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Intensified synthesis of structured lipids from oleic acid rich moringa oil in the presence of supercritical CO_2

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ABSTRACT

Structured lipids have been synthesized using different medium chain fatty acids based on the reaction with long chain triglyceride viz. moringa oil, in the presence of supercritical CO_2. Effect of different operating parameters related to supercritical CO_2 approach on the product yields has been studied also establishing the effect of type of biocatalyst (using two forms of commercial lipases as Novozym 435 and Lipozyme RM). It was observed that 100 bar pressure at 50 °C temperature with 300 rpm speed, molar ratio of 4:1 (medium chain fatty acids: long chain triglycerides), reaction time as 5 h and Novozym 435 as the catalyst were the best conditions giving 63.2% yield which was much higher as compared to the conventional approach where only 28.2% yield was obtained. After acidolysis, the residual unreacted triglycerides were extracted using hexane and allowed to react again with free corresponding medium fatty acids under same reaction conditions. After complete reaction with medium fatty acids, product obtained was structured lipids with medium chain fatty acids at 1,3 position and long chain fatty acid at 2-position. The enzyme reusability studies confirmed that enzyme was efficient up to 15 cycles providing a cost effective proposition. Overall, it was established that structured lipids possessing significant nutritional benefits could be effectively synthesised using green intensified approach based on supercritical CO_2.

1. Introduction

Structured lipids (SL) are reorganized form of lipids offering added nutritional benefits, and hence find applications in various food and nutraceutical products. SL can be produced either enzymatically or chemically (Akoh, 2002). Chemical method is inexpensive compared to enzymes but lacks specificity. Enzyme catalysed synthesis of structured lipids is favourable over chemical method because of its specificity and green nature (Kim and Akoh, 2006). Biocatalysts offer high specific activity and a low impact on the environment (Basri et al., 1996). Structured lipids can be synthesized by interesterification, acidolysis, and alcoholysis methods (Kapoor and Gupta, 2012). In the present work, structured lipids have been synthesized by acidolysis of long chain triglyceride (LCT) with medium chain fatty acids (MCFA) catalyzed by lipase with intenification studies based on supercritical carbon dioxide.

SL are generally synthesized from Medium chain triacylglycerols (MCT) and long chain triglycerides. MCT are easily absorbed in the body cells and break down quickly which drives the important applications in the areas of treatment of malabsorption syndrome cases, infant care and also offer as a high energy nutrient source (Babayan, 1987). The ease of solubility and rapid metabolism of MCT provide health benefits when incorporated into SL (Jennings and Akoh, 2009). MCT are utilized quickly as energy source, not stored in adipose tissue as fat and are metabolized

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through the portal system instead of the lymphatic system (Jennings and Akoh, 2000). Even though MCT is a fast energy source with wide variety of applications, it lacks presence of essential fatty acids. Considering this lacunae, combination of MCT with essential fatty acids can be a excellent way of obtaining very effective SL. One such source of fatty acids is moringa oil (MO), which is rich in oleic acid (OA) and posses various health benefits. Oleic acid reduces blood pressure, aids weight loss and protects cells from free radical damage (Teres et al., 2008). MO is extracted from the seeds of the tree. *Moringa oleifera* seeds contain a high proportion of oil that makes the plant as a major source of oil for edible as well as non-edible purposes. MO typically consists of 70–78% oleic acid similar to that of olive oil (Nestel et al., 1994). The high percentage of oleic acid in the oil makes it desirable in terms of nutrition, health friendly nature and the offered high stability makes it ideal for use as cooking and frying oil (Tsaknis et al., 1998). The high oleic acid content of the oil also confers it a functional food property as oils containing high oleic content have been shown to reduce risks of coronary heart disease. MO is highly resistant to autoxidation and can be used as a replacement of synthetic oxidants (Nadeem et al., 2014). MO also provides very good oxidative and thermal stability because of presence of high oleic acid and can be used as a substitute to commercial raw and refined edible oils. MO is hence considered as very stable and healthy substitute for commercial groundnut oil as a cooking and frying medium (Babatunde et al., 2014). *M. oleifera* is food crop typically with all the parts of the plant considered useful for human and animal consumption after some processing. In India, the annual production of *M. oleifera* fruits is 1.1–1.3 million tonnes, which can yield about 9500 kl of oil. Thus, it can be said that MO is also available in good amount (though the cost is higher as compared to other oils like groundnut, sunflower or palm; off course the quality attributes can compensate the cost) and hence it can prove as a potential source of value addition especially if used in small proportions in SL giving significant quality attributes (Mathur, 2014). MO contains all the main fatty acids similar to that of olive oil, and therefore, can be used as a possible substitute to the expensive olive oil (Abdulkarim et al., 2005), which is commonly used currently. The properties of MO can be highly advantageous particularly with the current trend of replacing polyunsaturated vegetable oils with the better substitutes in the production of commercial products with value addition (Corbett, 2003). Considering this analysis, MO was selected as rich source of oleic acid in the present work aimed to utilize MCT and oleic acid rich MO in synthesis of SL that can offer combined health and nutritional benefits.

There has been an increased interest in the biosynthesis of SL especially the form containing long chain fatty acids, at the sn-2 position, as well as MCFA at the sn-1,3 positions, often described as MLM-SL (Ingle et al., 1999). Additional health benefits such as improved nitrogen balance and reduction in cancer risk are shown by these types of SL (Akoh and Kim, 2008). Use of SLs in diet also causes significant reduction in accumulated body fat and serum cholesterol (Kasai et al., 2003). Thus, structured lipids synthesized using medium chain fatty acids and essential fatty acids offer increased health benefits and hence the importance of the present work is established.

Conventional method of synthesis of SL includes use of organic solvents like hexane (Jennings and Akoh, 2000) typically requiring reaction time of more than 24 h. Although organic solvents in the presence of enzymes show good results in terms of yield compared to solvent free conditions but the adverse effect on environment confine the use of such SL in food and health related applications. Use of supercritical CO_2 (SC-CO_2) to replace the organic solvents is a green approach for synthesis of structured lipids. SC-CO_2 mediated enzymatic reaction offers an intensification approach because of advantages of high diffusivity, low surface tension, and low viscosity that induces accelerated mass transfer (Habulin et al., 1999; Oliveira and Oliveira, 2000). A review of literature revealed that there are some reports dealing with use of SC-CO_2 in synthesis of structural lipids. Kim et al. (2004) investigated enzymatic alcoholysis of Palm Kernel Oil in SC-CO_2 and reported 26.4% as the obtained yield after 4 h of reaction. Sellappan and Akoh (2001) studied synthesis of SL from trilinolein and caprylic acid catalyzed using lipase Lipozyme IM. Analysis of the literature also revealed that the use of MCT and moringa oil combination has not been investigated at all though it offers significant benefits as discussed earlier. Also the comparison of different types of lipase enzymes is lacking in the literature for the synthesis of SL. Considering these aspects, the novelty of the work dealing with intensified synthesis of SL from MCT and moringa oil in the presence of different lipases is clearly established. The work also presents kinetic analysis which gives important design related information for possible commercial scale operations.

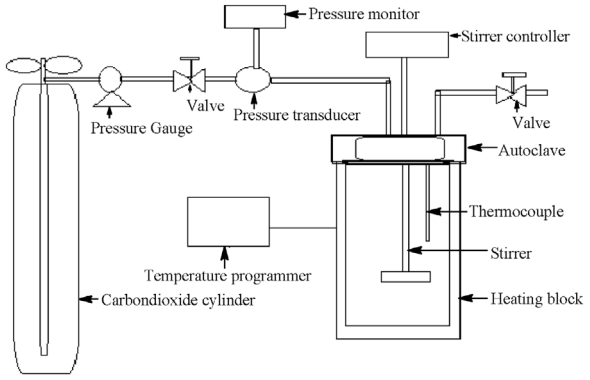

Fig. 1 – Schematic representation of the experimental setup.

2. Materials and methods

2.1. Materials

The different medium chain fatty acids used such as caprylic acid (99.9%), capric acid (99.9%) and lauric acid (99.9%) were procured from Hi-Media, (India) Ltd. Mumbai. Samples of enzymes were obtained from Brenntag India Pvt., Ltd. Mumbai as gift samples. Moringa oil was obtained from Earth Expo Pvt., Ltd. (India), Gujarat. Sodium thiosulphate, potassium iodide, potassium hydroxide and starch were procured from S.D Fine-Chem Pvt., Ltd., Mumbai, India.

2.2. Reaction scheme

The reaction considered for the present study is an acidolysis reaction between medium chain fatty acids (caprylic acid-CpA, capric acid-CA and lauric acid-LA) with moringa oil in the presence of enzymes (*Novozym* 435 (N435) and *Lipozyme RM* (LRM)) as the catalyst. Supercritical carbon dioxide is applied as green solvent and as process intensification approach. The reaction scheme can be represented as follows:

$$\text{Medium chain fatty acids} + \text{Moringa Oil} \xrightarrow[\text{Supercritical } CO_2]{\text{Bio-catalyst}} \text{Structured Lipid}$$

2.3. Experimental setup

The reactor used in the work is a jacketed stainless steel vessel with 600 ml capacity (A2560HC13EE, Parr Instruments Co., USA) as depicted in Fig. 1. The temperature of reaction vessel, pressure and rotating speed were controlled using Parr 4848 controller with the values being shown on digital display. The sampling line for the liquid phase was located at the top of the reactor and the withdrawn samples were used for HPLC analysis. The desired pressure was maintained by pumping CO_2 into the reactor and temperature was achieved by heat-