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Optimization of enzymatic hydrolysis of triglycerides in soy deodorized distillate with supercritical carbon dioxide

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Abstract

Enzymatic hydrolysis of triglycerides of soy deodorized distillate (DOD), using immobilized *Candida rugosa* lipase under supercritical carbon dioxide (SC-CO_2) medium, was carried out. Optimization of the reaction parameters using response surface methodology based on Box-Behnken model at three levels of pressure (120–180 bar), temperature (40–60 °C) and moisture content (40–80% of triglyceride content) for maximum hydrolysis of triglycerides was arrived by multilinear regression of the experimental results. The optimum conditions for maximum degree of triglyceride hydrolysis (94%) were found to be: pressure of 180 bar, temperature of 43 °C and moisture content of 40% to the triglyceride content. Maximum degree of hydrolysis was achieved with short incubation time of 1.5 h under SC-CO_2. Whereas conventional method of hydrolysis in hexane under similar reaction conditions of temperature, moisture and enzyme concentration, needs 5 h to achieve 88% of triglyceride hydrolysis.

Keywords: Soy deodorized distillate (DOD); Supercritical carbon dioxide (SC-CO_2); Lipase; Immobilization; Zeolite; Optimization

1. Introduction

The soy deodorized distillate (DOD) is a by-product (0.3–0.5%) of soybean oil refining process. Soy DOD is a complex mixture of free fatty acids (FFA), triglycerides, sterols, sterile esters, tocopherols and hydrocarbons [1]. Tocopherols and sterols find extensive applications in cosmetic, pharmaceutical industries and as natural antioxidants in processed foods. Tocopherol content of the soy DOD varies widely (0.5–15%) depending on the raw material source and the conditions employing during deodorization process and soy DOD is one of the principle source for the manufacture of natural tocopherols [2].

Preparation of tocopherol concentrates using deodorized distillates involves a series of physical and chemical processes, by separating FFA from the distillate in the initial step, followed by separation of sterols to yield a rich tocopherol concentrate. Some of the processes established for the manufacture of tocopherols from the DOD of different vegetable oil sources are urea adduct formation, liquid–liquid extraction with polar and non-polar solvent pairs, double distillation, alkali saponification, molecular distillation and supercritical fluid extraction [3–7]. Among these, supercritical carbon dioxide (SC-CO_2) extraction is found to be as a potential technology for the enrichment of tocopherols from deodorized distillates of various vegetable oils sources [8–11]. Soy DOD as such will not be feasible to work with SC-CO_2 for the tocopherol enrichment, owing to its poor selective solubility for the components of soy DOD. Hence, soy DOD has to be modified to obtain fatty acid esters (FAME) from FFA and fatty acids associated with triglycerides, to improve the selective solubility and preferential extraction of fatty acids in the form of fatty acid esters with SC-CO_2. For that, triglycerides the major component (55–60 wt%) of soy DOD need to be hydrolyzed to obtain FFA. Attempts have been made to successfully hydrolyse the triglycerides of DOD both chemically, i.e., with acid hydrolysis [8] and enzymatically, using commercially available lipases with shake flask method [12].

Our objective is the enzymatic modification of soy DOD using lipases under SC-CO_2 medium, as a preliminary step towards the recovery of tocopherols. Enzymatic modification of soy DOD involves both the hydrolysis and the esterifcation reactions using suitable lipases. The present work covers first part of the modification process of soy DOD that is, enzymatic hydrolysis of triglycerides under SC-CO_2 medium. SC-CO_2 with a critical pressure of 7.4 MPa and temperature of 31 °C has been used for the extraction and fractionation various food and pharmaceutical components, particularly lipids [13–18]. Recently,

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it has also been exploited as a medium to conduct enzymatic reactions involving lipids; better reaction rates were reported due to high diffusivity, low viscosity, low surface tension of SC-CO_2 and hence low resistance to mass transfer [19]. The marked temperature and pressure dependence of its solvent power eases the post-reactional separation of products. Also, enzymes have been shown to exhibit sustainable activity at pressures as high as 400 MPa in SC-CO_2 medium [20].

A number of factors such as reaction pressure, temperature, moisture content of the reaction mixture and enzyme concentration play a critical role in determining the rate of the reaction, that is the effect on yield of FFA by triglyceride hydrolysis. It is difficult to arrive at the optimum conditions for maximum FFA yield, by trial and error because multiple factors exhibit interaction effects.

Response surface methodology (RSM) is one technique that offers a solution to multivariable optimization problems. So, Box-Behnken design consisting of three variables as reaction pressure, temperature and moisture content (weight percent of the triglyceride content) at three levels have been employed to optimize the hydrolysis of triglycerides. Although enzyme concentration is one of the factor which influences the degree of hydrolysis, it does not show influence beyond its optimum concentration, and hence the experimental design does not include enzyme concentration as one of the variable.

2. Experimental

2.1. Materials

Soybean deodorizer distillate was procured from M/s Shakthi Soya Ltd., Pollachi, Tamilnadu. Soy DOD was subjected to high-speed centrifugation (Shimadzu, 15,000 × *g* at 15 °C for 30 min) to remove the solid impurities. Then the sterol content (7 wt%) was removed from soy DOD by subjecting it to cold crystallization at 4 °C for overnight and centrifugation (10,000 × *g* at 15 °C for 15 min).

Candida rugosa lipase powder (activity, 30,000 U/g) was a gift from Amano Enzyme Inc., Japan. Zeolite (100–750 μm), an anion exchanger obtained from Howard & William Fine Chemicals, England was used for enzyme immobilization.

Tocopherols standards kit (α-, β-, γ- and δ-isomer, ≥95% pure) was purchased from Calbiochem (Darmstadt, Germany), and was used for tocopherol analysis as reference. Sterols and saturated hydrocarbons were purchased from Sigma Chemical Co (St. Louis, USA). Sodium hydroxide, petroleum ether, oxalic acid and phenolphthalein indicator were purchased from Ranbaxy Chemicals, Mumbai, India and all of them were analytical grade.

2.2. Enzyme immobilization

The enzyme was immobilized by the method of Lisa et al. [21]. One gram of lipase (*C. rugosa*) was dissolved in 1 ml of distilled water. Then the pretreated zeolite (washed thrice with 5 ml of distilled water and dried in an oven at 85 °C) was added to the enzyme preparation, mixed well with a stirring rod, and spread on to filter paper. It was then dried in a vacuum desiccator overnight at room temperature, and stored in glass vials under refrigeration until use.

2.3. Activity of the immobilized enzyme

Activity of the immobilized enzyme was checked by conducting the hydrolysis of soy refined oil (source of triglycerides), by the shake flask method, at a temperature of 45 °C and a speed of 150 rpm. The activity of the immobilized enzyme was found to be 4500 U/g, using acid value determination [22] to measure FFA content in the sample.

2.4. Enzymatic hydrolysis of triglycerides in soy DOD with SC-CO_2

Hydrolysis reactions were carried out in a 500 cm^3 high pressure reactor unit (Berghof Autoclave, Germany, Fig. 1),

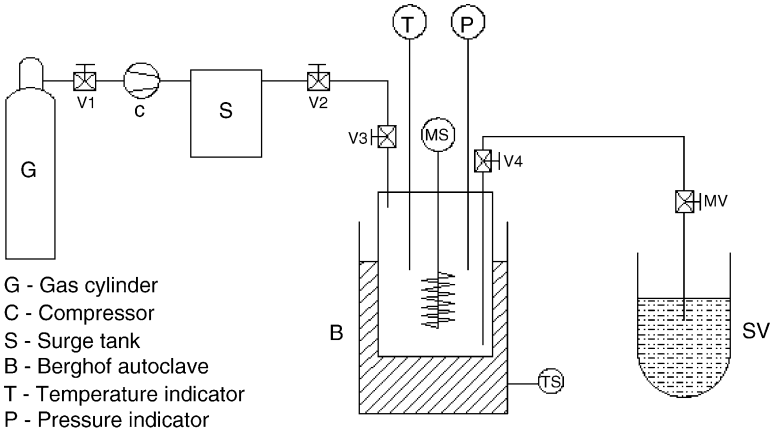

Fig. 1. Schematic diagram of the supercritical carbon dioxide reaction unit.