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# Partial purification and biochemical characterization of an alkaline esterase from *Sorghum bicolor*

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**ABSTRACT.** Esterases are enzymes that present good potential for industrial applications since they catalyze the formation or cleavage of ester bonds in water-soluble substrates, and sorghum seeds can represent an alternative source of this enzyme. The extraction of esterase from sorghum seeds is an economical alternative to obtain an enzyme of great interest. Esterases may improve the quality or accelerate the maturation of cheeses, cured bacon and fermented sausages and may also resolve racemic mixtures. Recently, seed esterases have been the focus of much attention as biocatalysts. In some cases, these enzymes present advantages over animal and microbial lipases due to some quite interesting features such as specificity and low cost, being a great alternative for their commercial exploitation as industrial enzymes The esterase studied here was extracted from sorghum seeds and some of its biochemical properties determined using synthetic substrates (p-nitrophenyl butyrate, caprylate, laurate and palmitate). The enzyme presented optimum activity at pH 8.0 and was stable in all the pH ranges studied. The optimum temperature for its activity was 40°C but it showed low stability at this temperature (40% relative activity). The values derived for Km and Vmax were 0.67mM and 125 $U.mg^{-1}$, respectively, obtained using *p*-nitrophenyl butyrate as the substrate. The enzyme showed an increase in activity when $K_2HPO_4$ was added to the reaction medium, but the ions $Mn^{2+}$, $CO^{+}$, $Hg^{+}$ and $Fe^{2+}$ strongly inhibited the enzyme activity. This enzyme showed a preference for the hydrolysis of short chain fatty acids. The characteristics of sorghum esterase are very similar to those of the microbial esterases used in detergent processing.

**Keywords:** *Sorghum*; esterase alkaline; biochemical characterization.

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## Introduction

Esterases or carboxylesterases (carboxylic–ester hydrolases; EC 3.1.1.1) belong to the international enzymatic class of hydrolases and comprise a group of "lipolytic enzymes" able to hydrolyze hydrophobic short and long chain carboxylic acid esters (Singh, Gupta, Goswami, & Gupta, 2006; Barros, Celligoi, & Macedo, 2016). Due to their ample availability esterases are of interest for application in industrial processes. Esterases may improve the quality or accelerate the maturation of cheeses, cured bacon and fermented sausages. In addition, the esterases have the catalytic potential to form ester bonds in water soluble substrates and are also widely used in the resolution of racemic mixtures of compounds in order to produce pure anantomers (Houd, Kademi, & Leblanc, 2004; Singh et al., 2006; Fahmy et al., 2008; Su, Zhou, You & Wei, 2010).

The growing interest in esterases is due to the fact that these enzymes can be applied in many sectors (dairy products, wine production, fruit juices, beer, alcohol, the synthesis of low molecular weight esters, cosmetics, detergents, the transformation of low molecular weight fats into oils with greater commercial value and the resolution of racemic compounds, amongst others) in addition to presenting some advantages in comparison to esterases from microorganisms in some cases, they can be applied without being purified (Barros & Macedo, 2011). Besides, these biocatalysts present other advantages due to their specificity and low cost, being a great alternative for their commercial exploitation as industrial enzymes (Barros, Fleuri, & Macedo, 2010; Tuter, Secundo, Riva, Aksoy, & Ustun, 2003).

Esterases can be of animal (pancreatic, hepatic or gastric), microbial (bacterial, fungal or yeast) or vegetable origin, with variations in their catalytic properties (Mohamed, Mohamed, Mohamed, & Fahmy,

2000; Barros ety al., 2010). The possibility of using enzymes from seeds is an economic industrial alternative (Paques & Macedo, 2006).

To exploit esterases for industrial use, it is important to characterize them biochemically. Fahmy et al. (2008) studied the biochemical characteristics of the esterase from *Cuncurbita pepo* cv and observed some interesting characteristics such as high stability, alkaline pH optimum and high affinity towards short-chain esters like some microbial esterases used in the maturation and flavor development of cheeses. Purified esterases from soybeans (*Glycine max*) were also characterized and showed great potential for application in the production of low molecular weight esters for use in the food industry and in chemical products. Regarding substrate affinity, the enzyme showed greater activity for substrates containing short-chain fatty acids, especially p-nitrophenyl acetate. (Barros & Macedo, 2015).

Sorghum (*Sorghum bicolor* L.) is the fifth most produced cereal throughout the world, after wheat, rice, corn and barley and is widely used as human and animal feed (Ramatoulaye, et al., 2016). Some researchers have studied sorghum as an alternative to replace barley malt. Nwanguma, Eze, and Ezenowa, (1996) detected lipase-like lipolytic activity in non-germinated seeds during grain malting and mashing in three sorghum species studied. These authors observed a slight decrease in enzyme activity during steeping for 24 hours, but it increased several-fold during the course of germination. This variation could be due to the differential abilities of the varieties to synthesize the lipase or due to the presence of different levels of lipase activity regulation. Uvere and Orji (2002) studied lipase activities during the malting and fermentation of sorghum for the production of Burukutu, an alcoholic beverage produced by the germination of sorghum seeds Between 24% and 60% of the lipolytic activity was retained after cooking at 48°C, but no activity was found after mashing at 65°C. About 68% of the lipase activity of 72 hours old malt was detected in the plumule, while 29% and 3% were found in the endosperm and radicle, respectively.

No recent study has been carried out to characterize the physicochemical properties of the esterase enzyme present in sorghum seeds, and to date only the presence of lipolytic activity has been determined. A carboxylesterase from sorghum seed was partially purified by $(NH_4)_2SO_4$ fractionation and Sephadex G-100 gel filtration. The enzyme was identified as carboxylic ester hydrolase, (E.C. 3.1.1.1) by studying the inhibition response to DFP (diisopropylphosphofluoridate), serine and PCMB (*p*-chloromercuribenzoate) (Sae, Kadoum, & Cunningham, 1971). The present study aimed to observe and quantify the presence of esterase activity in non-germinated sorghum seeds, assess the specificity and study the biochemical characteristics of the esterase.

## Material and methods

### Materials

The sorghum seeds studied were donated by VITAO Polinutri Industry LTDA, Brazil, and the commercial lipase used was Lipozyme TLIM obtained from *Thermomyces lanuginosus* (Novozymes®) and used at a concentration of 2 $mg.mL^{-1}$. The substrates *p*-nitrophenyl butyrate (pNPB), *p*-nitrophenylcaprylate (pNPC), *p*-nitrophenyl-laurate (pNPL) and *p*-nitrophenylpalmitate (pNPP) were purchased from Sigma Chemical (St Louis, MO). Triacetin and triolein were obtained from Fluka (Swizerland) and the other chemicals were of analytical grade.

### Esterase extraction

The esterase was extracted from the sorghum using a modification of the procedure described by Aizono, Funatsu, Sugano, Hayashi, and Fujiki (1973). Non-germinated and post germinated seeds (150 g of each type) were suspended in a $1x10^{-3}$M $CaCl_2$ solution and blended for 3 min. at 4°C in a Waring blender. The sample was transferred to a conical flask, stirred mechanically at 4°C for 30 min. and then subjected to ultrasound (125W at 200 kHz) for 1 min. in an effort to release the bound enzyme. The resulting suspension was centrifuged at 2000x*g* for 45vmin. at 5°C and the supernatant collected and cooled to 0°C. Solid ammonium sulphate was added with constant stirring to obtain 80% saturation. Similarly, the enzyme was precipitated by the addition of 40% acetone. The resulting precipitates were separated by centrifugation at 2000x*g* for 45 min. and then resuspended in a minimum amount of 1 X $10^{-3}$M $CaCl_2$ solution. The $CaCl_2$ suspensions were dialyzed against the same solution at 4°C until all traces of ammonium sulfate had been removed, and then re-centrifuged to eliminate any insoluble material (Kermasha, Van de Voort, & Metche, 1986). The resulting extracts were assayed for their protein contents using the modified Bradford (1976) method.

### Enzyme assay

The esterase activity was determined spectrophotometrically at 405 nm following hydrolysis of the substrate *p*-nitrophenyl-butyrate (*p*NPB). An aliquot (0.070 mL) of the purified enzyme extract was added to 3.43 mL of a reaction mixture containing the following: 1.12mM pNPB dissolved in 50mM phosphate buffer, pH 7.0, plus 0.2% (N/P) Triton X-100 and 0.42M tetrahydrofuran. The enzymatic activity was determined for a period of 5 min. at 37°C, determining the absorption against a blank solution at one-minute intervals. One unit of esterase activity was defined as the amount of enzyme required to release one micromole of *p*-nitrophenol per minute under the standard assay conditions (Macedo & Pio, 2005).

The esterase activity was also determined by a titrimetric method using triacetin and triolein as the substrates. One milliliter of the purified enzyme extract was added to a reaction mixture containing the following: 5mL of 0.1M phosphate buffer, pH 7.0 and 1 mL of triacetin. The solution was incubated at 37°C for 6h with shaking at 120 rpm. After incubation, the reaction was stopped by adding 15mL ethanol:acetone 1:1 (v $v^{-1}$) and the fatty acids released titrated with 0.05 M sodium hydroxide. One unit of esterase activity was defined as the amount of enzyme required to release one µmol of oleic acid per minute per mL under the specified conditions (Paques, Pio, Carvalho, & Macedo, 2008).

The substrate specificity of the enzyme was determined by evaluating its activity with different *p*-nitrophenyl-esters. Several *p*-nitrophenyl-esters were analyzed: *p*-nitrophenyl butyrate (pNPB), *p*-nitrophenylcaprylate (pNPC), *p*-nitrophenyllaurate (pNPL) and *p*-nitrophenyl-palmitate (pNPP). The specificity was also tested using different triacylglycerols (triacetin, tributyrin, tricaproyn, tripalmistin and triolein) by titration with NaOH as previously described.

### Optimum temperature and heat stability

The effect of temperature on the esterase activity was studied by carrying out assays at different temperatures in the range from 30-90°C at pH7.0, using *p*NPB in 0.1M phosphate buffer as the substrate.

The thermostability of the esterase was tested by pre-incubating the enzyme for 1h at different temperatures ranging from 30 to 60°C. After pre-incubation, the residual activity was measured at 37°C and expressed as a percentage of the relative esterase activity.

### pH optimum and stability

The optimum pH for esterase activity was determined using the following buffers: 0.1M acetate in the range from pH 3.6 to 5.6, 0.1M phosphate from pH 6.0 to 7.0, 0.1M Tris-HCl from pH 7.2 to 9.0 and 0.1M borax-NaOH from pH 9.5 to 10.0.

The pH stability was evaluated by incubating the enzyme with different buffers at pH values ranging from 3.6 to 10.0 for 24h at room temperature. After incubation, the residual activity was determined (37°C, phosphate buffer pH 7.0) and expressed as a percentage of the relative esterase activity. All the tests were carried out in duplicate.

### Effects of salts and chemical agents

The effects of different salts ($CaCl_2$, $KCl_2$, $HgCl_2$, $MnCl_2.4H_2O$, $CoCl_2.6H_2O$, $K_2HPO_4$, $NaNO_3$, $FeSO_4.7H_2O$, $MgSO_4.7H_2O$, ZnSO4.$7H_2O$, $MnSO_4.H_2O$, $K_2SO_4$, $Na_2SO_4$, $NaHSO_3$, $HgCl_2$, $NaH_2SO_4$, $CuSO_4$ and $NaCl_2$) on the esterase activity were studied. The salts were added to the reaction buffer at concentrations of 1 and 10 mM. The relative activity was expressed as a percentage of the relative esterase activity as measured in the reactive medium without the added salts. The effects of different chemical agents (EDTA, urea, sodium lauryl sulphate, sodium bisulfate, glutathione (reduced), ascorbic acid, sodium citrate, cysteine, Tris and sodium persulfate) were also evaluated at concentrations of 1 and 10mM.

### Effect of substrate concentration

The sorghum esterase was assayed in the reaction buffer (pH 7.0) with different concentrations of *p*NPB (*p*-nitrophenyl butyrate) as the substrate (0.5 – 2mM). The values for Vmax (maximum velocity) and Km (Michaelis constant) were calculated from the Lineweaver–Burk plot.

### Statistical analysis

The data from the experiments were presented as the mean ± standard error (SEM) and analyzed using a one-way analysis of variance (ANOVA), with the differences analyzed by the Tukey test at the 5% probability level (Statistical Analysis System [SAS], 2009).

## Results and discussion

The esterase activity was higher in the non-germinated (dormant) than in the post-germinated *Sorghum bicolor* grains, therefore dormant seeds were chosen for the purification process.

### Study of the semi-purification of the esterase from sorghum

Semi-purification, independent of the enzyme source, aims at concentrating the protein contents, thus favoring an increase in enzyme activity and making it possible to improve the study of the physicochemical properties of the enzyme. Semi-purification can be carried out using vacuum evaporators, precipitation or ultrafiltration. Precipitation is a simple way of concentrating an enzyme, which can be done by adding salts, organic solvents, polymer solutions or by altering the pH value (Freire & Castilho, 2008). The best way used to precipitate an enzyme depends mainly on the characteristics of the medium in which the enzyme is located.

In the case of lipolytic seed enzymes, they are usually precipitated by adding salts or organic salts to the medium in which the enzyme is located, although in some cases organic solvents can lead to enzyme inactivation (Bom et al., 2008). The esterases and lipases of oleaginous seeds are generally precipitated by the addition of ammonium sulfate (Yesiloglu & Baskurt, 2008; Sagiroglu & Arabaci, 2005; Sana, Hossin, Haque, & Shaha, 2004; Abigor et al., 2002; Kermasha, Van de Voort, & Metche, 1986).

The semi-purification of sorghum esterase was studied using different percentages of ammonium sulfate saturation and 40% acetone (v:v), and the results obtained for the activity of the semi-purified esterase can be seen in Table 1.

**Table 1**. Enzyme activity of the crude extract of the esterase fraction (CEEF) from sorghum seeds, semi-purified by fractionation with ammonium sulfate and concentrated by precipitation with 40% acetone[1,2,3]

| Semi-purification steps | Protein (mg.mL$^{-1}$) | Total activity (U mL$^{-1}$) | Specific activity (U mg$^{-1}$) | Purification factor | Yield (%) |
|---|---|---|---|---|---|
| CEEF-0% ammonium sulfate | 0.88±0.003$^{a}$ | 32.04±1.18$^{d}$ | 36.22±1.34$^{d}$ | 1 | 100 |
| CEEF-40% ammonium sulfate | 0.92±0.007$^{c}$ | 59.52±3.11$^{c}$ | 65.22±3.36$^{c}$ | 1.80 | 180 |
| CEEF-60% ammonium sulfate | 0.94±0.01$^{b}$ | 177.78±10.11$^{a}$ | 188.30±10.76$^{a}$ | 5.19 | 519 |
| CEEF-80% ammonium sulfate | 1.08±0.006$^{b}$ | 121.70±4.47$^{b}$ | 113.55±4.13$^{b}$ | 3.13 | 313 |
| CEEF-40% acetone | 1.08±0.00$^{b}$ | 34.64±1.57$^{d}$ | 31.53±1.45$^{d}$ | 0.88 | 88 |

[1]Mean of six repetitions each determined once ± standard deviation. [2]Means accompanied by the same letters do not differ significantly $p \leq 0.05$. [3] Substrate 4-*p*-nitrophenyl-butyrate (1.12mM); reaction time 15 minutes.

A significant increase in enzyme activity can be seen in Table 1 when the enzyme was semi-purified with ammonium sulfate. The highest value for specific activity (188.30 U mg$^{-1}$) was found in the crude extract semi-purified with 60% saturated ammonium sulfate, but when the enzyme was concentrated using 80% saturated ammonium sulfate there was a fall in enzyme activity (113.55 U mg$^{-1}$). Sae, Kadoum, and Cunningham (1971) used ammonium sulfate precipitation and molecule exclusion chromatography (Sephadex G-100) to purify a carboxylesterase from sorghum, resulting in an overall 72-fold purification. No method chromatographic was used in the present study.

Abigor et al. (2002) used 60 and 80% saturated ammonium sulfate to semi-purify the esterase extracted from jatropha seeds. On using 60% saturated ammonium sulfate, the authors found esterase activity of 8.14 U mL$^{-1}$ and specific activity of 0.58 U mg$^{-1}$, whereas with 80% saturated ammonium sulfate the esterase activity was 13.34 U mL$^{-1}$ and the specific activity 1.40 U mg$^{-1}$. These results were different from those obtained in the present study, where the enzyme activity decreased when the degree of saturation of the ammonium sulfate was increased from 60% to 80%.

Yesiloglu & Baskurt (2008) studied the semi-purification of almond seed lipase and observed an increase in activity with increase in the ammonium sulfate concentration in the extract containing the enzyme, reaching maximum activity with 90% saturation of the salt. In the case of the crude sorghum extract studied in the present work, 60% saturation of the ammonium sulfate was sufficient for the partial purification of the enzyme, a decrease in activity being observed with higher salt concentrations.

When 40% (v:v) acetone was used as the precipitating agent, there was a significant increase in protein content, but the same was not observed for the enzyme activity when compared with that of the crude extract. In this case the addition of acetone to the enzyme extract did not result in an increase in esterase activity. Probably the acetone caused a partial inactivation of the enzyme. According to Freire and Castilho (2008) acetone is widely used to concentrate enzymes of interest, but in some cases it can cause enzyme inactivation.

Thus the best ammonium sulfate concentration for the partial purification of sorghum esterase was 60% of saturation, both with respect to enzyme activity (U $mL^{-1}$) and specific activity (U $mg^{-1}$).

## Study of the specificity of the sorghum esterase

The specificity of the sorghum esterase was studied using different substrates and the results are shown in Figure 1.

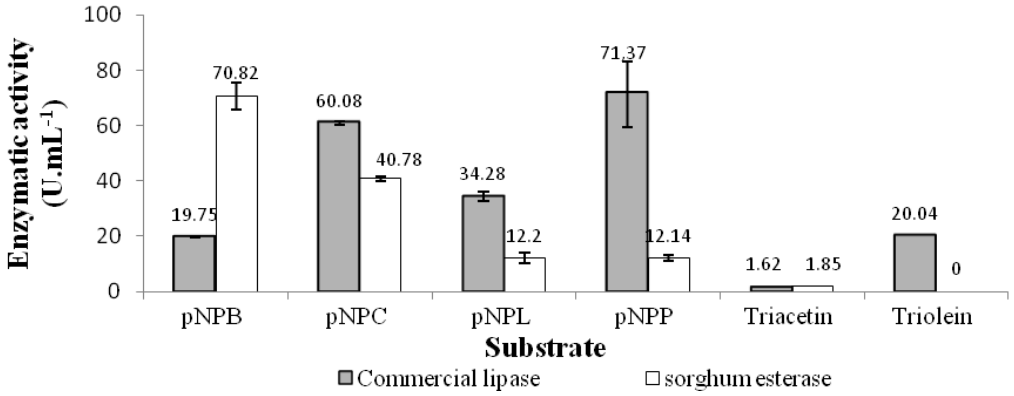


**Figure 1.** The activities of sorghum esterase and commercial lipase (*Thermomyces lanuginosus*) in different substrates (pNPB, pNPC, pNPL, pNPP, Triacetin and Triolein)

For activity, the highest value was observed with pNPB (70.82 U $mL^{-1}$) after 30 minutes of reaction, with pNPC in second place, the maximum activity being 40.78 U $mL^{-1}$. The preference of the sorghum esterase for the substrates tested was in the following order: pNPB>pNPC>pNPL>pNPP. According to Marten, Pfeuffer, and Schrezenmeir (2006) and Osborn and Akoh (2002), fatty acids with 2 to 6 carbons in their structure are classified as short chain fatty acids, for example butyric acid (4 carbons), and fatty acids containing from 6 to 12 carbons are classified as medium chain fatty acids (e.g. caprylic acid). The sorghum esterase showed an affinity for short chain fatty acids, this being the expected behavior for an esterase.

Kubicka, Grabska, Jedrychowski, and Czyz (2000) evaluated the activity of barley esterase and detected a greater affinity for short chain fatty acids, especially for acetic acid esters. Liaquat and Apenten (2000) studied the syntheses of different esters by the lipase from corn seeds and observed a preferential hydrolysis of short chain fatty acids by the enzyme in the following order: acetic acid (2C) > butyric acid (4C) > caproic acid (6C) in an organic medium using isopentanol, after 72 hours of reaction.

Pollizelli, Tiera, and Bonilla-Rodriguez (2008), studying the lipase from *Pachira aquatica* seeds with 90 minutes of reaction time, found the following values for activity – 2.5; 5 and 4.5 U $mL^{-1}$ in *p*NPB, pNPC and pNPP respectively, values lower than those found in the present study. In addition, the activity of the sorghum esterase was evaluated in triacetin and triolein and showed greater activity in triacetin.

## Effect of temperature on the activity and stability of sorghum esterase

The structure and shape of the enzyme's active site are due to its three-dimensional structure, and can be affected by environmental agents such as temperature (Marzzoco & Torres, 2016).

Figure 2 shows the effect of temperature on the activity and thermostability of the sorghum esterase.

Catalysis increases as the temperature rises until a maximum velocity is achieved, and then declines with further increases in temperature due to some inactivation process. (Thomas & Scopes, 1998). The highest value for the sorghum esterase activity was at a temperature of 40ºC, and there was a significant decrease in enzyme activity at temperatures above 40ºC. Below the optimum temperature the molecules have less kinetic energy and consequently collide less frequently, resulting in less reactions. A rise in temperature increases the reaction speed while the enzyme conserves its native structure. However, most enzymes are denatured at temperatures above 50-55ºC and lose their catalytic power (Marzzoco & Torres, 2016). Staubmann, Ncube, Gübitz, Steiner, and Read (1999), studying the esterase from physic nut (*Jatropha curcas* L.) observed optimal activity at 50ºC in a substrate of *p*NPB. The lipase from the kapok tree nut (*P. aquatica*), studied by Polizelli, Facchini, Cabral, and Bonilla-Rodriguez (2008), showed optimum activity at 40ºC using pNPP as the substrate, and Isbilir, Ozcan, and Yagar (2008), studying the lipase from bay seeds (*Laurus nobilis* L.) found the optimum activity at 50ºC in triolein.

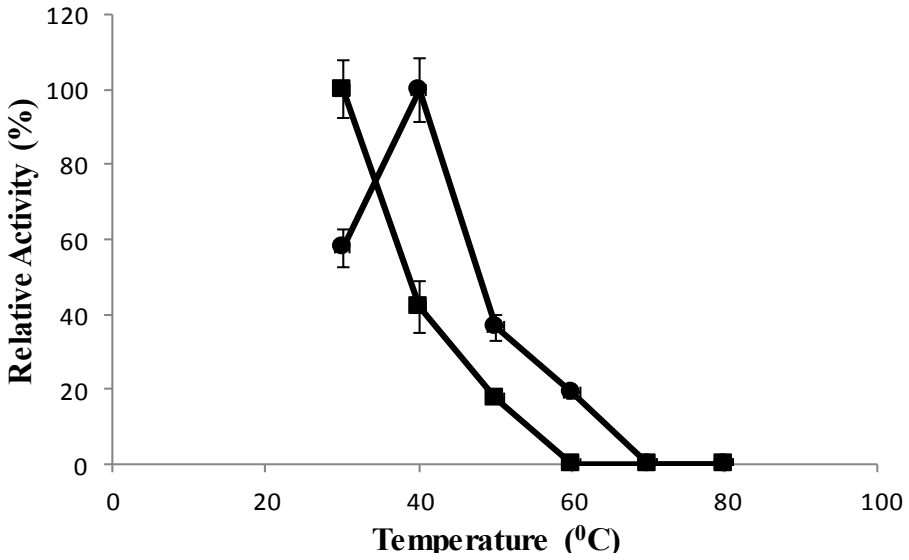


**Figure 2.** Optimum temperature (●) and thermostability (■) for sorghum esterase activity

The sorghum esterase was more stable at 30ºC. Although the enzyme showed its greatest activity at 40ºC, it only presented 40% of residual activity at this temperature after one hour of incubation. Figure 3 shows the thermostability of the sorghum esterase.

### Effect of pH on the activity and stability of the enzyme

Figure 3 shows the effect of pH on the activity of the sorghum esterase.

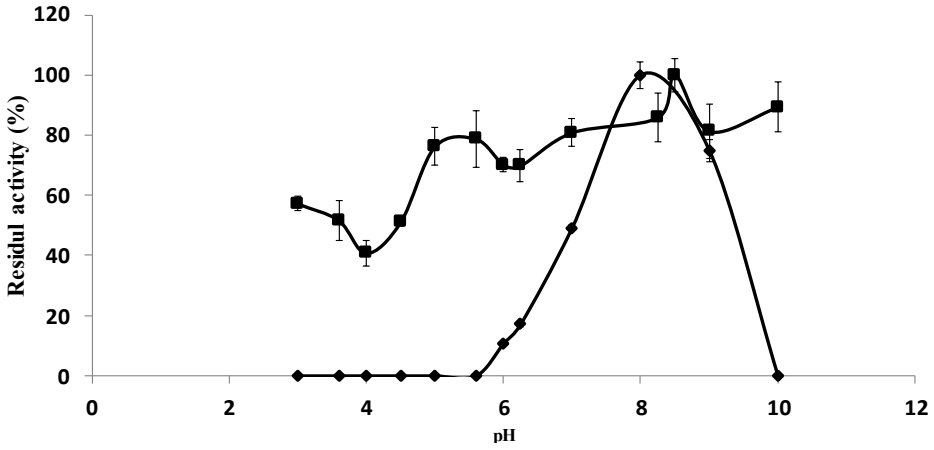


**Figure 3.** Effect of pH on the activity (●) and pH stability (■) of the sorghum seed esterase

The sorghum esterase showed its greatest activity at pH8.0, similar to soybean esterase, and is thus an alkaline enzyme (Barros & Macedo, 2011). This value is similar to that shown by the esterases from physic nut (*J. curcas*) studied by Staubmann et al. (1999) and by Abigor et al. (2002), and by the bay seed lipase (*J. curcas*) studied by Isbilir, Ozcan, and Yagar (2008), which showed their maximum activities at pH 7.5.

With respect to pH stability, the sorghum esterase was stable at all the pH values studied, as shown in Figure 3. It was most stable at pH 8.5, but there were no significant differences between the values found at pH values (Tris-HCl) of 7, 8 and 9. The lowest value for residual activity was found at pH 4, with only 40% of residual activity. The sorghum esterase was shown to be stable at all the pH values studied.

### Effects of some salts, activators and inhibitors on the activity of esterase

The effects of some salts, activators and inhibitors on the activity of sorghum esterase can be seen in Tables 1 and 2.

**Table 1.** Activity of sorghum esterase in different salt solutions[1, 2]

| Salts | Final concentration (1mM) | Final concentration (10mM) |
|---|---|---|
| | Relative activity (%) | Relative activity (%) |
| Control | 100±2.42$^{bcde}$ | 100±2.42$^{bcde}$ |
| $K_2HPO_4$ | 95.50±5.66$^{bcdef}$ | 130.77±6.02$^{a}$ |
| $NaNO_3$ | 111.29±8.67$^{ab}$ | 111.47±9.87$^{ab}$ |
| Mn $Cl_2$ | 84.91±3.79$^{def}$ | 109.05±7.90b$^{c}$ |
| $CaCl_2$ | 106.32±1.03$^{bc}$ | 105.70±3.05$^{bcd}$ |
| $Fe_2SO_4$ | 102.99±6.54$^{bcde}$ | 0.0$^{h}$ |
| $Na_2SO_4$ | 88.95±4.07$^{cdef}$ | 101.48±11.04$^{bcde}$ |
| $KCl_2$ | 91.45±5.71$^{bcdef}$ | 98.71±7.16$^{bcde}$ |
| $K_2SO_4$ | 75.42±2.13$^{f}$ | 93.81±8.55$^{bcdef}$ |
| $MnSO_4$ | 90.28±7.11$^{cdef}$ | 0.0$^{h}$ |
| $CoCl_2$ | 83.64±4.18$^{ef}$ | 0.0$^{h}$ |
| $MgSO_4$ | 83.18±2.98$^{ef}$ | 75.33±9.82$^{f}$ |
| $ZnSO_4$ | 52.75±10.23$^{g}$ | 0.0$^{h}$ |
| HgCl | 0.0h | 0.0$^{h}$ |

[1]Means of four repetitions with one determination each ± standard deviation. [2]Means accompanied by the same letters do not differ significantly at p ⩾ 0.05.

Of the salts studied in this trial, the only one that favored an increase in enzyme activity was potassium phosphate ($K_2HPO_4$) at a concentration of 10mM. On the other hand, the salts $CoCl_2$, HgCl, $Fe_2SO_4$ and $ZnSO_4$ strongly inhibited the enzyme activity. The same occurred with the lipase from the kapok tree nut (*P. aquatica*) studied by Polizelli et al. (2008).

For the esterase of physic nut (*J. curcas)* studied by Staubmann et al., (1999) only $BaCO_5$, at a concentration of 10Mm, showed a positive effect on enzyme activity. The author mentioned that the ions $K^{2+}$, $Ca^{2+}$, $Mg^{2+}$ and $Mn^{2+}$ frequently showed a positive effect on esterase activity, but this was not observed with the physic nut esterase. However, in the present study potassium did have a positive effect on the enzyme activity.

Isbilir et al. (2008), studying the lipase from bay seeds (*L. nobilis*), observed a significant increase in enzyme activity in the presence of the ions $Ca^{2+}$, $Co^{2+}$, $Cu^{2+}$, $Fe^{2+}$ and $Mg^{2+}$ in the reaction medium, results similar to those observed for the lipase from almond seeds (*Amygdalus communis* L.). The lipase from Kapok seeds (*P. aquatica*) also showed an increase in activity in the presence of the ions $Ca^{2+}$ and $Mg^{2+}$.

Table 2 shows the effect of different chemical agents, causing activation or inhibition, on the activity of the esterase from sorghum seeds.

Urea showed a positive effect on the enzyme activity increasing by 20%, but reduced glutathione, sodium bisulfate and L-cysteine at concentrations of 10mM inhibited the enzyme activity. Polizelli et al. (2008), studying the effects of some chemical compounds on the activity of the lipase from the kapok tree nut (*P. aquatica*), also observed the negative effect of reduced glutathione at a concentration of 10Mm, suggesting that the enzyme requires one or more intact disulfide bonds to maintain its native conformation as postulated for other lipases.

The values for $K_m$ and $V_{max}$ of the enzyme were 0.67mM and 125 U.mg$^{-1}$, respectively (Figure 4) using *p*-nitrophenyl butyrate as the substrate, values higher than found for two esterases (JEA and JEB) obtained from the seeds of *J. curcas* JEA showed a $K_m$ of 0.02 mM and a $V_{max}$ of 0.26 U mg$^{-1}$ and under the same conditions JEB showed a $K_m$ of 0.07 mM and a $V_{max}$ of 0.24 U mg$^{-1}$(Staubmann et al., 1999).

**Table 2.** Activity of the sorghum esterase in the presence of different chemical agents

| Chemical agents | Final concentration (1mM) | Final concentration (10mM) |
|---|---|---|
| | Relative activity (%) | Relative activity (%) |
| Control | 100±5.33$^{bc}$ | 100±5.33$^{bc}$ |
| Urea | 105.04±11.67$^{ab}$ | 120.80±11.46$^{a}$ |
| Sodium citrate | 87.39±3.18$^{bcde}$ | 90.67±3.40$^{bcd}$ |
| EDTA | 85.74±9.89$^{cde}$ | 69.14±0.35$^{efgh}$ |
| Reduced glutathione | 79.82±4.68$^{def}$ | 0.0$^{j}$ |
| Sodium bisulfate | 75.37±5.56$^{defg}$ | 0.0$^{j}$ |
| L-cisteine | 69.58±7.68$^{efgh}$ | 0.0$^{j}$ |
| Ammonium persulfate | 64.28±3.55$^{fgh}$ | 35.09±5.58$^{i}$ |
| TRIS | 63.00±1.60$^{fgh}$ | 101.68±9.65$^{bc}$ |
| Ascorbic acid | 60.80±2.33$^{gh}$ | 56.36±7.07$^{h}$ |

[1]Means of four repetitions with one determination of each ± standard deviation. [2]Means accompanied by the same letters do not differ significantly at p ⩾ 0.05.

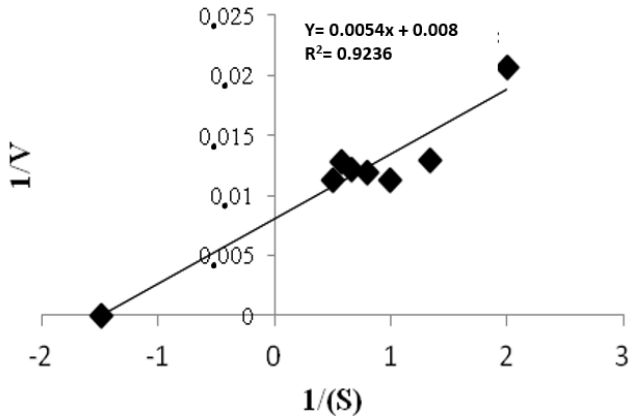


**Figure 4.** Lineweaver-Burk plot of sorghum esterase using *p*-nitrophenyl butyrate as the substrate for the determination of Km and Vmax

## Conclusion

The sorghum esterase showed some very interesting characteristics such as its specificity, activity and catalytic stability at an alkaline pH value (pH 8.0). These characteristics demonstrate the potential for the application of this enzyme in various industrial sectors, such as in the synthesis of low molecular weight esters (aromatic esters), the production of industrial detergents and other applications.

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