P-108 **Biosynthesis and encapsulation of aroma compounds in miniemulsion**

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INTRODUCTION AND OBJECTIVES

Synthesis processes based on lipase and esterase enzymes, as alternative catalysts to chemical route, produce fatty acid esters and other similar flavors. Esterification is frequently encountered in the fine chemistry industry in view of the diverse applications of esters in a variety of industries such as intermediates, pharmaceuticals, fine chemicals, fragrance and flavour chemicals, plasticizers, solvents, food, etc. (Schrader J. et al., 2004).
As a lipolytic enzyme, lipase and cutinase have been presented as a versatile enzyme showing several interesting properties for applications in industrial products and processes. Potential use of cutinase is developed recently in the reaction of transesterification of fat or oils and esterification reactions of alcohol with organic acids at low water activities (Carvalho et al., 1999).
The aim of this study is to create an efficient enzymatic process for the production of aroma ester in a miniemulsion system. Miniemulsions are heterophase systems where small droplets with high stability in a continuous aqueous phase are created by using high shear force such as ultrasonication in order to obtain droplets in the size range of 50 nm to 500 nm (Landfester, 2006). These miniemulsions have proven to be a suitable and convenient reaction system for acid and lipase catalyzed reactions (de Barros et al., 2010).
In the miniemulsion system, the water produced during esterification in the dehydrative droplet (Manabe et al., 2002) is expelled to the continous aqueous phase, favoring the product formation inside of droplet. The other very important factors that favor the formation of esters are the huge interfacial area, readily available for interfacial catalysis, and the stability of the miniemulsion throughout the reaction, which is increased with increasing hydrophobicity of oil phase (Aschenbrenner et al., 2009).
The esterification reaction was performed with three different enzymes, lyophilized *Fusarium solani pisi* cutinase produced by *Sacharomyces cerevisiae* SU50; commercial Lipase type VII from Candida rugosa, and Amano lipase PS from *Burkholderia cepacia*. The effect of the chain length of the alcohol and of the acids and influence of substrates concentration were investigated. A miniemulsion system shows a excellent potential as a system for the synthesis of flavour esters (e.g. for hexyl octanoate 96.2% with Amano lipase PS) and better stability for higher substrates concentration comparing with organic media system.

MATERIAL AND METHODS

Commercial lipase type VII from *Candida rugosa* (SIGMA) (1.150 U/mg against olive oil as substrate), Amano lipase PS from *Pseudomonas cepacia* (ALDRICH) (30,000 U/g against olive oil as substrate) and cutinase *Fusarium solani pisi* (170 U/mg against p-nitrophenyl butyrate as substrate) (Almeida et al., 2004) were used for enzymatic esterification.
Reagents including hexanoic, heptanoic, octanoic (99%, SIGMA, Germany), decanoic and oleic acid (FLUKA,Germany) and ethanol abs. and hexanol (Merck, Germany) were used for esters synthesis while hexadecane 99% (SIGMA,Germany) and Lutensol® AT-50 (donation, BASF, Germany) were used to obtain miniemulsions. NaOH (Merck, Germany) as reagent and tetrahydrofuran (THF) (Merck, Germany) as solvent for titration were used. Paranitrophenyl butyrate (SIGMA, Germany) used was of analytical grade. All other chemicals used were of analytical grade.

Enzymatic esterification reaction in miniemulsion

Relative weight amounts of substrates (16.5%), hexadecane (0.7%), water(81.6%) and Lutensol (1.6%) constitute the esterification reaction media and were homogenized by magnetic stirring for 1 h. The two phase system was ultrasonicated for 120 s in pulses of 5 s and pauses of 10 s at 70% amplitude (Branson sonifier W450 digital, tip size 6.5 mm) with ice cooling.

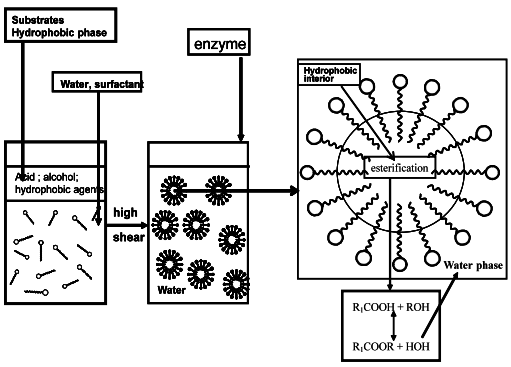

Fig. 1. Principle of esterification in miniemulsion

20 ml of the miniemulsion was added into the reaction vessel containing the appropriate amount of enzyme (5 mg of enzyme preparation per ml of reaction mixture)

Fig. 1. The esterification reactions were performed in a thermostated incubator (Advanced ChemTec PLS 4x4, 400 rpm) at 40 °C unless otherwise stated. Samples were withdrawn periodically (20 µl) using a needle and dissolved in DMSO-d_6 (0.5 ml) prior to ^{1}H-NMR analysis.

RESULTS AND DISCUSSION

The ability of the lipase C*andida rugosa* type VII and *Fusarium solani pisi*. cutinase to catalyze the synthesis of ethyl- and hexyl esters were compared with the esterification activity of Amano lipase PS in the miniemulsion system.

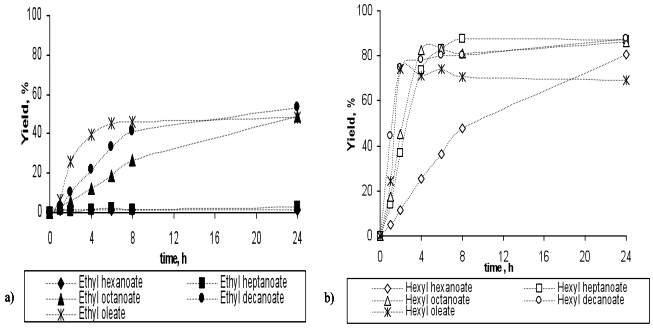

Fig. 2. Effect of chain length of acids and alcohols

The effect of the chain lengths of the acids on the synthesis of ethyl and hexyl esters catalyzed by Amano lipase PS in miniemulsion was assessed by using various linear carboxylic acids with increasing chain length from C_6 to C_{18}, i.e. hexanoic, heptanoic, octanoic, decanoic and oleic acid.

The time courses of the esterification reactions are shown in Fig. 2. The equilibrium for ethyl oleate (C_{18}) was reached after 6 h with an ester yield of 46% and after 24 h for octanoate (C_8) (49%) and decanoate (C_{10}) (54%). The esterification yield of ethyl hexanoate (C_6) and ethyl heptanoate (C_7) was insignificant (Fig. 2a). For hexyl esters the equilibrium was attained after about 4 h for oleate (C_{18}) (71%), decanoate (C_{10}) (78%) and octanoate (C_8) (83%) and about 8 h for heptanoate (C_7) (88%) and more than 24 h for hexanoate (C_6) (81%).

A consecutive alcohol supply during the enzymatic production of alkyl esters in miniemulsion system improves the esterification yield, especially in the case of hexyl esters. The enhancement of the ester yields in fed-batch operational mode was of 19%, 11%, and nearly of 6% for hexyl octanoate, hexyl hexanoate and ethyl octanoate, respectively, which were significant values according to the experimental error.

For *C. rugosa* lipase the maximum ester yields of 79, 67 and 62% were obtained for hexyl -octanoate, -decanoate, and -oleate, respectively (Fig. 3).

A similar esterification yield profile was observed with *F.s. pisi* cutinase showing also a maximum yield of 76% for hexyl decanoate and then slightly decreasing for hexyl oleate but yet to a surprisingly high value of 69%, contrarily to the previously reported cutinase selectivity

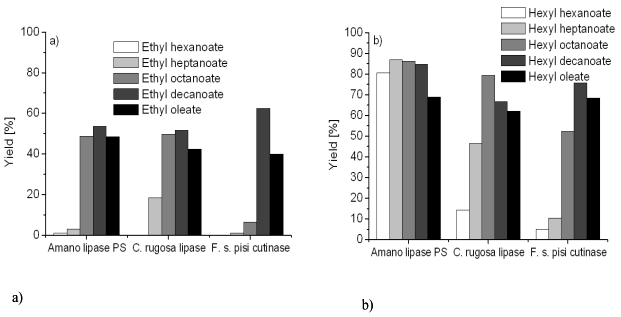

Fig. 3 Esterification yields for ethyl (a) and hexyl (b) esters at 24 h for three different enzymes

CONCLUSIONS

To summarize, the present study shows the high potential and advantages of using water-based miniemulsions for the biosynthesis of ethyl- and hexyl acid esters (acid chain length from C_6 to C_{18}) by two lipases (Amano Lipase PS and *Candida rugosa* lipase) and *Fusarium solani pisi* cutinase. A high stability of miniemulsions, with high esterification yields was especially attained with hexyl esters (e.g. hexyl heptanoate 88%, Amano Lipase PS). However, it was possible to obtain stable miniemulsions even with ethanol and acids of C_8, C_{10} and C_{18} chain length which proves that it is possible to carry out esterification reaction in miniemulsions with, at least one highly water soluble substrate. Amano lipase PS from *Burkholderia cepacia* showed good selectivity for the synthesis of hexyl esters. Cutinase show selectivity for longer chain of acid. Longer chain of acids and alcohol stabilize mininemulsion and provide better conversion to ester. Fed-batch operational mode with consecutive supply of alcohol showed as a good strategy to improve esterification degree

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