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Performance of two immobilized lipases for interesterification between canola oil and fully-hydrogenated canola oil under supercritical carbon dioxide

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ABSTRACT

The performance and stability of Lipozyme RM IM and TL IM for the interesterification between canola oil and fully-hydrogenated canola oil (FHCO) in the presence of $SCCO_2$ were studied using a high pressure batch stirred reactor at 65 °C/17.5 MPa. The influence of exposure time (4, 8, and 12 h) in $SCCO_2$ and pressurization/depressurization cycles of up to 12 times on the enzyme's resultant activity were studied. There was no significant difference ($p > 0.05$) in the performance of the two enzymes over reaction time, reaching a constant degree of interesterification of about 23% after 2 h. Although FE-SEM images illustrated some morphological changes on the surface of the enzymes after 4 cycles of 7 h each, similar degrees of interesterification were achieved after each cycle. However, the amount of reaction intermediates decreased by 50–60% in the product obtained by using $SCCO_2$-treated enzymes after 12 pressurization/depressurization cycles compared to untreated enzymes, while there were no significant changes in the conformational and morphological structure of the treated enzymes based on FTIR and FE-SEM analysis. Findings enhance our understanding of enzymatic conversions of lipids under high pressure CO_2, targeting production of base-stock for zero-*trans* margarines.

1. Introduction

The functional, physicochemical and nutritional properties of triglycerides (TG) are determined by the nature and type of fatty acids in their structures. The most abundant TG in canola oil is triolein (Ratnayake & Daun, 2004). As is also the case for other vegetable oils, canola oil is sometimes modified in order to enhance its physicochemical properties to meet the specifications for certain food applications. For example, it may be desirable to produce a solid fat, which can be achieved by partial hydrogenation of an unsaturated oil. However, the hardened fats produced in this manner contain *trans*-isomers, which are known to be undesirable from nutritional standpoint since they have been shown to be a major risk factor for cardiovascular disease (Combe, Clouet, Chardigny, Lagarde, & Leger, 2007; Roos, Schouten, Scheek, van Tol, & Katan, 2002). Interesterification is an alternative approach for hardening oils by incorporating fully hydrogenated or stearin fractions, thereby eliminating the formation of *trans*-isomers. Although the presence of saturated fatty acids can have a negative effect on blood lipid profile, and their consumption is associated with a higher risk of cardiovascular diseases, some reports suggest that stearic acid is an exception, having a lower level of intestinal absorption and hence not affecting blood lipid profile negatively (Valenzuela, Delplanque, & Tavella, 2011).

The most common type of interesterification employed for margarine and confectionary fat production is chemical interesterification, in which metal alkylate catalysts are used. This process leads to a fully random TG structure because there is no selectivity for fatty acid position on the glycerol backbone. Also, the catalysts used in this process are toxic and lead to darkening of the final products; therefore, these color compounds and catalysts need to be removed by downstream processes. On the other hand, lipase-catalyzed interesterification can take place under milder conditions with fewer side reactions, leading to products with less refining requirement (Marangoni & Rousseau, 1995; Xu, 2003).

Conducting reactions in supercritical fluids (SCF), especially supercritical carbon dioxide ($SCCO_2$), has been shown to have advantages. This is due to several factors: (1) SCF allow high mass transfer rates due to their high diffusivity and low viscosity; (2) the solvation power of SCF and their performance as reaction media are

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a strong function of temperature and pressure; and (3) SCF are also easily removed after reaction by reducing pressure (Jenab, Rezaei, & Emam-Djomeh, 2006; Nakamura & Hoshino, 1992; Ramsey, Sun, Zhang, Zhang, & Gou, 2009; Rezaei, Temelli & Jenab, 2007). Supercritical fluids can be used as reaction media in lipase-catalyzed lipid reactions since the low viscosity and high diffusivity of SCF enhance transport of substrates and products through the pores of enzyme support. This leads to easier access of substrates and removal of products to and from the active sites of the enzyme, resulting in higher reaction rates in SCF compared to those in organic solvent or solvent-free reaction systems (Rezaei, Jenab & Temelli, 2007; Rezaei, Temelli & Jenab, 2007). However, the solubility of vegetable oils in $SCCO_2$ alone is such that high pressures and temperatures are required in order to conduct the reaction in a supercritical solution, making the process less feasible. Alternatively, solvent-free enzymatic reactions can be conducted by dissolving the $SCCO_2$ in the liquid lipid phase and having a CO_2-expanded lipid (CX-lipid) phase to improve the mass transfer properties (Jessop & Subramaniam, 2007; Seifried & Temelli, 2010; Subramaniam, 2010).

Lipase-catalyzed reactions are desirable because of enzyme specificity and their selectivity towards fatty acid positions on the glycerol backbone (Marangoni & Rousseau, 1995). However, the stability and activity of enzymes under high pressure CO_2 depends on many parameters including the type of enzyme, pressure and temperature of the reaction system, exposure time, depressurization rate, the number of pressurization/depressurization steps, and the nature of enzyme support (Wimmer & Zarevucka, 2010). In high pressure batch stirred reactors, the exposure time to $SCCO_2$ and the number of pressurization/depressurization steps can have an important effect on enzyme efficiency (Hlavsova et al., 2008). However, such parameters have not been investigated for the interesterification between canola oil and fully-hydrogenated canola oil (FHCO).

The objectives of this study are: (a) to determine the performance of two immobilized lipases, Lipozyme TL IM and RM IM, under $SCCO_2$ for interesterification between canola oil and FHCO, (b) to assess the reusability of both enzymes for 4 cycles of 7 h of interesterification using $SCCO_2$ at 65 °C and 17.5 MPa, (c) to investigate the effects of incubation of immobilized lipases in $SCCO_2$ (4, 8 and 12 h) and pressurization/depressurization cycles (4, 8 and 12 times) at 65 °C and 17.5 MPa on their efficiency of lipid interesterification and morphology. This study is complementary to our previous work (Jenab & Temelli, 2011, 2012) in which the physical properties of CO_2-expanded canola oil and its blend with FHCO were investigated. The long-term objective is to explore interesterification between canola oil and FHCO using $SCCO_2$ for the production of zero-*trans* margarines.

2. Materials and methods

2.1. Materials

Lipozyme RM IM, purchased from Sigma Aldrich (Oakville, ON, Canada) and Lipozyme TL IM, generously provided by Novozymes (Novozymes North America Inc., Franklinton, NC, USA) were used for the interesterification between canola oil and FHCO. Lipozyme RM IM is derived from lipases of *Mucor miehei* immobilized on Duolite ES 562, a macro-porous ion-exchange resin based on phenol-formaldehyde copolymer as a support matrix. The moisture content of Lipozyme RM IM was 3.3 ± 0.2 g/100 g, determined by using a gravimetric method based on drying in an oven at 110 °C for 5 h. The average particle size of Lipozyme RM IM was 590 ± 170 μm (d_{10} = 370 μm, d_{90} = 810 μm) measured by a laser diffraction particle size analyzer (LS 13 320, Beckman Coulter, CA, USA). Lipozyme TL IM is from lipases of *Thermomyces lanuginosus*, immobilized on a granulated silica carrier with a moisture content of 4.7 ± 0.5 g/100 g and average particle size of 510 ± 180 μm (d_{10} = 280 μm, d_{90} = 750 μm). These two *sn*-1,3 stereo-specific enzymes are immobilized in large scale production and easily accessible. 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 compositions of canola oil and FHCO have 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. Lipase-catalyzed interesterification

The experimental apparatus (Phase equilibria unit, Sitec Sieber Engineering, Zurich, Switzerland) used for conducting the enzymatic reactions in equilibrium with $SCCO_2$ has been illustrated in detail elsewhere (Jenab & Temelli, 2011). Immobilized lipase (10 g/100 g of initial reaction mixture of canola oil and FHCO) was added to the previously blended mixture of canola oil and FHCO (70 g of canola oil/100 g of blend) (≈2 g) in a stainless steel cylindrical basket. Then, the basket containing the oil blend, enzyme, and a magnetic bar was placed into the 10 mL high pressure cell that had been previously heated to 65 °C. Then, the pressure of the cell was raised to the target pressure of 17.5 MPa with CO_2 using a syringe pump (Model 260D, Teledyne Isco, Lincoln, NE, USA) and monitored by a pressure transducer. The temperature of the cell, measured by a thermocouple located at the inner wall of the cell, was maintained at 65 °C and controlled by circulating hot water in the jacket surrounding the cell. The temperature of 65 °C was selected in order to ensure a homogeneous liquid oil mixture in the reactor throughout the reaction, whereas the pressure of 17.5 MPa was selected as an intermediate pressure between 10 and 30 MPa used commonly for enzymatic reactions. At the end of the targeted reaction time, the reactor was depressurized carefully and the sample was filtered (Whatman filter paper no. 4) to remove the enzyme. Then, hot (≈65 °C) hexane was used to wash any oil residue from the enzyme.

In order to assess the stability and reusability of the enzymes, residual hexane was removed by purging a gentle stream of nitrogen through the filtered enzyme and then the enzyme was placed again inside the reactor and the reaction protocol was repeated using the same amount of oil blend. The enzymes were reused 4 times for 7 h each at 17.5 MPa and 65 °C for the interesterification of canola oil and FHCO.

2.3. Activity of immobilized lipases exposed to $SCCO_2$ and pressurization/depressurization cycles

In order to study how the exposure time to $SCCO_2$ affects the activity of immobilized lipases, both Lipozyme RM IM and TL IM were incubated in $SCCO_2$ at 17.5 MPa and 65 °C for 4, 8 and 12 h using the same high pressure cell described above. Immobilized enzyme, about 0.4 g, was placed in a stainless steel basket prior to putting it in the reactor for ease of removal at the end of a run. The basket was placed inside the cell, which was already heated to