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Optimisation and economic assessment of lipase-catalysed production of monoesters using *Rhizomucor miehei* lipase in a solvent-free system

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ABSTRACT

Enzymatic production of glycerin monolaurate (GML) was carried out by lipase catalysed esterification of lauric acid and glycerin in a solvent-free media. Response surface methodology (RSM), based on 5-level and 3-variable composite design, was employed to study the interactive effects of reaction temperature (48–60 °C), enzyme load (1–4% w/w), and glycerin to lauric acid molar ratio (1:1–4:1) on glycerin laurate yield. The optimum conditions obtained were a temperature of 60 °C, an enzyme load of 4%, and a glycerin to lauric acid molar ratio of 4:1. The maximum predicted and experimental conversion values were 92.26% and 93.23%, respectively. Utilisation of Lipozyme RM IM (*Rhizomucor miehei* lipase) allowed the formation of a mixture consisting of 50% monoglyceride, 34.6% diglyceride and 8.4% triglyceride, which fulfills the requirements established by the World Health Organization (WHO) for use as a food emulsifier. In addition, the Lipozyme RM IM maintained more than 90% of its original activity after being used for six cycles. Finally, an economic study was performed to interrogate the feasibility of the proposed enzymatic manufacturing process. The obtained results were compared with the traditional catalysed chemical process. The economic feasibility study revealed that the needed amount of Lipozyme RM IM was 2.5 kg/t of feedstock. The high conversion achieved in a short reaction time (1 h), as well as the proven operational stability of Lipozyme RM IM, revealed a promising potential for this green and sustainable route in practical applications.

1. Introduction

The use of green monoesters as emulsifiers in the food, cosmetic, and pharmaceutical industries has recently become a growing research area (Abdelmoez et al., 2013, 2016; Abdelmoez and Mustafa, 2014). Monoesters are considered as high value added and fine oleochemicals. Although monoesters are produced in low quantities, they are expensive enough to have high profit margins (Keng et al., 2009). Glycerin monolaurate, as a monoester, has versatile applications in different areas such as an emulsifier in food products and a surfactant in cosmetics. Moreover, glycerin laurate has antibacterial, antiviral, and other antimicrobial influences in vitro (Freitas et al., 2010).

Two methods are used mainly for glycerin laurate production. The first is carried out by transesterification of oils such as palm kernel. The second is carried out by a direct esterification of lauric acid and glycerin using chemical or enzymatic catalysed processes (Kapoor and Gupta, 2012). Industrially, glycerin laurate is produced by the conventional chemical approach. This method is well developed and has been commercialized worldwide. It involves fatty acid esterification or oil transesterification at high temperature in the range of 170–220 °C by using chemical catalysts such as mineral acids, tin salts, organo-titanates, silica gel, or cation exchange resins (Rajendran et al., 2009). Recently, the chemical method has been improved by employing a reactive distillation technique. This development integrates both the chemical reaction and distillation steps into one unit and eliminates the product separation steps from the reaction mixture (de Jong, 2010). Although great efforts have been carried out in the improvement of this process, it still suffers from high production costs and many

Abbreviations: GML, glycerin monolaurate; RSM, response surface methodology; ANOVA, analysis of variance; FOB, free on board; SBE, spent bleaching earth; BE, bleaching earth.

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environmental concerns. Furthermore, the resulting dark products (due to the high reaction temperature employed) and the non-selective mechanism toward monoesters production represent the main drawbacks of the chemical method (Abdelmoez et al., 2013; Jegannathan and Nielsen, 2013). It has been reported that, in most applications, the monoglyceride portion in the final preparation would impact the texture and mouth feel of the processed food product (Kapoor and Gupta, 2012). For these reasons, many approaches have been investigated for the selective enzymatic synthesis of monoesters in the last few decades (Basri et al., 2013).

As lipases display a high degree of specificity in esterification and transesterification reactions, they became a principal biocatalyst for the production of several fine oleochemicals. The use of lipases for catalysing esterification reactions may also offer several advantages over the chemical route. These include lower energy consumption, enhanced product quality, and a cleaner environmentally friendly production process. Reasonably good results have been reported by many workers in this regard (Ghamgui et al., 2006; Freitas et al., 2010).

Most previous research activities used different organic solvents such as hexane, heptane and tert-butanol. The main reason behind using solvents is their ability to offer good homogenisation between the different reactants in the reaction mixture at lower temperature. However, few studies have considered solvent-free esterification of fatty acids (Radzi et al., 2011; Hu et al., 2013; Bucio et al., 2015). Here, the proposed monoester has a major use in the food and cosmetic sectors. Accordingly, the elimination of solvents is strongly preferable during the production process (Freitas et al., 2007). In addition, it decreases the production cost through eliminating many complex industrial purification and evaporation steps. Furthermore, the utilisation of solvent-free systems will dramatically reduce the process hazards accompanied by solvent exposure, toxicity and flammability. Such development will shift the manufacturing process towards a green and environmentally friendly route (Freitas et al., 2010).

Generally, for a successful enzymatic reaction in a solvent-free system, there should be a driving force for the reaction to proceed in the desired direction until completion (Won and Lee, 2001). In enzymatic esterification reactions, water content is one of the key factors that affect the activity of the enzyme. In this regard, decreasing the water content could shift the reaction equilibrium toward ester synthesis. However, below a certain water content, enzyme activity decreases due to the insufficient hydration of the enzyme. Such low hydration levels could lead to a dramatic deterioration in enzyme performance. The water generated during the reaction can be removed or controlled through different techniques. These include the addition of molecular sieves in a closed reactor (Li et al., 2011), reaction in an open reactor (Li et al., 2011; Mostafa et al., 2013), reaction under reduced pressure (Aguieiras et al., 2011), or removal of water by pervaporation (Ziobrowski et al., 2009).

Economic assessment is a key driving force supporting the development of any new process technology. Such an assessment can be utilised to give an approximate estimation for the cost of a process plant, product manufacturing as well as comparing the different proposed technologies (Jegannathan et al., 2011). Most published works state only that the use of enzymatic processes is costly compared to chemical processes, but without both deep and detailed economic assessment. Therefore, a comparison of the economic assessment of monoester production using chemical and enzymatic processes can give an idea about the cost difference range, and may lead to developing biocatalyst processes having a cheaper production cost (Jegannathan et al., 2011). To the best of our knowledge, there is no economic study in the literature that reports the cost of monoester production using the solvent-free enzymatic method.

Here, both technical and economic feasibilities of producing glycerin laurate using direct stereoselective enzymatic esterification of lauric acid and glycerin in a solvent free system were studied. The used enzyme was 1, 3 specific immobilised *Rhizomucor miehei* lipase (commercially named Lipozyme RM IM). In addition, a process flow diagram based on the semi-continuous production of glycerin laurate was proposed, to encourage the applicability of this method in industry further.

2. Materials and methods

All experiments were carried out in an open batch reactor containing 5 g of media (lauric acid and glycerin). All experiments were performed in triplicate.

2.1. Materials

Lipozyme RM IM, 150 IUN/g (*Rhizomucor miehei* lipase supported on a macro porous anionic resin) was provided by Novozymes (Denmark). Lauric acid >99%, tert butanol, ethanol and acetone were purchased from Merck. Pharmaceutical glycerol >99.7% was purchased from a local pharmacy in Portugal. 1-lauroyl-rac-glycerol (monolaurin) > 99%, glycerol tridodecanoate (glycerol trilaurate) > 99% and dodecanoic acid >99% for gas chromatography (GC) analysis were purchased from Sigma. All other reagents were of analytical grade.

2.2. Time progress curve

The appropriate enzymatic reaction time between lauric acid and glycerol to produce glycerin laurate was investigated. An open batch reactor containing 5 g of media (3:1 glycerin/lauric acid molar ratio) was reacted in an incubated shaker adjusted at 50 °C and 200 rpm. Periodically, aliquots were withdrawn every 0.5 h up to 5 h. The percentage of conversion was measured by determining the remaining fatty acids in the reaction mixture by titration against 0.1 M NaOH.

2.3. Glycerin laurate production

Esterification reactions between glycerol and lauric acid were performed in an open 50 mL batch reactor (without stopper) containing 5 g of media and containing 1–4% (w/w) Lipozyme RM IM. The glycerol to lauric acid molar ratio ranged from 1:1–4:1. The reaction was carried out in an incubated shaker at temperatures of 48–60 °C at constant stirring speed of 200 rpm. Aliquots of 200 μL were withdrawn periodically. Then, the reaction was terminated by the addition of 10 mL of ethanol: acetone mixture (50:50, v/v). Control experiments were carried out in the absence of lipase. The percentage of conversion was calculated by determining the remaining unreacted free fatty acids in the reaction mixture. A titration method against 0.1 M NaOH to the end point at pH 10 using thymolphthalein as an indicator was carried out. The conversion was calculated using Eq. (1).

$$\text{Conversion of monoester } (\%) = \frac{N - N^{o}}{N} \times 100 \quad (1)$$

Where N is the volume of NaOH consumed without adding lipase. N^{o} is the volume of NaOH consumed with adding lipase.

It should be mentioned here that all experiments were performed in triplicate and the conversion values are represented by calculating the average mean value. The minimum experimental error was ±0.5%, while the maximum experimental error was