Partial Purification and Properties of Lipase from Germinating Seeds of *Jatropha curcas* L.

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ABSTRACT: Lipase present in the seeds of *Jatropha curcas* L. was isolated and some of its properties studied. Lipase activity was detected in both dormant and germinating seeds. The lipase was partially purified using a combination of ammonium sulfate precipitation and ultrafiltration, which increased the relative activity of the lipase by 28- and 80-fold, respectively. The lipase hydrolyzed palm kernel, coconut, and olive oils at comparable rates (approximately 5 μg FFA/μg protein/min); palm—*Raphia hookeri* and *Jatropha curcas* L.—oils at about twice the rate of the first group of oils; and palm and fish oils at a higher rate than all other oils. The lipase, however, had the highest activity with monoolein. Optimal pH and temperature for maximal lipase activity were 7.5 and 37°C, respectively. The addition of ferric ion (15 mM) to the lipase assay medium caused 90% inhibition of lipase activity, whereas calcium and magnesium ions enhanced lipase activity by 130 and 30%, respectively.

Paper no. J10304 in *JAOCS 79,* 1123–1126 (November 2002).

KEY WORDS: *Jatropha curcas* L. seed, lipase, protein purification, *Raphia hookeri.*

Jatropha curcas L., commonly known as either physic nut, purging nut, or Barbados nut, belongs to the family Euphorbiaceae. It is an ornamental shrub that grows as tall as 5 m, is native to tropical America, and is cultivated in various parts of the tropics, including Brazil, Central America, the Cape Verde Islands, and West Africa (1,2). Although the plant bears a large number of seeds, producing as much as 200 to 500 kg oil per hectare (3), the seeds are usually not harvested and are thus underutilized. The shrub produces encapsulated seeds weighing about 5 to 7 g each and having a thin, dark brown shell and a kernel consisting mainly of a fatty endosperm surrounding a small embryo. The kernel amounts to 58 to 65 wt% of the intact seed and contains 50 to 60% oil (4).

Although lipases from various oil seeds have been studied extensively, only recently has work on the lipase from *J. curcas* L. seeds commenced (5). In this paper, we report the detection, partial purification, and some properties of the lipase present in the dormant and germinating seeds of *J. curcas* L. This was done with a goal of developing the use of this underutilized domestic oilseed (6,7).

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MATERIALS AND METHODS

Materials. Mature *J. curcas* L. seeds were harvested from trees at the Isiuwa quarters of the Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Nigeria. The seeds were rinsed with water and stored at 4°C prior to analysis. Palm, palm kernel, coconut, *Raphia hookeri,* and *J. curcas* oils were kindly donated by the Nigeria Experiment Mill, NIFOR. The oils were refined before use by methods described previously (8). Menhaden and olive oils were obtained from Sigma-Aldrich (St. Louis, MO). All solvents and reagents used were of analytical grade and were obtained from Aldrich Chemical (Milwaukee, WI).

Seed germination. Jatropha curcas L. seeds were soaked in running tap water for 20 min, transferred to a solution of benomyl fungicide (Rockland Corporation, West Caldwell, NJ; 4 mL in 4 L of distilled water), and left to soak for 5 min. The seeds were removed from the fungicide solution, spread onto paper towels, covered with transparent wrap, and allowed to germinate under room light at room temperature (about 25°C). The day of soaking was taken as the first day of germination. The seeds were harvested for enzyme preparations on the sixth day of germination.

Enzyme preparations. All operations for the isolation of the enzyme were carried out at 4°C. Entire germinating or ungerminated seed tissues were used for enzyme preparations. Enzyme preparation was carried out as described previously (9): The seed tissues were washed with distilled water and homogenized for 10 min in a grinding medium (4 mL/g fresh weight) using a Polytron homogenizer (Kinematica, AG, Littau, Swizerland). The grinding medium contained 0.6 M sucrose, 1 mM EDTA, 10 mM KCl, 1 mM $MgCl_2$, 2 mM DTT, and 0.15 M tricine {*N*-[tris(hydroxymethyl)methyl]-glycine} buffer adjusted to pH 7.5 with KOH. The homogenate was filtered through one layer of cheesecloth and centrifuged for 30 min at 10,000 × *g*, yielding a fat layer, a supernatant layer, and a pellet. The supernatant layer was used for the assay of lipase activity.

Protein determination. Protein concentrations were determined by the bicinchoninic acid assay (BCA; Pierce, Rockford, IL) (10). BSA was used as the standard.

Assaying for enzyme activity. Lipase activity was determined by continuous titration of FFA released by lipolysis of emulsified oils (11). The reaction was started by the addition

of 0.58 mL of the enzyme supernatant to 5.0 mL of substrate emulsion containing 20 μM $CaCl_2$. A titrating pH meter (Radiometer, Copenhagen, Denmark) was used to maintain the pH at a preset value by the addition of 0.1 M KOH. Unless stated otherwise, incubations were carried out at 37°C and a preset pH of 7.5. Enzyme activity was calculated from the maximal rate of base consumption. Under these conditions a unit of enzyme activity was defined as the release of 1 μg FA/μg protein/min.

In determinations of the pH and temperature dependence of lipase activity, the set point pH of the pH meter was adjusted to the values of interest. A thermostated, water-jacketed reaction chamber was employed in determining the temperature dependence of lipase activity. For the investigation of the effect of cations on lipase activity, 0.5% (wt/vol) polyvinylpyrrolidone (PVP) replaced gum arabic as the emulsifier and calcium chloride was omitted from the assay mixture. Therefore, to prepare the substrate emulsion, 0.25 g PVP in 21 mL of water, 20 mL of deionized water, and 9 mL of oil were sonicated.

Partially purifying the enzyme. The crude enzyme extract was fractionally precipitated with ammonium sulfate using a method described previously (12). The extract was taken up to 60% saturation with solid ammonium sulfate (398 g/L), and after standing for 1 h in an ice bath the suspension was centrifuged at 2500 × *g* for 10 min. The precipitate and supernatant were assayed for enzyme activity. The precipitate was discarded, and the supernatant taken to 80% saturation with solid ammonium sulfate (129 g/L). After standing for 1 h in an ice bath, the suspension was centrifuged at 2500 × *g* for 10 min, the precipitate discarded, and the supernatant assayed for lipase activity. The enzyme was further purified by ultrafiltration as follows: 2 mL of the 80% ammonium sulfate supernatant was placed into the sample reservoir of a Centricon-100 ultracentrifuge (Amicon, Inc., Beverly, MA) and centrifuged at 5000 × *g* for 5 h. A rapid and sensitive qualitative assay, in which hydrolysis of olive oil by lipase induces a change in the fluorescence of rhodamine B, was used to track lipase activity in the retentate and filtrate (13). The retentate showed about 90% of the lipase activity, whereas the filtrate showed about 10% of the total activity using the rhodamine plate method. The unit was then inverted and centrifuged at 1000 × *g* for 5 min to transfer the concentrate into the retentate vial, which was saved for further lipase assay.

RESULTS AND DISCUSSION

Lipase activity was detected in both ungerminated (dormant) and germinating *J. curcas* L. seeds. Most oilseeds examined for lipase activity show little if any detectable lipase activity in extracts from dormant seeds (14), except for castor beans (*Ricinus communis*) (15,16). In this study, lipase activity was detected in both the dormant and germinating seeds of *J. curcas* L. (Table 1). Previously, lipase activity was reported only in germinating of *J. curcas* L. seeds (5).

Table 1 lists the results of the lipolysis of palm, palm kernel, coconut, *R. hookeri*, *J. curcas* L. seed, olive, and fish oils by the lipase isolated from germinating *J. curcas* L. seeds. The enzyme hydrolyzed palm kernel, coconut, and olive oils at comparable rates; *R. hookeri* and *J. curcas* L. seed oils at twice the rate of the former oils; and palm and fish oils at a higher rate than all the other oils. This result showed that the lipase hydrolyzes long-chain TAG at a faster rate than medium-chain TAG, in contrast to results found in previous work (5), which reported that lipase from germinating *J. curcas* L. seeds hydrolyzed short- and long-chain TAG at the same rate. The lipase also exhibited a preference for partial acylglycerol hydrolysis in that it hydrolyzed monoolein at a much faster rate than triolein and was slightly faster for diolein (Table 1).

TABLE 1
Lipolysis of Different Oils and Acyglycerol Substrates by Lipase Isolated from Dormant and Germinating *Jatropha curcas* L. Seeds[a]

Substrate	Lipase activity[b] (μg FFA/μg protein/min)	Substrate	Lipase activity (μg FFA/μg protein/min)
Palm oil	11.29 ± 0.01[c]	*J. curcas* L. seed oil	9.41 ± 0.15[d]
	13.59 ± 0.01[d]	Olive oil	3.96 ± 0.01[d]
Palm kernel oil	4.47 ± 0.01[c]	Fish oil (menhaden)	11.88 ± 0.11[d]
	5.19 ± 0.10[d]	Triolein	2.80 ± 0.01[d]
Coconut oil	5.13 ± 0.11[c]	Diolein	4.20 ± 0.01[d]
	4.97 ± 0.11[d]	Monoolein	20.00 ± 0.02[d]
Raphia hookeri oil	8.68 ± 0.12[c]		
	9.94 ± 0.12[d]		

[a]Germinating seeds were harvested on the sixth day of germination.
[b]The lipase assay was conducted by the addition of 0.58 mL of enzyme supernatant to 5 mL of emulsified oil substrate and 20 μL of 15 mM $CaCl_2$, employing a titrating pH meter to maintain a constant pH (7.5) by the addition of 0.1 M KOH. Data are the mean ± SD of three determinations.
[c]Lipase isolated from dormant seeds.
[d]Lipase isolated from germinating seeds.

Sarada and Joseph (17) found that the lipase from *Propionibacterium acidipropionici* was capable of hydrolyzing the oils from groundnut, olive, castor, palm, and coconut, with palm oil being hydrolyzed with maximum efficiency. Lin *et al*. (18) reported that seed lipases often have higher activities with TAG that contain the major FA of the seed storage TAG of that species. The results of this study are in agreement with those findings in that palm, *R. hookeri,* and *J. curcas* L. seed oils all have palmitic acid as one of their major FA, which results in a high rate of hydrolysis of these oils by the lipase from *J. curcas* L. seed (6).

Figures 1A and 1B, respectively, show the effects of pH and temperature on the activity of the lipase from *J. curcas* L. seed. The data suggest that the pH optimum is near neutrality, similar to that of lipases from potato tuber (pH 7.5), rice bran (pH 7.5), and peanut (pH 8.5) (19). Several lipases, however, have been found to be active at an acid pH, e.g., castor bean lipase (pH 4.2) (16) and oil palm mesocarp lipase (pH 4.2) (20), whereas some are active at neutral pH values, e.g., castor bean lipase found in the oil-body membrane (16). The optimal temperature of 37°C for this lipase parallels earlier findings on lipases from *Sesamum indicum* (21), oat (20), and *Cucumeropsis edulis* (22).

Calcium and magnesium ions enhanced lipase activity by 130 and 30%, respectively, whereas ferric and zinc ions

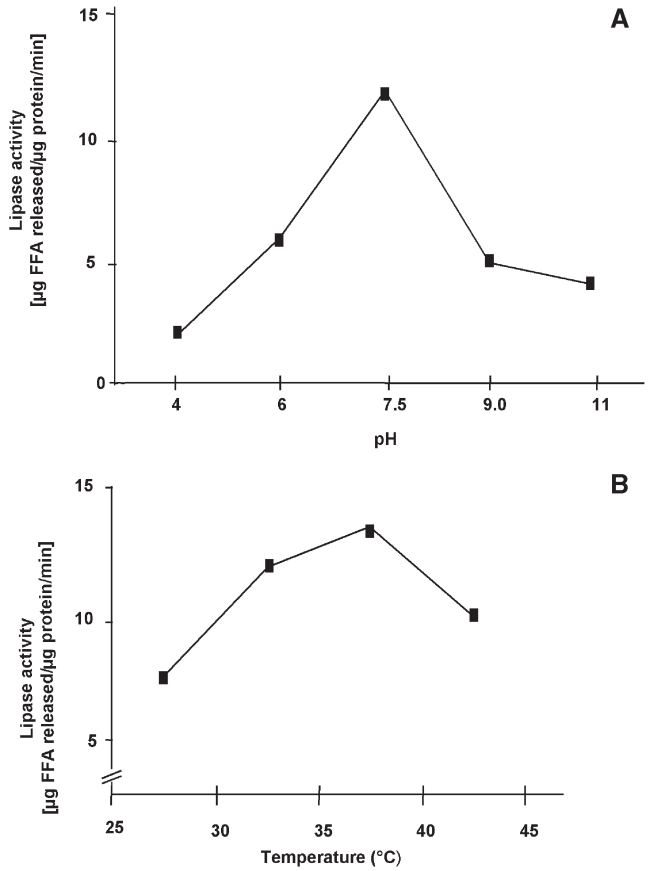

FIG. 1. *Jatropha curcas* L. lipase activity isolated from germinating seeds as a function of (A) pH and (B) temperature. The lipase assay medium contained 0.58 mL enzyme supernatant, 5 mL of substrate emulsion, and 20 µL of 15 mM calcium chloride. (A) Lipase activity was determined at the indicated pH values using a titrating pH meter. (B) Solution pH was first adjusted to 7.5. A thermostated, water-jacketed assay chamber attached to the titrating pH meter was used to assay lipase activity at the indicated temperatures.

brought about 100 and 92% inhibition, respectively (Table 2). These results are similar to the effect of metal ions on activity of esterases present in *J. curcas* L. seeds (5). Calcium ion previously has been shown to have a stimulatory effect on some lipolytic enzymes (23), but its effect varies from enzyme to enzyme. With some fungal lipases, calcium ions were observed to have different effects from those that were expected. For example, Linfield *et al*. (24) observed that calcium ions inhibited lipolysis reactions catalyzed by *Candida rugosa* lipase, whereas Haas *et al*. (13) reported a threefold increase in the activity of the lipase from the fungus *Rhizopus delemar*.

TABLE 2
Effect of Added Salts on the Activity of Lipase from *J. curcas* L. Seed

Salt	Concentration (mM)	Relative activity (%)[a]
None	—	100.00
$CaCl_2$	15	231.00 ± 0.10
$MgCl_2$	15	130.00 ± 0.10
$FeCl_3$	15	0.00
$ZnCl_2$	15	8.00 ± 0.01

[a]The effect of cations on *J. curcas* L. lipase activity was measured using 0.5% (wt/vol) (0.25 g in 21 mL H_2O) polyvinylpyrrolidone as the emulsifier in the substrate emulsion and omitting $CaCl_2$ from the lipase assay. The lipase assay mixture contained 5 mL of substrate emulsion, 20 µL salt solution (15 mM), and 0.58 mL enzyme supernatant, employing a titrating pH meter to maintain a constant pH (7.5) by the addition of 0.1 M KOH. Data are the mean ± SD of three determinations.

In another study, both magnesium and calcium ions (at a concentration of 1 mM) brought about an 80% inhibition of the lipase from the endosperm of germinating castor beans (15). EDTA reversed the inhibition. Sarada and Joseph (17) found that magnesium and barium ions caused significant inhibition of the lipase from *P. acidipropionici*, whereas copper ions caused inhibition. Calcium and sodium chloride increased the activity of the lipase present in the lipid bodies of cotyledons from rape and mustard seedlings by about 10–20% (8).

Partial purification of the *J. curcas* L. lipase by 80% ammonium sulfate precipitation achieved a 28-fold increase in the relative activity of the lipase in the supernatant, and subsequent ultrafiltration of this fraction resulted in an additional threefold increase in its relative activity (Table 3).

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TABLE 3
Partial Purification of *J. curcas* L. Seed Lipase

Fraction no.	Purification step	Volume (mL)	Protein (µg/mL)	Total protein (µg)	Total activity (µg FFA/min/mL)	Specific activity (µg FFA/min/µg)	Relative activity
1	Crude extract	30.00	32.33	969.10	1.50	0.05	1
2	60% $(NH_4)_2SO_4$ supernatant	30.00	14.14	424.00	8.14	0.58	11.60
3	80% $(NH_4)SO_4$ supernatant	30.00	9.50	285.00	13.34	1.40	28.00
4	Ultrafiltration	20.00	4.76	9.52	19.00	40.00	80.00

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[Received April 17, 2002; accepted July 13, 2002]