AIDIC *Conference Series*, Vol. 10, 2011
Editor Sauro Pierucci
DOI: 10.3303/ACOS1110034

ISBN 978-88-95608-58-7 ISSN 2036-5969

STUDY OF THE INFLUENCE OF ALCOHOLS IN THE SYNTHESIS OF SHORT CHAIN ESTERS

Maria Dolores Romero, Jose María Gómez, Beatriz Díaz-Suelto, Alicia García-Sanz*

Chemical Engineering Department, Universidad Complutense Madrid
Av. Complutense s/n, 28040, Madrid, Spain. *email: aliciagarcia@quim.ucm.es

The effect of the type of alcohol and the alcohol/acyl donor was explored on the synthesis of short chain esters by enzymatic catalysis in n-hexane. The reactions studied were esterification and transesterification. First, the effect of the alcohol chain length was investigated. The differences in primary alcohols are inappreciable, but among secondary alcohols, those with a larger hydrocarbon chain gave the best yields for esterification reactions, while the shortest ones led to get higher esterification extents in transesterification reactions. Then, the position of the –OH group was analyzed, concluding that the steric hindrance leads to a worse esterification extent. To impel reaction yield, excess alcohol was used. The results obtained validate this assumption.

1. INTRODUCTION

According to the report made by Van Beuzekom and Arundel (2009), biotechnology plays a very important role in industry, health and agriculture. The main industrial applications are the production of bulk and specialty chemicals, enzymes, plastics, biofuels, and bioremediation. Also, it is used in the development, production, and prescribing of therapeutics, in vivo diagnostics, and vaccines for humans. These applications are complemented with those in the agricultural sector, which include diagnostics, vaccines and therapeutics for animal health and genetic modification to develop improved plant and animal varieties. Esterification is the most widely used reaction in the organic chemistry industry (Otera, 2003), because it presents many applications in the natural products synthesis production ranging from lab to industrial scale.

1.1 Esterification in biotechnology

Esters of different alcohols and acids are used for a wide variety of applications in industry as flavors, emulsifiers, lubricants, and additives in cosmetics (Chand et al., 1997). Traditionally, this kind of compounds have been isolated from natural sources or produced by chemical synthesis (Karra-Châabouni et al., 2006). The chemical route often suffers from poor reaction selectivity leading to undesirable side reactions and low yields. However, commodity chemicals and specialty chemicals can be produced by biotechnological processes, in which the same chemicals can be produced in a more economical and environmentally friendly way. Besides the advantages of mild operating conditions, availability of enzymes from different microbial sources possessing specificity of action and the fact that their catalytic activity can be easily regulated are some of the profound virtues that most chemical catalysts do not possess.

1.2 Enzymes as biocatalysts

The importance of lipase-catalyzed synthesis of such esters in presence of several solvents has been reported in many publications (An et al., 2010; Chiang et al., 2003; Habulin and Knez, 2009; Leblanc et al., 1998). Esters from a variety of alcohols and acids can be synthesized by various lipases (EC 3.1.1.3), according to their specificity in aqueous medium (De Barros et al., 2010). However, there are many problems in establishing such a reaction system, the main problem being that an excess of water in the reaction mixture tends to favour hydrolysis rather than esterification (Tewari and Bunk, 2001). So, the interest to non-aqueous solvents, as organic solvents, SC-CO2 and ionic liquids, is growing due to its capability of overcoming the difficulty of

Please cite this article as: Romero M.D., Gomez J.M., Diaz-Suelto B. and Garcia-Sanz A., (2011), Study of the influence of alcohols on the synthesis of short chain esters, AIDIC Conference Series, 10, 305-314 DOI: 10.3303/ACOS1110034

dissolving hydrophobic substances and shifting the equilibrium to the formation of the desired products (Ghanem, 2007; Kragl et al., 2002; Romero et al., 2005a).
Candida antarctica lipase B is one of the most commonly used lipases. It is commercially available in both free and immobilized form. The application of lipase to synthesize esters has been studied for many years since the work of Iwai (1964).

2. MATERIALS AND METHODS

2.1 Reagents and Materials

Vinyl acetate, hexanoic acid, 2-propanol, 2-butanol, n-hexane and n-decane were from Fluka with GC purity (> 99%). Butyric acid, 1-propanol, 1-butanol, 2-hexanol and 3-hexanol were purchased from Sigma-Aldrich with at least 98% purity. The enzyme was Novozym 435® from Candida Antarctica hydrolase B, immobilized on a macro porous acrylic resin with a water content of 1-2 % w/w, and kindly provided by Novo Nordisk, Denmark.

2.2 Method for esters synthesis

Reactions in n-hexane were carried out in a shaker, equipped with a temperature controller. In all the synthesis remained constant the agitation rate (200 rpm), the atmospheric pressure and the amount of enzyme (13,8 g/mol substrate in defect). All the experiments were carried out at equimolar concentrations of reactants except when the opposite is indicated. Reaction mixture volume was 20 ml. n-Decane was used as internal standard (1% w/w). After different reaction times, 200 µl of sample were taken in order to follow the reaction. Samples were analyzed by GC.

2.3 Analytical Methods

Substrates and products concentrations were determined by Gas Chromatography with a Varian Gas Chromatograph equipped with a hydrogen flame ionization detector and a Chiraldex™ B-PM column (30 m length x 0.25 mm i.d.). Helium was used as carrier gas at a flow rate of 1 $ml \cdot min^{-1}$. In all cases the initial temperature of the column oven was 40ºC with a heating rate of $2ºC \cdot min^{-1}$ to 54ºC and after one minute of stabilization the temperature was increased up to 150ºC (at $15ºC \cdot min^{-1}$). Injector temperature was 200ºC and detector temperature was 250ºC.
All the samples were analyzed three times to determine the experimental error, which was less than 4% in all cases.

3. RESULTS AND DISCUSSION

The purpose of the present study was to investigate the effect of the type of alcohol, as well as the alcohol/acyl donor ratio, using two types of acyl donors, which let us to study also the effect of the type of reaction. The effect of the alcohol chain length was studied for primary and secondary alcohols. Also, the position of the –OH group was analyzed. Esterification extent here was defined as the amount of ester produced to initial substrate in defect (acyl donor or alcohol). The most significant findings are summarized below.

3.1 Effect of alcohol chain length: primary and secondary alcohols

In order to study the effect of the alcohol chain length esterification and transesterification reactions using butyric acid and vinyl acetate, respectively, as acyl donors were developed. The equimolar substrates concentration was 0,5 M.
Figure 1 shows the results obtained when primary alcohols are tried using butyric acid as acyl donor, while Figure 2 depicts those obtained when vinyl acetate was used as acyl donor.
The results depicted in both Figure 1 and Figure 2 do not show any noticeable influence on the esterification extent when 1-propanol, 1-butanol, 1-hexanol and 1-octanol are used. There is only a slight difference between the slopes of the largest chain alcohols (1-hexanol and 1-octanol) and those of the shortest (1-propanol and 1-butanol) when butyric acid is used as acyl donor, but not when vinyl acetate.

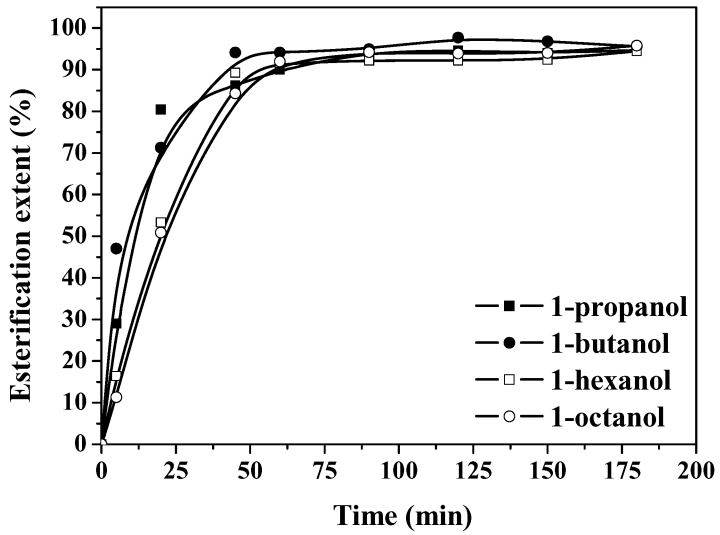

Figure 1. Esterification of butyric acid using short primary alcohols

Romero et al. (2002) studied the esterification of acetic anhydride using several alcohols (from ethanol to 1-dodecanol), one of the main conclusions obtained was that esterification rate increased when the carbon number of the alcohol grew. Butyric acid and acetic acid (acetic anhydride used as acyl donor can split up to give two acetic acid molecules) are both short chain carboxylic acids, but the inhibiting properties of acetic acid (Romero et al., 2005b) do not benefit the reaction, therefore the differences between the extents obtained in that case are clearly more marked than these obtained with butyric acid and vinyl acetate.

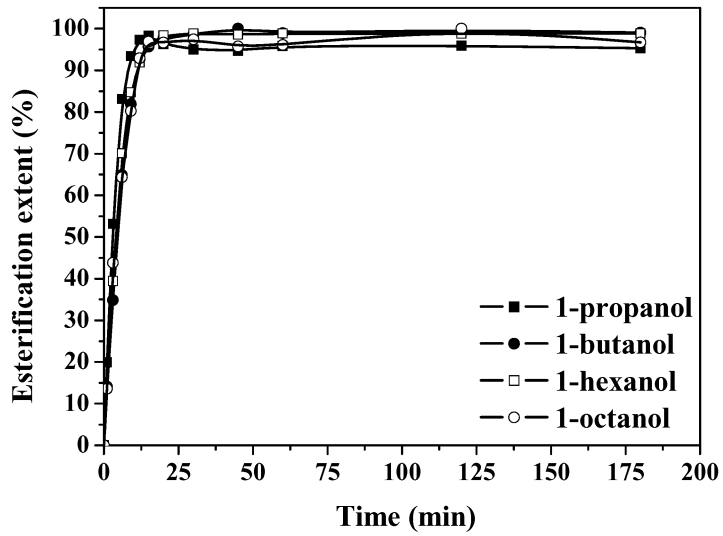

Figure 2. Transesterification of vinyl acetate using short primary alcohols

However, there are differences when secondary alcohols are used. The esterification extent obtained with 2-hexanol is much higher than with 2-butanol (Figure 3), using butyric acid as acyl donor. So, this means that large

chain alcohols lead to get a more productive esterification, with less reactive consumption, when an acid is used as acyl donor.

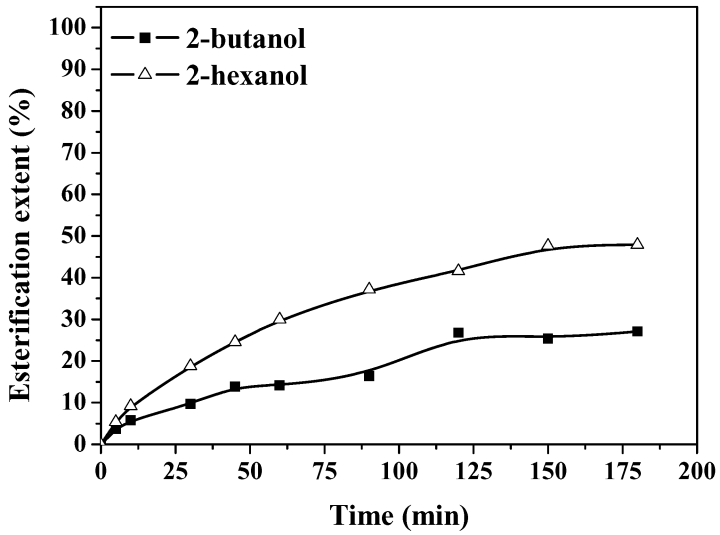

Figure 3. Esterification of butyric acid using short secondary alcohols

On the contrary, when an ester is used as acyl donor (Figure 4), and a transesterification happens, the results are different, providing the shorter alcohol higher esterifications extents at shorter times, reaching both an equilibrium at 100 min (~ 2 hours).

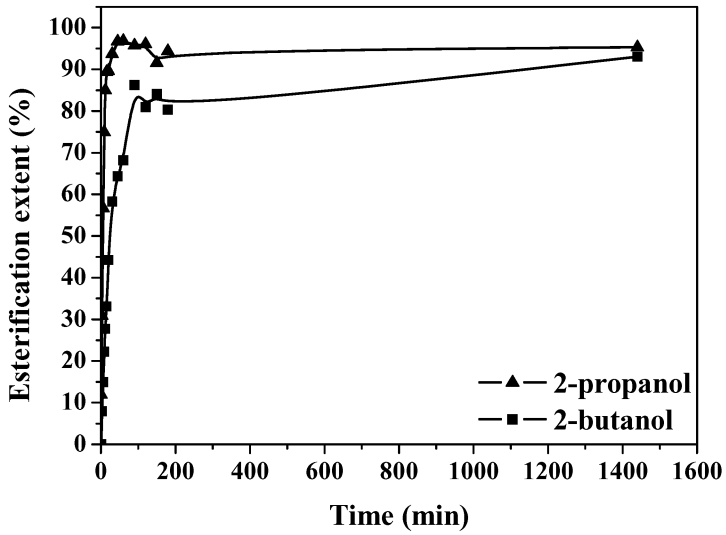

Figure 4. Transesterification of vinyl acetate using short secondary alcohols

Comparing the results in Table 1, can be concluded that vinyl acetate is highly recommended as acyl donor to get esters in a productive and fast way. Not only with secondary alcohols but also with primary ones.

Table 1: Time to reach the highest esterification extent for both types of reactions using primary and secondary alcohols (1 / 2-butanol).

		Esterification extent (%)	Time (min)
Esterification (Butyric acid)	1 – butanol	~ 95	60
	2 - butanol	~ 25	180
Transesterification (Vinyl acetate)	1 – butanol	~ 100	20
	2 - butanol	~ 80	100

3.2 Effect of the position of –OH group

In order to study the effect of the position of the –OH group, three different alcohols (1-hexanol, 2-hexanol and 3-hexanol) were employed for the butyric acid esterification. The transesterification reaction was carried using 1-butanol and 2-butanol and vinyl acetate as acyl donor. The equimolar substrates concentration was 0,5 M.

The results are showed in Figures 5 and 6. As expected, the esterification extent obtained is much higher when 1-hexanol and 1-butanol are used, due to the less steric hindrance. The worst extent is obtained with 3-hexanol because of the high steric resistance that the hydrocarbon chains, which surrounds the –OH group, create.

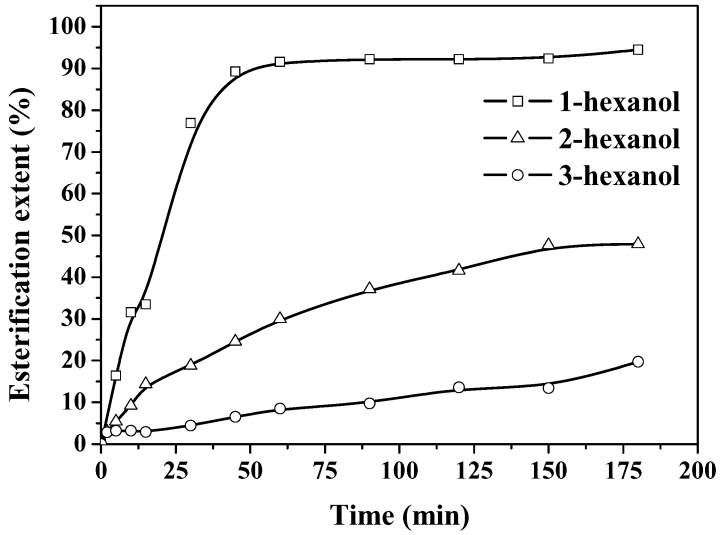

Figure 5. Esterification of butyric acid using 1 / 2 / 3- hexanol alcohols

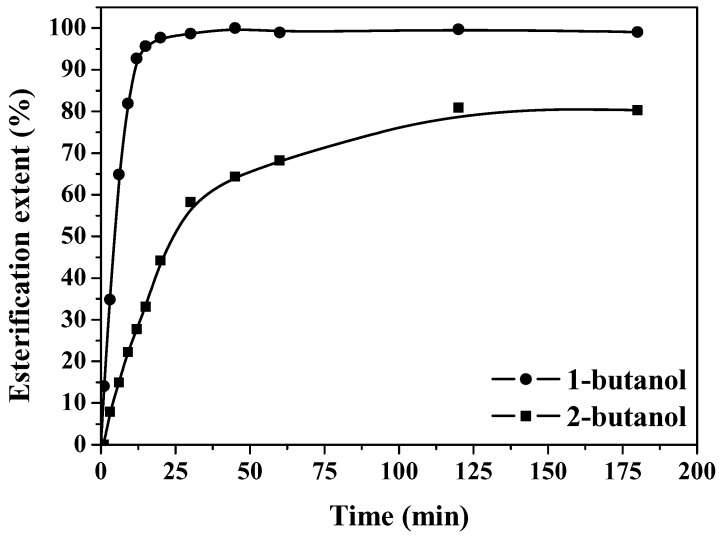

Figure 6. Transesterification of vinyl acetate using 1 / 2-butanol alcohols

3.3 Effect of alcohol/acyl donor ratio

The effect of alcohol/acyl donor ratio was also studied. Hexanoic acid was used as acyl donor and 2-butanol as alcohol. A set of experiments were made using a fixed concentration of acyl donor (0,5 M) and several alcohol/acyl donor ratios (1; 1,6; 2; 3; 8). The results are depicted in Figure 7 and it shows that an excess of alcohol leads to obtain better results.

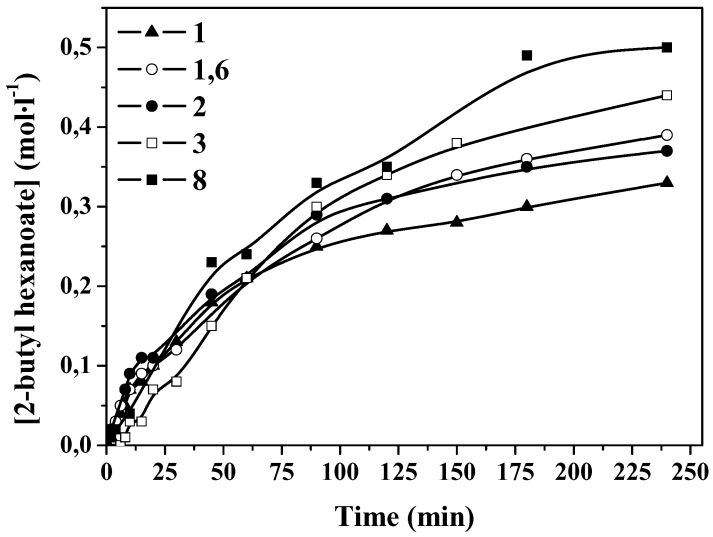

Figure 7. Esterification of hexanoic acid and 2-butanol at several alcohol/acyl donor ratios

The effect of excess alcohol on ester yield is shown in Table 2. As alcohol to acyl donor ratio increased so did ester yield, because the equilibrium of the reaction was pushed toward the product formation as the acyl acceptor (alcohol) free concentration raised.

Table 2: Results obtained at a fixed time of 90 minutes for the esterification of hexanoic acid with 2-butanol. Different alcohol/acyl donor ratios used.

Alcohol/Acyl donor ratio	[2-butyl hexanoate] ($mol \cdot l^{-1}$)	Esterification extent (%)
1	0,25	49,10
1,6	0,26	51,84
2	0,29	57,00
3	0,3	59,40
8	0,33	66,40

Transesterification reactions were carried using vinyl acetate as acyl donor and 2-butanol as alcohol. A set of experiments were made using a fixed concentration of alcohol (0,5 M) and several alcohol/acyl donor ratios (0,13; 0,33; 0,50; 0,63; 1). The results are depicted in Figure 8 and it shows that an equimolar alcohol/acyl donor ratio leads to obtain better results.

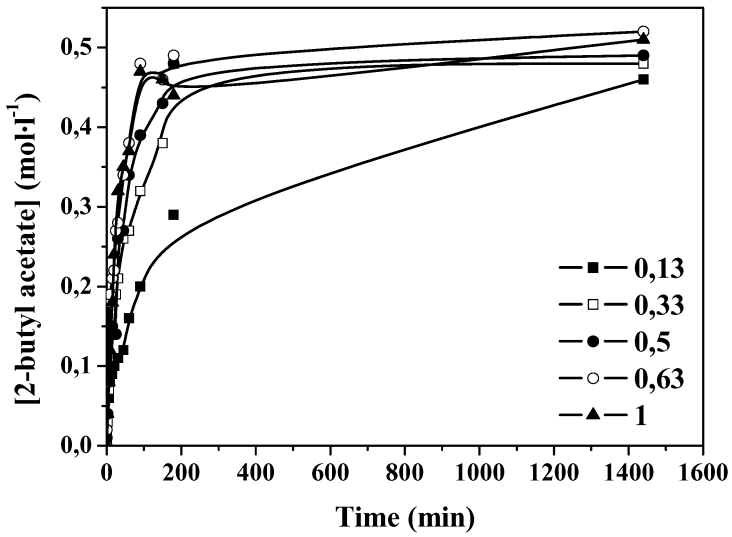

Figure 8. Transesterification of vinyl acetate and 2-butanol at several alcohol/acyl donor ratios

The effect of excess alcohol on ester yield is shown in Table 3. As alcohol to acyl donor ratio increase so did ester yield, because the equilibrium of the reaction was pushed toward the product formation as the acyl donor free concentration raised, this can be easily noticed at short reaction times. For long reaction times (> 24 hours), the reaction is completed for all the alcohol/acyl donor ratios tried, and thus no influence is observed.

Table 3: Results obtained at a fixed time of 90 minutes for the transesterification of vinyl acetate with 2-butanol. Different alcohol/acyl donor ratios used.

Alcohol/Acyl donor ratio	[2-butyl acetate] ($mol \cdot l^{-1}$)	Esterification extent (%)
0,13	0,20	40,40
0,33	0,32	63,40
0,5	0,39	78,60
0,63	0,48	96,80
1	0,47	94,00

As mentioned previously, the acyl transfer is affected by the concentration of free alcohol available. Hari Krishna et al. (2001) studied the synthesis of isoamyl acetate, propionate and isobutyrate, obtaining the same effect of the alcohol/acyl donor molar ratio. Also Romero et al. (2005b) found the same effect in the synthesis of isoamyl acetate. Higher concentration of alcohol (acyl acceptor) usually leads to higher levels of equilibrium conversions due to the availability of excess nucleophile for acyl transfer. This effect indicates competitive nature of alcohol and acid binding, the initial preferential binding being that of the acid.

4. CONCLUSIONS

The synthesis of short chain esters using lipases as biocatalysts has been developed. The use of larger chain alcohols brings higher esterification extents in esterification reactions, this effect is more marked when secondary alcohols are used. However, in transesterification reactions larger chain alcohols do not lead to get higher esterification extents. The steric hindrance effect is clearly noticed in esterification and transesterification reactions, reaching higher exterification extents for 1-hexanol and 1-butanol, respectively. The optimal alcohol/acyl donor ratio is the one that fulfils with the requirements of a high esterification yield and less chemical consumption. According to that, a higher alcohol/acyl donor ratio benefits the esterification yield but does not meet the saving approach. This effect is noticed in both types of reactions, not only when the alcohol initial concentration increase, but also when the acyl donor.

5. REFERENCES

An I., Onyeozili E.N. and Maleczka Jr. R.E., 2010, Enzymatic kinetic resolution of α-hydroxysilanes, Tetrahedron: Asymmetry, 21, 527–534.

Chand S., Adlercreutz P. and Mattiasson B., 1997, Lipase-catalyzed esterification of ethylene glycol to mono- and diesters. The effect of process parameters on reaction rate and product distribution, Enzyme Microbial Technology, 20, 102-106.

Chiang W.D., Chang S.W. and Shieh C.J., 2003, Studies on the optimized lipase catalyzed biosynthesis of cis-3-hexen-1-yl acetate in n-hexane, Process Biochemistry, 38, 1193–1199.

De Barros D.P.C., Lemos F., Fonseca L.P. and Cabral J.M.S., 2010, Kinetic cutinase-catalyzed esterification of caproic acid in organic solvent system, Journal of Molecular Catalysis B: Enzymatic, 66, 285–293.

Ghanem, A., 2007, Trends in lipase-catalyzed asymmetric access to enantiomerically pure/enriched compounds, Tetrahedron 63, 8, 1721–1754.

Habulin M. and Knez Ž., 2009, Optimization of (R,S)-1-phenylethanol kinetic resolution over Candida antarctica lipase B in ionic liquids, Journal of Molecular Catalysis B: Enzymatic, 58, 24–28.

Hari Krishna S., Divakar S., Prapulla S.G. and Karanth N.G., 2001, Enzymatic synthesis of isoamyl acetate using immobilized lipase from Rhizomucor miehei, Journal of Biotechnology, 87, 3, 193-201.

Iwai M., Tsujisaka M. and Fukumoto J., 1964, Studies on lipase II. Hydrolytic and esterifying actions of crystalline lipase of Aspergillus niger, Journal of General and Applied Microbiology, 10, 13-22.

Karra-Châabouni M., Ghamgui H., Bezzine S., Rekik A. Gargouri Y., 2006, Production of flavour esters by immobilized Staphylococcus simulans lipase in a solvent-free system, Process Biochemistry 41, 1692–1698.

Kragl U., Eckstein M. and Kaftzik N., 2002, Enzyme catalysis in ionic liquids, Current Opinion in Biotechnology, 13, 565–571.

Leblanc D., Morin A., Gu D., Zhang X.M., Bisaillon J.G., Paquet M. and Dubeau H., 1998, Short chain fatty acid esters synthesis by commercial lipases in low water systems and by resting microbial cells in aqueous medium, Biotechnology Letters., 20, 1127–1131.

Otera J., 2003, Esterification: Methods, Reactions, and Applications. Wiley-VCH, Weinheim, Deutschland.

Romero M.D., Calvo L., Alba C. and Luis M., 2002, Enzymatic synthesis of flavor esters in n-hexane and supercritical carbon dioxide, Contribution to the 9th Mediterranean Congress of Chemical Engineering.

Romero M.D., Calvo L., Alba C., Habulin M., Primožič M. and Knez Z., 2005a, Enzymatic synthesis of isoamyl acetate with immobilized Candida antarctica lipase in supercritical carbon dioxide, Journal of Supercritical Fluids, 33, 77–84.

Romero M.D., Calvo L., Alba C., Daneshfar A. and Ghaziaskar H.S., 2005b, Enzymatic synthesis of isoamyl acetate with immobilized Candida antarctica lipase in n-hexane, Enzyme and Microbial Technology, 37, 42-48.

Tewari Y.B. and Bunk D.M., 2001, Thermodynamics of the lipase-catalyzed esterification of glycerol and n-octanoic acid in organic solvents and in the neat reaction mixture, Journal of Molecular Catalysis B: Enzymatic, 15, 135–145.

Van Beuzekom B. and Arundel A., 2009, OECD Biotechnology Statistics 2009, Paris.