ENHANCING PRODUCTION EFFICIENCY: ENZYMATIC SYNTHESIS OF MLM-TYPE STRUCTURED LIPIDS IN CONTINUOUS FIXED-BED REACTORS

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ABSTRACT

The objective of this study was to produce MLM-type dietary lipids in fixed-bed reactors through the reaction of capric acid with grape seed oil. Immobilized *Rhizopus oryzae* lipases on hydrophobic particles of styrene-divinylbenzene (DIAON-HP20) were utilized under conditions optimized by neural networks. The synthesis achieved approximately 61 mol% of capric acid incorporation at the sn-1 and sn-3 positions of grape seed oil triacylglycerol structure. The reaction system operated for 192 hours, yielding a maximum productivity of 5.05 g of new TAG / h.g_{enzyme}. Furthermore, the sustained operation of the reaction system for 192 hours demonstrates its stability and efficiency in producing structured lipids. These findings underscore the potential of this approach for industrial-scale production of MLM-type structured lipids.

Keywords: Lipase. Structured lipids. MLM-type lipid. Grape seed oil. Acidolysis

1 INTRODUCTION

In the quest of innovative food production technologies, the utilization of modified oils and fats has emerged as a pertinent strategy for crafting healthier food options. These modified lipids offer nutraceutical properties and play a role in various physiological processes essential for disease prevention and treatment. MLM-type lipids, characterized by long-chain fatty acids at the *sn*-1 and *sn*-3 positions of glycerol, boast enhanced solubility and accelerate metabolism, preventing fat accumulation in the body and aiding in weight management and obesity prevention [1-4].

Grape seed oil, often discarded as industrial waste by wine and juice industries, was chosen for its nutritional richness, including high levels of unsaturated fatty acids such as linoleic acid at the *sn*-2 position, surpassing those in corn and soybean oils [5]. Additionally, grape seed oil contains antioxidants like vitamin E and tocotrienols, with potential in preventing cardiovascular diseases [6-9]. This eco-conscious choice for structured lipid synthesis adds value to industrial byproducts, fostering sustainability in food production.

In the food and biotechnology sectors, synthesizing structured lipids, particularly MLM-type triacylglycerols, poses a significant challenge due to the limited selectivity of chemical catalysts [1,5]. This study focuses on leveraging lipases derived from *Rhizopus oryzae*, highly selective enzymes capable of catalyzing specific positions in triacylglycerol molecules. By employing these lipases, enhanced efficiency in synthesizing MLM-type triacylglycerols is anticipated, enabling precise and controlled production of these compounds. This approach holds promise for producing structured lipids with diverse applications in food, medicine, and other fields linked to human health and well-being.

2 MATERIAL & METHODS

2.1. Lipase immobilization

The polymeric support Diaion™ HP-20 was initially dried in an oven at 60°C for 12 hours and subsequently incubated in 10 mL of hexane (1g of support: 10 mL of hexane), and 300 mg of enzyme powder was gradually added with stirring. The immobilized material was then left to rest under refrigeration at 4°C for 12 hours, following the procedure outlined by [10]

2.2. Acidolysis reactions in fixed-bed reactor

The reaction system consisted of a glass column (height of 210 mm, inner diameter of 14 mm, and total volume of 32 cm^3). The column was filled with lipases immobilized on Diaion™ HP-20 (15 g). A mixture of grape seed oil and capric acid in proportions of 1:6.7 mol was maintained at 41°C, optimized by an artificial neural network system, and kept in a feeding tank under magnetic stirring (150 rpm). The substrate was pumped by a peristaltic pump (SJ-1211 Pump, Atto Bioscience & Biotechnology, Tokyo, Japan) to the filled column corresponding to residence times of 24 hours. Samples were collected daily at the reactor outlet, and analyses of capric acid incorporation degree were performed using gas chromatography.

2.3. Determination of fatty acid composition of SLs and incorporation degree (ID, %)

Incorporation degree (%ID) was calculated according to Eq. (1) [10], where MFA is the number of moles of medium-chain fatty acids (C10:0) in triglyceride and MT is the number of total moles of fatty acids in triglyceride.

$$ID\ (\%) = \frac{MFA}{MT}.100 \quad (1)$$

2.4. Specific productivity

The productivity of the continuous process was calculated according to Eq. (2), where New TAG corresponds to the mass of MLM after incorporation of capric acid., $t_{reaction}$ corresponds to the reaction space time in hours and g_{enzyme} corresponds to the mass of enzyme bed.

$$P = \frac{New\ TAG_{(g)}}{t_{reaction} \cdot g_{enzyme}} \quad (2)$$

3 RESULTS & DISCUSSION

3.1. Structured Lipids Produced in Fixed-Bed Reactor

The results obtained from this system are presented in Fig.1. In this setup, samples were taken every 24 hours, and the incorporation degree of capric acid into the triacylglycerol structure was analyzed. Initially, the addition of capric acid to the triacylglycerol structure was low (4.0 ± 0.35 ID%). This low incorporation degree was expected, as the reactor reaches a steady state after three space times, as demonstrated by [11], where maximum conversion of the bioproduct catalyzed by lipase is achieved after passing through three space times. For this fixed-bed reactor system, a higher incorporation degree of capric acid (≈ 60 %ID) was achieved after 96 hours, corresponding to the fourth space time, and these values were maintained until the seventh space time. After 192 hours of steady-state reaction, a decrease of approximately 5% was observed. This decrease in the incorporation degree may be associated with enzyme deactivation.

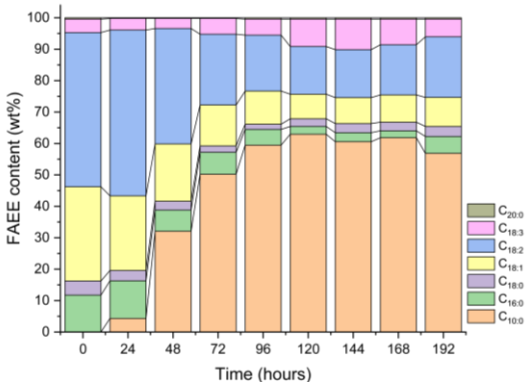

Figure 1. Fatty Acid Profile of Structured Lipids Over Time

The results in Fig.1 show a promising trend, with an increase in capric acid (%) accompanied by a decrease in other fatty acids. Notably, linoleic and linolenic acids remain prominent, at 15% and 9% respectively. This increase in linolenic acid may be attributed to its fixation at the *sn*-2 position of the triacylglycerol, facilitated by the preferential action of *Rhizopus oryzae* lipase at *sn*-1 and *sn*-3 positions. As capric acid gradually replaces side carbon chains, the presence of linolenic acid diminishes in the structured lipid composition.

3.2. Productivity of Structured Lipids

The productivity analysis, depicted in Fig. 2, illustrates the synthesis efficiency of MLM-type structured lipids. As the fixed-bed reactor progresses, productivity steadily increases beyond the third space time, remaining consistently above 4.5 g of NEW TAG / h.genzyme from the 96th hour onwards. However, a decline in productivity is observed after 192 hours, possibly due to enzyme immobilization loss. When compared to other studies, such as [12], our work demonstrates the effectiveness of using laboratory-immobilized lipases versus commercially immobilized ones, such as Lipozyme RM IM. [13] investigated the production of low-calorie structured lipids from olive pomace oil through acidolysis with medium-chain fatty acids (caprylic and capric acids). They utilized commercially immobilized lipases from *Thermomyces lanuginosus* (Lipozyme TL IM) and *Rhizomucor miehei* (Lipozyme RM IM) at 40°C in solvent-free conditions in continuous fixed-bed reactors. Their results showed approximately 30% conversion of the new TAG using caprylic acid and approximately 50% conversion using capric acid after 70 hours of reaction catalyzed by Lipozyme TL IM. Similarly, approximately 40% conversion using caprylic acid and approximately 60% using capric acid were achieved after 70 hours of reaction catalyzed by Lipozyme RM IM, with a maximum productivity of 1.87 g of NEW TAG / h.genzyme.

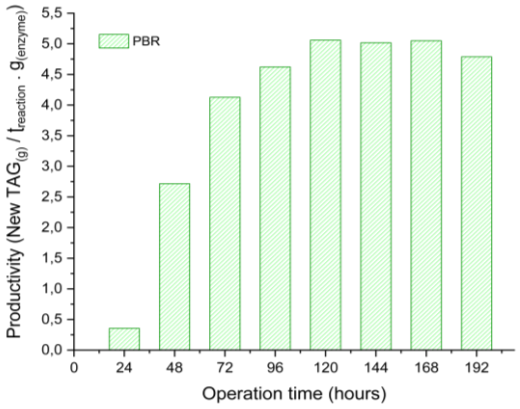

Figure 2. Productivity of MLM-type lipid in fixed-bed reactor

4 CONCLUSION

The fixed-bed reactor has proven to be a functional reaction system for the efficient synthesis of structured lipids. The results obtained using conditions optimized by the neural network demonstrated an incorporation of approximately 60% of capric acid, corresponding to a conversion of 90% of the substrate into new TAG. The biocatalyst showed promise in producing these lipids, surpassing the effectiveness of commercially immobilized biocatalysts. Additionally, the reaction system operated continuously with a high degree of incorporation for 120 hours. These findings are promising in the quest to implement this reaction system on an industrial scale.

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