Effect of acid and alcohol chain length on the enzymatic synthesis of short-chain esters in supercritical carbon dioxide.

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The enzymatic synthesis of short-chain esters was studied in supercritical carbon dioxide ($SCCO_2$). Novozyme 435® was used to catalyze these syntheses due to its demonstrated efficiency. Esterifications were achieved for different chain length of acids and alcohols, in order to study the effect of acid and alcohol chain lengh on the yield of esters. Substrates were fed to the reactor in equimolar ratio. Reaction conditions used were 313 K, 140 bar and 13,8 g enzyme/mol alcohol. Results in $SCCO_2$ showed that reaction rate increases with the chain length of the acid, becoming even faster than reactions in n-hexane. This can be explained because the use of CO_2 avoids diffusional problems. Acetic and propionic acid presented a different behaviour because of their low solubility in $SCCO_2$ due to its high polarity. In this case, yields in $SCCO_2$ were similar to those obtained in organic solvent. A similar trend was observed for the alcohol chain length, although a maximum yield was observed for hexanol.

INTRODUCTION

Most of short-chain esters are characterized by their pleasant flavour, so they have lots of applications in different industries like food, cosmetic and pharmaceutical. Nowadays there is a tendency towards the labelled "natural products" that has led to an increasing interest towards biotechnology. Lipases are being successfully used for the synthesis of these esters [1, 2]. These reactions must be carried out in non-aqueous solvents, like organic solvents or supercritical fluids (SCF), in order to avoid hydrolysis reactions. However, the use of toxic organic solvents for food and pharmaceutical products is being progressively restricted. SCF have properties between those of gases and liquids: they have a good solvent capacity like liquids, and show high diffusivities like gases. One of the most used supercritical fluids is carbon dioxide [3, 4], since CO_2 is gas at room conditions and can be easily removed without leaving any residues in the product. In addition, CO_2 is non toxic and cheap. When using $SCCO_2$, the downstream processing might be much simpler than in the case of liquid solvents, because the product fractionation can be achieved by operating a cascade of depressurisations and temperature changes [3, 5, 6].

The purpose of this work was to synthesize esters by esterification of several carboxylic acids with alcohols of different chain length. These reactions were carried out in $SCCO_2$, using the immobilized lipase Novozym 435® from *Candida antartica*. The effect of the substrate chain length was studied using different acids at initial equimolar substrates concentrations.

MATERIALS AND METHODS

Materials

All chemicals: acetic acid (Merck), propionic and butyric acids (Aldrich), caproic acid (Fluka), propanol and butanol (Merck), hexanol (Aldrich) and octanol (Lancaster), were of analytical grade. The enzyme was Novozyme 435® from *Candida Antarctica* lipase B, immobilized on a macroporous acrylic resin with a water content of 1-2% w/w, and it was kindly provided by Novo Nordisk, Denmark. Pressurized liquid carbon dioxide at about 5MPa, >99.998% purity, was supplied by Air Liquide.

Synthesis method of esters in supercritical carbon dioxide

Synthesis of esters was carried out in a high-pressure stirred-batch reactor of a 0.05 L of capacity, equipped with agitation, temperature and pressure reading devices, inlet and outlet connections and a rupture disk set at 25 MPa (Figure 1). Temperature was controlled by a heating jacket. The CO_2 was fed by a membrane pump with a coupled cooling system in order to guarantee the liquid state.
Initially, the reaction mixture formed by the acid and the alcohol was introduced in the reactor. Then the desired amount of Novozyme 435® was added. After the reactor was closed, CO_2 was pumped into the reactor up to the working pressure (140 bar). The system was kept at constant temperature (40ºC) during the reaction. Agitation rate was fixed at 500 rpm. Samples were taken from the reactor through the sampling valve in a tube, during the reaction. The taken samples were solved in n-hexane and analysed by gas chromatography. The pressure of the system was set to the operational conditions by pumping fresh CO_2. This amount did not significantly affect the composition of the reaction mixture.

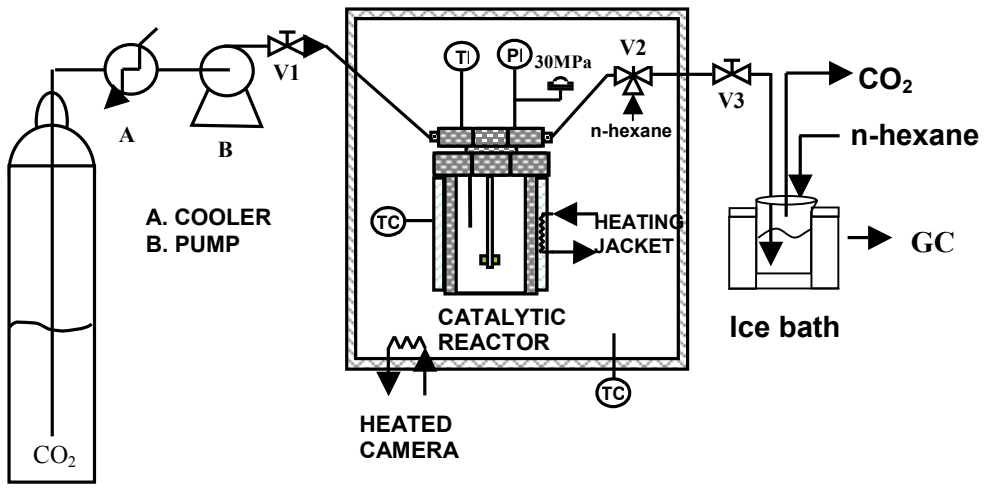


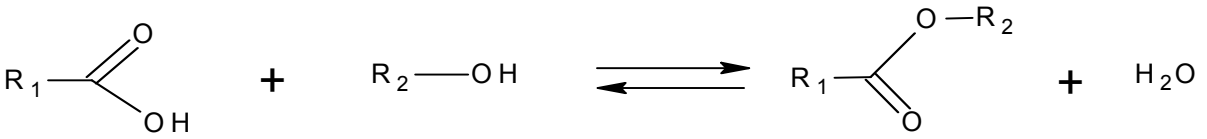
Figure 1: Experimental high-pressure stirred-batch reactor for the enzymatic synthesis of esters in supercritical CO_2

Analytical methods

Products and reactants concentrations were determined by Gas Chromatography with a Varian Gas Chromatograph equipped with a hydrogen flame ionization detector and a TR-FFAP column (60 m length x 0.32 mm i.d.). Helium was used as carrier gas. Column oven temperature was 100ºC. Injector and detector temperatures were 200ºC and 250ºC respectively.

RESULTS AND DISCUSSION

The efficiency of Novozyme 435® for enzymatic synthesis of flavour esters has been studied in previous works [7, 8, 9, 10]. Several esterification reactions have been carried out with this enzyme in supercritical CO_2. Four different hexyl esters and four different butyrates have been synthesized in order to study the effect of the chain length of the acid as acyl donor and the alcohol. The general reaction scheme is:



Carboxylic acid Alcohol Ester Water

The reported percentage of esterification was defined as amount of ester produced. Calculations based on alcohol or acid conversion were found to be in good agreement. Average uncertainty in reported data was ± 7%.

Effect of acid chain length

Different carboxylic acids have been tested: acetic, propionic, butyric and caproic. The effect of the chain length of these acids on esterification reaction is shown in Figure 2.

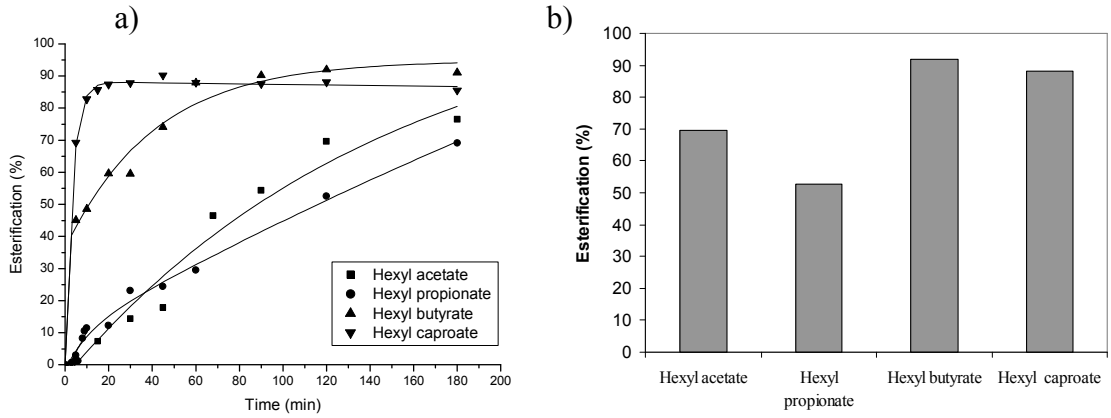


Figure 2: Effect of acid chain length on percentage of esterification a), esterification percentage at 2h of reaction b).

Reaction rate increased with the chain length of the carboxylic acid. A great difference was observed between acetic and propionic acids, and the longest chain acids. This behaviour can be explained by the low solubility of these acids in carbon dioxide due to their high polarity [11]. Several studies in organic solvents had also demonstrated that acetic and propionic acids present strong inhibition on enzyme activity [12-14]. Yields of butyric and caproic acids reached their highest value at two hours of reaction and they were similar, while acetic and propionic acid did not reach their maximum value at this time.
The use of carbon dioxide avoided diffusional problems when compared with n-hexane as solvent in the synthesis of hexyl esters, as for example in the case of hexyl butyrate and hexyl caproate (Figure 3). Although the reaction rates were higher in supercritical carbon dioxide, final yields were similar.

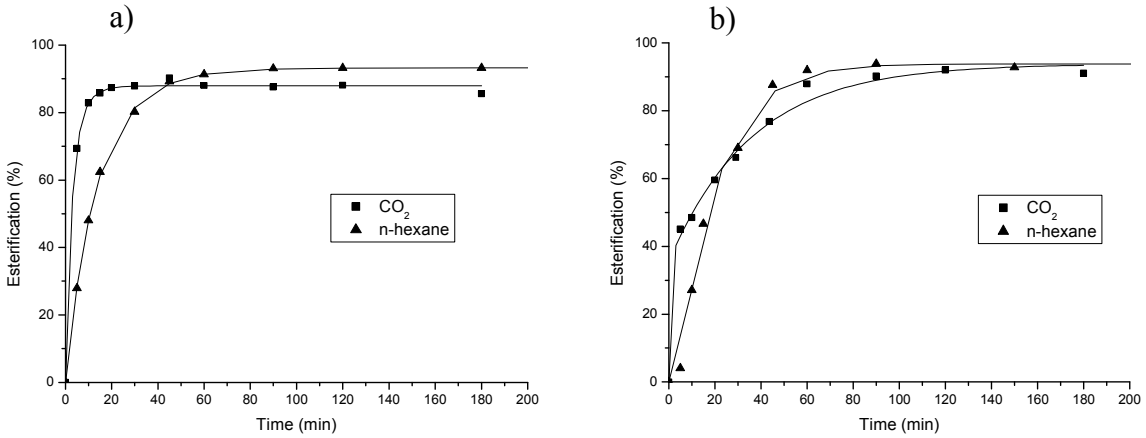


Figure 3: Comparison of the effect of acid chain length between $SCCO_2$ and n-hexane as solvents for the esterification of hexyl caproate a) and hexyl butyrate b)

Effect of alcohol chain length

Several alcohols have been tested in the synthesis of butyrates: propanol, butanol, hexanol and octanol. The effect of the chain length of these alcohols is shown in Figure 4.

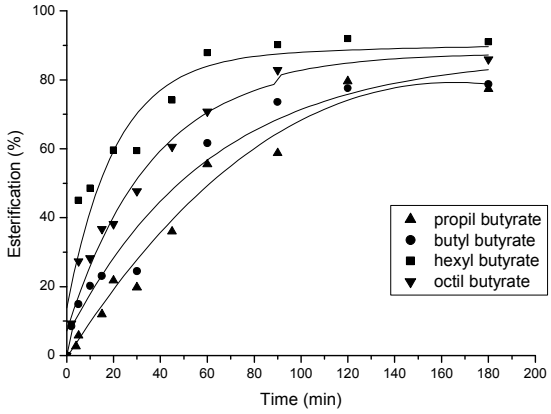


Figure 4: Effect of alcohol chain length on the synthesis of butyrate esters

As the alcohol chain length increased, so did the percentage of esterification and the reaction rate. However, it seems to be a maximum when hexanol was used in the synthesis of butyrates. With all the alcohols tested, the reactions reached their higher yield at two hours. Similar behaviour was also observed with soluble lipase of *Mucor miehi, Candida rugosa* and *Rhizopus arrhizus* [15]. On the other hand, other enzymes as soluble *Aspergillus* or immobilized *Mucor sp* lipases [12, 15] showed a decrease in yields with longer chains of alcohols.

CONCLUSIONS

Novozyme 435® was found a very efficient catalyst for the synthesis of flavour esters in supercritical carbon dioxide. This enzyme seemed to present better affinity for the longest acids and alcohols in $SCCO_2$ (yields around 90%). In general, reaction rates increased with the chain length of the carboxylic acid. Acetic and propionic acids showed great inhibition on enzyme activity, and they presented also bad solubility in carbon dioxide due to their high polartity.
Carbon dioxide avoided diffusional problems in the case of the longest acid tested for the synthesis of hexyl esters.
The percentage of esterification increased as the chain of alcohol became longer, although it seemed to reach a maximum when hexanol was used. This fact could be due to the different affinity of the enzyme of each alcohol.
Supercritical carbon dioxide can be considered as a good solvent in the synthesis of flavour esters, obtaining reasonably high yields and reaction rates. It could be a good alternative to organic solvents for the synthesis of food and health products, where solvent residues are forcefully restricted. Carbon dioxide also facilitates downstream separations.

Acknowledgements

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