EXTRACTION OF SAPUCAIA NUT OIL (*Lecythis pisonis Cambess)* AIMING ITS APPLICATION TO OBTAIN DIETARY TRIGLIACYLGLYCEROLS

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ABSTRACT

Sapucaia (*Lecythis pisonis Cambess*.) is a native brazilian plant. Its seeds are oilseed nuts which are used for consumption and food preparation. These nuts have a high lipid content (51 - 64% m/m) which determines a high potential for extracting edible oil rich in unsaturated fatty acids. Due to these characteristics, this oil is an interesting raw material for applications such as structured lipids synthesis with nutraceutical applications. The production of medium-long-medium (MLM) triacylglycerols, via Brazilian native oils and fats modification, represents an opportunity for the food industry. The present study seeks to develop structured lipids from the oil extracted from Sapucaia's seed and capric acid, using Lipozyme RM IM via acidolysis reaction. A 2^2 central composite design was used to optimize capric acid incorporation in sapucaya nut oil. The independent variables were enzyme loading (5-10% w/w) and sapucaia nut oil to capric acid molar ratio (1:3–1:9). The temperature was set at 45 ◦C. The best conditions, namely a 1:9 oil to acid molar ratio and enzyme loading 10% w/w, provided a degree of incorporation of about ~37 mol%, as shown by compositional analysis of the modified oil.

Keywords: Sapucaia nut oil. Enzyme. Dietary trigliacylglycerols. Acidolysis. Bioprocess.

1 INTRODUCTION

Consumer demands for healthier products, coupled with new scientific discoveries about the beneficial effects of certain foods on health, have motivated the search for suitable processes capable of producing these specific foods. Structured lipids are oils and fats with basic nutraceutical properties which, in addition to being nutritious, have metabolic or physiological effects used in the prevention and treatment of diseases[1].

Among these lipids, we can mention MLM-type trigliacylglycerols, which have medium-chain fatty acids (M), in the *sn-1* and *sn-3* positions, and long-chain fatty acids (L), in *sn-2* position of the trigliacylglycerol molecule. There is great interest in the production of this kind of trigliacylglycerol because of its low-calorie content, which makes it suitable for people with metabolic deficiencies. MLM trigliacylglycerols can be synthesized chemically or enzymatically, catalyzed by lipases (glycerol ester hydrolases - E.C. 3.1.1.3) using interesting oil and medium-chain fatty acids[2].

Lipases are biocatalysts that can present regioselective. Lipases sn-1,3 regioselective typically do not exchange acyl groups at the sn-2 position due to steric hindrance[3], thus allowing the control of fatty acids incorporation at the *sn-1* and *sn-3* specific positions of the glycerol chain in the triacylglycerol structure[4,5]. For this reason, lipases are the most suitable catalysts for MLM lipids production[6].

New sources of oils have been studied for technological applications, particularly those with high concentrations of long-chain polyunsaturated fatty acids. Sapucaia seed oil has an excellent lipid profile and is rich in the essential fatty acid linoleic C18:2 n6 (39.93 - 46.85%), oleic acid C18:1 n9 (34.30 - 41.27%), and palmitic acid C16:0 (10.68 - 14.49%)[1,7]. The sapucaia (*Lecythis pisonis Cambess*) is a plant native to Brazil. Sapucaia nuts can be eaten or used in food preparation. Some studies have already identified that they are excellent sources of proteins, nutrients, minerals and have a high lipid content (51 - 64%)[8], making them a potential source of vegetable oil.

In order to turn sapucaia nut oil into a nutraceutical food, we propose to study the development of MLM-type structured TAGs through an acidolysis reaction using Lipozyme RM IM lipase as a biocatalyst.

2 MATERIAL & METHODS

The materials were: Capric acid (98%, Sigma-Aldrich), sapucaia nut oil (*Lecythis pisonis Cambess*) extracted in a Soxhlet system with hexane. Lipozyme RM IM® (*Rhizomucor miehei*) was kindly provided from Novozymes S/A (Araucária, PR, Brazil).

The reactions were carried out in a stirred tank reactor operating in batch mode (BSTR). The experimental apparatus was a jacked glass reactor (10 cm height and 4.5 cm diameter) connected to a thermostatic bath (Marconi, model MA 184/6) and a magnetic stirrer (Fisatom, model 752). A 2^2 central composite design (CCD) (Table 1) was used to determine the experimental

conditions needed to achieve maximum capric acid degree incorporation in the BSTR. Independent variables studied were enzyme loading (5-10% w/w) and sapucaia nut oil to capric acid molar ratio (1:3–1:9). After 24 h of reaction, aliquots of the reaction medium were collected.

Samples were first neutralized with a hydroalcoholic solution (30% v/v ethanol) containing 0.8 M potassium hydroxide[9] and then subjected to methylation[10] under sequential acid and alkaline conditions. Fatty acids were analyzed by gas chromatography according to method Ce 2-66[11].

The incorporation degree (ID, mol%) of fatty acids was calculated according to Equation (5)[12]:

$$ID = \frac{MFA}{TFA} x100 \quad (1)$$

where MFA is the number of mol of medium-chain fatty acids (C10:0) in triglyceride molecules and TFA is the total number of moles of fatty acids in triglyceride molecules.

3 RESULTS & DISCUSSION

A 2^2 CCD with axial points was applied to determine the experimental conditions that provide the maximum ID in BSTR. The CCD matrix and experimental results are presented in Table 1.

Table 1 2^2 central composite design matrix showing coded and actual values of independent variables and responses in terms of incorporation degree after 24 h of reaction.

Run	Oil to capric acid molar ratio	Enzyme loading (% w/w)	Incorporation degree (ID, %mol)
1	-1(1:3)	-1(5)	20.84
1*	-1(1:3)	-1(5)	20.03
2	1(1:9)	-1(5)	26.91
2*	1(1:9)	-1(5)	27.87
3	-1(1:3)	1(10)	26.35
3*	-1(1:3)	1(10)	24.63
4	1(1:9)	1(10)	37.18
4*	1(1:9)	1(10)	35.80
5	0(1:6)	0(7.5)	30.15
5*	0(1:6)	0(7.5)	30.15
5*	0(1:6)	0(7.5)	30.65
5*	0(1:6)	0(7.5)	30.02
5*	0(1:6)	0(7.5)	27.18
5*	0(1:6)	0(7.5)	28.86

*Replicate

Statistical analysis of experimental data showed that loading enzyme and oil/acid molar ratio had significant positive effects ($p < 0.05$) on the ID of capric acid in sapucaia oil. In other words, an increase in these variables resulted in an increase in the production of dietary triacylglycerols. Similar findings were reported by Remonatto et al.[6]; the authors investigated the production of structured lipids and found that the concentration of MLM lipids increased significantly with the increase in oil/capric acid molar ratio from 1:2 to 1:4. In acidolysis reactions, where fatty acids are esterified to glycerol, an excess of fatty acids is beneficial because these compounds shift the chemical balance of the reaction toward product formation, resulting in a high ID[13]. Using ID values, we estimated the regression coefficients and constructed first-order models.

$$Y = 28.33 - 4.49x_1 + 3.54x_2 + 1.01x1x_1x_2 \quad (2)$$

The mathematical model describing the ID of capric acid (y) as a function of oil/acid molar ratio (x_1) and enzyme loading (x_2) is described in Equation (2). Analysis of variance was performed to assess the adequacy of the adjusted model. The R2 was 0.9128, demonstrating that the model explained 91.24% of the variance in ID. The F-value was 34.7, higher than the F-critical (3.71), indicating that the first-order model (Equation 2) successfully described capric acid incorporation as a function of oil/acid molar ratio and enzyme loading. The equation 2 was used to generate 2D-contour plots, depicted in Figure 1.

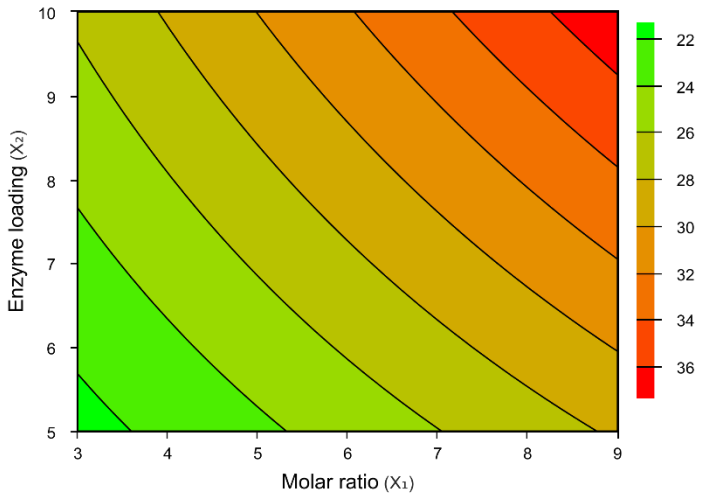

Figure 1 2D-Contour (b) plots of the degree of incorporation (%, w/w) of capric acid as a function of sapucaia oil/capric acid molar ratio and enzyme loading in a BSTR using Lipozyme RM IM as catalyst.

The CCD runs that provided the highest ID (~37 mol%) were runs 4 and 4*, carried out using high oil to acid molar ratios and a high enzyme loading. Run 4 was performed using a 1:9 oil to acid molar ratio and 10% (w/w) of Lipozyme RM IM. This run resulted in a capric acid ID of ~37 mol%, and was considered to be the better result in this acidolysis reaction design matrix.

4 CONCLUSION

Relevant values of incorporation degree were obtained ~37% w/w in sapucaia nut oil modified. However, these are preliminary results; studies must still be carried out to better understand the reaction kinetics and achieve greater incorporation of capric acid in sapucaia nut oil.

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