Chapter 5

ENZYMATIC MODIFICATION OF OILS AND FATS (continued)

In the previous chapter, basics of lipases and lipase-mediated syntheses of MAGs and DAGs were shown. This chapter, the continuation of CHAPTER 4, deals with TAGs. Functions and production of specialty structured TAGs are reviewed. In addition, some strategies to enhance the efficiency of the lipase-mediated reactions are outlined.

5-1 Structured TAGs

5-1-1 What is Structured TAGs?

Structured TAGs (sTAGs) are defined as TAGs which are modified chemically or enzymatically to change the fatty acid composition and/or positional distribution in the glycerol backbone. In a stricter definition, sTAGs are referred particular molecular species of TAGs with defined molecular structure (i.e. specific fatty acid residues in specific positions).

Molecular structure of TAGs influences their metabolic fate in organisms (i.e. digestion and absorption) as well as their physical characteristics (e.g. melting points) and crystallinity. Consequently, designing sTAGs with particular chemical structure, it is possible to control the behavior of TAGs, thereby improving the nutritional and pharmaceutical properties of TAGs.

5-2 Examples of sTAGs

5-2-1 Low calorie fat

Long chain saturated fatty acids have higher melting points. In addition, they easily form insoluble calcium salts in body. Therefore, saturated fatty acids are relatively less absorbable than unsaturated fatty acids. From this reason, saturated fatty acids have relatively lower calories than others.

Hence, it would be expected that TAGs having only long chain saturated fatty acids can be used as low-calorie fats. However, since such saturated TAGs have very high melting point (= too hard), and its use for food would be limited.

Attaching long and short or long and medium chain fatty acids into one glycerol molecule gives TAGs with low calorie and appropriate consistency. Salatrim and Caprenin are typical examples of such low-calorie fats.

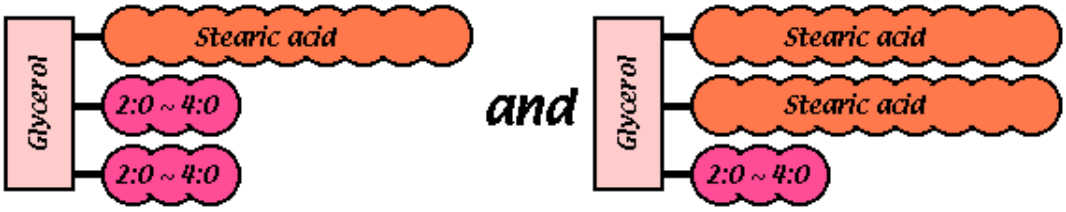

Figure 5-2-1: Salatrim.

Salatrim (short and long acyl triglyceride molecule) is TAG consisting of C2-4 short chain fatty acids and long chain saturated fatty acids (Figure 5-2-1). This was developed by Pfizer and Nabisco, and commercialized as Benefat^{TM}. Because the short chain fatty acids themselves have low calorie and because the long chain saturated fatty acids are less absorbable, salatrim has lower calorie (4.5-5.5 kcal/g) than usual cooking oil (9 kcal/g). Salatrim is industrially produced by chemical interesterification between short chain TAG (triacetyl-, tripropionyl or tributyl-) and long chain saturated TAG (hydrogenated vegetable oil).

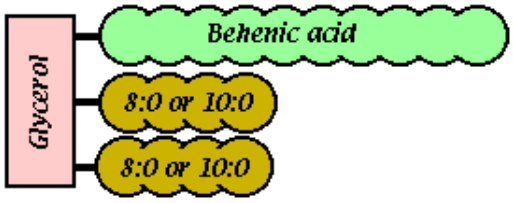

Figure 5-2-2: Caprenin

Caprenin^{TM}, developed by Proctor and Gamble (P&G), contains behenic acid (22:0) and medium chain fatty acid such as caprylic acid (8:0) or caprenic acid (10:0). Caprenin's calorie is about 5 kcal/g, and it is industrially produced by chemical acylation of behenic acid containing 1-MAG with medium chain fatty acid.

5-2-2 Easily absorbable oil.

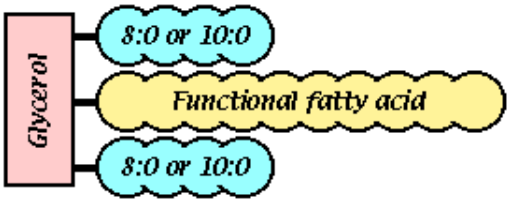

Figure 5-2-3: MLM-type sTAG

So-called "MLM-type sTAGs" are considered to be a easily absorbable oil. MLM-type sTAGs have medium chain (6-10 carbons) fatty acids at the 1 and 3-positions and long chain (12 carbons or more) fatty acids at the 2-position (Figure 5-2-3).

Mammalian pancreatic lipase has 1,3-positional-specificity and fatty acid preference on medium chain fatty acids. So it hydrolyzes the ester linkages at the 1 and 3-positions with a preference for medium chain fatty acids over long chain fatty acids. The resulting 2-MAGs

are well absorbable through the intestinal mucosa. Therefore, long chain fatty acids located at 2-position of MLM-type sTAGs are absorbed well.

Based on this expectation, MLM-type sTAGs can be used as effective carriers of long chain fatty acids, especially functional fatty acids such as polyunsaturated fatty acids.

5-2-3 Specialty fat for infant formula

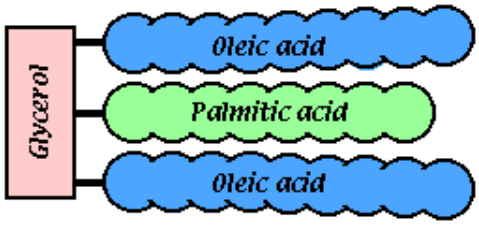

Figure 5-2-4: Betapol^{TM}

In infants, absorption of saturated fatty acids from mother's milk is better than from vegetable oils with similar fatty acid content. This is because most of saturated fatty acids (mainly palmitic acid) are located on the 2-position of TAG in mother's milk.

Betapol^{TM} is a sTAG designed to mimic the TAGs in mother's milk. It contains unsaturated fatty acid (mainly oleic acid) at 1 and 3-positions and saturated fatty acid (palmitic acid) at the second position (Figure 5-2-4). It was developed by Unilever, and used for infant formula (artificial milk for babies)

5-2-4 2.4 Specialty fats for chocolate making

The examples shown above are intended to control mainly nutritional properties (digestion and absorption) of oils and fats. Besides, there are some sTAGs developed to control the physical properties (melting points and crystallinity) of oils and fats.

Figure 5-2-5: Cocoa butter equivalent (CBE)

Cocoa fats, from which chocolates are made, are rich in TAG molecules with saturated fatty acid (mainly palmitic or stearic) at the 1 and 3-positions and unsaturated fatty acid (mainly oleic) at the 2-position. The physical properties of chocolate are owing to such TAG molecules. Cocoa butter equivalent (CBE) is a fat which imitates the cocoa fats. It is produced from palm oil and appropriate unsaturated fatty acid.

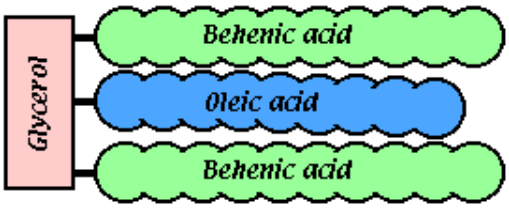

Figure 5-2-6: BOB, an anti-blooming agent for chocolates.

As another example of sTAGs for chocolate making, BOB (1,3-behenic 2-oleoylglycerol, Figure5-2-6) is shown. Keeping chocolates for a long period causes "blooming", a phenomenon in which the surface of the chocolate is covered with whitish material. Blooming is due to partial melting and recrystallization of chocolate fats, and it reduces the commercial value of the chocolates (although not harmful at all to humans' health). BOB was developed by Fuji Oil, and is used as an anti-blooming agent for chocolate.

5-3 Enzymatic synthesis of sTAGs

5-3-1 Symmetrical sTAGs

Remember the sTAGs shown in the previous section. The functionalities (with both low calorie and appropriate hardness) of Benefat^{TM} and Caprenin^{TM} come from their fatty acid composition rather than the positions of the fatty acids in the molecule. In other words, these fats can be "functional", if long and medium or long and short chain fatty acids are attached within one TAG molecule. Therefore, practically they are produced by chemical reactions but not by enzymatic reactions (though possible).

In contrast, the other sTAGs (MLM, Betapol^{TM}, CBE and BOB) have symmetrical structure, i.e. two identical (or of the same type) fatty acids at 1 and 3-positions and another fatty acid at the 2-position. Importantly in these cases, such specific structure is inevitable for the functionalities of them. To synthesize sTAGs with this specific structure, ordinary chemical reactions are not suitable because of the random nature. Therefore, lipase-catalyzed position-specific reactions are necessary, and in fact, mainly employed. Three methods for the production of the symmetrical sTAGs are shown here. A key point for all the strategies is the use of 1,3-position-specific lipase.

5-3-2 Interesterification of two TAGs

In this strategy, a mixture of two different mono-acid TAGs is reacted with a 1,3-position-specific lipase (Figure 5-3-1).

In the reaction, all the substances in the reaction mixture (i.e. the interesterified products and unreacted substrates) are TAGs theoretically. Thus, the product as a "TAG fraction" is recovered very easily just by removing the catalyst. However, the reaction always gives a complicated non-homogeneous mixture of many TAG species, each of which is very difficult to isolate by practical means. As far as this strategy is used, there is no way to make only the target sTAG. Therefore, this strategy might be preferable for preparation of a mixture of TAGs containing the target sTAG by a simple and economical method rather than for preparing only the defined sTAG species.

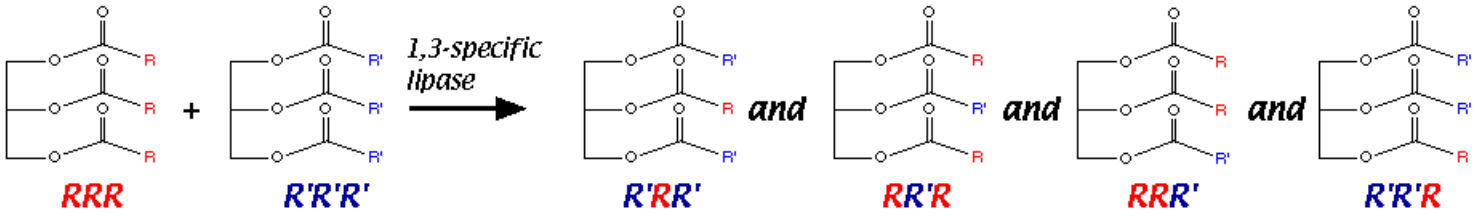

Figure 5-3-1: Synthesis of symmetrical sTAGs by enzymatic interesterification of two mono-acid TAGs, RRR and R′R′R′. Suppose that the target sTAG is R′RR′. This reaction gives R′RR′, but also RR′R, RRR′, and R′R′R.

5-3-3 Acidolysis of TAG with fatty acid

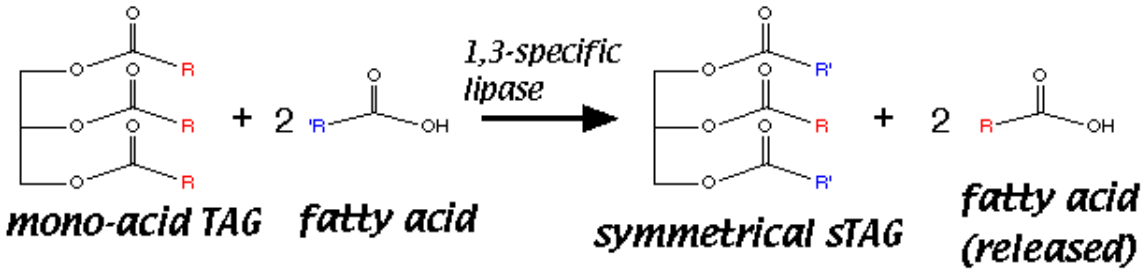

Figure 5-3-2: Synthesis of symmetrical sTAGs by enzymatic position-specific acidolysis.

The second strategy is acyl exchange of oils with excess of fatty acid (acidolysis, Figure 5-3-1). The strategy is to substitute the fatty acid residues specifically at the 1,3-positions of the oils with desired ones by a 1,3-position-specific lipase, leaving the residues at the 2-position unchanged. Theoretically, these reactions give mixtures of TAGs (containing the target sTAGs) and fatty acids. The fatty acids can be removed after the reaction by molecular distillation or alkali extraction. Therefore, if all the acyl groups at the 1,3-positions are replaced with the desired one, eventually the target sTAG species with specific structure can be obtained with quite high purity. Most of the symmetrical sTAGs (MLM, Betapol^{TM} , CBE and BOB) are industrially produced by this method.

5-3-4 Acylation of 2-MAG

The third method is esterification of 2-MAG with the desired fatty acid. The 2-MAG can be prepared by ethanolysis of a TAG as shown in CHAPTER 4. The driving force to promote the reaction is the removal of the generated water, which is achieved by performing the reaction under reduced pressure.

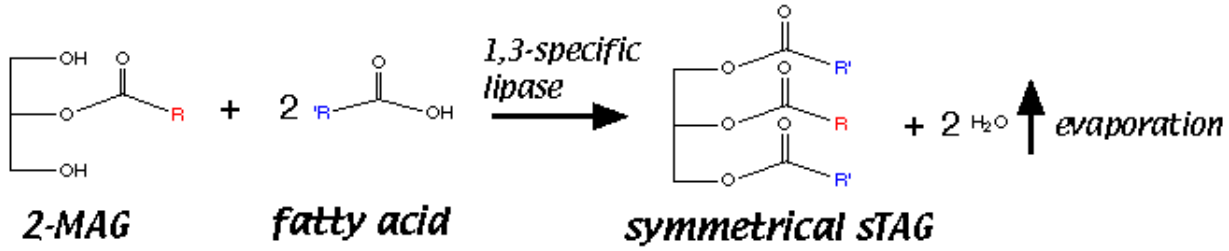

Figure 5-3-3: Synthesis of symmetrical sTAGs by direct esterification of 2-MAG.

5-4 Strategies to increase the product yield

5-4-1 Shifting the equilibrium

The lipase-catalyzed esterification and transesterification are reversible reaction. Therefore, the reactions do not complete (stop at the equilibrium) by just mixing the substrates and the enzyme. To make the reaction run nearly completely, it is important to shift the equilibrium towards the product formation. Hence, how to shift the equilibrium? Some strategies are shown below.

5-4-2 Use of excess reactant

All the the lipase-mediated reactions are, in principle, two-substrate reactions. Therefore, the most simple strategy is to use either one of the two reactants (usually the cheaper one) This shifts the equilibrium towards the right side of the scheme, thereby the product yield will relatively increase.

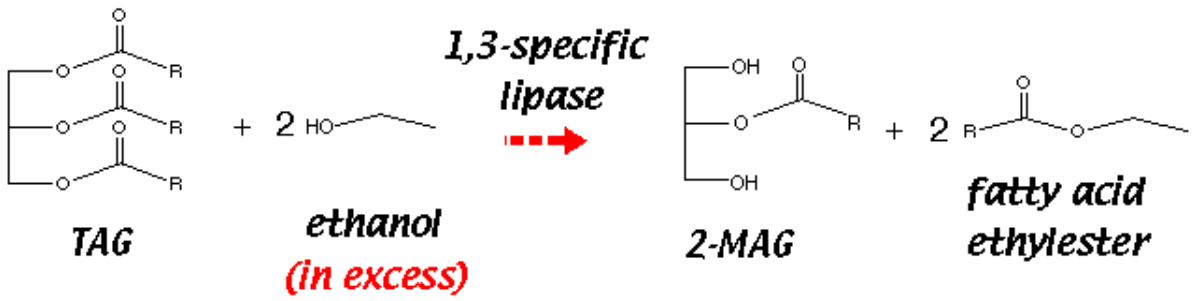

Figure 5-4-1: Excess ethanol drives the reaction towards the formation of 2-MAG in ethanolysis of TAG.

An example of use of this strategy is the ethanolysis of TAG for 2-MAG production (Figure 5-4-1). In this case ethanol used both as one of the substrate and as the solvent, in excess because it is the substrate used as the solvent as well as one of the substrates, exists in large excess, allowing the complete conversion of the TAG.

Another example is acidolysis of TAG with fatty acid to make symmetrical sTAGs (Figure 5-4-2). Use of excess fatty acid allows the complete conversion of the substrate TAG into the target sTAG.

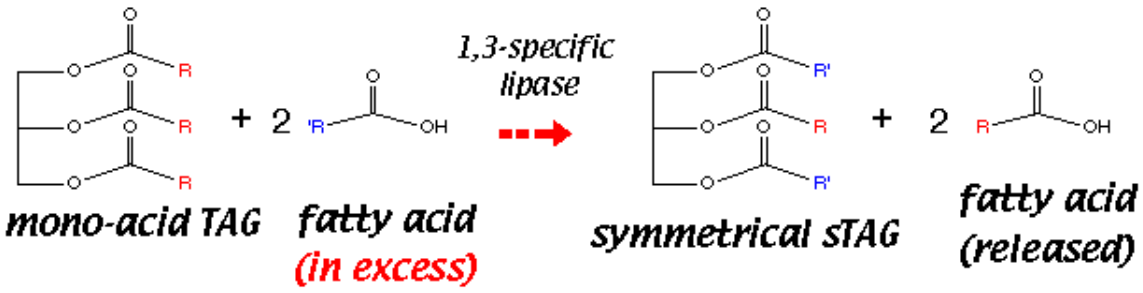

Figure 5-4-2: Use of excess fatty acid enables complete conversion of the substrate TAG into the target sTAG.

5-4-3 Removal of the target product

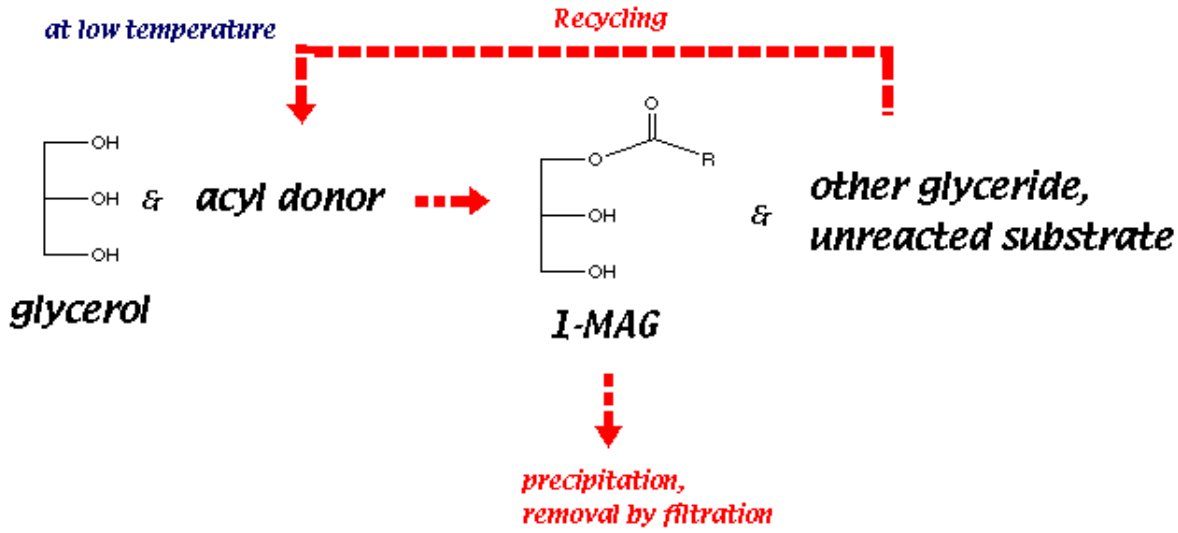

Figure 5-4-3: Removal of the crystallized 1-MAG out of the reaction vessel shifts the equilibrium.

Removing the target product also increase the yield. It is already mentioned that the crystallization of 1-MAG or 1,3-DAG is the key point in enzymatic glycerolysis. In the reaction scheme shown in Figure 5-4-3, the solidified 1-MAG is removed out of the reaction vessel, thereby the equilibrium is shifted.

Alternative examples are solid phase glycerolysis for production of 1-MAG (Figure 5-4-4) and 1-3-DAG (Figure 5-4-5). In these cases, the solidified target products are not taken away from the reaction vessel. The solidified 1-MAG or 1,3-DAG are no more recognized by the enzyme, thereby the equilibrium is shifted.

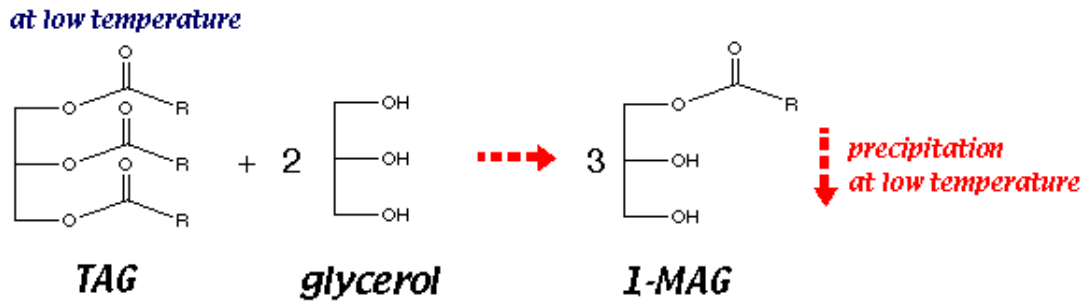

Figure 5-4-4: Crystallized 1-MAG is no more reacted, therefore the equilibrium is shifted.

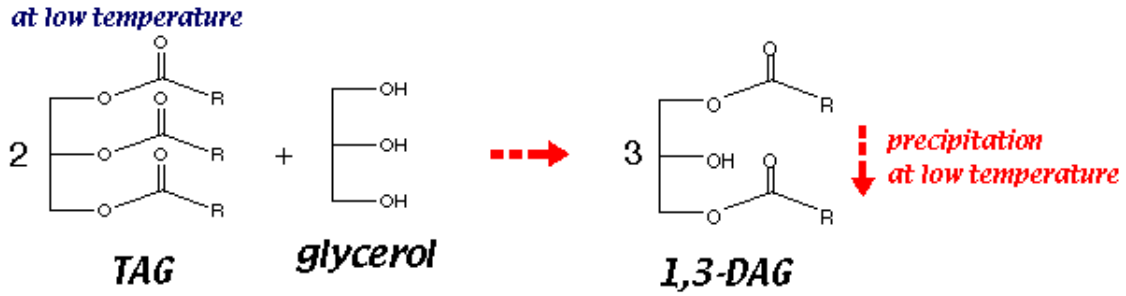

Figure 5-4-5: Crystallization of 1,3-DAG protects it from the enzyme's action, shifting the equilibrium.

5-4-4 Removal of the by-products

Removal of water: In the case of ester synthesis, a condensation reaction, water is generated during the reaction. Therefore, removing the generated water drives the reaction towards the product formation.

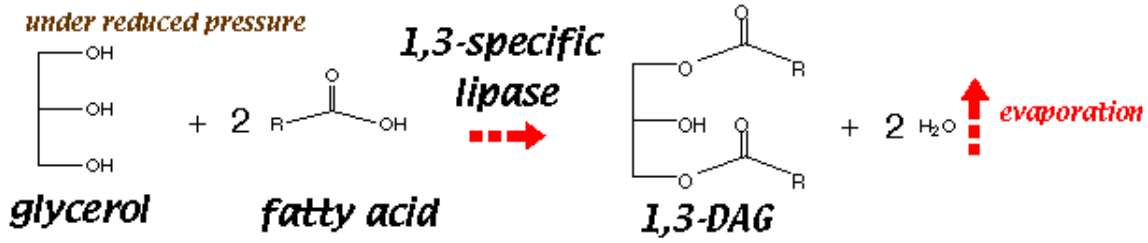

Figure 5-4-6: Synthesis of 1,3-DAG by direct esterification with removal of water under reduced pressure.

This can be achieved by performing the reaction under reduced pressure by a vacuum pomp connected to the reaction vessel. The reactions should be done in solvent-free system (if organic solvent is used, the solvent would be also evaporated together with water), and

all the reactant should not be evaporated by the operation. Figures 5-4-6, 5-4-7 and 5-4-8 illustrate esterification of glycerol, 1,2-isopropylideneglycerol and 2-MAG, respectively, with fatty acids.

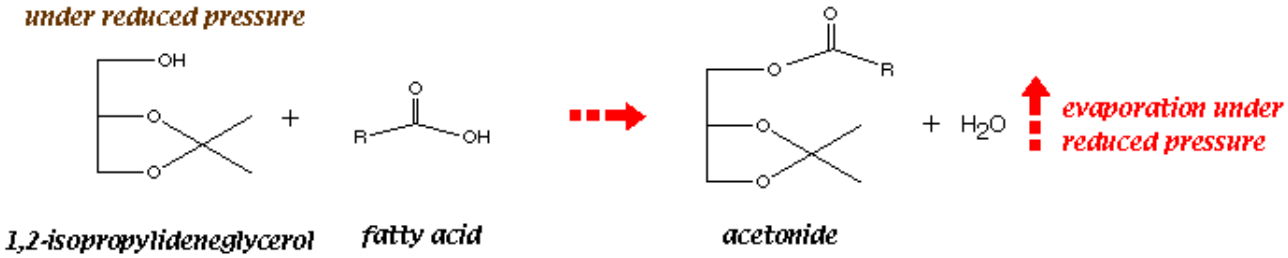

Figure 5-4-7: Esterification of 1,2-isopropylideneglycerol under reduced pressure to afford acetonide.

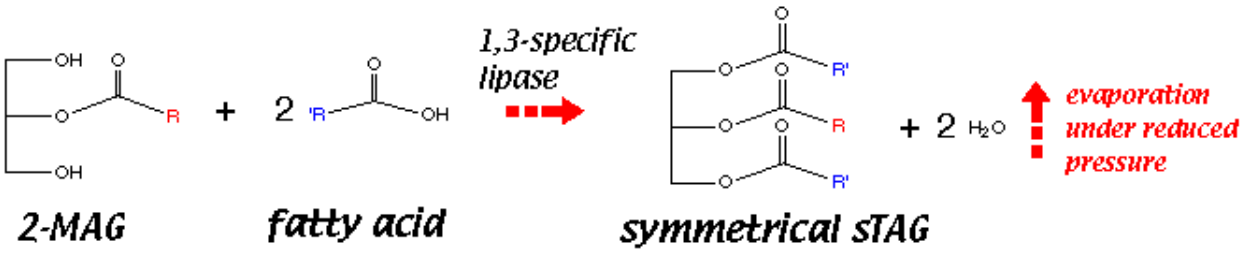

Figure 5-4-8: Esterification of 2-MAG with fatty acid under reduced pressure to afford symmetrical sTAG.

Removal of alcohol: The acylation of 1,2-isopropylideneglycerol is all so possible by using fatty acid alkylester (e.g., ethylester) (Figure 5-4-9). In this case an alcohol is generated as a by-product, which is removed by evaporation.

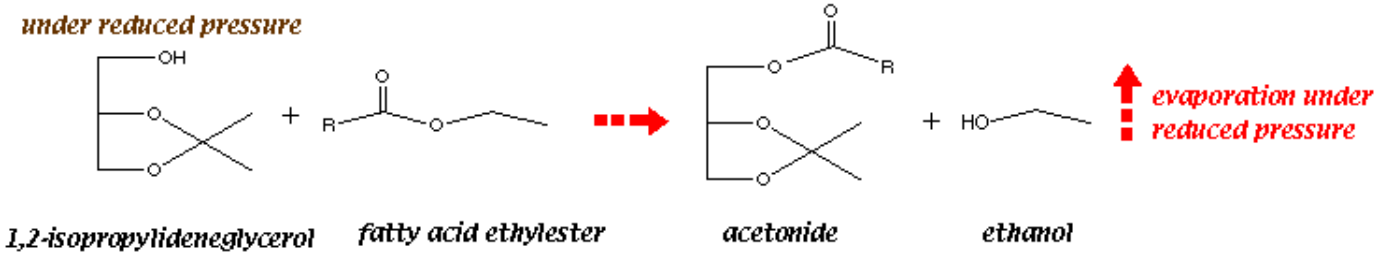

Figure 5-4-9: Synthesis of acetonide by acylation of 1,2-isopropylideneglycerol with fatty acid ethyl ester.

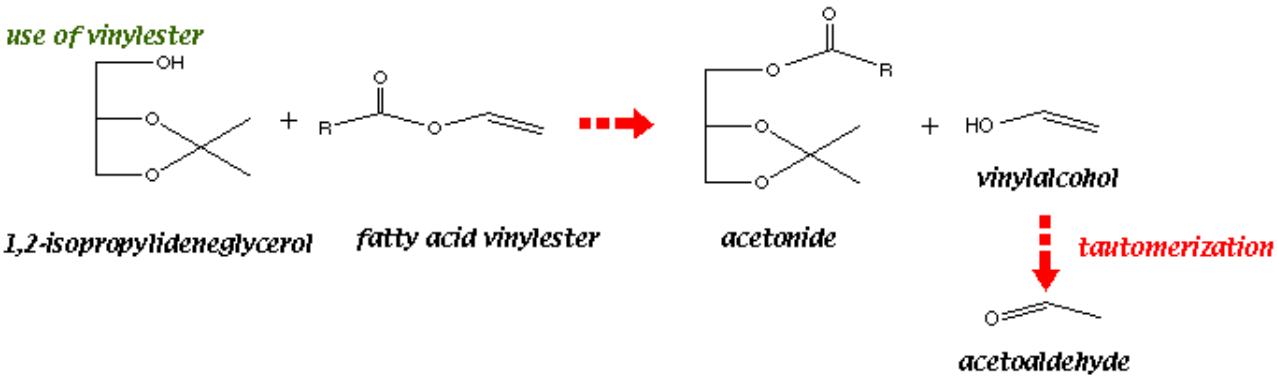

Figure 5-4-10: Synthesis of acetonide by acylation of 1,2-isopropylideneglycerol with fatty acid vinyl ester.

Use of vinylesters: For acylation of an alcohol, use of fatty acid vinylester, if available, as the acyl donor is also promising (Figure 5-4-10). The by-product is vinylalcohol, which is spontaneously isomerized by keto-enol tautomerism into acetoaldehyde, resulting in equilibrium shift.