

Characterization of Apple Juice Clarified by Tannase from *Serratia marcescens* IMBL5 Produced using Agro-industrial Waste Materials

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Abstract

In the present study, clarification of apple juice with tannase from *S. marcescens* IMBL5 produced using various agro-waste materials was carried out. Sugarcane bagasse was found to be the most suitable source for the augmented production of tannase enzyme by response surface methodology with the temperature at 40 °C, pH 4.5 and the incubation period of 96 hrs. The enzyme was quantified and partially purified through protein precipitation. The partially purified tannase with gelatin clarified about 62% of the apple juice in 3 hr of incubation at room temperature and it was gently increased with the incubation period. The detannification was characterized by estimating tannin content of the clarified juice. The amount of total reducing sugar in the juice was increased almost 50 % after 5 hours of incubation period. FTIR spectrum of the clarified juice revealed that the conformational changes that occurred in the functional groups. The spectrum absorptions between 500 and 1700 cm^{-1} mainly reflected the C=O stretch of the pectins and acids and C–O modes of the carbohydrates that correspond to the absorption zones of the sugars. The HPLC analysis of the clarified apple juice indicate the presence of phenolic compounds and sugar derivatives such as gallic acid, catechin, caffeic acid, epicatechin, glucose and sucrose which confirms the quality and clarity of the apple juice using the tannase enzyme.

Keywords: Tannase, Agro-industrial wastes, Sugarcane bagasse, Response surface methodology, Apple juice clarification, High performance liquid chromatography

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INTRODUCTION

The growing human population builds a high demand for food products and consequently agro-industrial wastes are elevated. All over the world, it has been reported that millions of tons of agricultural wastes are produced yearly.¹ Most of the agro-industrial wastes are left unused or disposed into environment without proper disposal process which affects human and animal health. It also causes environmental pollution and climate change by generating greenhouse gases.² Generally, agro-industrial wastes contain high carbon and nitrogen content and are the better alternative for many hydrolytic enzyme productions at a lower cost.³ Number of various agro-industrial wastes such as coconut powder, rice bran, coffee husk, sugarcane bagasse, rice straw, groundnut waste, wheat bran, cashew apple bagasse, fruit, and vegetable pulps are utilized by bacteria and fungi as a substrate for the production of industrially important enzymes like tannase, cellulase, amylase, endoglucanase, and exoglucanase.^{4,5} Among these enzymes, tannase is the most exploited enzymes which holds a significant role in feed, food, beverage and pharmaceutical and cosmetics production industries. The enzyme tannase is an extracellular enzyme that produced from bacteria and fungi using most of the agro-industrial residues.⁶ Fruits may have astringency and bitter taste due to the presence of tannins and so tannase was highly utilized in fruit juice clarification process since they have effective tannin degradation and polyphenol stabilization properties.^{7,8}

Apple juice is the highly consuming and revenue generating fruit juice in the global market. The raw apple juice is brown in color and contains turbid due to the presence of different tannins, proteins, and polysaccharides which are tend to settle down during storage. The production of clear apple juice is a challenging factor and the removal of suspended materials prevents turbidity development during storage.⁹ The conventional apple juice clarification process consists of vast time-consuming methods and also expensive compared with the novel clarification methods using microbial enzymes. The removal of tannin, pectin and starch and elimination of haze formation is the key process of juice clarification, which can be achieved effectively only through

enzymatic degradation.^{10,11} In this study, enhanced tannase production from the gut microbe *S. marcescens* IMBL5 was attempted using different agro-industrial waste materials, and its efficiency in apple juice clarification was perceived through validating multiple analysis.

MATERIALS AND METHODS

Materials

Tannic acid, Sodium acetate and Ammonium sulphate were obtained from sigma. All the solutions were prepared using deionized water.

Maintenance of microbial strains

S. marcescens IMBL5 a potent tannase producer isolated previously from the gut of *Onthophagus babirusa* IMBL, a dung beetle was used for tannase production.¹² The culture was stored with glycerol (15 % v/v) in a deep-freezer (-80 °C).

Tannin containing agro-waste materials

Different Tannin-containing agro-waste materials were co-utilized in this study as a sole carbon source for the growth of the *S. marcescens* IMBL5 and as the inducer for tannase as well. Agro-industrial wastes used in this study included wheat bran, rice bran, sawdust, corn waste, sugarcane bagasse, orange pulp and coffee husk collected from various agro fields. The ground substrates were washed with purified water then dried in hot air oven at 60 °C for about 48 hr and stored in vials at room temperature before their incorporation into the fermentation broth.

Selection of agro-industrial waste through one-factor-at-a-time (OFAT) method

The influence of different agro waste material on tannase production was primarily studied through the traditional OFAT method. The agro-industrial wastes such as rice bran, wheat bran, sugarcane bagasse, sawdust, corn straw, coffee husk were used as sole carbon sources in the basal medium to find the tannase activity. The tannase enzyme was quantified using a method prescribed by Pan et al.¹³ The optimized concentration of the chosen agro-waste material was used as a key ingredient for further experiments to optimize the culture condition factors by RSM. The origin software (ver. 2018) was used to analyze the experimental data.

Optimization of the factors using Central composite design (CCD)

RSM was employed to achieve optimized tannase production using an independent variables, such as sugarcane bagasse (A), temperature (B), pH (C), and incubation time (D at five coded levels (Table 1). All experiments were performed in triplicate, and the results were analyzed in Design Expert 11.0 software (Stat-Ease Inc., Minneapolis, USA). The experimental results of CCD were fitted into the following regression equation.

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j$$

Where Y is the predicted response, X_i and X_j are coded input variables of tannase production. The intercept is β_0 , and β_i & β_{ii} denotes the linear and quadratic coefficient respectively. The interaction coefficient is termed by β_{ij} . The significance and adequacy of the obtained model were tested through F-test (Fisher's test) and ANOVA (analysis of variance). The relationship between responses are illustrated by the two-dimensional contour plots.

Apple juice preparation and clarification

Two kilogram apples obtained from the local market for the juice preparation were washed thoroughly with water and surface sterilized with 150 ppm sodium hypochlorite solution. Then they were cut into small pieces and the juice was extracted from the apples using a juice extractor. Then the raw, unclarified juice was filtered using cheesecloth and the filtered juice was stored at 4 °C.

The clarification of the apple juice was done as described by Andrade et al.¹⁴ with minor modifications. Five milligram of gelatin was pre-swelled in 500 μ l of distilled water and melted in microwave oven. The prepared gelatin was mixed in 1:1 ratio with tannase enzyme just before the

treatment. A volume of 50 mL of apple juice was taken in a flask and 5.0 mL of tannase mixture was added and then kept for incubation. One ml aliquot of fruit juice sample was taken from flask at 1 h interval. After incubation, the samples were kept in a water bath at 90 °C for 1 min for enzyme denaturation and then centrifuged at 10,000 \times g for 8 min. Apple juice with water instead of the enzyme was used as control. The transmittance of the supernatant was analyzed using UV-Vis spectrophotometer at 600 nm (Ultraspec pro 2100). Juice clarification was calculated by using the formula

$$\text{Percentage of juice Clarification} = \frac{\text{Tr. t} - \text{Tr. c}}{\text{Tr. c}} \times 100$$

Where, Tr. t = Transmittance of Test, Tr. c = Transmittance of control

Determination of tannin content in apple juice

The total tannin content of the clarified apple juice was estimated by the protein precipitation procedure previously described by Makkar et al.¹⁵

Estimation of reducing sugars

The amount of reducing sugars in the juice was determined using the Di-nitrosalicylic acid method as demonstrated by Lanza et al.¹⁶ Here glucose is used as standard and untreated apple juice is used as control. The absorbance was noted after one hour of incubation of juice with enzyme gelatin mixture at 40 °C.

(2, 2-diphenyl-1-picrylhydrazyl) DPPH antioxidant assay

The antioxidant property of the clarified apple juice was measured as described by Rydzak et al.¹⁷ Four milliliters methanol containing DPPH radicals was added to 0.5 mL of clarified juice. Then it was vigorously shaken and incubated in the dark chamber for 30 minutes. Then the absorbance was noted at 517 nm and of methanol was used

Table 1. A four factor variables and levels of the CCD experiment

Variables	Units	Coded values	Range of levels				
			$-\alpha$	-1	0	+1	$+\alpha$
Sugar bagasse	g/l	A	1.0	1.5	2.0	2.5	3.0
Temperature	°C	B	30	35	40	45	50
pH	-	C	3.5	4	4.5	5	5.5
Incubation time	h	D	46	72	96	120	144

as blank. The anti-radical activity of the apple juice was calculated by

$$\text{DPPH free radical scavenging ability \%} = (1 - \text{OD of sample} / \text{OD of control}) \times 100$$

Characterization of juice clarification through spectral analysis

Infrared spectroscopic analysis was employed to identify the major phenolic groups and sugar derivatives present in the apple juice clarified through tannase of *S. marcescens* IMBL5 following the methodology of Dhaulaniya et al.¹⁸ The infra-red spectrum was obtained between 500 – 4000 cm^{-1} frequency range using Fourier-transform infrared spectrometer (Perkin Elmer, Inc., Llantrisant, UK).

Chromatographic characterization of clarified apple juice

The tannase clarified apple juice was centrifuged and then the supernatant was used for the analysis. The occurrence of phenolic compounds and sugar derivatives were determined by HPLC according to the procedure by Onsekizoglu et al.¹⁹ The C18 column was used for the separation at a flow rate of 1 mL/min at 25 °C.

RESULTS AND DISCUSSION

Effect of agro waste raw materials as carbon source in tannase production

The key ingredients from the agro-industrial waste materials which influence the biosynthesis of tannase were identified using traditional OFAT method. The production of tannase evaluated by employing diverse agro-industrial waste materials as sole carbon source and sugarcane bagasse exhibited the highest tannase production amongst the others (Fig. 1) followed by wheat bran and coffee husk at 3% (w/v) concentration. Tannase production observed in sugarcane bagasse, wheat bran and coffee husk were calculated as 34.81 U/ml, 23.87 U/ml and 19.69 U/ml respectively.

There are some reports fairly agreed with the present observations that the wheat bran, pomegranate rind, oil mill waste and palm kernel powder as best substrates for tannase production.^{3,20-22} Paranthaman et al.²³ stated that the agro-industrial wastes sugarcane bagasse and rice straw were the better alternatives for the enhanced tannase production.

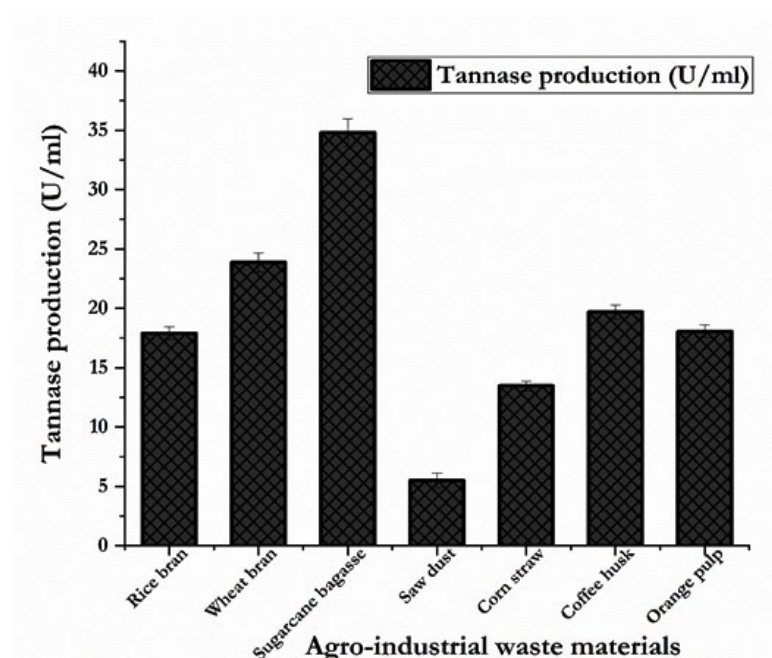


Fig. 1. Effect of agro-industrial waste materials for the optimization of tannase production from *S. marcescens* IMBL5 using OFAT approach.

RSM analysis

To determine the optimal value of the factors that affects tannase production, experiments were designed using uniform design method, which include the experiment with four factors (sugarcane bagasse, temperature, pH and incubation time) and the experimental design is given in Table 1. The tannase production was enhanced after the analysis of CCD data (102.7 U/ml and 103.1 U/ml at run 13 and 20 respectively, Table 2) when all the four factors were remained the same as of preliminary experiments (center point values). When the pH was lower and all the other factors i.e., sugarcane concentration, temperature and incubation time were maintained at 0 levels, augmented tannase production was observed more or less same as that of center point values (100.3 U/ml, run 5, Table 2). Similarly, when temperature and incubation time were retained at +1 with pH at -1 level, increased tannase

production was detected (96.4 U/ml, run 8, Table 2) which indicates that the lower pH favored tannase production. When we compared the runs where sugarcane concentrations were maintained at levels +1 and + α with varying pH, temperature (-1 and +1), it was found that the tannase production was lower (87.4 U/ml, 85.4 U/ml and 83.6 U/ml, run 6, 16 and 17 respectively, Table 2) which denotes that higher concentration of carbon source may affect the enzyme production. Similarly reduction in tannase production i.e., 81.1 U/ml and 84.9 U/ml were observed at - α and + α levels of temperature and with other three parameters at 0 levels which described the dependence of tannase production on temperature. The effects of the four variables on tannase production using 'Design Expert11' software was described below.

$$Y = 103.1 - 0.462*A + 1.40*B - 0.323*C - 2.217*D - 3.701*A^2 - 4.822*B^2 - 2.379*C^2 - 4.176*D^2 - 1.216*AB + 1.879*AC + 0.069*AD + 0.764$$

Table 2. Experimental design and corresponding responses of the RSM analysis

Run	Sugarcane bagasse (g/l)	Temp. (°C)	pH	Incubation time (h)	Response Tannase activity (U/mL)	
					Actual values	Predicted values
1.	25 (+1)	30 (- α)	4 (-1)	120 (+1)	93.40	92.76
2.	25 (+1)	35 (-1)	5 (+1)	72 (-1)	82.60	84.21
3.	15 (-1)	35(-1)	5 (+1)	72 (-1)	79.60	79.08
4.	15 (-1)	45 (+1)	5 (+1)	72 (-1)	89.80	88.47
5.	20 (0)	40 (0)	4 (-1)	96 (0)	100.30	101.08
6.	30 (+ α)	40 (0)	4.5 (0)	96 (0)	87.40	87.50
7.	10 (- α)	40 (0)	4.5 (0)	96 (0)	87.40	89.25
8.	15 (-1)	45 (+1)	4 (-1)	120 (+1)	96.40	93.02
9.	20 (0)	30 (- α)	4.5 (0)	96 (0)	81.10	81.04
10.	20 (0)	40 (0)	5.5 (+ α)	96 (0)	92.20	92.97
11.	20 (0)	40 (0)	4.5 (0)	48 (- α)	80.90	81.99
12.	15 (-1)	35 (-1)	3.5 (- α)	120 (+1)	86.20	87.38
13.	20 (0)	40 (0)	4.5 (0)	96 (0)	102.70	103.13
14.	25 (+1)	45 (+1)	5 (+1)	120 (+1)	91.10	91.46
15.	15 (-1)	45 (+1)	4 (-1)	120 (+1)	92.20	93.02
16.	25 (+1)	45 (+1)	4 (-1)	120 (+1)	85.40	86.04
17.	25(+1)	35 (-1)	4 (-1)	72 (-1)	83.60	83.40
18.	15 (-1)	35 (-1)	4 (-1)	72 (-1)	87.80	85.79
19.	25 (+1)	45 (+1)	5 (+1)	72 (-1)	90.70	88.73
20.	20 (0)	40 (0)	4.5 (0)	96 (0)	103.10	103.13
21.	15 (-1)	35 (-1)	5 (+1)	120 (+1)	87.30	86.78
22.	25 (+1)	45(+1)	4 (-1)	72 (-1)	85.40	84.86
23.	15 (-1)	45 (+1)	4 (-1)	72 (-1)	91.20	92.12
24.	20 (0)	40 (0)	4.5 (0)	144 (+ α)	90.10	90.86
25.	25 (+1)	35 (-1)	5 (+1)	120 (+1)	93.30	92.18
26.	20 (0)	50 (+ α)	4.5 (0)	96 (0)	84.90	86.64

$$*BC - 1.311*BD + 0.386*CD$$

Where Y is the tannase production from *S. marcescens* IMBL5 and A, B, C and D are the values

of the four independent factors such as sugarcane bagasse, temperature, pH, and incubation time respectively. The significance and competence

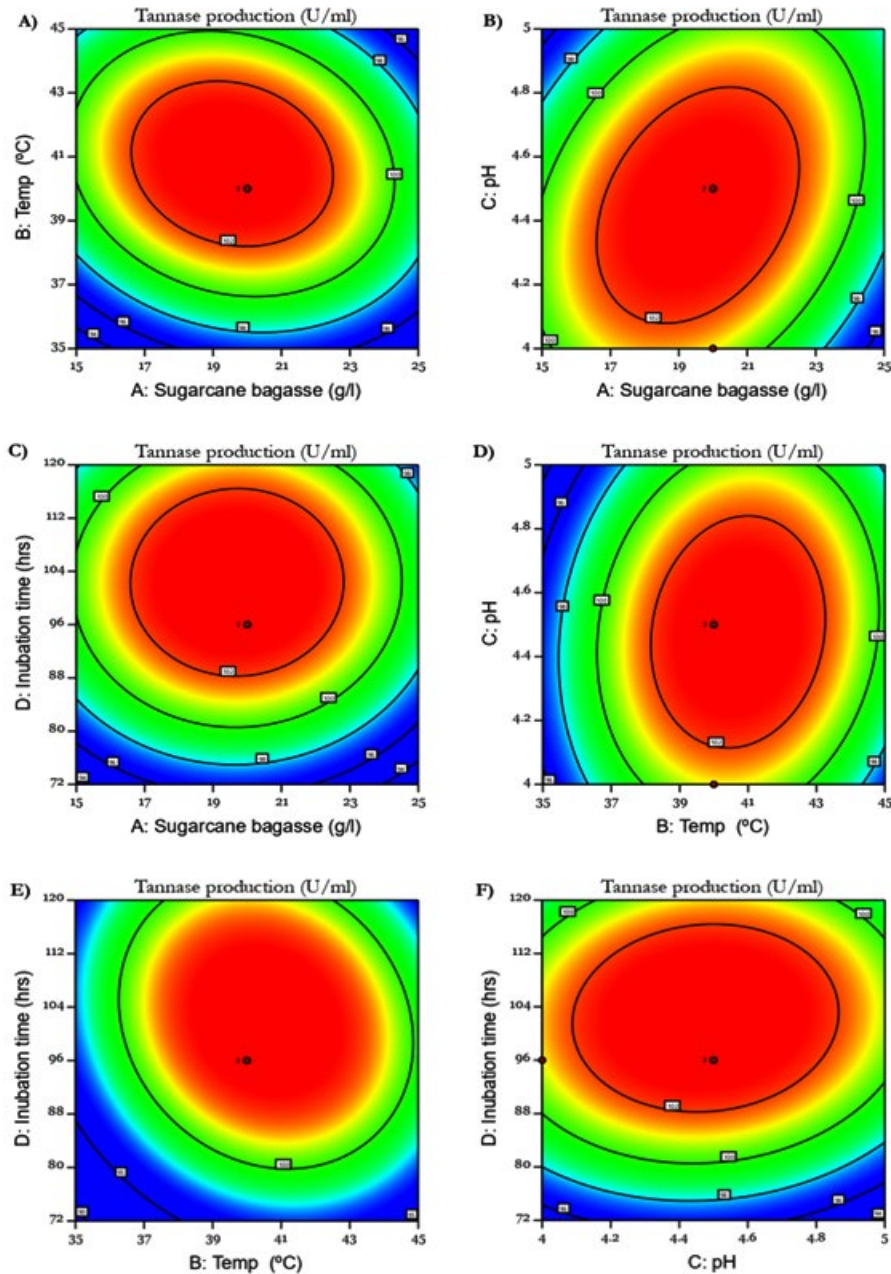


Fig. 2. Optimization of sugarcane bagasse and physical parameters on the production of tannase by RSM using CCD. (a) Influence of temperature and sugarcane bagasse on tannase production, (b) Influence of pH and sugarcane bagasse on tannase production, (c) Influence of incubation time and sugarcane bagasse on tannase production, (d) Influence of pH and temperature on tannase production, (e) Influence of incubation time and temperature on tannase production, (f) Influence of incubation time and pH on tannase production

of the regression model was verified by ANOVA (Table 3) authenticate that the model is significant for tannase production using agro waste material. In this study, B, D, AB, AC, BD, A², B², C² and D² are predicted as a significant model terms.

The two dimensional contour plots showed in the Fig. 2A- 2F are the graphical representations of the analytic model showing the four factor interaction effect of tannase production. The contour lines characterize the interaction of any two variables upon the response when the remaining variables are maintained at optimum. The smallest ellipse region is known to be the vital response area and the intersection point of the minor and major axes of the ellipse is described as the optimal point. Fig. 2A demonstrate the effect of pH (x1) and incubation time (x2) on enzyme production while sugarcane

bagasse and temperature are maintained at their optimal value. The elliptical contours in Fig. 2A designate the interface between two variables x1(pH) and x2 (incubation time) on tannase production. The perfect elliptical contours in Fig. 2B, 2D and 2F states the ample interaction on the responses where the effect of sugarcane bagasse (x1) and pH (x2), temperature (x1) and pH (x2), pH (x1) and incubation time (x2) were analyzed respectively.²⁴ Virtually a circular plot in Fig. 2C implies that the interaction between the represented variables, sugarcane bagasse (x1) and incubation time (x2) are less.²⁵ In Fig. 2E, the interaction effect of temperature and incubation time on tannase production was measured and the elliptical plot indicate the substantial interaction

Table 3. ANOVA results for the response surface quadratic model for tannase production

Term	Values
Standard deviation	1.87
Mean	89.49
CV%	2.09
R ²	0.9608
Adjusted R ²	0.911
Predicted R ²	0.714
Adeq Precision	16.89
Model F- value	19.27
Lack of fit F- value	1.16
PRESS	282.02

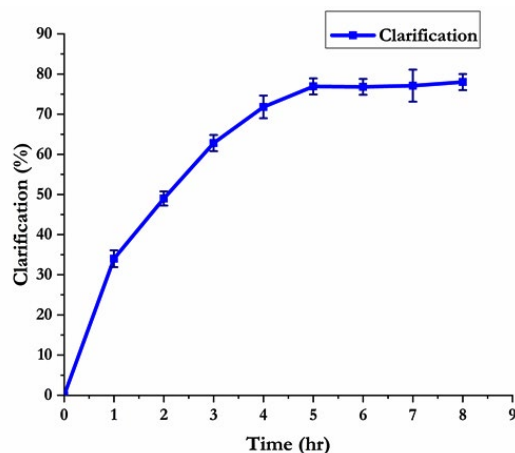


Fig. 3. Apple juice clarification using tannase enzyme from *S. marcescens* IMBL5 along with gelatin.

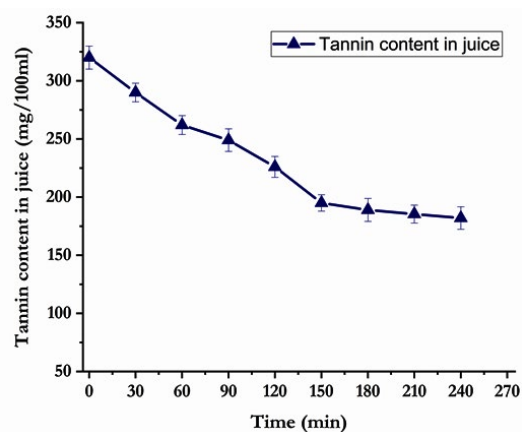


Fig. 4. Estimation of tannin content in the clarified apple juice.

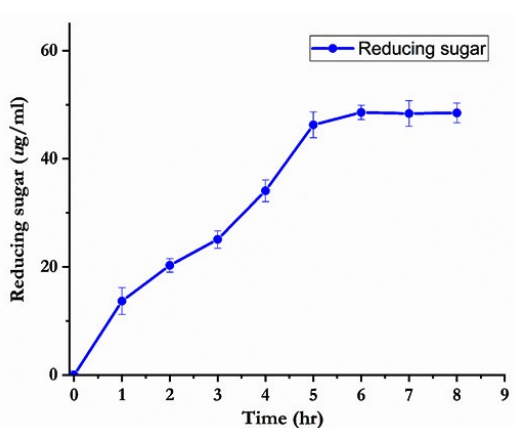


Fig. 5. Estimation of total reducing sugar in the clarified apple juice.

among the factors. RSM was effectively utilized to optimize the tannase production by *A. oryzae* using agro-waste pomegranate rind extract.²²

Validation of the analysis model

The predicted values obtained from the analytical model were 19.2 g/l, 40°C, 4.3 and 96 for x1, x2, x3 and x4 with the tannase production of 102.98 U/ml. This was correspond to the response obtained from experimental value of 100.72 U/ml with <2 % error which was 3.3 fold higher than that of the enzyme production through OFAT analysis.

Apple juice clarification

Microbial enzymes with stability in acidic pH range are suitable candidates for fruit juice

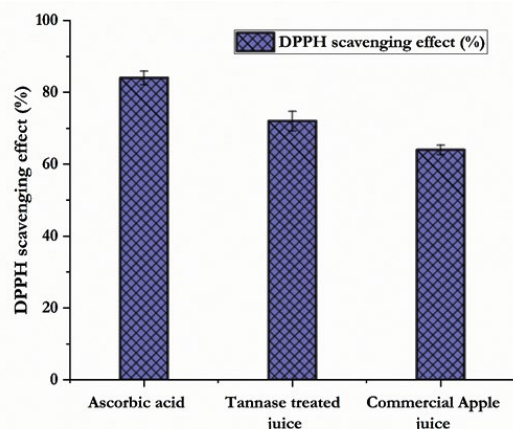


Fig. 6. DPPH radical scavenging activity of tannase on the clarified apple juice.

clarification as most of the fruit juices are acidic.²⁶

The effect of tannase on the clarification of apple juice is shown in Fig. 3, here 1 ml (253 U/ml) of tannase enzyme was used for the clarification of 10 ml apple juice. Apple juice clarification up to 60% was observed in 3 hours of incubation with tannase enzyme and gelatin mixture. The enzyme mixture gave up to 80% juice clarification with five more incubation hours. The yield of clarified juice was also increased about 5% and visually the enzyme treated juice showed maximum clearance. The pectin present in fruits leads to colloid formation, which decreases the commercial value of juices. During the enzymatic clarification process, removal of the colloidal materials occur and this leads to the presence of more soluble materials.²⁷ The reduction of turbidity can be related to the degradation of cell wall components such as tannins and pectin which indicate the efficiency of the clarification process.¹⁴

Detannification of apple juice

Tannins like plant polyphenols are astringent compounds and bitter in taste. The eco-friendly and healthy method of detannification by using the microbial tannase was showed in Fig. 4. About 45% of tannin content decrease was observed within 3 hr of incubation with enzyme-gelatin mixture. A gradual decrease in total tannin content represented the effective tannin degradation by tannase which evidenced the

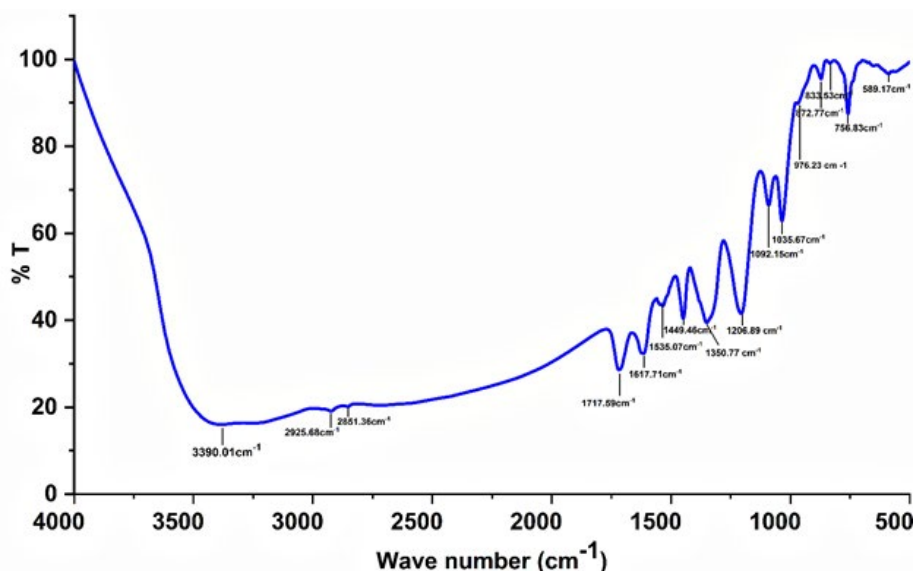


Fig. 7. FT-IR spectrum of clarified apple juice using tannase enzyme from *S. marcescens* strain IMBL5.

active clarification. Then, the tannin concentration was further decreased slowly with extending the incubation hours. Generally, addition of tannase into fruit juice could aid the tannin degradation but the addition of gelatin along with tannase gives commendable result. The tannase-gelatin treatment may be attributed the precipitation of tannins, which in turn the bitterness of the juice was reduced. The results were most consistent with previous reports.^{28,29}

Determination of reducing sugar in juice

The reducing sugar level in juices are the best indicative of the quality juices, and monitoring their levels during juice preparation could aid to improve the market quality. In the Fig. 5, the amount of reducing sugar was exponential and significantly increased by nearly 50 µg/ml after 5 h of incubation time. This may lead to increase the sweetness of the clarified juice. The percentage of reducing sugar observed was higher than that of the juice clarified using enzyme alone. Suryawanshi et al.³⁰ stated that fruit juice treated with mixture of enzymes increased the reducing sugar level.

Antiradical scavenging assay

The antiradical scavenging activity is a very significant characteristic feature of fruit juices and it is measured by DPPH scavenging assay which is a standard method to estimate the stable radicals. The scavenging activity of tannase clarified apple juice was presented in Fig. 6. Tannase from *S. marcescens* IMBL5 significantly enhanced the clarification and maximum scavenging activity was obtained for the tannase treated apple juice with 74.16 % and

83.67 % for ascorbic acid. Compared with the treated one, lower level of scavenging activity was noticed in the commercial apple juice with 66.08 %. Generally, the cloudy juices show greater ability to scavenge free radicals might be attributed to its high tannin content and so neutralizing the free radicals through transferring the hydrogen atom or an electron. Due to the free radical scavenging capacity, the phenolic compounds like flavonoids and tannins are denoted as the main group of antioxidant phytochemicals with remarkable properties.³¹ The results supports the findings of Abdullah et al.²⁹ and Jana et al.⁷ that the tannase treated fruit juices showed 78.44 % and 87.7 % of DPPH radical scavenging activity respectively.

Infrared spectra of a clarified apple juice

FTIR spectroscopy is a cost-effective more rapid method for the investigation of phenolic derivatives and carbohydrates in fruit juices and it can potentially provide facts about their presence and proportions. The IR spectrum of tannase clarified apple juice exhibited a diverse absorption peak patterns from 3390 to 590 cm^{-1} was presented in Fig. 7. The presence of intermolecular bonded hydroxyl groups (OH) was identified by means of broad and deeply stretched peak at 3390.01 cm^{-1} confirming the existence of a tannins polymerization.³² The region of aliphatic stretching is diagnosed as a methylation of hydrolysable and condensed tannins. The CH₂ stretching of a peak at 2925.68 cm^{-1} provide information about the methylene substituents.³³ A specific, strong peak at 1717 cm^{-1} denotes the C=O --- H bond interaction of catechin occurring in polymers include condensed tannins.³⁴ An

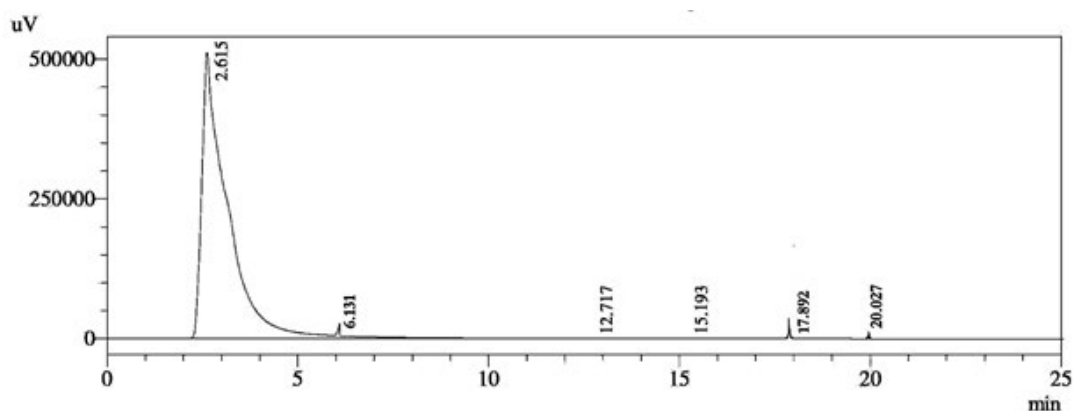


Fig. 8. HPLC analysis of sugar derivatives and phenolic compounds from the tannase treated apple juice.

aromatic - C = C - stretching are also detected in the spectral region of 1449.46 cm^{-1} which is typically characterized by an envelope of strong bands with the combinations of C-O stretching, C-H aromatic bending and C-OH deformations. Generally, bands of main carbohydrates were spotted between 900 and 1400 cm^{-1} spectral range. The absorption peaks patterned at 1092.15 and 1035.67 cm^{-1} represented C-C stretching C-O modes may be ascribed to the band of glucose. The presence of minor peak at 976.23 cm^{-1} revealed by the C-O-H bending vibrational mode of fructose.³⁵ The FT-IR result exhibited that tannin present in the apple juice was degraded efficiently by the tannase of *S. marcescens* strain IMBL5 with varied modification pattern and also the fruit juice quality and authenticity was firmly evaluated.

Chromatographic characterization of apple juice

HPLC system is an ample technique used to detect the presence of phenolic compounds and the rapid characterization of many nutritionally significant polyalcohol and major sugars found in food products. The chromatogram of tannase clarified apple juice was presented in Fig. 8. Phenolic compounds and sugar derivatives in the clarified juice were identified as gallic acid, catechin, caffeic acid, epicatechin, quercetin, sucrose, fructose and sorbitol in C18 column. The compounds spotted in the spectrum were identified through retention time and confirmed by the spectral data of the resultant peaks of previously reported chromatographic investigations of apples juices. The major peak was detected at the retention time of 2.6 min on a chromatogram indicates the presence of gallic acid, an important derivative of tannin degradation and it is also present in apple juice. This peak observation evidently designates the metabolic reaction of tannase on the juice clarification. The peak detected at the retention time of 6.43 min and 12.71 min are indicating the occurrence of phenolic compounds like quercetin and catechin respectively.^{36,37} These peaks also confirms the presence of carbohydrates such as sucrose and fructose in the clarified apple juice.³⁸ A mild peak observation shown at retention time 15.19 min confirms the presence of the sugar derivative sorbitol. Another peak detected at 17.947 min indicating that the presence of caffeic acid.³⁹ Du et al.⁴⁰ stated that the caffeic acid, catechin and epicatechin are the leading

polyphenols present in apple juice. A peak attained at 20.01 min evidently demonstrating the presence of epicatechin, the predominant byproduct of tannin degradation.⁴¹ Similar to our investigation, many other researchers have reported the study of polyphenols in apple juice in various centuries.^{17,42-44} The chromatogram study indicate the occurrence of polyphenols and sugar molecules were identified evidently and the significance of tannase in apple juice clarification was also proved.

CONCLUSION

A key concern in the food industry is the cost effective production and utilization of microbial enzymes. In this context, the use of agro-industrial wastes and RSM technology provides the augmented production of tannase and also encourage their consumption on industrial applications. Generally, the bacterial enzymatic clarification has an edge over the other methods. The present investigation inferred that the tannase along with gelatin clarified apple juice fruitfully. This clarification method was effective, less expensive and we can employ this method for large scale juice production in industrial level.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

BT and MK designed the experiments and prepared the manuscript. BT and RA performed the experiments. MK supervised and reviewed the manuscript. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

Not applicable.

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