Enzyme processing,

a speculative look into the future

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Speculating about the future

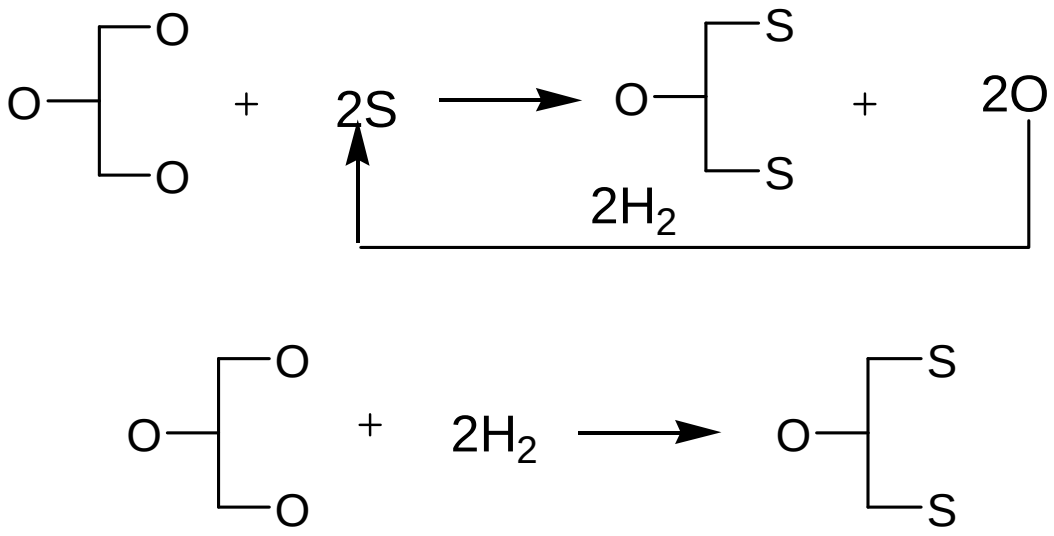
- *"Prediction is very difficult, especially about the future",* Niels Bohr (1885-1962)
- So let's have a look at what lessons the past can teach us
  - Which developments have been successful, and why?
  - Which developments failed, and why?
  - Are there general conclusions to be drawn?
  - Can they tell us something about the future?
- Above all, let's aim at a balanced view and look at both advantages and (potential) disadvantages of these developments

Production of lysolecithin

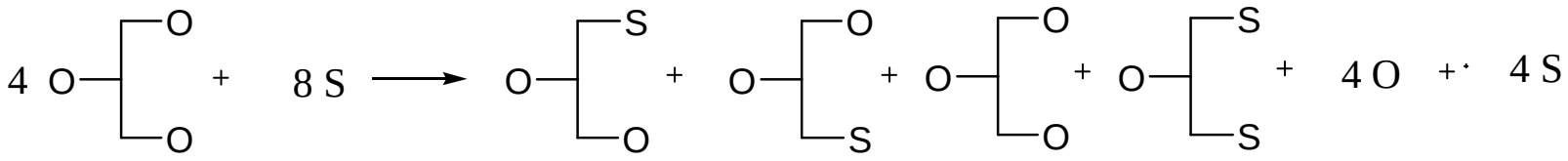
- Lysolecithin was fist mentioned in 1923
  - Was made by using snake venom
- Started being made industrially in the 1970s (?)
  - By treating gums originating from the water degumming of soya bean oil with phospholipase A
  - Used as emulsifier in calf milk replacer
  - Quality control was by a kind of creaming test
  - Is it still made and used for this purpose?
- Lessons:
  - Pro: Application makes use of enzyme specificity
  - Pro: Final product not very critical

Production of cocoa butter equivalents

- Developed (Unilever, Fuji Oil) to produce symmetrical monounsaturated triglycerides that are compatible with cocoa butter (as mentioned by Mr Holm)
- Relies on 1,3 specificity of lipase enzyme
- On the face of it, the process looks quite attractive



Less attractive in practice



- With stoichiometric S, the SOS-yield is only 25%
- Large excess of stearic acid is required to improve the SOS-content of reaction mixture at equilibrium
- High oleic oils also have linoleic acid on the 2-position
  - Consequently, SOS will also contain SLS
- Isomerisation of partial glycerides leads to stearic acid insertion at the 2-position; loss of selectivity
  - Formation of trisaturated triglycerides with poor mouthfeel; their removal by fractionation decreases overall CBE yield
  - Similarly, SOS fraction also contains SSO

Possible improvements

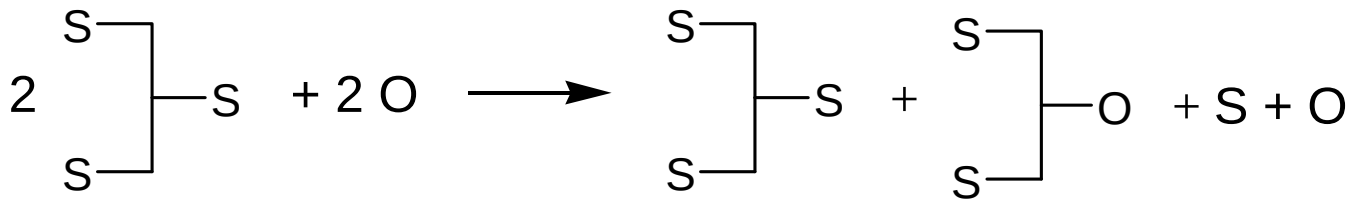
- Reacting by-product shea olein (high SOO-content) with stearic acid or methyl stearate
  - Requires less substitution and liberates less oleic acid
- Reacting palm mid fraction (high POP content) with stearic acid or methyl ester
  - Gives POS and SOS (as explained yesterday by Dr Gibon)
  - Further reduction in liberation of oleic acid or its methyl ester
- Even so, both processes yield trisaturated triglycerides
  - And thus require additional fractionation step to remove trisaturated triglycerides, which reduces CBE yield

Lessons from enzymatic CBE manufacture

- Pro: Process makes use of specificity of enzyme
- Con: But process reckons too little with chemistry
  - Water in enzyme leads to hydrolysis
    - Formation of diglycerides and FFA
    - Diglycerides affect crystallisation behaviour
  - Kinetics leads to by-products
  - Acyl migration in partial glycerides leads to loss of specificity
  - Subsequent fractionation diminishes CBE yield
    - Little use for fractionation by-products
- Con: Finally, EU Chocolate Directive prohibited use of enzymatic CBE in chocolate

No hope for enzymatic CBE process?

- Yes there might be: Use of 2-specific lipase for CBE
  - Existence of 2-specific lipase has been reported
    - X. Song *et al. EJLST,* **110**, 1095-1101, 2008
  - Better yield than use of 1,3-specific lipase
    - 50% when using equivalent amount of fatty acid and 2-specific lipase as opposed to 25% when using the 1,3-specific lipase



Other interesterification applications making use of enzyme specificity

- Anti-bloom adjuvant (BOB)
  - Used in small amounts so loss of specificity will hardly be noticed (has been mentioned by Dr Gibon)
- Fat for infant formula (as mentioned by Mr Holm)
  - Just like lard, fat in human milk has palmitic acid predominantly on 2-position
    - 1-position 18.7%; 2-position 57.1%; 3-position 5.3%
  - So a kosher substitute can be made by enzymatic interesterification of tripalmitin with unsaturated fatty acids
  - Physical properties are not critical
  - Loss of specificity is not critical either
- Both products command attractive price
  - Consumers have little choice

More interesterification applications relying on enzyme specificity

- Value added fish oils (Dr Bruheim)
  - High added value product but small volume
  - Might face competition from single cell oil
- Diacylglycerol (Mrs Noor Lida, Ir De Clercq)
  - Process has to be compared with extensive patent literature (P&G) dealing with synthetic CBEs
- Low-calorie fats (Dr Gibon)
  - Advantages of enzymatic route are not clear
  - Market did not materialise (commercial flop)

And what about enzymatic randomization?

- Conducted as continuous process with enzyme filled reactors in series
  - Not suitable for frequent type changes
- The reactor with the oldest enzyme is regularly taken out of service
- Enzyme life has large influence on interesterification cost, but is still unpredictable (Dr Diks)
- Physical properties of enzymatically interesterified fat are quite similar to those of chemically esterified fat

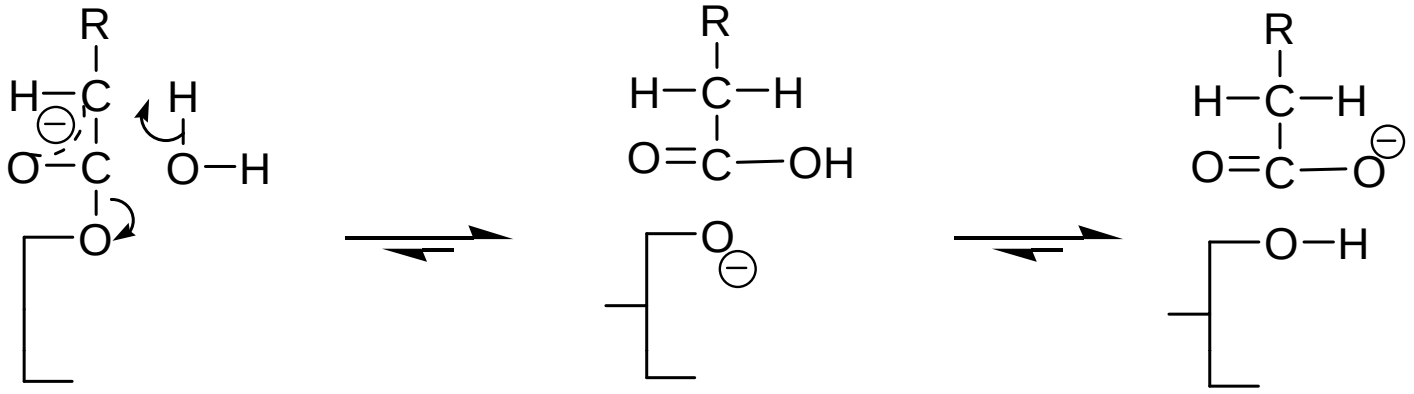
Aspects that are hardly mentioned

- Difference in activity:
  - 0.5 kg $NaOCH_3$ randomises 1 ton of oil within a few minutes
  - 600 kg enzymes require 1 hour to randomise 1 ton of oil
  - $NaOCH_3$ costs some 4 $/kg; enzyme costs about 40 $/kg
  - Lauric oils require even more enzyme (Dr Cowan)
- Hydrolysis:
  - Enzymes need some water to be active (Dr Diks); this water causes FFA (how much?) to be formed that are lost
- Potential yield improvement in chemical process
  - FAME formation and FFA formation are proportional to the amount of $NaOCH_3$ used
  - **Both FAME and FFA formation can be avoided**

How to avoid formation of FAME?

- Avoid the presence of methanol
- Examples of methanol-free catalysts:
  - Na and K metals or their low-melting alloy have been used
    - Preferably used at rather low temperature (~50°C)
    - Rather hazardous
  - Sodium hydride, sodium amide have been mentioned in literature
  - Water (US Patent 2,378,005); requires temperature of 260°C
  - **Sodium glycerolate**
- This catalyst is produced by drying a solution of caustic soda in glycerol under vacuum.
  - Cheap and totally innocuous

How to avoid formation of FFA



- Enolate anion reacts with **water** to give FFA and glycerolate anion
- In alkaline or neutral environment, this gives soap and a partial glyceride
- In acid environment this gives FFA and partial glyceride
- FFA or soap formation is quantitative and equivalent to the amount of sodium methanolate
- FFA formation can be prevented by anhydrous inactivation of the enolate anion

Experimental support

- Interesterification with large excess of catalyst
  - Take 50 g soya bean oil; measure FFA (0.12%)
  - Dry under vacuum and add 0.5 g sodium methanolate (1%)
  - Heat under vacuum to 100°C
  - Inactivate with 5 g glacial acetic acid
  - Wash with water until neutral
  - Measure FFA in interesterified oil (0.82%)
- Amount of FFA formed: 0.82 – 0.12 = 0.70 %
- Amount of FFA equivalent to 1% $NaCH_3O$
  - 282 : 54 = 5.22 % FFA
- Far less FFA formation by anhydrous inactivation

Lessons from the enzymatic randomization saga

- Enzymatic process does not rely on specificity of enzyme
  - Does not generate a unique product
- Enzymatic process looked like having advantages by highlighting presumed shortcomings of chemical process
  - Proper knowledge of industrial practice reduced these advantages
  - An improved insight into the mechanism of the chemical reaction made it more competitive by reducing yield loss
- When considering an enzymatic process, reckon with possible improvements of chemical process

Chlorophyllase

K. Carlson *et al.* Presentation at 102nd AOCS Annual Meeting, Cincinnati, 2011

- The standard way to remove chlorophyll from oil is by using acid activated bleaching earth
- Costs involved:
  - Purchase of bleaching earth
  - Oil retention in spent earth (50% of fresh earth)
  - Disposal spent earth
- Treating the oil with chlorophyllase splits chlorophyll into porphyrin (water soluble) and phytol (oil soluble)
  - Treatment can be combined with enzymatic degumming
- Sounds great, but

C.R. Beharry *et al.* US Patent 5,315,021 (1994) (Lapsed in Europe since 2004 by non-payment of fees)

1. A process for removing up to 98% of the chlorophyll color impurities from vegetable oils comprising:
   - dispersing a source of phosphoric acid in a vegetable oil which has a moisture content of less than 0.1% by weight at a temperature of 70-160°C and at a pressure of less than 10 Torr, for a time sufficient to develop a precipitate of said color impurities;
   - subjecting the vegetable oil containing the chlorophyll color precipitates to further processing steps selected from the group consisting of degumming, water washing, neutralization or bleaching, and
   - removing the precipitated chlorophyll color impurities during the subsequent processing.

- Lesson: Always reckon with possible chemical alternatives

But I do dislike misleading publicity, (as for enzymatic degumming)

- Industrial enzymatic degumming processes claiming low residual phosphorus are always preceded by an acid refining process (Mr Dayton)
  - Addition of citric acid, reaction time, partial neutralisation
  - Enzymatically catalysed reactions are an order of magnitude slower than chemical reactions
  - On an industrial scale, the fine dispersion that enables the reaction between NHP and a reagent in the water phase cannot be maintained sufficiently long
  - Enzymes therefore only interact with hydrated phosphatides
- On an industrial scale, enzymes do not degum the oil
- but they can be used to de-oil the gums

Yield loss on enzymatic degumming (soya bean oil; P = 1000 ppm)

Degumming process	Removed in gums (% on oil)		Yield loss (%)
	phosphatides	oil	
Acid refining (AR)	2.5	1.5	4.5
PLC + AR	0.8	0.9	2.4
AR + PLA	1.6	0.4	3.4
AR + PLC + PLA	0.5	0.1	1.8

Comments

- Data in table based on quite optimistic guesswork
  - and assume maximum DAG formation
- Yield improvements are proportional to phosphatide content of crude oil
  - Also depend on phosphatide composition
- Yield improvement with PLC on its own is higher than with PLA on its own (2.1% *vs* 1.1%)
- Additional yield improvement by combining the two enzymes is low at 0.6%
  - Results from reduced oil retention by LPI and LPA
- Is additional PLA treatment still worth it?

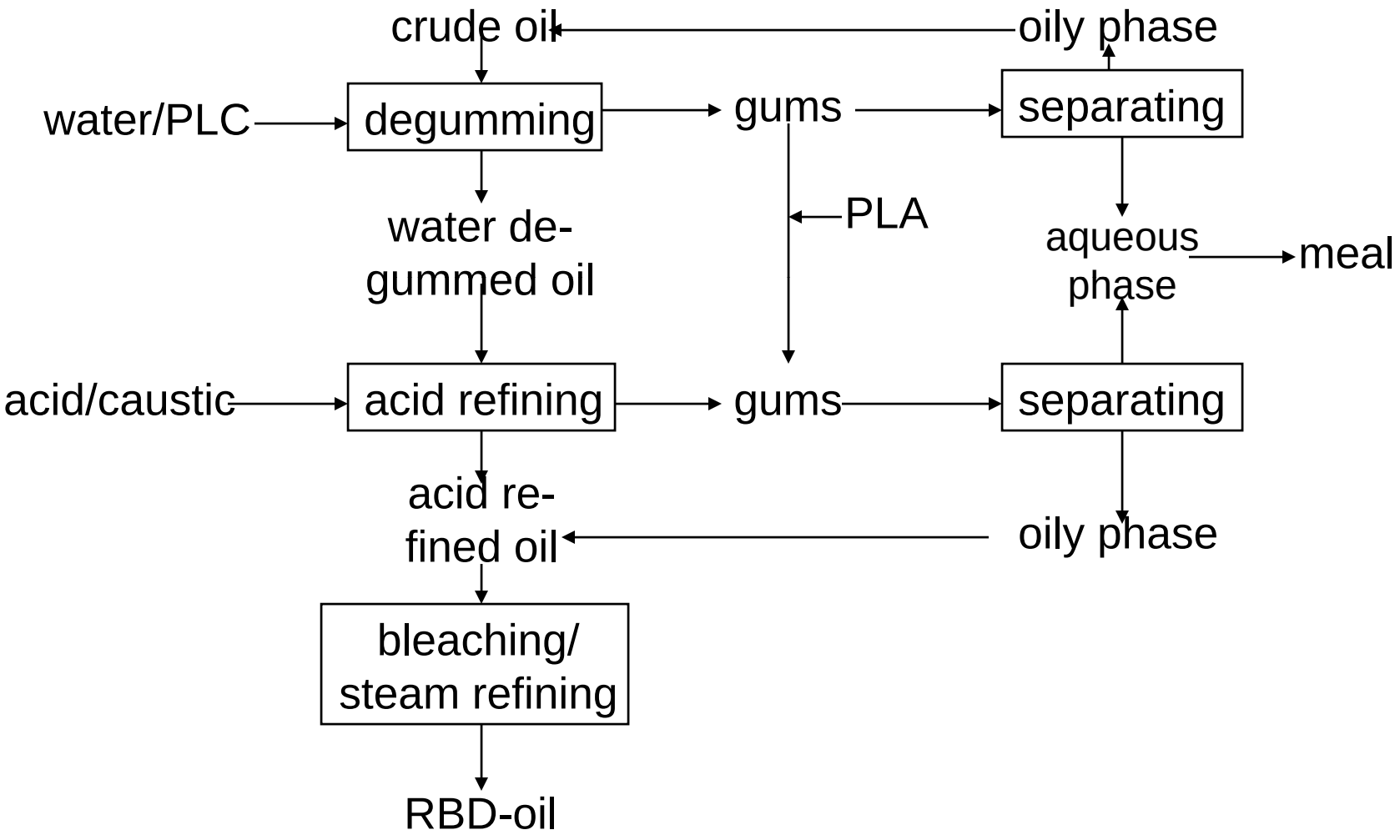
What enzymatic de-oiling process would be most suitable?

- Combined process in which the oil is acid refined, enzymes are added to catalyse the hydrolysis of hydrated gums, a holding time is provided and finally the gum phase is separated
- Consecutive process in which oil is acid refined, the gum phase is separated and treated with enzymes to hydrolyse phosphatides and liberate oil
- Multi-stage process in which the oil is first water degummed and the resulting gums are first treated with PLC to avoid PC and PE from being hydrolysed by PLA and then treated with PLA to reduce oil retention even further

Combined edible oil processes

- Alkali refining
  - Combines degumming with neutralisation
- Dry degumming
  - Combines phosphatide removal with bleaching
- Bleaching
  - Combines colour removal with filtration
- Physical refining
  - Combines neutralisation with deodorisation
- Lesson: the two processes proceed simultaneously
- That is not the case in enzymatic degumming where acid refining precedes enzymatic gum hydrolysis

Possible flow diagram



Explanation of flow diagram

- Crude oil is water degummed with PLC solution
  - PLC continues to react in intermediate gum storage
  - Hydrolysed gums are separated if not further treated
  - DAG-rich fraction to crude oil
  - Or hydrolysed gums are mixed with acid refining gums.
  - Mixture is treated with PLA
- Water degummed oil is acid refined so the resulting oil can be bleached and physically refined
  - Hydrolysed combined gums are separated and oily phase is mixed with acid refined oil
- Pro: Maximum DAG yield by consecutive treatment
- Pro: Optional PLA treatment if cost effective

How about re-use of enzyme?

- Enzyme is present in aqueous phase
  - Aqueous phase also contains sugars and phosphatide hydrolysis compounds
- A simple process that isolates enzymes from aqueous phase might enable them to be re-used
- Enzyme manufacturers may develop re-use process when process without re-use is not viable
  - They will not do so for viable processes

- Lesson: it is up to oil refiners to develop processes in which enzymes are re-used.

Summary of lessons from the past

- Aim for applications that make use of the uniqueness of the enzyme capabilities
- Look for shortcoming of chemical processes
- But beware of chemistry-based retaliation
  - When pushed, chemists can be quite inventive
- Be honest and non-biased
  - If you repeat a biased statement often enough, you will start to believe it yourself and forget/deny that it is still biased
- Disregard arguments with clear marketing flavour
  - “Natural”, “organic”, “eco-friendly”, “sustainable”, etc.
- Be creative, but from a knowledge base
  - Please make sure that you know what you are talking about
  - Ask experts to comment, and shoot down your ideas

Side reactions of chemically catalysed esterification are another shortcoming

- At Vandemoortele, we wanted to reduce the DAG content of crude shea butter by esterification with FFA
  - Esterification without a catalyst (*cf* esterification of terephthalic acid with ethylene glycol) required too high a temperature
  - Using zinc or tin salts led to randomisation
  - Using acid activated bleaching earth as esterification catalyst led to *trans* isomer formation
  - Using Nafion® (a sulphonated perfluorocarbon) worked, but was too expensive at that time
- Using lipase enzymes would not have suffered from any of the above disadvantages (except cost?)

This is now called remediation or deacidification by esterification

- On hydrolysis of TAG, free hydroxyl groups in partial glycerides and FFA are formed in equivalent amounts
- On remediation, both carboxyl and hydroxyl groups disappear (Dr Cowan)
  - This causes a quadratic decrease of the rate of esterification
- To diminish this rate decrease, one of reagents has to be in excess
  - Adding glycerol leads to fat with high Hydroxyl Value (HV)
  - Adding FFA leads to low HV and residual FFA that can be removed during subsequent deodorisation
  - Distillate can be recycled to next esterification reaction
- US Patent 5,061,498 uses two or more enzymes

However,

- Enzyme activity decreases when water content drops
- Esterification kinetics demand low water content to arrive at low residual hydroxyl group content
- So esterification cannot but take long time
- Using packed columns increases rate of reaction
- But combining packed columns with water removal is not that obvious
- Lesson: this requires some inspired engineering

Even so,

- Enzyme catalysed esterification could be used for high value-added products, but then their sale is strongly marketing dependent
  - Cosmetics (Mr Holm)
  - Drug modification (Dr Zeng Guo)
- DHA/EPA-concentrates (Dr Bruheim)
  - Using a DHA/EPA specific lipase would lead to just these fatty acid ester bonds being hydrolysed
  - That would allow these fatty acids to be isolated in reasonably pure form
  - Using same lipase for esterification with glycerol would lead to DHA- or EPA-concentrates

What about commodity oils?

- Palm oil tends to have appreciable FFA content
- Rice bran oil has even higher FFA content
- Can these oils profit from enzymatic remediation?
- In principle yes, but in practice technological breakthroughs are required
  - Minimization of enzyme usage per ton oil
  - Reactor design to increase reaction rate
- (Re-)esterified oils are synthetic and may not be allowed in food
  - May require authorisation according to Novel Food legislation

Enzyme-assisted oil milling
(Ideal subject for obtaining research grant)

- Project proposals can list many strong arguments:
  - Exposure to *n*-hexane can lead to neuropathy
  - *n*-Hexane is highly flammable and its use has led to several oil mill explosions and fires with loss of life
  - Explosion proof plants are expensive
  - Oil seeds contain compounds that have been synthesized enzymatically so enzymes can also selectively break them down
  - Large return on effort since oil mills operate on a large scale
  - Governments compete(d) in subsidizing biotechnology

Economics of oil milling

- It pays to extract to low residual oil content
  - Enzymatic processes have low oil yields
  - Isolating oil from the skim has proven to be very difficult
- Modern extraction plants are near effluent-free
  - Enzymatic processes produce large amounts of high BOD aqueous effluent
- In solvent extraction, energy is main cost factor
  - Used for meal desolventising and miscella evaporation
  - That is why isopropanol (IPA) cannot compete with hexane
  - Water has an even higher heat of evaporation than IPA
  - If meal is not dry, it is prone to microbial attack
- Solvent extraction requires no adjuvants
  - Enzymes invariably cost money

So what about fruit oils?

- When olive paste is separated into olive oil and pomace, the latter contains some oil
  - By drying the pomace it can be solvent extracted to yield pomace oil, which has lower value than virgin olive oil
  - Using pectinase increases the virgin oil yield but
  - use of enzyme causes the oil to “lose its virginity”
- Palm oil press cake also contains some oil
  - This oil is normally not recovered
  - Use of enzymes might reduce this loss of palm oil

What about using enzymes for the production of single cell oils?

- These cells occur in aqueous environment
  - Their concentration is low
- Enzymatic hydrolysis of membranes and cell walls could avoid cell harvesting and drying
  - Oil would just float to surface and could be isolated by decantation
- Non-oil residues could feed next batch
- Sounds great or rather ….
- reflects my ignorance of this particular subject
  - Perhaps enzyme requirement would be so large as to be prohibitively expensive

Take-home messages

- In edible oil processing, enzymes will continue to be used because
  - They have unique advantages (phytase)
  - Persistent R&D and marketing effort by enzyme producers
- In viable uses, enzymes achieve what chemical catalysts cannot (yet?) achieve and/or save money
  - PLC-catalysed hydrolysis of phosphatides to form DAG
- Because of enzyme cost and necessary R&D support it makes sense to start with niche applications
  - EPA/DHA-concentrates;
- Major industrial use of enzymes (larger palm oil yield; oil extraction from algae?) is not impossible, but
  - should reckon with the possibility of chemical retaliation

Further take-home messages

- Current development is dominated by enzyme producers who may also cooperate with engineering companies
  - Enzyme producers want to sell enzymes
  - Contractors want to sell equipment
- By cutting down on internal R&D, edible oil processors are at the mercy of their suppliers
  - For which they have only themselves to blame
- Suppliers have no incentive to develop processes in which enzymes are re-used
- So making the most of enzyme-assisted processes and realising their full potential requires the oil processing industry to carry out its own process R&D

DIXI

(I have spoken)