

# Enzymatic Catalysis of Xylitol Monoesters by Using Ultrasound and Microwave

Alessandra Rodrigues Rufino, Sérgio Luiz de Lucena, Acácio Raphael Aguiar Moraes

**Abstract**— The increasing social and political pressures related to the environmental problems generated by traditional chemical processes involving the use of toxic solvents, strong acids, and high energy demand suggest utilization of clean methodologies that follow the precepts established by green chemistry. Enzymatic catalysis promotes enantioselective syntheses operating under mild conditions of temperature and pressure. However, the process requires elevated times of reactions and the employ of high-cost immobilized derivatives. These consequences usually become obstacles to industrial application of such syntheses. With the use of ultrasound and microwaves as alternative heating sources in the enzymatic catalysis, it is possible to overcome these problems besides leading to satisfactory yields and allowing the reduction of solvent volume. In this work, esterification reactions of oleic acid were performed to obtain xylitol monooleate by using alternative methodologies which agree with the precepts of green chemistry. The results showed that, through these studies, it was possible to reduce the mass of the catalyst, the volume of the solvent, and the energy consumed in the process without any damage related to the reaction yield.

**Index Terms**— Enzyme catalysis, Esterification, Green chemistry, Immobilized enzyme, Lipase, *Penicillium camembertii*, Surfactant

## 1 INTRODUCTION

The use of enzymes as catalysts reduces environmental damage by promoting more selective reactions under milder conditions of temperature, pressure, and pH, what is in accordance with the principles listed by green chemistry. Allied to this, enzymes can bring better yields to production and reduce expenses in the purification stage of the final product, thus constituting an interesting material not only from the environmental point of view, but also economically. Owing to these characteristics, there has been an increase in the use of enzyme catalysis in industrial process, especially in recent years when governmental incentives and international pressures have arisen for searching and applying methodologies less aggressive to the environment [1].

Lipases are a subgenre of enzymes characterized by the interaction between the polar and apolar phases of a heterogeneous mixture, allowing the application of enzymatic catalysis in new segments and industrial processes emphasizing on the synthesis of surfactants through the esterification of sugars. Literature records of *Penicillium camembertii* lipase (lipase G) point out it as a potential catalyst,

leading to high activity in direct esterification of glycerol using fatty acids with different chain sizes [2]. The esters produced have potential use as emulsifiers in the food and cosmetic industries. In addition, likely antitumor and antimicrobial actions are reported for this material, enabling its use in drug formulations. Traditionally, these compounds are produced by high-temperature reactions with toxic reagents and acid catalysts, what is not a clean methodology [3].

Enzymatic catalysis applied in organic synthesis takes place under milder conditions of temperature and pressure. Chemoenantioselectivity promoted by enzymes also favors their use, thus increasing the end product yields and reducing the financial cost and environmental impact, which are characteristic at the purification steps, mainly for the cosmetic, food, and drug industries. Nevertheless, in addition to the high cost of the immobilized derivative, enzymatic reactions require elevated reaction times, making their industrial application almost unviable [4].

The technique of enzymatic catalysis associated with the alternative heating methodologies by microwaves or ultrasound can be favorable to the reaction yield with the benefit of allowing the reduction of the solvent volume and, mainly, the reduction of the reaction time [5,6]. Both alternative technologies promote heating in a more direct way in the reactional environment and become advantageous to reduce energy losses, which occur typically in heat transmission by conduction, guaranteeing therefore a greater energy use [7,8]. In their work, Da Rós et al. [9] presented a conversion result higher than 80% in the synthesis of ethyl esters

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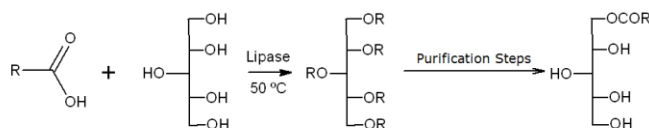
from palm oil fatty acids. According to the authors, microwave irradiation does not affect the enzymatic activity directly, only increase the heating of the medium [9]. Paiva et al. [10] report in their publication a conversion of about 97% of babassu oil in ethyl ester after only 10 minutes of reaction. The positive results reveal an optimistic outlook for the use of such heating technologies in esterification reactions.

In this work, reactions of esterification of oleic acid to obtain xylitol monooleates were carried out with studies focusing on reaction parameters, such as the solvent volume, the enzymatic catalyst mass (*Penicillium camembertii*, lipase immobilized on POS-PVA support), and alternative heating sources with use of different methodologies that fit the precepts of green chemistry, thereby reducing maximally environmental impacts.

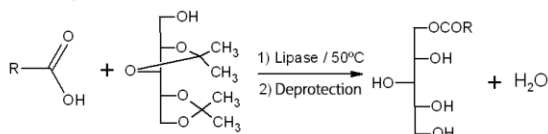
## 2 MATERIAL AND METHODS

The esterification reactions were carried out using lipase immobilized derivatives of *Penicillium camembertii* (Lipase G) by the sol-gel technique on POS-PVA support [11]. Initially, a protection step of four of the five sugar hydroxyls was carried out according to the methodology described in the literature [4], thus improving the xylitol solubility in organic medium and providing greater selectivity for  $\alpha$ -monoester synthesis. Although protection reactions are not recommended by green chemistry, the guarantee of the absence of undesirable by-products in the form of mono-, di-, and triesters justifies their application by eliminating the environmental impact caused by  $\alpha$ -monoester purification, besides allowing reduction of the solvent volume due to polarity reduction of the initial polyalcohol, thus increasing its solubility in the reaction medium. Figure 1 exhibits comparatively esterification schemes of syntheses through both enzymatic and chemoenzymatic routes.

(a) Enzymatic Route



(b) Chemoenzymatic Route



**Fig.1** Esterification reactions by enzymatic route (a) and chemoenzymatic route (b).

### 2.1 Determination of the immobilized derivative mass

For the study of the mass of the immobilized derivative in the synthesis of xylitol monooleate, a 1:4 molar ratio of protected xylitol / oleic acid was utilized starting from 50 mmol of the polyalcohol, 200 mmol of protected xylitol, and 10 mL of *tert*-butanol (*t*-BuOH) as solvent. The reaction occurred under a temperature of 50 °C in a shaker with stirring. The mass of catalyst employed in the reaction was varied in 100 mg at an interval from 100 mg to 500 mg. The reactions were followed for 24 h, and GC analysis was performed at 6-h intervals using a Varian CP 3380 chromatograph, CPSil8CB capillary column. The final product was extracted and purified on a column chromatography and characterized by <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, IR spectroscopy.

### 2.2 Study of the influence of the volume of solvent and use of ultrasound and microwaves as alternative heating sources for the reaction medium to obtain xylitol monooleate

The esterification reactions were initially performed in an Anton Paar model microwave reactor Monowave 300 MAS 24 (software version 3.20) under heating at 50 °C and stirring at 200 rpm, where 50 mmol of the protected xylitol and 200 mmol oleic acid were added. 400 mg of the immobilized derivative of lipase G and volumes of *t*-BuOH as the solvent varying from 2.5 mL to 10 mL were used. The reaction was conducted for 9 h with 100  $\mu$ L-aliquots analyzed at every 3 h.

Afterwards, the reactions were performed to obtain xylitol monooleate in an ultrasonic bath by using a model Kerry Pulsatron apparatus with 20 kHz frequency waves for 9 h. The same reaction conditions were kept using 50 mmol of protected xylitol and 200 mmol of oleic acid in *t*-BuOH volumes varying between 2.5 mL and 10 mL, and utilizing 400 mg of lipase G as a temperature-controlled catalyst maintained around 50 °C. The reaction was conducted for 9 h with 100  $\mu$ L-aliquots analyzed every 3 h of reaction.

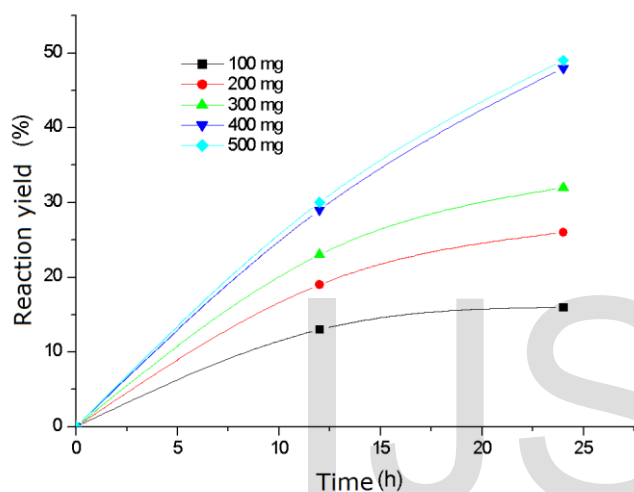
## 3 RESULTS AND DISCUSSIONS

### 3.1 Study of the mass variation of the immobilized derivative required to catalyze the reaction

The results of the reactions in the study of the mass variation of the immobilized derivative were observed along 24 h of reaction, and are displayed in Table 1.

**Table 1** Reaction yield results observed for the change of lipase G catalyst immobilized by sol-gel technique for obtaining xylitol monoester

Catalyst mass (mg)	Reaction yield (%)	
	t = 12 h	t = 24 h
100	13	16
200	19	26
300	23	32
400	29	59
500	30	60



**Fig. 2** Yield vs time curves: influence of the catalyst mass — lipase G immobilized by the sol-gel technique — on the reaction yield to obtain xylitol monoester in a period of 24 h.

The analysis of the graph allows one to verify that the results increase significantly with the increase in mass of the immobilized derived employed up to a 400 mg range. The change in mass between 400 mg and 500 mg did not lead to a significant difference to the results. The ideal mass of lipase corresponds to approximately 0.6% of the total mass of the reactants, considering 50mmol of xylitol and 200 mmol of oleic acid.

### 3.2 Reaction assisted by microwaves and ultrasound with variation of the volume of the solvent

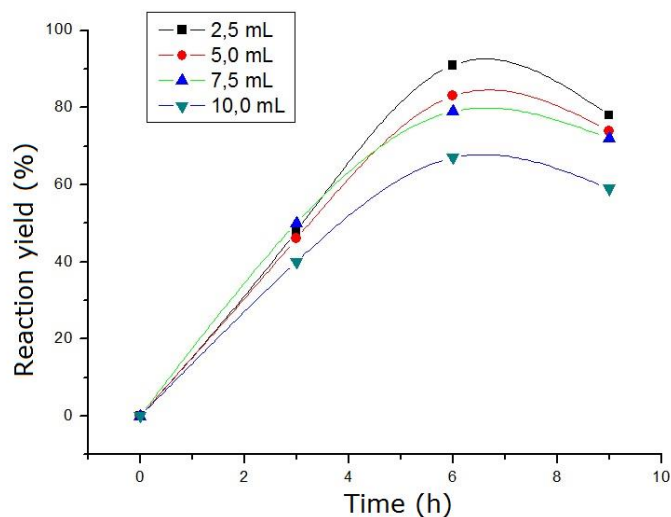
Table 2 presents the results of reaction yields obtained from the reactions carried out in a microwave reactor with variation in the volume of solvent used in the reaction medium.

**Table 2** Yield results for the solvent volume change in the esterification of protected xylitol, catalyzed by lipase G in a microwave reactor.

Solvent volume (mL)	Reaction yield* (%)		
	t = 3 h	t = 6 h	t = 9 h
2.5	48	91	78
5.0	46	83	74
7.5	50	79	72
10.0	40	67	59

\*determined by potentiometric neutralization titration

With the data obtained in 9 h of reaction, the graph was generated as Figure 3. The reaction time required to achieve yields of 91% in the reaction represents a reduction of about 75% of the time described in the classical methodologies of this synthesis, conducted in 48 h and up to a maximum yield in 24 h. The obtained results still show that the reduction of solvent volume is favorable to the yield. The use of 2.5 mL of *t*-BuOH afforded a 91% yield, whereas the use of 10.0 mL of solvent — an increase of 400% — led to a yield of only 67%. Taking also into account the financial and environmental advantages derived from the use of smaller quantities of organic material and energy savings promoted by reducing the reaction time, the use of microwaves can be characterized as an advantageous application technique in these aspects.



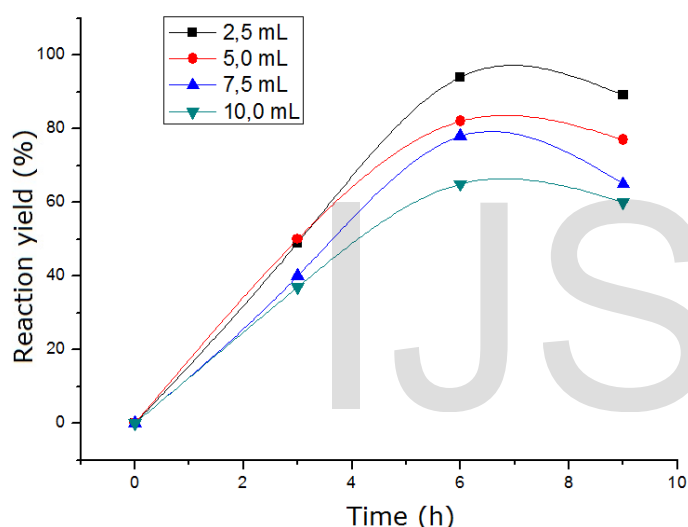
**Fig. 3** Yield vs time curves: influence of the solvent volume on the esterification of protected xylitol catalyzed by lipase G immobilized on POS-PVA support, conducted in a microwave reactor.

The influence of the volume of solvent studied with ultrasonic heating led to the results shown in Table 3.

The graph in Figure 4 represents the yield data as a function of the reaction time.

**Table 3** Results of yield from solvent volume changes in the esterification of the protected xylitol catalyzed by lipase G, conducted in an ultrasonic bath

Solvent volume (mL)	Reaction yield (%)		
	t = 3 h	t = 6 h	t = 9 h
2.5	49	94	89
5.0	50	82	77
7.5	40	78	65
10.0	37	65	60



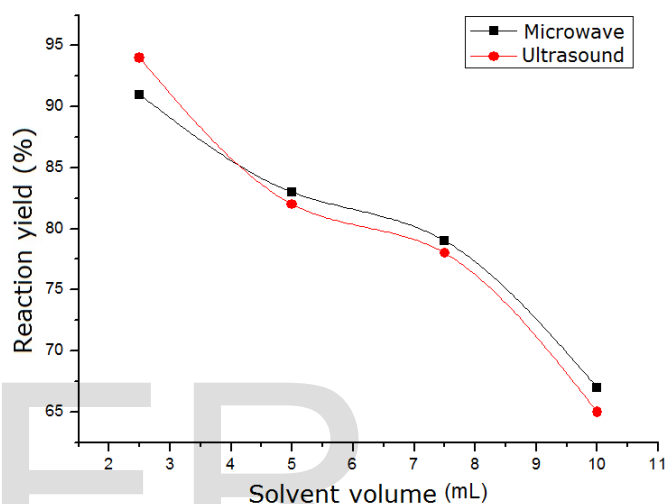
**Fig. 4** Influence of solvent volume on the esterification of protected xylitol catalyzed by lipase G and assisted by ultrasound.

The results show that, in the same way as in microwave heating, it is possible to reduce the reaction time of the enzymatic reactions in only 75% with the heating source change utilized. Reduction of the solvent volume was also favorable to increasing the yield with the use of ultrasound. It was observed, however, that this technique partially destroyed the immobilized derivative, probably due to the mechanical stirring promoted by the ultrasonic waves.

Table 4 shows the best yields for the solvent volumes employed in both bath heating sources of microwaves and ultrasound. The graph in Fig.5, generated from this table, exhibits the comparative results.

**Table 4** Yields for solvent volumes used under microwave emission and ultrasound bath for a reaction time of 6 h.

Solvent volume (mL)	Reaction yield (%)	
	Microwaves	Ultrasound
2.5	91	94
5.0	83	82
7.5	79	78
10.0	67	65



**Fig. 5** Influence of the heating source on the esterification of protected xylitol catalyzed by lipase G in several solvent volumes.

Both methods showed very similar behavior for all volumes of solvent studied and afforded close results. For the smallest volume of the solvent, 2.5 mL, the ultrasound method presented the highest yield: 94%. In relation the other solvent volumes, due to a more efficient stirring generated by the technique itself, which improves the mixture between reagents and catalyst, microwave method displayed results slightly higher than those obtained by ultrasound. Therefore, in the microwave oven, the magnetic stirring enhanced the reaction medium. On the other side, in the ultrasound-assisted reactions, there was mechanical damage caused to the support of the immobilized derivative, and that did not occur under the microwave heating, where the integrity of the support was maintained, making this more suitable for reuse. NMR analyses of xylitol monooleates provided the following data:

$^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ,  $\delta$ ) 5.45(m, 2H,  $\text{H}_{9-10}$ ); 4.25(dd, 2H,  $\text{H}_{1a-1b}$ ); 1.87(m, 4H,  $\text{H}_{8-11}$ ); 1.39(m, 22H,  $\text{H}_{3-7}$ , and  $\text{H}_{12-17}$ ); 0.98(t, 3H,  $\text{H}_{18}$ ).



<sup>13</sup>C-NMR (300 MHz, CDCl<sub>3</sub>, δ) 175.6 (C = O); 131.0-128.5(C<sub>9</sub>=C<sub>10</sub>); 71.4(C<sub>3</sub>); 69.2(C<sub>2</sub>); 71.1(C<sub>4</sub>); 64.4(C<sub>1</sub>); 64.1(C<sub>5</sub>); 35.1(C<sub>2'</sub>); 32.8(C<sub>8'</sub>, 11' ou 16'); 29.7-29.1(C<sub>4'-6'</sub> e C<sub>12'-15'</sub>); 25.1(C<sub>3'</sub>); 22.9(C<sub>17'</sub>) and 14.50(C<sub>18'</sub>).

#### 4 CONCLUSION

The use of ultrasound and microwave irradiations has been shown to be promising tools for conducting enzymatic reactions. In the synthesis of xylitol monooleate, both alternative heating sources were able to raise the reaction yield with reduction in the solvent volume and in the reaction time. Energy savings and reduction of solvent use with increased reaction yields satisfy the key parameters in green chemistry. The microwave technique was considered better because it did not cause damage to the immobilized derivative. Maintenance of the physical integrity of enzymes immobilized on POS-PVA support is interesting to later exploit the reuse of these immobilized derivatives in subsequent and consecutive cycles of reaction. In addition, it was possible to reduce the mass of the catalyst used in the synthesis, reflecting, consequently, material savings and lowering of the production cost.

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