Precipitation Using Organic Solvents for Purifying Lipase from *Preussia africana*

ABSTRACT

Aims: The present study aimed to purify a new lipase from the endophytic *fungus Preussia africana (P. africana).*
Study design: Lipases were produced through fermentation and precipitation using different organic solvents and characterized at optimal temperature and pH conditions.
Place and Duration of Study: Assays were carried out at the Laboratory of Bioprocess at the Federal University of Alfenas between January and August, 2023.
Methodology: Enzymes were produced via submerged fermentation. After fermentation, the broth was filtered and lyophilized so that enzymes were concentrated. Organic solvents (methanol, ethanol, isopropanol and acetone) were used at 10 and 25 °C for lipase precipitation. A second precipitation step was investigated in a supernatant:solvent ration ranging from 1:3 to 1:2 (v/v) and temperatures between 10°C and 25 °C. Optimal temperature and pH conditions for the hydrolytic activity of precipitated lipase were also found.
Results: Acetone and isopropanol were more efficient in precipitating lipase while maintaining its catalytic activity, as specific activities of 211.34 ± 0.05 and 179.50 ± 0.08 U/mg were achieved, respectively. For the second precipitation step, acetone in a supernatant:solvent ratio of 1:2 (v/v) at 10°C achieved optimal performance, as purification factor of 1.89 ± 0.01 and recovered activity of 93.45% were found. Maximum hydrolytic activity of 72.80 ± 1.29 U/mL was observed using olive oil as substrate at pH between 7 and 8 and 37°C.
Conclusion: The use of acetone at 10°C in two precipitation steps proved to be a more robust strategy for *P. africana* lipase precipitation in the purification process.

Keywords: Lipase, Preussia africana, Purification, Organic solvents, Acetone

1. INTRODUCTION

It is estimated that fungi have been combining with plant species approximately for 400 million years ago [1]. Although the first endophyte was discovered in 1904, these microorganisms did not receive much attention until a recent discovery of its ecological importance and potential for producing secondary metabolites having different biological functions [2-5]. However, endophytic fungi are also able to produce a wide variety of enzymes, such as cellulases, amylases and lipases, but there are few studies aimed at investigating enzymes produced by these fungi, as well as their possible applications in different industrial sectors [6,7].

Lipases are enzymes capable of hydrolyzing ester bonds of fatty acids present in triacylglyceride molecules, releasing free fatty acids and glycerol. In media where there is reduction or absence of water, lipases can also carry out synthesis reactions [8]

In recent decades, the use of lipases in industrial processes has been growing and becoming increasingly popular in different sectors, such as food, pharmaceuticals, fine chemicals, biofuels, among others, thus increasing their importance in the contemporary world [9,10].

However, for such a purpose, a purification step is usually required after their production in order to remove impurities present in the culture medium, while at the same time maintaining enzyme activity [11,12]. The process of purifying an enzyme is often the most costly and significant. Just one step is often insufficient, thus there must be a cascade of operations so as to achieve the greatest degree of purity. Selecting the method to be used depends on several factors, such as source of enzyme and its molecular size [13].

Precipitation of enzymes by organic solvents is widely reported in literature. The use of these substances neither requires a sophisticated apparatus, nor specific equipment for their use [14]. The selection of organic solvent to be used depends on the enzyme structure, its tolerance to solvents, its toxicity level and availability. An appropriate choice can lead to a fast and efficient purification process, otherwise it causes denaturation and consequent loss of activity, thus requiring specific studies on different enzymes [15].

In the present work, different organic solvents were used for precipitating lipases produced by submerged fermentation from the endophytic fungus *P. africana* aiming at reaching greater enzyme recovery and maintaining the catalytic capacity of lipases.

2. MATERIAL AND METHODS

2.1 Microorganism and lipase production

The endophytic fungus *P. africana* was previously isolated and identified by Souza et al [16], (*GenBank access code: SAMN 06350896; CPQBA 1304/16 DRM - 01*). Lipases were produced by submerged cultivation. The inoculum medium had the following composition: 2% (m/v) casein peptone, 0.1% (m/v) yeast extract, 0.05% (m/v) sodium nitrate, 0.05% (w/v) magnesium sulfate heptahydrate and 1% (v/v) sunflower oil [16].

The production medium had a similar composition to that described by Souza et al. (2018) [16]: 2% (w/v) casein peptone, 0.1% (m/v) yeast extract, 0.05 % (m/v) sodium nitrate, 0.05% (m/v) magnesium sulfate heptahydrate, 0.05% (m/v) calcium nitrate tetrahydrate, 0.05% (m/v) monobasic sodium phosphate and 1% (v/v) sunflower oil.

The microorganism was incubated at 30°C in a plate containing Sabouraud Dextrose Agar (SDA) for 7 days. Afterwards, 5 mm in diameter of the plate containing the developed colony was removed and transferred to a 1 L Erlenmeyer flask containing 100 mL of the inoculum medium incubated for 48 h in an orbital shaker at 30°C and 250 rpm agitation. In the production stage, 10 mL inoculum was transferred to 100 mL of the previously autoclaved production medium in a 1 L Erlenmeyer flask. These were kept at 30°C and 250 rpm agitation for 60 hours. Afterwards, the broth was vacuum filtered using filter paper and the supernatant was lyophilized until it reached one third of its initial volume, thus the initial hydrolytic activity was 19.53 ± 0.44 U/mL.

2.2 Precipitation using different solvents

The organic solvent precipitation method was used for the previously lyophilized broth. The evaluated solvents were: acetone, anhydrous ethanol, 95% ethanol, isopropanol and methanol [17].

Precipitation assays were carried out at two different temperatures (10 and 25 °C) in a culture broth:solvent ratio of 1:3 (v/v). After adding the solvent, the system was homogenized and centrifuged for 15 minutes at 3,000 rpm for protein precipitation. The precipitated enzymes were resuspended in a phosphate buffer pH 7 100 mM and subjected to hydrolytic activity analysis and protein quantification [18].

In posterior assays, isopropanol and acetone were used at temperatures of 10 and 25°C in a culture broth:solvent ratio of 1:3 (v/v). The system was homogenized and centrifuged for 15 minutes at 3,000 rpm. The precipitation step was repeated, the supernatant removed and the solvent was once again added. Variation in the proportion of solvent for this precipitation step was carried out in supernatant:solvent ratios of 1:2 and 1:3 (v/v). The system was homogenized and centrifuged under the same conditions described above. The precipitated lipases were resuspended in a 100 mM phosphate buffer at pH 7 and submitted to hydrolytic activity analysis and protein quantification. All assays were performed in triplicate.

2.3 Parameters of lipase precipitation from *P. africana*

The parameters described in the equations below were calculated according to Menegotto et al [19].

Relative recovered activity (RA) is given by equation I:

$$AR(\%) = \frac{FA}{IA}\ x\ 100 \qquad (I)$$

Where FA is the final enzyme activity (U) obtained after precipitation and IA is the initial enzyme activity (U).

The recovered protein (RP) percentage was calculated by equation II:

$$RP\ (\%) = \frac{FP}{IP}\ x\ 100 \qquad (II)$$

Where IP is the initial amount of protein (mg) and FP is the final amount of precipitated protein (mg)

The initial specific activity (SIA) and final specific activity (SFA) of the precipitated enzyme were calculated by dividing the measured activity (U/mL) by the amount of protein (mg/mL). Thus, the purification factor (PF) was calculated by a division of these specific activities:

$$PF=SFA/SIA \qquad (III)$$

2.4 Determination of optimal pH and temperature for lipase from *P. africana*

The determination of optimal pH conditions for the enzyme activity of purified lipase was carried out at pH ranging between 4.0 - 9.0 (pH 4.0 and 5.0 – 100 mM sodium acetate buffer; pH 5.0 – 8.0 – 100 mM sodium phosphate buffer; pH 9.0 - 100 mM sodium bicarbonate buffer). Assays were carried out at 37 °C for 5 min using olive oil emulsified with gum arabic according to details describend on the ‘Determination of hydrolytic activity’ section.

The effect of temperature on hydrolytic activity was evaluated at temperatures ranging from 25 to 80°C at 5 min of reaction using olive oil emulsified with gum arabic and 100 mM sodium phosphate buffer at pH 7 (see section 'Determination of hydrolytic activity').

2.5 Determination of specificity for different substrates

The lipase specificity for different substrate was carried out using different vegetable oils (olive, soybean, cotton, sunflower, corn, palm, canola, linseed) and tributyrin. Lipase hydrolytic activity was determined according to details described on the "Determination of hydrolytic activity" section, using pH 7.0 and 37 °C, by replacing olive oil with those mentioned above.

2.6 Determination of protein concentration

The determination of the amount of protein was carried out according to the Bradford method [20] using bovine serum albumin (BSA) as standard.

2.7 Determination of hydrolytic activity

The hydrolytic enzyme activity was determined by the method described by Castro et al [21] through olive oil hydrolysis using olive oil emulsion and distilled water in the ratio of 1:1, and 7% (m/V) of Arabic gum as an emulsifier.

3. RESULTS AND DISCUSSION

The results obtained for the enzyme activity recovery (relative) regarding the different solvents used in the purification process of lipases from *P. africana* are shown in Table 1. Optimal results of activity recovery were found for acetone at 10 °C (69.80%) and for isopropanol at 25 °C (52.10%).

Table 1 – Initial broth activity, precipitate activity after precipitation and relative activity obtained for each solvent at 10 and 25°C.

Solvent	Temp. 10 °C			Temp. 25 °C		
	IA (U)	FA (U)	RA (%)	IA (U)	FA(U)	RA (%)
Methanol	39.06 ± 0.90	1.89 ± 0.47	4.84	39.06 ± 0.90	1.08 ± 0.03	2.76
Absolute Ethanol	48.42 ± 0.50	15.30 ± 1.53	31.60	48.42 ± 0.5	11.79 ±1.82	24.35
Isopropanol	64.76 ± 0.99	29.39 ± 0.18	45.38	64.76 ± 0.99	33.74 ± 2.10	52.10
Acetone	73.29 ± 0.36	51.15 ± 0.96	69.80	73.29 ± 0.36	37.13 ± 1.77	50.66
Ethanol 95%	69.66 ± 1.17	19.65 ± 0.44	28.21	69.66 ± 1.17	15.96 ± 0.99	22.92

Temp. = Temperature; IA = Initial activity of the broth; FA = Final precipitate activity; RA = Relative recovered activity

While adding organic solvents to the previously lyophilized enzymatic broth, water molecules present in the broth, which were previously solvating proteins, were mobilized and start interacting with the solvent. Thus, surface charges of enzymes become available to interact with each other. As a consequence, there is the formation of large agglomerates that precipitate due to their great molecular weight [11].

Ferreira et al. [14] observed a similar behavior while purifying lipase from *G. candidum*. The maximum yield found for recovered enzyme activity was 87.9% using acetone at 0 °C in the purification process.

Table 2- Initial protein, protein present in the precipitate after precipitation, and relative precipitated protein obtained for each solvent at 10 and 25°C.

Solvent	Temp 10 °C			Temp 25 °C		
	IP (mg)	FP (mg)	RP (%)	IP (mg)	FP(mg)	RP (%)
Methanol	0.33 ± 0.01	0.33 ± 0.01	100.00	0.33 ± 0.01	0.30 ± 0.01	91.85
Absolute Ethanol	0.33 ± 0.01	0.30 ± 0.01	90.08	0.33 ± 0.01	0.32 ± 0.01	97.52
Isopropanol	0.33 ± 0.01	0.26 ± 0.01	79.45	0.33 ± 0.01	0.31 ± 0.01	92.56
Acetone	0.33 ± 0.01	0.24 ± 0.01	73.07	0.33 ± 0.01	0.21 ± 0.01	62.44
Ethanol 95%	0.33 ± 0.01	0.29 ± 0.02	87.24	0.33 ± 0.01	0.25 ± 0.01	76.97

Temp. = Temperature; IP = Initial protein; FP = final precipitated protein; RP = Relative recovered protein

An interaction of the solvent with water molecules explains recovery results in terms of proteins (Table 2). Highly polar solvents, such as methanol and absolute ethanol, performed better, as they precipitated 100% (10°C) and 97.52% (25°C) of protein, respectively, if compared to acetone (73.07 and 62 .44% of precipitated enzyme for tests carried out at 10 and 25 °C, respectively), which has lower polarity. However, water molecules also move across internal areas, such as the active site of the enzyme. Thus, interactions within the molecule itself can lead to enzyme distortion and loss of its three-dimensional structure, in addition to loss of its catalytic activity, thus explaining the low values of relative recovered activity found for these solvents (see Table 1). Although lower yields of protein precipitation of 73.07 and 62.44% were found during assays carried out at 10 and 25 °C for acetone, greater relative recovered lipase activity was observed (69.80 % in tests at 10 °C and 50.66% at 25 °C).

By observing specific recovery activity values (Table 3), a better performance of acetone is verified, as its maximum value was 211.34 ± 0.05 U/mg in the assay carried out at 10°C. However, despite presenting superior results compared to other solvents, acetone was unable to actually increase enzyme purification through only one purification step, since purification factor was less than 1, and it was found that the specific activity of the culture broth was greater than the one observed after precipitation using acetone. Therefore, another precipitation step was performed for acetone and isopropanol, which were the solvents able to achieve the highest relative recovered activity (see Table 1).

Table 3- Initial specific activity, specific activity after purification with solvents at 10 and 25°C and purification factor.

Solvent	Temp 10 °C			Temp 25 °C		
	SIA (U/mg)	SFA (U/mg)	PF	SIA (U/mg)	SFA (U/mg)	PF
Methanol	117.91 ± 0.26	5.75 ± 0.27	0.05	117.91 ± 0.05	3.55 ± 0.05	0.03
Absolute Ethanol	146.17 ± 0.04	51.27 ± 0.13	0.35	146.17 ± 0.04	36.50 ± 0.18	0.25
Isopropanol	195.50 ± 0.04	111.66 ± 0.04	0.57	195.50 ± 0.05	110.04 ± 0.09	0.56
Acetone	221.23 ± 0.04	211.34 ± 0.05	0.96	221.23 ± 0.4	179.50 ± 0.08	0.81
Ethanol 95%	210.28 ± 0.05	68.00 ± 0.05	0.32	210.28 ± 0.5	62.61 ± 0.01	0.30

Temp. =Temperature; SIA = Initial specific activity; SFA = Final specific activity; PF = Purification factor

Thus, new assays were carried out to investigate the efficiency of a second precipitation step involving organic solvents in different ratios between the resulting supernatant and organic

phase (1:3 and 1:2 v/v) for acetone and isopropanol which showed better performance for relative recovery activity. The effect of temperature, 10 and 25 °C, was also evaluated in these assays.

Table 4 presents the results of two precipitations obtained in a 1:3 broth:solvent ratio (v/v) in initial and second precipitation steps.

Table 4 - Recovery of enzyme and protein activity in initial and second precipitation steps using isopropanol and acetone at 10 and 25 °C in ratios of 1:3 (v/v, broth: solvent) for the initial precipitation and 1:3 (v/v, supernatant:solvent) for the second precipitation.

Solvent	**Temp 10 °C**			**Temp 25 °C**		
	IA (U)	**FA(U)**	**RA (%)**	**IA (U)**	**FA (U)**	**RA (%)**
Isopropanol	71.91 ± 1.54	70.11±3.4	97.50	57.97 ± 0.01	43.35 ± 2.6	74.78
Acetone	64.81 ± 0.42	62.67±2.0	96.70	70.79 ± 1.28	48.48 ± 1.2	68.48
	Temp 10 °C			**Temp 25 °C**		
	IP (mg)	**FP (mg)**	**RP (%)**	**IP (mg)**	**FP (mg)**	**RP (%)**
Isopropanol	0.91 ± 0.01	0.91 ± 0.01	100.00	0.91 ± 0.01	0.85 ± 0.01	93.79
Acetone	0.91 ± 0.01	0.84 ± 0.01	93.07	0.91 ± 0.01	0.77 ± 0.01	84.48

Temp. = Temperature; IA = Initial activity of the broth; FA = Precipitate activity; RA = Relative recovered activity; IP = Initial protein; FP = Precipitate protein; RP = Relative recovered protein

Optimal lipase recovery activity was found using solvents at 10°C (Table 4), and it was observed that there was no great activity loss, since relative recovered activity was close to 100% for both solvents. When solvents are used at low temperatures for precipitation, the enzyme activity loss usually decreases, since there is less solvent penetration into the molecule [11]. However, when solvents are used at higher temperatures, the three-dimensional structure of the enzyme might be compromised by breaking the chemical bonds composing it, thus leading to its catalytic activity losses [22].

Protein recovery was higher using isopropanol at 10°C, thus leading to total precipitation of lipases present in the culture broth. Such high precipitation led to an increase in specific activity of the precipitated enzyme (Table 5), consequently a higher purification factor was found (1.89 ± 0.01). Acetone showed very similar performance, as it reached purification factor of 1.88 ± 0.01.

Table 5 - Initial specific activity, specific activity after two purification steps and purification factor using isopropanol and acetone at 10 and 25 °C in a 1:3 ratio (v/v, broth:solvent) for the initial precipitation and 1:3 (v/v, supernatant:solvent) for the second precipitation.

Solvent	**Temp 10 °C**			**Temp 25 °C**		
	SIA (U/mg)	**SFA (U/mg)**	**PF**	**SIA (U/mg)**	**SFA (U/mg)**	**PF**
Isopropanol	79.20 ± 0.03	150.04 ± 0.06	1.89 ± 0.01	63.85 ± 0.01	88.24 ± 0.07	1.38 ± 0.01
Acetone	71.39 ± 0.02	134.20 ± 0.04	1.88 ± 0.01	77.98 ± 0.03	100.68± 0.04	1.29 ± 0.01

Temp. =Temperature; SIA = Initial specific activity; SFA = Final specific activity; PF = Purification factor

Afterwards, a second precipitation step was performed with a smaller amount of solvent and 1:2 (v/v, supernatant: solvent). The same analyzes were also performed in order to

determine the relative enzyme activity and recovered protein. Table 6 shows the results obtained for enzyme activity and amount of proteins recovered.

Table 6 - Enzyme and protein recovery activity for initial and second precipitation steps using isopropanol and acetone at 10 and 25 °C in a 1:3 ratio (v/v, broth: solvent) for the initial precipitation and 1:2 (v/v, supernatant:solvent) for the second precipitation.

Solvent	**Temp 10 °C**			**Temp 25 °C**		
	IA (U)	**FA (U)**	**RA (%)**	**IA (U)**	**FA (U)**	**RA (%)**
Isopropanol	61.09 ± 0.34	45.81 ± 1.4	74.98	61.14 ± 1.02	44.20 ± 0.9	72.29
Acetone	61.39 ± 0.53	57.37 ± 0.8	93.45	66.06 ± 1.25	49.95 ± 3.2	75.61
	Temp 10 °C			**Temp 25 °C**		
	IP (mg)	**FP (mg)**	**RP (%)**	**IP (mg)**	**FP(mg)**	**RP (%)**
Isopropanol	0.90 ± 0.01	0.90 ± 0.01	100.00	0.90 ± 0.01	0.80 ± 0.01	88.76
Acetone	0.90 ± 0.01	0.76 ± 0.01	84.71	0.90 ± 0.01	0.79 ± 0.01	87.46

Temp. = Temperature; IA = Initial activity of the broth; FA = Precipitate activity; RA = Relative recovered activity; IP = Initial protein; FP = Precipitate protein; RP = Relative recovered protein

Optimal performance was achieved using acetone at 10°C, thus obtaining 93.45% relative recovered activity. If compared to the relative activity recovered for acetone at the same temperature, as shown in Table 4 (96.70%) in which a larger amount of solvent was used, it was verified that there was a small drop in recovered activity (from 3.35%), however, such a drop was more pronounced for isopropanol at 10°C.

For the assay using acetone at 25°C, there was an increase in relative recovered activity by around 7%, and a concomitant increase in the amount of recovered protein (around 3%), thus explaining the increase in relative activity found at 25°C.

Table 7 - Initial specific activity, final specific activity after two purification steps and purification factor using isopropanol and acetone at 10 and 25 °C, in a 1:3 ratio (v/v, broth:solvent) for the intial precipitation and 1:2 (v/v, supernatant:solvent) for the second precipitation.

Solvent	**Temp. 10 °C**			**Temp. 25 °C**		
	SIA (U/mg)	**SFA (U/mg)**	**PF**	**SIA (U/mg)**	**SFA (U/mg)**	**PF**
Isopropanol	67.89 ± 0.45	79.90 ± 0.04	1.18 ± 0.01	68.05± 0.03	104.99 ± 0.03	1.54 ± 0.01
Acetone	68.33 ± 0.02	128.82 ± 0.02	1.89 ± 0.01	73.53± 0.03	103.48 ± 0.08	1.41 ± 0.01

Temp. =Temperature; SIA = Initial specific activity; SFA = Final specific activity; PF = Purification factor

Regarding the purification factor, acetone was the most efficient solvent, as 1.89 ± 0.01 was obtained for assays carried out at 10°C, in addition to specific activity of 103.48 ± 0.08 U/mg. Isopropanol at 10 °C showed lower specific activity (79.90 ± 0.04) and purification factor (1.18 ± 0.01) compared to tests performed at 25 °C, in which it was observed specific activity of 104.99 ± 0.03 (similar to that obtained for acetone at the same temperature range, 103.48 ± 0.08) and purification factor of 1.54 ± 0.01.

By comparing the results concerning the highest purification factor values found using acetone at 10 °C in the second precipitation step, it is possible to observe that a greater amount of solvent was unnecessary during the purification process to reach greater purification of lipases produced, nor more efficient.

In the second precipitation step the purification factor obtained was 1.88 ± 0.01 for acetone in a ratio of 1:3 (v/v) at 10 °C and using a a ratio of 1:2 (v/v), it was very similar (1.89 ± 0.01).

However, isopropanol reached optimal results in assays carried out at 10°C in a ratio of 1:3 (v/v) in the second precipitation step (1.89 ± 0.01), since a lower purification factor of 1.18 ± 0.01 was reached in a 1:2 ratio (v/v).

These results showed that acetone presented a better performance among the solvents under study in the purification of lipases from *P. africana*, as it obtained one of the highest purification factor values (1.89 ± 0.01), one of the highest specific activities obtained from the precipitate (103.48 ± 0.08 U/mg) using a smaller volume of solvent in the second precipitation step (1:2 v/v).

3.1 Partial characterization of the precipitated enzyme

After enzyme precipitation, it was possible to characterize it in terms of optimal pH and temperature.

For optimal pH assays, 37°C was set and olive oil was used as substrate in gum arabic emulsion. Figure 1 shows the effect of pH on the hydrolytic activity of lipase from *P. africana*.

Optimal pH is between 7 and 8 (72.80 ± 1.29 - 72.07 ± 1.61 U/mL), since maximum hydrolytic activity was found under such conditions. This result is close to that observed by Ramos et al. [23] who studied lipase from *G. candidum* and observed optimal pH of 8. The effect of temperature on enzyme activity is shown in Figure 2. In these assays, pH was set at 7 and activity assays were carried out in olive oil emulsion

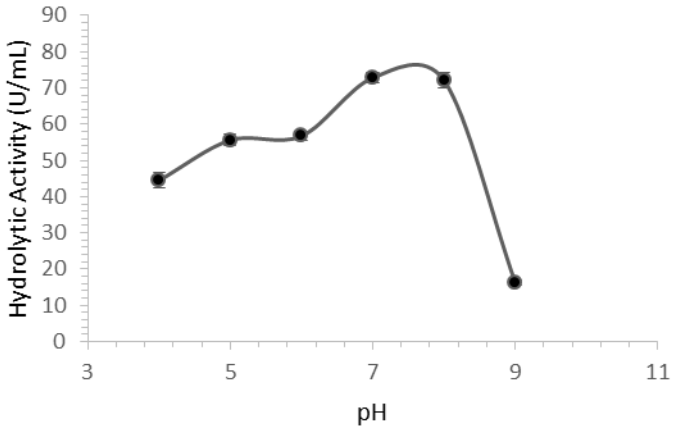

Fig. 1. Effect of pH on the hydrolytic activity of *P. africana* lipase

The optimal temperature for lipase was 37 °C, and hydrolytic activity of 72.80 ± 1.29 U/mL was reached.

The lipase produced from *Bacillus altitudinis* studied by Nimkande et al. [24], which comes from Antarctic soil, has very similar optimal temperature, and maximum activity was reached at 40°C.

Thus, the maximum catalytic activity of lipase was reached at 37°C and pH 7, and maximum activity of 72.80 ± 1.29 U/mL was obtained under these conditions

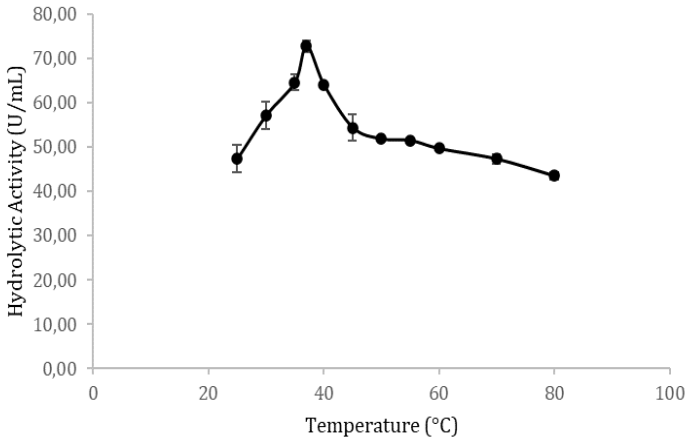

Fig. 2. Effect of temperature on the hydrolytic activity of *P. africana* lipase

Lipases could possess different substrate specificities. It means that they are able to recognize and hydrolyze certain ester bonds present in specific fatty acids bound to triacylglycerides more rapidly. The purified lipase from *P. africana* showed greater specificity for hydrolyzing ester bonds of fatty acids with long carbon chains present in sunflower, olive, soybean, corn, cotton and linseed oil and there was less activity for palm kernel, canola oils and tributyrin (Figure 3).

This type of substrate specificity allows the application of this lipase in relevant biotechnological transformations, such as the enrichment of certain polyunsaturated fatty acids (PUFAS) important for different physiological functions [25,26].

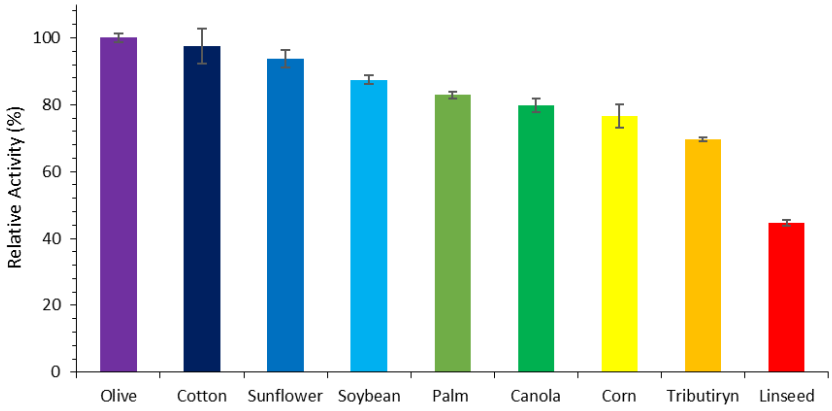

Fig. 3. Specificity of the lipase from *P. africana* using different substrates

4. CONCLUSION

Among the organic solvents assayed to investigate precipitation, isopropanol and acetone achieved optimal performances, and were selected for further studies. After two precipitation steps, the use of acetone at 10°C proved to be the most robust strategy, since it a purification factor of 1.89 ± 0.01 could be reached, even by using smaller amounts of this solvent (1:2 v/v ratio). By characterizing this enzyme, it was observed that the highest hydrolytic activity was achieved using olive oil at 37°C and pH between 7 and 8.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

REFERENCES

1. Jagannath S, Konappa N, Lokesh A, Bhuvaneshwari, Dasegowda T, Udayashankar AC et al. Bioactive compounds guided diversity of endophytic fungi from *Baliospermum montanum* and their potential extracellular enzymes. Anal Biochem. 2020;614(1):114024-39. http://dx.doi.org/10.1016/j.ab.2020.114024.

2. Gunatilaka AAL. Natural products from plant-associated microorganisms: Distribution, structural diversity, bioactivity and implication of their occurrence. J.Nat.Prod. 2006;69(3):509-26. https://doi.org/10.1021/np058128n.

3. Mattoo AJ, Nonzom S. Endophytic fungi: understanding complex cross-talks. Symbiosis. 2021;1(1):1-28.

Adeleke BS, Babalola OO. Pharmacological potential of fungal endophytes associated with medicinal plants: A review. J Fungi. 2021;7:1–16. https://doi.org/10.3390/jof7020147

Bajaj S, Fuloria S, Subramaniyan V, Meenakshi DU, Wakode S, Kaur A et al. Chemical characterization and anti-inflammatory activity of phytoconstituents from Swertia alata. Plants. 2021:31;10(6):1109. https://doi.org/10.3390/plants10061109

4. Yasser MM, Mousa AM, Marym A, Tagyan AI. Molecular identification, extracellular enzyme production and antimicrobial activity of endophytic fungi isolated from *Solanum*

tuberosum L. in Egypt. Biosci Biotechnol Res Asia. 2019;16(1):135-42. http://dx.doi.org/10.13005/bbra/2731.

5. Seddouk L, Jamai L, Tazi K, Ettayebi M, Alaoui-Mhamdi M, Aleya L et al. Isolation and characterization of a mesophilic cellulolytic endophyte *Preussia africana* from *Juniperus oxycedrus*. Environ Sci Pollut Res. 2022;29:45589-600. http://dx.doi.org/10.1007/s11356-022-19151-9.

6. Pereira AS, Souza AH, Fraga J, Villeneuve P, Torres AG, Amaral PF. Lipases as effective green biocatalysts for phytosterol esters' production: a review. Catalysts. 2022;12(1)88: 1-24. https://doi.org/10.3390/catal12010088.

7. Liu S, Bilal M, Rizwan K, Gul I, Rasheed T, Iqbal H. Smart chemistry of enzyme immobilization using various support matrices – A review. International J Biol Macromol. 2021; 190(1):396-408. https://doi.org/10.1016/j.ijbiomac.2021.09.006.

Chandra P, Enespa, Singh R, Arora P.K. Microbial lipases and their industrial applications: a comprehensive review. Microb Cell Fact. 2020;19:1–42. https://doi.org/10.1186/s12934-020-01428-8

8. Kilikian BV, Junior AP. Biotecnologia industrial: Engenharia Bioquímica. 4th ed. São Paulo: Blücher; 2001.

Malviya R, Fuloria S, Verma S, Subramaniyan V, Sathasivam KV, Kumarasamy V, et al. A. Commercial utilities and future perspective of nanomedicines. PeerJ. 2021;19(9):12392. https://doi.org/10.7717/peerj.12392

9. Bharathi D, Rajalakshmi G. Microbial lipases: an overview of screening, production and purification. Biocatal Agric Biotechnol. 2019;22(1)101368:1-7. http://dx.doi.org/10.1016/j.bcab.2019.101368.

10. Ferreira MM, Santiago F, Silva N, Luiz J, Fernadéz-Lafuente R, Mendes A et al. Different strategies to immobilize lipase from *Geotrichum candidum*: kinetic and thermodynamic studies. Process Biochem. 2018;67(1):55-63. http://dx.doi.org/10.1016/j.procbio.2018.01.028.

11. Preczeski KP, Dalastra C, Czapela FF, Kubeneck S, Scapini T, Camargo AF et al. *Fusarium oxysporum* and *Aspergillus* sp. as keratinase producers using swine hair from agroindustrial residues. Front Bioeng Biotechnol. 2020;8(1): 327-45. http://dx.doi.org/10.3389/fbioe.2020.00071.

12. Souza IM, Bassi GJ, Luiz JHH, Hirata DB. Isolation and screening of extracellular lipase-producing endophytic fungi from *Handroanthus impetiginosus*. Asian J Biotechnol Bioresour Technol 2018;4(2):1-10. http://dx.doi.org/10.9734/ajb2t/2018/43014.

13. Preczeski K, Kamanski A, Scapini T, Camargo AF, Modkoski T, Rossetto V et al. Efficient and low-cost alternative of lipase concentration aiming at the application in the treatment of waste cooking oils. Bioprocess Biosyst Eng. 2018; 41(6):851-57. http://dx.doi.org/10.1007/s00449-018-1919-y.

14. Ota Y, Sawamoto T, Hasuo M. Tributyrin specifically induces a lipase with a preference for the sn-2 position of triglyceride in Geotrichum sp. FO401B. Biosci Biotechnol Biochem. 2000;64(11):2497-99. https://doi.org/10.1271/bbb.64.2497.

15. Menegotto AL, Fernande IA, Steffens J, Valduga E. Protein purification of Arthrospira platensis using aqueous two-phase system composed of polyethylene glycol and potassium phosphate/sodium citrate. J Appl Phycol. 2021;34(1):311-20. https://doi.org/10.1007/s10811-021-02652-4.

16. Bradford MM. A Rapid and Sensitive Method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72(1):248-54. . https://doi.org/10.1006/abio.1976.9999

17. Castro PF, Moreira NC, Esperança MN, Oliveira LM, Badino AC, Tavano OL, et al. High lipase production from *Geotrichum candidum* in reduced time using cottonseed oil: optimization, easy purification and specificity characterization. J Chem Eng Res Updates 2016(3):60–69. http://dx.doi.org/10.15377/2409-983X.2016.03.02.1.

18. Janson JC. Protein Purification: Principles, High Resolution Methods, and Applications. New Jersey: John Wiley & Sons, Inc. 2011.

19. Ramos EZ, Júnior RH, Castro PF, Tardioli PW, Mendes AA, Fernanés-Lafuente R et al. Production and immobilization of *Geotrichum candidum* lipase via physical adsorption on eco-friendly support: Characterization of the catalytic properties in hydrolysis and esterification reactions. J Mol Catal B: Enzym. 2015; 118(89):43-51. http://dx.doi.org/10.1016/j.molcatb.2015.05.009.

20. Nimkande VD, Sivanesan S, Bafana A. Screening, identification, and characterization of lipase-producing halotolerant *Bacillus altitudinis* Ant19 from Antarctic soil. Arch Microbiol. 2023;205(4):327-45. http://dx.doi.org/10.1007/s00203-023-03453-8.

21. Yan J, Liu S, Hu J, Gui X, Wang G, Yan Y. Enzymatic enrichment of polyunsaturated fatty acids using novel lipase preparations modified by combination of immobilization and fish oil treatment. Biores Technol. 2011;102(14):7154–58. https://doi.org/10.1016/j.biortech.2011.04.065

22. Ward OP, Singh A. Omega-3/6 fatty acids: Alternative sources of production. Process Biochem. 2005;40(12): 3627–52. https://doi.org/10.1016/j.procbio.2005.02.020