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Juice clarification with tannases from *Aspergillus carneus* URM5577 produced by solid-state fermentation using *Terminalia catappa* L. leaves

Vanilla Mergulhão Alves da Silva[1], Roberta Cruz[2], Julyanna Cordoville Fonseca[1], Cristina Maria de Souza-Motta[1], Amanda Reges de Sena[2] and Keila Aparecida Moreira[2*]

[1]Department of Mycology, Federal University of Pernambuco, Recife, Pernambuco, Brazil.
[2]Academic Unit of Garanhuns, Federal Rural University of Pernambuco, Garanhuns, Pernambuco, Brazil.

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Tannase is an inducible extracellular enzyme produced by filamentous fungi, yeasts, and bacteria by solid-state fermentation (SSF) or submerged fermentation (SmF). Among the filamentous fungi, *Aspergillus* and *Penicillium* are recognized as the most efficient producers of tannase. The aims of this study were to evaluate the production of tannase under SSF by isolation from *Aspergillus* and *Penicillium* preserved in the Collection of Cultures Micoteca - URM (WDCM604); select the best enzyme producer, and use the crude extract to clarify mangaba and tamarind juices. The optimal conditions were determined by using the Placket-Burman Planning (PB) and response surface methodology (RSM). All tested crops produced activity between 2088.19 and 238.93 U/gds, and *Aspergillus carneus* URM5577 was the best producer. Through MSR, the best parameters for producing tannase were found to be 70 h of cultivation at pH 6.0, 7% tannic acid at 28°C and, as the response variable, 5449.31 activity U/gds. The optimum purification conditions were the molecular weight of PEG 8000 (g/mol), concentration of PEG 15% (w/w), 25% citrate (w/w), and pH 8.0. Its application in mangaba juice reduced the tannin content by 49.66% after 90 min and in tamarind by 51.82% at 120 min incubation at 37°C.

Key words: Tannase, agroforestry waste, *Aspergillus, Penicillium.*

INTRODUCTION

Fungi are organisms responsible for manufacturing various highly important products, such as food, beverages, organic acids, drugs etc. Among the fungi commonly preserved in culture collections are those belonging to the genera *Aspergillus* and *Penicillium* (Bon et al., 2008).

Tannins occur in a wide variety of vegetables and can be found in every part of the plant. They represent the fourth most abundant constituent of plants after cellulose, hemicellulose, and lignin - compounds that are degraded by the action of tannase (Yadav et al., 2008). Tannin acyl hydrolase (TAH) (EC 3.1.1.20), or tannase, is an inducible

*Corresponding author. E-mail: moreirakeila@hotmail.com.

enzyme that catalyzes the hydrolysis of ester bonds and hydrolysable tannins in peptide such as tannic acid, releasing glucose and gallic acid (Pinto et al., 2005; Costa et al., 2008). The filamentous fungi are recognized as great producers of this enzyme, and species of the genera *Aspergillus* and *Penicillium* stand out in this respect (Costa et al., 2008; Cruz et al., 2013; Lima et al., 2014).

The filamentous fungi are featured in fermentation processes, as they can secrete substantial quantities of proteins in culture media (Gomes et al., 2007). There are two types of fermentation, submerged fermentation (FmS), in which free water is present and solid-state fermentation (SSF), wherein the microorganism is usually inoculated waste or solid substrates in the absence of free water.

Several types of substrates are used in the production of fungal tannase, which aim at reducing production costs. The "castanhola" leaves (*Terminalia catappa* L.) is rich in tannins but has never been used as a substrate to produce tannase. When used in the production of these enzymes, these substrates can achieve a high market value (Selwal and Selwal, 2012; Cruz et al., 2013).

The presence of tannin gives food a bitter taste, decreasing its consumption and consequently, its commercial value. The use of tannase to clarify tannin-containing drinks may be a solution to these problems (Lima et al., 2014; Banerjee et al., 2005).

Depending on the final application, methods of tannase purification may be sought as alternatives to extraction with organic solvents, such as a pre-purification aqueous two-phase system (ATPS). The aqueous two-phase system is formed by the meeting of certain polymers, polyelectrolytes, or polymers in combination with low-molecular-weight solutes. This process can be used to purify tannase for use in the clarification of juices or in the production of animal feed (Luccarini et al., 2005).

Therefore, the purpose of this work was to evaluate the production of tannase under SSF by isolation from *Aspergillus* and *Penicillium* preserved in the Culture Collection Micoteca - URM using "castanhola" leaves (*T. catappa* L.) as a substrate and to select the best producer, optimize the production, and apply the enzyme to the clarification of mangaba (*Hancornia speciosa* Gomes) and tamarind (*Tamarindus indica* L.) juices.

MATERIALS AND METHODS

Substrate

"Castanhola" leaves (*T. catappa* L.) were obtained on the campus of the Federal University of Pernambuco, located in the city of Recife in Pernambuco - Brazil. The leaves were previously washed with sterile distilled water and dried at 55°C for 72 h (Lima et al., 2014).

Microorganisms and inoculum preparation

In the present study, 30 species were used. Of these, 15 were species of *Aspergillus,* and 15 were of *Penicillium* preserved in mineral oil (Sherf, 1943) and maintained by the Collection of Cultures Micoteca - URM (WDCM604), from the Center of Biosciences of the Federal University of Pernambuco, Brazil.

Each strain was inoculated on malt extract agar (MEA) and incubated at 30°C. Subsequently, spores of each culture were transferred to a test tube containing 10 ml of sterile distilled water and 0.1% (v/v) Tween 80. The spore suspension was used as inoculum. The spores were quantified by the plate count technique, at a concentration of 5×10^8 spores/ml (Sabu et al., 2005).

Production of Tannase by solid-state fermentation (SSF)

Five grams of "Castanhola" leaves (*T. catappa* L.) were placed in 250-mL Erlenmeyer flasks and sterilized at 121°C for 30 min in flowing steam. The substrates were moistened with 5 mL of sterile salt solution containing 0.5% w/v NH_4NO_3, 0.1% w/v $MgSO_4.7H_2O$, and 0.1% w/v NaCl, pH 5.0. The moisture content was adjusted to 50%. Each vial was inoculated with 1 mL of spore solution (5×10^8 spores/mL). The contents were mixed and incubated at 30°C for 96 h. After this period, to each bottle was added 50 mL of distilled water containing 0.01% previously sterilized Tween 80. Then, the vials were shaken in a rotary shaker (Tecnal TE421, Sao Paulo, Brazil) at 150 rpm for 10 min. Then, the contents were filtered using Whatman number 1 filter paper, and the filtrate was regarded as crude enzyme extract, packaged in conical vials, and preserved at 4°C for later analysis (Sabu et al., 2005).

Determination of tannase activity

Tanase activity was measured by spectrophotometry (Sharma and Gupta, 2013). This method is based on the formation of a chromogen from gallic acid (released by the esterase activity of tannase) and rhodanine (2-thio-4- ketothiazolidine). To determine the gallic acid concentration, 100 µL crude enzymatic extract was incubated with tannic acid (0.3 mM) and sodium phosphate buffer (10 mM, pH 5.5) for 30 min at 30°C. Then, 300 µL of the methanolic solution of rhodanine (0.667% w/v rhodanine in 100% methanol) and 100 μL of 500 mM KOH were added to the mixture, which was diluted with 8.6 mL distilled water and incubated for 10 min at 30°C. After this period, samples were read in a spectrophotometer (Hitachi-U5100) at a wavelength of 520 nm. A standard curve was generated using gallic acid in different concentrations. All assays were performed in triplicate. One unit of tannase activity (U) was defined as the amount of enzyme required to release one micromole of gallic acid per minute under the defined reaction conditions. Enzyme yield was expressed in U/gds.

Statistical analysis

Optimization of the production of tannase

To optimize the conditions for the production of tannase from *A. carneus* URM5577 Plackett-Burman (PB) planning and response surface methodology (RSM) design were used.

Identification and selection of the most important variables in the optimization using Plackett-Burman design planning (PB)

For selection of medium components to produce tannase, a PB design was used, in which the variables time (h), temperature (°C), pH, and tannic acid (%) were evaluated. Each component was examined on two levels: "−1" down and level "+1" tall, using the statistical software package STATISTICS 8.0 (Statsoft, 2008), which generated a set of 12 experimental tests.

Table 1. Levels of factors used in the experimental design of the selected type 24 for the extraction and purification of tannase using the ATPS PEG/phosphate.

Variable	Levels		
	Low (-1)	Centric (0)	High (+1)
M_{PEG}[a]	3350	6000	8000
C_{PEG}[b]	15	20	25
C_{FOS}[c]	15	20	25
pH	6.0	7.0	8.0

[a]PEG molecular weight (g/mol); [B]concentration of PEG (%); [C]concentration of phosphate (%).

Optimization of the selected components using response surface methodology (RSM)

To determine the optimal level of the two variables selected by Plackett-Burman design planning [time (h) and tannic acid (%)], a central composite design (CCD) was applied using the statistical software STATISTICS 8.0 (Statsoft, 2008). The experiments were conducted in a 250-ml Erlenmeyer flask containing 5 g of "Castanhola" leaves (*T. catappa* L.) moistened with saline solution (pH 6.0) prepared in accordance with the design, at 28°C.

Pre-purification of tannase

Preparation of an aqueous two-phase system

For tannase extraction, the system was prepared with polyethylene glycol (PEG) of different molecular weights (3350, 6000 and 8000 g/mol) and phosphate salts. The phosphate solution (40% w/w) was prepared by mixing appropriate amounts of dibasic potassium phosphate (K_2HPO_4) at different pH values (6.0, 7.0 and 8.0) at 25 ± 1°C. The desired amounts of PEG and salt were placed in graduated centrifuge tubes (15 ml). The crude enzyme extract containing PG represents 20% of the total system volume and was added to the tubes, and water was added to a final weight of 10 g. After 1 min of vortexing, the tubes remained at rest for 60 min to separate the phases. The volume of each phase was measured, and the phases were separated by using automated pipettes. The enzyme activities and dosage of the protein were determined, allowing the calculation of the partition coefficient, yield, and purification factor of tannase.

Experimental design

The effects of the molar mass of PEG (MPEG), the PEG concentration (CPEG), phosphate concentration (CFOS), and pH of the system response variables (partition coefficient (K), performance at work (Y), and purification factor (FP) were evaluated based on the results of a type-24 experimental design, another central point that was carried out twice to allow estimation of experimental error (Vieira Neto, 2002). The selected values for these variables (Table 1) were chosen based on the binodal diagrams in published reports (Vernau and Kula, 1990; Assis et al., 2001). All statistical analyses and graphs were generated by using Statistica 8.0 software (Statsoft, 2008).

Determination of partition coefficient, activity yield, purification factor, and selectivity

K was defined as the ratio of the volumetric enzyme activity in the top phase (At) to that in the bottom phase (Ab):

$$K = At/Ab$$

PF was calculated as the ratio of the specific activity in the top phase to the specific activity in the cell extract before partition:

$$PF = (At/Ct)/(Ai/Ci)$$

Where, Ct and Ci are the total protein concentrations, expressed in mg/mL, in the top phase and initial extract, respectively.

Y was defined as the ratio of total activity in the top phase over that in initial extract expressed as a percentage:

$$Y = [(At \times Vt)/(Ai \times Vi)] \times 100$$

Where, Vt and Vi are the volumes of the top phase and the initial extract, respectively.

Application of crude enzyme extract to mangaba juice and tamarind for clarification

For the preparation of juice, fruit mangaba (*H. speciosa* Gomes) and tamarind (*T. indica* L.) was washed in running water, the seeds were removed and pulp was liquefied (Black & Decker, LF910) and then filtered with the aid of a particle-size sieve (strainer in stainless steel–ASTM 1/4 inch opening 6.30 mm). The juice was stored at -4°C for further analysis (Sabu et al., 2005).

To clarify juice in 125-mL Erlenmeyer flasks, different aliquots of crude enzymatic extract: (0.5, 1.0, 1.5 and 2.0 mL), which contained 5449.31 U/gds tannase activity, were added separately to 10 mL of mangaba and tamarind juices. As a control, we used mangaba and tamarind juice separately, without the addition of crude enzyme extract. Then, the vials were placed on a rotary shaker at 150 rpm and 37°C for 120 min and analyzed every 30 min (0, 30, 60, 90 and 120 min). Assays were performed five times. After stirring, the flasks were incubated in a water bath for 10 min at 50°C. After this period, 1 mL juice treated with crude extract was removed to determine the concentration of enzyme extract tannins. The tannin content present in grapes was determined by the method of protein precipitation by tannins (Hagerman and Butler, 1978).

RESULTS

All 30 strains tested produced tannase through SSF (Table 2). The three best producers were *Aspergillus aureolus* URM 7034 (2088.19 U/gds), *A. carneus* URM 5577 (1128.59 U/gds) and *Penicillium implicatum* URM 6233 (1055.75 U/gds), respectively.

Two species have excelled in the production of the enzyme, *A. aureolus* URM 7034 and *A. carneus* URM 5577, these being selected to optimize the production of tannase.

Tannase production conditions for the two species mentioned above were optimized using the PB experimental design and after selecting the optimal conditions, RSM was applied, to select important variables in the production of tannase and to verify their significant levels. Four variables were analyzed: time (h), temperature (°C), pH, and tannic acid (%) (Table 3) in relation to the production of tannase. The effect of each

Table 2. Tannase activity (U/gds) of strains of *Aspergillus* and *Penicillium* in SSF, using "castanhola" leaves (*Terminalia catappa* L.) as substrate, after 96 h of fermentation.

Species	N° URM	U/gds
Aspergillus aureolus	7034	2088.19
A. carneus	5577	1128.59
Penicillium implicatum	6223	1055.75
P. brasilianum	6892	974.16
P. melinii	6463	933.37
A. viride-nutans	7033	929.49
P. corylophilum	6491	925.60
P. brevicompactum	6833	881.90
P. aurantiogriseum	6844	867.33
P. fellutanum	6472	855.67
A. candidus	6607	810.02
A. flavus	7028	802.25
A. flavo-furcatis	6142	774.09
P. griseofulvum	6846	773.12
A. versicolor	7029	749.81
A. carbonarius	6613	738.15
A. tubingensis	6991	683.76
P. adametzii	7015	662.39
P. janczewskii	6672	599.26
P. citrinum	7030	588.58
A. terreus	3420	523.50
A. aculeatus	7013	518.65
A. caespitosus	5938	511.85
A. sclerotiorum	6619	506.02
P. commune	6671	427.35
P. purpurogenum	6634	372.96
A. oryzae	5638	303.03
P. citreonigrum	6458	255.44
P. restrictum	6135	253.50
A. niveus	5461	238.93

Table 3. Experimental Plackett-Burman Matrix planning (CP) for producing tannase from *Aspergillus aureolus* URM 7034 and *Aspergillus carneus* URM 5577 through SSF using "castanhola" leaves.

Test	pH	Incubation temperature (ºC)	Tannic acid (%, p/v)	Fermentation time (h)
1	6 (+)	28 (-)	5 (+)	48 (-)
2	6 (+)	32 (+)	1 (-)	96 (+)
3	4 (-)	32 (+)	5 (+)	48 (-)
4	6 (+)	28 (-)	5 (+)	96 (+)
5	6 (+)	32 (+)	1 (-)	96 (+)
6	6 (+)	32 (+)	5 (+)	48 (-)
7	4 (-)	32 (+)	5 (+)	96 (+)
8	4 (-)	28 (-)	5 (+)	96 (+)
9	4 (-)	28 (-)	1 (-)	96 (+)
10	6 (+)	28 (-)	1 (-)	48 (-)
11	4 (-)	32 (+)	1 (-)	48 (-)
12	4 (-)	28 (-)	1 (-)	48 (-)

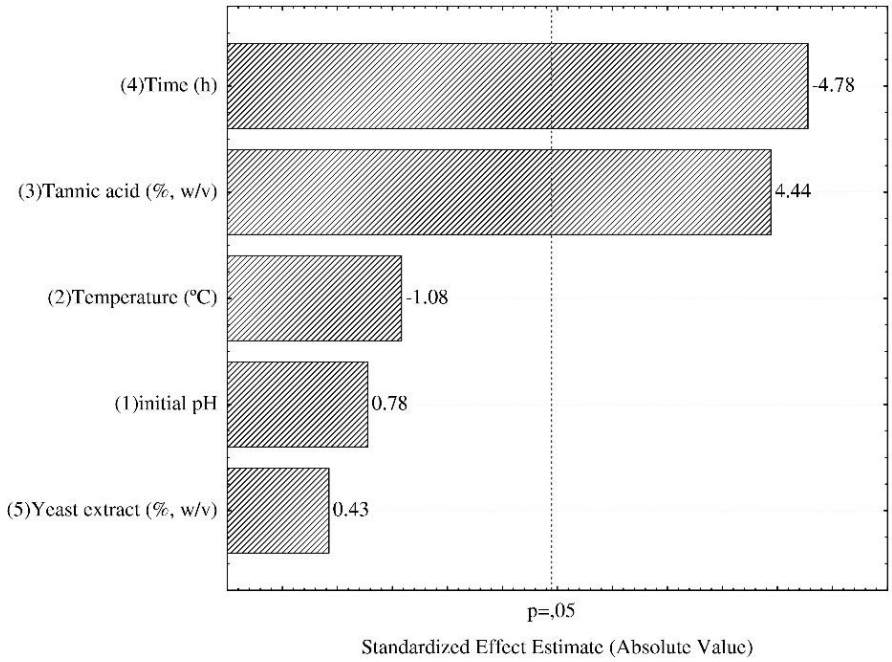

Figure 1. The effect and coefficient for tannase production presented by the variables used in the Plackett-Burman design (PB).

Table 4. Significant variables used in response surface methodology (RSM).

Independent variables	-1	-0.866	-0.5	0	0.5	0.866	1
Time (h)	22	-	34	46	58	-	70
Tannic acid (%, w/v)	-	6	-	7	-	8	-

variable and the corresponding coefficient are presented in Figure 5. The significant variables were time and tannic acid, which shows that the influence of these variables was higher on the parameters tested in the production of tannase from *A. carneus* URM 5577.

The fermentation time and tannic acid concentration have been identified as the most significant variables for producing tannase from *A. carneus* URM 5577 in SSF using the Plackett-Burman model (Figure 1). For *A. aureolus*, only tannic acid was significant for fermentation: The other variables did not influence tannase production. These variables were selected to be optimized using the central composite design (CCD) parameters provided by the statistical program (Table 4).

Data were subjected to analysis of variance (ANOVA), with the tannase production levels and experimental data shown in Table 4. The quadratic regression equation better explained the optimization of environmental variables to produce tannase, with an R^2 of 0.98, explaining 98% of the variability of the model and showing its quality.

The interactions between variables generated three-dimensional graphs showing increased production of tannase, suggesting an optimal production (5571.09 U/gds) in culture medium with 7% tannic acid added and incubated at 28°C. Therefore, there was an increase of approximately 4% in the production of the enzyme, when compared with the maximum activity obtained in PB planning (5449.31 U/gds) and approximately 500% compared with the initial screening (1128.59 U/gds), proving the validity of the optimization model. According to the RSM planning, while simultaneously analyzing the factors time and tannic acid concentration, it was observed that the production of tannase from *A. carneus* URM 5577 increases according to the increase in tannic acid content and incubation temperature (Figure 2).

For this study, PEG of different molecular weights (3350, 6000, and 8000) were tested at pH 6, 7, and 8, and random points were chosen in the two-phase region according to the phase in which the tannase is set, in which case was the PEG. Tables 5 to 7 show the results obtained for the purification factor (PF), total activity (Y), and partition coefficient (K).

The effects of PEG concentration, potassium citrate, pH, and concentration of PEG on the purification factor are shown in Figure 4.

All variables showed significant effects. AMPEG was the independent variable that most influenced the

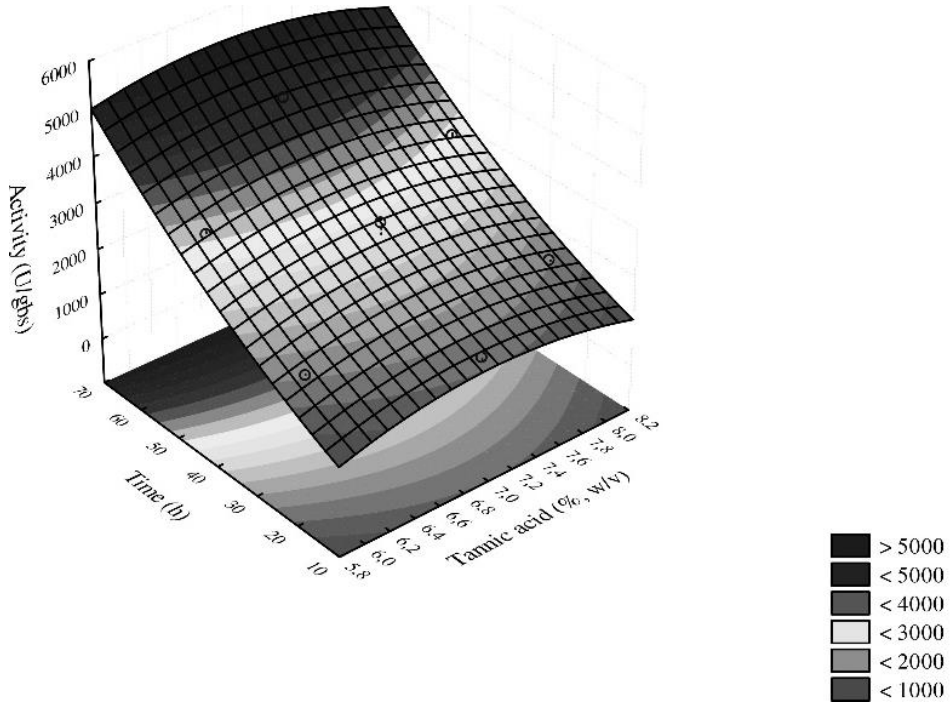

Figure 2. Response surface for the activity of tannase considering tannic acid and time.

Table 5. Tannase activity by ATPS based on PEG 3350 pH 6 and 8.

Run	PEG (%)	pH	Citrate potassium	PF	K	Y (%)
1	15	6	15	3.72	23.14	2166.38
3	25	6	15	2.13	17.84	1981.65
5	15	8	15	2.21	14.44	1280.49
7	25	8	15	1.36	16.29	2122.61
9	15	6	25	3.82	15.62	1941.95
11	25	6	25	3.58	21.82	2269.33
13	15	8	25	1.56	25.51	1749.58
15	25	8	25	2.44	21.18	1941.43

Table 6. Tannase activity by ATPS based on PEG 6000 pH 7.

Run	PEG %	pH	Citrate potassium	PF	K	Y (%)
17	20	7	20	1.93	7.89	1440.41
18	20	7	20	1.77	9.01	1382.69

response variables (K, Y, and FP). The interaction among the variables of the molecular weight of PEG, the pH of potassium citrate with the molecular weight of PEG and the concentration of PEG, and between pH and the concentration of PEG had significant positive effects: When levels of both variables were increased, the purification factor increased (Figure 3). pH will adversely interact with citrate, and PEG concentration, interacting with other variables, will also have a negative effect, namely, when the others are increased, the concentration of PEG will decrease.

In the application, the crude enzymatic extract of *A. carneus* URM 5577 containing tannase is shown in Figures 5 to 8. The pure mangaba juice presented a

Table 7. Tannase activity by ATPS based on PEG 8000 pH 6 and 8.

Run	PEG %	pH	Citrate potassium	PF	K	Y (%)
2	15	6	15	1.83	8.14	1906.95
4	25	6	15	1.73	10.58	599.64
6	15	8	15	2.75	11.44	1518.05
8	25	8	15	8.07	12.60	2327.76
10	15	6	25	4.95	10.35	1366.20
12	25	6	25	6.42	14.22	1514.90
14	15	8	25	8.15	17.03	1389.52
16	25	8	25	7.39	18.90	2229.30

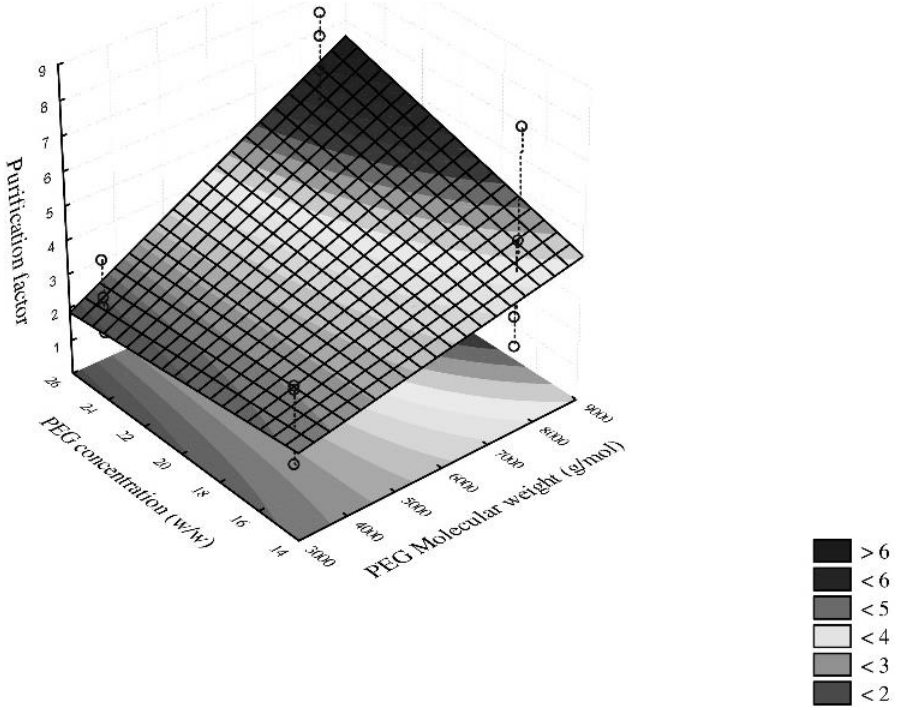

Figure 3. Response surface for the purification factor considering CPEG and MMPEG.

tannin concentration of 126.21 U/mL. Clarification was tested in different crude extract concentrations (Figure 5) and over different time intervals (Figure 6). These data were subjected to regression analysis, polynomial equations of the second degree were selected with an R^2 = 0.94 and statistical analysis was performed. A further increase in the volume of crude enzyme extract (2 mL) improved the point of clarification, since according to the prediction equation of the data, the maximum point was with a volume of crude extract of 63.53 U/mL tannase (Figure 6), whereas the minimum quantity of tannin showed no significant difference when applied to different concentrations of crude extract (Figure 5). The tannin content in the juice was reduced to 49.66% (63.53 U/mL) after 90 min of incubation with the crude enzyme extract in 2 ml at 37°C.

Pure tamarind juice contained 161.21 U/ml tannins (Figure 7) and underwent the same conditions of analysis as mangaba juice: R^2 = 0.99 for time and R^2 = 0.92 for extraction concentration. It also showed a further increase in clarification according to the volume of crude enzyme extract (2 ml), where its peak was at a volume of crude extract containing 77.67 U/ml tannase (Figure 8). The tannin content in the juice was reduced to 51.82% (77.67 U/mL) after 120 min of incubation with the crude enzyme extract in 2 ml at 37°C.

DISCUSSION

SSF has been proven to be of enormous benefit to the production of tannase by numerous authors (Gupta et al.,

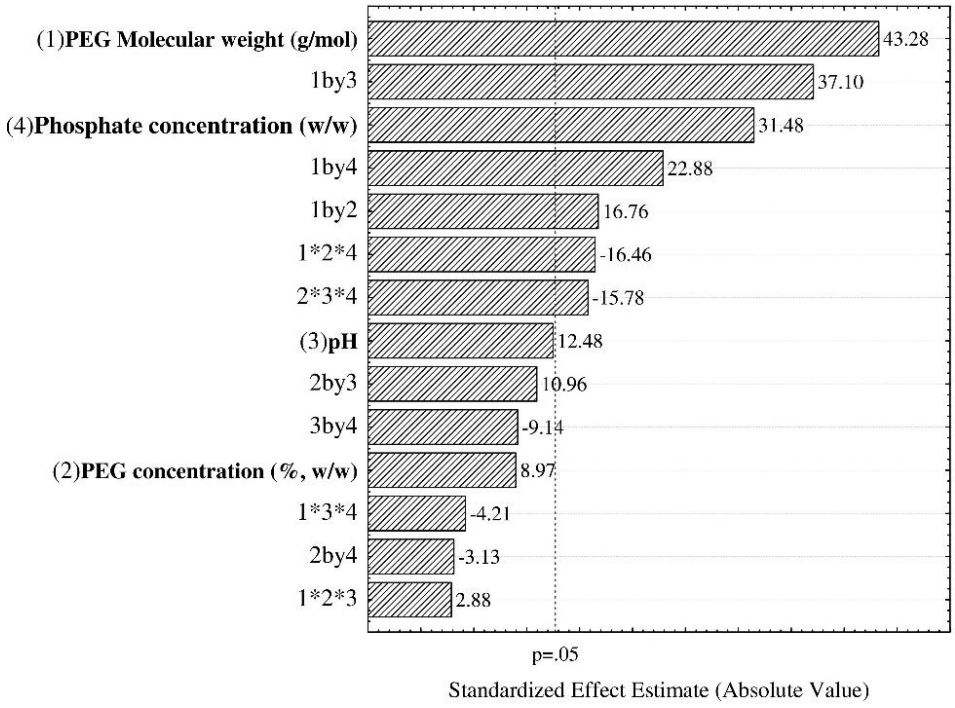

Figure 4. Comparison chart of the effects of purification factors.

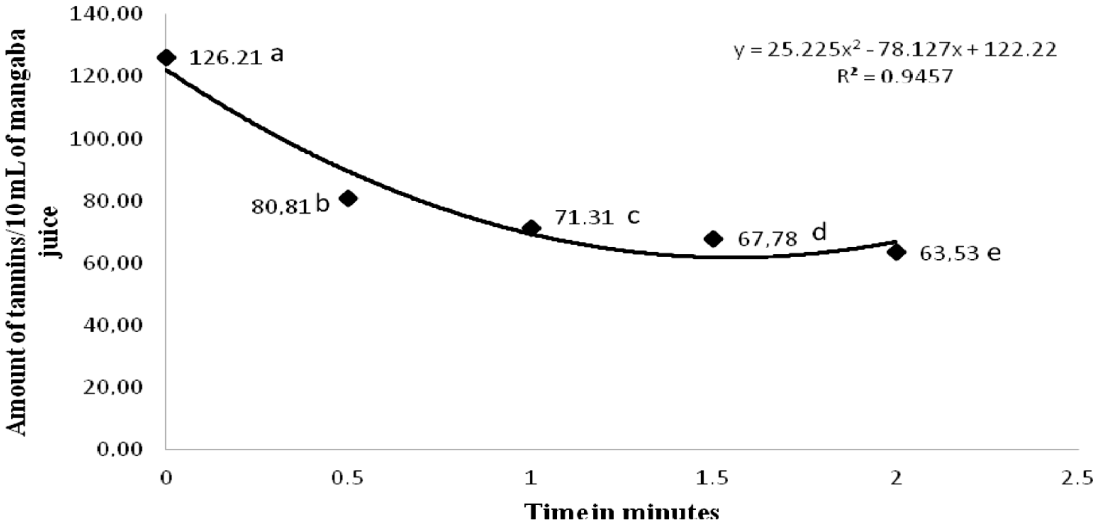

Figure 5. Effect of different volumes of crude extract on the degradation of tannins in mangaba juice after 90 min incubation.

2008; Wang et al., 2013) due to several factors, such as the low cost of energy and water and the reuse of sheets as a substrate (Selwal and Selwal, 2012).

In Brazil, especially in coastal regions, castanets (*T. catappa* L.) easily adapts to conditions of high salinity and strong winds (Thomson and Evans, 2006). Its leaves are rich in tannins, thus presenting an excellent carbon source for the production of tannase by SSF.

The possibility of using of agricultural waste as a source of carbon to produce enzymes by fungi has aroused the interest of scientists (Sabu et al., 2005). Selwal et al. (2011) used different leaves as substrates, such as amla (*Phyllanthus emblica*), ber (*Ziziphus mauritiana*), jamun (*Syzygium cumini*), Jamoa (*Eugenia cuspidate*), and keekar (*Acacia nilotica*) in SSF. In this study, the authors obtained a maximum activity of 170.75

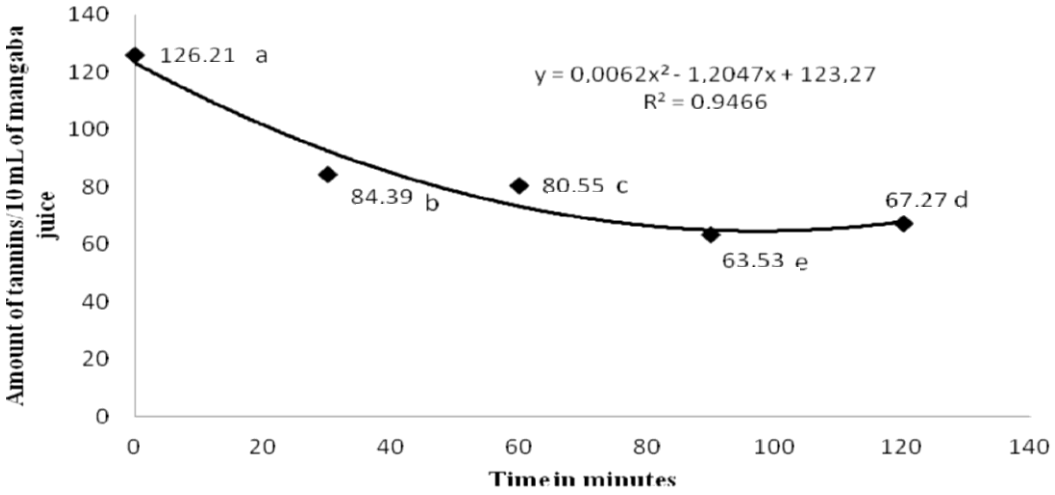

Figure 6. Effect of 2 ml of crude extract on the degradation of tannins from the juice mangaba at different times.

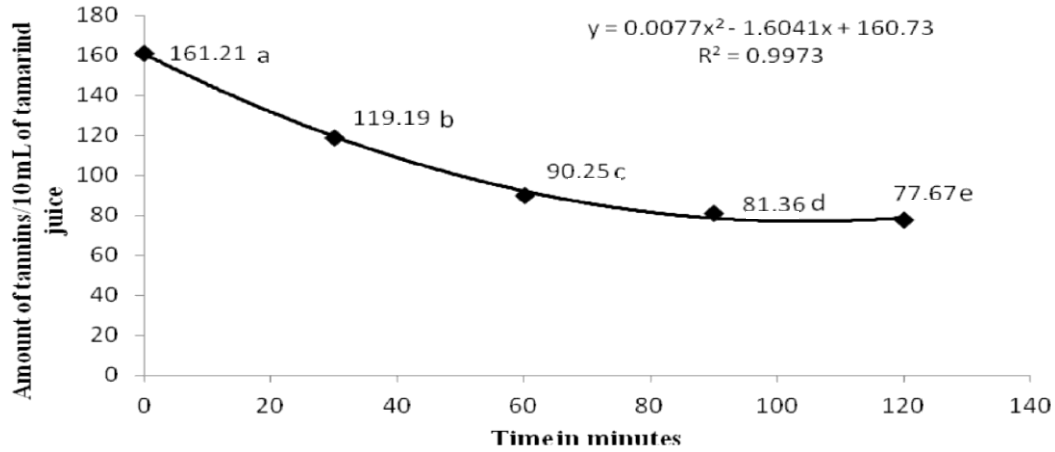

Figure 7. Effect of different volumes of crude extract on the degradation of tannins present in tamarind juice after 90 min incubation.

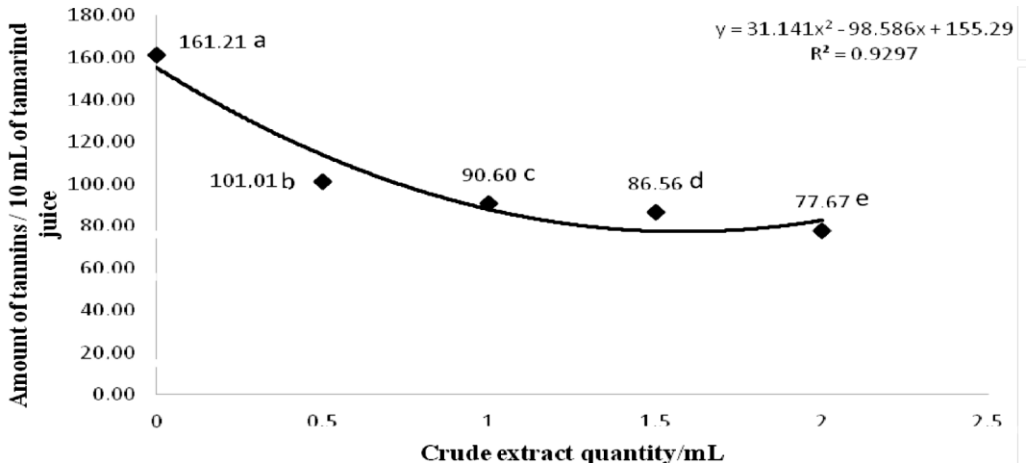

Figure 8. Effect of 2 ml of crude extract on the degradation of tannins present in tamarind juice at different times.

and 165.56 U/gds in jamun and keekar, respectively, after 96 h at 28°C. Macedo and Madeira Jr. (2012) evaluated the production tannase using orange pulp as a substrate by SSF using *Paecilomyces variotii* as biocatalyst. In this study, after optimization, an enzymatic activity of 5,000 U/gds was obtained using 3% tannic acid for 96 h.

The "castanhola" leaves used in this study produced significant results when compared with the literature, potentially increasing their activities by almost 5 times after optimization, with an activity of *A. carneus* URM5577 of 5571.09 U/gds. This is the first report of tannase production in this species. Thus, it is proven that the use of agro-forestry waste as a substrate for filamentous fungi is effective for producing tannase and is of great industrial interest for its potential to minimize the costs of enzyme production.

pH and time are very important variables in the production of metabolites (Selwal and Selwal, 2012). In this study, the greatest activity was shown at pH 6.0 for the 70 h shorter fermentation time. Shorter incubation times will be of interest to the enzyme production industry, as this will decrease the cost of production. Therefore, tannase production by *A. carneus* URM 5577 in a relatively short time is feasible and has proven itself highly efficient.

After applying PB design, time and percentage of tannic acid were identified as significant variables. According to PB, the shorter fermentation (48 h) enabled increased activity. However, after application of RSM, it was observed that the optimum time is 70 h for producing tannase by *A. carneus* URM 5577. In addition, RSM showed that the optimal concentration of tannic acid is 7%. Anwar (2007) obtained a better optimization of tannase production with 7% tannic acid by *A. niger*.

The purification factor showed significant results when compared with the literature. Rodríguez-Durán et al. (2011) used different concentrations of PEG 400, 600 and 1000 with potassium phosphate to optimize the purification of tannase produced by *A. niger* and obtained a recovery of 96%, which was recovered from in the lower phase of the system consisting of PEG 1000, and increased the purification factor by 7-fold.

All test results exceeding 100% may have been due to enzyme activation analysis, for example, upon removal of secondary metabolites during purification, enzyme activity could be inhibited, or otherwise increasing the concentration of salt and/or protein, which helps to maintain the conformation of the protein in its active form (Pan et al., 2001).

Evaluating the partition coefficient (K), it was observed that all K values were greater than one (1), demonstrating that the enzyme exhibits different partition trends between phases. Marcos et al., (1999) reported that the salt-rich phase (bottom phase) has hydrophilic characteristics, and the PEG-rich phase (top phase) has hydrophobic characteristics. Thus, partitioning of the enzyme showed a greater affinity for the PEG-rich phase (top phase), favored by a hydrophobic interaction in the system.

The juices from some fruits may have some undesirable characteristics, such as astringency, darkening, and bitter taste due to the presence of tannins in these fruits. The use of enzymatic treatment is growing in industries due to its low cost and high efficiency (Aguilar et al., 2007).

Sharma et al. (2014) observed the production of tannase by SSF in *A. niger* in the treatment of guava juice (*Psidium guajava*), where there was a reduction of 59.23% in tannin content after 60 min using 2% crude enzyme extract.

In another study, Lima et al. (2014) tested the activity of grape juice by using *Penicillium montanense* URM 6486, and found that it was more efficient at reducing tannin content by 46% after 120 min of incubation at 37°C and 2 ml of the crude extract. This study showed that the enzyme produced by *A. carneus* URM 5577 contained in 2 ml of the crude enzyme extract when applied to both juices showed efficiency when compared to the literature, thus demonstrating the relevance of fungal tannase in the clarification of juices rich in tannins.

Conclusions

A. carneus URM5577 is described for the first time to produce tannase by SSF using as a substrate "castanhola" leaves. The aqueous, two-phase system is a promising and cost-effective alternative in the purification of these enzymes. However, optimization of the growth parameters in a bioreactor is essential for the commercial viability of tannase and its possible application in the clarification of juices such as tamarind and mangaba.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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