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Optimization of enzymatic synthesis of cocoa butter analog from camel hump fat in supercritical carbon dioxide by response surface method (RSM)

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ABSTRACT

Supercritical carbon dioxide (SC-CO_2) has been studied as a medium for esterification of camel hump fat and tristearin in producing cocoa butter analog using Immobilized *Thermomyces lanuginosus* lipase (Lipozyme TL IM) as a biocatalyst. Process conditions (pressure, temperature, tristearin/camel hump fat ratio, water content, and incubation time) were optimized by conducting experiments at five different levels using the response surface method (RSM). A second-order polynomial response surface equation was developed indicating the effect of variables on cocoa butter analog yield. Contour maps generated using the response surface equation showed that all the experimental variables significantly affected the yield. The pressure, 10 MPa; temperature, 40 °C; SSS/CHF ratio, 1:1; water content, 10% (w/w); and incubation time, 3 h were found to be the optimum conditions to achieve the maximum yield of cocoa butter analog.

1. Introduction

Fat constitutes one-third of the chocolate content and its properties affect certain parameters of the chocolate manufacturing process, especially at the tempering and cooling stages [1]. Melting fast and completely in mouth while hard and brittle at room temperature, cocoa butter is the fat phase in chocolate and is characterized by sharp melting point, desirable physicochemical properties, and fatty acid components [2]. It is composed predominantly (>70%) of symmetrical triacylglycerols, 1,3-dipalmitoyl-2-oleoylglycerol (POP), 1(3)-palmitoyl-3(1)-stearoyl-2-monoolein (POS), and 1,3-distearoyl-2-oleoylglycerol (SOS), with oleic acid in the sn-2 position [3]. The typical fatty acid composition of cocoa butter in mole percentage is: C16:0 24.4%, C18:0 33.6%, C18:1 37.0%, C18:2 3.4%, and others 1.6%. Cocoa butter melts at temperatures between 32 and 35 °C and is able to re-crystallize during processing to a stable crystal mode [4]. World production of cocoa butter was about 675,000 metric tons in 2001. Numerous factors, however, including the degree of uncertainty in supply, variability in quality, and price premium compared to other fats have driven the search for alternatives [5]. Cocoa butter replacers (CBRs) are those fats which replace cocoa butter partially or wholly in chocolate products. CBRs are further classified as (i) cocoa-butter-equivalents (CBE), which behave like cocoa butter and are able to blend in cocoa butter in any proportion without altering the physicochemical characteristics of cocoa butter; and (ii) cocoa-butter-substitute (CBS), which are fats that can be blended with cocoa butter to a limited extent, without significantly altering its physicochemical characteristics [6].

Procedures of chemical interesterification for analogs have been developed for years from cheap fats and oils and are found to be practical to industries [7]. Recently, preparation of CBE through 1, 3-specific lipase-catalyzed interesterification has received much attention because lipases offer certain advantages over other chemical catalysts [8]. While chemical catalysts will randomize all of the fatty acids in a triacylglycerol mixture, 1, 3-specific lipase can incorporate fatty acids into the sn-1, 3-positions without changing the fatty acid residues in the sn-2-position [9]. Other advantages include the mild conditions under which the reactions take place, thereby requiring minimal energy inputs, reduced levels of by-products generated during the reaction, and more efficient conversion of labile substrates. In order to improve the reuse and stability of lipase, immobilization of the lipase is necessary [10].

However, CBE prepared through the lipase-catalyzed interesterification of triacylglycerols in organic solvent systems are suffering from some major drawbacks such as final solvent removal, which is usually costly and lengthy. Supercritical fluids, with less mass transfer resistance than the conventional liquid solvents, have been used as solvents for enzymatic reactions [11]. In addition, the solubility of most organics in supercritical fluids is higher than that in gaseous phase and is comparable with liquid solvents. Among supercritical fluids, SC-CO_2 is especially advantageous: low viscosity, high diffusivity and low surface tension, which allow it to penetrate easily throughout macro- and microporous materials

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