

Enzymatic methods to produce EPA/DHA concentrates

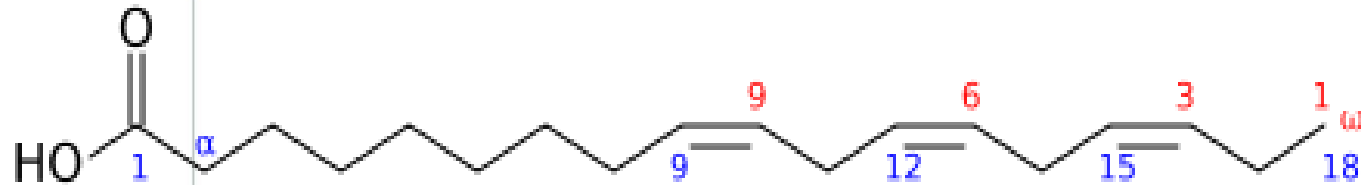
Inge Bruheim

Olympic Seafood

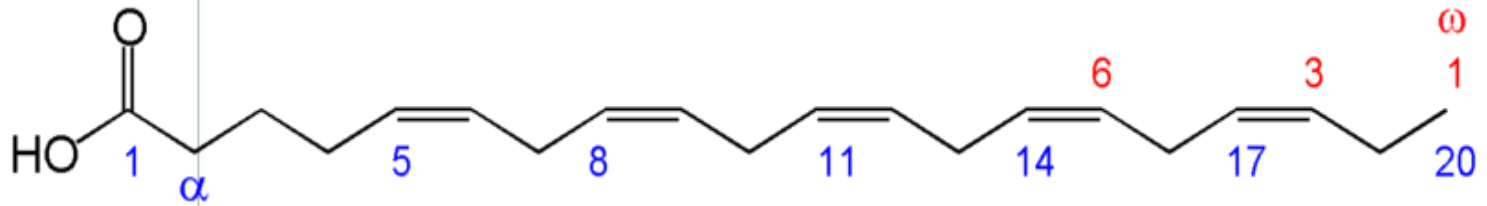


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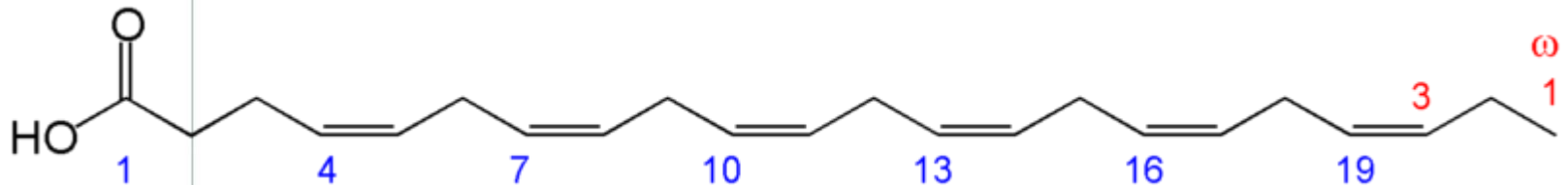
Omega-3 fatty acids



α linolenic acid



EPA



DHA



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Nutritional effects of EPA/DHA

- Cardiovascular effects EPA/DHA
- Cognitive effects in elderly high EPA
- Joint effects high EPA
- Anti-inflammatory effects high EPA
- Cognitive development high DHA
- Eye health high DHA



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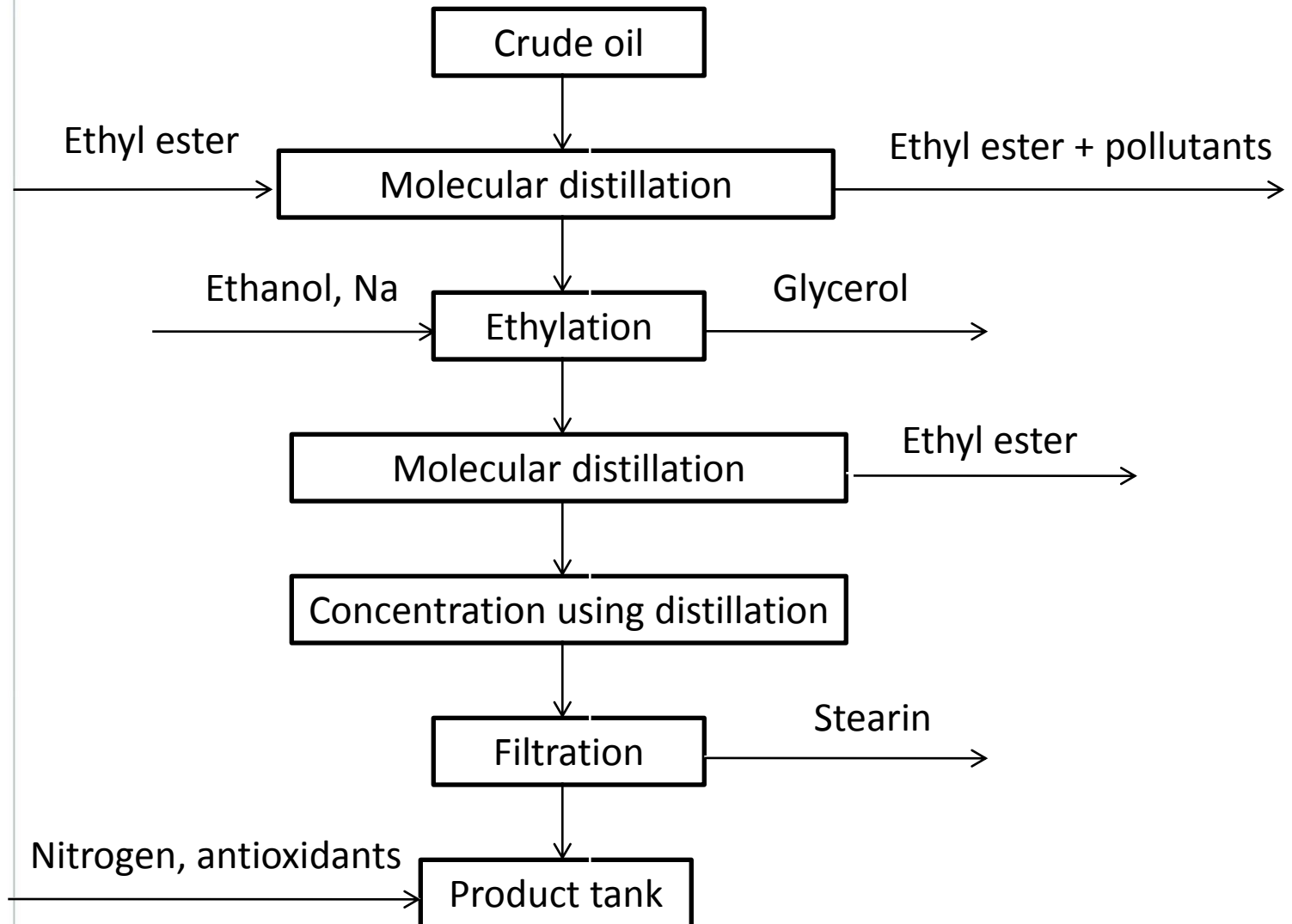
Methods to concentrate EPA/DHA

- Molecular distillation
- Urea fractionation
- Supercritical fluid extraction
- Chromatography
- Selective enzymatic reactions

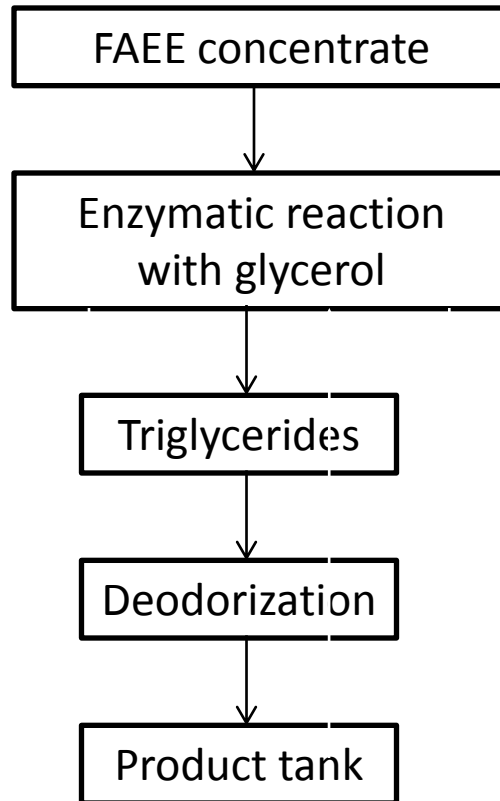


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Omega-3 concentrate production

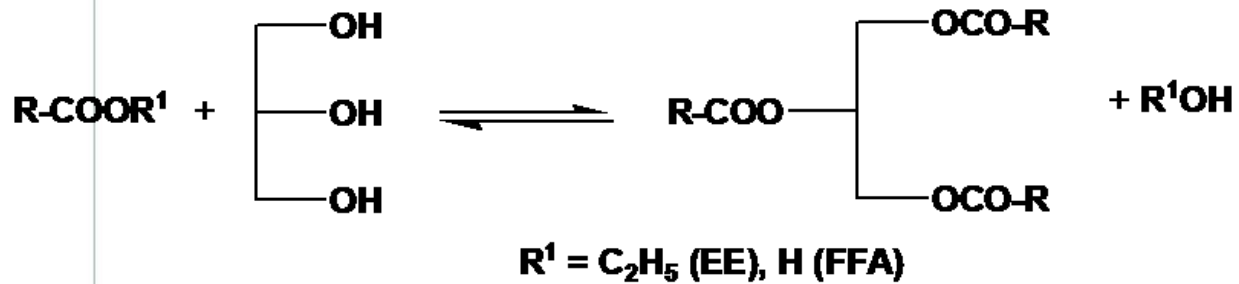


Enzymatic TG production



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Condensation reaction



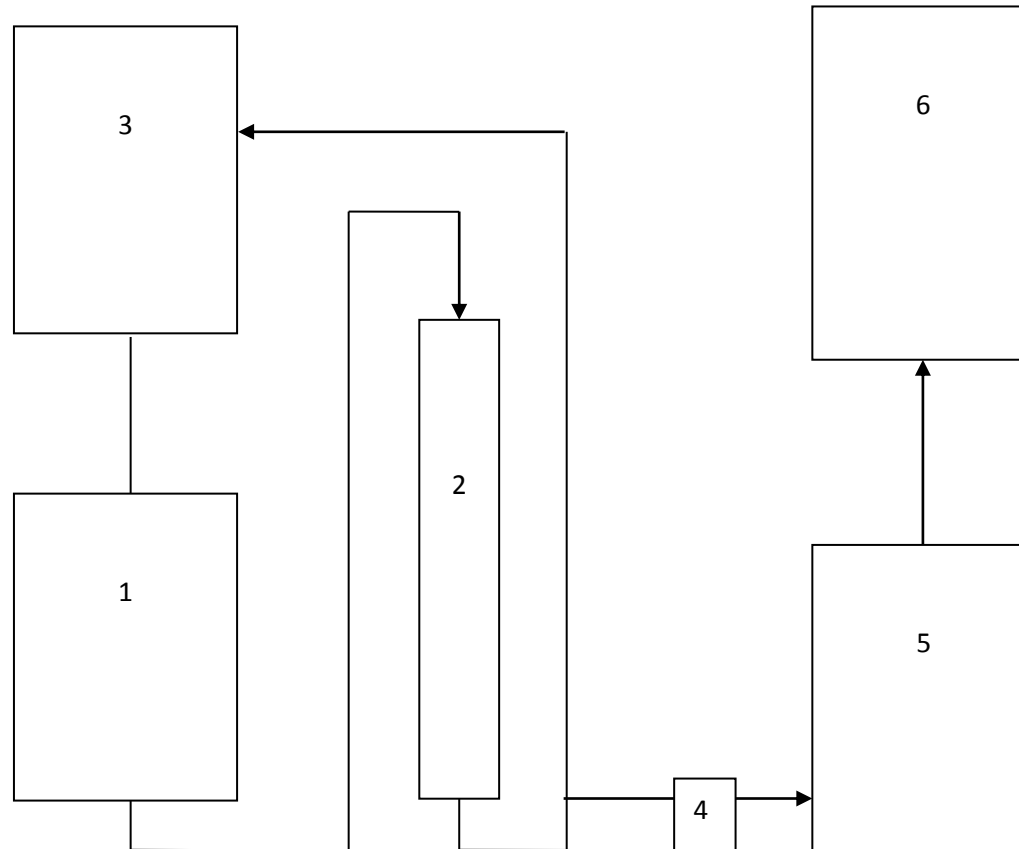
Advantages of enzymatic packed bed

- Continuous operation
- Efficient removal of ethanol
- Increased life time of enzymes
- Improved overall economics



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Schematic setup



1=Feed tank, 2=Enzyme column, 3=Flash tank,
4=Valve, 5=Storage tanks, 6=Molecular distillation

Conditions for TG production

- Run reaction as close as possible to equilibrium
- Lipozyme 435 from Novozymes
- High vacuum (40-70 mbar)
- Flow rate 3.5 bar
- Temperature 70°C



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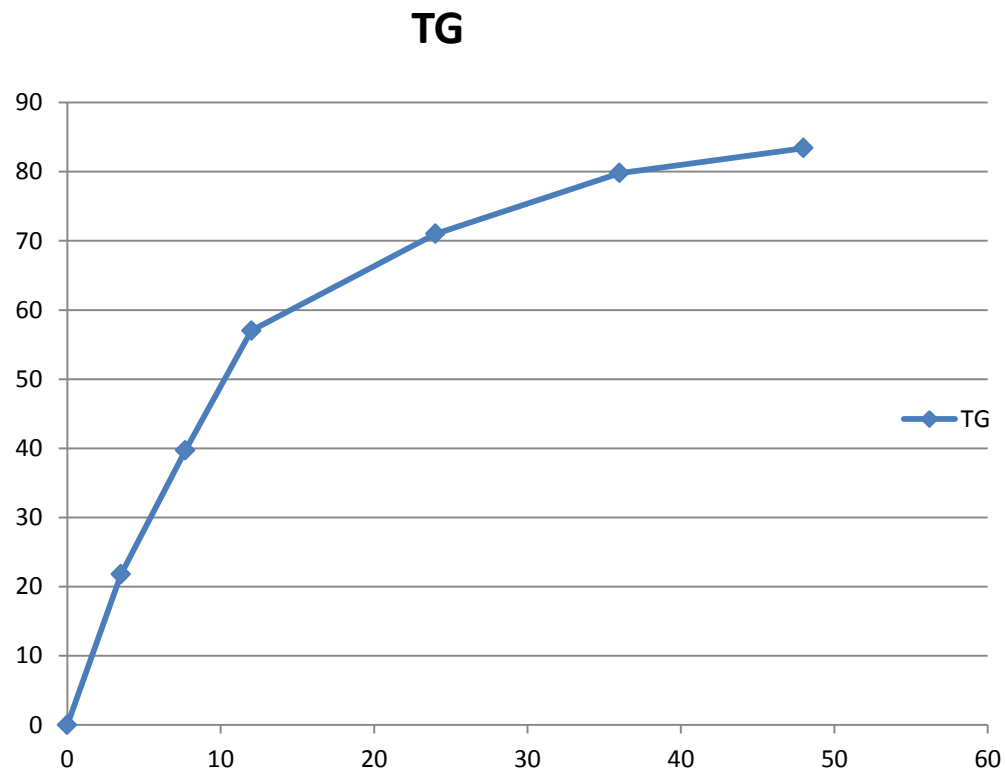
Specification TG concentrate

- TG >60%
- FAEE <7%
- PX=5
- AV=20
- Cold test (3 h at 0 °C)
- Color=6 Gardner



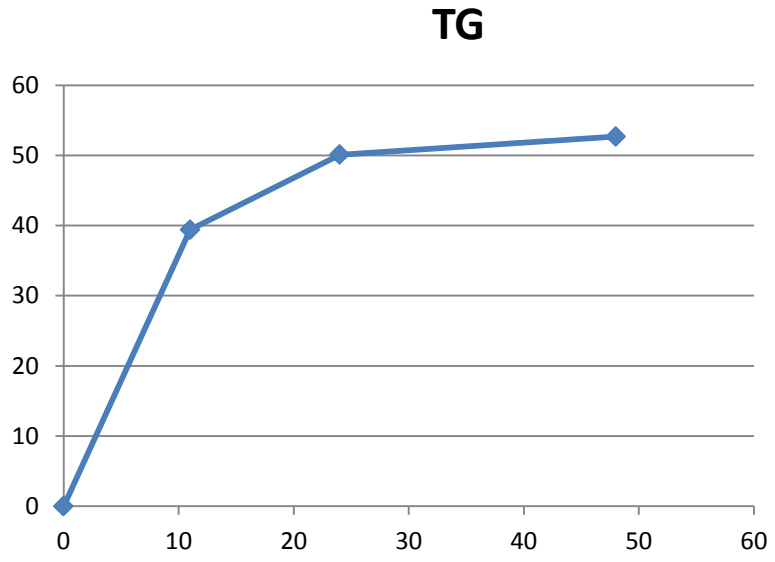
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FAEE : Glycerol ratio=3:1

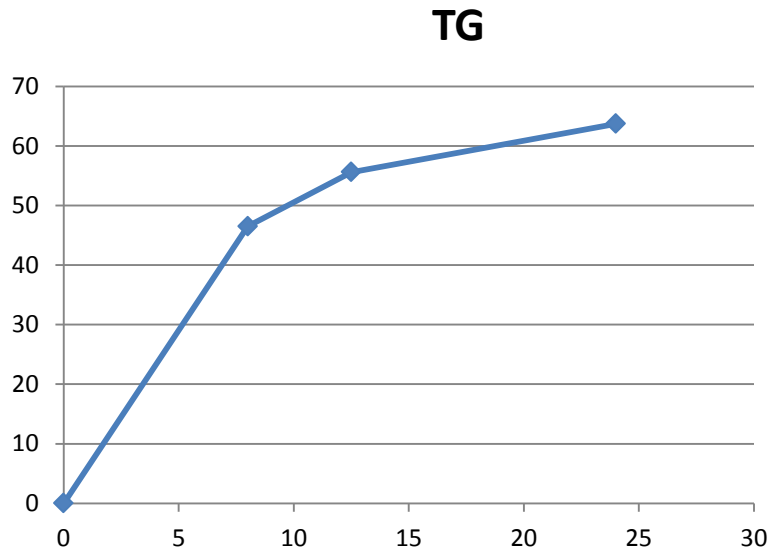


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Reactant relationship depends upon the final specification



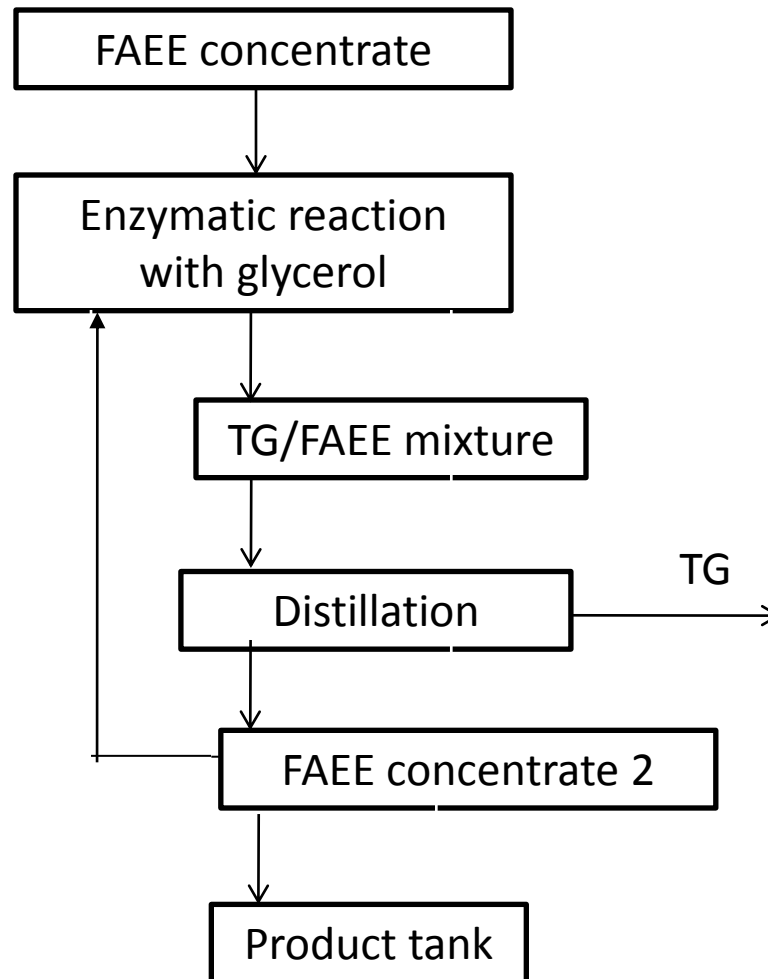
**FAEE:Glycerol
ratio=1.5:1**



**FAEE : Glycerol
ratio=4.5:1**



Enzymatic EPA/DHA production



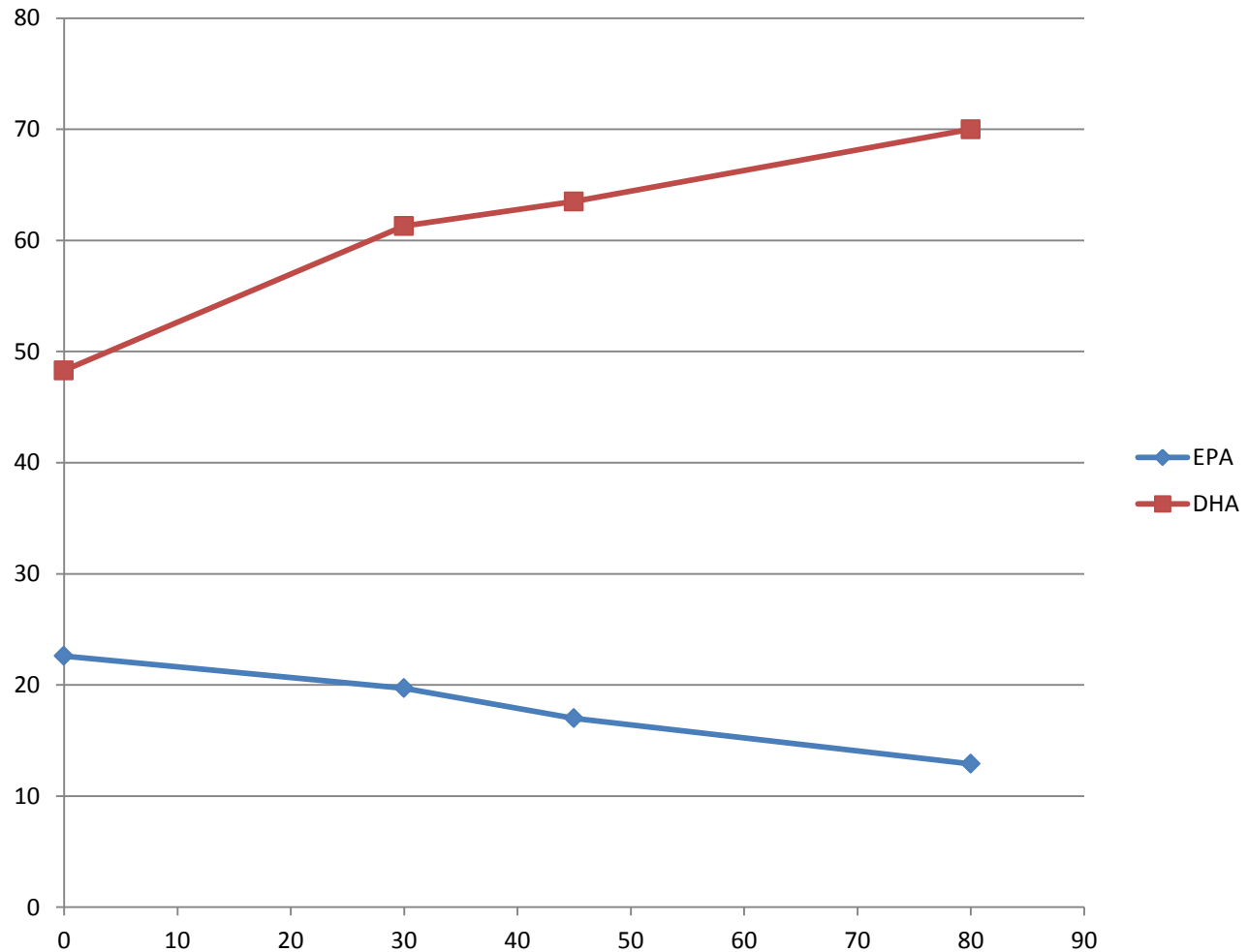
Conditions

- Stop reaction before equilibrium
- RM-IM from Novozymes
- 40°C
- Vacuum as low as possible 40-70 mbar
- Flow rate=3.5 bar
- Starting with a high DHA source in 2:1 glycerol ratio



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EPA/DHA concentration starting from 20-50 EE

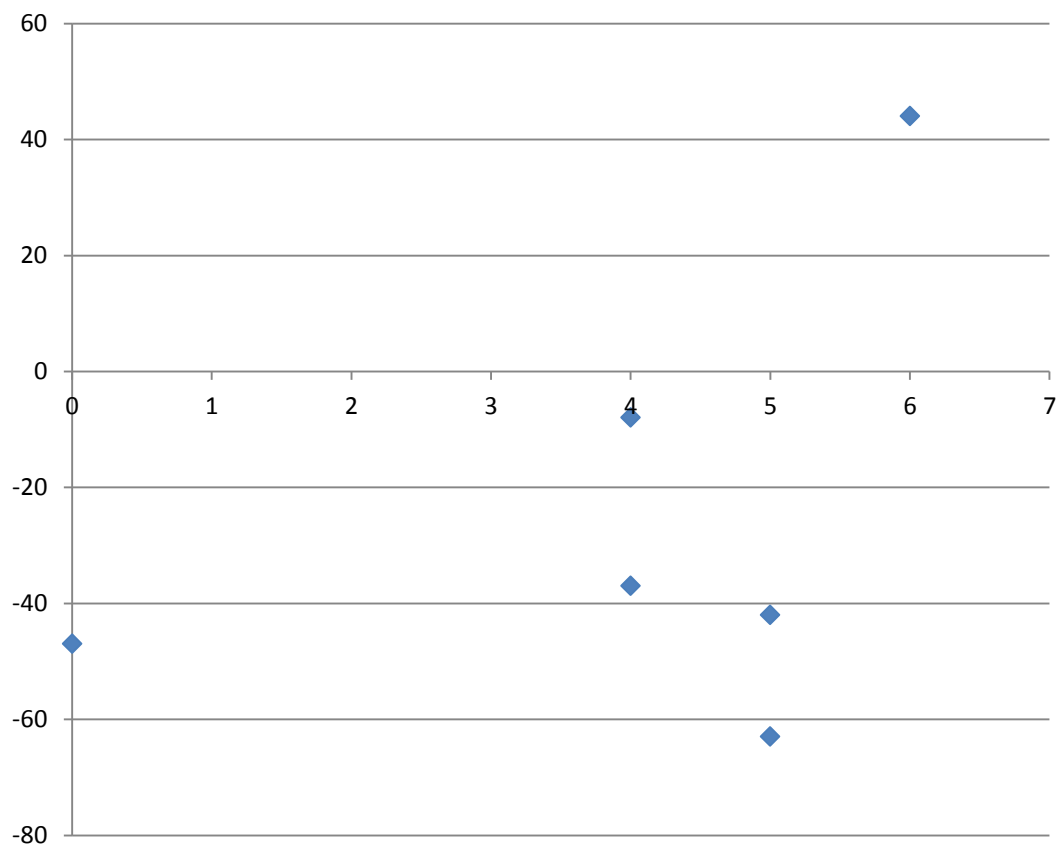


GPC profile

Hours	MG	DG	TG	Esters
30,0	9,8	32,6	4,1	47,0
45,0	11,0	33,8	4,1	51,3
80,0	13,6	39	4,5	42,7

Yield determined by the level of esters!

Relative change as a function of number of double bonds



EPA/DHA concentration starting from 300-300

Hours	EPA	DPA	DHA	EPA + DHA	EPA/DHA
0	35,20	6,45	31,90	67,10	1,10
0,75	34,10	6,26	31,70	65,80	1,08
2,25	35,00	5,90	33,90	68,90	1,03
3,25	35,00	5,80	34,55	69,55	1,01
4,6	35,00	5,60	35,50	70,50	0,99

Hours	AA	SDA	Oleic	Stearic
0	1,9	1,04	1,56	2,21
0,75	1,84	1,08	1,43	1,99
2,25	1,85	1,17	1,35	1,86
3,25	1,85	1,15	1,3	1,75
4,6	1,86	1,18	1,22	1,63



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Observations

- Water addition
- Physical effect on immobilized enzymes
- Vacuum
- Flow rate



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Conclusions

- Can use water in enzymes to run the reaction
- No sign of deactivation
- No sign of physical destruction of enzymes
- Importance of vacuum
- Difficult to scale up



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Acknowledgements

- Novozymes
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