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DE-TANNIFICATION OF PUNICA GRANATUM L. JUICE USING TANNASE PRODUCED FROM A LOCALLY ISOLATED MUCOR ELLIPSOIDEUS126271

Sunanda Joshi and Arindam Kuila*

Department of Bioscience and Biotechnology, Banasthali University, Banasthali- 304022, Rajasthan, India.

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***Corresponding Author**
Arindam Kuila
Department of Bioscience and Biotechnology, Banasthali University, Banasthali- 304022, Rajasthan, India.

ABSTRACT

Bioprocesses for synthesizing tannase enzyme help add value to agro-industrial waste. This study aimed to investigate a new filamentous fungus strain isolated from the intestines of *Odontotermes obesus* (white Indian termite) for tannin acyl hydrolase (Tannase) production and characterize it using 16S rRNA gene sequencing technology. The synthesis of tannase was boosted in a submerged fermentation medium combining household and industrial agro-wastes, i.e., *Ananas comosus* and *Citrus limetta* peel substrate, in a 1:1 ratio. After four days of incubation at 35°C and a 6 % substrate concentration, the optimal tannase yield (0.690 IU/mL) was obtained. A sequential optimization strategy based on a statistical research design was utilized to optimize the result of tannase enzyme by ***Mucor ellipsoideus* (*M.ellipsoideus*) strain CBS 126271** by submerged fermentation. The enzymatic saccharification was modified using central composite method (CCD)-based response surface methodology (RSM), and statistical analysis of variance (ANOVA) revealed a satisfactory correlation between the experimental parameters chosen. It was discovered that purification with 60% ammonium sulphate treatment was sufficient for maximum tannase activity. For clarity and quality enhancement, pomegranate (*Punica granatum L.*) juice was treated with crude extracts of tannase enzyme generated in the lab. When different amounts of tannase were treated with pomegranate juice at 35°C, a 25% decrease in tannin was detected without losing biological attributes such as pH, sugar content, or protein content.

KEYWORDS:- Tannase, Tannin, Termite, Submerged fermentation, Juice Clarification, *M. ellipsoideus*

1. INTRODUCTION

Tannins are a type of water-soluble polyphenolic compound that is abundantly secreted by plant defence mechanisms in wood, fruits, roots, and seeds. They are the fourth most prevalent class of plant biomass components. (Maisetta *et al*. 2019). Throughout millions of years of biological history, enzymes such as tannin acyl hydrolases, also known as tannases, have evolved to exploit tannins as carbon and energy sources for growth and development (Lekha *et al.* 1997) (Aguilar *et al*. 2007). Tannases (EC 3.1.1.20) release glucose and gallic acid by hydrolyzing ester and depside links in Gallotannins, ellagitannins, condensed tannins, complex tannins, and phlorotannins are the five primary categories of tannins (Govindarajan *et al*. 2016; Panzella *et al*. 2017; Serrano *et al*, 2009; Khanbabaee *et al*. 2001; Sieniawska *et al*. 2007; Sieniawska *et al*. 2017). The tannases are most commonly used in the commercial manufacturing of instant tea and acorn wine, as well as the antioxidant gallic acid, beer and fruit juice clarification, and coffee-flavored soft drinks (Banerjee *et al*. 2007; Lokeswari *et al*. 2007; Hota *et al*. 2007; Lokeswari *et al*. 2007) catechin gallates (Raab *et al*. 2007), and propyl gallate (Yu *et al*. 2007; Yu *et al*. 2008) Fig1.

Fig. 1: Most common commercial application of tannase enzyme.

Bacteria (KOSTINEK *et al*. 2007)), yeast (ZHONG *et al*. 2004), and fungus such as *Aspergillus versicolor* (BATRA *et al*. 2005), *Aspergillus niger* (MATA-GÓMEZ *et al*. 2009; VENTURA *et al*. 2009), *Paecilomyces variotii* (MAHENDRA *et al.* 2006) and *Aspergillus ruber* (KUMAR *et al*. 2007) have all been found to produce tannin acyl hydrolase. Alternative producers may be discovered for future or inventive purposes, making the promise of uncovering new sources of tannases with distinct biotechnological properties enticing.

Fungal tannases account for the majority of biochemically studied and described tannases (Aboubakr *et al*. 2013). Although *Aspergillus* and *Penicillium* have been used to manufacture tannase in the past (Zarate *et al*. 2014), nothing is known about tannase produced by bacteria or yeasts living in harsh settings, which are regarded to have the potential to produce tannases with unexpected features (Dhiman *et al*. 2018; Dhiman *et al*. 2018).

Extracellular and inducible enzymes make up the majority of microbial tannase. They are now made using submerged fermentation and solid-state fermentation processes (Zhang *et al*. 2011; Gurung *et al*. 2013)

Selwal *et al*. (21) used natural tannin from Terminalia chebula fruit to optimise the development of extracellular tannase. Similarly, numerous researchers have found success with natural tannin-rich agro-residual substrates for production optimization (Selwal *et al*. 2011).

Tannases are used to remove unwanted properties from fruit juices in animal feed and grape wine production (Kanpiengjai *et al*. 2020).

Clarification is an important step in the processing of fruit juice, and it's commonly done with enzymes. Polysaccharides (pectin, cellulose, hemicellulose, lignin, and starch), protein, tannin, and metals are all found in fruit juices (Vaillant *et al*. 2001). Tannins are prevalent in several juices, including apple, grape, pomegranate, and berry (Smeriglio *et al*. 2017).

The presence of tannins in pomegranate and cashew apple juices resulted in astringent mouthfeel, and the high concentration of tannins altered organoleptic aspects of the juices, such as astringency, brown colour, and turbidity. To ensure consumer acceptance and storage stability, tannins must be removed from fruit juice.

This is the first report of tannase production by the novel *M.ellipsoideus* strain CBS 126271 (sequence recovered from termite stomach). This study provides a new fungal tannase with detailed biochemical and structural characterization, paving the path for tannin manipulation in food and beverage items as well as the transformation of tannins into biologically active products to benefit human health based on specific demands.

2. MATERIALS AND METHODS

2.1 Sample collection

The white Indian termite (*O.obesus*) was collected from the ground field of Banasthali Vidyapith, Rajasthan, India using proper sterilized protocols. Dead termites (50) were collected from dry wood, moist termite soil and decaying tree trunk (Fig 2).

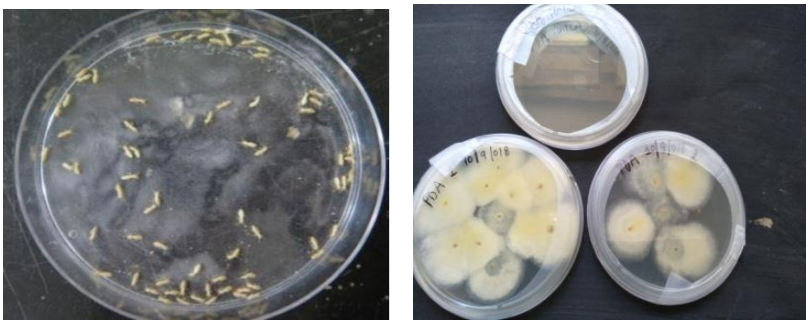

Fig. 2: Showing culture of fungus on potato dextrose agar isolated from *Odontotermes obesus.*

2.2 Isolation and Molecular identification of fungi

Under aseptic conditions, 100 ml of potato dextrose agar (PDA) media was prepared, and 0.33 µl of streptomycin sulphate (35mg/litre) antibiotic was added to the media to avoid contamination. Dead termites were carefully dissected, and crushed gut parts spread on the media plates containing PDA media simultaneously positive controls without gut part were incubated for four days at 28^0C. The colonies of potential fungal isolates obtained later examined using lactophenol blue under a light microscope followed by molecular ITS (Internal Transcribed Spaces) rRNA sequencing at National Center for Microbial Resources (NCMR) Pune, Maharashtra, India.

2.3 Identification of isolated strain

The identification of fungi was made at the two levels:

2.3.1 Microscopic analysis using lactophenol blue

Some previous studies based on morphological recognized the fungi *Aspergillus niger* (Matthew *et al*. 2016). The pale blue background of the lactophenol stain fungi is dark blue. Lactic acid functions as a preservative fungus in the lactophenol cotton blue. The part of the phenol kills the fungi; the fungi are stained with cottage blue.

Staining with lactophenol and cotton blue, morphological characteristics of the *M. ellipsoideus* strain were explored using light microscopy. A drop of lactophenol and the

cotton blue stain were put in the center of a smooth slide. Using an inoculating or teasing needle, a tiny tuft of the fungus (2-3 mm) was transmitted to the drop and teased softly. Preparation was examined for the existence of distinctive mycelia and other structures under low and high dry magnification (Fig 3).

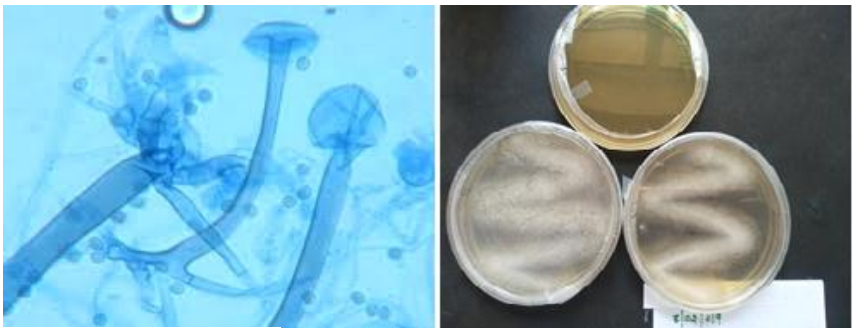

Fig. 3: Microscopic analysis of the cellulase-producing fungal strain *M. ellipsoideus.*

2.3.2 Molecular identification of fungi by ITS (Internal Transcribed Spaces) rRNA Sequencing

For their ITS-based microbial identification, the fungal isolates with cellulase production potential were transferred to the National Center for Microbial Resources (NCMR) Pune, Maharashtra.

Isolates were identified using ITS sequencing of the 16S ribosomal RNA gene, and the identification report was created using the NCBI Database. The availability and level of homology revealed by our sample's 550-bp sequence with its database's nearest neighbor restrict the confidence in identification. Identified strain is *M. ellipsoideus* strain CBS 126271, Accession no is MH863952.1 and similarity is 100%. The 16S rRNA gene sequences were compared using BLAST (http://www.ncbi.nih.gosv) to those in the Genebank database (Boratyn *et al*. 2013). To further characterize MH863952.1, we constructed a phylogenetic tree as shown in Fig 4, based on comparison of the 16S rDNA gene sequence of the isolate by using the Neighbor-Joining method.

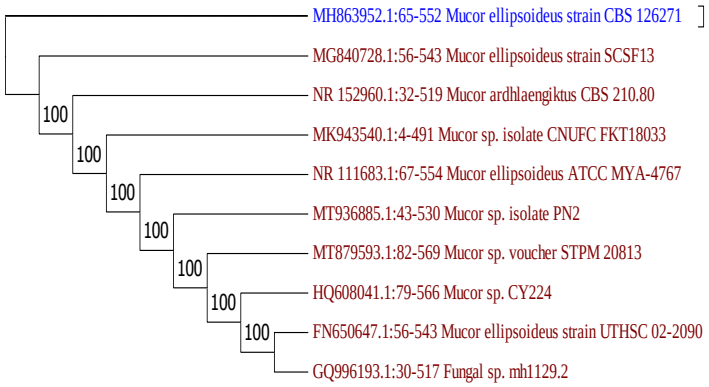

Fig. 4: Phylogenetic analysis of newly isolated *M. ellipsoideus* using the Neighbor-Joining method.

Sequence text (in FASTA format)

>A_JAN_19_151

ATTTTGGAGGCCATCCATTTCGGTCGCTTTCTATCTTTTTTATATTAAAAAAAATA
TAGGCAGTTAAAACTTAATAGCCATAGTAGATTTAAAGCGTCTCAAGATATCGTT
ACGCTCAGATCTATTCAAACAAAATATTTGAATAAGGGTTGTTTTTGATACTGAA
ACAGGCGTACTCATTGGAATACCAATGAGTGCAAGTTGCGTTCAAAGACTCGAT
GATTCACTGAATATGCAATTCACACTAGTTATCGCACTTTGCTACGTTCTTCATCG
ATGCGAGAACCAAGAGATCCATTGTTAAAAGTTGTTTTATAGATTTTTTAGGTCT
ATGTTACAATATTAAAACTGAATTCTTTTGGTAAATAATAATTGGGTACCAAGCA
TCAAGCTTGATTATGACTAGGTTAACATTCTATATACCTACCCTTATAGTATATAG
TCATCCCCTTATATGTCATAAATAAAACAGTTCACAGTAAATAAGA

2.4 Tannic acid agar medium (TAA) for screening of Tannase

Tannic acid agar medium (TAA) was utilized to isolate tannase-producing fungus and for preliminary screening Fig 5 (Pinto *et al*. 2001).

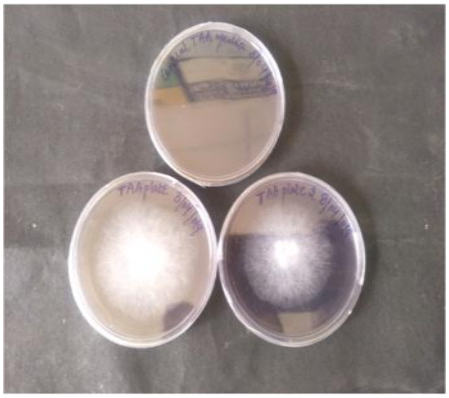

Fig. 5: Screening of *M. ellipsoideus* strain on Tannic Acid Agar medium (TAA).

2.5 Substrate- The mixture of fruit waste (*Ananas comosus and citrus limetta peel*) was gathered in sterile bags from the local market of Banatshali University, Rajasthan, and distributed on trays. Surface sterilized and rinsed with water to remove surface soil, the substrates were shredded into small parts and oven-dried for 24 hours at 70°C. The dried samples were broken into pieces with mortar and pestle (Omojasola *et al*. 2008). The dried waste was then placed at room temperature in autoclaved polythene bags or sterile storage containers until further use.

2.6 Enzyme production by submerged fermentation

Submerged fermentation was used to tannase production in a 250 mL Erlenmeyer flask containing 100 mL of sterile medium Fig 6. Media was prepared according to the table, all the components were dissolved, pH was set, and volume was made up to 100 ml. 8 ml sterile distilled water was taken and transferred into the six-day-old slant of *M.ellipsoideus*. The slant was shaken vigorously so that spores of *M.ellipsoideus* drew closer to the water. 0.1 ml of inoculum was transferred into the 10 ml of media containing boiling tube. Boiling tubes with substrate concentration (2-6%) were placed at different temperatures (25-35^{0}C) for additional incubation days (4-6 days).

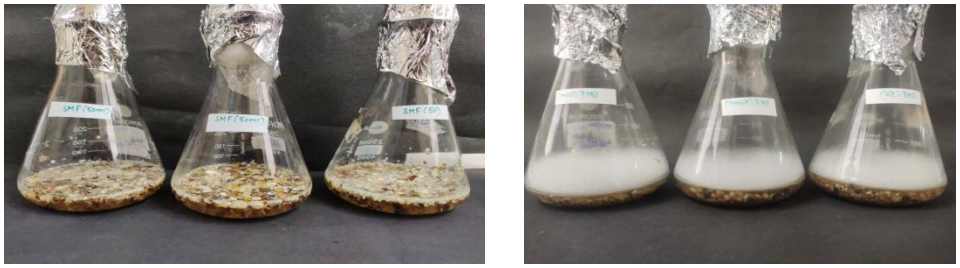

Fig. 6: Tannase enzyme production by submerged fermentation.

2.7 Tannase assay - It was developed a new method for assessing the activity of tannin acyl hydrolase (tannase). The change in optical density of the substrate tannic acid at 310 nm was used to develop this approach. Because of its simplicity, this approach had a measurement error of roughly 3% and could examine a large number of samples at once.

The steps were as follows

One part enzyme solution to four parts substrates (0.350 w/v percent tannic acid dissolved in 0.05M acetate buffer, pH 7.0). (5.5). To terminate the reaction after 60 minutes at 300C, 0.1 part of the mixture was put to 10 parts 90 percent ethanol.

At 310 nm, the optical density of the ethanol solution was measured. The tannase activity (unit/ml) was calculated using the equation below.

$$u = 114 \times Et1 - Et2 / t2 - t1$$

Where E t1 and E t2 denote the optical density of the ethanol solution at 310 nm prepared after t1 and t2 minutes of reaction, respectively, and one unit of enzyme denotes the quantity of enzyme capable of hydrolyzing one mole of the ester link in tannic acid in one minute.

2.8 Partial purification by ammonium sulphate fractionation

Ammonium sulphate crystals were gradually added to the crude extract for enzyme concentration, stirring continuously for 4 hours till solubilization with a 30-90 percent saturation ratio Fig 7.

At 40°C, centrifuge at 10,000 rpm for 15 mints. The enzymatic activity and concentration of Protein in the filtrate and residue were estimated in each step (Sharma *et al*. 2014). A concentration of Protein was measured according to (Lowry *et al*. 1951).

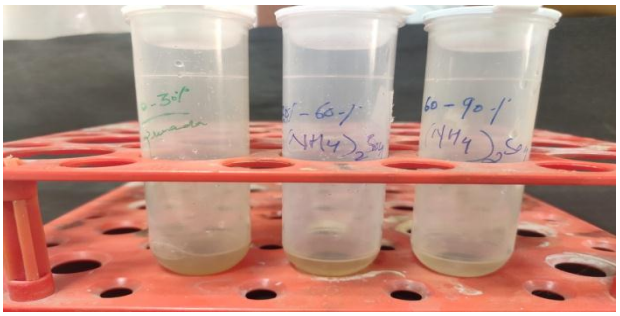

Fig. 7: Purification of tannase produced from the culture of *M.ellipsoideus* under optimum fermentation conditions.

3. Juice preparation

To eliminate any clinging particles, the fruits were rinsed in water. The fruit sacs' rind, rag, and membrane were mechanically removed. The juice was taken from the arils by blending them together in a blender and then filtering them through cheesecloth. Until it was used, the extracted juice was kept at 4°C.

3.1 Treatment of juice with tannase

1 mL of tannase was added to 10 mL of fruit juice in a test tube. Instead of tannase, the control test tube received 1 mL of water. The test tubes were then incubated at 37°C for 120 minutes with gentle shaking. To deactivate the enzyme, the test tube was immersed in a water

bath at 50°C for 10 minutes. At various time intervals, 1 mL aliquots of fruit juices were obtained from each test tube and their tannin concentration was determined.

3.2 Tannin Content Estimation

Tannin concentration in fruit juice was determined using tannins' protein precipitation technique (Hagerman *et al*. 1981).

4. RESULTS AND DISCUSSION

4.1 Optimization of culture conditions using response surface methodology (RSM)

The present study used three-factors and five-level face-centred cube design, requiring 20 studies. The independent variables studied were substrate concentration (X^1), temperature (X^2) and incubation time (X^3) and a quadratic model (8) was used for the central composite design (CCD). Table 1 represents the coded(x) and actual (X) variables' experimental design of the study, and Table 2 represents individual linear, quadratic, and interaction terms that are subjected to variance analysis (ANOVA) and the effect and regression coefficients calculated. The contour maps are based on regression models using statistical calculations to generate regression coefficients.

***significane at p>0.05, R-Sq = 97.95% R-Sq (pred) = 83.12% R-Sq (adj) = 96.10%**

Model summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0224227	97.95%	96.10%	83.12%

To find the best circumstances for tannase production, researchers looked at the interaction of the independent factors (substrate concentration, temperature, and incubation period). By using the response surface regression method, ANOVA analysis (Table 2) yielded the following second-order polynomial model (Chakraborty *et al*. 2011):

Table 1: Central composite designs for the experimental design and predicted results for Tannase activity (IU/mL) by *M. ellipsoideus.*

Run No.	Substrate Concentration %	Temperature ^{0}C	Incubation days	Actual value	Predicted value
1	2	25	4	0.460	0.467
2	6	25	4	0.390	0.394
3	2	35	4	0.420	0.420
4	6	35	4	0.690	0.704

5	2	25	6	0.510	0.516
6	6	25	6	0.350	0.356
7	2	35	6	0.410	0.419
8	6	35	6	0.610	0.616
9	2	30	4	0.430	0.433
10	6	30	4	0.520	0.538
11	4	25	4	0.360	0.355
12	4	35	4	0.480	0.486
13	4	30	4	0.390	0.390
14	4	30	6	0.280	0.293
15	4	30	5	0.280	0.293
16	4	30	5	0.300	0.293
17	4	30	5	0.290	0.293
18	4	30	5	0.310	0.293
19	4	30	5	0.310	0.312
20	4	30	5	0.310	0.312

Table 2: Analysis of variance.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	0.240072	0.026675	43.05	0.000
Linear	3	0.040471	0.013490	26.83	0.000
Substrate concentration	1	0.008167	0.008167	16.24	0.002
Temperature	1	0.025627	0.025627	50.97	0.000
incubation days	1	0.006677	0.006677	13.28	0.005
Square	3	0.124121	0.041374	82.29	0.000
Substrate concentartion*Substrate concentration	1	0.023753	0.023753	47.24	0.000
temperature*temperature	1	0.004346	0.004346	8.64	0.015
incubation days*incubation days	1	0.003789	0.003789	7.54	0.021
2-Way Interaction	3	0.066083	0.022028	43.81	0.000
Substrate concentartion*temperature	1	0.061250	0.061250	121.82	0.000
Substrate concentartion*incubation days	1	0.003527	0.003527	7.01	0.024
temperature*incubation days	1	0.001307	0.001307	2.60	0.138
Error	10	0.005028	0.000503		
Lack-of-Fit	5	0.004228	0.000846	5.28	0.046
Pure Error	5	0.000800	0.000160		
Total	19	0.245100			

[d]egree of freedom [b]Sum of square [c]Mean squares

Regression equation in uncoded units

Tannase enzyme	=	3.554 - 0.3922 Substrate concentration - 0.1117 temperature - 0.369 incubation days + 0.02403 Substrate concentartion*Substrate concentration + 0.001644 temperature*temperature + 0.0454 incubation days*incubation days

		+ 0.008750 Substrate concentartion*temperature - 0.00958 Substrate concentartion*incubation days - 0.00233 temperature*incubation days

The F value was 43.05, and the P-value was 0.000, according to the ANOVA Table. The obtained F value was lower than the table F value, resulting in a modest P-value (less than 0.05). The RSM model accurately explains the relationship between the dependent variables (tannase activity) and the response (tannase activity). The F value was 43.05, and the P-value was 0.000, according to the ANOVA Table. The obtained F value was lower than the table F value, resulting in a modest P-value (less than 0.05). The RSM model accurately explains the relationship between the dependent variables (tannase activity) and the response (tannase activity).

Using 3D response surface plot analysis, the interacting effect of independent factors was discovered Fig 8. The effect of two independent factors on tannase production is depicted in each picture, while other parameters are held constant at their optimum levels. The effect of substrate concentration and incubation duration on *M. ellipsoideus* tannase was shown in Figure 1. Tannase activity was shown to be increased by increasing substrate concentration and incubation time. Maximum tannase activity (0.690 IU/mL) was obtained using 6 % substrate concentration and after 4 days of incubation time. After 4 days of incubation, further increase in tannase production decreased significantly. The influence of temperature and substrate concentration on tannase synthesis was demonstrated. At 35°C, the highest tannase activity was seen. It has been shown that temperature and incubation time have an interaction influence on tannase production. Tannase activity was shown to be increased by increasing temperature and incubation time, however tannase production was found to be suppressed when a specific value increased or decreased in concentration.

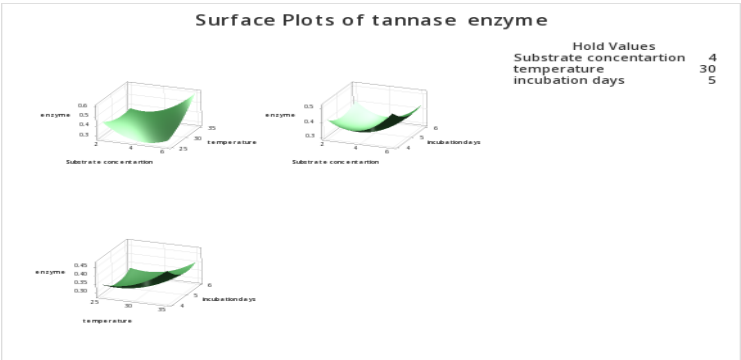

Fig. 8: Interactive effect of independent variables using 3D response surface plot analysis.

The ideal projected conditions for tannase production based on 3D response surface plot analysis were: substrate concentration 6%, temperature 35°C, and incubation duration 4 days. The greatest experimental tannase activity was 0.69 IU/mL under the conditions described above, which was close to the predicted response (0.70 IU/mL). The current study can be expanded upon by gradually increasing tannase production to increase enzyme production. Following the optimization of tannase production, the tannase was utilized to clarify and increase the quality of pomegranate juice.

4.2 $(NH4)_2SO4$ precipitation method

Table 3 reveals that among the three precipitates (30%, 60%, and 90%), the enzyme precipitated at 60% saturation had the highest tannase activity 1.01, compared to the initial crude protein lysate's specific activity of 5.04.

Table 3: Summary of purification of tannase produced from the culture of *M.ellipsoideus* under optimum fermentation conditions.

The percentage saturation of $(NH4)_2SO4$	Tannase activity U mL-1)	Protein content (U mL-1)	Specific activity (U mg-1)	Fold purification	Percentage yield
Crude enzyme extract	2.18	5.04	0.819	1	100
30%	0.79	0.65	1.107	1.35	37.04
60%	**1.01**	**1.23**	**1.24**	**1.53**	**17.43**
90%	0.47	0.93	0.88	1.08	19.85

$(NH_4)_2SO_4$ precipitation resulted in 1.53-fold purification at 60% saturation. As a result, while a low ionic strength (30% $(NH_4)_2SO_4$) is insufficient to disrupt this interaction, a higher ionic strength (70% $(NH_4)_2SO_4$) concentration is sufficient to disrupt the solvated layer. The hydrophobic interaction of enzyme molecules is favored under these conditions, leading in tannase aggregation and precipitation. The present results support the study of Jaya Prakash and Ebenezer (2012), who reported that 70% $(NH_4)_2SO_4$ precipitation saturation resulted in the highest lipase activity by *Aspergillus sp.* Precipitation with ammonium sulfate 60% saturation showed high tannase activity (1.01U/ml) and specific activity (1.24 U/mg protein) compared with crude tannase (0.819 U/ml), as reported in the above table (Jayaprakash and benezer; 2012). After 60% ammonium sulfate precipitation, the enzyme was almost 1.53folds, and the yield was 17.43%.

With ammonium sulphate precipitation, Rajkumar and Nandy were able to achieve a yield of 69 percent (Rajakumar *et al*. 1983). For tannase precipitation, Hamdy found that a 65% ammonium sulphate solution was best (Hamdy *et al*. 2008).

The obtained results agreed with many of the studies that pointed to the importance of the use of ammonium sulfate as the first step of tannase purification (Nandi *et al*. 2016) was used of ammonium sulfate at a saturation rate of 60% to a concentration of the enzyme with a specific activity 1.24 (unit/mg) with purification fold of 1.53 and yield 17.53, while (Philip *et al*. 2015) were showed the possibility of enzyme precipitation by ammonium sulfate with saturation 75% from *Aspergillus niger* with purification a fold of 1.4.

4.3 Applications of tannase from *M. Ellipsoideus* in pomegranate juice clarification

Fig 9 and 10 show the influence of tannase on the clarity of pomegranate juices. The data presented is an average of five replicated tests. To clear 10 mL of fruit juice, 1.5 mL of tannase (0.690 Iu/mL) was utilized. The level of clarity did not improve with a further drop or increase in tannase concentration. The effect of incubation (0, 60, 120, and 180 minutes) and temperature[25,30,35,40] of the fruit juice on the enzyme was also evaluated at various intervals. After 120 minutes of incubation with the enzyme at 35°C, the tannin content had decreased by 25%. This demonstrated that the efficiency of debittering is dependent on the enzyme concentration utilized and the incubation period. The juice was tested for sugar, pH, and protein to see if there was a difference in quality due to the use of debittering aids. In these areas, there was no significant difference between the non-treated and treated samples. However, total sugar level decreased somewhat from 956 (g/ml) in untreated juice to 924 (g/ml) in treated juice table 4. Quality measures such as flavor appearance, color, and acceptability were used to evaluate the fluids before and after treatment. After treatment, it was determined that the juice's acceptance had increased.

Table 4: Effect of enzyme pretreatment on properties of pomegranate juice.

Parameters	Fresh Juice	Treated Juice
Total Sugar (µg/ml)	956	924
Total Protein (µg/ml)	491	485
pH	5.54	4.29

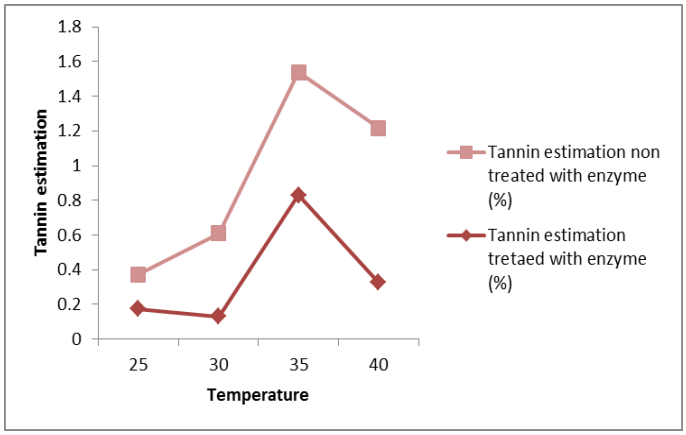

Fig. 9: Tannin estimation with temperature from *M.ellipsoideus* under optimized and unoptimized culture conditions.

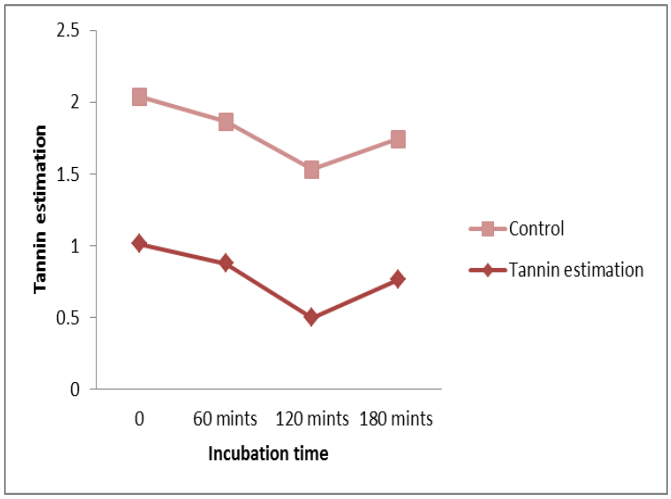

Fig. 10: Tannin estimation with the incubation period from *M.ellipsoideus* under optimized and unoptimized culture conditions.

M. ellipsoideus, a newly identified fungus, was used as an inoculum for the bioconversion of a tannin-rich substrate to tannase. Lima *et al*. (2014) tested the activity of grape juice using *Penicillium Montanans* URM 6486. (Lima *et al*. 2014). They discovered that after 120 minutes of incubation at 37°C with 2 ml of crude extract, the tannin concentration was reduced by 46 %. Rice husk was employed by Nandi and Chatterjee (2016) to generate the enzyme from *Aspergillus niger* MTCC 2525. With an activity of 173U/mg of the purified enzyme, the enzyme was used to detannify fruit juice, resulting in a 56% reduction in tannin concentration. Sharma *et al*. (2014) used SSF to synthesize tannase in *A. niger* to treat guava juice (Psidium guajava) and found that the tannin concentration was reduced by 59.23% after 60 minutes using a 2% crude enzyme extract.

Tannase catalyses the hydrolysis reaction of the ester linkages present in the hydrolysable tannins and gallic acid esters, resulting in a 25% drop in tannin concentration after 120 minutes of incubation. The enzyme was used in this research to try to breakdown tannin, which is responsible for the pomegranate juice's bitterness. According to the findings, using partially purified tannase in the $(NH_4)_2SO_4$) precipitation process could help to clear fruit juices.

On the basis of the quality measure, a sensory evaluation of the juices was performed before and after the treatment.

5. CONCLUSION

Tannase was extracted from the midgut of a dead Indian termite with high tannase utilization ability using the fungal strain *M.ellipsoideus* CBS 126271. With 1.53-fold purification using the partial purification $(NH_4)_2SO_4$) precipitation technique, the purified enzyme was recovered with a final yield of 17.43. The use of this enzyme for juice decalcification also indicates that it can be used in fruit juice processing to efficiently remove tannin.

When 10ml of juice was treated with 1.5ml tannase for 120 minutes, a maximum tannin reduction of 25% was achieved. The effectiveness of each approach in removing tannin varied depending on the type, parameters, and fruit juices used. The tannin degradation efficiency can be a useful tool in a variety of industrial settings, such as tannery effluent treatment, fruit juice debittering, wine clarifying, and so on.

Declaration

Author's contribution

SJ has performed the experimental procedures along with data compilation under the guidance of AK. AK has designed the entire concept and and finalized the manuscript. VV has helped in.

Conflict of interest

Authors declare no conflict of interest.

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