Food Chemistry 135 (2012) 155–160

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

# A study on parameters of potential cocoa butter analogue synthesis from camel hump by lipase-catalysed interesterification in supercritical $CO_2$ using response surface methodology

Hajar Shekarchizadeh, Mahdi Kadivar *

*Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran*

ARTICLE INFO

*Article history:*
Received 23 October 2010
Received in revised form 2 August 2011
Accepted 7 April 2012
Available online 17 April 2012

*Keywords:*
Enzymatic interesterification
Camel hump fat
Lipozyme TL IM
Supercritical carbon dioxide ($SC–CO_2$)
RSM

ABSTRACT

A potential cocoa butter analogue was prepared from camel hump fat and tristearin by enzymatic interesterification in supercritical carbon dioxide ($SC–CO_2$) using immobilised *Thermomyces lanuginosus* lipase (Lipozyme TL IM) as a biocatalyst. Response surface methodology (RSM) was used to investigate the effects of pressure, temperature, tristearin/camel hump fat ratio, water content, and incubation time on TAG distribution of cocoa butter analogue. The process conditions were optimised by conducting experiments at five different levels. A second order polynomial response surface equation was developed to indicate the effect of variables on TAG distribution of cocoa butter analogue. Overlaid contour plots generated using the response surface equations showed that all TAG components of cocoa butter analogue are significantly affected by the experimental independent variables. The pressure of 10 MPa; temperature of 42 °C; SSS/CHF ratio of 1.15:1; water content of 10% (w/w); and incubation time of 3 h were found to be the optimum conditions to achieve the most similar cocoa butter analogue to the corresponding cocoa butter.



## 1. Introduction

Cocoa butter is currently the selected fat in chocolate and other confectionery industries based on its appropriate organoleptic characteristics and physical properties in these kinds of applications (Torbica, Jovanovic, & Pajin, 2006). The main triacylglycerol (TAG) composition of cocoa butter is: 1,3-dipalmitoyl-2-oleoylglycerol (POP) 13.6–15.5%, 1(3)-palmitoyl-3(1)-stearoyl-2-monoolein (POS) 33.7–40.5%, and 1,3-distearoyl-2-oleoylglycerol (SOS) 23.8–31.2%. Numerous factors, however, including the degree of uncertainty in supply, variability in quality, and price premium compared to other fats have driven oil industry for alternatives (Hartmann, Meyer, & Scheper, 2001). Much attention has been drawn to the production of cocoa butter in comparison to the fats and oils of lower value via interesterification with lipase in organic solvents (Liu, Cheng, Chang, & Shaw, 1997). However, organic solvent systems are suffering from some major drawbacks such as final solvent removal, which is usually costly and time consuming. Supercritical fluids, with less mass transfer resistance than the conventional liquid solvents, have been extensively used as solvents for enzymatic reactions (Habulin, Primožič, & Knez, 2007). 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 due to low toxicity, viscosity, cost, surface tension, and high diffusivity whose impact on environment is negligible (Knez & Habulin, 2002).

The camel stores its energy and reserves it in the form of fat in different depots of its body in which the hump comprises a considerable amount of the adult body weight; therefore, camels can survive for a long period of time without having food and drinking water. The hump contains mixtures of fatty acids and most of them are C16:0, C18:0 and C18:1 (Kadim, Mahgoub, Al-Maqbaly, Annamalai, & Al-Ajmi, 2002).

Response surface methodology (RSM) is a mathematical tool, which can help in arriving at optimum conditions for a reaction with minimum number of experiments to obtain statistically acceptable results. By using this method, it becomes possible to evaluate the effects of multiple parameters, alone or in combination, on response variables and to predicts their behaviour under given sets of conditions (Nagesha, Manohar, & Udaya Sankar, 2004).

In the present work, it was found that cocoa butter analogue could be efficiently synthesised by interesterification with immobilised *Thermomyces lanuginosus* lipase (Lipozyme TL IM) as biocatalyst in $SC–CO_2$. The conditions of this process including pressure, temperature, tristearin/camel hump fat ratio, water content, and incubation time were optimised by RSM.

* Corresponding author. Tel.: +98 311 3913382.
*E-mail address:* mak120@mail.usask.ca (M. Kadivar).

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http://dx.doi.org/10.1016/j.foodchem.2012.04.033