Biotechnological complex based on lipase and β-cyclodextrin on hydrolysis of acylglycerides in plant oils and fats

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INTRODUCTION

In recent years, fatty stains (butter, oil, solid fats, sauces with fats) are very resistant to treatment and cleaning from different surfaces. Combination of surfactants isn't capable to remove plant oils and fats fully. For cleaning purpose, there are natural biocatalysts – thermophilic enzymes and oligosaccharides – with high efficiency, selectivity and low environmental impact. **Lipases** (EC 3.1.1.3) are hydrolases that break down long chain fatty acid esters of glycerol at the water-oil interface.

A completely new approach based on combining lipase with natural β-cyclodextrines as an additive was suggested. **β-cyclodextrins** (β-CD) are cyclic oligosaccharides that were attracted attention for enhance stability and efficiency of enzymes. CDs can form various inclusion complexes with esters and lipids and act as phase transfer additive in water-oil interface reactions. In present study, the effects of β-CD on enzymatic hydrolysis of acylglycerides by thermophilic lipase were investigated by modern methods.

AIM OF STUDY

The present study aimed to evaluate in vitro interaction of compounds and enzymatic activity of a complex based on thermophilic lipase and β-CD for hydrolysis of acylglycerides in plant oils and fats.

MATERIALS AND METHODS

Lipase (Lipex® Evity® 200 L from Novozymes A/S)
β-cyclodextrins (Zhongbao Chemicals Co., Limited)

1. Lipase Assay Activity

It was performed according to the method described in [3]. 5 g of glyceryl oleate (substrate) was emulsified in 95 mL of distilled water with 2% polyvinyl alcohol, homogenized for 6 minutes. 1 ml of solution containing lipase (40-80 g/L) and β-CD (0-49.94 mg/mL) was added to 5 mL of substrate and diluted to 10 mL with phosphate buffer (pH 7,0-7,4). Conditions: 25±2*C for 15-180 minutes. Ethanol (15 mL) was added to finish the reaction. After all, mixtures was titrated with NaOH (50 mM) using phenolphthalein. The rate of hydrolysis (%) was calculated using formulas:

C – the concertation of NaOH (0.05 mol/L)
M – the sample weight (g)
V – the NaOH solution volume for titration (mL)
AV – the acid value (mg NaOH/g)
SV – the saponification value (mg NaOH/g)

$$AV = 39.99 * C * M/V$$

$$X\% = \frac{AV_1 - AV_0}{SV - AV} \times 100\%$$

2. Ultraviolet (UV) Spectroscopy Analysis

The UV spectrum of the homogenous dispersion of lipase (1.67 mg/ml) and β-CD (0-4.54 mg/ml) was measured by a Specord 250 Plus ultraviolet spectrophotometer (190-340 nm).

3. Fluorescence Spectroscopy Analysis

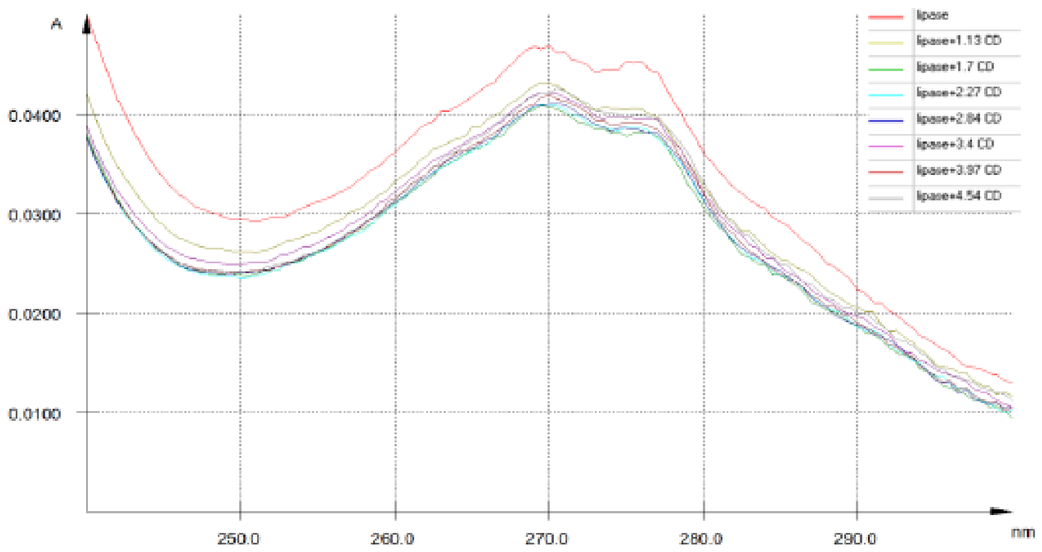
The fluorescence spectrum was measured with Fluorolog-3 fluorescence spectrophotometer at 280 nm (excitation) and 300-500 nm (emission). Lipase (1.67 mg/ml) and β-CD (0-4.54 mg/ml) were dissolved into Tris-HCl buffer (0.05 mol/L, pH 7.7).

4. Transmission Electron Microscopy Analysis

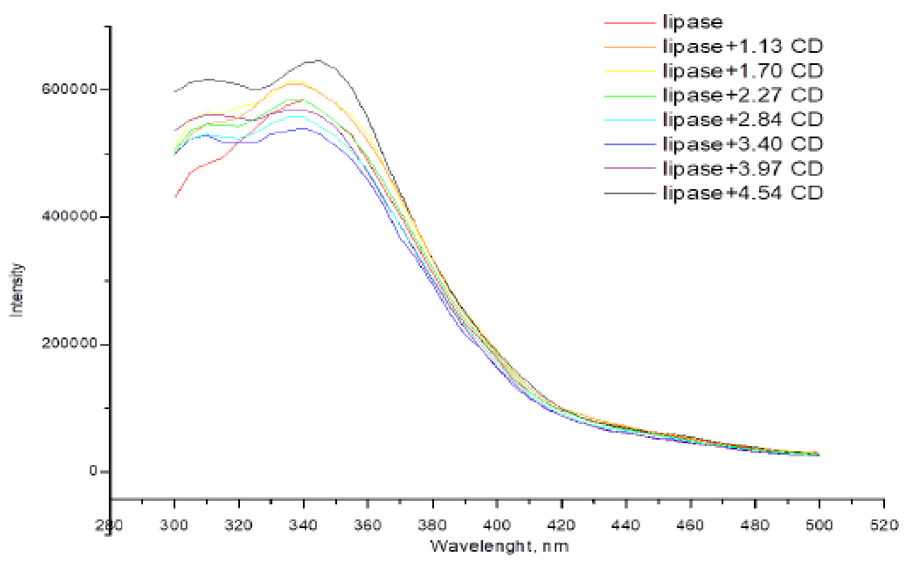
Morphological analysis of the dried complex lipase with β-CD was carried out using FEI Tecnai G2 F20 S-TWIN TMP equipped with a STEM detector. Accelerating voltage 80, 200 kV, extraction voltage 4500 V.

RESULTS

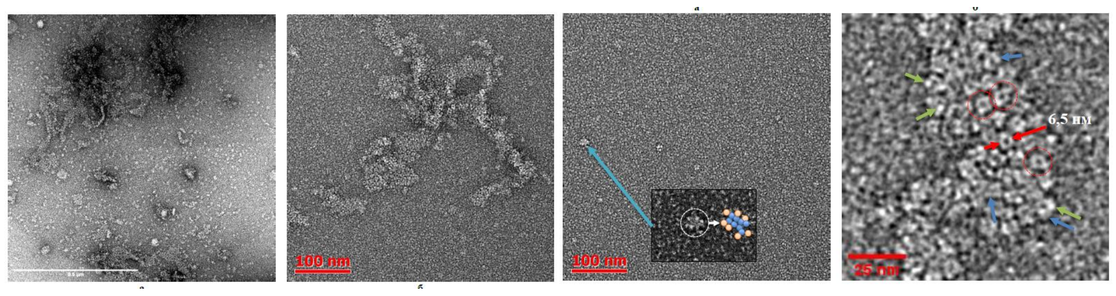
UV absorption revealed about surface interaction and formation of clathrate-like inclusion complex of lipase with β-CD (4.54 mg/mL).



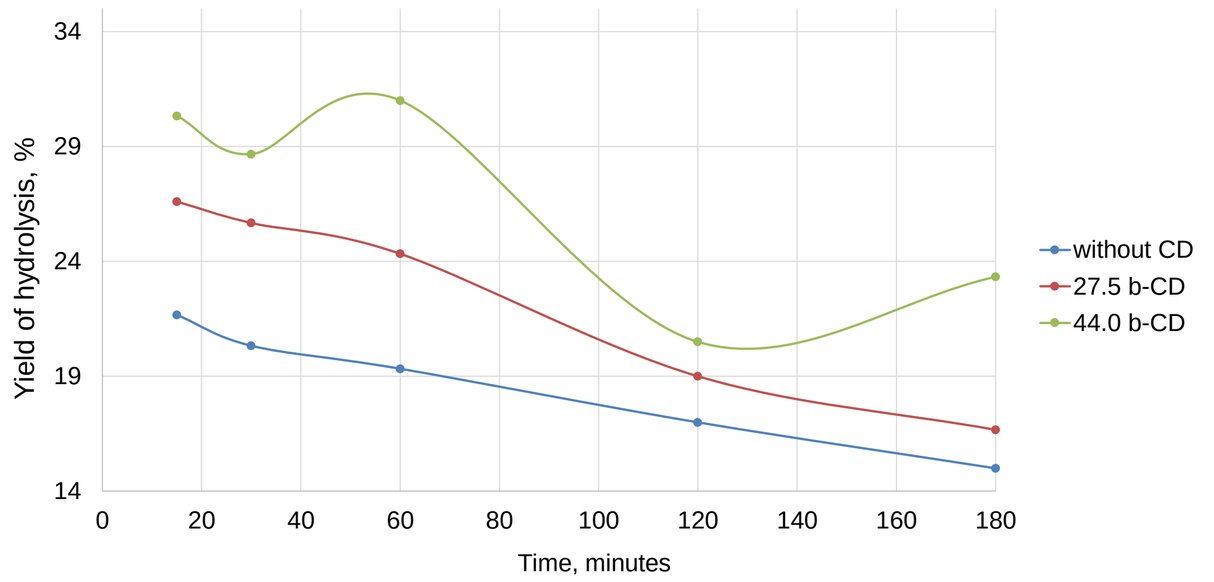
Fluorescence analysis showed increase the emission intensity of lipase in presence of β-CD (0-1.7 mg/mL and 3.4-4.54 mg/mL) due to interaction between tryptophane in active center of lipase with β-CD.



Lipase forms grape-like active conglomerate with β-CD molecules for maintaining stability and improving lipase activity.



β-CD increased lipase activity up to 138% compared to β-CD-free lipase samples. Lipase (80 mg/mL) produced the highest yield of oleic acid in presence of β-CD (44.0 mg/mL) after 1 and 3 hours.



CONCLUSION

1. Spectroscopy showed that β-CD interacted with surface and active center of lipase in different concentrations.
2. Lipase formed a special clathrate-like complex with β-CD for improving hydrolysis and stability.
3. The enzymatic activity of the lipase against acylglycerides of plant oils and fats increased in presence of β-CD molecules that can be useful for surface cleaning with low environmental impact.

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