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Synthesis of Food Flavors by Enzymatic Esterification Process

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Abstract: ***Flavors and fragrances play an important role in food, pharmaceutical and cosmetic industries. This has led to the development of nature identical artificial flavoring compounds which can be used in different products to compensate the loss or boost the flavor. Although the natural flavor may contain nearly a hundred different compounds, but certain single esters resembles the natural odors and are often used in the food industry as artificial flavors and fragrances. Most of the flavor compounds are produced by traditional methods as chemical synthesis or extraction from natural sources. Recent interest for "natural" products led the flavor industry to seek new methods like biocatalytic flavor synthesis, to obtain flavor compounds naturally. These reactions can be carried out under mild operating conditions with high specificity and reduced side reactions. High purity flavor compounds can be produced without expensive separation techniques. In the present study, (CALB) Candida antartica lipase B has been used for the production of different flavoring such as geranyl acetate (Rose), citronellyl acetate (Lemon), isoamyl acetate (Banana). The results indicate that up to 79 % conversion could be obtained under the specified parameters. The process can be suitably scaled up for commercial production of food flavors.***

Keywords: CALB, Esterification, Food flavor, Lipase enzyme, Eco-friendly

1. Introduction

Enzyme technology offers a very promising option for natural flavor biosynthesis. A number of enzymes catalyze the production of aroma-related compounds from precursor molecules. Currently, most of the flavor compounds are produced by traditional methods as chemical synthesis or extraction from natural sources. Chemically production flavor esters are very common but not eco-friendly in nature. The biotransformation of these compounds is potential of considerable interest for application in the food flavor industry. Lipases are amongst most versatile enzymes and have a broad variety of industrial applications due to the multiplicity of reaction they catalyze. Versatility of lipase leads to multiple industrial application in food and flavor making, pharmaceutical, synthesis of carbohydrate esters, amines and amides, biodetergent and recently cosmetics and perfumery[1]. However, esterification of short chain fatty acids and alcohols has not received much attention [2]

Nowadays, many researchers and industries have switched to biocatalytic flavor synthesis due to consumer's inclination towards natural flavors over chemical ones. These reactions use mild operating conditions, have high specificity with reduced side reactions, and produce high purity flavor compounds by avoiding the expensive separation techniques [3]

Among different enzymes, lipase is the most widely used for the flavor development. Lipases were employed for esterification in organic solvents to produce flavor esters such as Isoamyl acetate [4], [5], Isoamyl butyrate [6], Geranyl acetate [7], citronellyl acetate [8].

There are different flavors produced earlier by enzyme lipase, etc method like, Lipase mediated synthesis of flavor esters under solvent-free conditions (in which the reaction medium involves a reactant itself (i.e., an alcohol) as a solvent) has significant importance in different food and pharmaceutical industries due to the avoidance of toxic solvent and elimination of its recovery during the operation [9].

Lipase catalyzed production of flavor esters by esterification reactions is influenced by a number of esterification variables such as molarity of alcohol, reaction time, addition of water, temperature, agitation speed, and amount of immobilized enzyme.

Several researchers reported the application of immobilized lipases for the flavor ester synthesis. Lipases were employed for esterification in organic solvent to produce flavor esters such as Isoamyl acetate [10],[11], Isoamyl butyrate [12], Geranyl acetate [13], citronellyl acetate [8], octyl acetate [14], and methyl butyrate [15].Akoh and Yee studied the lipase catalyzed esterification of primary terpene alcohols with vinyl esters in organic media as a solvent.

Many works were performed by using immobilized lipases and solvent-free conditions for the synthesis of flavor esters to overcome the problems associated with free enzyme separation and solvent toxicity. Immobilized lipase from C. rugosa and porcine pancreatic lipase were employed for the synthesis of isoamyl acetate (banana flavor), ethyl valerate (green apple flavor), and butyl acetate (pineapple flavor) in n-hexane [16]. Several authors assessed the immobilized lipases for esterification ability to produce various flavor esters [17], [18].

Solvent-free synthesis of ethyl oleate reported by Foresti et al. [19], results in a 78.6% conversion in 7 h using Candida antarctica B lipase adsorbed on polypropylene powder. In another study Ye et al. [20] synthesized saccharide fatty acid esters in solvent-free conditions and reported 88% yield of fructose oleate.

Based on the present demand and inclination of customers towards natural flavors, the present study has intended to synthesize the flavor esters, namely, methyl butyrate and octyl

acetate, through immobilized lipase mediated esterification under solvent-free conditions.

2. Objectives of Flavor Production by Lipase Enzyme

1. High yield at low cost.
2. Reusability of enzyme.
3. Make commercially important product.
4. No side effect of alcohol and any essential oil, chemicals or solvent.

3. Materials and Method

3.1. **Enzyme**: Immobilized enzyme from Candida antarctica, Provided by FERMANTA BIOTECH (specific activity 10000((Propyllaurate unit) (PLU)/gram, dry weight: water content 1- 2% w/w).

3.2.Chemicals: Standard Terpineol,Linalool, Citronellal, Geranial are procured from Gogia chemicals New Delhi,Acetic Acid, Butyric Acid, Phenyl Acetic Acid,Isoamyl Alcohol from MERK, Benzoic Acid, Tiglic Acid from CDH supplier New Delhi.

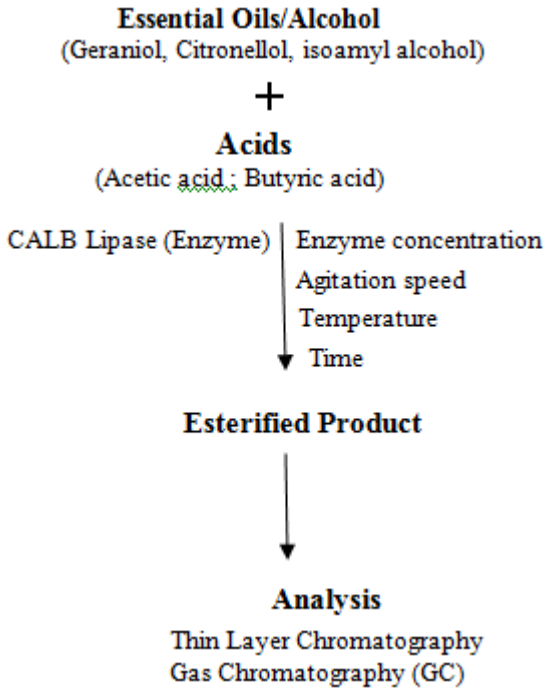
3.3. Standard Esters For Analysis:
Terpinyl Acetate, Geranyl Acetate, Isoamyl Acetate, Citronenyl Acetate, Isoamyl Butyrate, 3 are obtained from Gogia fragrances New Delhi.

3.4. Instrument: Shaking incubator (LABTech), Chromatography (Nucon 5765), Weighing balance (Mattler Toledo)(RSI-2005RL).

4. Reaction Setup for Esterification

Ester synthesis was carried out in flask containing essential oils/ isoamyl alcohol in acetic acid to a total volume of 20-25 ml, where acid act as an acyl donor. The reaction was initiated by the addition (g of enzyme per ml of reaction mixture) of immobilized lipase. Samples were placed in an orbital shaker along with the respective controls (samples with no enzyme). Esterification reactions were carried out at various temperature and shaking speed. All the experiments were performed in triplicate and the results were reported as the mean ± standard deviation.

4.1. Steps for Esterification Reaction



5. Result and Discussions

Process optimization was done with the help of biocatalyst, by optimizing the Geranyl acetate ester formation from geraniol and acetic acid using enzyme lipase 435 which is best known from the review for the terpene alcohol. Different studies were carried out and data obtained is reported subsequently.

5.1. Time dependence on ester formation

Ester formation were found to increase from 20 hours to the 24 hours, after that there was a gradual decrease in the ester formation with increase in time and finally least ester formation was noticed at time interval of 72 hour.

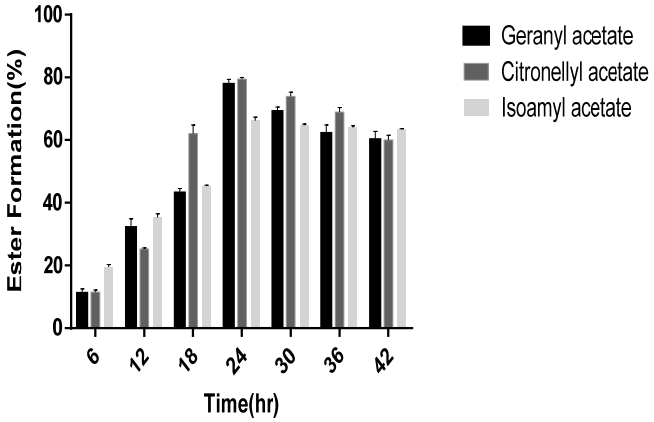


Figure 1: Effect of time on the synthesis of ester formation

5.2 Temperature dependence on ester formation

The lipase activity was noticed to increase up to the 30^0C, then there was a gradual decrease in the activity of lipase with respect to ester formation and minimum amount of ester formed at the temperature at 70^0C, because at this temperature

enzyme active site got denatured and was no more available to recognize substrate.

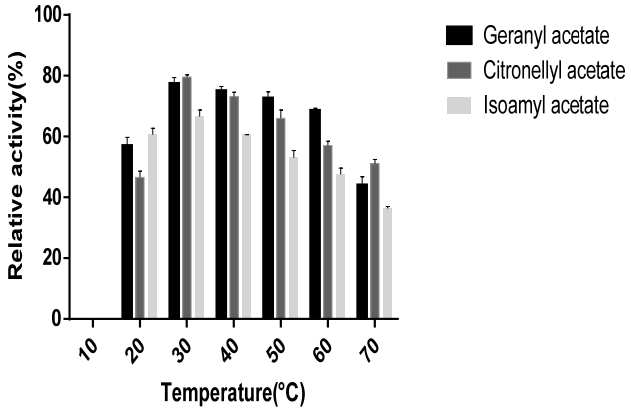


Figure 2: Effect of Temperature on the synthesis of ester formation

5.3 Effect of enzyme concentration on Ester production

Here, three different enzyme concentration were taken (5%, 10%, 15%), initially rate of ester formation increased with increase in enzyme concentration for all three percentage. Highest conversion rate was obtained with the 10% at 24 hour time interval i.e. nearly 75-80%.

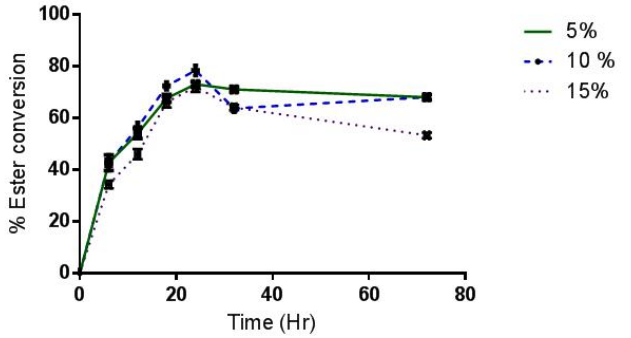


Figure 3: Effect of Enzyme Concentration on the synthesis of ester formation

5.4. Effect of substrate concentration on ester formation

The effect of substrate concentration on ester formation was carried out by using four different molar concentration (0.4,0.8,1.0,2.0).There was gradual increase with increase in molar concentration of substrate up to 1.0 molar concentration, after that there was rapid decrease on ester formation.

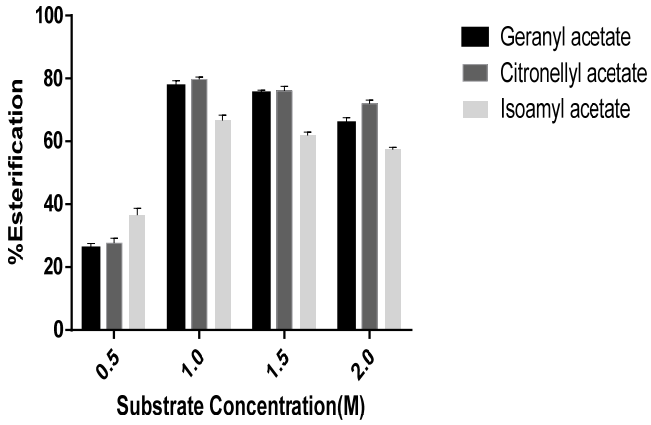


Figure 4: Effect of Substrate Concentration on the synthesis of ester formation

6. GAS Chromatography Analysis for the Formation of Esters

The sample and standard was analyzed using an Nucon 5765 gas chromatograph equipped with a flame ionization detector (Nucon Engineers, Delhi, India), with fused silica capillary column BPX-70, 60m x 0.25 mm x 0.25 µm (SGE, India). The column temperature was programmed to increase from 180°C to 240°C at 4°C/min. The detector and injector temperature was set at 240 °C and 230 °C respectively. The carrier gas used was Nitrogen (40 psi) at a flow rate of 45.0 mL/min and air and hydrogen were used at flow rates 30 mL/min and 300 mL/min respectively. The sample injection volume was 1.0µl with a split flow of 60 mL/min.

(I). Showing retention time and area percentage of different esters. (GC results)

S.N	Ester name (concentration)	Retention Time	Enzyme conc. (%)	Molar conc.	Ester %
1.	Geranyl Acetate	4.93	10	1:1	76.457
2.	Geranyl Acetate	4.82	10	1:4	25.9096
3.	Citronellyl Acetate	4.61	10	1:1	79.1682
4.	Citronellyl Acetate	4.65	10	1:4	26.529
5.	Isoamyl acetate	8.6	10	1:1	65.402
6.	Isoamyl acetate	8.6	10	1:4	49.201

7. Conclusion

Our study shows that efficient synthesis of Geranyl acetate, Citronenyl acetate, and Isoamyl acetate is possible by lipase catalysis without organic solvents containing high

concentrations of substrate up to 1.0 M. Substrate concentration has a significant contribution in enhancing both rates and yields giving high conversions (around 79%) even at high acid concentrations. The high operational stability at optimum conditions also indicates the efficiency of the Process. Using immobilize lipase from Candida antartica showing best result is also reported in the literature. However, the results indicate that the maximum yield was obtained at temperature 30^0C, optimum speed for shaking is 200 rpm, with 10% enzyme concentration which is applicable for all 3 esters. In this process, the enzyme can be reused a number of times making the process cost effective. The results obtained can be used for scaling up the process at pilot or higher level.

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