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# Lipase-catalysed interesterification between canola oil and fully hydrogenated canola oil in contact with supercritical carbon dioxide

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ABSTRACT

The processing parameters in enzymatic reactions using $CO_2$-expanded (CX) lipids have strong effects on the physical properties of liquid phase, degree of interesterification, and physicochemical properties of the final reaction products. CX-canola oil and fully hydrogenated canola oil (FHCO) were interesterified using Lipozyme TL IM in a high pressure stirred batch reactor. The effects of immobilised enzyme load, pressure, substrate ratio and reaction time on the formation of mixed triacylglycerols (TG) from trisaturated and triunsaturated TG were investigated. The optimal immobilised enzyme load, pressure, substrate ratio and time for the degree of interesterification to reach the highest equilibrium state were 6% (w/v) of initial substrates, 10 MPa, blend with 30% (w/w) of FHCO and 2 h, respectively. The physicochemical properties of the initial blend and interesterified products with different FHCO ratios obtained at optimal reaction conditions were determined in terms of TG composition, thermal behaviour and solid fat content (SFC). The amounts of saturated and triunsaturated TG decreased while the amounts of mixed TG increased as a result of interesterification. Thus, the interesterified product had a lower melting point, and broader melting and plasticity ranges compared to the initial blends. These findings are important for better understanding of CX-lipid reactions and for optimal formulation of base-stocks of margarine and confectionary fats to meet industry demands.



## 1. Introduction

Partial hydrogenation of vegetable oils for the production of base-stock for margarine and shortening leads to the formation of *trans*-fatty acids, which are known to increase the risk of cardiovascular diseases. Therefore, considerable effort has been made to find suitable alternative methods to produce *trans*-free base-stocks for various food applications. One alternative method can be blending of a fully hydrogenated oil rich in tristearin with a vegetable oil rich in triolein, followed by chemical or enzymatic interesterification (Ahmadi, Wright, & Marangoni, 2008; Gavriilidou & Boskou, 1991; List, Mounts, Orthoefer, & Neff, 1995; Seriburi & Akoh, 1998). However, in enzymatic interesterification, using regiospecific (1,3- or 2-specific (Rogalska, Cudrey, Ferrato, & Yerger, 1993; Sugihara, Shimada, & Tominaga, 1991)) and fatty acid specific lipases as catalysts, the positioning of acyl groups on the glycerol back bone of triacylglycerols (TG) is controlled and a desired acyl group can be guided to a specific position. This results in products with predictable composition in contrast to chemical interesterification. Therefore, enzymatic interesterification with lipases of different properties is becoming more attractive to convert some commodity oils such as soybean oil, rapeseed oil, lard and tallow, among others, to high value products and modified fats (Liu, Cheng, Chang, & Shaw, 1997; Macrae, 1983; Miller, Blanch, & Prausnitz, 1991; Xu, 2003).

Enzymatic interesterification of canola oil and fully hydrogenated canola oil (FHCO) can be used to produce base-stocks for margarines. In contrast to myristic and palmitic acids, which raise serum cholesterol levels, stearic acid is viewed as being neutral on serum cholesterol levels (Bonanome & Grundy, 1988; Kris-Etherton et al., 2001). Stearic acid is a long chain fatty acid (C18) with a melting point of 70 °C. Therefore, TG with high amounts of this fatty acid have poor absorption in the human body due to their melting point being higher than the body temperature and poor formation of emulsion and micellar solubilization in the intestinal tract (Bonanome & Grundy, 1988; Hashim & Babayan, 1978). Thus, stearic acid is an excellent substrate for making structured lipids with reduced calories (Finley, Klemann, Leveille, Otterburn, & Walchak, 1994) and some products are taking advantage of this approach.

Sustainable technologies have emerged in lipid processing because of strict environmental regulations related to the use of organic solvents as well as the increase in consumer demand for

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'natural' products. Supercritical $CO_2$ ($SCCO_2$) is an alternative technology to reduce the use of organic solvents (Seifried & Temelli, 2010b) that has several advantages. The transport properties of $SCCO_2$, including viscosity and diffusion coefficient, are intermediate between those of gases and liquids. These properties lead to greatly reduced mass transport limitations for reactions conducted in $SCCO_2$. In addition, the non-flammability and low cost of $CO_2$ have made it the most popular medium for investigation (Chi, Nakamura, & Yano, 1988; Miller, Blanch, & Prausnitz, 1990; Rezaei, Temelli, & Jenab, 2007).

The solubility of triacylglycerols in $SCCO_2$ at moderate temperatures and pressures are relatively low, but it is still possible to benefit from the properties of $SCCO_2$ by dissolving it in the liquid lipid phase. As the pressure is increased, $CO_2$ dissolves in the liquid lipid phase. Lipids saturated with $CO_2$ under moderate pressure expand in volume and their physical properties, such as viscosity, density, and interfacial tension, change substantially in a way to enhance mass transfer properties (Jenab & Temelli, 2011; Seifried & Temelli, 2009, 2010a, 2011).

Studying the effect of processing parameters on the interesterification of $CO_2$-expanded (CX) canola oil and FHCO is important in order to find the optimal reaction conditions and to better understand interesterification under $SCCO_2$ conditions. Furthermore, the physicochemical properties of the final product need to be investigated to assess whether these are suitable for commercial applications. There have been some studies about enzymatic interesterification of oils under $SCCO_2$, such as for producing a cocoa butter analogue (Shekarchizadeh, Kadivar, Ghaziaskar, & Rezayat, 2009), structured lipids (Kim et al., 2004) and interesterification of vegetable oils and fatty acids (Liang, Chen, & Liang, 1998; Yu, Rizvi, & Zollweg, 1992). However, the literature lacks information on the enzymatic interesterification of canola oil and FHCO under $SCCO_2$ and the physicochemical properties of the final products. Therefore, the objectives of this study were: (a) to determine the effects of enzyme load, pressure, and the ratio of initial reactants on the degree of interesterification of the reaction under $SCCO_2$; (b) to determine chemical composition of the enzymatically interesterified products; and (c) to assess physical properties such as melting behaviour and solid fat content of the products obtained at optimal reaction conditions.

## 2. Materials and methods

### 2.1. Materials

Lipozyme TL IM, generously provided by Novozymes (Novozymes North America Inc., Franklinton, NC, USA) was used for the interesterification between canola oil and FHCO. Lipozyme TL IM is a lipase from *Thermomyces lanuginosus*, immobilised on a granulated silica carrier. FHCO was kindly provided by Richardson Oilseed Ltd. (Lethbridge, AB, Canada) and canola oil manufactured by the same company was purchased from a local market. The fatty acid composition of this canola oil and FHCO has been reported previously (Jenab & Temelli, 2012).

$CO_2$ (bone dry, Syphon UN 1013 Class 2.2) and $N_2$ (extra dry, UN 1046 Class 2.2) were purchased from Praxair Canada Inc. (Mississauga, ON, Canada). All analytical grade solvents were from Fisher Scientific (Ottawa, ON, Canada) and GC and HPLC lipid standards and internal standards were purchased from Nu-Chek Prep Inc. (Elysian, MN, USA).

The prepared oil mixtures and the enzymes were kept in a desiccator at 4–5 °C to prevent further moisture absorption. The reaction products, blanketed with nitrogen, were kept at −20 °C prior to analysis.

### 2.2. Process optimization

#### 2.2.1. Experimental set up and reaction protocols

Lipase-catalysed interesterification of $CO_2$-expanded canola oil and FHCO was conducted in batch mode in a Nova Swiss (Nova-Werke AG, Effretikon, Switzerland) high pressure, electrically heated, magnetically stirred 200 mL autoclave setup as shown in Fig. 1. Lipozyme TL IM (2%, 6%, and 10% (w/v) of initial reactants) was loaded inside the vessel, which was heated to around 65 °C. A total volume of 70 mL of substrates, consisting of canola oil and FHCO blend prepared at different ratios (10, 30, and 50 wt.% of FHCO) was added to the autoclave using a syringe. Once the autoclave was sealed, it was gently purged with $CO_2$ and then the autoclave was pressurized to 10, 17.5, and 25 MPa using a syringe pump (Model 260D, Teledyne Isco, Lincoln, NE, USA). After reaching a stable temperature and pressure the magnetic stirrer was turned on. The sampling tube was filled with the reaction mixture ($\leqslant$0.2 mL) using a dip tube with a filter at the end after closing valves 12 and 13 and opening valve 11 (Fig. 1). The sampling port on the system made sample collection possible without significantly affecting the temperature and pressure inside the autoclave. During sample collection, the sampling tube was first washed by injecting hexane at 50 °C through the washing port to remove any residual material left and then dried by blowing air prior to taking the next sample.

In order to see the effect of $CO_2$ pressure, interesterification of canola oil and FHCO at optimal enzyme load and FHCO level in the blend was also conducted at atmospheric pressure using the same reactor but without the sampling part and under a blanket of $N_2$. The samples were taken at different time intervals throughout the 8 h of reaction using a syringe through one outlet port of the reactor after stopping the stirrer for 1 min to let the immobilised enzymes settle to the bottom of the cell. The time intervals for the six samples were adjusted for each treatment depending on the time needed to reach equilibrium.

#### 2.2.2. Determination of degree of interesterification

The degree of interesterification between canola oil and FHCO was defined as the number of moles of 1,3-distearoyl-2-oleoyl-glycerol

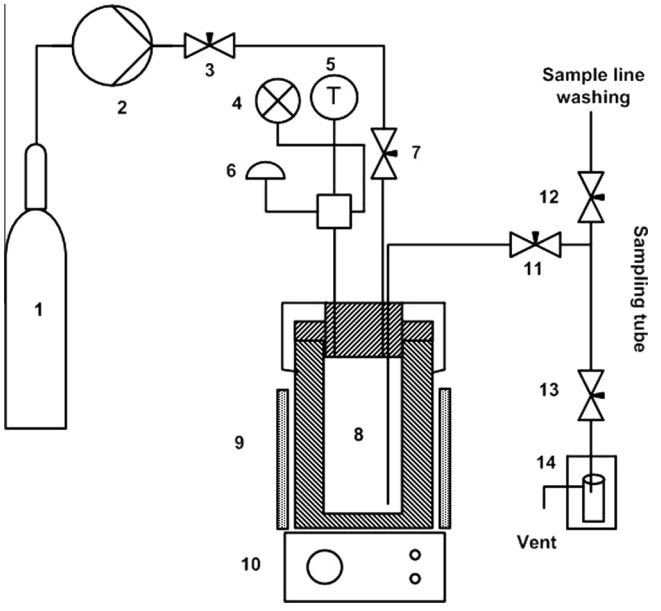


**Fig. 1.** High pressure batch stirred reactor: 1, $CO_2$ cylinder; 2, syringe pump; 3, 7, 11, 12, and 13, shut-off valve; 4, pressure gauge; 5, temperature controller; 6, Rupture disc; 8, high pressure vessel; 9, heating jacket; 10, stirrer; 14, collector.