

Biosynthesis of ethyl caproate and other short alkyl esters catalyzed by cutinase in organic solvent

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Fatty acid esters belong to a large group of flavor compounds that are very important gradients of natural aromas largely used in various food, beverage, cosmetic and pharmaceutical industries. Fatty acid esters are commonly produced by chemical synthesis of an alcohol with an organic acid in the presence of an acid catalyst or by extraction from natural sources. Natural aromas obtained by the extraction are very expensive due to low concentration in the natural product and low extraction yield. The chemical synthesis of these fatty acid esters is cheap but, in general, they are characterized by low purity and not classified like as natural products.

Biotechnology processes based on enzyme synthesis, as alternative catalysts to chemical route, produce also those fatty acid esters and other similar flavors. These enzymatic processes have technological and economical interesting characteristics as use mild reaction condition, synthesize flavors with high quality and purity due to the regio- and stereo-specificity of enzymes. The high purity of these flavors allows to be classified as *natural* by food regulatory agencies and better public acceptance as ingredients for food industry in relation to those synthesized by chemical processes.

Cutinase are a group of enzymes that can be considered as a link between esterase and lipase. In recent years, the esterolytic activity of cutinase has been largely exploited and several applications in different industrial field have been presented. In the reaction of esterification cutinase showed selectivity for short-chain fatty acid esters.

The goals of these studies are to determine and optimize the conditions of the synthesis of fruit flavours by cutinase in organic media, in particular isooctane an organic solvent recognized as safety ingredient in food and beverage industrial processes. Enzyme stability in esterification reactions in non-conventional media and efficiency in the synthesis of short chain alkyl esters such as valerate, caproate and octanoate were evaluated. In order to avoid substrate inhibition and to improve the esterification yield a discontinuous feed pulse of substrates strategy (pulse fed-batch mode of substrate supply) was tested which achieved concentration of ethyl caproate of 1.5M in reaction media. According these experiments this type of cutinase from *S. cerevisiae* strain shows excellent properties for synthesis of short alkyl esters and eventual industrial applications is only dependent to find the right encapsulation strategy to obtain an economical enzyme immobilization form.