# New applications for enzymes in oil processing

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Customer Solutions

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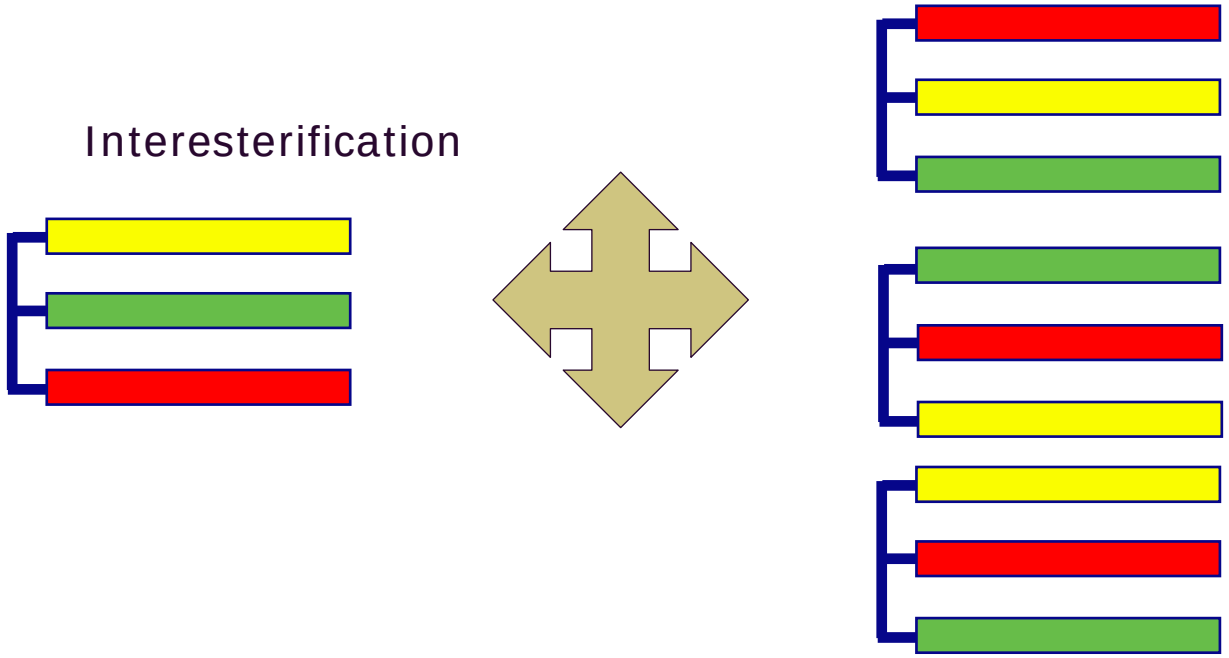
# Agenda

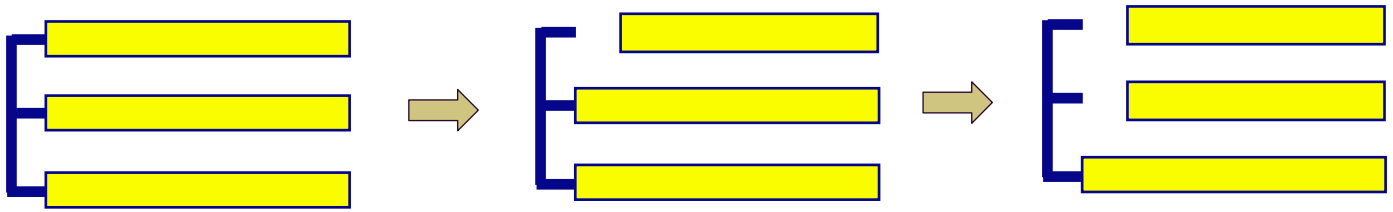
- Current Applications
- Where to from here – my perspective

# Mechanisms of Current Applications

## Interesterification

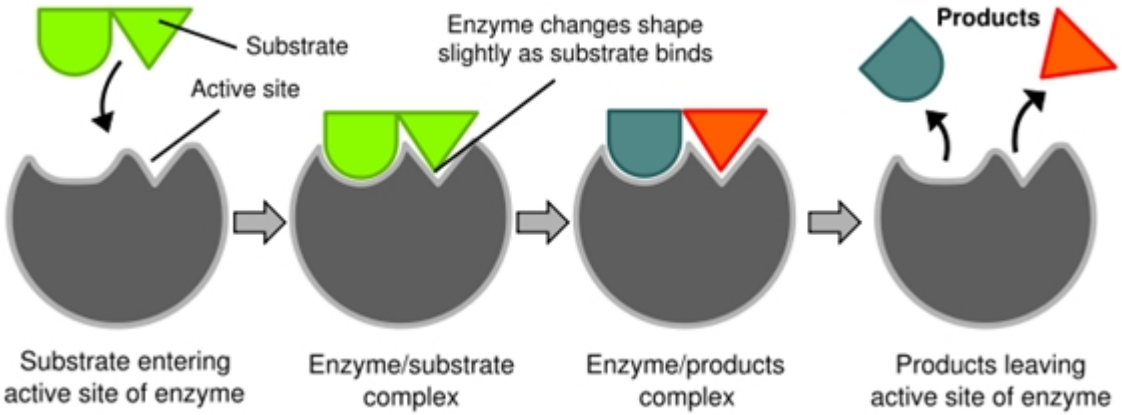


## Hydrolysis

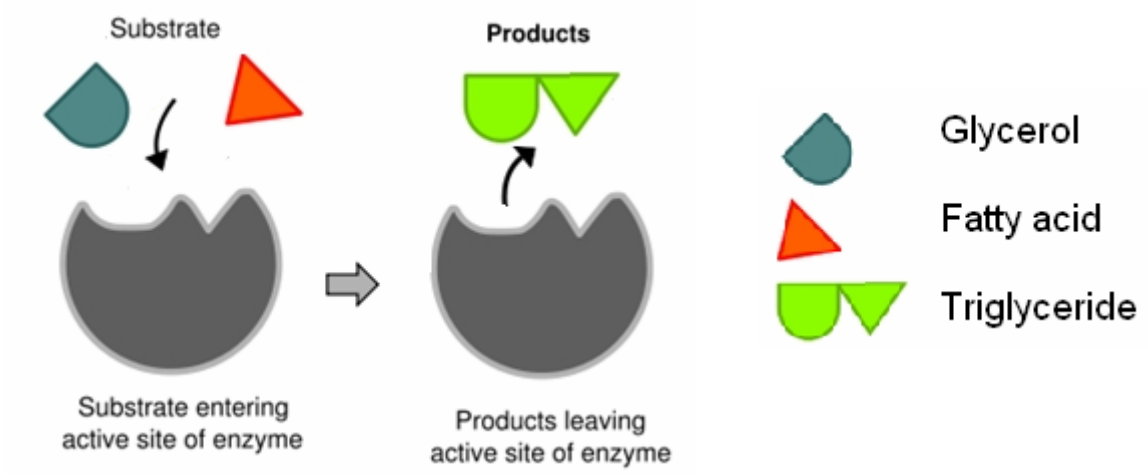


# Several near future applications based on synthesis and modification – 2 examples

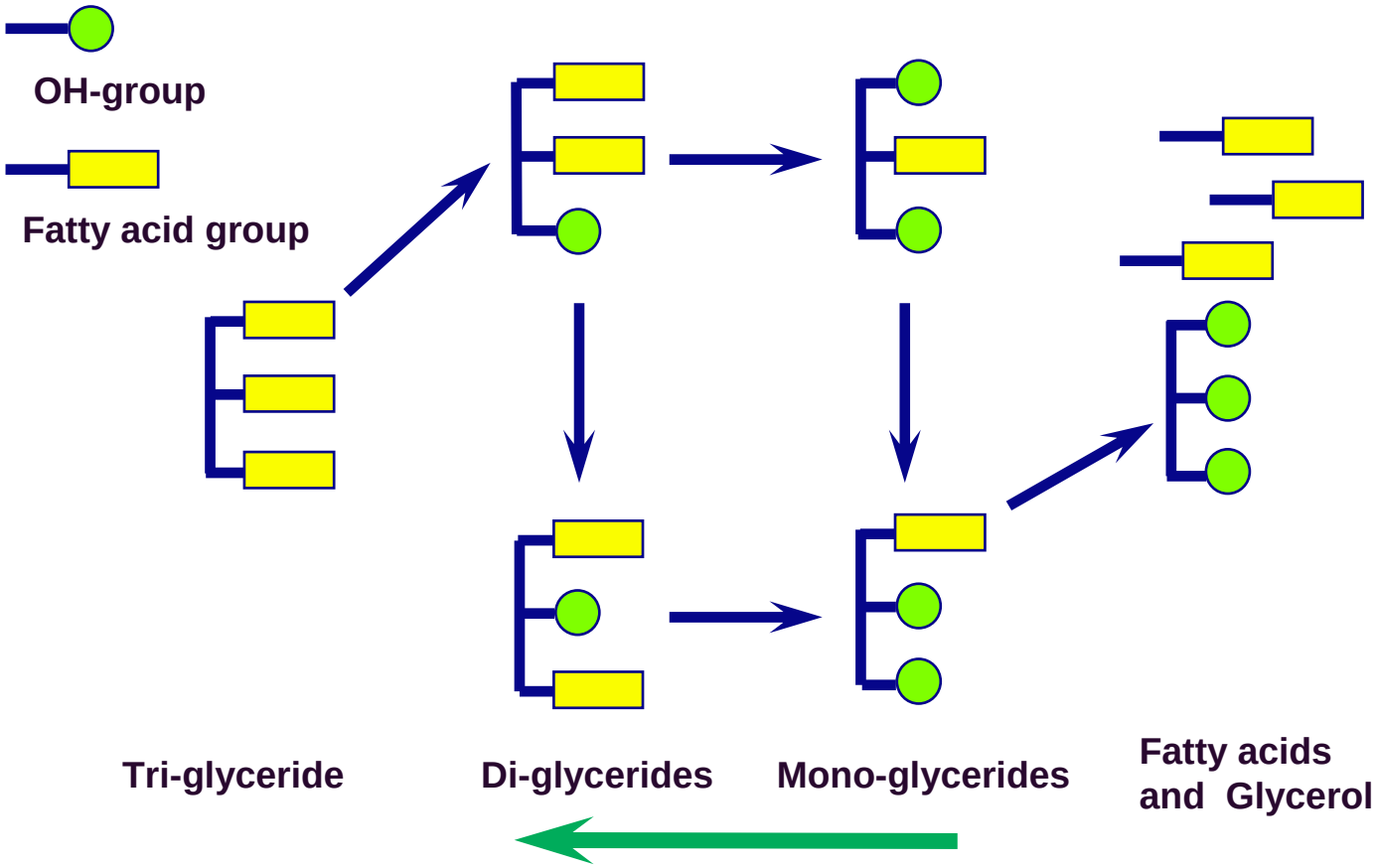
*If an alcohol or fatty acid is substituted for water we can make esters or exchange fatty acids*




*Mixing esters or fatty acids with glycerol allows for condensation reactions to occur under vacuum*

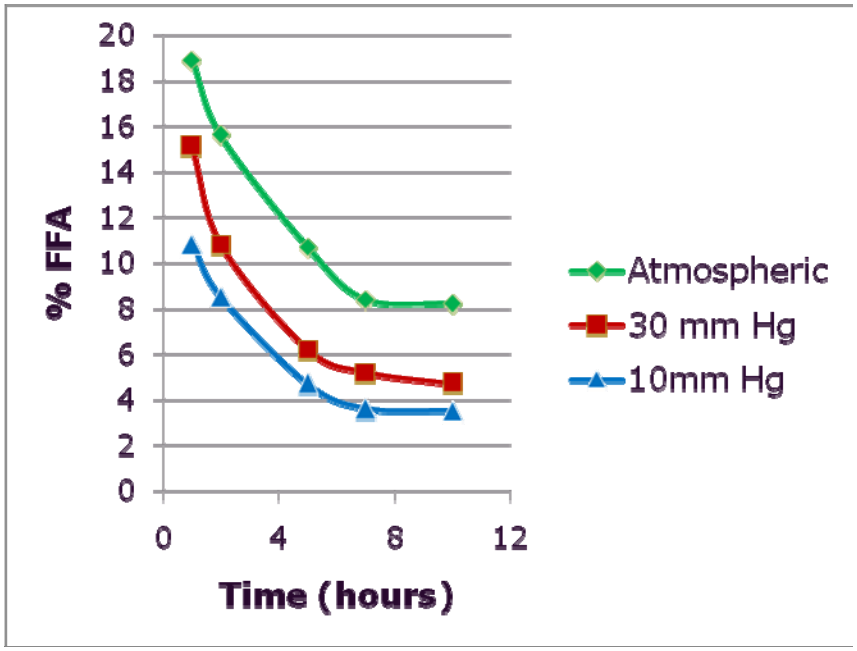


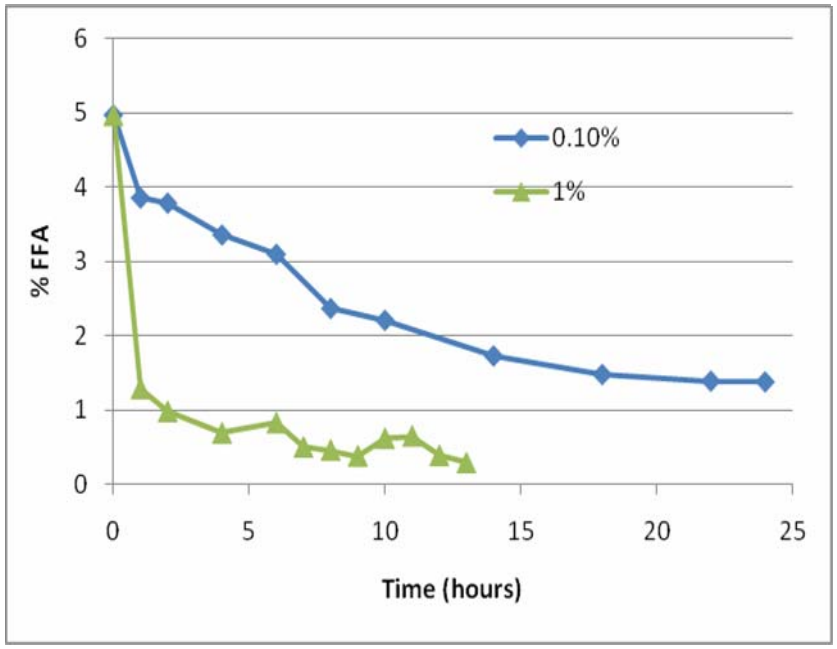
# Application 1: removal of FFA




**REMEDIATION BY CONDENSATION**

# Conditions for reaction



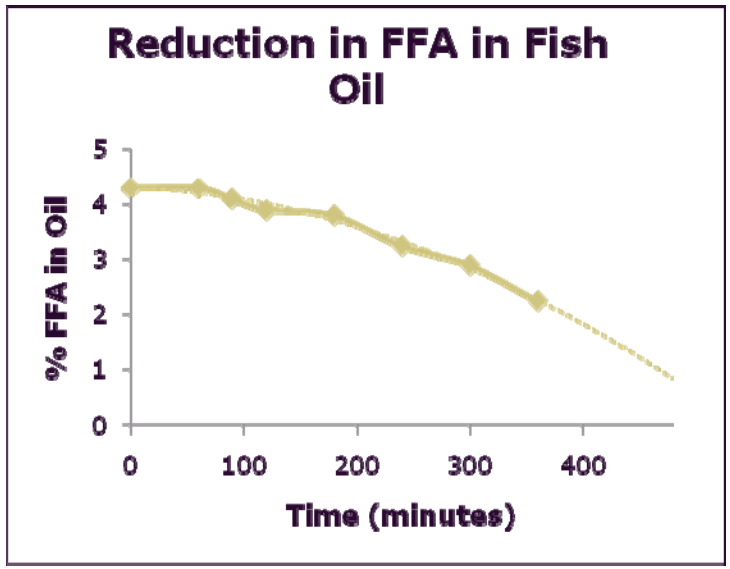




Operating under vacuum to allow removal of generated water increases reaction rate and moves the equilibrium to FFA removal

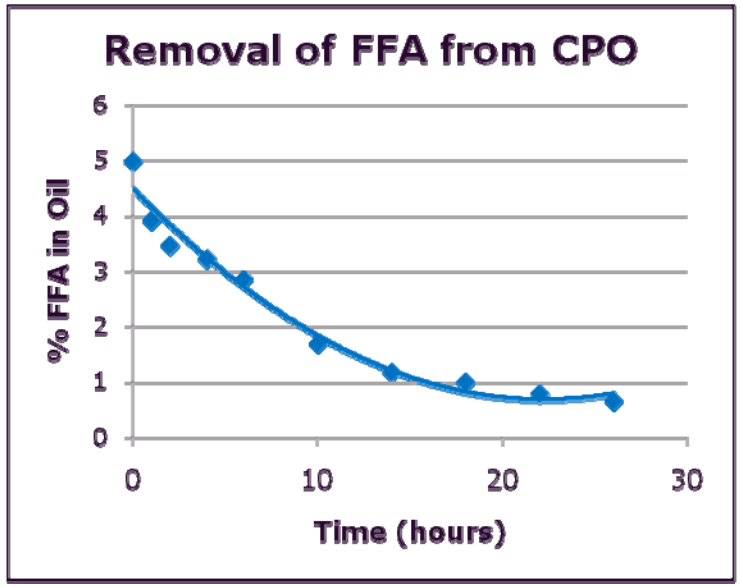
Increased enzyme dosage increases reaction speed and as enzymes (liquid and immobilized) can be recovered, re-use is possible

# New applications

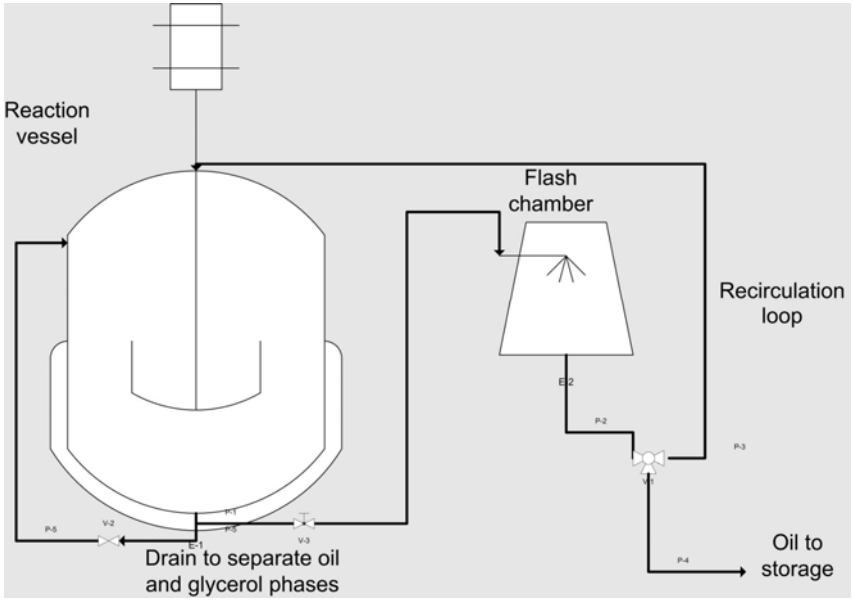
Fish oil for use in high DHA/EPA products contains 5-10% FFA. Removing this by alkali neutralization gives a double loss due to oil entrained in soaps.
Conversion back to TAG reduces losses and makes a better utilization of scarce resources.

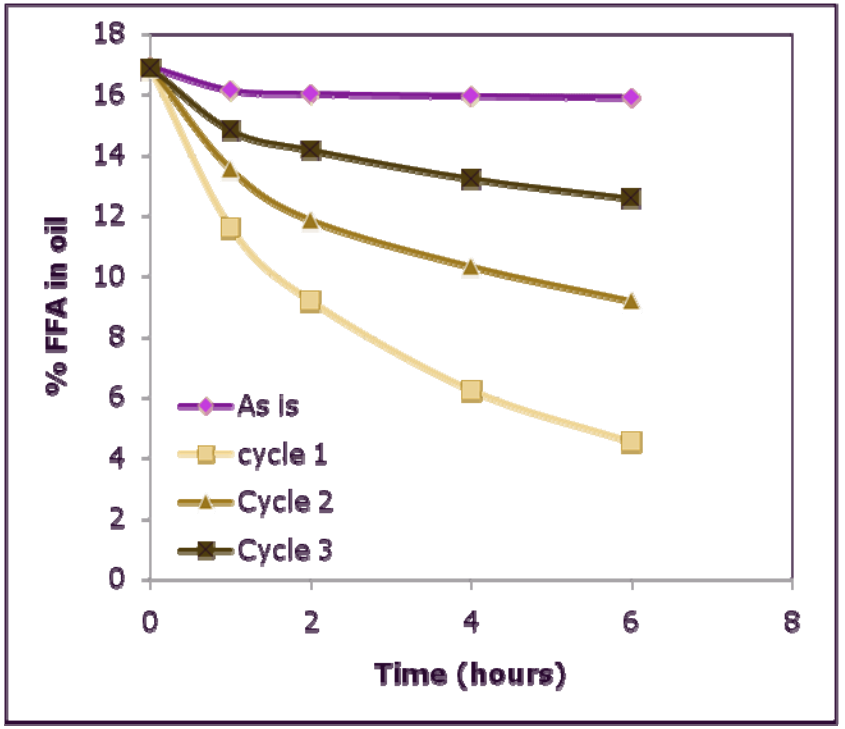



Palm oil extracted from fruit bunches also contains FFA coming from microbial degradation of the lipids. MAG & DAG in this oil is associated with 3-MCPD formation, so condensation of FFA back onto these could reduce the pre-cursors for the formation of this unwanted compound.



# Non Food grade oils

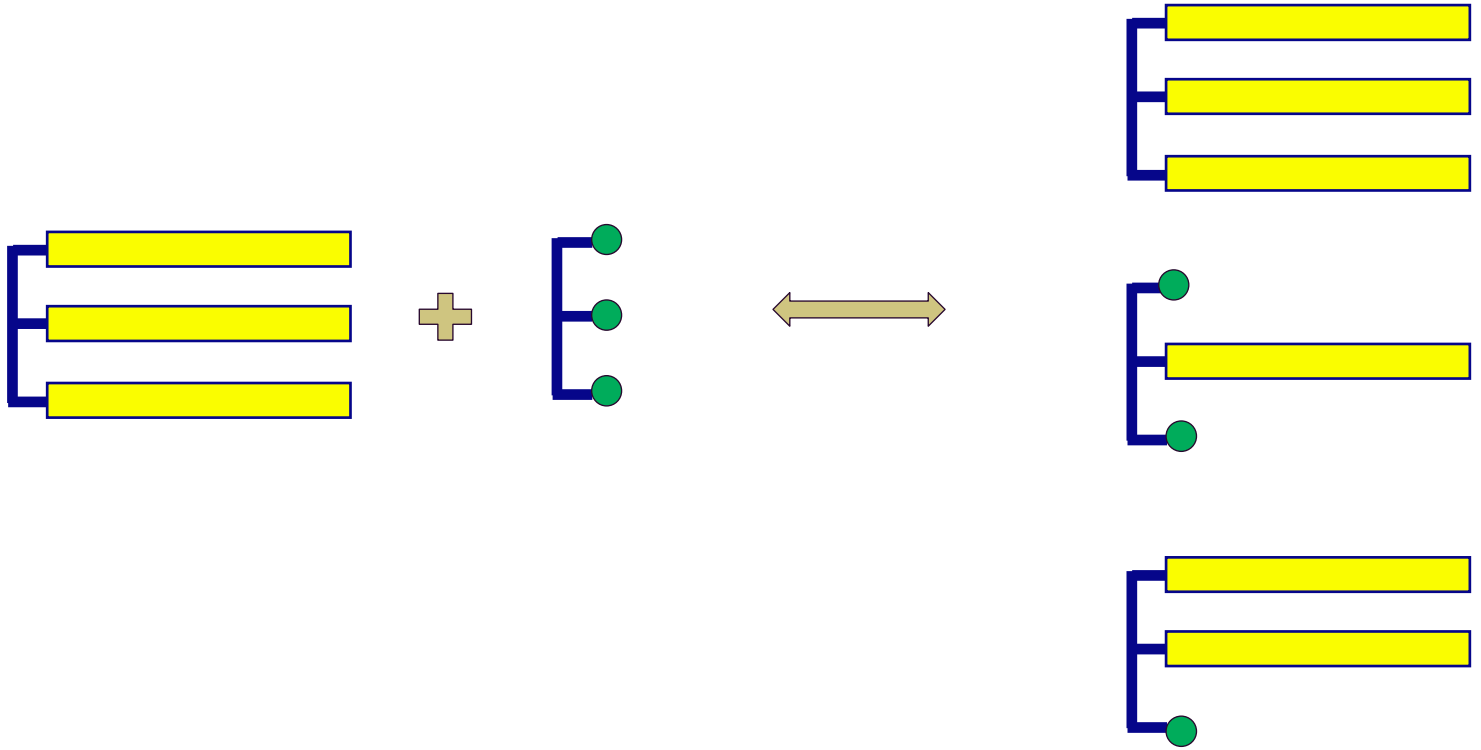



*Condensation of waste oil using Lipozyme Calb at 70°C, 0.1% w/w enzyme and 2% w/w glycerol*

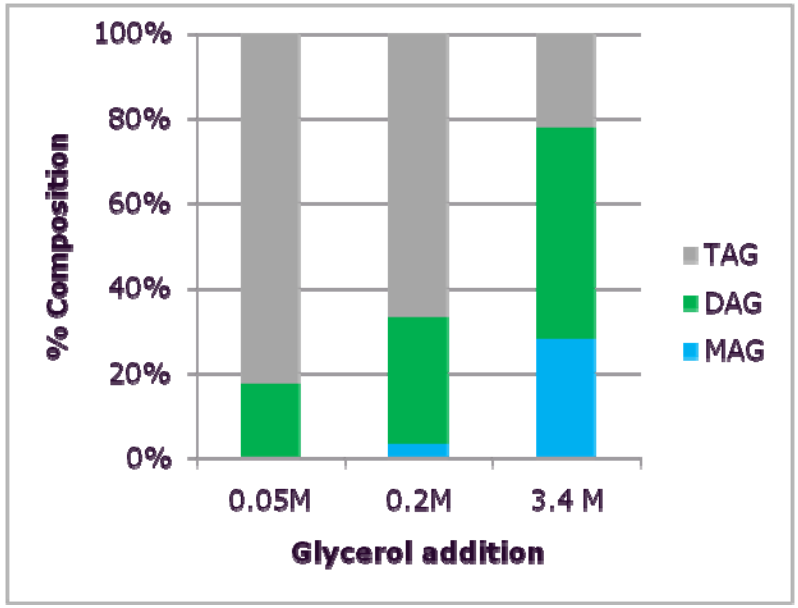
- Waste oils (brown grease) can be readily converted in a batch system but oil quality needs to be considered. Oils often contain high levels of acidity (Non FFA) and oxidation products which can reduce enzyme activity. Pre-treatments may be required to reduce these to get acceptable enzyme working life.

# Application 2: Production of DAG

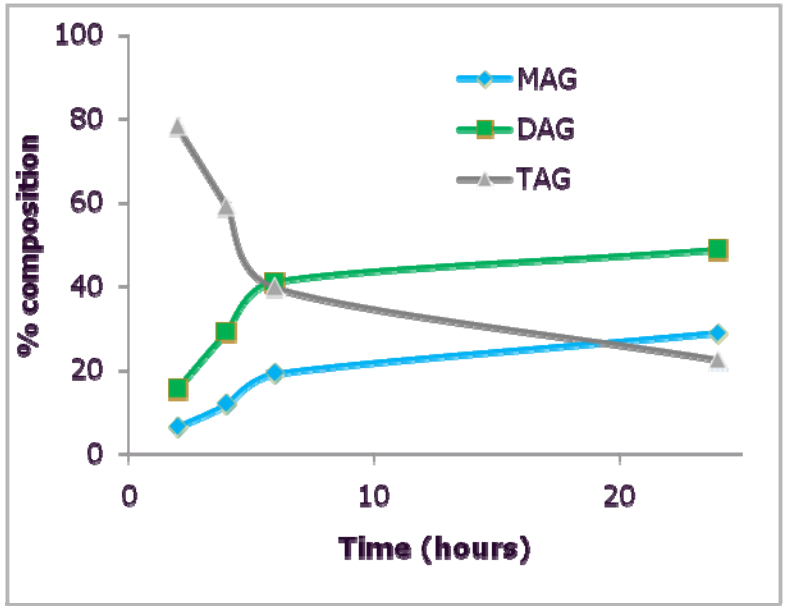


- Glycerolysis of a tri-glyceride can be used to re-distribute fatty acids to produce a mixture of MAG, DAG and TAG. Lipase used can be non-specific or with Sn1,3 specificity
- Immobilised enzyme may be preferred due to high glycerol addition rate.

# Production of DAG/MAG



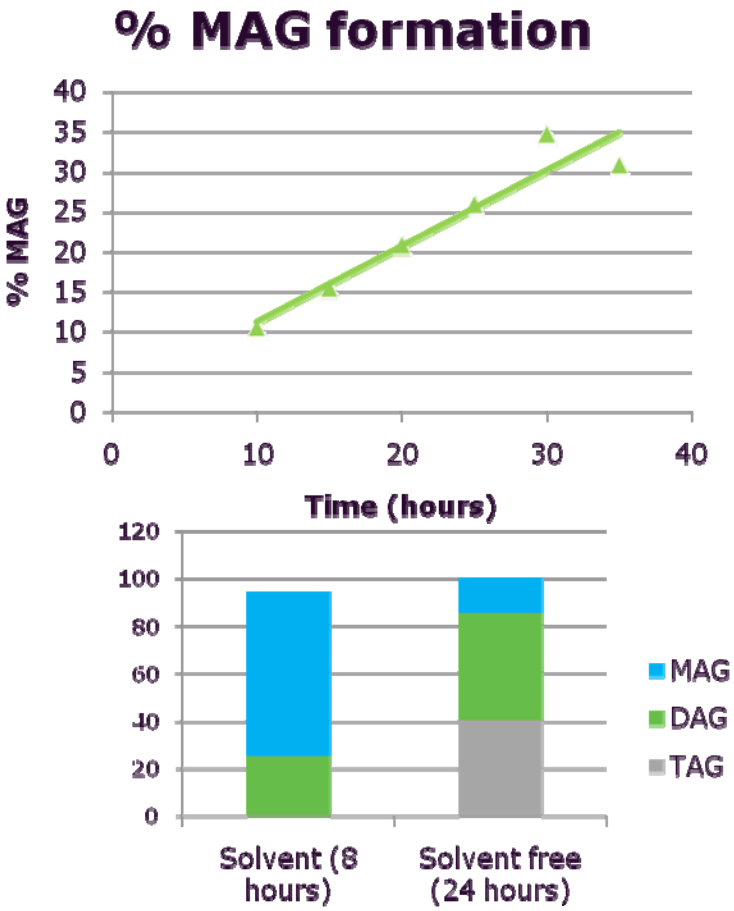

- Glycerolysis with Lipozyme 435 (2%) and various glycerol addition rates at 80°C/24 hours




Glycerolysis with Lipozyme 435 (2%) and glycerol (3.4M) at 80°C, 0-24 hours

# Alternative routes

- MAG and DAG can also be synthesised by combining glycerol and fatty acids
- Using liquid enzymes reduces the conversion to DAG but does not eliminate it .
- Presence or absence of a solvent alters the relative proportions of MAG and DAG




Yang, Rebsdorf, Engelrud, and Xu,
Monoacylglycerol synthesis via enzymatic glycerolysis in an efficient reaction system,
*J. Food Lipids* 12, 299-312, 2005.

# Reactions involving alcohols


Conversion of oils from low to high grade to methyl or ethyl esters (Biodiesel)

Masking of FFA in high acidity waste oils

Enzymatic ethylation of fish oils avoiding losses due to neutralization

# Previous observations for Biodiesel production

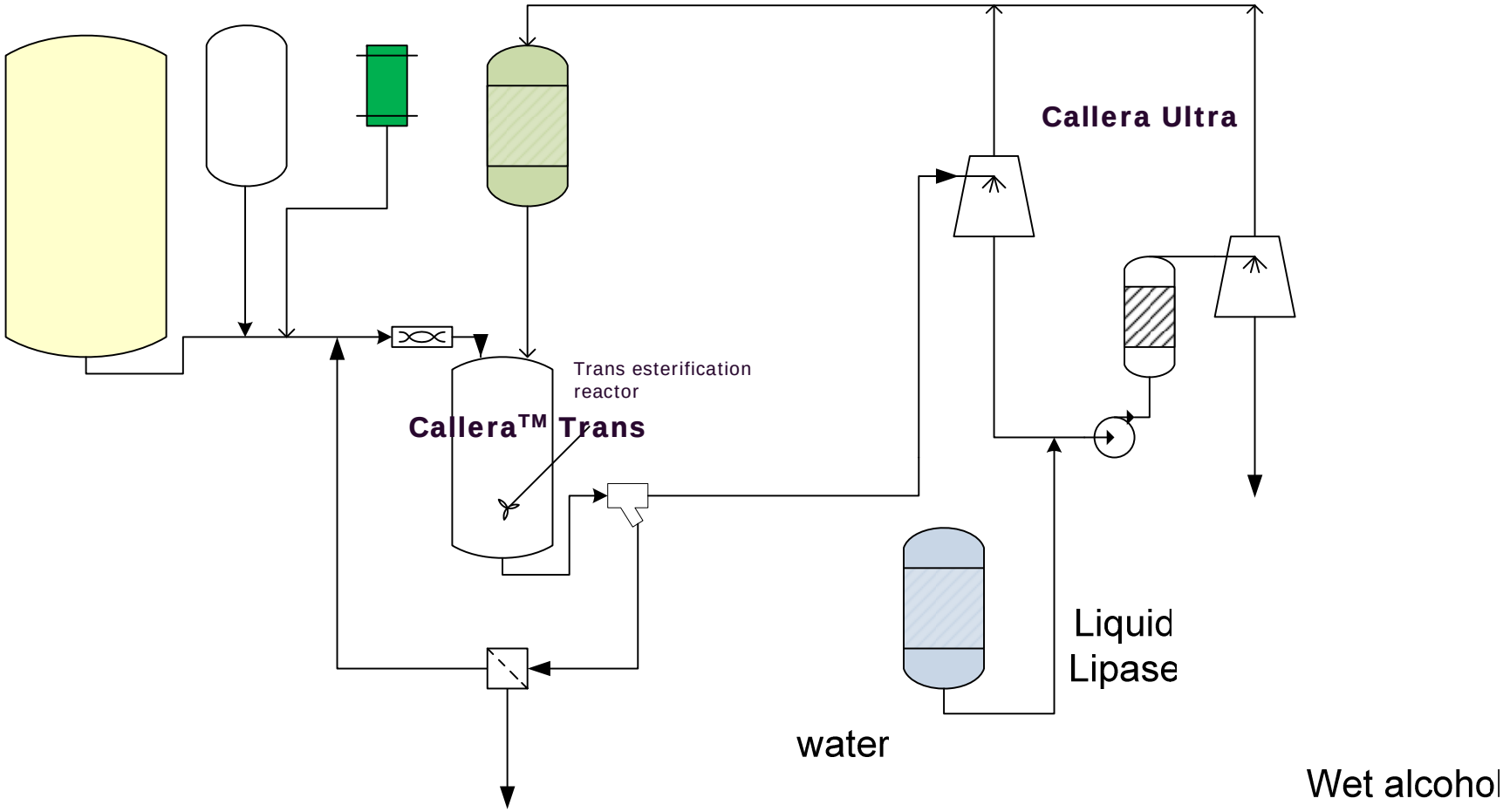
## Observations

- Methanol toxic to immobilised enzyme
- Ethanol gives higher yield as more bulky alcohol
- Enzyme re-cycling required for process economy
- Prospects limited for enzymatic bioiesel

## Remedies

- Systems utilising phased addition or co-solvents
- Methyl esters are generally regarded as being biodiesel
- Normally immobilised enzymes suggested but these are normally high cost due to support.
- Joint research with external partners to resolve problems

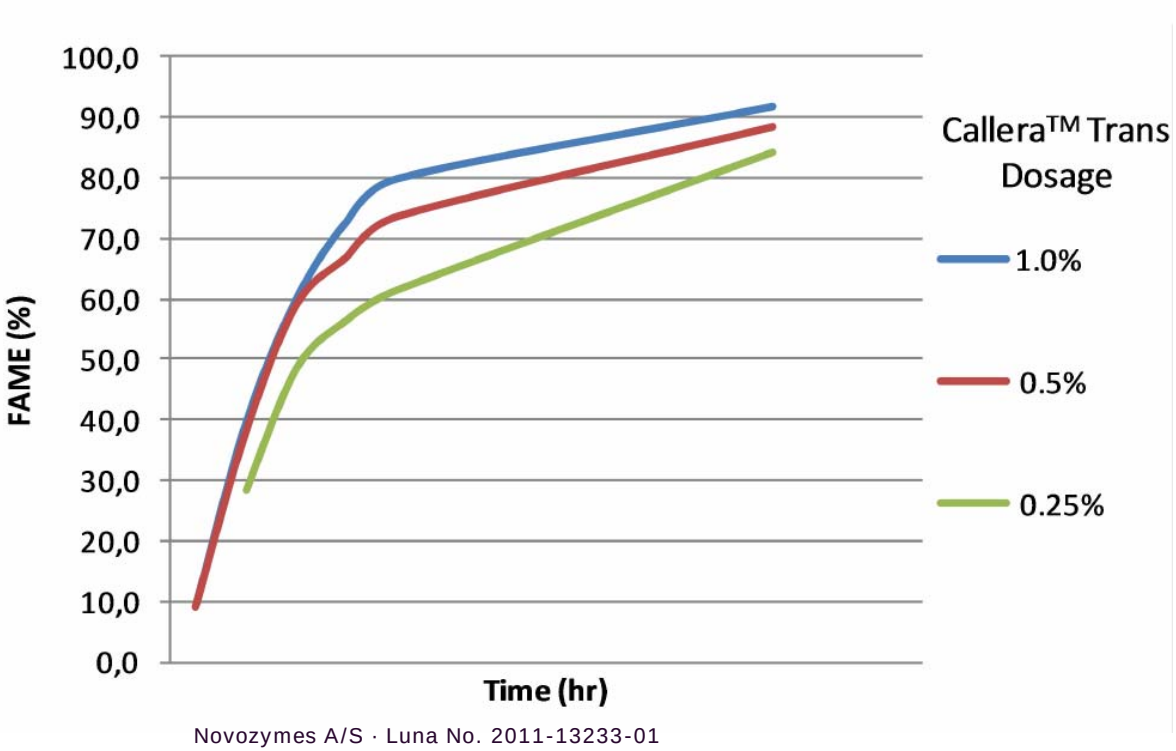
# Full enzymatic process




- Two stage system using liquid lipase for trans-esterification followed by immobilised enyme for FFA removal

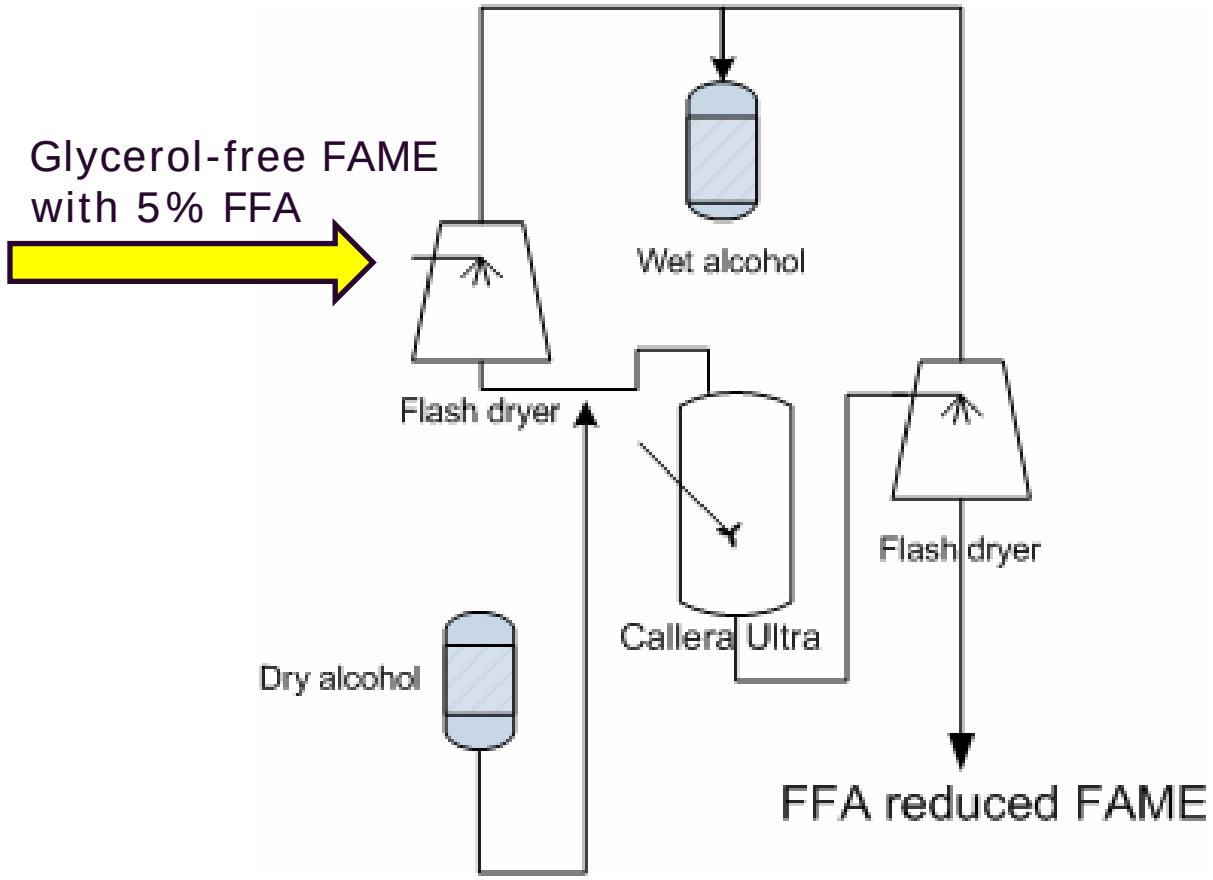
# Outline for batch system

- Liquid formulated lipase (Callera Trans) is added at 0.5% (w/w) of oil
- 1.5 molar mass equivalents of methanol to fatty acids, both FFA and fatty acids bound in triglycerides (alternatively, ethanol can be used)
- Temperature: 35–40 °C (95–104 °F)
- Reaction time:0-6 hours




NB: A CSTR continuous system is also available

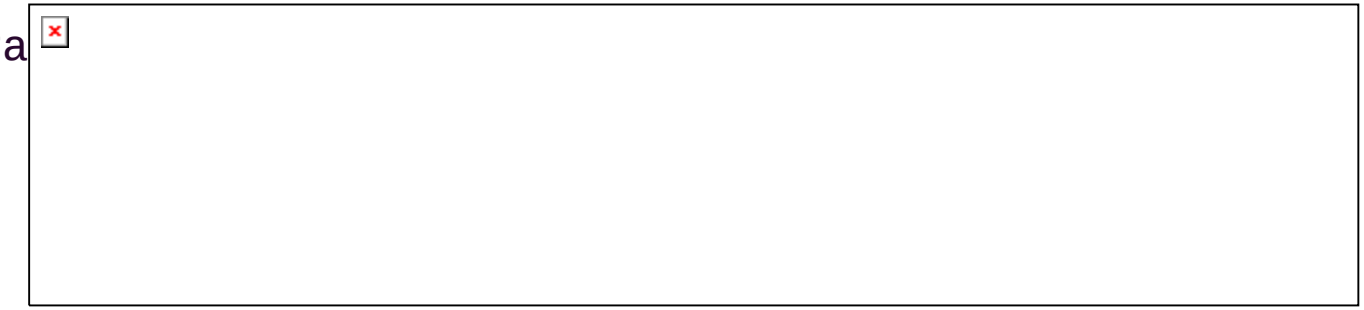
# The FFA esterification process




**…and turn it into a FFA-esterification pretreatment unit for chemical plants**

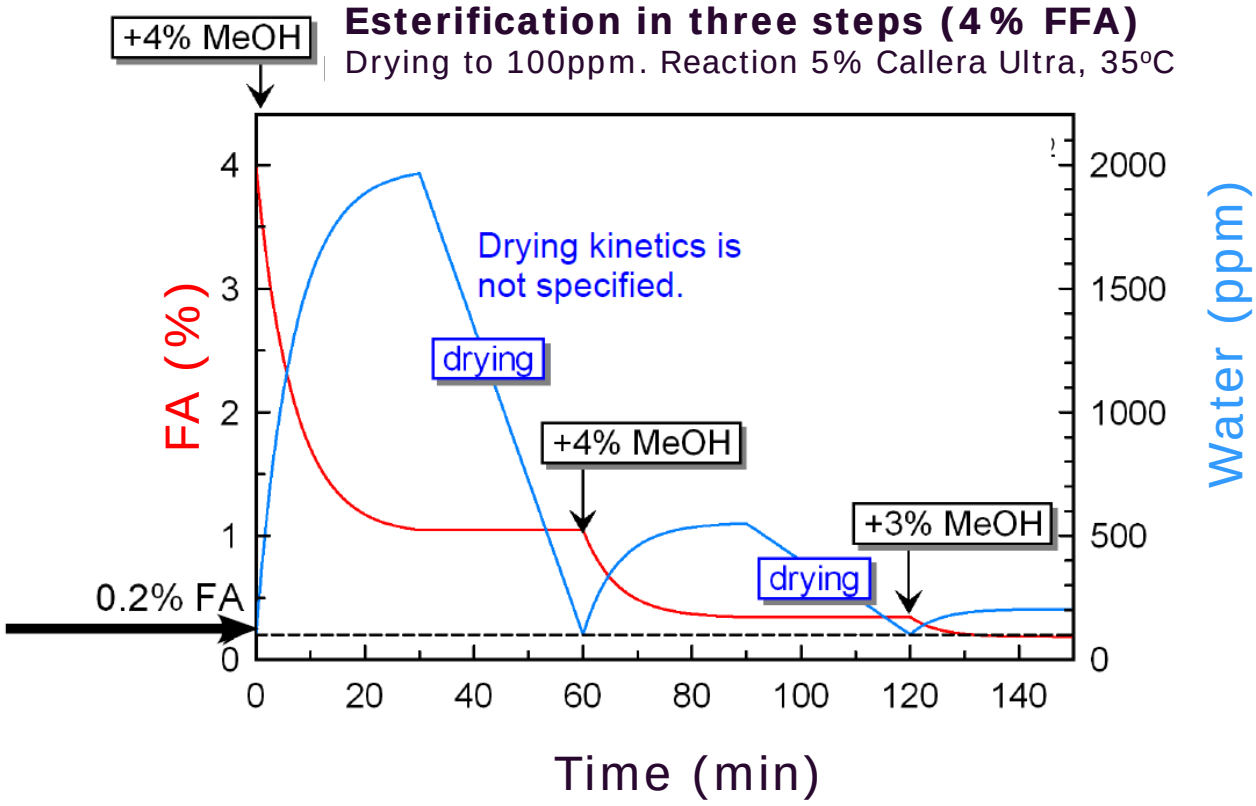
# The work with FFA esterification showed us that:

- FFA in raw materials equals YIELD
- We have an enzyme that loves FFA
  - *C.antarctica* lipase B
    - Callera™ Ultra



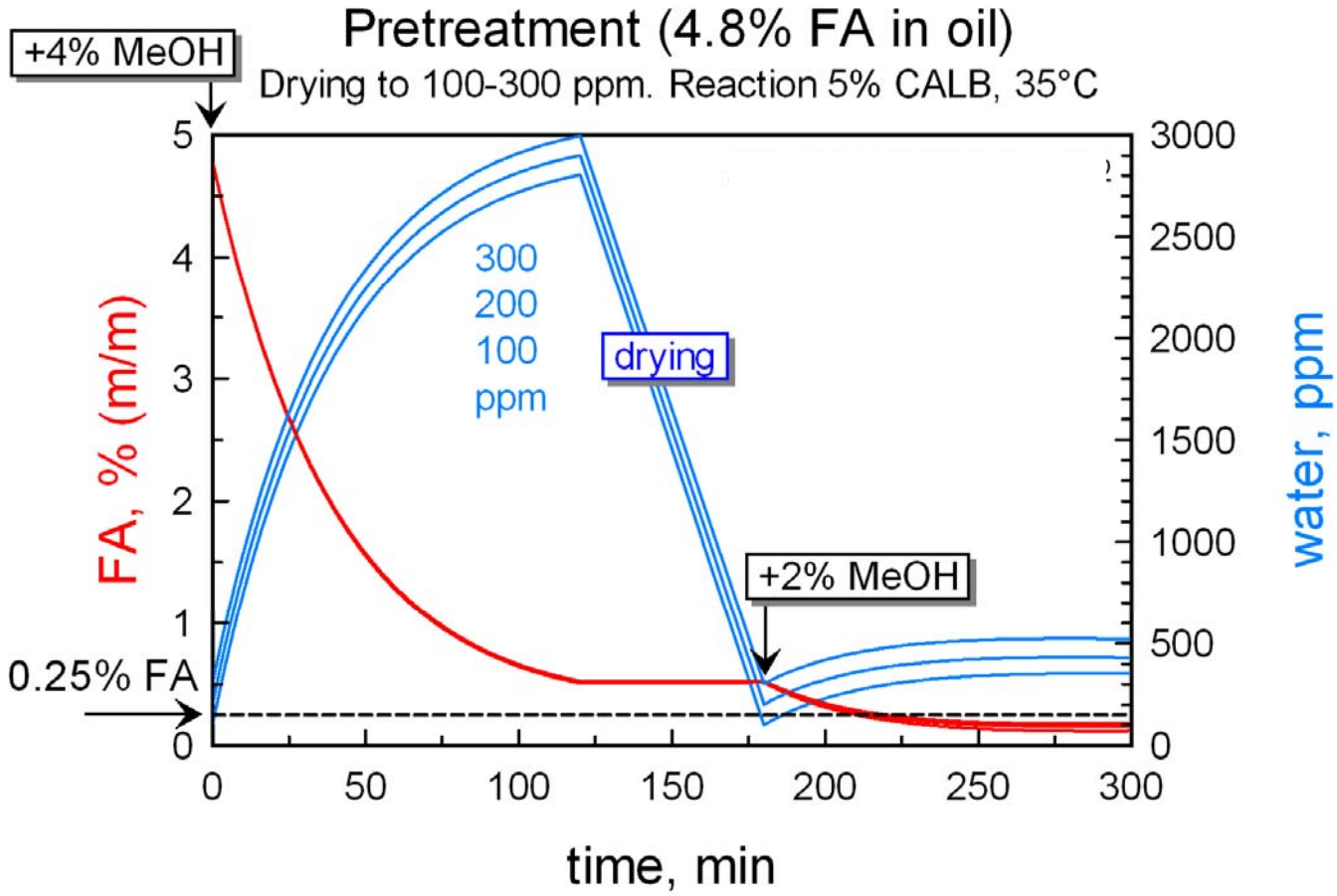
- To drive the reaction the water needs to be removed. 1 kg FFA produces approximately 1.05 kg FAME and 0.06 kg water
- We need to remove the water
  - FAeSTER process eliminate water continuously
  - Air loop drying technology
  - Flash drying

# Principles of the water flash removal



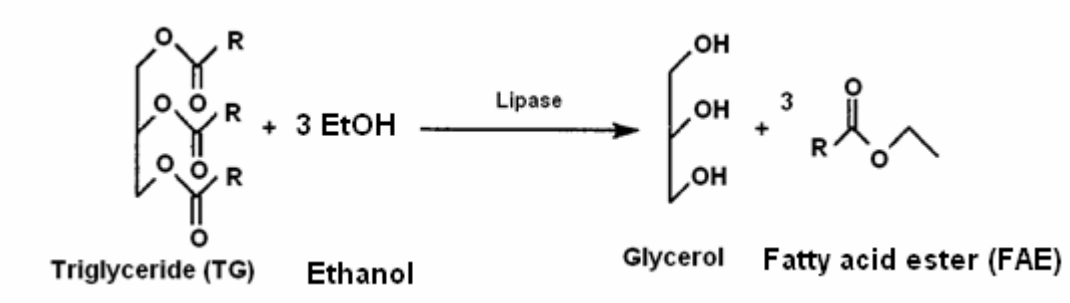

Ref. S.Fedosov. Aarhus University Unpublished data

# Adapting the FFA removal step for oil pre-treatment




Ref. S.Fedosov. Aarhus University, unpublished data

# Ethylation of Fish oils

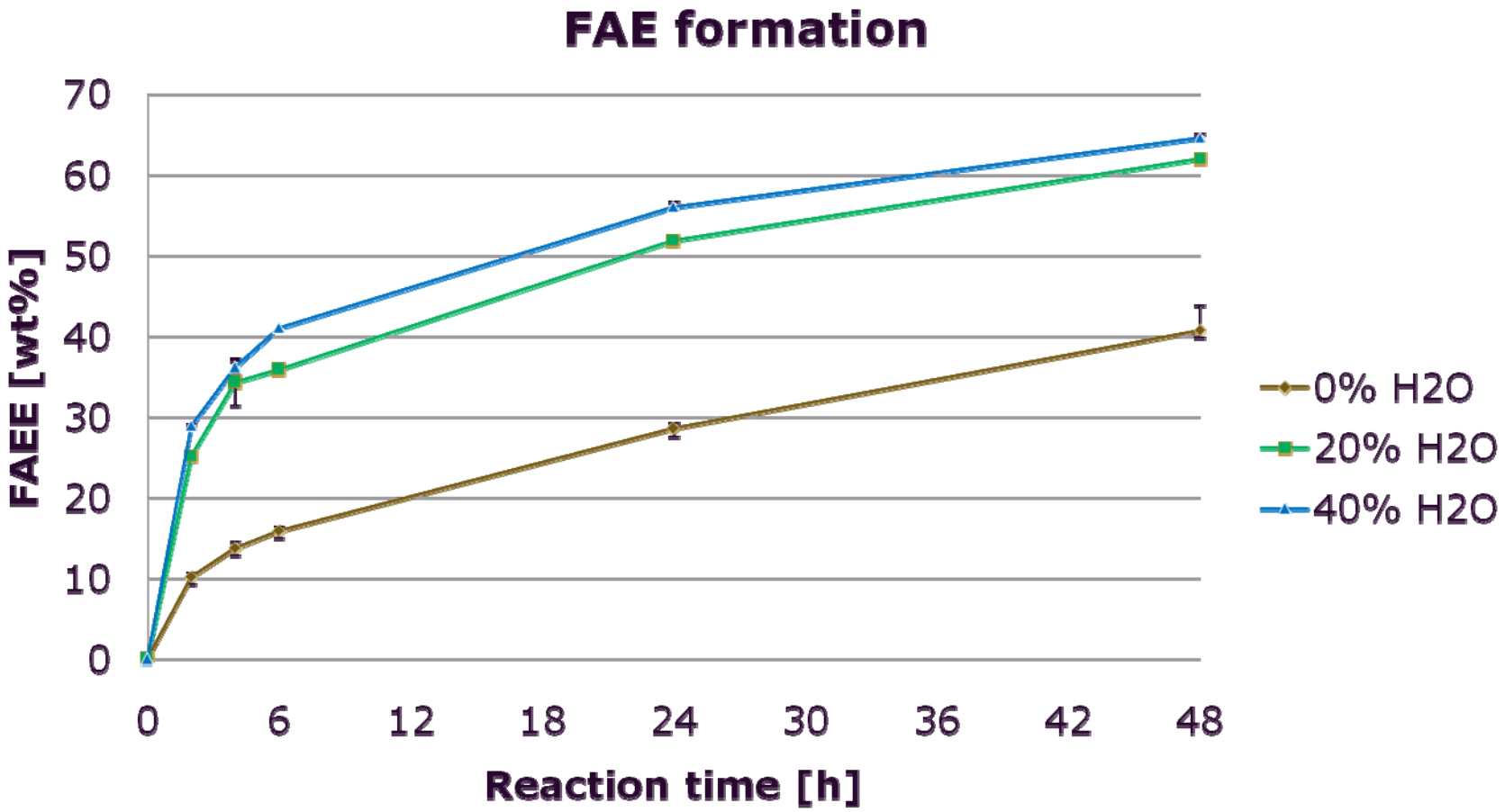



In order to separate DHA & EPA from other fatty acids in fish oils, the tri-glyceride is first converted to ethyl esters and then the separation made using short path distillation

Residual FFA in the oils will neutralise the catalyst producing soaps and yield loss

The high temperature used in the reaction can promote polymerization and tans fat production

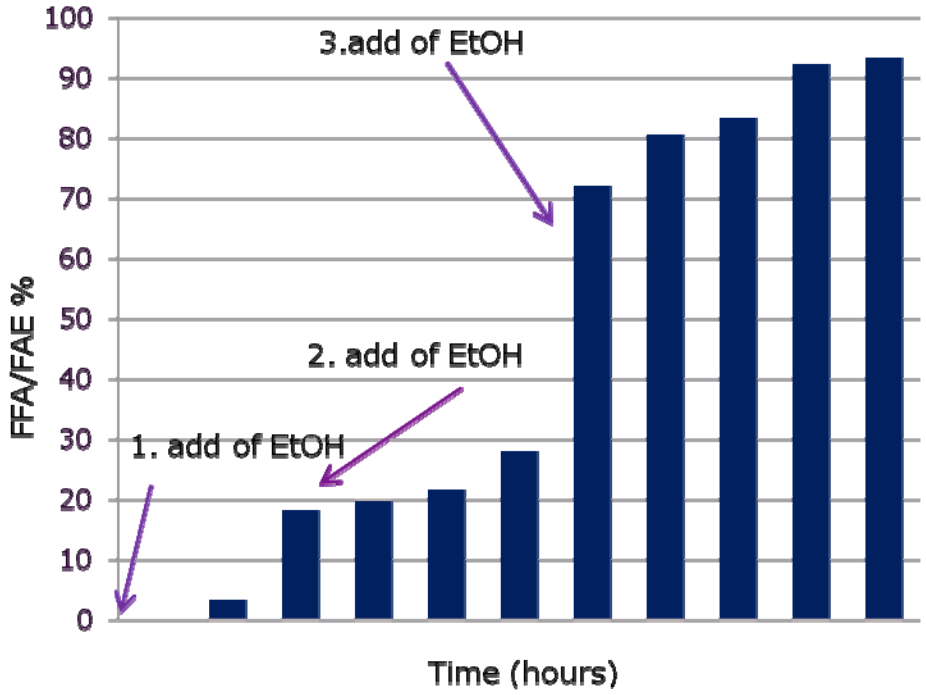
# Adaptation of Biodiesel process




**Reaction conditions:** 35°C, 5 eqv. ethanol to triglyceride, EtOH added in 5 portions over 1 h. 1.2% Lipozyme TL 100 of oil, neutralized with 0.025 M KOH (1 ml per 100 g oil). FAE content measured by GC. Fish oil kindly provided by Berg Lipid Tech AS. Ref. PRBA

# Optimisation by enzyme switch

- Use of alternative enzyme improves overall yield of FAEE, indicating different fatty acid preferences for lipases
- Step wise addition of ethanol avoids enzyme inactivation
- Plateaus in the conversion indicate possibility to reduce reaction time to an acceptable level

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# Conclusion & Summary

- Enzyme applications are more widespread than degumming and interesterification and new possibilities are under development
- Renewed interest in yield improvement and improving sustainability is driving many of these developments
- We can also expect the development and introduction of new enzymes within the degumming, speciality fats and interesterification applications as success there drives new developments and attracts new players