Oleochemistry

Application of biotechnology in fats, oils and oleochemical

by
Shamsul Bin Zakaria
Faculty Industrial Science and Technology
shamsulzakaria@ump.edu.my

The students should be able to understand:

- How biotechnology can be applied in Oleochemistry.
- Lipase as the main enzyme in Oleochemistry
- Application of biotechnology in production of commercial oleochemical based products.

Chemical VC Enzymatic Catalyst

- Physical process involves high pressure and high temperature (250°C)
- Undesirable side reaction suc highly unsaturated fatty acids and hydrocarbons

- Enzymatic fat splitting is carried out at ambient pressure and temperature 40-60°C ensure minimal thermal degradation
- Better odor and color and are usually purer
- Specific products

Enzyme

- **Enzymes are catalysts which accelerate the rate of chemical reactions & most proteins, present in all living cells of humans, animals, plants and microorganisms.**
- **Exhibit a high specificity**
- **3,000 different enzyme have been isolated**

Production of enzyme/Lipase

- Lipase
  - Plant → Oilseed lipases → Relatively specific for native triacylglycerols
  - Animal → Human and other pancreatic lipases
  - M.O.
    - Fungi
    - Bacteria
      ↓

only a few of lipase-producing bacteria are commercially exploited as wild or recombinant strains

Microorganisms-greater Industrial Importance

- More stable
- Can be obtained in bulk at low cost- high growth rate and larger proportion of enzymes in relation to their body mass than plants or animals
- Less time consuming in harvesting
- Easily cultivated-not affected by climate

Typical enzymes used in industrial processes

CLASS	INDUSTRIAL ENZYMES
1. Oxidoreductases	Peroxidases Catalases Glucose oxidases Laccases
2. Transferases	Fructosyl-transferases Glucosyl-transferases
3. Hydrolases	Amylases Cellulases Lipase Pectinases Proteases Pullulanases
4. Lyases	Pectate lyases Alpha-acetolactate Decarboxylases
5. Isomerases	Glucose isomerases
6. Ligases	Not used at present

Lipase selectivity

a. Towards different classes of lipid-
Triglyceride, diglyceride and monoglyceride

b. Regioselectivity- Non Specific and 1,3 Specific

c. Fatty acid specificity- Saturated (Short, medium and long chain fatty acids) unsaturated fatty acids (Mono/polyunsaturated fatty acids

d. Alcohol specificity- sn-3 and sn-1 positions

e. Stereo and chiral specificity

Lipase: E.C 3.1.1.3

Known as acylglycerol acylhydrolases

Hydrolyse or synthesis esters of long- chain fatty acids from acylglycerol at oil/water interfaces

Hydrolysis/Synthesis

$$\mathbf{TAG} \underset{-H_2O}{\overset{+H_2O}{\rightleftharpoons}} \mathbf{DAG+FFA} \underset{-H_2O}{\overset{+H_2O}{\rightleftharpoons}} \mathbf{MAG\ +\ 2FFA} \underset{-H_2O}{\overset{+H_2O}{\rightleftharpoons}} \mathbf{3FFA}$$

TAG :Triacylglycerol

DAG:Diacylglycerlglycerol

MAG:Monoacylglycerol

FFA:Free Fatty Acid

Lipase Catalyzed Reactions- Interesterification

1. Direct Esterification

$$RCOOH + R'OH \rightleftharpoons RCOOR' + H2O$$

2.Transesterification

$$RCOOR' + R''COOR^* \rightleftharpoons RCOOR^* + R''COOR'$$

3. Alcoholysis (Glycerolysis)

$$RCOOR' + R''OH \rightleftharpoons RCOOR'' + R'OH$$

4. Acidolysis

$$RCOOR' + R''COOH \rightleftharpoons RCOOR'' + R'OH$$

Commercial Lipases

- **Novozyme (Denmark)**
  - Lipozyme RM IM
  - Lipozyme TL IM
  - Novozyme 435
  - Lecitase® Ultra

- **Amano Pharmaceutical Co. Ltd. (Nagoya, Japan)**
  - A Amano 6 (from *Aspergillus niger*)
  - M Amano 10 (from *Mucor javanicus*)
  - R Amano (from *Penicillium roqueforti*)

Positional Specificity

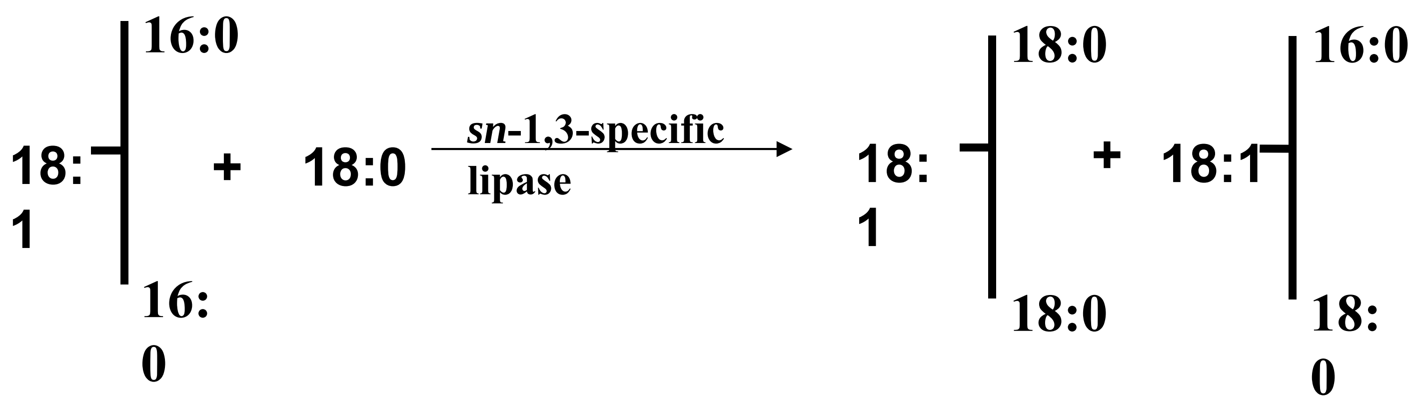
There are 2 types

- 1,3-specific
- **Non-specific**

Position Specific

a) <u>1,3-specific</u>

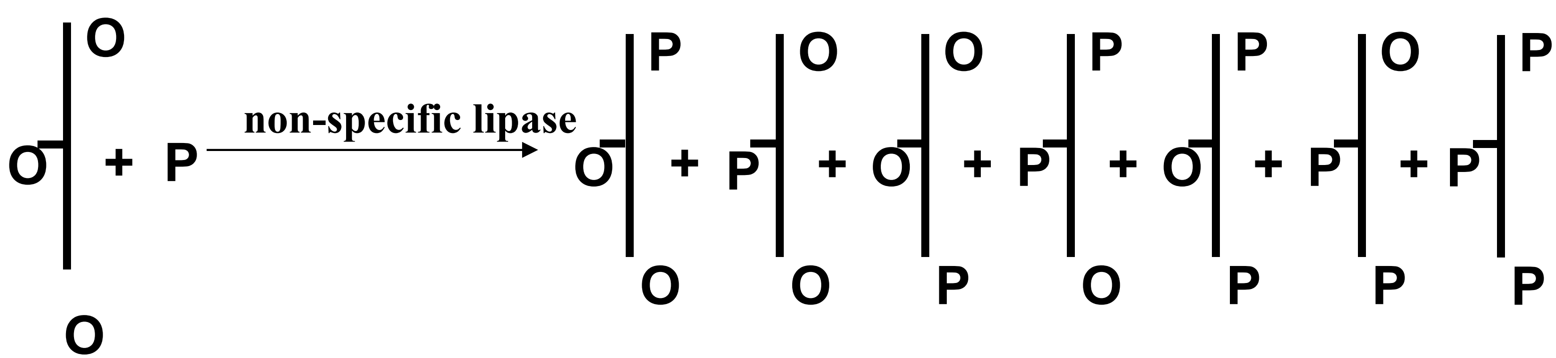
- Eg: lipases from *Rhizopus delemar, Rhizomucor miehei (Lipozyme), Rhizopus arrhizus, Aspergillus niger*



Position Specific

b) <u>Non-specific</u>

- Eg: Lipases from *Pseudomonas sp, Candida rugosa*



Fatty Acid Specific

- *Penicillium cyclopium* prefer short chain FA
- *A. niger* and *R. delemar* prefer FA of medium chain length.
- Lipase from *Geotrichum candidum* prefers long chain FA with a cis double bond in the 9 position of the TG (Eg: oleic acid, linoleic and linolenic acid) .

Example for Fatty acid specificity

Lipase B from *Geotrichum candidurn* → fatty acids with a double bond between C9 and C10

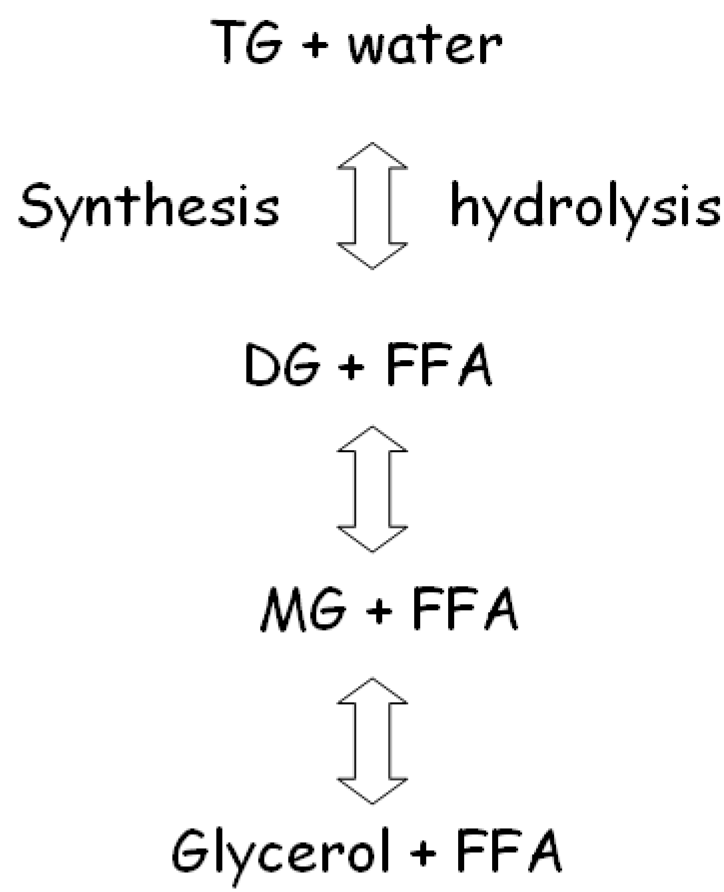
Substrate specificity:

lipase from *Pseudomonas* sp. LP7315 showed:

- Is not able to hydrolyze di- and triglycerides
- Hydrolyzes all the monoglycerides tested depending on the type of monoglyceride

Reactions for Lipase

- at high water activity, *hydrolysis* of TAG will occur
- at low water activity, *synthesis* of TAG will occur.



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Hydrolysis

Triglycerides → Triglycerides + diglycerides + monoglycerides + FFA

Position-specific and -non specific lipases →

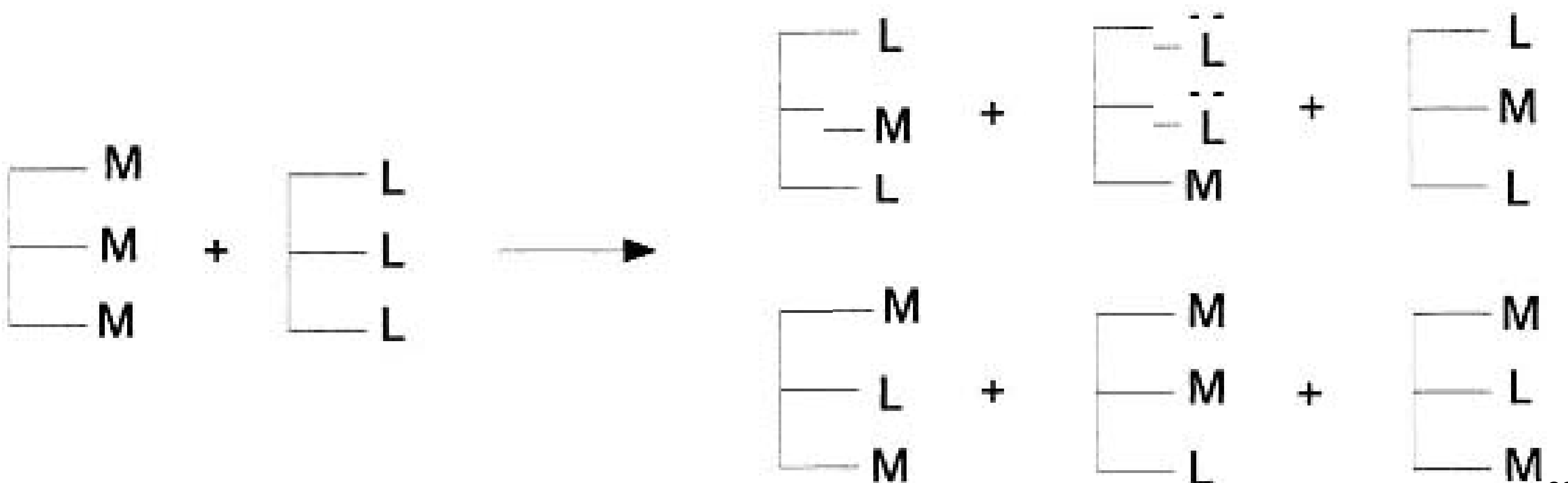
- Monoacylglycerol
- Diacylglycerol
- Polyunsaturated fatty acid-enriched mono- and diacylglycerols
- free fatty acids
- Phospholipids modification

Interesterification

- Rearrange the fatty acids so they become distributed randomly among triacylglycerol molecules of the fat
- Improves consistency of fats
- Applications:
  - Manufacture of shortenings
  - Lard (want ~10% tri-saturated glycerides)
    - Forms large and coarse crystals
    - Shortenings posses grainy consistency and poor baking performances
    - Randomization improves plastic range
  - Production of high stability margarine blends and hard butters with desirable melting qualities and crystallization behavior

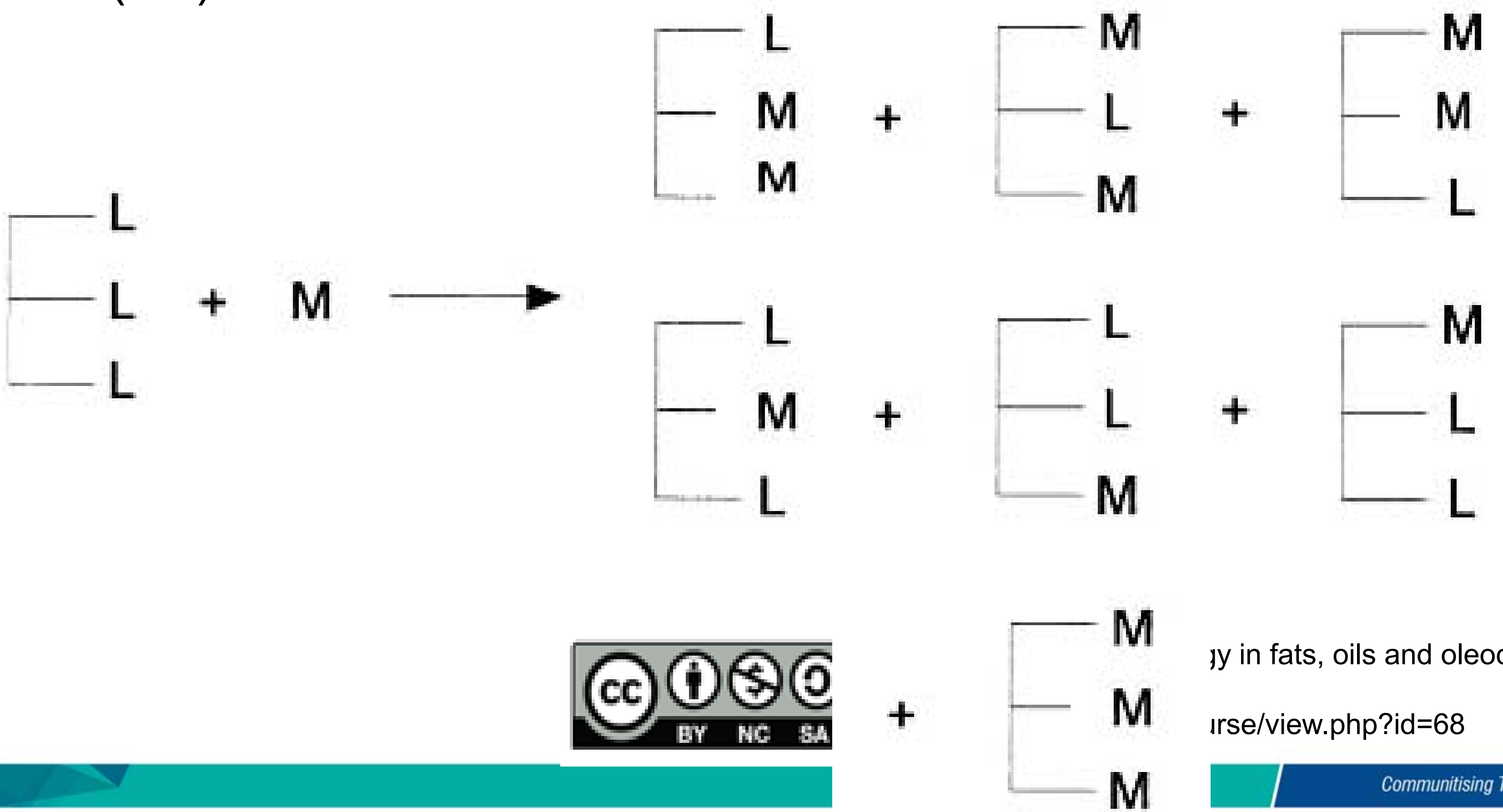
Interesterification

a) <u>Transesterification</u> – reaction between an ester (TAG) with another ester (TAG).



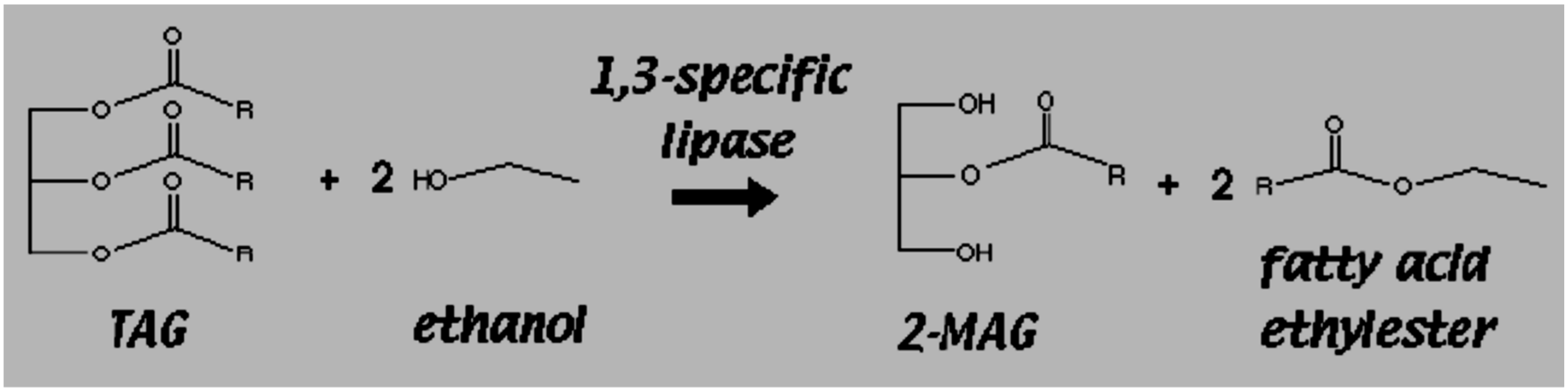
Interesterification

b) <u>acidolysis</u> – reaction between an ester and an acid (FA)



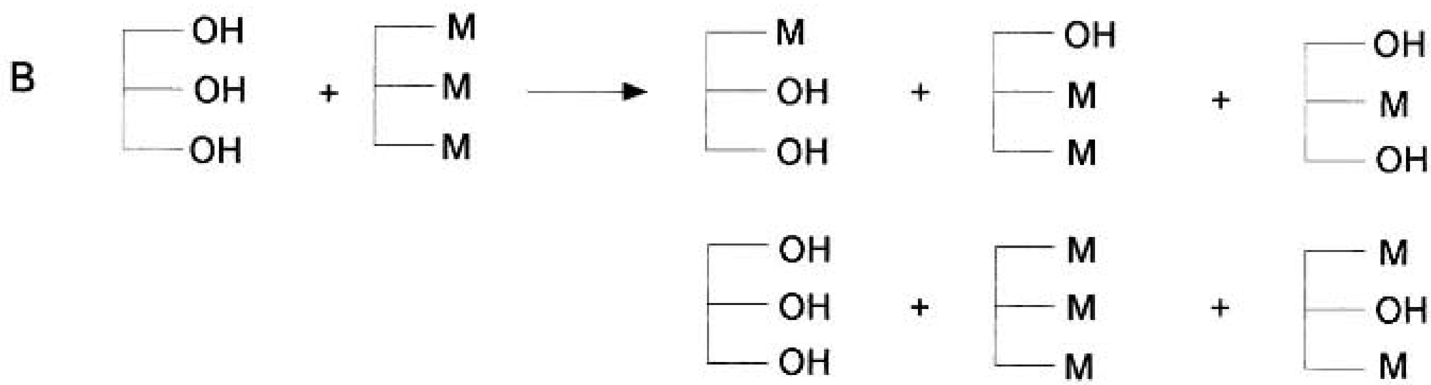
Interesterification

c) alcoholysis – reaction between an ester and an alcohol



Interesterification

d) glycerolysis – reaction between an ester and glycerol



Interesterification can be affected by using either chemical catalysts like alkali metal alkoxides or enzymes.

Application of Lipases

1) processing of fats and oils

2) detergents industry

3) the synthesis of fine chemicals and pharmaceuticals

4) paper manufacture

5) production of cosmetics

6) food processing
- Structured lipid
- cheese ripening
- vegetable fermentation
- meat products curing
- fish processing

Products Produces Through Biocatalyst

a) Dairy Industry

b) Cocoa butter substitutes

c) Trans-free margarine

d) DIMODAN (MG & DG emulsifiers)

e) Low calorie fats

f) Nutraceutical fats

Dairy Industry

Extensively used for the hydrolysis of milk fat
Current applications:

1. a) flavour enhancement
   b) cheese ripening
   c) lipolysis of butterfat and cream

2. The free fatty acids generated - specific flavour characteristics.

- release short chain (mainly $C_{4:0}$ and $C_{6:0}$) FA - sharp,tangy flavour
- release of medium chain ($C_{12:0}$,$C_{14:0}$) FA - soapy taste

- FFA - initiates the synthesis of flavour ingredients such as aceto acetate, beta-keto acids, methyl ketones, flavour esters and lactones.

Commercial Lipases Developed for the Cheese Manufacturing Industry

a) ***Mucor miehei***

- Palatase M (Novo Nordisk)

b) *Aspergillus niger* and *A.oryzae*

- Palatase A (Novozymes)
- Lipase AP (Amano)

Trans-free Margarine

- Margarine is a water in oil emulsion which normally produced through Hydrogenation

Unsaturated oil (soybean oil, sunflower oil, Canola oil —— **Ni, 180 ºC** ——→ **Margarine (Trans-fatty acid)**

Adverse Effect of Trans Fatty Acid

- Act like saturated FA
  - increase cholesterol level in blood
- Causing artherosclerosis, trombosis and coronary heart disease

Enrichment of Palm Oil with EPA & DHA

- Fats that are devoid of DHA and EPA are enriched using lipases with DHA, EPA, linoleic and linolenic acid (EFA)
- All the above fats will improve the bioavailability of the essential FA (EFA) in our diet.
- It is best to have TG that have the EFA at the *sn*-2 position and short chain (C8 and C10) FA at the *sn* 1 and *sn*-3 positions.

Advantages & Disadvantages of Enzyme Catalyst

- Widely found in nature
- Specific and selective
- Offer greater control over position distribution of FA
- Formation of little of no side products
- Enzymes (immobilized) can be recovered by simple filtration
- Low and mild reaction condition
- Reduced environmental pollution
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- Cost of production is very expensive

  e.g.: Lipozyme RM IM => RM 3,000/kg

Conclusion

- Lipase have been used in production of various oleochemical based product
- Lipase specifics in selecting the products, easy to tailor the product based on industry requirements.

Chapter description

All pictures/photographs/diagrams/figures used in this chapter is subjected to common creative that for education purposes