LIPASE-CATALYZED INTERESTERIFICATION OF TRIGLYCERIDE WITH SUPERCRITICAL CARBON DIOXIDE EXTRACTION

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Key Words: Extraction, Bioreactor, Supercritical Fluid, Carbon Dioxide, Enzyme, Triglyceride, Interesterification

Introduction

Biologically active triglycerides have come to be used in the nutrient food manufacturing and detergent industries. Modification through interesterification with lipase is one of the promising methods for producing these thermally labile materials.[2,5] However, a high degree of incorporation of required fatty acid into triglyceride cannot be attained because of its reverse reaction. We applied supercritical carbon dioxide (SC–CO_2) extraction to the removal of products from a liquid-phase reaction system as a means of solving the problem. SC–CO_2 is nontoxic, and its critical temperature (304.2 K) is both sufficiently low for dealing with thermally labile materials and close to the optimal temperature for the enzymatic reaction. In this process, a reflux column with temperature distribution was employed for the selective extraction.

The objective of this study is to demonstrate experimentally that the equilibrium of interesterification between tricaprylin and methyl oleate is effectively shifted forward by the *in situ* extraction of by-product, methyl caprylate, using SC–CO_2.

1. Experimental

1.1 Materials

Methyl oleate and tricaprylin from Nippon Oil Ltd. were used. The methyl oleate had a purity of 77%; most impurities were esters having molecular weights lower than that of methyl oleate. The tricaprylin had a purity of 98%. Carbon dioxide (99.9% purity) used was from Nippon Sanso Products. 1,3-regiospecific lipase from *Mucor miehei* which was immobilized on a macroporous anion exchanger (0.4 mmϕ, NOVO Industry Japan) was used as the enzyme for interesterification.

1.2 Experimental apparatus and procedure

A schematic diagram of the apparatus is shown in Fig. 1. The apparatus consisted of a high-pressure reactor (316 stainless steel) and a reflux column[1,4]. The volume of the reactor was about 800 cm^3. Glass windows set into opposite sides of the reactor allowed visual observation. The reflux column (10.6 mm ID × 1 m) was connected to the reactor. Eight pairs of thermocouples were set in the inner pipe along the column. Twenty channels of external heaters were controlled by a decoupling control system[3] to obtain the required temperature distribution through the column. According to the results of preliminary experiments the following condition was chosen in this study. The temperature of the reactor was set at 313.2 K and the pressure of the reaction system was 10 MPa. The linear temperature distribution of the reflux column was controlled from 313.2 K at the bottom of the column to 373.2 K at the top.

One hundred grams of a mixture of tricaprylin (20 g) and methyl oleate (80 g) were loaded with 20 g of the immobilized lipase in the reactor. The reaction mixture was stirred at 150 rpm. SC–CO_2 was introduced from the bottom of the reactor at a feed rate of 5 Ndm^3/min.

About 0.5 g the reaction mixture was intermittently sampled from the bottom of the reactor. Compositions of the samples were analyzed by a gas chromatograph, Hewlett-Packard Model HP-5890A, with a

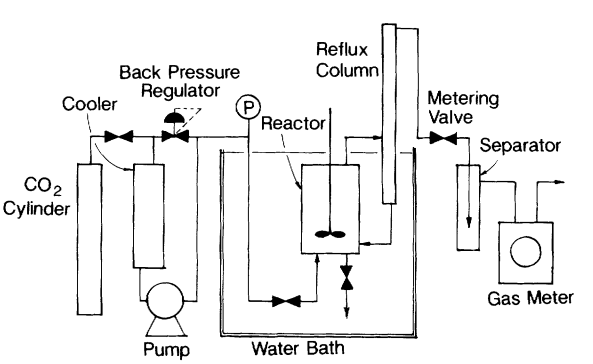

Fig. 1. Schematic diagram of experimental apparatus for extractive reaction

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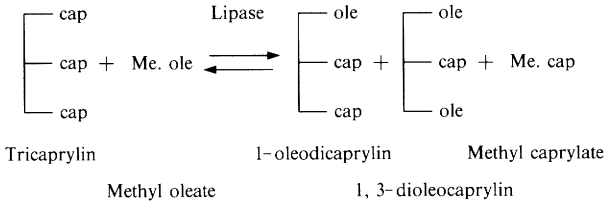
fused silica capillary column (0.32 mm ID × 25 m, GL Sciences) equipped with a hydrogen flame ionization detector.

2. Results and Discussion

The reaction scheme in this system is shown below.



Products in the reaction system were mainly 1-oleodicaprylin, 1,3-dioleocaprylin and methyl caprylate. In none of the experiments was triolein (ole-ole-ole) detected. This was because of the 1,3-regiospecificity of the lipase employed in the experiments. The amount of hydrolytic products was negligibly small. Therefore, the degree of incorporation of oleic acid into triglyceride was evaluated as follows.

$$\text{Incorporation degree} = \frac{C_1 + 2C_2}{2(C_0 + C_1 + C_2)} \text{ [—]}$$

where C_0, C_1 and C_2 [mol/cm^3] are the concentrations of tricaprylin, 1-oleodicaprylin and 1,3-dioleocaprylin, respectively.

Figure 2 shows change of incorporation degree of oleic acid to triglyceride with time. The results obtained from an experiment conducted at atmospheric pressure are also shown in this figure. At atmospheric pressure the incorporation degree approached the equilibrium value of about 60%, while extraction of methyl caprylate elevated the equilibrium incorporation degree to as high as 87.3% in 14 hrs.

Figure 3 shows the composite amounts of methyl caprylate and of methyl oleate extracted. It is clear that selective extraction of methyl caprylate from the liquid mixture during reaction could be achieved.

The results obtained demonstrate that the proposed process effectively shifts the equilibrium forward by the selective extraction of products from the reaction system.

A chromatogram of the reaction mixture after an extractive reaction showed fewer peaks than that of the atmospheric reaction. This may be because impurities contained in the methyl oleate fed were also extracted from the reaction system. Thus the reactants were purified by *in situ* extraction, which is another specific feature of this process.

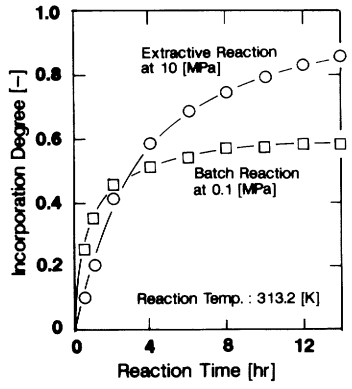


Fig. 2. Change of incorporation degree of oleic acid into triglyceride with time

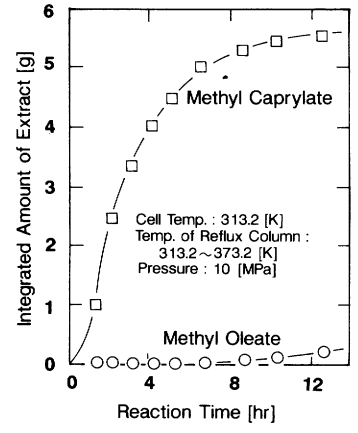


Fig. 3. Composite amounts of extracts

Acknowledgement

The authors wish to express their thanks to a Grant-in-Aid for General Scientific Research (01550724) of the Ministry of Education, Science and Culture, Japan.

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