Fatty acid preference of mycelium-bound lipase from a strain of 

_Penicillium italicum_

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ABSTRACT

Mycelium-bound lipase was prepared using a strain of _Penicillium italicum_ AT4421. At the time of maximum lipase activity (72 h), the mycelia to which the lipase was bound were harvested yielding 34.1 g L⁻¹ and lipolytic activity of 200 U g⁻¹. The activity of free (extracellular) lipase in the culture supernatant (after removal of mycelia) was less than 20.0 U mL⁻¹. The fatty acid preference of the _P. italicum_ mycelium-bound lipase was assessed in the hydrolysis of vegetable oils having different fatty acids profiles. The lipase demonstrated high selectivity for canola oil (elevated oleic acid content) and low specificity for soybean oil, rich in linolenic acid. Hydrolysis reaction rate of canola oil was increased from 50 to 80% by combining ultrasonic irradiation with mechanically stirred. This can be probably associated with better homogenization of the medium, which favored the lipase activity. The resulting hydrolysate was composed by lauric acid (2.1%), myristic acid (0.8%), palmitic acid (7.8%), oleic acid (49.0%) and linolenic acid (13.6%).

1. INTRODUCTION

In comparison to chemical procedure, the enzymatic hydrolysis of triacylglycerols by lipases has several advantages, including: (i) High fatty acid selectivity that is of priority importance for the proposed application; (ii) Mild reaction conditions in terms of pH and temperature are also important in processes that involve highly labile polysaturated fatty acids (Freitas et al., 2007). Most of lipases used in biotechnological processes are purified and/or immobilized enzymes obtained from microorganisms, included fungus. The genus _Penicillium_ is known as good producer of extracellular lipase, inclusive with commercial availability of this enzyme from some species, as for example, Amano Lipase G from _P. camemberti_. However, the use of whole cells (mycelium bound lipase) from...
Penicillium in biotransformation process is still scarce in the literature. There are many advantages in using mycelium-bound lipase as biocatalyst as it eliminates the expensive and laborious operations of isolation, purification, in addition of co-factor and immobilization (Cortez et al., 2017). Based on this, Marotti et al. (2015; 2016) evaluated ten Penicillium species isolated from different habitats, as source of mycelium bound lipase to be used for modifications of oil and fats. With high hydrolytic activity (200 U g\(^{-1}\)) and high retention of activity on the whole cells, \(P. italicum\) AT4421 was considered as a potential biocatalyst to be used in the hydrolysis of vegetable oils. In the present study, the hydrolysis of different vegetable oils having different fatty acids profiles by whole cells of Penicillium italicum was evaluated aiming at determining its specificity. Assays were carried out under conventional heating and ultrasonic irradiation.

2. MATERIAL AND METHODS

\(Penicillium italicum\) AT4421 was kindly provided by the Laboratório de Processos Biotecnológicos e Purificação de Macromoléculas of Universidade Federal de São João del Rei (São João del Rei – MG). The strain was cultivated and the biomass recovered as described by Marotti et al. (2016). Batch hydrolysis reactions were performed in 100 mL jacketed glass reactor under mechanical stirring (300 rpm) at 40 °C and atmospheric pressure, containing 50 mL emulsion of vegetable oil (25%) in phosphate buffer (100 mmol L\(^{-1}\), pH 7). The reaction was started by the addition of biomass to attain 400 units of activity per gram oil. Fatty acids released were titrated with standard 0.02 mol L\(^{-1}\) potassium hydroxide solution. The effect of ultrasonic irradiations on the reaction medium under hydrolysis process was also verified. Batch reactions were performed in 125 mL Erlenmeyer flasks immersed in ultrasonic bath (mode of frequency of 40 kHz and power of 132 W) containing substrate (25, 35 or 50% canola oil) in phosphate buffer (100 mmol L\(^{-1}\), pH 7) maintained fixed the others conditions. Periodic samples were taken to quantify the formed free fatty acids and the percentage of hydrolysis calculated as reported by Rooney and Weatherley (2001). The profile of fatty acids was identified in samples previously purified and methylated as described by Freitas et al. (2007).

3. RESULTS AND DISCUSSION

3.1. Hydrolysis of Vegetable Oils

\(P. italicum\) mycelium bound lipase was able to hydrolyze all the evaluated vegetable oils, although at different rates depending on the fatty acids profile. With the exception of canola oil, the maximum formation of free fatty acids was attained within 8 h reaction ranging from 20% (for soybean oil) to 40% (for palm and coconut oils). The released of fatty acid in the hydrolysis of canola oil achieved its maximum at 24 h reaction yielding about 80%. Based on the results displayed in Figure 1, it is possible to state that \(P. italicum\) AT4421 showed low preference for linoleic acid (soybean oil) and higher
selectivity for oleic acid (canola oil) than palmitic acid (palm oil) and lauric acid (coconut oil), indicating that this lipase displayed high substrate selectivity for unsaturated fatty acid containing a cis-9 double bond.

![Graph showing hydrolysis of vegetable oils by mycelium bound lipase of P. italicum at different incubation times](image1)

*Figure 1. Hydrolysis of vegetable oils by mycelium bound lipase of P. italicum at different incubation time*

### 3.2. Ultrasonic wave Irradiation on Hydrolysis of Canola Oil

To enhance the reaction rate, ultrasound irradiation coupled with mechanical stirring was used in the hydrolysis of canola oil by mycelium bound lipase of *P. italicum*. Figure 2 shows that in the irradiated system, the production of free fatty acids was 42% higher than that obtained in the conventional heating system, achieving 79.9 ± 0.4% of conversion in 8 h.

![Graph showing effect of ultrasonic irradiation on the hydrolysis of canola oil by whole cells of P. italicum](image2)

*Figure 2. Effect of ultrasonic irradiation on the hydrolysis of canola oil by whole cells of P. italicum.*

This results is relating to the requirement of high oil/water interface to a full lipase catalytically performance, thus ultrasound irradiation (low frequency) promoted better emulsification and stabilization of the medium in detrimental of high stirring, favoring the reaction rates (Huang et al., 2010).
The chromatography analysis carried out on the FFA fractionated from the lipid materials obtained following the hydrolysis of canola oil with *P. italicum* revealed that the hydrolysate was composed by lauric acid (2.1%), myristic acid (0.8%), palmitic acid (7.8%), oleic acid (49.0%) and linolenic acid (13.6%).

Further studies carried out to determine the effect of substrate concentration on the reaction rate assisted with ultrasound irradiation demonstrated that the hydrolytic activity was decreased with increment in the substrate concentration from 35 to 50%. This behavior is usually related with coalescence effect, which decrease the interface area and consequently, decrease the reaction rates (de Castro et al., 2004).

### 4. CONCLUSIONS

Whole cells of *P. italicum* have potential application to split vegetable oils to obtain hydrolysates rich in polyunsaturated fatty acids (C18:1). Ultrasound irradiation coupled with mechanical stirring favored the hydrolysis process in about 40%. Additional studies are required to evaluate the use of the whole cells in fixed bed reactors operating under continuous flow.

### 5. REFERENCES


