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Modeling lipase-catalyzed interesterification of flaxseed oil and tricaprylin for the synthesis of structured lipids

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ABSTRACT

The biosynthesis of structured lipids (SLs) in organic solvent media (OSM) was carried out by the interesterification of flaxseed oil (FO) TAGs and tricaprylin (TC) using Lipozyme TL-IM from *Thermomyces lanuginosus*. The bioconversion yield (BY, %) of medium-long-medium type SLs (MLM-SLs), including CLnC (C-caprylic and Ln-linolenic acids), CLaC (La-linoleic acid) and COC (*O*-oleic acid), was monitored. Response surface methodology (RSM) was used to obtain significant models for the responses and to optimize the interesterification reaction, on the basis of a three level, five variable fractional factorial design (FFD) with center points. For the optimization of the interesterification reaction significant parameters, including reaction time (R_t), reaction temperature (T_r), TC to FO molar ratio (M_r), enzyme concentration (E_c), and agitation speed (A_s, 100–300 rpm), were considered. The optimal conditions generated for the maximum synthesis of CLnC, CLaC and COC, were found to be, 4.00–4.01 h for R_t, 41.49–50.00 °C for T_r, 1.5% for E_c, 5.00–5.13 mol/mol for M_r and 260–300 rpm for A_s. Under these optimal conditions, the BY of CLnC, CLaC and COC were predicted to be 35.34–35.45, 4.09–4.19 and 8.44–8.53%, respectively.

1. Introduction

Structured lipids (SLs) are triacylglycerols (TAGs) that have been restructured in terms of fatty acids (FAs) composition and/or positional distribution, thus are broadly referred to as modified or synthetic oils and fats with functional or nutraceutical applications [1]. SLs can be synthesized differently according to their type. SLs that require specific distribution of FAs, namely MLM-SLs (M-medium chain and L-long chain FAs), cannot be synthesized chemically, but rather enzymatically, using regio-specific lipases [2].

Although the generally high price of commercial enzymes limited their use in industrial applications, the development of new cost-effective immobilized lipases provided more opportunities for the industry to produce economy-balanced products with better properties [3]. In this regard, immobilized *sn*-1,3 specific lipase from *Thermomyces lanuginosus*, Lipozyme TL-IM, has gradually gained attention in lipid research [4–6].

Nutritional studies [7–10] have suggested that the use of structured and randomized medium/long chain TAGs (MLCTs) in the diet could result in a significant reduction in accumulation of body fat and serum total cholesterol, as well as the improvement of nitrogen balance and lipid clearance. Moreover, MLM-SLs have shown to be hydrolyzed rapidly by pancreatic lipase and absorbed efficiently, which makes them preferable for treatment of lipid malabsorption [11–13].

With the growing recognition of the nutritional importance of dietary ω-3 and ω-6 FAs in human nutrition, flaxseed oil (FO) is being used increasingly for food and nutraceutical purposes. Since ω-3 and ω-6 FAs in most vegetable oils including FO, are prevalent at the *sn*-2 position in TAGs [14], they have been found to be ideal substrates for the catalysis of MLM-SLs via transesterification using medium chain FAs (MCFAs) and their esters in order to alter their physiological effects [15–17].

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for optimization of stochastic functions [18]. This methodology is based on approximation of the stochastic objective function by a low order polynomial on a sub-region of the domain. The coefficients of the polynomial are estimated by ordinary least square estimate. The precision of estimating the unknown parameters of the polynomial model depends to a large extent on the design used in the experiment. The tools needed for adequate selection of a design and the subsequent fitting and evaluation of the model, using the data generated by the design, have been developed in an area of experimental design known as RSM [19].

The aim of the present study, which is a part of an ongoing research work [20,21] was to establish a model for the

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optimization of the biosynthesis of MLM-SLs in hexane medium, by the interesterification of FO and tricaprylin (TC) using Lipozyme TL-IM as the catalyst. Significant parameters, investigated in the preliminary trials, including reaction temperature (T_r), substrate molar ratio (M_r), enzyme concentration (E_c), reaction time (R_t) and agitation speed (A_s), have been optimized by RSM. The bioconversion yield of MLM-SLs of the unsaturated long chain fatty acids (LCFAs), linolenic acid (CLnC), linoleic acid (CLaC) and oleic acid (COC), was monitored as the response for the optimization of the process.

2. Materials and methods

2.1. Materials

Flaxseed oil (FO) was a gift from Arista Industries, Inc. (Wilton, CT). Commercial immobilized lipase, Lipozyme TL-IM from *T. lanuginosus*, was acquired from Novozymes Nordisk A/S (Bagsværd, Denmark). Tricaprylin, with a minimum purity of >99%, was purchased from Sigma-Chemical Co. (St-Louis, MO). All high-performance liquid chromatography (HPLC) grade organic solvents were purchased from Fisher Scientific (Fair Lawn, N.J.).

2.2. Interesterification reaction

The interesterification reaction of flaxseed oil (FO) and tricaprylin (TC) was performed in a 30-mL reactor flask. Prior to each enzymatic reaction, stock solutions with well determined concentrations of FO and TC were prepared in *n*-hexane. Appropriate amounts of FO and TC stock solutions were introduced into the flasks to acquire a 3 mL total reaction volume with a final concentration of 40 mM FO and variant concentrations of TC, according to the substrate molar ratios generated by RSM. The enzymatic reaction was initiated by the addition of the defined amount (%, w/v) of Lipozyme TL-IM. The reactor flasks were incubated in obscurity under vacuum, at different temperatures and agitation speeds using an orbital shaker (New Brunswick Scientific Co., Inc.; Edison, N.J.) for different periods of time, obtained by RSM. The experiments were run randomly and control trials, without lipase, were carried out in tandem with the enzymatic assays under the same conditions. Sample mixtures of 100 μL volume were recovered, dried under vacuum, flushed with a gentle stream of nitrogen and stored at −80 °C for further analysis.

2.3. Characterization of structured lipids (SLs)

Hundred μL solution was withdrawn from the reaction mixture and subjected to high-performance liquid chromatography (HPLC) analysis. The characterization of the synthesized MLM- and MML-SLs, was performed, with the use of a ChromSpher 5 Lipids column (250 × 4.6 mm, Varian Inc.; Lake Forest, CA). Beckman HPLC system (Model 126, Beckman Instruments Inc.; San Ramon, CA), was equipped with an evaporative laser scatter detector (ELSD) (Model II A, Varex Corporation; Rockville, MD) and computerized data handling as well as integration analysis system (Karat 32, Beckman). The peaks were identified by the use of chemoenzymatically synthesized standard TAGs, according to a modification of the method described by Halldorsson et al. [22].

The separation and quantification of the synthesized SLs were carried out, using two gradient elution programs described previously [21]. The first method aimed at the quantification of the MLM and MML isomers of oleic and linolenic acid with caprylic acid (COC, CCO, CLnC and CCLn), while the second method was performed to quantify the MLM and MML isomers of linoleic acid with caprylic acid (CLaC, CCLa). The fatty acyl chains of the synthesized SLs were identified by atmospheric pressure chemical ionization/mass spectrometry (APCI/MS) analysis [21].

Table 1
Fractional factorial design (FFD) arrangement of the actual and coded experimental variables and the observed responses for the interesterification reactions catalyzed by Lipozyme TL-IM, using flaxseed oil and tricaprylin as substrates.

	Independent variables					Bioconversion yield[a] (%)		
Run No	T_r[b]	M_r[c]	E_c[d]	R_t[e]	A_s[f]	CLnC[g]	CLaC[h]	COC[i]
1	50(+1)	5(−1)	0.5(−1)	4(−1)	300(+1)	28.96	3.69	6.82
2	30(−1)	10(+1)	0.5(−1)	4(−1)	300(+1)	31.05	3.51	6.48
3	50(+1)	10(+1)	0.5(−1)	4(−1)	100(−1)	34.20	4.04	7.78
4	50(+1)	5(−1)	0.5(−1)	10(+1)	100(−1)	25.82	3.50	6.37
5	30(−1)	10(+1)	1.5(+1)	10(+1)	300(+1)	30.66	4.22	7.37
6	30(−1)	5(−1)	0.5(−1)	10(+1)	300(+1)	28.13	4.10	6.82
7	30(−1)	5(−1)	1.5(+1)	4(−1)	300(+1)	30.99	3.38	7.73
8	30(−1)	10(+1)	1.5(+1)	4(−1)	100(−1)	34.66	4.24	8.12
9	50(+1)	10(+1)	1.5(+1)	10(+1)	100(−1)	23.40	3.33	6.21
10	50(+1)	10(+1)	0.5(−1)	10(+1)	300(+1)	35.61	4.70	8.05
11	50(+1)	10(+1)	1.5(+1)	4(−1)	300(+1)	36.94	4.16	8.66
12	30(−1)	5(−1)	0.5(−1)	4(−1)	100(−1)	27.20	3.55	5.92
13	50(+1)	5(−1)	1.5(+1)	4(−1)	100(−1)	30.74	2.99	6.64
14	30(−1)	5(−1)	1.5(+1)	10(+1)	100(−1)	27.12	3.71	6.25
15	50(+1)	5(−1)	1.5(+1)	10(+1)	300(+1)	26.81	3.23	5.56
16	30(−1)	10(+1)	0.5(−1)	10(+1)	100(−1)	33.22	5.12	7.48
17	40(0)	7.5(0)	1(0)	7(0)	200(0)	23.48	2.67	4.84
18	40(0)	7.5(0)	1(0)	7(0)	200(0)	24.63	2.63	5.28
19	40(0)	7.5(0)	1(0)	7(0)	200(0)	26.25	2.52	5.55
20	40(0)	7.5(0)	1(0)	7(0)	200(0)	28.06	2.32	4.96

[a] Bioconversion yield (%) is defined as the synthesized SLs (M) divided by limiting substrate (FO, M) in the blank at time *t*, multiplied by 100.
[b] Reaction temperature (°C).
[c] Substrate molar ratio (mol/mol, tricaprylin/flaxseed oil).
[d] Enzyme concentration (% w/v).
[e] Reaction time (h).
[f] Agitation speed (rpm).
[g] 1,3-Dicaprylyl-2-linolenyl glycerol.
[h] 1,3-Dicaprylyl-2-linoleyl glycerol.
[i] 1,3-Dicaprylyl-2-oleyl glycerol.