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Valorization of Palm Oil Industrial Waste as Feedstock for Lipase Production

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Abstract The use of residues from the industrial processing of palm oil as carbon source and inducer for microbial lipase production can be a way to add value to such residues and to contribute to reduced enzyme costs. The aim of this work was to investigate the feasibility of using palm oil industrial waste as feedstock for lipase production in different cultivation systems. Evaluation was made of lipase production by a selected strain of *Aspergillus niger* cultivated under solid-state (SSF) and submerged fermentation (SmF). Lipase activity levels up to 15.41 IU/mL were achieved under SSF. The effects of pH and temperature on the lipase activity of the SSF extract were evaluated using statistical design methodology, and maximum activities were obtained between pH 4.0 and 6.5 and at temperatures between 37 and 55 °C. This lipase presented good thermal stability up to 60 °C and higher specificity towards long carbon chain substrates. The results demonstrate the potential application of palm oil industrial residues for lipase production and contribute to the technological advances needed to develop processes for industrial enzymes production.

Keywords Lipase · Agro-industrial wastes · Palm oil residues · Biorefinery · Submerged fermentation · Solid-state fermentation

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Introduction

The use of enzymes in different industrial sectors is increasing as an alternative to conventional chemical processes due to the technical-economic and environmental advantages afforded by these biocatalysts. Enzymes are capable of catalyzing a wide range of chemical transformations with high stereoselectivity, regioselectivity, and chemoselectivity, producing almost exclusively a single major product, without undesired byproducts [1]. Among the various industrial enzymes, lipases (triacylglycerol hydrolase, EC 3.1.1.3) stand out as the main biocatalysts with proven industrial applications and can make an important contribution to the multi-billion-dollar lipid biotechnology industry [2]. Lipases are highly selective enzymes that catalyze the hydrolysis of a wide range of esters, especially those that are insoluble in water, such as triglycerides. Furthermore, lipases catalyze esterification and transesterification reactions, making them attractive catalysts in organic synthesis [3, 4]. An important industrial application of lipases is the modification of fats and oils to produce biodiesel and other structured lipids.

Lipases can be produced by several different organisms (mammals, plants, and microorganisms), although microbial lipases are the class of enzymes most widely used in biotechnological applications. Filamentous fungi, including those from the genera *Aspergillus*, *Penicillium*, and *Fusarium*, are valuable organisms for the production of these biocatalysts [2]. However, the main obstacle to the wider application of lipases in the industrial sector is related to the cost of the enzymes [4]. Solid-state fermentation (SSF) is a promising alternative for the production of industrial enzymes, offering the potential to reduce production costs by using renewable agricultural residues as feedstocks [5–8]. Nevertheless, large-scale industrial production of enzymes still mainly uses submerged fermentation (SmF) technology due to easy monitoring and process control. In addition to the cultivation technology used, the selection of a suitable lipolytic microorganism (preferably one that produces an extracellular enzyme in order to avoid purification steps), together with a low-cost lipase inducer that enables high enzymatic production, is required to advance in the development of bioprocesses for lipase production suitable for large-scale industrial applications.

Palm oil is an oil source with considerable potential for application in the biofuels industrial sector due to the high oil productivity per hectare (3–6 tons of oil per hectare). An important characteristic of palm oil is that it has similar proportions of saturated (43.7 % palmitic acid, C16:0) and monounsaturated fatty acids (40.2 % oleic acid, C18:1) [9]. This characteristic results in a biodiesel with high stability towards oxidation, which is a desirable property of biofuels. Since the palm is becoming a very important economic crop in various countries, the generation of residual waste from the oil processing industry is expected to increase [10]. Hence, the use of waste from the palm oil processing industry as inducer and carbon source for microbial cultivation is a potential strategy for the production of lipases and can also contribute to adding value to the associated industrial waste streams.

The aim of this work was to investigate the feasibility of using palm oil industrial wastes as feedstock for lipase production under different cultivation systems. Evaluation was made of lipase production by a selected strain of *Aspergillus niger* cultivated under both solid-state fermentation (SSF) and submerged fermentation (SmF). A systematic characterization of the lipase produced, in terms of the effects of pH and temperature, thermal stability, and specificity towards different substrates, was also carried out in order to evaluate its potential for use in different biotechnological applications.

Methods

Microorganisms

An initial screening of 18 filamentous fungal strains from the Embrapa microorganism collections was carried out for selection of the most promising strain for lipase production under different cultivation systems. The strain selected for use in the fermentation processes was *Aspergillus niger* C, BRMCTAA 82, from the Embrapa Food Agroindustry Microorganisms Collection (Rio de Janeiro). Fungal spores were kept under freezing conditions (−18 °C) in 0.9 % NaCl and 20 % glycerol solution. Microorganism revitalization was carried out in plates containing potato dextrose agar (PDA) incubated for 4 days at 30 °C before starting the fermentation for enzyme production.

Substrates Used for Fermentation Processes

Agro-industrial wastes from the palm oil industry (palm fiber and palm alkaline soapstock) were used as feedstocks to produce lipase under both SSF and SmF. These wastes were kindly donated by Agropalma S.A. (Belém, Brazil). The palm fiber samples were milled and sieved, and the particle size selected was $1.0 \leq dp \leq 2.0$ mm.

Fungal Screening for Lipase Production

Three different methodologies were used for the screening of potential lipase producer microorganisms. Firstly, 18 filamentous fungi from the Embrapa collection were submitted to screening on plates, using tributyrin as substrate. The diameter of the transparent halo formed around the colony was measured and used to calculate the enzymatic index, EI, which is the ratio between the halo size and the colony size. This assay was carried out for 96 h, measuring the colony and halo diameters at intervals of 24 h. Subsequently, for the second screening step using olive oil as substrate and Rhodamine B [11], eight strains were selected from these fungi: three with higher EI, two with intermediate EI, and three negative controls (with no halo around the colony). In this plate assay, the lipase-producing strains showed fluorescent halos when subjected to UV light. Finally, the screening results using plating assays were confirmed by carrying out preliminary SSF cultivations with wheat bran as substrate and olive oil as inducer [12]. These cultivations were carried out in 250-mL flasks containing 10 g of wheat bran and 3 % (*v*/*w*) of olive oil, which were sterilized by autoclaving at 121 °C for 20 min before inoculation. A spore suspension volume corresponding to a final concentration of 10^7 spores/g of wheat bran was inoculated into the solid medium. The moisture content of the medium was adjusted to 80 % by the addition of 8 mL of 1.2 % (*w*/*v*) ammonium sulfate solution at pH 7.0 and then incubated under static conditions at 30 °C for 96 h. After this period, the enzymes were extracted by the addition of 1:5 (*w*/*v*) 100 mM sodium phosphate buffer solution (pH 7.0), with 150 rpm agitation at 30 °C for 30 min. The final enzymatic extracts were vacuum-filtered, centrifuged at 12,000 rpm for 15 min at 4 °C, and kept frozen at −18 °C prior to the analytical assays. All experiments were performed in triplicate, and the data were calculated as means ± standard deviations.

Lipase Production Under Solid-State Fermentation (SSF)

The SSF for lipase production was conducted in 250-mL flasks containing 10 g of solid substrate (palm fiber). A volume corresponding to 3 % (*v*/*w*) of palm alkaline soapstock was added after heating the soapstock until it melted. The solid medium was sterilized by autoclaving at 121 °C for 20 min before inoculation. A spore suspension volume corresponding to 10^7 spores/g of dry palm fiber was inoculated into the solid medium. The moisture content was adjusted to 80 % by the addition of 8 mL of 1.2 % (*w*/*v*) ammonium sulfate solution at pH 7.0, followed by incubation under static conditions at 30 °C for 96 h. After this period, the enzymes were extracted by the addition of 1:5 (*w*/*v*) 100 mM sodium phosphate buffer solution (pH 7.0), with 150 rpm agitation at 30 °C for 30 min. The final enzymatic extracts were vacuum-filtered, centrifuged at 12,000 rpm for 15 min at 4 °C, and kept frozen at −18 °C prior to the analytical assays. This enzymatic extract was used to quantify the lipase produced under SSF, which was expressed as activity units per volume (IU/mL). All experiments were performed in triplicate, and the data were calculated as means ± standard deviations.

Lipase Production Under Submerged Fermentation (SmF)

For the SmF cultivation for lipase production, a pre-culture step was carried out in 500-mL flasks using 100 mL of the liquid nutrient medium described by Toscano et al. [13], supplemented with 30 g/L of glucose. The pH was adjusted to 7.0 and the liquid medium was then sterilized by autoclaving at 121 °C for 20 min before inoculation. The pre-culture was initiated by inoculation of a spore suspension volume corresponding to 10^7 spores/mL of liquid medium, and the cultivation was performed at 30 °C for 48 h in an orbital shaker incubator with continuous stirring at 220 rpm. A volume of pre-culture suspension corresponding to 10 % (*v*/*v*) was transferred to the SmF culture medium in 500-mL flasks, as described above but supplemented with 10 g/L of glucose and 1 % of inducer (palm alkaline soapstock), which were kept for 96 h at 30 °C in an orbital shaker incubator, with continuous stirring at 220 rpm. Samples of the cultures were taken every 24 h, filtered, centrifuged at 12,000 rpm for 15 min at 4 °C, and kept under freezing conditions (−18 °C) for later analysis. All experiments were performed in triplicate, and the data were calculated as means ± standard deviations.

Determination of Lipase Activity

Lipase activity was determined by the hydrolysis of emulsified olive oil method, according to the procedure described by Soares et al. [14]. In this assay, after the enzymatic hydrolysis reaction at standard conditions (pH 5.0 and temperature of 45 °C), the medium containing the released free fatty acids (product of the enzymatic reaction) was titrated with 0.02 N KOH solution until pH 11 was reached. One unit of lipase activity was defined as the amount of enzyme that released 1 μmol of fatty acid per minute, under the assay conditions.

Determination of Effects of pH and Temperature on Lipase Activity

A full factorial design followed by response surface analysis was used to evaluate the effects of temperature and pH, and their possible interaction, on the lipase enzymatic activity. The experimental design selected was a central composite design comprising 11 runs,

corresponding to four cube points, four axial points, and three central points, with the experiments carried out in random order. The dependent variable (response) was the lipase activity. Statistica v. 10 software (Statsoft) was used to analyze the experimental data, perform analysis of variance (ANOVA), and plot the response surfaces. These assays were carried out with the crude enzymatic extract obtained after 72 h of cultivation under SSF using the wastes from the palm oil industry as feedstock.

Lipase Thermal Stability

The thermal stability of the lipase was evaluated by measuring the residual enzymatic activity of the crude enzymatic extract obtained after 72 h of SSF using the palm oil industry waste as feedstock. Temperatures of 40, 45, 50, 60, 65, and 80 °C were evaluated for a total period of 24 h, with sampling at 3, 6, 12, and 24 h. At the end of the incubation period, the samples were immediately submitted to lipase activity assays. Measurement of enzymatic activity was performed under standard conditions (pH 5.0 and temperature of 45 °C). All the experiments were carried out in triplicate, and the data were calculated as means ± standard deviations.

Specificity Towards Different Substrates

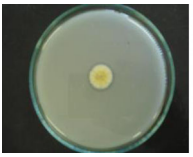
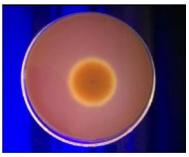
The crude enzymatic extract obtained after 72 h of SSF cultivation using the palm oil industry waste as feedstock was characterized in terms of its specificity for the hydrolysis of various emulsified oily substrates with different proportions of fatty acids: palm oil (43 % palmitic acid (C16:0) and 40 % oleic acid (C18:1)), soybean oil (22 % oleic acid (C18:1) and 53 % linoleic acid (C18:2)), sunflower oil (20 % oleic acid (C18:1) and 68 % linoleic acid (C18:2)), corn oil (30 % oleic acid (C18:1) and 52 % linoleic acid (C18:2)), canola oil (61 % oleic acid (C18:1) and 22 % linoleic acid (C18:2)), and tributyrin (C4). The activities were expressed relative to the control (olive oil). Measurement of lipase activity was performed under the standard conditions described previously, using pH 5.0 and temperature of 45 °C.

Results and Discussion

Screening of Fungal Strains for Lipase Production

For the selection of a potential fungal strain to be used in the fermentation processes for lipase production, an initial plate screening of 18 strains of filamentous fungi was carried out in a medium containing tributyrin as substrate. The lipase-producing microorganisms were identified from the formation of a transparent halo around the colony. For each strain, the enzymatic index (EI) value (halo diameter/colony diameter) was calculated after 96 h of incubation (Table 1). The fungi that showed higher EI in this plate assay with tributyrin were the strains P77C5, P50B2, and *A. niger* 12. From this initial plate assay, eight strains were selected for further evaluation in another plating assay, with olive oil as substrate for lipase production and Rhodamine B reagent for revealing the lipolytic halo under UV radiation. The relevance of the Rhodamine B test is that tributyrin (a water-soluble lipid) can be hydrolyzed by other esterases and is therefore considered a non-specific lipase substrate that can generate a false-positive result. Hence, a further evaluation using insoluble lipids as substrate is usually required for the screening of lipase producer microorganisms.

Table 1 Screening plate assays of different filamentous fungal strains in the presence of substrates with different carbon chain sizes

	Tributyrin				Rhodamine B
					
Fungal strains	**Øc**	**Øh**	**E.I.**	**St. deviation**	**Revealing under U.V.**
P77C5	6.67	11.67	1.75	0.10	-
P50B2	11.67	19.33	1.66	0.02	+
A. niger 12	21.67	29.00	1.34	0.02	+
A. niger 3T5B8	22.33	29.33	1.31	0.01	
P45C3	17.67	22.33	1.26	0.09	
P12P3	20.33	23.67	1.16	0.02	
A. niger C	26.33	29.67	1.13	0.04	+
A. niger 11T53A14	21.67	24.00	1.11	0.03	+
P27C3	23.33	25.33	1.09	0.00	
P6B2	22.33	23.67	1.06	0.03	
P47C3	20.33	21.33	1.05	0.00	
P21C3	22.33	23.33	1.04	0.00	
P34P9	23.33	24.00	1.03	0.02	
P41C1	23.00	23.67	1.03	0.03	
P1M1	23.00	23.33	1.01	0.03	
P75P1	27.00	0.00	0.00	0.00	-
P18E2	19.33	0.00	0.00	0.00	+
T. harzianum	48.67	0.00	0.00	0.00	+

Øc = colony diameter (mm);
Øh = halo diameter (mm);
E.I. = enzymatic index (Øh / Øc).

For the screening using the plating assay with olive oil as substrate and Rhodamine B, which is a qualitative test that only indicates the presence or absence of lipase, the following strains were evaluated: P77C5, P50B2, and *A. niger* 12 (because they presented the highest EI values in the tributyrin assay); *A. niger* C and *A. niger* 11T53A14 (because they presented intermediate EI values); and P75P1, P18E2, and *Trichoderma harzianum* (which were used as negative controls). The results of this plating assay were then compared with the results obtained using the assay with tributyrin as substrate (Table 1).

In the Rhodamine B assay, only the P75P1 and P77C5 strains presented negative results for lipase production, with all the other strains showing positive results. The two plating assays therefore showed some conflicting results: P77C5 presented a halo when plated on tributyrin, with the highest EI value, but showed no fluorescence in the Rhodamine B assay; the strains P18E2 and *T. harzianum* showed fluorescence in the Rhodamine B assay, but no hydrolytic halo when grown in tributyrin. These findings suggested different lipase and esterase secretion profiles of the fungal strains: the P77C5 strain only showed a hydrolysis halo for the short-chain substrate (tributyrin, C4), indicating a predominant production of esterases, while the P18E2 and *T. harzianum* strains showed hydrolysis halos for the long-chain substrate (olive oil, C18), indicating that these strains predominantly produced lipases. The other fungal strains probably synthesized both types of enzyme, with the exception of the P75P1 strain, which presented no hydrolytic activity for any of the substrates used. Similar findings have been reported previously, with different results obtained using these two plating assays with tributyrin and Rhodamine B as substrates for the screening of lipase-producing fungal strains [7].

Following these two screening steps using plating assays, SSF cultivations were carried out using wheat bran as substrate and olive oil as inducer. These conditions are widely employed for lipase induction [15–17] and were used here to confirm the potential production of lipases by the eight fungal strains evaluated in the previous step, under cultivation conditions more representative of fermentation processes. Titration of fatty acids released from hydrolysis of the olive oil was carried out to compare the lipolytic activities of all eight strains (Fig. 1). From compilation of all the previous results, especially those for the SSF cultivation, the *A. niger* C fungus was selected for use in further experiments because this strain showed the highest

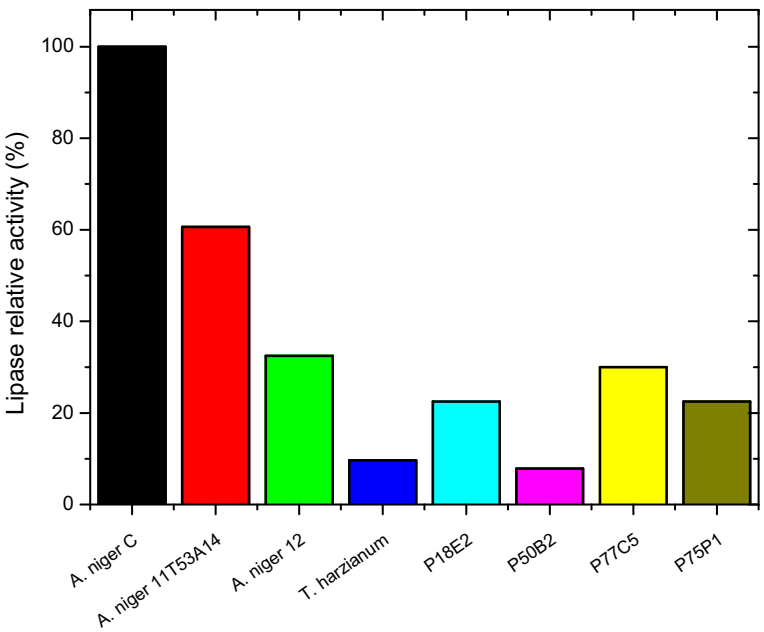


Fig. 1 Relative lipase activities in the screening of different fungal strains carried out under SSF cultivation for 72 h using wheat bran as solid substrate and olive oil as inducer

levels of lipase activity. The following cultivations under SSF and SmF were therefore carried out using the *A. niger* C strain and the palm oil industry waste as feedstock.

Fermentation Processes for Lipase Production

Figure 2 shows the time courses of lipase production in SSF and SmF cultivations with *A. niger* C, using the palm oil industry waste as feedstock. In the SSF cultivations, the maximum lipase activity was obtained after 72 h of cultivation (15.41 IU/mL, which is equivalent to 77.04 IU/g of dry substrate), while for cultivations performed under SmF, the activity peak was reached after 48 h (10.46 IU/mL). It is interesting to compare these results with previous findings reported in the literature. For example, in the work carried out by Toscano et al. [13], lipase activity measurements were also based on the olive oil titration method, using an *A. niger* wild-type strain and another *A. niger* mutant strain cultivated under SmF. The wild-type strain produced an activity of 7.5 IU/mL, while the value for the mutant strain was 15.5 IU/mL. However, these levels were only reached after 96 h of cultivation, with both organisms grown in a liquid SmF nutrient medium similar to the one employed in the present work, using olive oil as inducer and carbon source. In comparison of the present results with those of another study of lipase production by two *Aspergillus* strains, under SmF and SSF with an optimized medium [17], the lipase activity values obtained here using the palm oil industry waste as feedstock were up to 2-fold higher. The lipase activities obtained in the present study were similar or higher, compared to those achieved using other fungal stains and a variety of other raw materials as carbon sources and inducers [2, 12, 18–20]. The results therefore provide a clear indication that the fatty acids contained in the palm oil agro-industrial waste were suitable for inducing lipase production by *A. niger*.

The palm is an economically important crop in several countries, and palm fiber materials constitute an abundant waste generated by the oil processing industry [10]. Furthermore, the

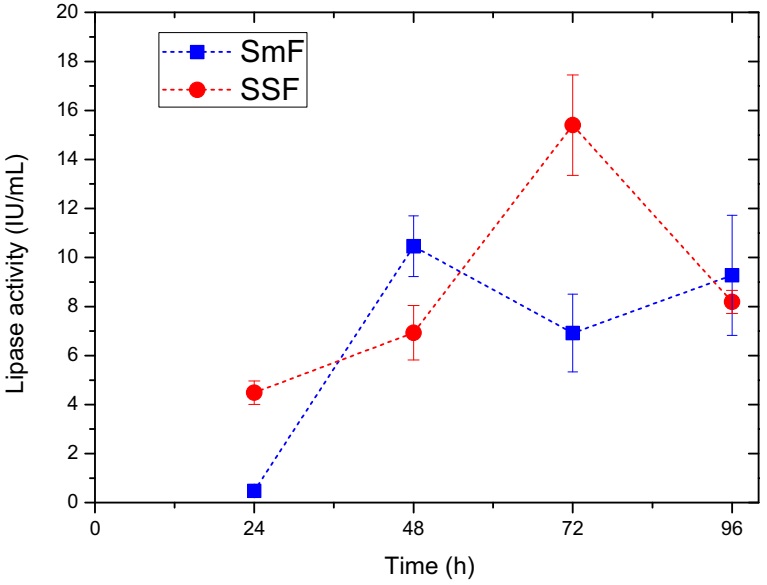


Fig. 2 Time profile of lipase production by *A. niger* cultivated under SSF and SmF using the palm oil industry wastes as feedstock

Brazilian government plans to extend palm plantations in deforested areas, for the purpose of biofuel production, which will lead to the generation of very large amounts of agro-industrial waste with potential biotechnological applications [21]. In the present work, the focus is on use of both of the main palm oil industry wastes (fiber and soapstock), adding value to these residues by using them to produce lipases under different fermentation conditions. This utilization of renewable agricultural residues as feedstock for lipase production can potentially contribute to reducing the overall costs of enzyme production [18].

Based on the previous results, the enzymatic extract obtained after 72 h of cultivation of *A. niger* C under SSF, using the palm oil industry waste as feedstock, was selected for use in the lipase biochemical characterization assays. Even though both SmF and SSF cultivations resulted in lipase productivity values of similar magnitudes, the use of SSF as the selected cultivation method was based on the fact that this technology is more suitable for filamentous fungi growth and, from the environmental perspective, enables the use of solid agro-industrial waste substrates as sources of carbon and energy for microorganism growth and enzyme production [6]. Besides the advantage of using a low-cost agro-industrial waste as substrate, SSF is also reported to result in enzymes with improved characteristics in terms of stability as well as such process usually requires less downstream operations steps, which makes SSF a promising technology [2, 6].

Effects of pH and Temperature on Lipase Activity

The effects of pH and temperature on lipase activity in the crude enzymatic extract obtained after SSF using *A. niger* C grown on the palm oil waste were evaluated using statistical design of experiments methodology and response surface analysis. Table 2 presents the results of the full factorial design for lipase activity. The ANOVA analysis of lipase activity (Table 3), with F value 3.53 times higher than the listed F value (at the 90 % confidence level), showed satisfactory prediction of the model used to generate the response surface plot of enzyme activity as a function of pH and temperature (Fig. 3).

Table 2 Experimental conditions and results of the statistical experimental design for lipase activity in the extracts produced by *A. niger* cultivated under SSF using the palm oil industry wastes

Trial	pH	Temperature (°C)	Lipase activity (IU/mL) Mean values
1	3.6 (−1)	35 (−1)	7.90
2	3.6 (−1)	55 (1)	7.86
3	6.4 (1)	35 (−1)	9.93
4	9.8 (1)	55 (1)	9.41
5	3.0 (−1.41)	45 (0)	7.21
6	7 (1.41)	45 (0)	8.67
7	5.0 (0)	30.9 (−1.41)	9.03
8	5.0 (0)	59.1 (1.41)	7.30
9	5.0 (0)	45 (0)	11.00
10	5.0 (0)	45 (0)	10.43
11	5.0 (0)	45 (0)	11.20

Table 3 Coefficient values and statistical analysis of lipase activity in the extracts produced by *A. niger* cultivated under SSF using the palm oil industry wastes

Factor	Coefficient	*p* Value
pH	0.70456	0.041152
pH^2	−1.28240	0.003460
T^2	−1.17365	0.006480
R^2	0.54	
F value	8.54	
$F_{measured}/F_{critical}$	3.53	

R^2 coefficient of determination

The response surface plot indicated that higher lipase activity values were obtained for pH values in the range from 4.0 to 6.5, and for temperatures between 37 and 55 °C. An advantage of using the experimental design methodology was that pH and temperature ranges (rather than specific values) could be identified for optimum enzyme activity, thus allowing more flexibility during process operations. The highest enzymatic activity was found for the conditions of the central point of the experimental design (pH 5 and 45 °C). Nevertheless, it is important to note that such activity values are within the optimum range shown in the surface plot (Fig. 3), in which values are not statistically different.

In terms of optimum pH and temperature, this lipase showed properties in common with lipases from other *A. niger* strains because lipases from *A. niger* strains are usually active in a pH range between 4 and 7, and in a temperature range between 40 and 55 °C. Kamini et al. [22] produced a lipase from *A. niger* by SSF with sesame oil and obtained a lipase that showed

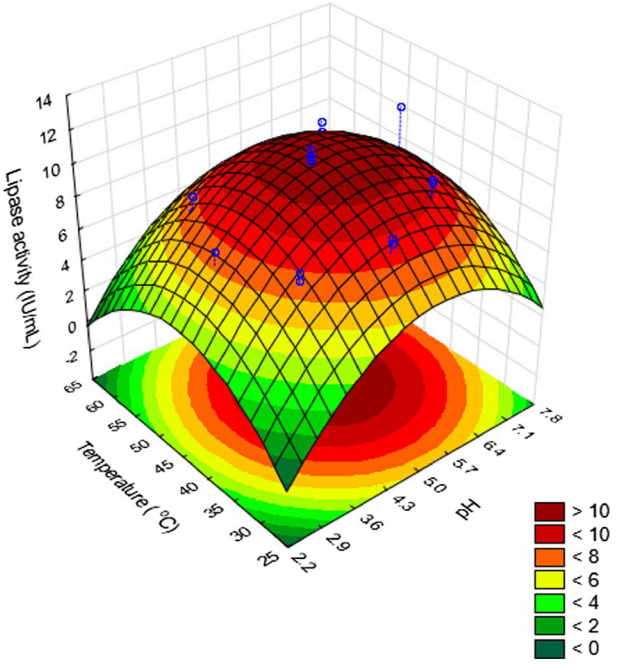


Fig. 3 Response surface for lipase activity, as a function of pH and temperature, in the extracts produced by *A. niger* cultivated under SSF using the palm oil industry wastes

optimum activity at pH 7 and 37 °C. Lipase from *A. niger* cultivated under SSF in wheat bran was reported to be most active at pH 6 and temperature around 40 °C [15]. The present data are therefore in agreement with previous findings for *A. niger* lipases.

Lipase Thermal Stability

The thermal stability of the lipase produced under SSF by *A. niger* C using the palm oil waste is shown in Fig. 4. Total incubation periods of 24 h at 40, 45, 50, 60, 65, and 80 °C were used. The lipase incubated at temperatures of 40, 45, and 50 °C remained highly stable throughout the 24-h period (Fig. 4), with around 80 % of the activity remaining at the end of the period. These temperatures lie within the optimal temperature range previously evidenced by the response surface (Fig. 3). However, a significant decrease in activity was observed at 60 °C, with approximately 10 % residual activity after 24 h. The lipase showed complete loss of hydrolytic activity after 3 h of exposure to 65 and 80 °C.

The lipases produced in this study showed encouraging thermal stabilities when compared to lipases produced previously using strains of *Aspergillus*. For instance, lipase from *A. niger* cultivated under SSF in wheat bran retained 60 % of its activity after exposure to 50 °C for 24 h [15]. The thermal stability of the lipase was also better when compared to enzymes from other fungal strains. Gutarra et al. [5] reported a rapid decay in the activity of lipases from *Penicillium simplicissimum* subjected to a temperature of 50 °C, with a half-life of 5 h at pH 5. It is widely acknowledged that an important characteristic required for enzymes used in industrial applications is that they should be stable at high temperatures due to the associated higher reaction rates, and for this reason there is a great demand for thermostable enzymes in industrial applications [3]. Nevertheless, most of the lipases produced by mesophilic fungi that have been reported in the literature have low thermal stability, with loss of most of the activity

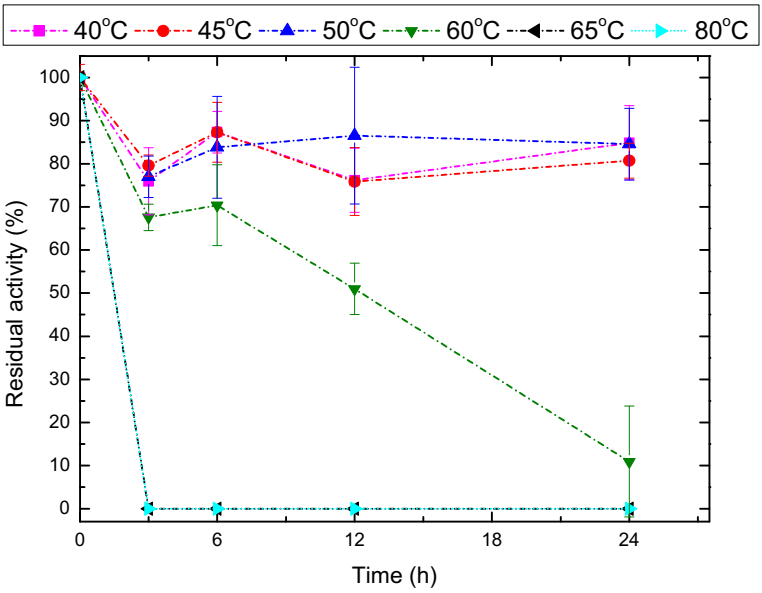


Fig. 4 Thermostability of lipase activity in the extracts produced by *A. niger* cultivated under SSF using the palm oil industry wastes

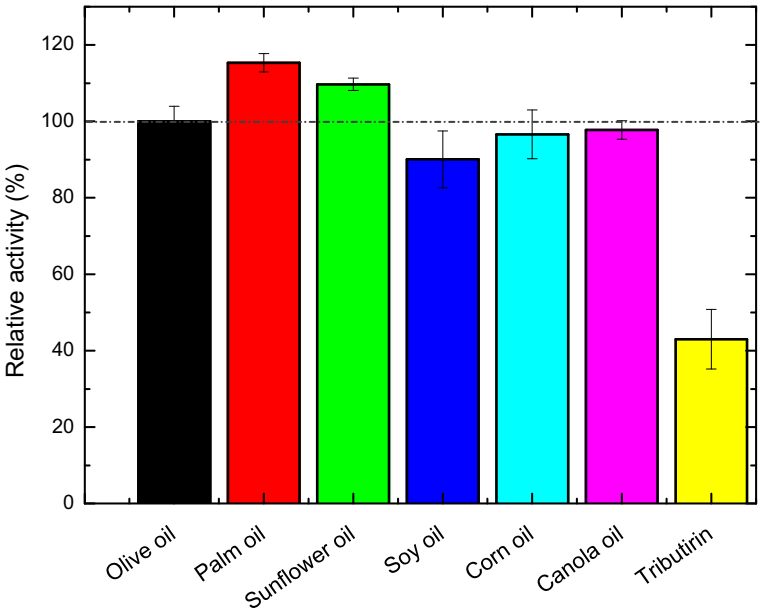


Fig. 5 Specificity of the lipase from *A. niger* cultivated under SSF with the palm oil industry wastes, using different substrates

at temperatures above 40 °C. In the present study, *A. niger* lipase produced using the palm oil waste exhibited very satisfactory thermal stability in the range from 40 to 50 °C, over a relatively long period (24 h), which could be of considerable interest in industrial applications.

Specificity Towards Different Substrates

The lipase produced under SSF by *A. niger* C using palm oil industrial wastes presented higher specificity towards hydrolysis of the long carbon chain fatty acids present in palm oil, sunflower oil, and olive oil, with lower activity towards a short carbon chain substrate such as tributyrin (Fig. 5). This is a very important property, confirming that this enzyme from *A. niger* C can be classified as a true lipase [4]. The high specificity of the *A. niger* lipase obtained from SSF cultivations towards long carbon chain fatty acids may be related to the substrate used as inducer because a characteristic of palm oil is that it contains similar proportions of saturated (43.7 % palmitic acid, C16:0) and monounsaturated fatty acids (40.2 % oleic acid, C18:1) [9]. The successful application of lipases in industrial processes also requires this specificity [3]. The lipase from *A. niger* produced under SSF using palm oil industrial waste is therefore a promising candidate for use in industrial applications involving biotechnological transformations.

Conclusions

The production of lipase using agro-industrial waste from the palm oil industry as feedstock was demonstrated to be a feasible process with high potential for obtaining marketable products. Lipase produced by the *A. niger* C strain cultivated under SSF showed a high level

of hydrolytic activity in the optimum temperature range between 37 and 55 °C and the optimum pH range between 4 and 6.5. Highly satisfactory thermal stability of the SSF extract was observed in the range from 40 to 50 °C, over a relatively long period of 24 h, and higher specificity towards long carbon chain substrates confirmed that it was a true lipase. This lipase therefore presents interesting catalytic properties and shows excellent potential for modifying fats and oils to produce different bio-products in industrial applications. In addition, this bioprocess technique provides a means of properly disposing of palm oil agro-industrial waste, while at the same time adding value to this material as a renewable feedstock for microbial enzyme production that is compatible with the biorefinery concept.

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