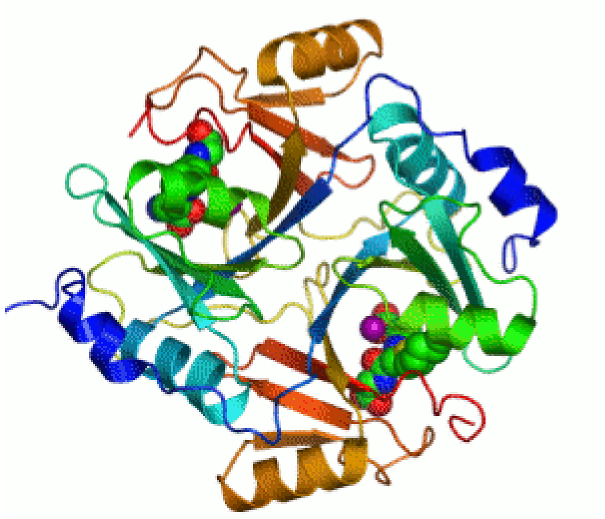
Enzyme Applications in Lipid Modifications



FE 461

Enzymes in Fats and Oil Industry

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Enzymes in Oil- and Lipid Based Industries

- Enzyme Application in Oil Extraction
- Enzyme Application in Oil Refining
- Enzyme Application in **Lipid Modification**

Enzymes in Oil- and Lipid Based Industries

Lipases and certain phospholipases are commonly used enzymes for lipid modification

Lipases

- Fats and oils are the natural substrates of lipases [enzyme class EC 3.1.1.3, triacylglycerol (TAG) hydrolases], the most often used biocatalysts in lipid modification.
- Lipases do not require cofactors and, most importantly, many of them, including immobilized versions, have been commercially available for decades.
- Lipases show distinct chemo- and regioselectivity
- They can be used for tailoring natural lipids to meet nutritional properties for humans.
- They can work at mild conditions and reduced waste

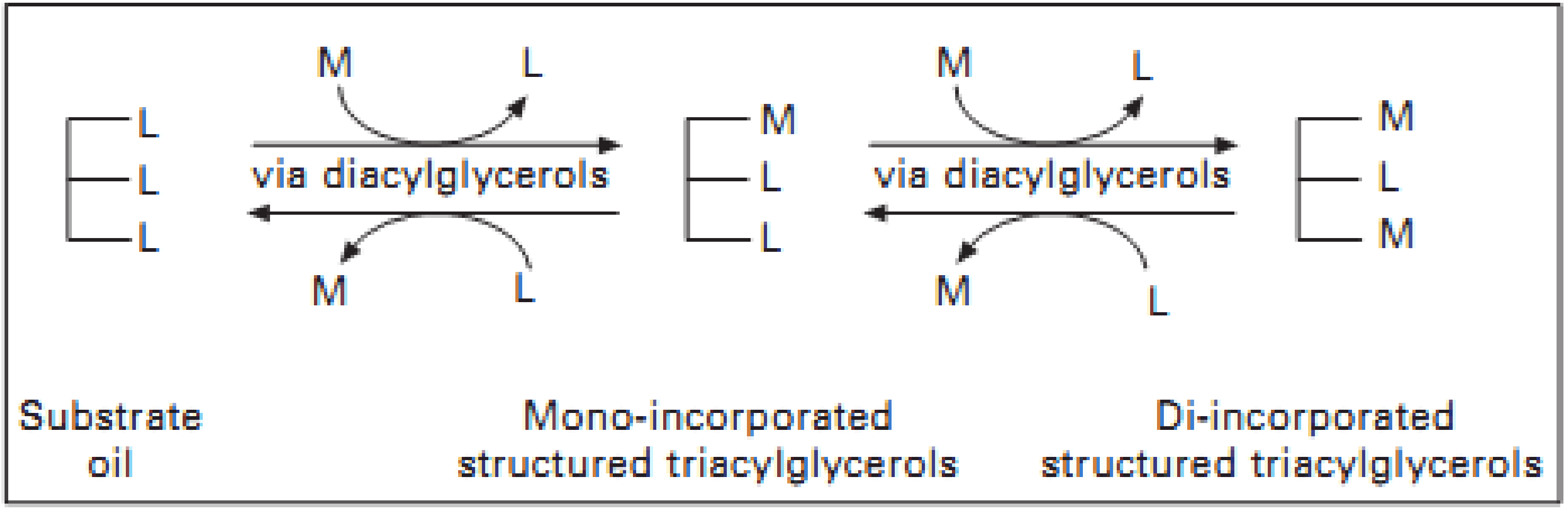
Lipases

- Lipases are classified as sn1-/sn3-specific and nonregioselective enzymes. Hence, sn1-/sn3-specific enzymes preferentially cleave fatty acids located at the outer positions of a triacylglycerol.
- The most common lipase sources are Rhizomucor miehei, Thermomyces lanuginosus, Pseudomonas fluorescens, Candida antarctica, and Rhizopus oryzae

STRUCTURED LIPIDS (MODIFIED LIPIDS)

Structured Lipids

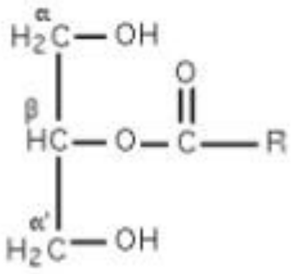
- Triacylglycerols (TAG) restructured or modified to change the fatty acid composition and/or their positional distribution in glycerol molecules by chemical or enzymatic processes.



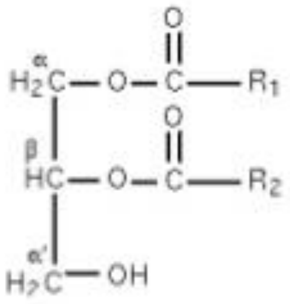
Structured Lipids

- Physical characteristics are influenced by:
  - Carbon chain length
    - Increased chain length = increased melting point
  - Degree of unsaturation
    - The more unsaturated a fatty acid is, the more liquid it will be at T_{room}
  - Distribution of fatty acids on glycerol

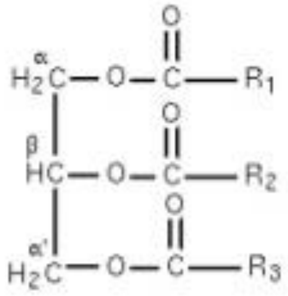
monoglyceride



diglyceride



triglyceride



Component Fatty Acids

- Important role in the nutritional and functional properties of TAGs

→Short Chain Fatty Acids

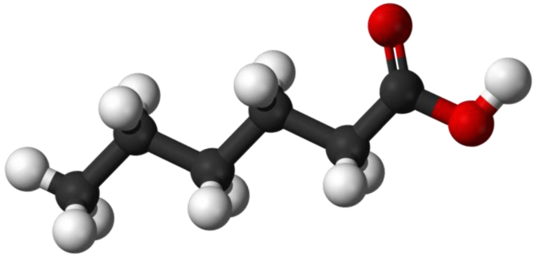
→Medium Chain Fatty Acids

→Long Chain Fatty Acids

→Poly/Mono unsaturated Fatty Acids

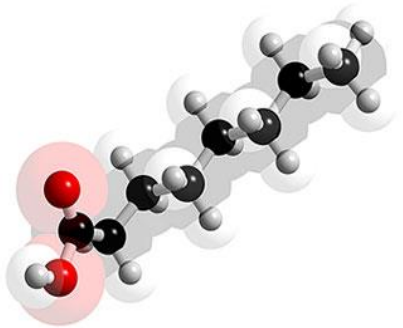
→Essential Fatty Acids

Short Chain Fatty Acids



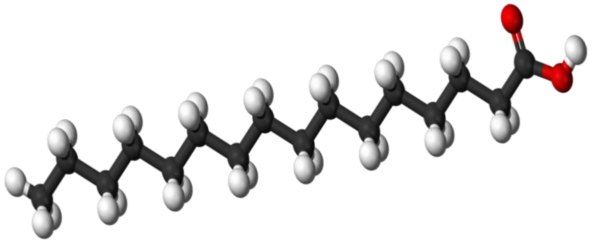
- C2:0 to C6:0
- Higher water soluble nature
- Smaller molecular size
- Rapidly absorbed in stomach
- Fewer calorie values per unit weight
- Butyric acid, Caproic acid, Propionic acid

Medium Chain Fatty Acids



- C8:0 to C12:0
- Higher water solubility than long chain fatty acids
- Quick energy and absorption
- Lower energy than LCFAs
- Caprylic acid, Capric acid, Lauric acid

Long Chain Fatty Acids



- C14 to C24
- Less water solubility, higher molecular size
- Absorbed and metabolized more slowly than either medium or short chain acids
- Highest energy source
- Palmitic Acid, Stearic Acid, Oleic Acid

Fatty Acids vs Melting Points

1) Number of C chain ↗ Melting Point ↗
2) If C numbers are equal ???

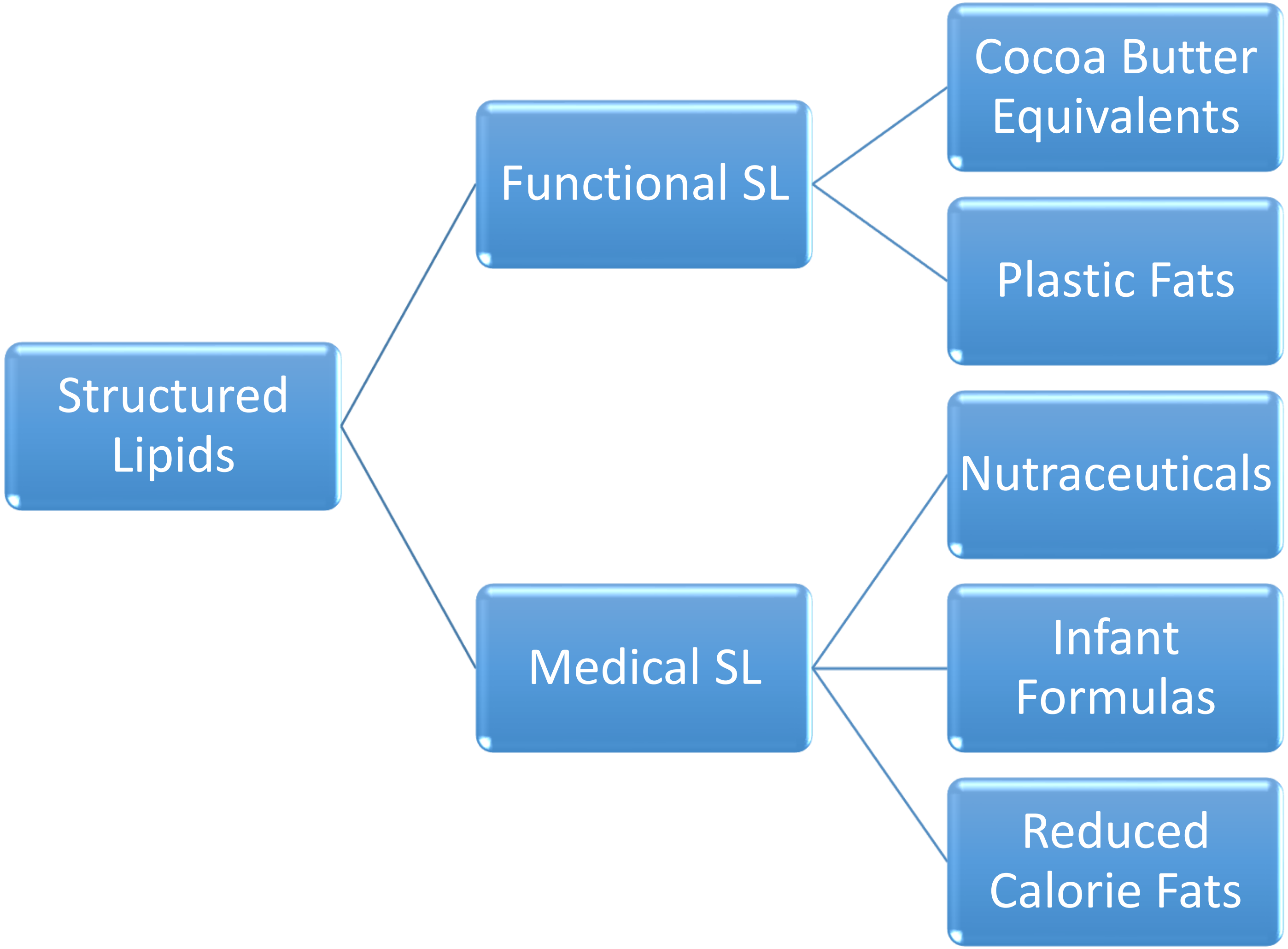
   Double bond ↗ Melting Point ↙

Palmitic Acid (16:0)

Stearic Acid(18:0)

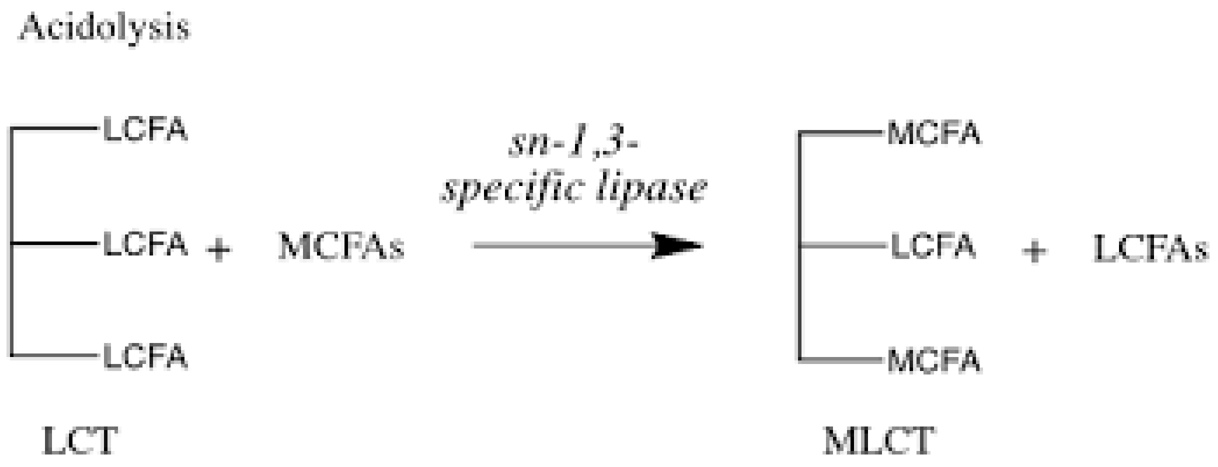
Oleic Acid (18:1)

Application of Structured Lipids



Reduced Calorie Fats

- Fats and Oils are the richest source of energy and high consumption causes diseases such as obesity, hypertension, cardiovascular diseases
- The reduced calorie fats are TAGs composed of mixtures of long chain fatty acids with medium or short chain fatty acids randomly arranged on glycerol molecule.
- This structured lipids contain fatty acids either are of a low energetic value or are poorly absorbable.



SALATRIM (BENEFAT)

short and long acyl triglyceride molecule

Production

SCFAs are chemically trans-esterified with vegetable oils such as highly hydrogenated canola or soybean oil.

Salatrim

$C_{18:0}$	$C_{18:0}$
SCFA	SCFA
SCFA	$C_{18:0}$

SCFA = $C_{2:0}$ and/or $C_{3:0}$ and/or $C_{4:0}$

5 kcal/g

Principle:

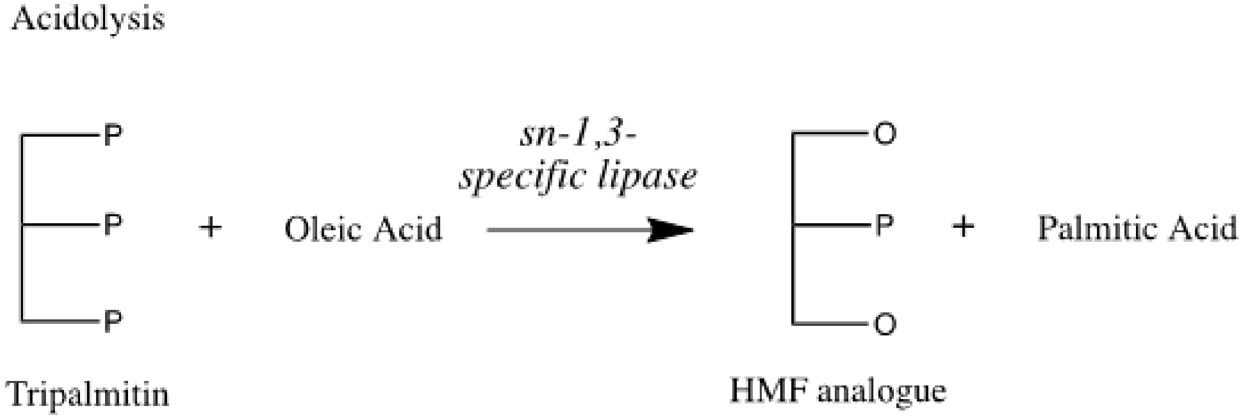
Short-chain fatty acids (e.g.,butyric) provide fewer calories per unit of weight than do longer chain fatty acids, and stearic acid (the primary long-chain fatty acid of Salatrim) is only partially absorbed by the body.

Properties:

It has the taste, texture, and functional properties of conventional fats and oils. It can be produced to have different melting profiles by adjusting the amounts of SCFAs and LCFAs used in their chemical synthesis.

Human Milk Fat Substitutes

- Production of human milk fat substitutes
- Lipids are one of the most important macronutrients for infants and comprise of 50% of the energy in human breast milk.
- For proper growth and function, important fatty acids need to be metabolized efficiently, and this is achieved in HMF due to the stereospecific distribution of the fatty acids.
- One of the most important fatty acids for the infant is palmitic acid because it is an important source of energy, and it is mainly located at the *sn*-2 position of HMF. This differs from many vegetable oils used in the production of infant formulas, which contain palmitic acid at the *sn*-1and *sn*-3 positions



Human Milk Fat Substitutes (Continued)

BetapolTM, which is used in infant nutrition as a milk-fat substitute. It contains oleic acid at the sn1 and sn3 positions and palmitic acid at the sn2 position [1,3-oleoyl-2-palmitoyl-glycerol (OPO)].

BetapolTM is manufactured by interesterification of tripalmitin with high oleic sunflower oil using RMIM.

Plastic Fats

- Margarines and shortenings usually contain high amounts of *trans* fatty acids due to the partial hydrogenation process of plant oils.
- FDA has banned the use of partially hydrogenated oils in processed foods.
- Enzymatic interesterification of saturated fats with unsaturated oils

Cocoa Butter Equivalents

The current concern for cocoa butter fat as major ingredients of chocolate intake in the World has raised the question of the high price of cocoa butter among all other vegetable fats.

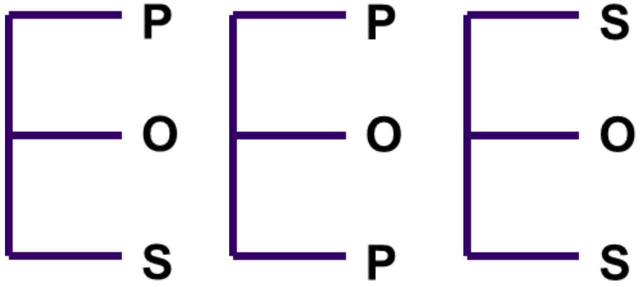
Productions of natural cocoa butter fats are decreasing day by day due to the decrease of cocoa cultivation worldwide;

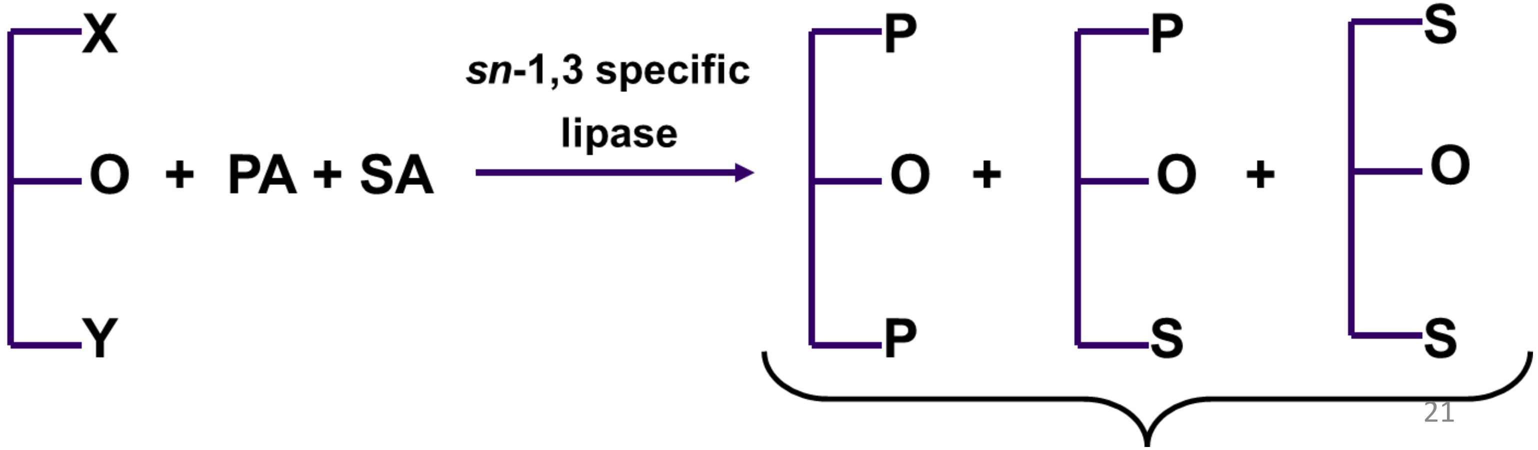
moreover, cocoa fruit contains only a little amount of cocoa butter.

Therefore, the food industries are keen to find the alternatives to cocoa butter fat and this issue has been contemplated among food manufacturers.

Cocoa Butter Equivalents

- the most valuable ingredient of chocolate industry
- crucial role in chocolate production
- gloss, snap, texture, cool melting in mouth, intense chocolate flavor
- Cocoa butter is expensive and limited
- Industry is looking for fats alternative to cocoa butter



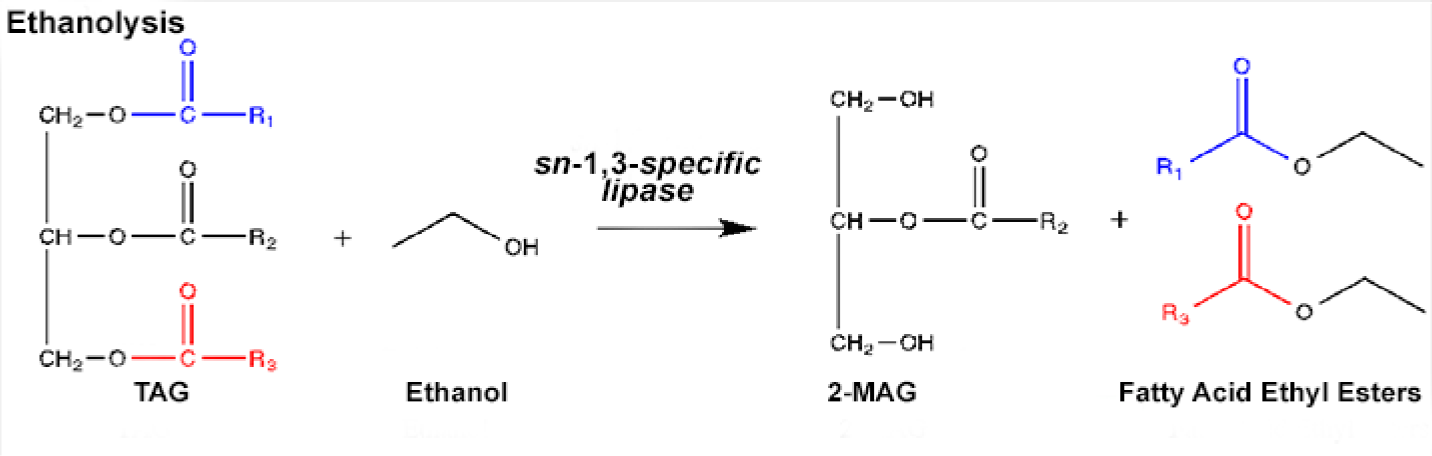


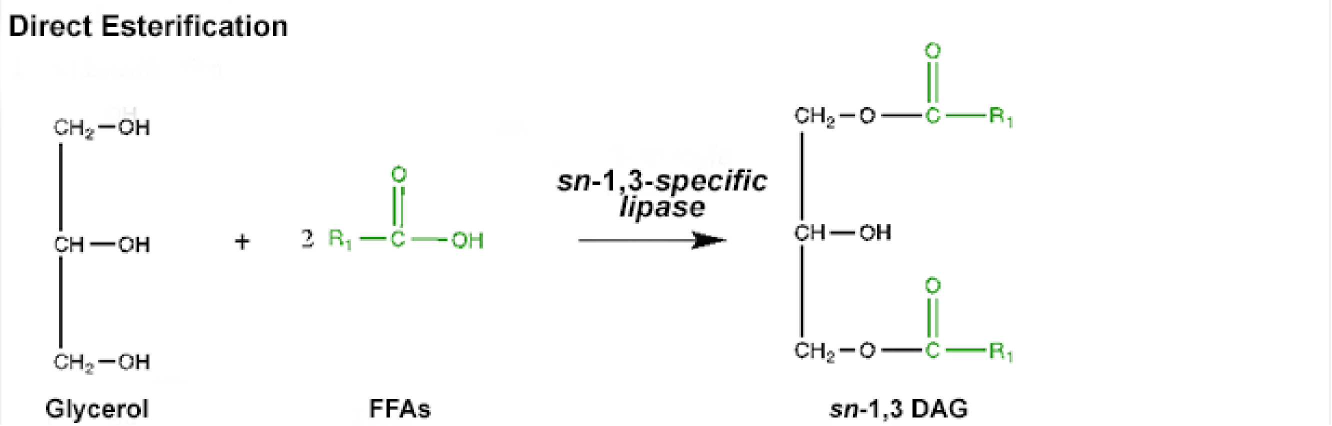
Cocoa Butter Equivalents (Continued)

- Processes were developed by Unilever and Fuji Oil using 1,3-regioselective lipases for the acidolysis of cheap plant oils and stearic acid as an acyl donor.

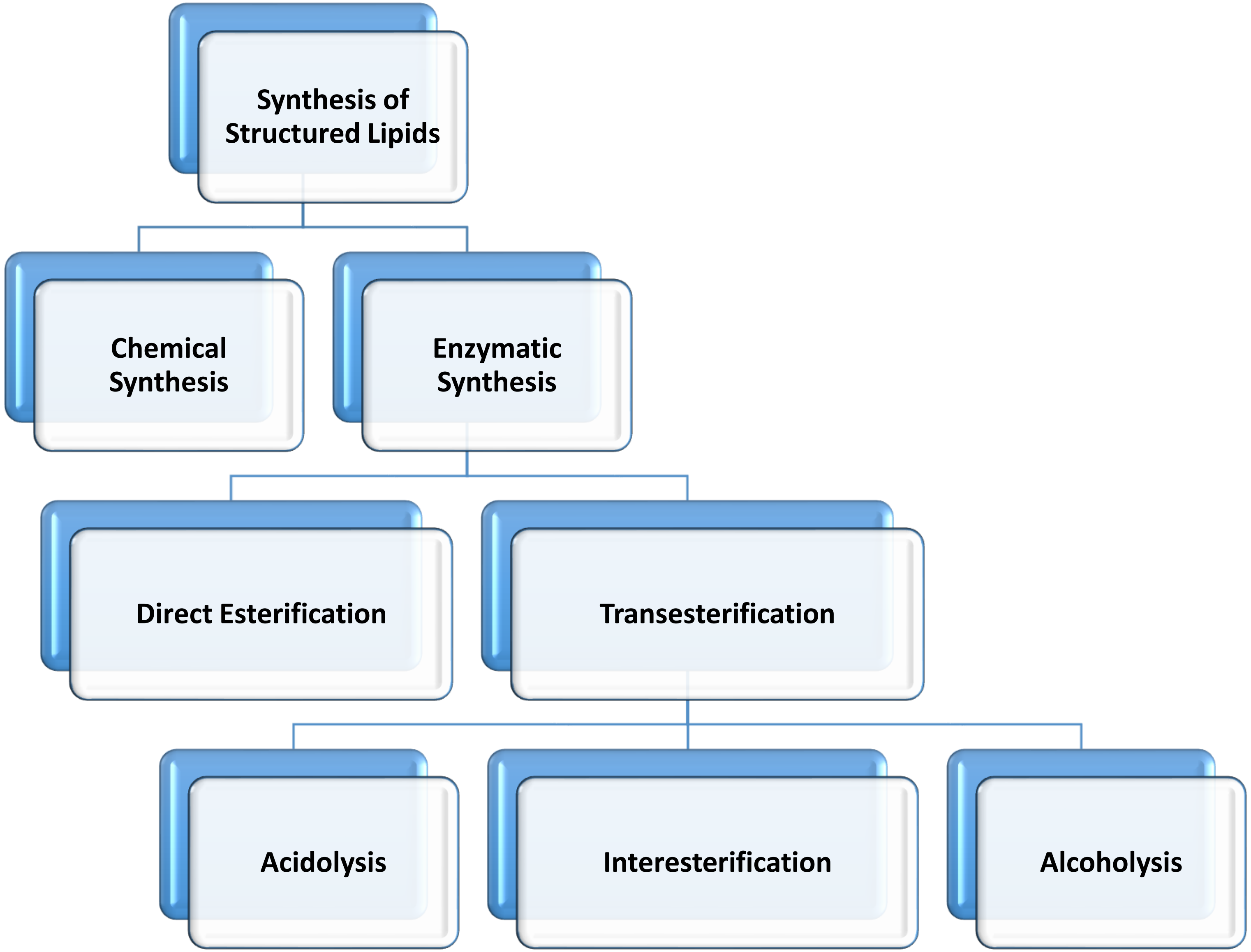
Production of Mono/Diacylglycerols

- 2-MAGs with unsaturated fatty acids to also serve as sources of dietary fatty acids because 2-MAGs are readily absorbed during digestion.
- DAGs can also be used in combination with MAGs as food grade emulsifiers.



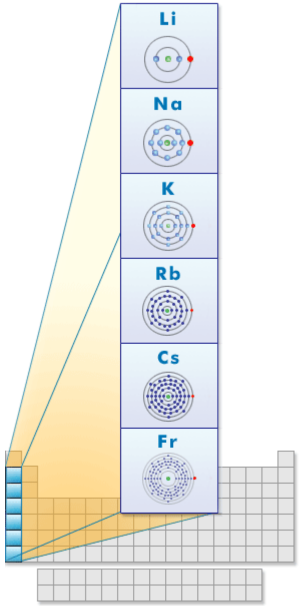


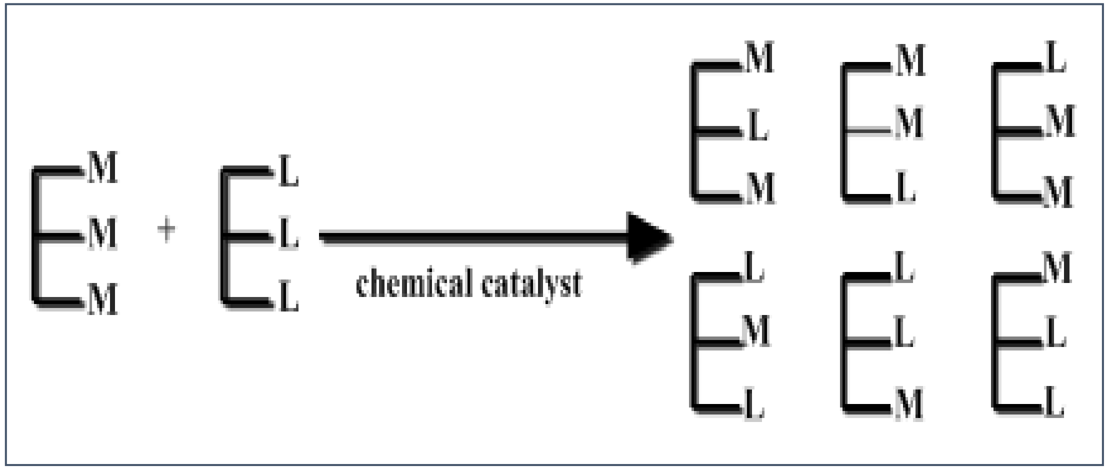
Synthesis of Structured lipids



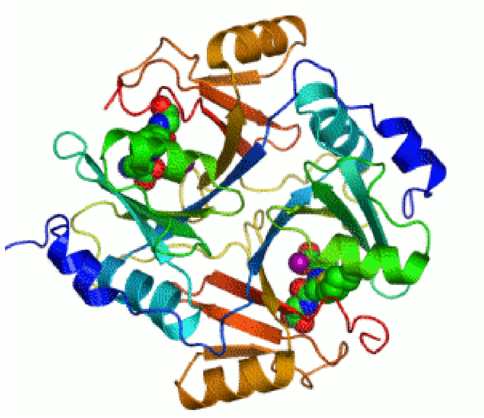
Chemical Synthesis

- Heating of fat/oil at 100-140°C
- Reaction temperature 50°C
- Reaction time 30 min
- Catalyzed by alkali metals or alkali metal alkylates





Lipase-Catalyzed Synthesis of Structured Lipids



Direct Esterification

- Reaction of free fatty acids with alcohol
- Lipase catalayzed
- Major problem: H_2O production

Hydrolysis

$$\text{Glycerol} + \text{MCFA} + \text{LCFA} \xrightarrow{\text{lipase}} \text{SL} + \text{Water}$$

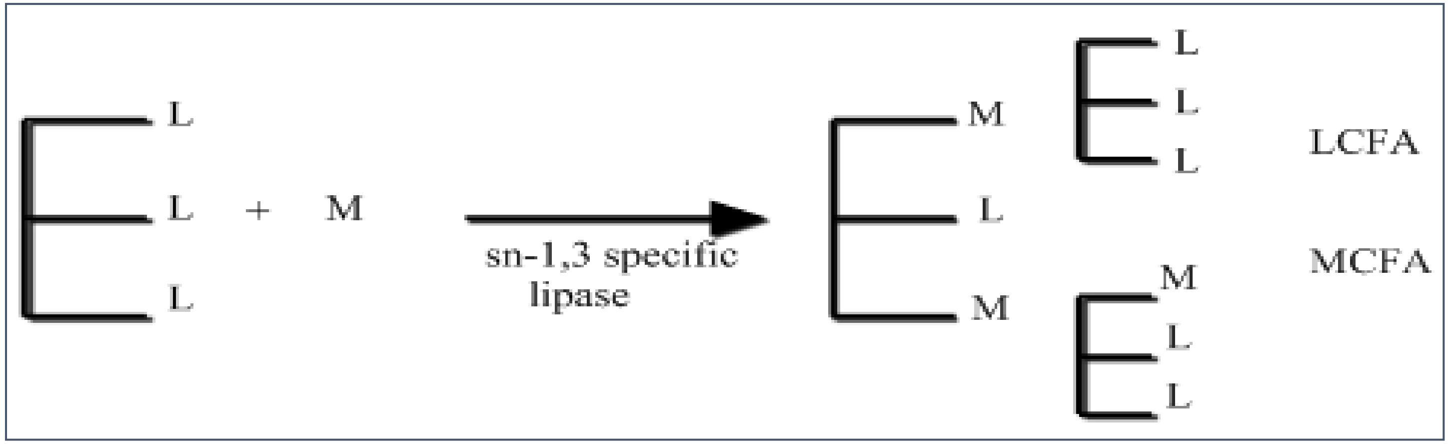
Transesterification

- Transesterification
  - Acidolysis
  - Interesterification
  - Alcoholysis

Acidolysis

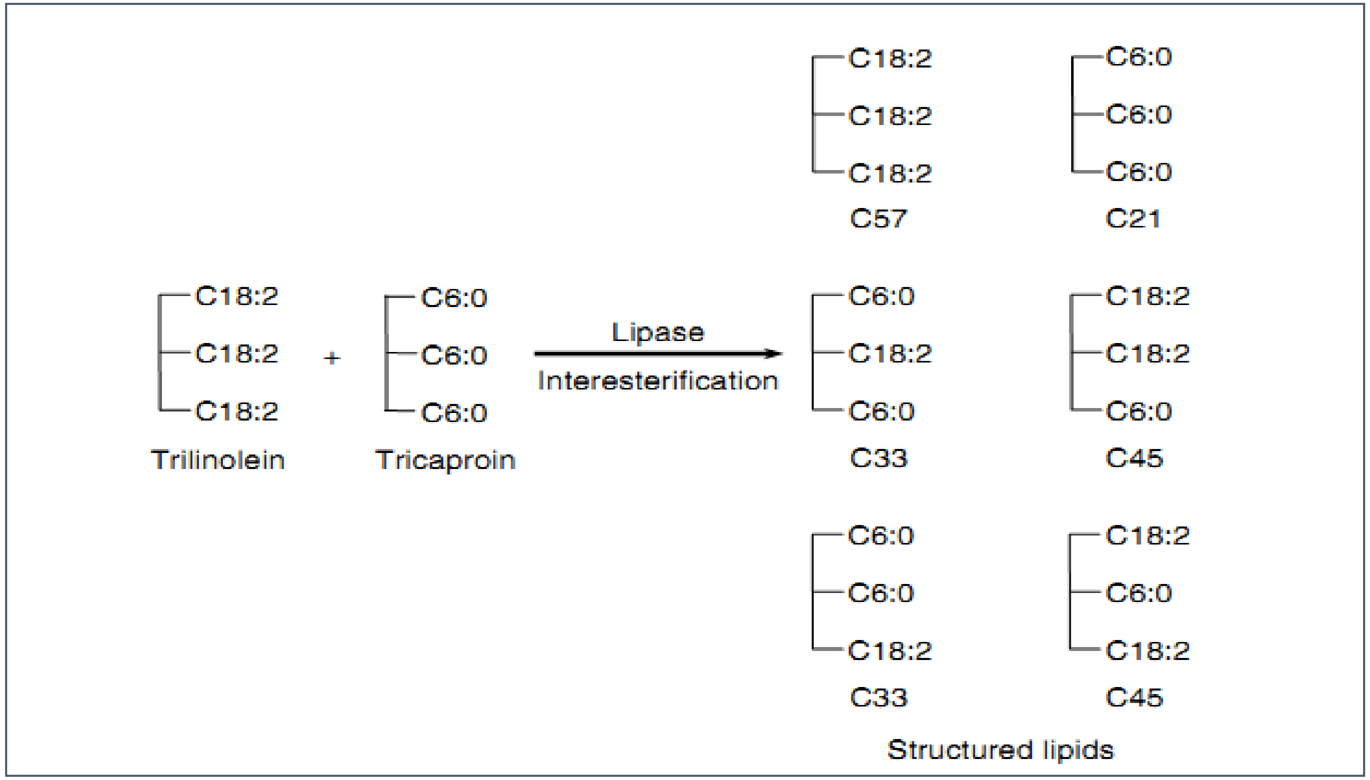
- Reaction of TAGs and free fatty acids
- Lipase catalyzed
- Side products: Mono-diacylglycerols

  Reaction products: Free fatty acids and TAGs



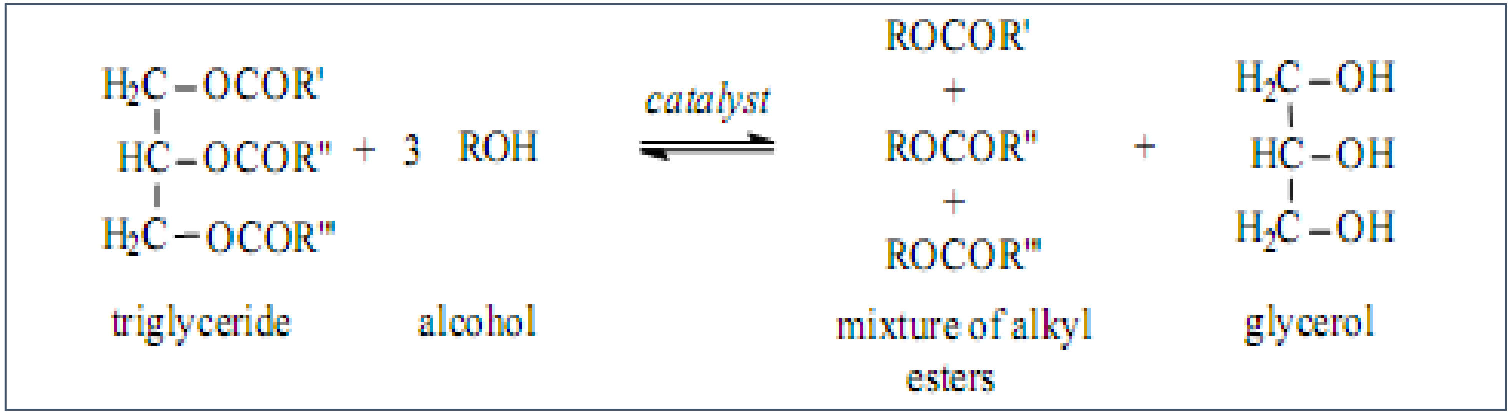
Interesterification

- Exchange of acyl residues between two TAGs
- Lipase catalyzed
- Products : New structured TAGs

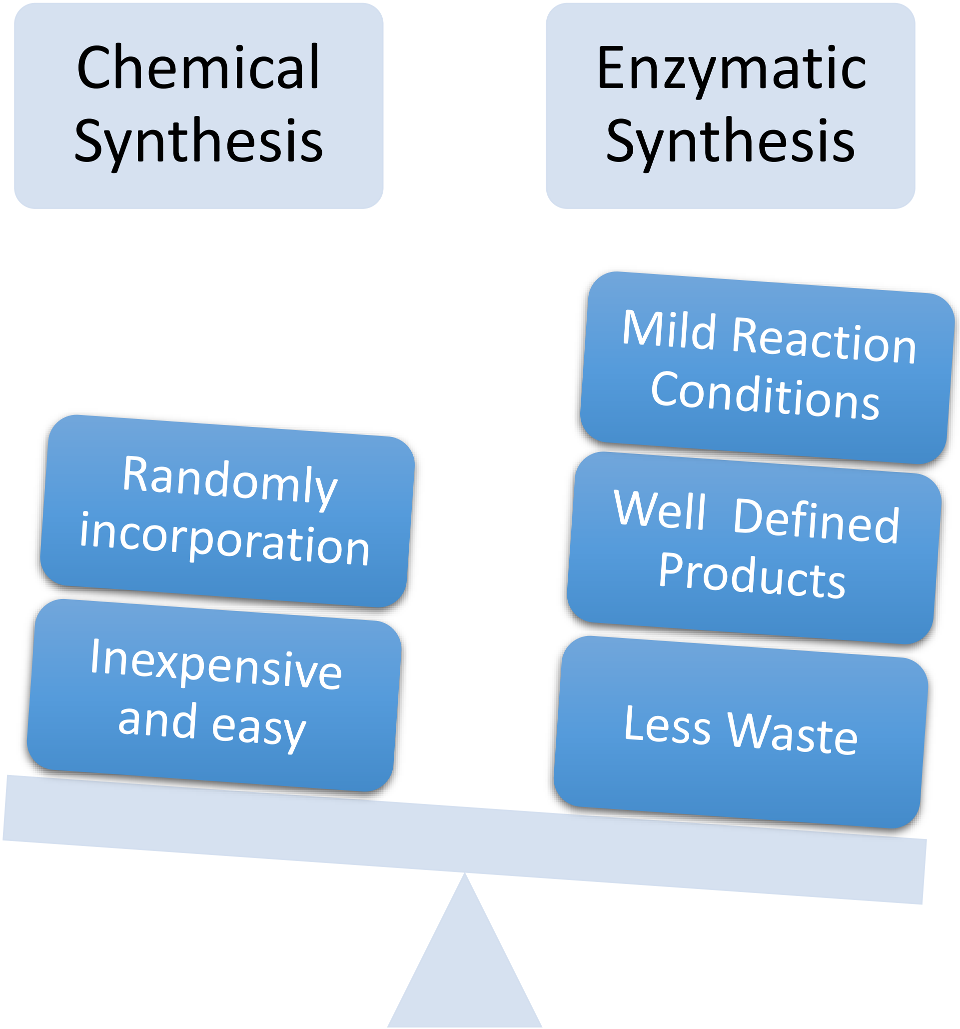


Alcoholysis

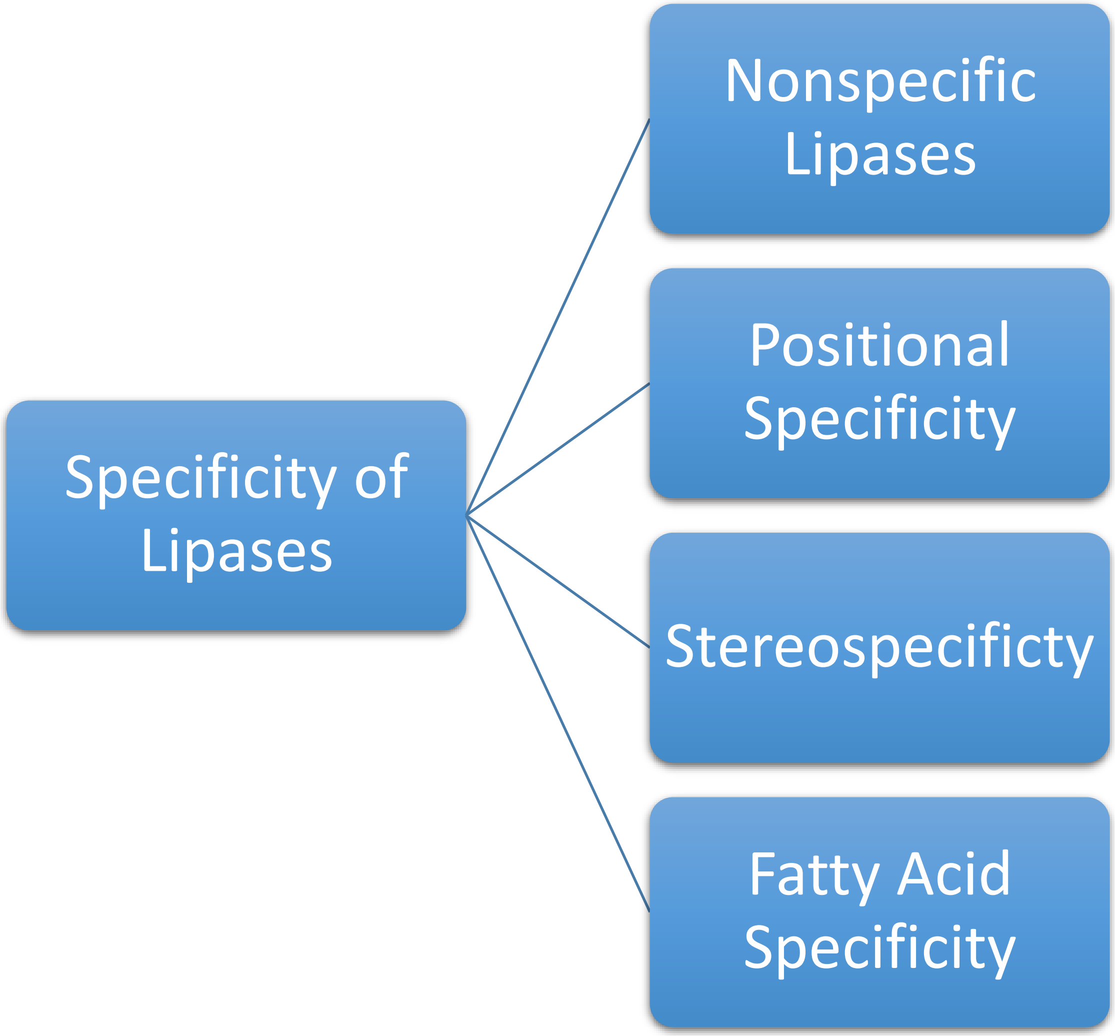
- Reaction of a triacylglycerol and an alcohol
- Lipase catalyzed



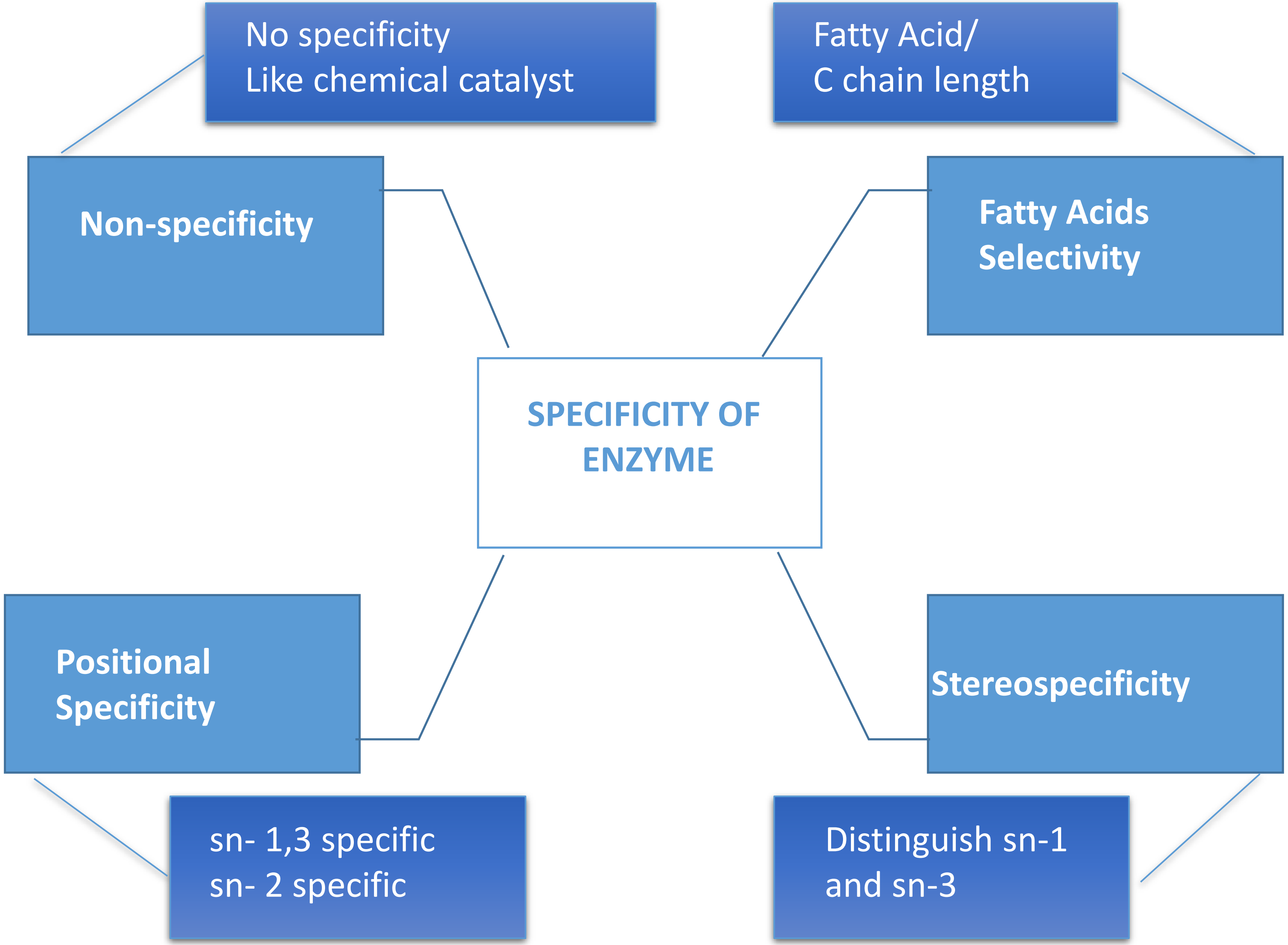
Chemical synthesis vs Enzymatic synthesis



Specificity of Lipases



Specificity of Lipases (Cont'd)



Factors affecting Lipase Activity In reaction systems

- **pH**

→Optimum pH

→ Stability of the tertiary or quaternary structure of enzymes is also pH dependent

- **Water Activity**

→Determine direction of hydrolysis or synthesis

→Water contents of <1% for effective interesterification

→presence of excess water decreases the catalytic activity

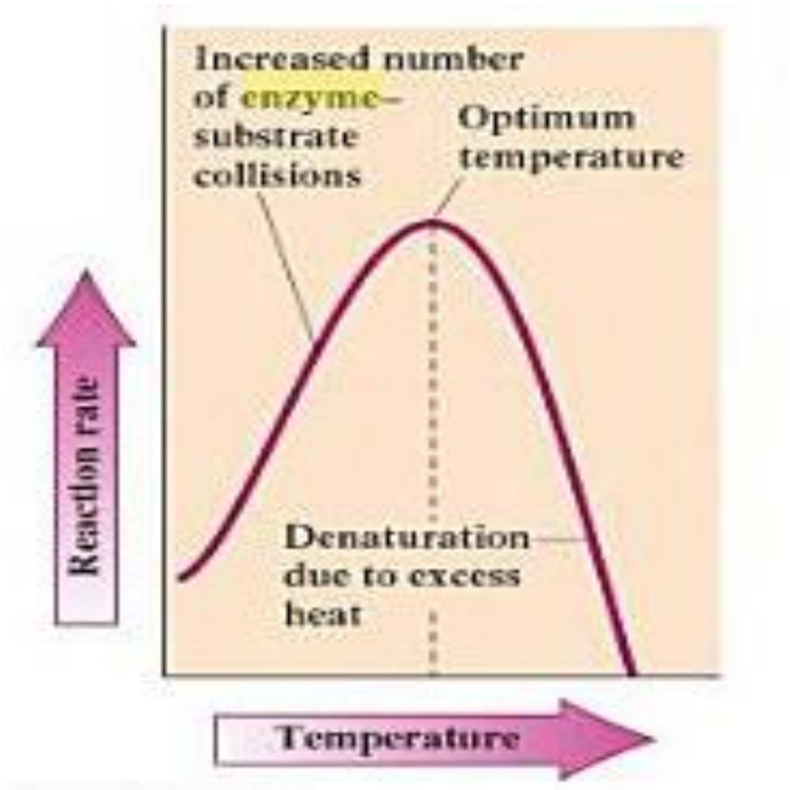
Factors affecting Lipase Activity In reaction systems (Cont'd)

- **Temperature**

→Molecules move faster and collides

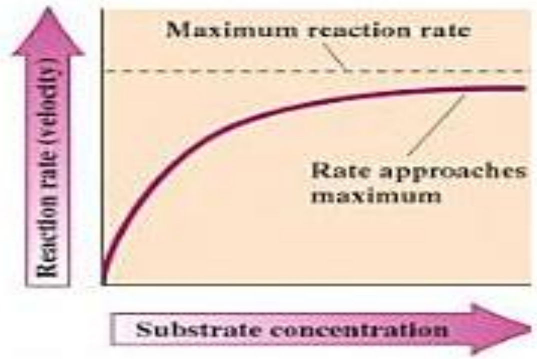
→Optimum temperature

→Denaturation

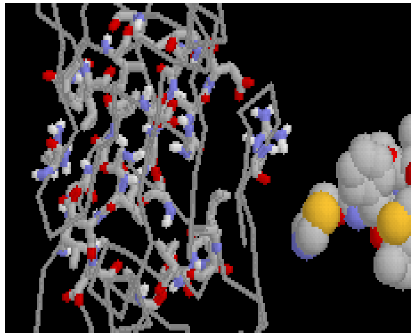


Factors affecting Lipase Activity In reaction systems (Cont'd)

- **Substrate Concentration**



- **Product Accumulation**



High levels of free fatty acids decreases rate of reaction due to acidification of enzyme medium

Factors affecting Lipase Activity In reaction systems (Cont'd)

- Solvent Type

Reason to apply enzymes in organic media

(1) to reverse enzymatic hydrolytic reactions

(2) to suppress side- reactions that require water

(3) to increase the substrate solubility

(4) to simplify product recovery

→ More efficient in nonpolar solvents than polar solvents