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# Preparation of structured lipids enriched with medium- and long-chain triacylglycerols by enzymatic interesterification for infant formula

Sameh A. Korma<sup>a,b</sup>, Xiaoqiang Zou<sup>a,\*</sup>, Abdelmoneim H. Ali<sup>a,b</sup>,  
Sherif M. Abed<sup>a,c</sup>, Qingzhe Jin<sup>a</sup>, Xingguo Wang<sup>a</sup>

<sup>a</sup> State Key Laboratory of Food Science and Technology, National Engineering Research Center for Functional Food, National Engineering Laboratory for Cereal Fermentation Technology, Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province, School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, Wuxi 214122, Jiangsu, PR China

<sup>b</sup> Department of Food Science, Faculty of Agriculture, Zagazig University, 44511 Zagazig, Egypt

<sup>c</sup> Food and Dairy Science and Technology Department, Faculty of Environmental Agricultural Science, El Arish University, 43511 El Arish, Egypt

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## ABSTRACT

Single cell oils (SCOs) are considered as important sources of the essential fatty acids (EFAs) such as arachidonic acid (ARA), which is of great importance for infant's growth and development. Medium-chain triacylglycerols (MCTs) induce the residual glyceride lipolysis, which improve fat absorption, and thus reduce its deposition in infants. Medium- and long-chain triacylglycerols (MLCTs)-rich structured lipids (SLs) combine these benefits, and they can be delivered to infants in an easy way. In this study, MLCTs-rich SLs were synthesized by lipase-catalyzed interesterification of ARASCO with MCTs in a solvent-free system. Four commercial immobilized lipases from different sources were compared for their efficiency in the production of MLCTs yield. The results indicated that the highest yield of MLCTs was achieved by Lipozyme 435 from *Candida antarctica* as a biocatalyst. The best reaction conditions were as follows; enzyme load 8% (w/w), reaction temperature 90 °C, reaction time of 3 h, and substrates mole ratio 1:1. Under these conditions, MLCTs were obtained in a yield of 53.75%. Thermoprofile displayed that the MLCTs-rich SLs melted below body temperature of 37 °C. The obtained MLCTs-rich SLs can be suggested as a nutritional and functional fat analogue with potential applications in infant formula industry.

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**Abbreviations:** SCOs, single cell oils; LC-PUFAs, long-chain polyunsaturated fatty acids; EFAs, essential fatty acids; ARA, arachidonic acid; FA, fatty acid; LCTs, long-chain triacylglycerols; MCTs, medium-chain triacylglycerols; MCFAs, medium-chain fatty acids; LCFAs, long-chain fatty acids; SLs, structured lipids; MLCTs, medium- and long-chain triacylglycerols; TAGs, triacylglycerols; MLM, medium-, long- and medium-chain-type MLCTs; MML, medium-, medium- and long-chain-type MLCTs; LMM, long-, medium- and medium-chain-type MLCTs; LML, long-, medium- and long-chain-type MLCTs; MLL, medium-, long- and long-chain-type MLCTs; LLM, long-, long- and medium-chain-type MLCTs; MAG, monoacylglycerol; DAG, diacylglycerol; LDL, low density lipoprotein; HDL, high density lipoprotein; FFAs, free fatty acids; RP-HPLC, reverse-phase high-performance liquid chromatograph; ELSD, evaporative light scattering detector; GC, gas chromatography; ECN, equivalent carbon number; CN, total carbon number without glycerol; DB, number of double bonds in the fatty acid; FID, flame ionization detector; DSC, differential scanning calorimeter.

\* Corresponding author.

E-mail address: [xiaoqiangzou@163.com](mailto:xiaoqiangzou@163.com) (X. Zou).

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## 1. Introduction

Single cell oils (SCOs) have attracted increasing attention due to their high content of the long-chain polyunsaturated fatty acids (LC-PUFAs) including essential fatty acids (EFAs) such as arachidonic acid (ARA), which is of great importance from the pharmaceutical, biological, and nutritional viewpoints. ARASCO is a triglyceride oil contains high amounts of ARA (20:4 n-6), the fungus *Mortierella alpina* is considered the most producer of it, and it has been extensively used in the industrial applications (Dyal and Narine, 2005; Ji et al., 2014). ARA is one of the omega-6 LC-PUFAs, exists in human milk, and has been added to infant formula for more than two decades as it is an important fatty acid (FA) for normal growth, visual acuity, and brain development of infants. Moreover, ARA plays a unique role in many physiological functions in human health, for instance a precursor of eicosanoid hormones (prostaglandins, leukotrienes, and thromboxanes). Meanwhile, it has a role in dealing with or preventing several human diseases (Archer et al., 2008; Ratledge, 2004; You et al., 2011). On account of its unique biological properties, it has been widely used in infant formula preparation, medicine, cosmetics, pharmacology, and food industry (Eroshin et al., 2000).

In spite of the essential properties and functions possessed by long-chain triacylglycerols (LCTs), they are metabolized slowly and mostly tend to be deposited in human adipose tissue. In contrast, medium-chain triacylglycerols (MCTs) regarded as a quick energy source via oxidation of the more hydrophilic medium-chain fatty acids (MCFAs), and have lower tendencies to be deposited in the adipose tissue due to their direct transportation through the portal vein to the liver instead of via the lymphatic system (Lee et al., 2012a). It was reported that MCFAs, such as capric acid provide infants with the protection against the harmful microorganisms, since they possessed antimicrobial and antiviral features (Álvarez and Akoh, 2015). However, the use of pure MCTs as a dietary MCFAs source for a long time may lead to gastrointestinal problems, for instance gastric discomfort, cramps, nausea, abdominal pain, and diarrhea. Furthermore, it cannot provide human with the EFAs (Akoh and Min, 2008; Yang et al., 2014). In order to overcome the undesired side effects of both LCTs and MCTs, it was imperative to produce specific structured lipids (SLs) retaining their benefits together. Therefore, medium- and long-chain triacylglycerols (MLCTs) were produced in this study by the enzymatic interesterification of ARASCO with MCTs in a solvent-free system.

Digestion of lipids requires firstly to be emulsified by bile salts in the intestinal to enable hydrolysis by pancreatic lipases. Compared with adults, the concentration of bile salts and the activity of pancreatic lipases in infants are very low. Thus, it was of great relevance to obtain SLs containing EFAs with easy absorption features for infants (Nguyen et al., 2015). MLCTs are a kind of modified lipids where the individual triacylglycerol (TAG) contains both MCFAs and LCFAs attached on the same glycerol backbone (Kasai et al., 2003). MLCTs mainly consisted of six types of TAG, namely MLM, MML, LMM, LML, MLL, and LLM (where M refers to medium-chain fatty acids, and L refers to long-chain fatty acids). The most typical type of MLCTs is MLM, in which MCFAs are esterified at sn-1,3 positions and LCFAs at the sn-2 position (Mu and Porsgaard, 2005). MLM-type is considered to be the desired structure of MLCTs since it can act as an effective carrier of LCFAs as com-

pared to the other types. That is, the 2-monoacylglycerols (2-MAG) retaining LCFAs are produced by pancreatic lipase digestion during the metabolism of MLM-type, and are well absorbed through the intestinal wall (Jandacek et al., 1987). Recently, numerous studies have reported various beneficial effects of MLCTs on human health. For example, the consumption of MLCTs diet could effectively reduce the body weight, fat accumulation, reducing the total cholesterol, the bad cholesterol (low density lipoprotein, LDL), and keeping the good cholesterol (high density lipoprotein, HDL), mainly due to the presence of MCFAs (Bourque et al., 2003; St-Onge et al., 2003; Zhao et al., 2014). As well, MLCTs have the ability to provide EFAs, which would be useful in the alleviation of malabsorption syndromes. Compared with the physical mixture of MCTs and LCTs, MLCTs have greater control in the release of MCFAs into the bloodstream (Koh et al., 2010). Most of the previous studies on MLCTs were conducted to provide the most effective means of delivering the required FAs for nutritional purposes, and for targeting specific diseases and metabolic conditions (Koh et al., 2010).

MLCTs can be produced using lipase catalysis via interesterification, acidolysis, or esterification processes (Lee et al., 2015; Öztürk et al., 2010; Zhao et al., 2007, 2014). Enzymatic acidolysis requires higher amounts of the free fatty acids (FFAs) as a substrate to increase the conversion of the starting oil or TAGs into MLCTs (Sahin et al., 2005; Zou et al., 2014). The use of high ratio of MCFAs creates an acidic condition in the reaction. As a result, the enzyme activity will reduce, and therefore decrease the yield of MLCTs. In addition, a high proportion of FFAs in the crude product. The remaining FFAs should be removed to get purified MLCTs, resulting in the loss of starting MCFAs and increasing costs. Likewise, the remaining FFAs are also high when esterification is used for preparing MLCTs with a high cost of FAs and glycerol (Lee et al., 2012b). Esterification processes lead to the formation of fractional glycerides, such as MAG and diacylglycerol (DAG) due to incomplete interaction. Commonly, the content of the fractional glycerides obtained via esterification is higher than that obtained via acidolysis and interesterification. It is well known that the process of removing the fractional glycerides is more complex than the elimination of FFAs from the crude product. Therefore, in comparison to acidolysis and esterification, interesterification is more preferred method of producing MLCTs in terms of its excellent selectivity, mild reaction conditions, lower byproducts (FFA, MAG and DAG), and easy recovery of catalysts (Yang et al., 2014).

Shimada et al. (1998) studied the enzymatic enrichment of ARA from *Mortierella* SCO by a two-step enzymatic method: (a) hydrolysis of ARA-containing oil with *Pseudomonas* sp. lipase to obtain a polyunsaturated-rich fatty acid fraction, and (b) selective esterification of the resulting FFA with lauryl alcohol using *Candida rugosa* lipase. As far as we know, this is the first study used ARASCO with MCTs as TAGs to prepare MLCTs-rich SLs by lipase-catalyzed interesterification in a solvent-free system. In order to achieve that purpose, a wide range of parameters, including the choice of enzyme type, enzyme load, reaction temperature, reaction time, and substrates molar ratio were investigated. After the enzymatic interesterification, reverse-phase high-performance liquid chromatograph (RP-HPLC) equipped with an evaporative light scattering detector (ELSD) and gas chromatography (GC) were applied to analyze the produced MLCTs-rich SLs. As well, the melting and crystallization profiles of the resultant SLs were determined.

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