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Enzymatic production of green epoxides from fatty acids present in soapstock in a microchannel bioreactor

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ABSTRACT

Soapstock containing high value of free unsaturated fatty acids was used for enzymatic production of natural epoxides in a solvent-free system. At a same reaction condition (36 °C, initial pH of 6.5, and H_2O_2/C=C molar ratio of 1 for 30 min), using a microchannel bioreactor were enhanced the yield of epoxidation by 2.8 and 1.7 folds in comparison with the stirred tank operated at batch and semi-batch, respectively. Further studies were performed for optimization of the microchannel bioreactor with statistical evaluations of the operational parameters namely temperature, molar ratio of H_2O_2 to C=C bond, and feed flow rate. Maximum epoxidation yield (85%) and specific epoxidation rate (1437.56 μmol g_{enzyme}^{-1} min^{-1}) were obtained at 36 °C, H_2O_2/C=C molar ratio of 1.61, and feed flow rate of 42 mL h^{-1}. The produced epoxides were analyzed by FTIR, ^{1}H NMR, and GC–MS analysis.

1. Introduction

Epoxidized vegetable oils find recently extensive industrial applications as plasticizer and additive in polymer industries such as Polyvinyl chloride (PVC) (Petrovic, 2008). It offers biodegradability and thermal stability in PVC products which most widely used in food packaging. In addition, the natural epoxides have been utilized as reactive diluents for paints, high-temperature lubricants and intermediates in polyurethane polyol production (Benaniba et al., 2003). In epoxidation reaction of vegetable oils, a single oxygen atom adds to each unsaturation (carbon=carbon bond) in the fatty acid chain using percarboxylic acids or organic/inorganic peroxides as an oxidizing agent. Epoxidation of vegetable oils was performed through several methods: the Prilezhaev reaction, catalytic epoxidation with acidic ion-exchange resin, metal-catalyzed epoxidation, and chemo-enzymatic epoxidation (Goud et al., 2006; Kim et al., 2015). Among them, the enzymatic epoxidation is preferred due to using of environmental-friendly raw materials, mild reaction conditions, high conversion, and less by-products (Wang et al., 2015). Lipases are versatile enzymes that can be used for the epoxidation reaction. On the basis of the extensive work reported elsewhere, the catalytic role of lipases in the chemo-enzymatic epoxidation involves two steps: first, the biocatalyst acts as a per-hydrolase to produce per-acids, then double bonds are epoxidized (Aouf et al., 2014). The chemo-enzymatic epoxidation of Jatropha oil using Novozym 435 was successfully reported by conversion of 100% along with 100% selectivity in 24 h (Rios et al., 2011). Also, Novozym 435 was used in the chemo-enzymatic epoxidation of Karanja oil and 80% conversion in 9 h was reported in the absence of any side reactions (Bajwa et al., 2016).

Biocatalytic epoxidation were studied in both organic-solvent and solvent-free systems. In solvent-free system, the reaction was usually not complete due to the formation of solid or highly viscous oily phase, thus solvents are used to decrease the viscosity of the reaction mixture and improve the mixing behavior between binary phases involved in the reaction (Zhang et al., 2014). However, some research demonstrated that the enzymatic epoxidation is performed better in solvent-free system. It seems that solvent remains in the oil phase as an inert substance, without influences on the epoxidation reaction, which occurs in the aqueous phase (Abdullah and Salimon, 2010). Elimination of the solvent is important for the enzyme activity, and also the environmental impacts, cost, safety and health issues of the process (Silva et al., 2013). Recently, Zhang et al. (2017) reported a chemo-enzymatic epoxidation of fatty acids in a solvent-free system using lipase sourced by *Candida* sp. They demonstrated that solvent-free epoxidation of free fatty acids yielded superior result when compared to the organic-solvent systems. Microchannel reactor, as a new type of reactors, has some attractive characteristics such as high surface-to volume ratio, short diffusion distance, less energy consumption, very efficient heat and mass transfer along with intrinsic safety, temporal and spatial control of reagents and products in solving the mixing and substrate inhibition

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problems in the enzymatic epoxidation reaction (Phimsen et al., 2017).

This study aims to establish a green and economically friendly approach for enzymatic production of natural epoxidized fatty acids (EFAs) through an epoxidation reaction of free fatty acids sourced by soapstock in a solvent-free system. Soapstock is a concentrated by-product solution of free fatty acids (FFAs) obtained in the refining of crude edible oils by reaction of FFAs with alkali and then centrifugal separation. Soapstock is an inherent result of the refining process of edible vegetable oils while its discharge into the environment has problematic negative effects. Soapstock while consisting in high contents of unsaturated fatty acids such as oleic, linoleic, and linolenic acids, it can be utilized as an economic raw substance in production of natural epoxides. To the best of our knowledge, no research has been reported in enzymatic epoxidation of free fatty acids from industrial wastes in a microchannel bioreactor. Response surface methodology (RSM) as a mathematical tool in evaluating the effects of the operating variables namely temperature, molar ratio of H_2O_2 to C=C bond, and feed flow rate was used for optimization of the microchannel bioreactor.

2. Materials and methods

2.1. Materials

Soapstock was obtained from Kermanshah's Mahidasht Agricultural Industrial Complex (Nazgol, Iran). The weight percent of total fatty acids in the soapstock was 16.7%. Commercial lipase from *Candida rugosa* (enzyme activity > 700 IU mg_{enzyme}^{-1}) was purchased from Sigma-Aldrich. The hydrogen peroxide solution (35 w%) was analytical grade, and purchased from Dr. Mojallali Chemical Complex Company. All others reagents were of Sigma-Aldrich and purchased from local suppliers.

2.2. Experimental setups

The microchannel bioreactor system consists of two adjustable syringe pumps that injected separately the organic (soapstock + lipase enzyme) and aqueous (hydrogen peroxide) phases into a microchannel. The microchannel was composed of T-type conjunction, for proper initial mixing of the phases, contacted to a channel with a length of 100 cm and an internal diameter of 0.8 mm. Prior to the reaction, 60 mg of *Candida rugosa* lipase was dissolved in 20 g of a pH-adjusted soapstock (pH = 6.5 using 2 M of hydrochloric acid) and then the solution mixed at 150 rpm for 30 min. This pre-treatment of the enzyme with the substrate had an important role in the stability of enzyme during the epoxidation reaction (Habibi et al., 2016). The effluent stream from microchannel bioreactor was collected in a product tank. The temperature of the reaction was controlled by using a thermostat chamber and in order to inactivate the enzyme after discharge from microchannel, the production tank was placed in hot water-bath controlled at 70 ± 2 °C.

In stirred tank experiments, the enzymatic epoxidation was carried out in a cylindrical glass reactor (height of 10 cm and 2.5 cm diameter) equipped with a mechanical agitator at batch and semi-batch operational modes for 30 min. In the experiments, the reaction conditions were kept constant as follows: agitation of 500 rpm, temperature of 36 °C, and molar ratio of H_2O_2/C=C equal to 1. At semi-batch operation, hydrogen peroxide was added dropwise to the reaction mixture with the flow rate of 0.3 mL min^{-1}.

At the end of all experiments, the product solution was centrifuged at 10000 × *g* for 10 min to separate the aqueous phase containing lipase, unreacted hydrogen peroxide and water. The upper organic phase was separated and then dried with anhydrous sodium sulfate (50 mg) to complete the elimination of trace water and also unreacted peroxide from the epoxidized fatty acids (EFAs) (He et al., 2013).

Table 1
Range of the independent variables and their coded levels used in design of the experiments by RCCD.

Variables	Levels[a]				
	$-\alpha$	−1	0	+1	$+\alpha$
Temperature (°C)	25	32	42	52	59
Feed flow rate (mL h^{-1})	9.5	30	60	90	110.5
Molar ratio of H_2O_2/C=C	1.24:1	1.61:1	2.15:1	2.68:1	3:1

[a] Actual levels of the each factor were converted to the coded values with following expression: $X = \frac{2x_i - (x_{max} + x_{min})}{(x_{max} - x_{min})}$, where, X id coded value, x_i is actual level, x_{max} is maximum actual level, and x_{min} is minimum actual level.

2.3. Statistical study of EFAs production in the microchannel bioreactor

The pretests showed that the reaction temperature (X_1), feed flow rate (X_2) and the molar ratio of H_2O_2 to C=C bond (X_3) were influential factors on the yield of epoxidation (Y_1) and the specific epoxidation rate (Y_2) in the microchannel bioreactor. In order to determine the importance of operational parameters in the microchannel bioreactor, a rotatable central composite design (RCCD, α = 1.68) was used. The levels of the variables with corresponding coded value for each parameter are listed in Table 1. The total number of the experiments were nineteen where the number of the repeated experiments in the center point of the design were equaled to five for determination of the sum squares of error. The "*Design Expert*" (version 8.07) software was used for the experimental design, empirical modeling, graphical analysis, and final optimization of the process.

2.4. Analytical methods

2.4.1. Characterization of FFAs present in soapstock

The free fatty acid composition of the soapstock was determined by gas chromatography- flame ionization detection (GC-FID) analysis with respect to the standard samples. The samples were prepared by pouring 300 µL of the soapstock in 10 mL of *n*-hexane and 2 mL of alkali methanol solution into a 50 mL vial and mixing for 60 s 2 µL of the solution was injected to GC–FID for analysis. The analyses were carried out with a TRACE GC-THERMO FINNIGAN gas chromatograph (Italy) equipped with BPX70 column (length 50 m and inlet diameter of 0.32 mm). The injector and detector temperatures were set to 220 °C and 250 °C, respectively. Oven temperature was programmed as follows: 190 °C for 1 min, raised with 1.5 °C min^{-1} to 215 °C, and isothermally was held for 10 min. Helium was used as carrier gas at a flow rate of 1.5 mL min^{-1} (Alipour et al., 2016).

2.4.2. Determination of iodine and oxirane values

Iodine value was determined using Wijs method (ASTM D5768) as described below: 1 g of sample was dissolved with 20 mL of iso-octane and 25 mL of the Wijs solution, and kept in a dark storage for 1 h, afterward 20 mL of KI solution and 100 mL of water were added and then titrated by sodium thiosulfate solution 0.1 N.

Theoretical oxirane oxygen content (OO_{th}) defined as the maximum oxirane content in 100 g of free fatty acids was determined from the following equation (Dinda et al., 2008):

$$OO_{th} = 100 \times A_0 \times \frac{IV_0/2A_i}{100 + (IV_0/2A_i)A_0} \quad (1)$$

A_i is the atomic mass of iodine, A_0 is the atomic mass of oxygen and IV_0 is the initial iodine value of the free fatty acids by the soapstock and equaled to 135.6.

Experimental epoxy content (OO_{exp}) was determined according to ASTM D1652. About 0.5 g of the epoxidized sample was measured in a 125-mL *Erlenmeyer* flask and dissolved with 10 mL of chlorobenzene, then titrated with 0.1 N of hydrobromic acid (HBr) solution in acetic