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ESTERIFICATION AND IN SILICO ANALYSIS OF LIPASE CATALYZED SYNTHESIS OF FLAVOUR ESTER

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PENGESAHAN

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LIST OF ABBREVIATIONS

%	Percent
℃	Degree Celsius
h	Hour
s	Second
min	Minutes
µl	Micro litre
kDa	Kilo Dalton
IR	Infrared
nm	Nanometer
rpm	Revolutions per minute
LB	Luria Bertani
IPTG	Isopropyl-beta-D-thiogalactopyranoside
APS	Ammonium persulfate
BSA	Bovine serum albumin
Ala	Alanine
Val	Valine
Leu	Leucine
Asp	Aspartate
Ser	Serine
His	Histidine

ABSTRACT

Flavour esters can be made artificially from the reaction between alcohols and carboxylic acids and this reaction is called esterification. Flavour refers to the combination of taste, touch and smell. Eighty percent (80%) of taste comes from smell. Esters have the ability to produce artificial fruit aromas. In this work, strawberry (ethyl hexanoate), banana (isoamyl acetate) and pear (propyl acetate) aromas are produced by esterification process, catalysed by the cold adapted Lipase AMS8. Esterification using cold adapted enzymes are not well studied. The use of cold active lipase would provide more advantages and economic benefits to the industry. Analysis using FT-IR and GC-MS showed the presence of esters at IR spectrum of ethyl hexanoate, isoamyl acetate and propyl acetate at 1704.30, 1706.25 and 1701.26 cm^{-1} respectively and the mass spectrum 144, 130 and 102 m/z respectively. Esterification reaction in the presence of toluene as an organic solvent showed higher ester conversions (ethyl hexanoate: 53.13%, isoamyl acetate: 50.77% and propyl acetate: 20.92%). In the absence of organic solvents, ester conversion has a lower yield (ethyl hexanoate: 29.45%, isoamyl acetate: 22.43% and propyl acetate: 10.05%). To support the experimental data, computational analysis was done via experimented docking between Lipase AMS8 and ligands which were toluene, hexanoic acid and ethanol. It showed multiple interactions between the lid and active sites of Lipase AMS8.

ABSTRAK

Perisa ester boleh dibuat secara buatan oleh tindak balas antara alkohol dan asid karboksilik dan tindak balas ini dipanggil pengesteran. Perisa merujuk kepada gabungan rasa, sentuhan dan bau. Lapan puluh peratus (80%) daripada rasa datang dari bau. Ester mempunyai keupayaan untuk menghasilkan bau buah-buahan. Dalam kajian ini, bau strawberi (etil hexanoat), pisang (isoamil asetat) dan lai (propil asetat) telah dihasilkan oleh proses pengesteran dengan menggunakan pemangkin Lipase AMS8 tahan sejuk. Kajian pengesteran dengan menggunakan enzim tahan sejuk masih kurang. Penggunaan lipase aktif sejuk memberi banyak kelebihan dan faedah ekonomi kepada industri. Analisis dengan menggunakan FT-IR dan GC-MS telah menunjukkan kehadiran ester pada spectrum IR etil hexanoat, isoamil asetat dan propil asetat di 1704.30, 1706.25 dan 1701.26 cm^{-1} dan spektrum jisim adalah masing-masing 144, 130 dan 102 m/z. Tindak balas pengesteran dengan kehadiran toluena sebagai pelarut organic menunjukkan penukaran kepada ester adalah lebih tinggi (etil hexanoat: 53.13%, isoamil asetat: 50.77% dan propil asetat: 20.92%). Jika tiada pelarut organik, penukaran kepada ester adalah lebih rendah (etil hexanoat: 29.45%, isoamil asetat: 22.43% dan propil asetat: 10.05%). Analisis komputer telah dilakukan melalui eksperimen percampuran(*docking*) antara Lipase AMS8 dan ligan iaitu toluena, asid hexanoik dan etanol dan ianya telah menunjukkan pelbagai interaksi antara penutup dan tapak aktif Lipase AMS8 dengan ligan.

CHAPTER 1

INTRODUCTION

Esterification is the process of forming ester bonds R'COOR'' from the reaction between carboxylic acids and alcohols in the presence of catalysts. The process is usually applied in food, cosmetic and pharmaceutical industries because esters have the ability to give off artificial fruit aromas to soft drinks, chewing gums, deodorizers and food flavourings. Methyl butyrate for example is an ester with the fruity odour of pineapple, apple and strawberry (Garlapati and Banerjee, 2013). This reaction can be carried out in and out of organic solvents, but for enzymatic catalysis, they are usually best produced in organic solvents (Zaks and Klibanov, 1988). In pharmaceutical industries, sulphuric acid (H_2SO_4), hydrogen fluoride (HF) and phosphoric acid (H_3PO_4) are usually used for acid catalysis reactions (Leng *et a*l., 2009). In cosmetic and food industries, lipase-catalysed reactions are usually used in place of chemical synthesis. An example is in the production of sugar fatty acid esters in ice cream, soup, and shampoo because it provides more advantages (Šabeder *et a*l., 2006). Nowadays, many studies have been made on lipase-catalysed reactions, for example, the lipase from *Candida antarctica* is able to produce fatty acid fructose esters (Šabeder *et al*., 2006). The application of lipase in esterification processes as a catalyst has become widely used and studied in the past decade due to the usefulness of organic esters in biotechnology and chemical industries. Many researchers are also interested to study on the factors that have the ability to influence the production of high yield esters, for example, the concentration of enzymes and substrates, free-solvent systems or in organic solvents and the water content among various microbial lipases (Stergiou *et al*., 2013).

A few cold active lipase enzymes and most lipases involved in esterification work best at medium to high temperatures. Cold active enzymes provide more advantages to commercial industries, for example, in the food industry as they prevent food from spoilage and maintain the original nutritional value and flavour. They also give economic benefits due to their relatively low energy consumption in the process to inactivate the enzymes after reaction (Cavicchioli *et al*., 2011). In spite of that, there are still very few studies using cold active lipases in food processing.

Recently, a cold active lipase (Lipase AMS8) from Antartic *Pseudomonas* was isolated and expressed in *E. coli* (Ali *et al*., 2013). The enzyme was shown to work in organic solvents. The current work describes the potential of the cold active lipase to synthesize flavour esters and its molecular interactions via computational approach. To achieve this, the following objectives were outlined as listed below.

1) To synthesize esters with fruit aroma using Lipase AMS8 as a catalyst.
2) To identify and characterize flavour ester conversion.
3) To analyse the protein solvent interaction by using computational method

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