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DoE oriented reaction optimization on the lipase-catalyzed monostearin synthesis

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ABSTRACT

Recognition that *trans* and saturated fats have negative health effects drive researchers to develop alternative systems that can structure liquid oils into semi-solid plastic pastes for food applications. Monoacylglicerols (MAG) can be used as a promising molecule to achieve this structuring so we have optimized a biocatalytic batch process to the esterification reaction between 1,2-O-isopropylidene glycerol and stearic acid, catalyzed by Lipozyme RM IM, using response surface methodology (RSM) in a laboratory setting with 95% of conversion after 4 h.

1. Introduction

A better understanding of the role of dietary lipids in the coronary heart disease (CHD) continues to give us insights into metabolic effects of individual fatty acids and their impact on surrogate markers of risk [1]. The recognition that *trans* and saturated fats have negative health effects drive researchers to develop alternative systems that can structure liquid oils into semi-solid plastic pastes for food applications because the traditional processes for manufacturing spreads, margarines, and shortenings uses hydrogenation and/or saturated fats to achieve the desired structuring [2,3].

In this way, monoacylglicerols (MAG) can be used as a promising molecule to achieve this structuring by utilizing the properties of gel phases (alpha gel and coagel) [4,5]. These MAG are commonly produced based on alkaline-catalyzed chemical glycerolysis of natural oil and fats at high temperatures (220–250 °C) and elevated pressure under nitrogen atmosphere leading to products with low yield, poor quality, dark-colored and burned-tasting characteristics [6,7]. To overcame this issues the use of enzymatic process can lead to an environmentally friendly approach, employing enzyme-catalyzed synthesis of MAG by selective hydrolysis or alcoholysis using 1,3-regiospecific lipases [8–10], esterification of glycerol with fatty acids [11,12], and glycerolysis of fats or oils [13].

In this work we have optimized a biocatalytic batch process to the esterification reaction between solketal and stearic acid using response surface methodology (RSM) in a laboratory setting [14]. The lipase-catalyzed esterification [15] has been investigated as a potential substitute to the traditional chemical glycerolysis, since lipases as biocatalysts demand milder reaction conditions which minimize energy costs, allow a better reaction control and consequently provide higher-quality products [16]. RSM is a statistical tool for developing and optimizing processes with one or more responses influenced by several variables. The RSM advantage is that it allows the user to gather large amounts of information from a small number of experiments. The RSM use also enables to observe the effects of individual variables and their combination of interactions on the response.

2. Experimental

Heptane was purchased from Tedia Co. (R,S)-1,2-isopropylidene glycerol from Sigma–Aldrich as well as all chromatofigureic

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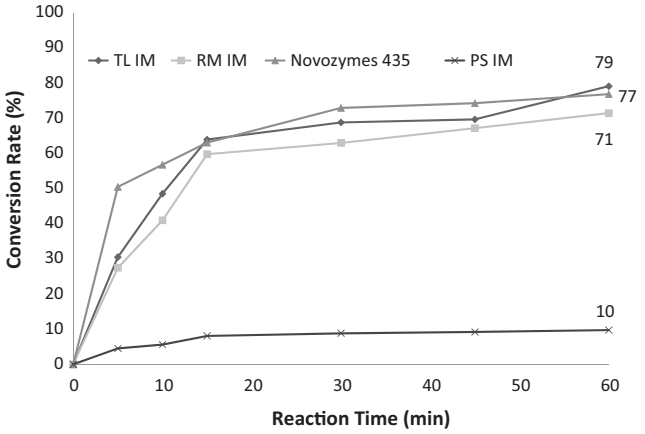

Fig. 1. Initial screening for immobilized lipases. Experimental conditions: 60 °C, 250 rpm and 1% (w/v) of enzyme (Lipozyme TL IM, Lipozyme RM IM, PS Amano IM and Novozym 435).

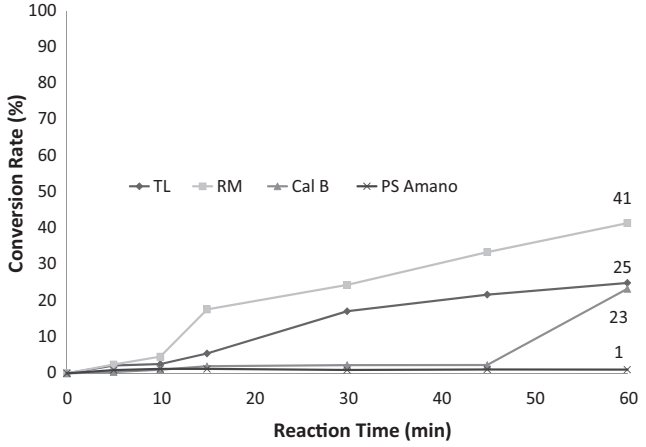

Fig. 2. Initial screening for free and lipases. Experimental conditions: 60 °C, 250 rpm and 1% (v/v or w/v) of enzyme (*Thermomyces lanuginosus, Rhizomucor miehei*, *Candida antarctica B* and PS Amano).

standards. Stearic acid (>98%) was purchased from Vetec Ltd. Lipases were purchased from Novozymes and Amano.

2.1. GC–MS analysis

The GC–MS analysis was performed by using modified method from EN 14105. Free fatty acids and solketal were transformed into more volatile silylated derivatives in presence of pyridine and N-methyl-N-trimethysilyltrifluoroacetamide (MSTFA). All GC–MS measurements were carried out in duplicate (Dizge & Keskinler; 2008) using a DB 5–HT (Agilent, *J & W. Scientific*®, USA) capillary column (10 m × 0.32 mm × 0.1 μm). The quantifying was done based on calibration curves with internal standards. The GC–MS samples were prepared by dissolving 0.1 g of the final product on 1 ml of n-heptane. 100 μl of this solution and pyridine solutions of butanetriol (1 mg/ml) and tricaprine (8 mg/ml), used as internal standards, were added on a flask forward by an addition of 100 μl of MSTFA. After 15 min, these reactants were dissolved on 8 ml de n-heptane. 1 μl of this sample was then injected into a Shimadzu CG2010 equipment.

2.2. Lowry–Tinsley analysis

The esterification rate was also measured using a modification of the Lowry and Tinsley assay [17]. The depletion of fatty acid was monitored as follows: 0.30 ml of the reaction solution, including the buffer solutions was added to a tube containing 0.6 ml of n-heptane and 1 ml of cupric acetate-pyridine (5%, w/v; pH 6.0). The final solutions were vigorously mixed for 30 s in vortex, and the upper organic phase was measured by a UV/visible spectrophotometer at 715 nm. Each reaction was analyzed in triplicate, and content conversion, calculated according to the percentage difference for the absorbance shown by the stock solution.

2.3. Batch procedure

A stock solution containing 1,2-O-isopropylidene glycerol and stearic acid in n-heptane in proportions of 2:1 (150 mM and 75 mM respectively) was prepared. In 2 ml cryotubes, were poured 1 ml of reaction medium, followed by the addition of appropriate enzyme (1%, w/w or w/v). The cryotubes were then incubated in shaker at 250 rpm and 60 °C. The esterification rate was measured by a modification of the Lowry and Tinsley assay, at time intervals between 0 and 60 min. The depletion of fatty acid was monitored as follows: 0.30 ml of the reaction solution was added to a cryotube containing 0.6 ml of n-heptane and 1 ml of cupric acetate–pyridine (5%, w/v; pH 6.0). The final solutions were vigorously mixed for 30 s in vortex, and the upper organic phase was measured by a UV/visible spectrophotometer at 715 nm. Each reaction was analyzed in triplicate, and content conversion, calculated according to the percentage difference for the absorbance shown by the stock solution. The results were confirmed by GC–MS analysis.

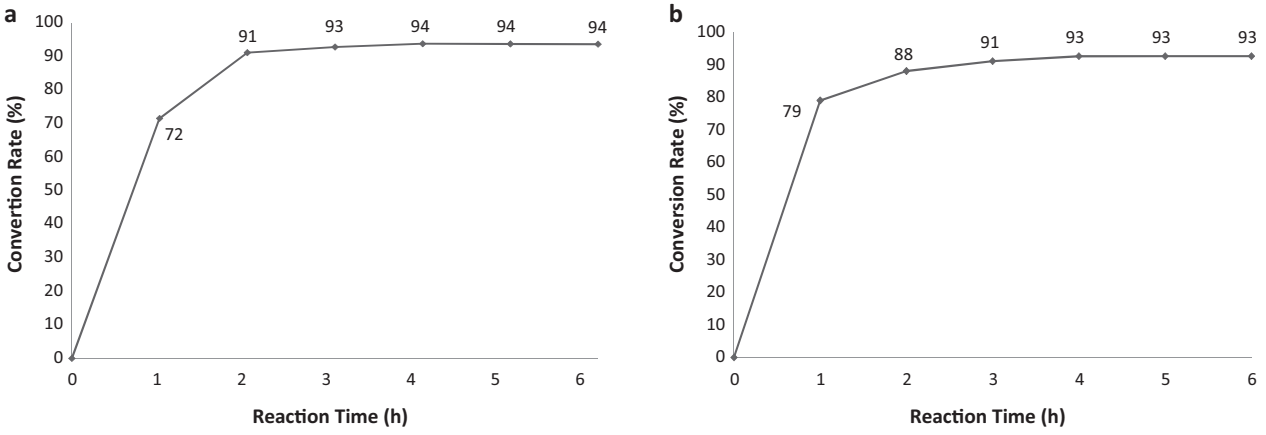

Fig. 3. Conversion rates of 1,2-O-isopropylidene glycerol stearate (2:1, 60 °C, 250 rpm and 1%, w/v) by using Lipozyme RM IM (a) and Lipozyme TL IM (b) as biocatalysts.