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Production of triacylglycerols rich in palmitic acid at *sn*-2 position by lipase-catalyzed acidolysis

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ABSTRACT

This paper studies the synthesis of triacylglycerols (TAGs) rich in palmitic acid (PA) at *sn*-2 position from palm stearin (PS), a vegetable oil highly rich in PA (60%, but only 12.8% of this is located at *sn*-2 position). These PA rich TAGs were obtained by lipase-catalyzed acidolysis of this oil with free fatty acids (FFAs) highly rich in PA, such as commercial PA (98% PA) and a FFA extract obtained by saponification of PS (60% PA). PA has a melting point of 63 °C and during the acidolysis reaction the substrates, highly rich in this acid, must remain liquid; therefore high temperatures or solvents must be used. An important objective of this work was to operate without solvent and at the lowest possible temperature. In this acidolysis reaction four factors were firstly studied: type of lipase, temperature, solvent amount and the intensity of treatment (IOT = lipase amount × reaction time/PS amount). The influence of these variables was studied in a stirred tank reactor (STR). The lipases tested were Novozym 435 from *Candida antarctica* (immobilized on a macroporous acrylic resin), and lipases QLC (immobilized on diatomaceous earth), and QLM (non-immobilized), both from *Alcaligenes* sp., and the one selected was lipase QLC. According to the manufacturer the optimum temperature for this lipase is 65–70 °C, which allows it to operate without solvent. The best results with lipase QLC (TAGs with 80% PA, both total and at *sn*-2 position) were obtained with commercial PA, at 65 °C, a 3:1 FFA/PS molar ratio (1:1, w/w), without solvent and an IOT = 7 g lipase × h/g PS (for example 2.5 g PS, 2.5 g commercial PA, 0.75 g lipase and 24 h). These results were the basis for establishing the operational conditions to obtain PA rich TAGs with the lipase immobilized in a packed bed reactor (PBR), operating by recirculation of the reaction mixture through the lipase bed. In this system TAGs with 75% PA were obtained at an IOT = 8 g lipase × h/g PS. This result and the apparent kinetic constants obtained in both reactors show that the reaction rate is lower in the PBR than in the STR. Subsequently, PA enriched TAGs were separated from FFAs by two procedures: the first one at room temperature and in presence of hexane and the second one at 65 °C and without hexane. Using the first procedure, 95% of TAGs in the acidolysis reaction mixture were recovered with a purity of 99%. Using the second one, 98% pure TAGs were obtained with a recovery yield of 80%. Therefore, these highly rich PA TAGs can be obtained by acidolysis of PS and PA rich FFAs in solvent-free media, and then these TAGs also can be purified to 98% in absence of hexane, using only a hydroethanolic KOH solution.

1. Introduction

The fatty acid composition of triacylglycerols (TAGs) in human diet, and especially their distribution in the TAG molecule, play an important role in the absorption of fatty acids and other nutrients [1–3]. The absorption of palmitic acid (PA) has been widely studied, since this fatty acid is important in infant nutrition [4–7]. The PA content of human milk is 20–25% of total fatty acids; 65–70% of PA at the central position of the TAG molecule [8,9]. Studies comparing PA absorption from human milk and from infant formulas with the absorption from formulas with PA mostly at the extreme positions, conclude that it is considerably higher in infants fed with human milk or infant formulas with PA at *sn*-2 position; this higher absorption also implies a decrease in the loss of calcium via faeces [10–12]. It is therefore important to synthesize TAGs resembling human milk fat substitutes (HMFS) with a composition and distribution of fatty acids similar to those of human milk; these TAGs are being developed from vegetable oils, lard or tripalmitin [13–16]. In particular there is considerable interest in the synthesis of 1,3-diolein-2-palmitin (OPO), which is the most abundant TAG in human milk.

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TAGs with a specific fatty acid composition can be obtained by chemical or enzymatic catalysis. Lipase-catalyzed processes have attracted attention because of the mild reaction conditions of temperature, pressure and pH under which enzyme operate, which made that they generally require less energy and are conducted in equipment of lower capital cost than many other chemical processes; also, under these milder conditions, the products are purer and less degraded than through alternative high-temperature reactions, so they are more easily purified and waste disposal is less of a problem [17]. Moreover, the regio-, acyl- and stereospecificity of enzymes result in products with better defined and more predictable chemical composition and structure [18].

Several types of enzymatic reactions appear in the literature to synthesize structured TAGs rich in PA at *sn*-2 position and in other fatty acids at *sn*-1, 3 positions (oleic acid, caprylic acid, etc.). The most direct procedure is the acidolysis of an oil rich in PA at *sn*-2 position and oleic or caprylic acid, catalyzed by a 1,3 specific lipase. Nielsen et al. [15] produced HMFS by acidolysis of lard and soybean oil fatty acids. Lard oil contains 29.5% PA (74% at *sn*-2 position, similar to human milk). In this acidolysis reaction PA contents were maintained, and the contents of linoleic and linolenic acids increased from 9.2 and 0.8% to 23.8 and 2.3%, respectively; at *sn*-2 position only the linoleic acid content increased from 3.2 to 4.8%. This reaction was catalyzed by Lipozyme RM IM immobilized in a packed bed reactor. Balcao and Malcata [19] also use the acidolysis reaction to increase the level of unsaturated fatty acid of a butterfat by acidolysis of this one with oleic acid; so decreases the level of saturated fatty acids, such as myristic and palmitic acids. Schmid et al. [16] synthesized the structured TAG oleic–palmitic–oleic (OPO) by a two-step enzymatic process: alcoholysis of tripalmitin with ethanol to produce 2-monopalmitin and esterification of the latter with oleic acid, catalyzing both reactions with 1,3 specific lipases. These authors obtained OPO with 96% PA at *sn*-2 position and 90% oleic acid at *sn*-1,3 positions. Lee et al. [20] obtained the structured TAG OPO by interesterification of tripalmitin and ethyl oleate, catalyzed by lipase Lipozyme TL IM from *Thermomyces lanoginosus*. OPO-rich human milk fat substitute was synthesized with 80.6% PA at *sn*-2 position and 64.9% of oleic acid at *sn*-1,3 positions.

Most of these methods are based on TAGs rich in PA at *sn*-2 position, such as tripalmitin or lard. However in vegetable oils (such as palm oil), the main constituents of infant formulas, PA is located predominantly at the extreme positions [21]. Methods to produce TAGs rich in PA at *sn*-2 position are therefore of great potential industrial interest, since these TAGs are not produced on an industrial scale. Chen et al. [22] synthesized tripalmitin from glycerol and ethyl palmitate catalyzed by Novozym 435 under vacuum; about 88% conversion with 91% molar of tripalmitin was attained after 36 h of reaction. The ethyl palmitate used was previously obtained by a three-step process: (i) saponification of palm oil, (ii) low temperature fractionation of palm oil fatty acids and (iii) transformation of palmitic acid into ethyl palmitate.

Moreover, it is desirable that these products could be obtained in solvent-free media, as they are intended for infant feeding. The products used (tripalmitin, palmitic acid, palm oil, etc.) have high melting points and the PA rich TAGs present a low solubility in hexane at near room temperature. For these reasons high temperatures must be used for obtaining PA rich STAGs both in presence or absence of solvents, although higher temperatures must be used in solvent-free media; in addition, the stability of the lipases is lower at the high temperatures needed to keep the reaction mixtures homogeneous and fluid. Some authors have already begun the search for suitable conditions to obtain these products in total absence of solvent. Thus, for example, Yang et al. [23] obtained TAGs with 71% PA at *sn*-2 position and 44% oleic acid at *sn*-1,3 positions, by acidolysis of lard and free fatty acids from soybean oil; this reaction was carried out at 61 °C and using Lipozyme RM IM as 1,3 specific lipase. Sørensen et al. [24] also produced TAGs with a molecular structure and fatty acid composition very similar to that of human milk fat, by acidolysis of butterfat and a mixture of rapeseed and soybean oil fatty acids, catalyzed by Lipozyme RM IM, at 65 °C; these TAGs contained 46–56% PA at *sn*-2 position and 31–35% oleic acid of total fatty acids. After the acidolysis reaction these TAGs were purified by short path distillation and, therefore, also in absence of solvent.

The acidolysis or interesterification reactions are being carried out in several reactor types, although the most commonly used are the batch stirred tank reactor (STR) and the packed bed reactor (PBR). The former is easy to operate but the volumetric throughput is relatively low and for most large-scale catalytic reactions have traditionally been used the second one [25], because it facilitates the contact and subsequent separation, allows reuse of the enzyme without prior separation and an easy use for a continuous operation mode [26,27].

The aim of this work was to produce TAGs rich in PA at *sn*-2 position by acidolysis of palm stearin (60% PA) and two PA enriched FFAs (commercial palmitic acid, with 98% PA, and a FFA extract obtained by saponification of palm stearin, 60% PA) in two batch reactors: STR and PBR. Three non-regioselective lipases were tested to catalyze this reaction: Novozym 435, from *Candida antarctica*, and lipases QLM and QLC, both from *Alcaligenes* sp. An important objective of this work was also to produce these PA rich TAGs in absence of solvent, both in the enzymatic reaction and in the subsequent TAG purification.

2. Materials and methods

2.1. Lipases and chemicals

The chemicals used were palm stearin (PS, kindly donated by Brudy Technology S.L., Barcelona, Spain), whose fatty acid composition is shown in Table 1, commercial palmitic acid (98% purity, Panreac S.A., Barcelona, Spain), hexane (95%) and other reagents of analytical grade (Panreac S.A., Barcelona, Spain). The lipases tested to catalyze the acidolysis reaction were: Novozym 435 from *C. antarctica* (kindly donated by Novozymes A/S, Bagsvaerd, Denmark) and lipases QLM and QLC from *Alcaligenes* sp. (kindly donated by Meito Sangyo Co. Ltd., Japan). Novozym 435 is supplied immobilized on a macroporous acrylic resin. Usually this lipase does not show positional specificity and its recommended operating temperature range is 40–60 °C. Lipase QLM was supplied as powder, it is non-specific in transesterification reactions and its optimum temperature is 65–70 °C. Lipase QLC is produced by immobilizing lipase QLM on diatomaceous earth. Pancreatic lipase (type II from

Table 1
Fatty acid composition (mol%) of palm stearin (PS) and a PA concentrate obtained by crystallization [26].

Fatty acid	Palm stearin (PS)			PA concentrate[b]
	TAGs		DAGs[a]	Total[c]
	Total[c]	Position 2[d]	Total[c]	
14:0	1.4	n.d.	n.d.	1.6
16:0	60.0	23.0	58.0	75.1
18:0	5.1	0.7	n.d.	6.7
18:1n9	29.0	65.2	39.5	14.5
18:2n6	4.5	11.1	2.5	2.1
Percentage of lipidic species (mol%)	95.5		4.5	

[a] Diacylglycerols.
[b] Obtained by saponification of PS and crystallization of FFAs.
[c] Fatty acid content of total fatty acids.
[d] Fatty acid content of total fatty acids at *sn*-2 position of TAGs (composition of 2-MAGs applying the pancreatic lipase method).