p_{spray} = 0.1 MPa

$\dot{m}(CO_2)$ = 10-12 kg/h

GPR = 30

S. Varona *et al.*, Ind. Eng. Chem. Res. 50 (2011) 2088–2097.



Schematic diagram
of the PGSS-drying apparatus

R. Melgosa *et al.*, Food Chem. 270 (2019) 138–148.

Formulation

Particles from Gas-Saturated Solutions (PGSS)-Drying

US-assisted emulsification

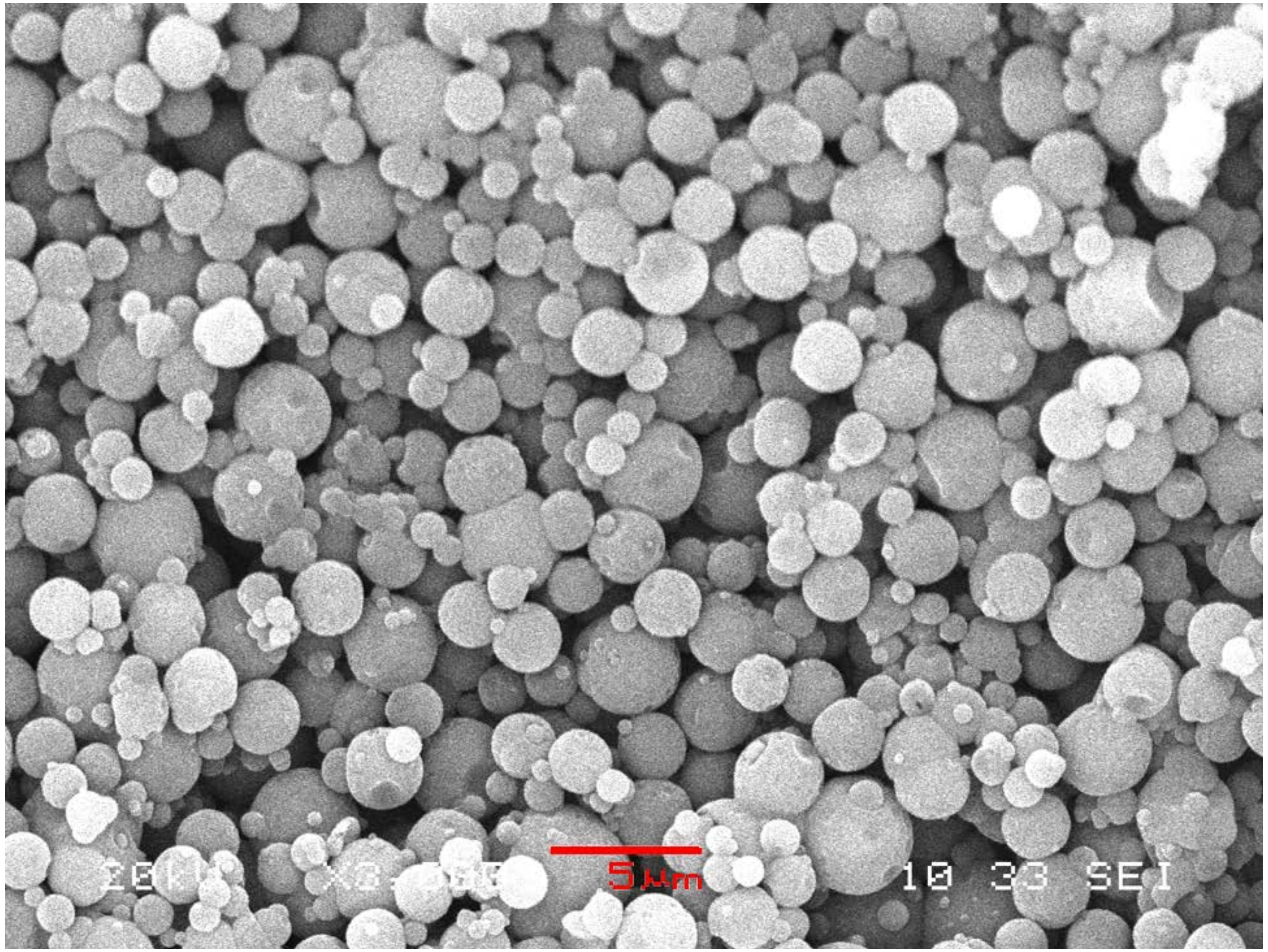
- 6 %wt. Omega-3 concentrate (70 %wt. DHA in TAG form)
- 24 %wt. OSA-starch
- 70 %wt. Water

PGSS-drying

Omega-3 (20 %wt.) microparticles

d_p = 2-5 μm

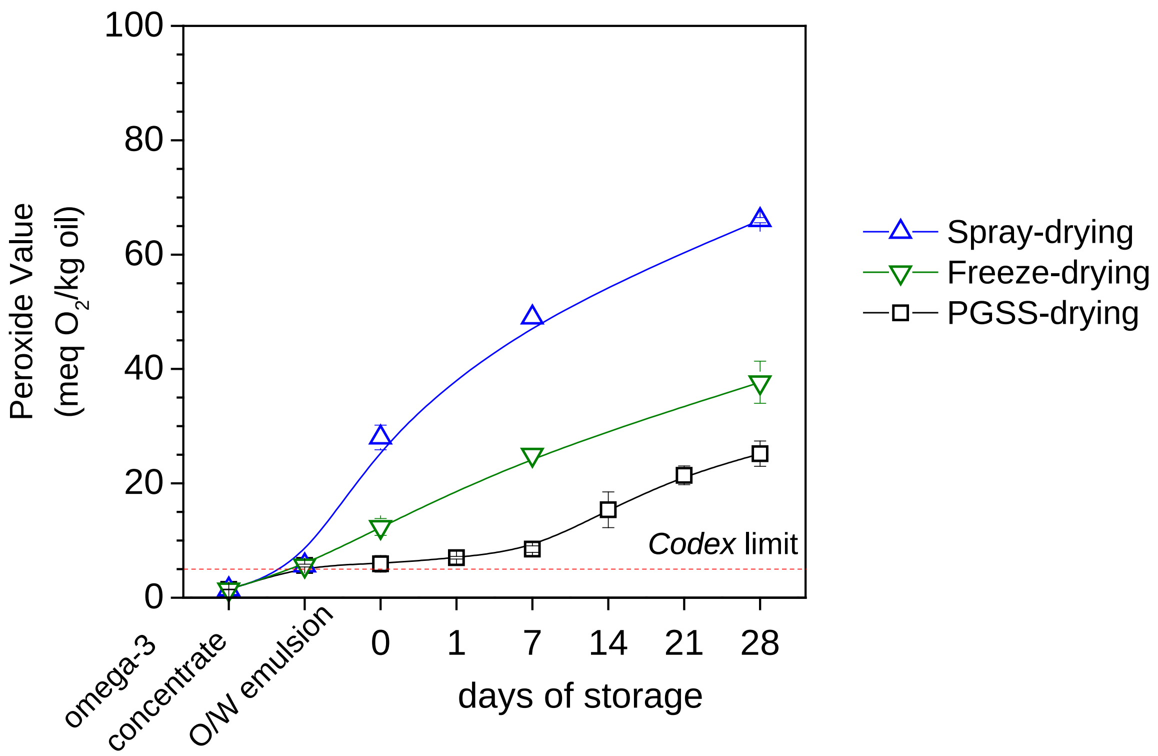
EE (%) = 97.9 ± 0.3



R. Melgosa *et al.*, Food Chem. 270 (2019) 138–148.

Product stability

Peroxide Value (AOAC Method), 28 days of storage at 4ºC

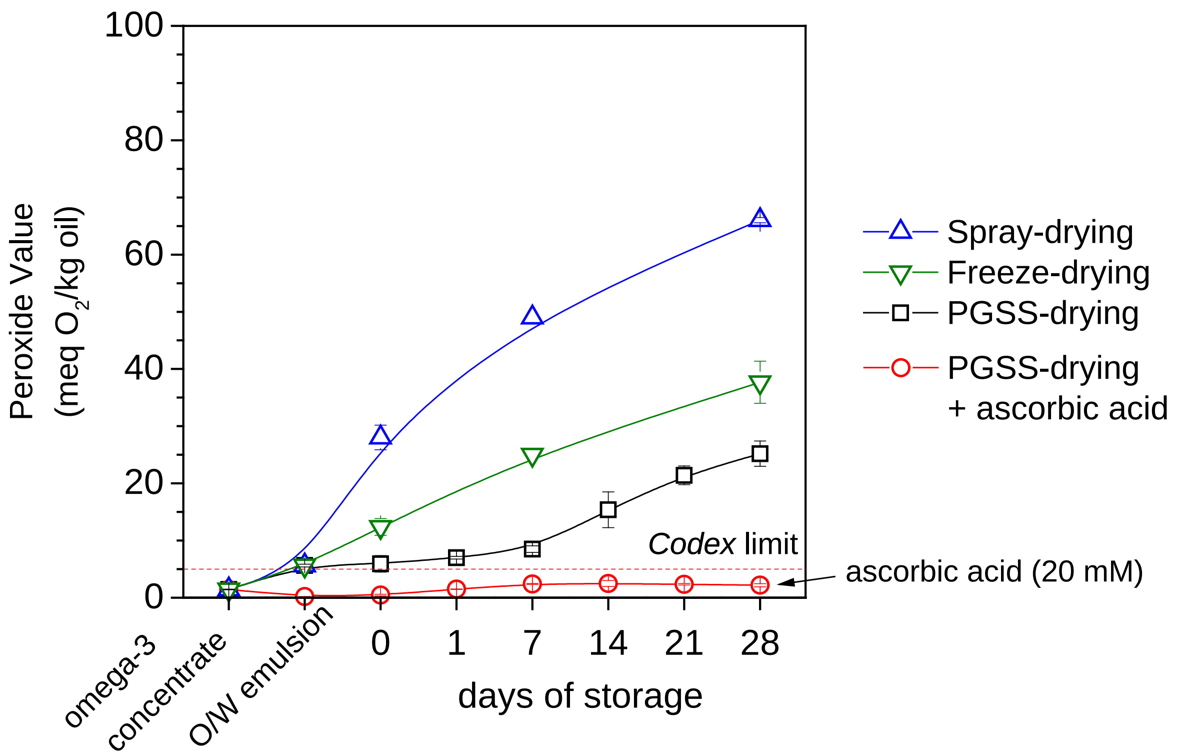


Compared to conventional spray-drying and freeze drying,
PGSS-drying produced less oxidized and more stable particles

R. Melgosa *et al.*, Food Chem. 270 (2019) 138–148.

Product stability

Peroxide Value (AOAC Method), 28 days of storage at 4ºC



Compared to conventional spray-drying and freeze drying,
PGSS-drying produced less oxidized and more stable particles

Ascorbic acid and PGSS-drying maintained PV
below recommended limits for 28 days of storage at 4ºC

R. Melgosa *et al.*, Food Chem. 270 (2019) 138–148.

Conclusion

$scCO_2$ affects the enzyme activity, being the effect different for free and immobilized enzymes.

In the ethanolysis of fish oil, $scCO_2$ allows to obtain a homogeneous mixture of the reactants, increasing the initial reaction rate

Ethanolysis reaction products (FAEE and MAG) can be fractionated using $scCO_2$ as separating agent. Results were acceptable for a one-step separation process

Less oxidized omega-3 microparticles can be obtained by PGSS-drying, compared to conventional drying methods

Future work

- Completing the countercurrent supercritical fractionation studies
- Implement the continuous reaction-fractionation process

Thank you for your attention

Research group **Industrial and Environmental Biotechnology (BIOIND)**

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