Oil & Fat Modification

Candida cylindracea lipase – A VERSATILE ENZYME FOR HYDROLYSIS & ESTERIFICATION REACTIONS.

Over the last 5 decades, use of lipases for various industrial application has significantly increased due to the commercial availability of different type of lipases. Lipase from *Candida cylindracea* was one of the first enzymes to be used in biotechnological processes and is still one of the most widely used enzymes for biotransformation today. It is used for its hydrolysis properties for applications such as dairy fat hydrolysis (milk, cheese, butter) in the production of dairy flavours. However, extensive studies of the properties of this lipase over the last 20 years has widened its range of applications to make use of its ability to perform esterification reactions on a wide range of substrates.

HYDROLYSIS

Candida cylindracea lipase (CCL) is a robust enzyme that can be used to hydrolyse a number of substrates from dairy fats to fish oils. The different applications CCL is applied to produces different outcomes from flavour enhancement in dairy applications to increased concentration of enriched fish oil like omega-3.

Dairy fat modification

Candida cylindracea lipase is a food grade enzyme which is widely used in dairy applications for flavour generation. The hydrolysis of the triglyceride generates free fatty acids; each of them possessing a different flavour (sharp, buttery or soapy flavours for example) depending on the chain length of the fatty acid. Therefore, CCL is used for example for milk hydrolysis, accelerated cheese ripening, production of Enzyme Modified Cheese (EMC) or for butter hydrolysis. CCL from Biocatalysts (Lipomod® 034MDP) is for example used as a cost-effective lipase for the production of EMC with a mild buttery / creamy flavour.

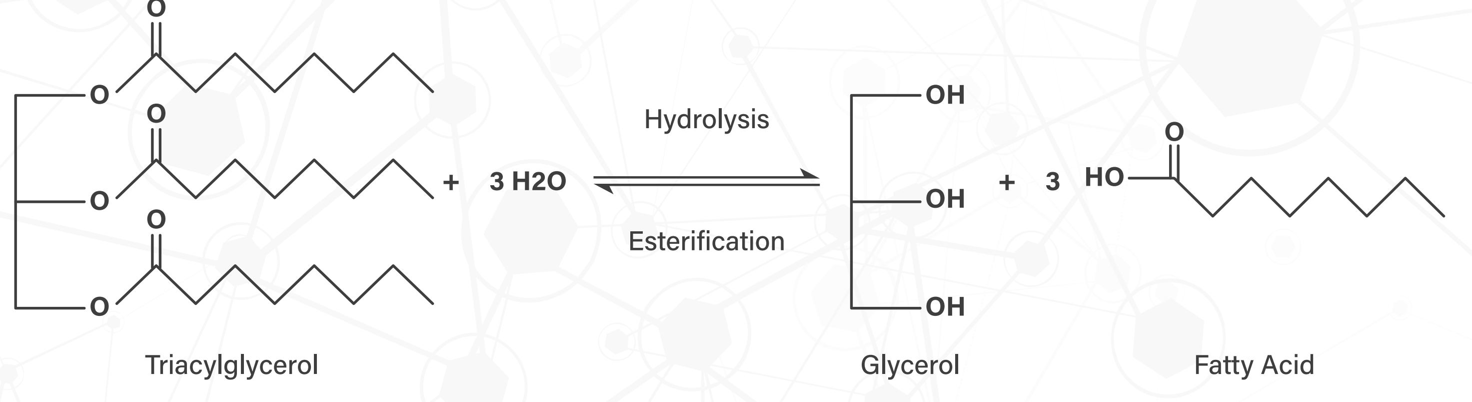

Figure 1 Lipase - Hydrolysis and esterification reactions.

Production of PUFA-enriched oils

Omega-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are recognised as beneficial fatty acids for human's health (reduction of inflammation, lower risk of chronic diseases such as heart disease, regulation of blood pressure). Fish (sardine, salmon, tuna) are the main sources of PUFAs. Lipase from *Candida cylindracea* is used to produce PUFAs-rich oil for nutritional complement; as CCL can selectively hydrolyse fatty acids other than DHA and EPA present in the oil (see Fig. 2). Literature has described the use of this lipase for concentrating omega-3 PUFA in fish oil such as tuna, salmon and sardine.

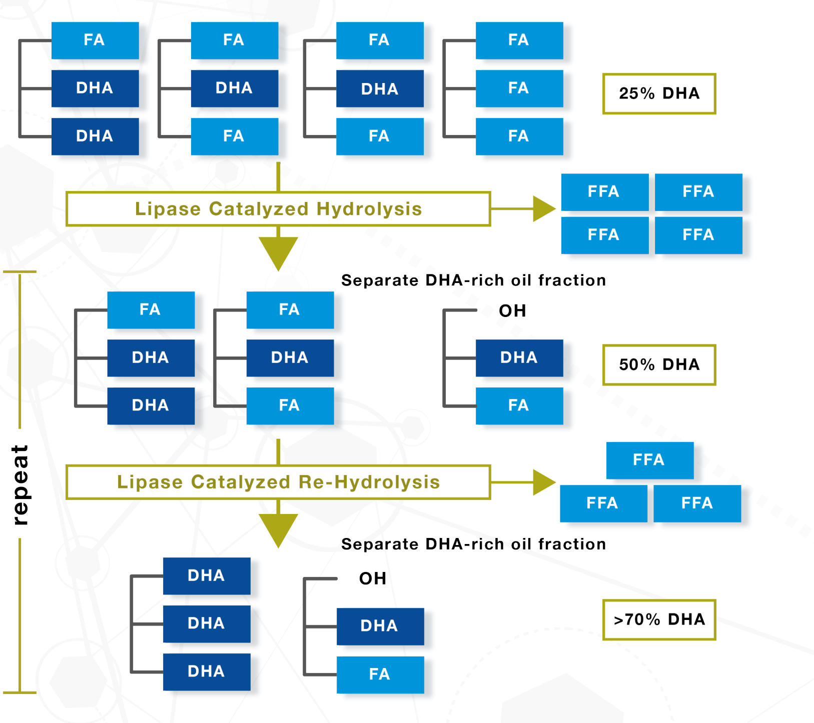

Figure 2 Production of PUFAs-rich oil with *candida cylindracea* lipase.

Esterification reactions of triglycerides with CCL

Although extensively used for hydrolysis reactions, *Candida cylindracea* lipase can also be used for esterification reactions with high regio-, stereo- and enantio-selectivity. Transesterification (reaction between a triglyceride and an alcohol) and interesterification (rearrangement of the fatty acids on the glycerol backbone of a triglyceride) can be performed using different types of fat or oils as the substrate and CCL. Figure 3 shows the substrates and resulting products from transesterification (also called alcoholysis) and interesterification reactions. Both of these reactions consist of 2 stages, with first hydrolysis of the ester bond and then esterification with an alcohol group. Lipomod® 767P is a high-strength, carrier-free CCL from Biocatalysts that can be used in esterification processes.

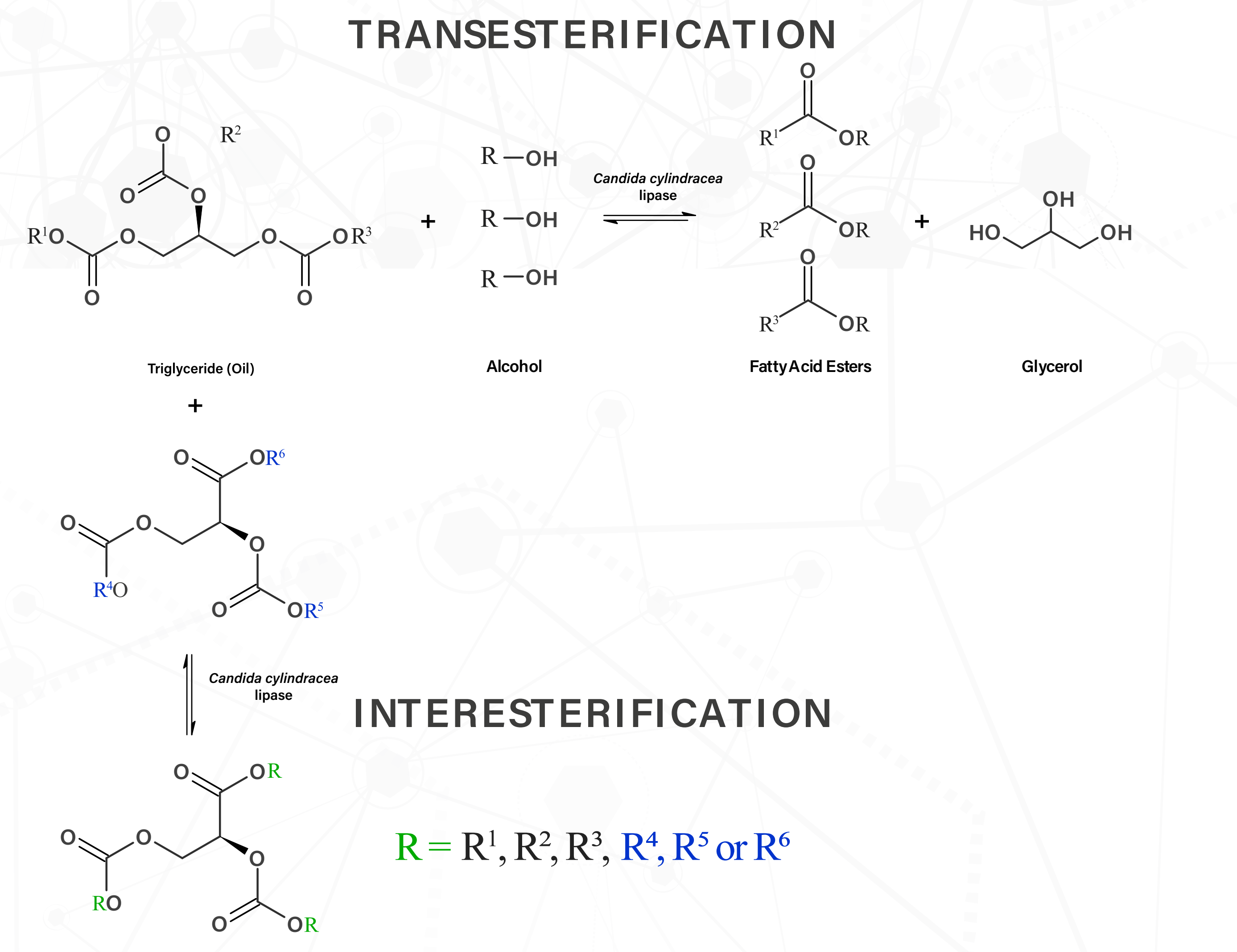

Figure 3 Transesterification and interesterification reactions

Transesterification

Biodiesel production can be done enzymatically using different sources of oil (waste oil, animal fats, vegetable oils), alcohol (usually methanol or ethanol) and a lipase . The enzymatic transesterification produces esters of the alcohol (i.e biodiesel) and glycerol as a by-product. If methanol is used, then the product of the reaction is FAME (fatty acid methyl esters).

CCL is currently not used in industrial processes for the production of biodiesel but has been shown in the literature to be able to produce different type of biodiesel. For example, CCL has been shown to be able to catalyse reaction between palmitic acid and methanol , fish oil and methanol , Eruca sativa oil and methanol and coconut oil with ethanol .

Although free lipase powder can be used for biodiesel production, immobilised *Candida cylindracea* lipase is most often a preferable alternative, in order to reduce cost in use. Immobilised *Candida cylindracea* has been shown to be able to produce biodiesel from waste cooking oil , Sunflower oil or Palm oil .

Esterification

Candida cylindracea lipase can be used for purification of fatty acid though esterification reactions. For example, Pinolenic acid (PLA) from pine nut oil can be enriched according to a two-step process involving CCL lipase-catalysed esterification and urea complexation . The first step for the preparation of highly purified PLA involved selective esterification of non-PLA fatty acids with lauryl alcohol. (Fig. 4). Under the optimum conditions of 0.1% enzyme loading, 10% additional water, and 15 °C, PLA was enriched up to 43 mol% from an initial value of 13 mol% in the pine nut oil.

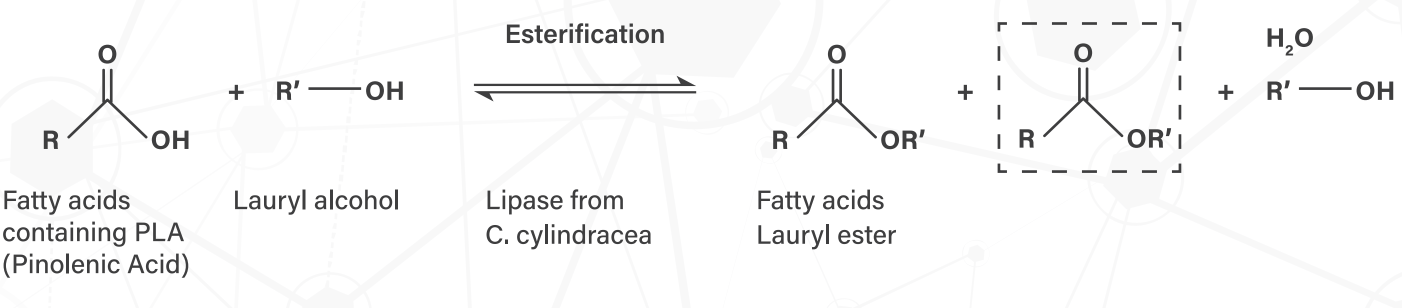

Figure 4 Production of highly purified Pinoleic acid (PLA) using CCL.

Interesterification

Enzymatic interesterification rearranges the position of fatty acids within triacylglycerols. The substrates for the reaction can be 2 different type of oils/fats or oil and free fatty acids. The redistribution of the fatty acids generates a fat with modified functionalities such as a higher melting point, texture or improved nutritional properties (reduced level of saturated fatty acids and trans fatty acids).

Although 1,3 specific lipases are mainly used for interesterification of oil (for example for the production of cocoa butter equivalent (CBE) through the enzymatic interesterification of the palm oil midfraction (POMF) with stearic acid), CCL has been shown to also perform this type of reaction. For example, interesterification between soybean oil and methyl stearate or palm oil with ethyl acetate or propyl acetate have been described with immobilised CCL.

Synthesis of intermediates

Due to its versatility, *Candida cylindracea* lipase has been described in the literature for non-food related applications; to replace chemicals for the synthesis of pharmaceutical intermediates, production of clean label emulsifiers and esterification of plant sterols for example. Compared to chemical reactions, enzymatic reactions can be performed under mild reaction conditions with higher selectivity and specificity, saving time and money and improving the viability and usability of the end products. Lipomod® 767 is a CCL with very high activity that can be used for various types of esterification.

CCL has been used for the enantioselective esterification of racemic (R,S)-ibuprofen with 1-propanol or 2-propanol (Fig. 5). The influence of reaction parameters (temperature, time, solvent) were investigated for the highest production yield of the (S) enantiomer; which is of interest for the pharmaceutical industry as it is 160 times more active than the (R) enantiomer.

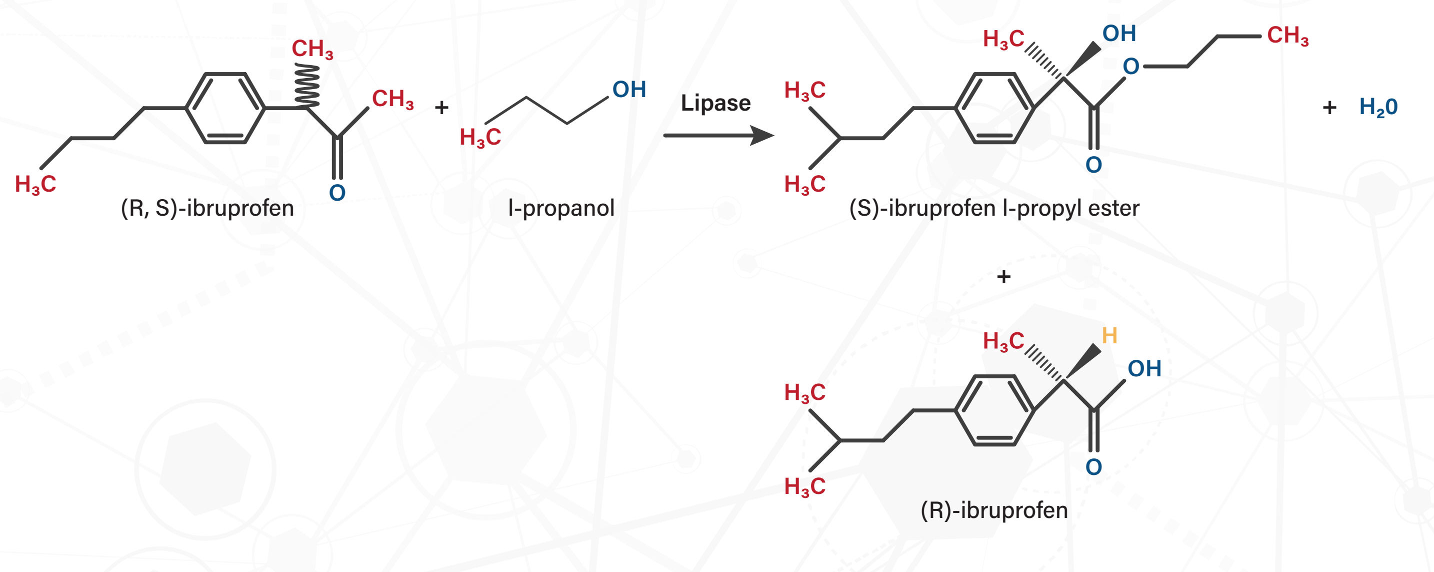

Figure 5 Enzymatic esterification of ibuprofen with *Candida cylindracea* lipase.

CCL has also been used for the esterification of glycerol with fatty acids from coconut oils (mainly free fatty acids with carbon chain length <16) or decanoic acid (Fig. 6). The glycerol-coconut fatty acid esters and glycerol-decanoic acid esters obtained showed activity as emulsifier for water in oil dispersion.

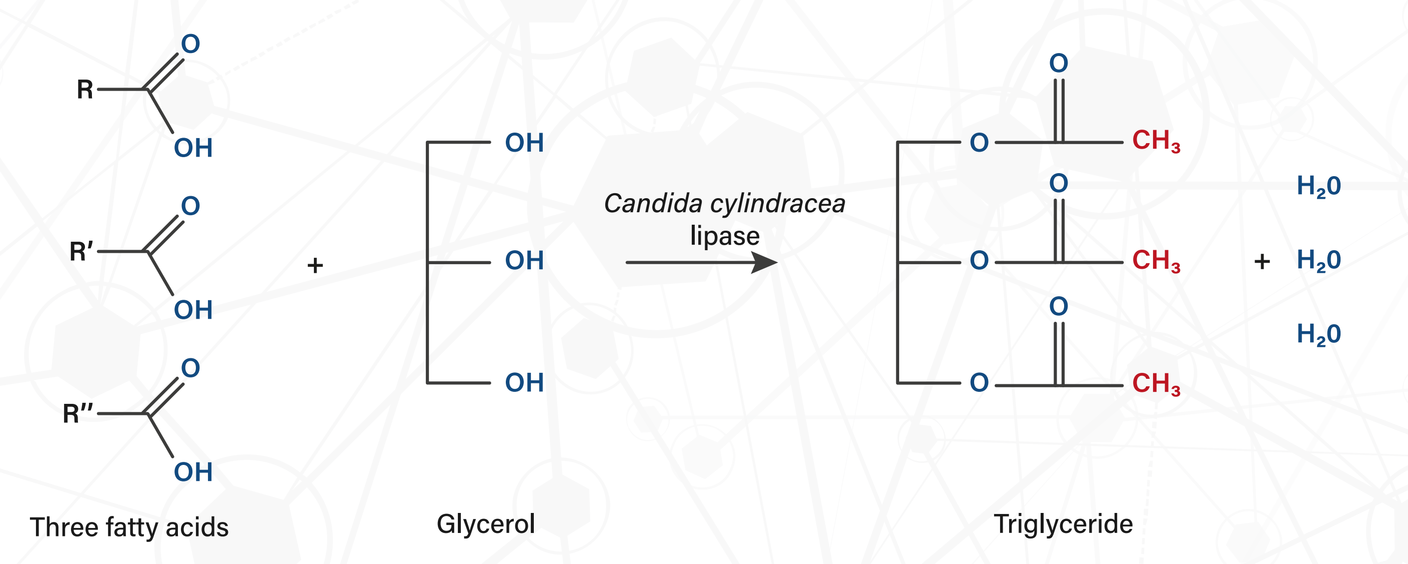

Figure 6 Enzymatic esterification of glycerol with coconut oil fatty acids.

CCL can also being used to develop novel enzymatic method for efficient synthesis of phytosterol linolenate through lipase-catalyzed transesterification of phytosterols with ethyl linolenate (Fig. 7). Phytosterols (plant sterols) and their esters have been shown to be of interest due to their potential beneficial effects on human health (cholesterol reduction for example).

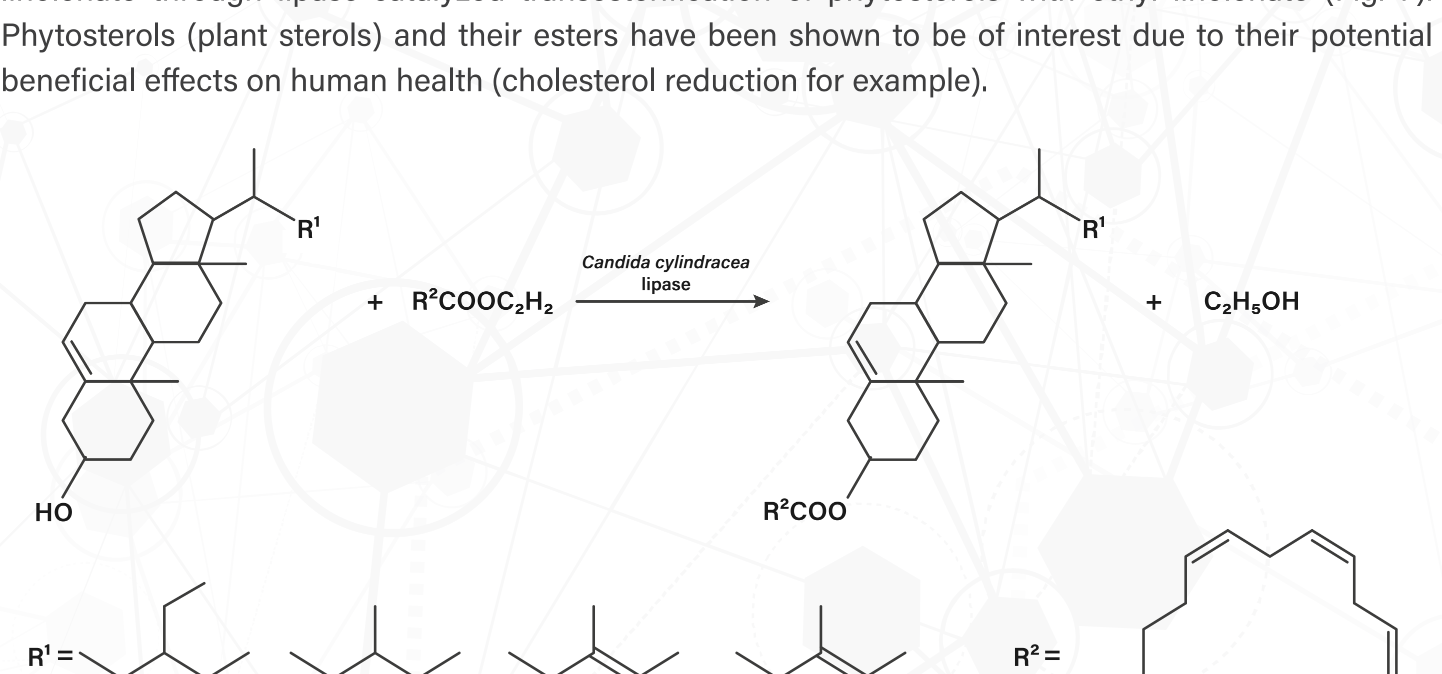

Figure 7: Enzymatic esterification of phytosterol with fatty acid ester.

CONCLUSION

The information in this paper demonstrates *Candida cylindracea* lipase's versatility as an enzyme for a wide range of applications. Its high hydrolysis efficiency makes this enzyme suitable for fat and oil hydrolysis. Biocatalysts has Lipomod® 34MDP in our product range for these types of applications. CCL can also be used in a wide range of applications for esterification reactions; due to its stereo- and regio-selectivity. The type of product produced and its corresponding yield will be dependent on the substrates used and condition parameters (time, temperature, solvent, water content) and usually will involve some optimisation work in order to obtain the highest yield. Biocatalysts offers Lipomod® 767MDP to customers looking for a lipase for esterification reactions. In some cases, use of an immobilised *Candida cylindracea* lipase is preferable in order to make the process cost effective. Biocatalysts can provide small sample of immobilised *Candida cylindracea* lipase to establish the best process parameters for your individual needs.