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Characterization of the catalytic properties of lipases from plant seeds for the production of concentrated fatty acids from different vegetable oils

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ABSTRACT

The aim of this work is to evaluate the catalytic properties of lipases from castor bean (*Ricinus communis*), corn (*Zea mays*), sunflower (*Helianthus annuus*) and passion fruit (*Passiflora edulis*) seeds for their application in oil hydrolysis to produce concentrated fatty acids. The influence of seeds germination time, pH and temperature in the catalytic activity of the lipases has been studied, together with the activity toward different oils. Among the biocatalysts tested, crude lipase extract from dormant castor bean seeds was the most active in the hydrolysis of oils in the absence of emulsifier agents. This lipase was then selected for the optimization of the hydrolysis reactions using factorial design. Under optimized conditions, complete hydrolysis of babassu, palm and soybean oils was reached after 60, 70 and 80 min of reaction, respectively, at pH 4.5. These results suggest that the use of lipase from dormant castor bean seeds has potential interest for the hydrolysis of different vegetable oils.

1. Introduction

Lipases (triacylglycerol ester hydrolases, EC 3.1.1.3) are enzymes that catalyze the cleaving of ester bonds in triglycerides to glycerol and free fatty acids in nature (de Castro et al., 2004; Hasan et al., 2006; Barros et al., 2010; Fernández-Lafuente, 2010; Rodrigues and Fernández-Lafuente, 2010a,b; Mendes et al., 2012). They are adapted to operate at the interfaces of biphasic systems, a phenomenon known as interfacial activation, in which the characteristic substrate is an aggregate, micelles or monomolecular film formed by ester molecules, interfacing an aqueous medium (Sarda and Desnuelle, 1958; Verger, 1997; Schmid and Verger, 1998). In the absence of interfaces, lipases have some elements in their secondary structure (the so-called "lid") covering their active sites thus making them inaccessible to substrates (closed conformation). However, in the presence of hydrophobic interfaces (e.g. an oil droplet or organic solvent), important conformational changes take place yielding the "open structure" of lipases. In organic medium, lipases also catalyze esterification, transesterification and interesterification reactions. These enzymes are applied in several industrial processes including the synthesis and degradation of engineering thermoplastics, production of pharmaceuticals, agrochemicals, cosmetics, flavors and fragrances, emulsifiers, structured lipids, pretreatment of lipid-rich wastewaters, biodiesel synthesis by transesterification of triglycerides with short-chain alcohols and concentrated fatty acids by hydrolysis of oils and fats (de Castro et al., 2004; Hasan et al., 2006; Barros et al., 2010; Fernández-Lafuente, 2010; Rodrigues and Fernández-Lafuente, 2010a,b; Mendes et al., 2012).

The hydrolysis of oils and fats from several sources for the production of concentrated free fatty acids and glycerol is relevant in the industrial processing of natural oils and fats. A significant number of high-value products such as coatings, adhesives, surfactants, and biofuels require fatty acids in their manufactures (Murty et al., 2002; Ting et al., 2008; Yiğitoğlu and Temoçin, 2010). Traditional oil hydrolysis is carried out by using a chemical catalyst at high temperature and pressure (250 °C and 70 bar) (Murty et al., 2002). Under these conditions, undesirable reactions such as oxidation, dehydration of the free fatty acids, or interesterification of the triglycerides are produced (Murty et al., 2002; Rooney and Weatherley, 2001). On the other hand, hydrolysis reactions catalyzed by lipases can be performed at lower temperatures to save

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Table 1
Fatty acid composition of the vegetable oils used in the present work.

Fatty acids	Composition (% m/m)					
	Babassu	Palm kernel	Palm	Olive	Soybean	Canola
Caprylic ($C_{8:0}$)	3.50	4.30	–	–	–	–
Capric ($C_{10:0}$)	4.50	3.50	–	–	–	–
Lauric ($C_{12:0}$)	44.7	47.2	0.10	–	–	–
Myristic ($C_{14:0}$)	17.5	15.6	1.20	–	–	–
Palmitic ($C_{16:0}$)	9.70	8.60	46.8	11.4	10.7	4.70
Palmitoleic ($C_{16:1}$)	–	–	–	0.65	–	0.14
Stearic ($C_{18:0}$)	3.10	2.00	3.80	2.60	3.00	1.65
Oleic ($C_{18:1}$)	15.2	15.9	37.6	80.6	24.0	66.0
Linoleic ($C_{18:2}$)	1.80	2.80	10.5	4.20	56.7	21.2
Linolenic ($C_{18:3}$)	–	–	–	0.60	5.40	5.20
Arachidic ($C_{20:0}$)	–	–	–	0.20	0.10	0.90
Average molecular mass (g/mol)	223.9	222.9	269.4	279.6	278.6	280.9

energy, and it exhibits high selectivity, leading to products with high purity and fewer side products (Murty et al., 2002; de Castro et al., 2004; Hasan et al., 2006; Sharma et al., 2009).

Lipases from plants, animals, and microorganisms have been used in the production of concentrated fatty acids (de Castro et al., 2004; Hasan et al., 2006; Barros et al., 2010; Fernández-Lafuente, 2010; Rodrigues and Fernández-Lafuente, 2010a,b; Mendes et al., 2012). Among them, plant lipases appear to be very attractive owing to their low cost, their high substrate specificity, and the fact that they are widely available from natural sources without the need for molecular genetic technology to produce them, making them a good alternative for commercial exploitation as industrial enzymes (Villeneuve, 2003; Barros et al., 2010). These enzymes have been isolated from leaves, stems, latex, oils, and seeds of oleaginous plants and cereals (Muto and Beevers, 1974; Lin et al., 1983; Villeneuve, 2003; Eastmond, 2004; Sagiroglu and Arabaci, 2005; Barros et al., 2010; Gu et al., 2011; Su et al., 2010; You et al., 2011; Avelar et al., 2013). Lipases from latex and seed of oleaginous plants are mainly used in biotransformations of oils and fats (Villeneuve, 2003; Barros et al., 2010; Gu et al., 2011). In some cases, it has been reported that lipase activity is absent in ungerminated (or dormant) seeds and increases rapidly when germination starts (Muto and Beevers, 1974; Lin et al., 1983; Villeneuve, 2003; Eastmond, 2004; Barros et al., 2010; Su et al., 2010; Gu et al., 2011). However, in some cases, the lipolytic activity has been found in dormant seeds (Villeneuve, 2003; Eastmond, 2004; Barros et al., 2010; Avelar et al., 2013). Although the use of plant lipases is well documented, their application in biotransformation reactions, including the production of concentrated fatty acids from vegetable oils is still scarce in the literature.

The objective of this work was to select plant lipases from castor bean, corn, sunflower and passion fruit seeds for the production of concentrated fatty acids by hydrolysis of different vegetable oils. The first aim of the present study was to investigate the influence of seed germination time on the production of the lipases and effect of the pH and temperature of reaction on their catalytic properties. The determination of the hydrolytic activity of plant lipases toward different vegetable oils has also been studied. Then, the biocatalysts were used in hydrolysis reactions of different vegetable oils in the absence of emulsifier agents. The plant lipase that presented highest hydrolysis percentage was then selected and a factorial design was used to evaluate the conditions that maximize the production of concentrated fatty acids by hydrolysis of vegetable oils containing different profiles of fatty acids in their composition such as babassu, palm and soybean oils. In a subsequent study, the effect of crude extract concentration for the selected plant lipase and buffer concentration on the hydrolysis reaction was also studied. Finally, hydrolysis reactions were performed under optimized conditions to determine maximum hydrolysis percentage. Factorial design has been widely used for the optimization of hydrolysis of vegetable oils catalyzed by lipases from several sources (Freitas et al., 2007; Gonçalves et al., 2012; Avelar et al., 2013). The use of this technique is advantageous over conventional methods and it includes fewer experiments, suitability for multiple experimental factors and easy search for common relationships between various factors resulting in the identification of the optimum process conditions. These are very hard to identify by optimizing each individual variable independently (Box et al., 1978; Cruz et al., 2012).

The direct application of crude lipase extracts from oil seeds is economically attractive in oleochemical industry due to their easy preparation and low-cost because purification and immobilization steps are not required because enzyme molecules are naturally "immobilized" onto solid materials from seeds, allowing easy recovery of the biocatalyst after sequential batches (You et al., 2011). Thus, in this paper crude lipase extracts have been utilized.

2. Materials and methods

2.1. Materials

Sunflower and corn seeds were acquired from Fecularia Piranguinho (Piranguinho, MG, Brazil), passion fruit seeds from Marajinho S.A. (Piranguinho, MG, Brazil), and castor bean seeds from BRSeeds Ltd. (Araçatuba, SP, Brazil). Germitest papers (28.0 cm × 38.0 cm) were acquired from Cial Artigos para Laboratórios (Paulínia, SP, Brazil). Refined olive oil (Carbonell), soybean and canola oils (Liza) were purchased at a local market. Babassu oil was kindly supplied by Pulcra Chemical (Jacareí, SP, Brazil), and palm and palm kernel oils from Agropalma (Belém, PA, Brazil). The composition in fatty acids was determined according to the Official Methods and Recommended Practices of American Oil Chemists Society (Table 1) (AOCS, 2004). Gum Arabic and acetone were acquired from Synth (São Paulo, SP, Brazil). All other chemical reagents were of analytical grade.

2.2. Germination procedure and preparation of lipase powder extracts

Initially, the seeds were selected, sterilized in 0.5% m/v sodium hypochlorite solution for 10 min and washed thoroughly with distilled water. The germination test was carried out with three replications of 50 sterilized seeds disposed along a line drawn longitudinally at 100 mm from the upper border of Germitest papers moistened with distilled water at the proportion water/seed (m/m) of 2.5-fold and kept in a germinator at the temperature of 25 °C for a maximum period of 96 h. All seeds were germinated according to the methodology described by Pego et al. (2012), with slight modifications. In periods of 0, 24, 48, 72 and 96 h, the seeds were removed