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Microbiota-Derived Pectinase from Bombyx Mori for Enhanced Fruit Juice Clarification and Wine Quality Modulation

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ABSTRACT: Pectinases play a key role in fruit juice processing by degrading pectic substances responsible for turbidity and low juice yield. In this study, a partially purified pectinase derived from Bombyx mori gut microbiota was evaluated for juice clarification, yield enhancement, and wine quality improvement, and compared with commercial Aspergillus niger-derived pectinase, in free and immobilized (calcium alginate bead) forms on orange, grape, and pomegranate juices. Enzyme treatment improved juice yield and clarity in all three fruits, with the greatest gains at 1% enzyme concentration, and produced a concentration-dependent reduction in turbidity at 540 nm. The commercial enzyme achieved comparable clarification at considerably lower concentrations (0.12-0.4%) than the test enzyme. Immobilized pectinase retained increasing catalytic activity with higher enzyme loading and effectively clarified orange juice, although residual absorbance was slightly higher than with the free enzyme. In wine fermentation trials, pectinase treatment increased colour intensity and red pigment proportion while reducing hue, indicating enhanced anthocyanin extraction, with only marginal change in total phenolic content across treatments. These findings indicate that Bombyx mori gut microbiota-derived pectinase is a promising, sustainable biocatalyst for improving fruit juice quality and wine processing, with its performance depending on enzyme source, concentration, and immobilization strategy.

1. INTRODUCTION

Fruits are an essential part of the human diet, providing vitamins, minerals, dietary fibre, and other bioactive compounds. They are widely consumed fresh or as juices, which retain much of their nutritional and functional value; fruit juices in particular are rich in antioxidants that neutralize free radicals, reduce oxidative stress, and support hydration (Dreher, 2018). Rising consumer preference for healthier alternatives to caffeine-containing beverages has driven strong growth in the natural fruit juice market, with demand increasingly favouring fresh, crystal-clear, low-viscosity juices such as orange, apple, mango, pineapple, cranberry, and pomegranate.

Despite their nutritional value, fruits are highly perishable, and the FAO estimates that approximately 45% are wasted annually due to spoilage (Hmar *et al.*, 2017). Processing fruits into value-added products such as juices helps reduce these losses while

preserving functional, sensory, and nutritional quality. However, conventional extraction methods—including diffusion, mechanical extraction, and decanter centrifugation—often yield turbid, viscous juices because of residual structural polysaccharides such as pectin, cellulose, hemicellulose, and starch, and are further associated with high costs, low yields, and contamination risks that limit consumer acceptability (Sikodia *et al.*, 2024). This has driven demand for more efficient clarification technologies (Nachal *et al.*, 2023).

Pectin presents major processing challenges by causing haze formation, increased viscosity, and reduced juice yield through interactions with phenolic compounds, proteins, and metal ions. Enzymatic degradation of pectin using pectinases has proven effective in mitigating these issues. In high-pectin food matrices, pectin modification is often essential for optimizing processing efficiency (Bélafi-Bakó and Nemestóthy, 2017). The rheological properties of pectin depend on structural factors such as molecular weight and degree of methylation, which can be tailored through enzymatic or thermal treatments (Zhang *et al.*, 2023).

Enzymatic clarification is increasingly recognized as an effective alternative to conventional physical and chemical methods (Anand *et al.*, 2020; Pinelo *et al.*, 2010). Enzymes including pectinases, cellulases, xylanases, amylases, proteases, fructozyme, dextranase, and amyloglucosidase enhance juice yield and clarity by degrading plant cell wall components (Panda *et al.*, 2021; Pandey *et al.*, 2022; Sikodia *et al.*, 2024). Among these, pectinases are widely used in the food, textile, and paper industries due to their efficiency in reducing juice viscosity and turbidity while improving nutritional value (Pandey *et al.*, 2022) However, large-scale application of pectinases is constrained by high production costs and limited enzyme stability under industrial conditions (Shrestha *et al.*, 2021; Rehman *et al.*, 2021; Patel *et al.*, 2022).

Microbial enzymes offer a promising solution for discovering novel and efficient biocatalysts. The gut microbiota of the domesticated silkworm, *Bombyx mori*, represents a unique and underexplored ecosystem with significant biotechnological potential. This microbiota comprises diverse bacterial and fungal communities capable of producing carbohydrate-degrading enzymes such as pectinases, cellulases, and xylanases. These microbes not only support digestion but also contribute to immune modulation and pathogen defense (Singh *et al.*, 2019). Advances in metagenomics and metaproteomics have enabled the identification of novel enzymes from this ecosystem, highlighting its potential as a reservoir of new industrial biocatalysts (Dhanjal *et al.*, 2020). Despite its promise, this microbial resource remains insufficiently explored (Banerjee *et al.*, 2022; Sharma, 2024).

To improve enzyme stability and economic feasibility, enzyme immobilization has emerged as a key strategy in industrial biotechnology. Immobilized enzymes exhibit enhanced stability, reusability, and resistance to deactivation, reducing operational costs and improving process efficiency (Attique *et al.*, 2023; Gupta and Modi, 2023). Among immobilization techniques, entrapment in calcium alginate beads is particularly advantageous due to its simplicity, low cost, biocompatibility, and suitability for food-grade applications (Ashkan *et al.*, 2023).

In this study, pectinases derived from the gut microbiota of *Bombyx mori* were investigated for their application in the extraction and clarification of orange (*Citrus sinensis*), grape (*Vitis vinifera*), and pomegranate (*Punica granatum*) juices, with the aim of improving clarity, reducing viscosity, and enhancing juice yield through a sustainable and biologically novel approach. Further its application was studied in wine quality improvement.

2. MATERIALS AND METHODS

2.1 Sample Collection

All the fresh, matured and ripened fruits such as Orange, Grapes and Pomegranate were purchased from a local grocery store and stored in the food processing laboratory. Other chemicals used were of analytical grade and purchased from Himedia, India.

Standard enzyme: Commercial *Aspergillus niger*-derived pectinase enzyme (3 U/mg) was procured from SRL, India (Product code: 90464).

Partially purified pectinase enzyme: The partially purified pectinase enzyme (ammonium sulphate precipitation) derived from *Bombyx mori L.* gut microbiota was used for the current study (Kishore Kumar *et al.*, 2026).

2.2 Juice clarification

Fresh, ripe oranges, grapes and pomegranate fruits were selected, thoroughly washed, peeled, and used in the following process. A sterile small-scale juice extractor was used to extract the juice. To deactivate the natural fruit enzymes, the extracted juice was pasteurized for three minutes at 85°C and then cooled to 4°C. Aliquots of juice (20 mL) were transferred into conical flasks and treated with varying concentrations of the partially purified pectinase enzyme (0%, 0.25%, 0.5%, 0.75%, and 1%). All the flasks were incubated at 30°C for 1 h. After incubation, the samples were again heated at 85°C for 3 min to inactivate the added pectinase enzyme. The juice treated with different enzyme concentrations was filtered using Whatman No. 1 filter paper. The volume of fruit juice obtained was measured using a 50 mL measuring cylinder. The juice was stirred to check for clarity, and then 10 mL of the liquid was centrifuged at 5000 rpm for 10 min. The supernatant was used to determine percentage of transmittance and absorbance was taken at 540 nm using spectrophotometer.

2.3 Analytical Methods

Clarification of fruit juices using commercial *Aspergillus niger*-derived pectinase (standard)

Fresh orange, grape, and pomegranate juices were used to investigate the effect of standard *A. niger*-derived commercial pectinase concentration on juice clarity. The enzyme was added to 20 mL aliquots of each juice at different concentrations. For orange juice, pectinase concentrations of 0.03%, 0.06%, 0.09%, and 0.12% were tested, corresponding to 6, 12, 18, and 24 mg of enzyme per 20 mL of juice, respectively. For grape juice, concentrations of 0.04%, 0.08%, 0.12%, and 0.16% were used, corresponding to 8, 16, 24, and 32 mg per 20 mL, respectively. For pomegranate juice, pectinase concentrations of 0.1%, 0.2%, 0.3%, and 0.4% were evaluated, corresponding to 20, 40, 60, and 80 mg of enzyme per 20 mL of juice, respectively. Additionally, the enzyme quantities were calculated in both mg/100 mL and mg/20 mL, with activity units proportionally adjusted accordingly. After treating all of the juices with standard pectinase enzyme, the same experimental methodology was used to measure the absorbance at 540 nm.

2.4 Immobilization of pectinase in alginate beads

Preparation of alginate beads: The preparation of alginate beads involved the addition of a standard pectinase enzyme and a partially purified enzyme to a 4% sodium alginate solution, which was continuously stirred on a magnetic stirrer for 30 minutes to ensure uniform mixing. This mixture was then transferred into a syringe and carefully dropped into a beaker containing a 5% CaCl2 solution. The beads were allowed to crosslink for 2 hours at ambient temperature while being stirred slowly. Beads prepared using sodium alginate without the addition of the enzyme served as the control. Each bead was carefully removed using a spatula, and the moist weight was measured. The average weight of three beads was used for estimation.

Protein content: Protein content of the immobilized enzyme was quantified using the Lowry method, with bovine serum albumin serving as the standard, following established protocols. For analysis, five enzyme-loaded beads were carefully washed and resuspended in 1 mL of distilled water. The protein concentration was then determined in the resulting suspension.

Pectinase activity: The DNS (3,5-dinitrosalicylic acid) method was employed to quantify the amount of reducing sugars produced from a pectin suspension (0.25% pectin in 50 mM citric-citrate buffer, pH 3.5). As a standard, galacturonic acid was utilized. The mixture of enzymatic extract and substrate (200 + 800 µL) was incubated at 28°C for 30 minutes. At 550 nm, the absorbance was measured. Under the assay conditions, the amount of enzyme activity required to release one micromole of reducing sugar per minute is one pectinase unit (U).

2.5 Application of immobilized enzyme in orange juice clarification

The freshly squeezed orange juice was prepared and filtered to remove coarse particles. The filtrate was used for the juice clarification process. Each bottle was filled with 20 mL of juice and subsequently pasteurized. A designated number of beads (10 beads) were added to each bottle, which were accurately labelled. The control group consisted of juice without beads, while the alginate control consisted of juice with alginate beads but no enzyme. A one-hour reaction was conducted at 30°C, after which the enzymes were inactivated by heating to 80°C for three minutes. Then, 10 mL of the liquid was centrifuged at 5000 rpm for 10 min. The supernatant was used to determine percentage of transmittance and absorbance was taken at 540 nm using spectrophotometer.

2.6 Application of pectinase in wine preparation and analysis

In this experiment 2 kg grapes were weighed and crushed properly, then divided into four fermentation containers, labelled as follows: Control (no enzymes), Group 1 (baker's yeast at 200 g/kg), Group 2 (baker's yeast at 200 g/kg plus 100 mg/kg of pectinase), and Group 3 (baker's yeast at 200 g/kg plus 200 mg/kg of pectinase). The grapes underwent maceration for 6 hours at a temperature of 18-20°C. Afterward, the pomace was separated by filtering through muslin cloth, and 250 mL of the extract was distributed with the corresponding enzyme supplements. The bottled wines were stored at 4-6°C for 5 days, after which an aliquot was drawn for analysis.

2.7 Colour intensity, hue and colour composition of the wines

The wine's colour intensity is quantified by the sum of absorbance at 420, 520, and 620 nm. The hue (H) is calculated as the ratio A420/A520, while colour composition is measured through proportions of yellow (%Ye), red (%Rd), and blue (%Bl), determined as follows: yellow proportion is (100 × A420/colour intensity), red proportion is (100 × A520/colour intensity), and blue proportion is (100 × A620/colour intensity). Additionally, the brilliance of red wines is calculated using the formula [100 × [1 − (A420 + A620)/2A520]], indicating that a higher brilliance value signifies a stronger red colour dominance.

2.8 Filtration speed and speed of sedimentation

The filtration speed was determined by the time required to filter a specific volume of mash sample (10 mL must) through the filtration paper. The speed of sedimentation was measured as the thickness of sediment generated within 30 minutes of mixing experimental bottles with samples (10 mL required).

2.9 Total phenolic index

The phytochemical profile of grape macerates was determined using calibration curves and standards. The total phenol (TP) concentration was evaluated using the Folin-Ciocalteu colorimetric technique. Gallic acid was used as a standard, and the results were represented in milligrams of gallic acid equivalent per millilitre (mg GAE/mL).

2.10 Statistical analysis

All experiments were performed in triplicate (n = 3), and results are expressed as mean ± standard deviation (SD). Data were analysed using SPSS. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare means among treatment groups, with differences considered statistically significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Effect of pectinase on juice clarity

Orange juice clarification

Enzyme treatment markedly improved orange juice yield and clarity relative to the control, as summarized in Table 1 and Figure 1. From an initial volume of 20 mL, the control sample yielded 14 mL of juice. In contrast, enzyme-treated samples showed a progressive increase in juice volume with increasing enzyme concentration. The highest yield was obtained at 1% enzyme concentration, producing 21.5 mL of juice and representing a 1.54-fold increase compared with the control. The lowest absorbance was observed at 0.5% enzyme concentration, suggesting maximum clarification at this level, while higher concentrations (0.75% and 1%) showed slightly increased absorbance but remained lower than the control (Figure 1). Overall, enzyme treatment enhanced juice yield and clarity, with increasing enzyme concentration generally improving extraction efficiency, while optimal clarification was observed at an intermediate enzyme level.

The increase in juice yield and reduction in absorbance may be attributed to enzymatic hydrolysis of pectic substances in the fruit cell wall. Pectinase degrades pectin polymers, reduces viscosity, and improves juice extractability, while the conversion of insoluble protopectin into smaller soluble fragments can destabilize colloidal suspensions of polysaccharides, proteins, and phenolic complexes. This promotes aggregation and sedimentation of suspended particles, thereby increasing light transmission and reducing absorbance at 540 nm. These findings are consistent with the established role of pectinases in improving juice extraction and clarification (Chinelate *et al.*, 2025; Mantovani *et al.*, 2005). Improved juice clarity is also relevant to industrial processing because it can enhance consumer acceptance, shelf stability, filtration efficiency, and product quality.

Table 1. Volume of orange juice after enzyme treatment

Sample	Volume obtained from 20 mL	Fold increase
Control	14	–
0.25%	16.5	1.18
0.5%	18.5	1.32
0.75%	20	1.43
1%	21.5	1.54

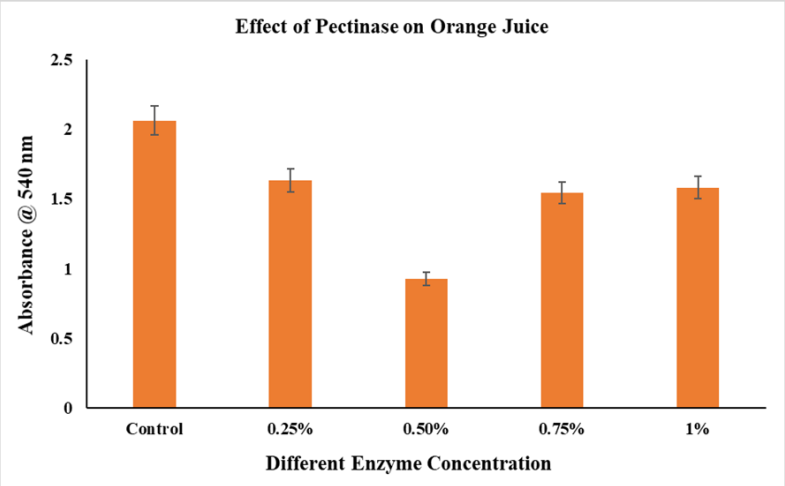

Figure 1. Effect of partially purified pectinase on the clarity of orange juice.

3.2 Grape juice clarification

Enzyme application markedly improved grape juice yield and clarity relative to the control, as presented in Table 2 and Figure 2. From an initial volume of 20 mL, the control yielded only 11.5 mL of grape juice, indicating lower extraction efficiency in the absence of enzyme treatment. The maximum juice yield was obtained at 1% enzyme concentration, producing 17.5 mL of juice and a 1.52-fold increase compared with the control. Enzyme-treated samples demonstrated progressively lower absorbance, with the lowest value observed at 1% enzyme concentration. This reduction in absorbance reflects improved clarification of grape juice due to enzymatic breakdown of pectic substances (Figure 2).

Similar enhancements in grape juice recovery following microbial pectinase treatment have been reported, including yield improvements of up to 31.6% (Nisha, 2016). Comparable improvements in juice extraction have also been documented in banana and pawpaw juices following enzymatic treatment.

Table 2. Volume of grape juice after enzyme treatment

Sample	Volume obtained from 20 mL	Fold increase
Control	11.5	–
0.25%	13.5	1.17
0.5%	15	1.30
0.75%	16.5	1.43
1%	17.5	1.52

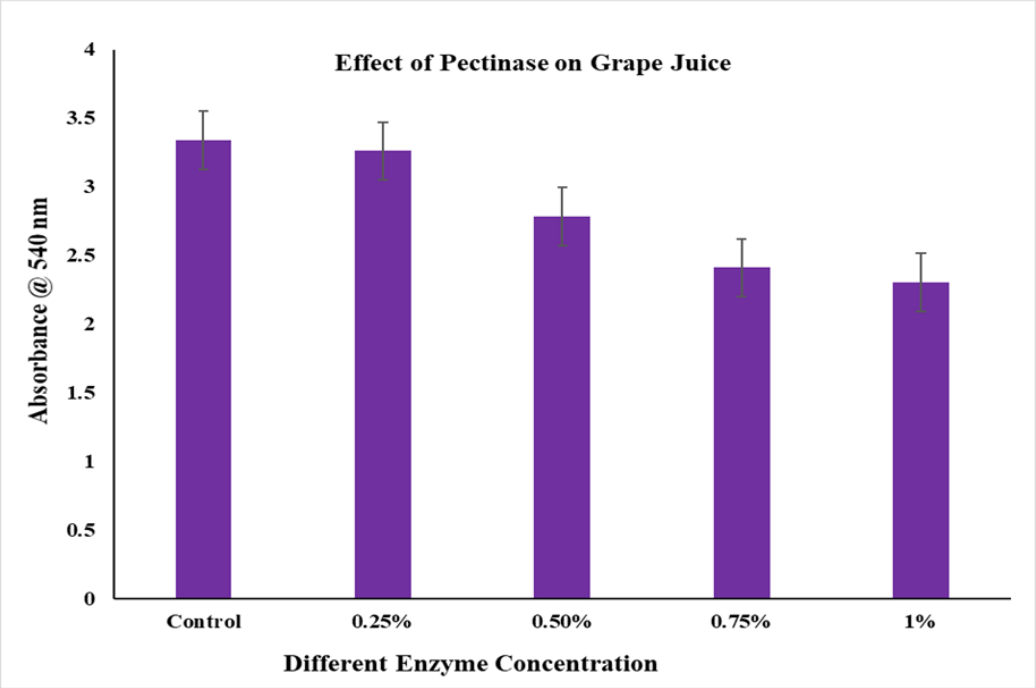

Figure 2. Effect of partially purified pectinase on the clarity of grape juice.

3.3 Pomegranate juice clarification

Pomegranate juice showed a comparatively modest response to enzyme treatment, as shown in Table 3 and Figure 3. The control sample yielded 17 mL of juice from an initial 20 mL, indicating relatively high extractability even without enzyme treatment compared with the other fruit juices. Increasing the enzyme concentration to 1% resulted in the highest juice yield of 19.5 mL, representing a 1.15-fold increase compared with the control. Enzyme treatment reduced absorbance progressively, with the lowest value observed at 1% enzyme concentration, suggesting improved clarification of pomegranate juice (Figure 3). Overall, enzyme treatment enhanced both juice yield and clarity in pomegranate juice, although the magnitude of improvement was lower than that observed for orange and grape juices.

Table 3. Volume of pomegranate juice after enzyme treatment

Sample	Volume obtained from 20 mL	Fold increase
Control	17	–
0.25%	18.5	1.09
0.5%	18.5	1.09
0.75%	18.5	1.09
1%	19.5	1.15

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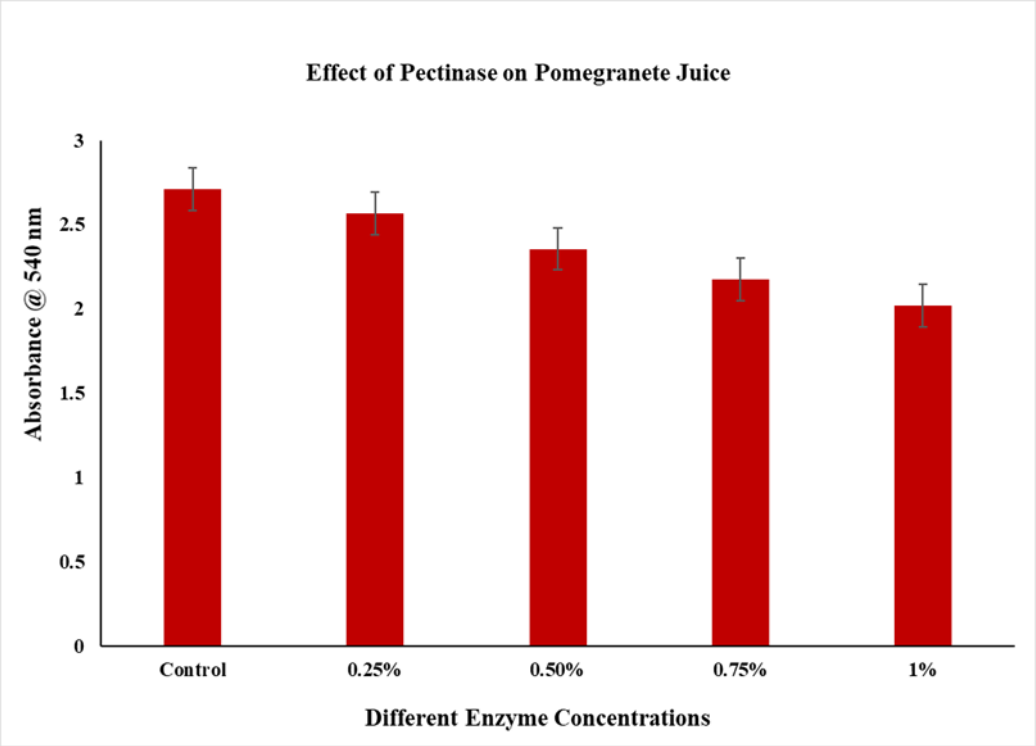

Figure 3. Effect of partially purified pectinase on the clarity of pomegranate juice.

3.4 Effect of standard commercial pectinase on juice clarification

The effect of standard pectinase (ex-*Aspergillus niger*, 3 U/mg powder) on juice clarification was evaluated for orange, grape, and pomegranate juices by measuring absorbance at 540 nm, as shown in Figure 4 and Table 4. In all three fruit juices, enzyme treatment resulted in a clear, concentration-dependent reduction in absorbance compared with the control, indicating enhanced clarification due to enzymatic degradation of pectic substances.

Although all juices responded positively to pectinase treatment, orange and grape juices showed effective clarification at comparatively lower enzyme concentrations, whereas pomegranate juice required higher enzyme levels to achieve similar clarity. The enzyme concentration, quantity of pectinase added, enzyme activity units, and corresponding absorbance values for each juice sample are presented in Table 4. Overall, these results confirm that pectinase from *A. niger* is effective in improving juice clarity across different fruit matrices, with clarification increasing with enzyme concentration and varying according to the intrinsic composition of each fruit juice.

Table 4. Standard pectinase conversion table for enzyme concentration and juice clarity

Juice type	Enzyme (%)	mg/100 mL	mg/20 mL	Units	Concentration	Clarity (Abs 540 nm)
Orange juice	Control	–	–	–	–	0.347
	0.03	30	6	18	0.03	0.227
	0.06	60	12	36	0.06	0.189
	0.09	90	18	54	0.09	0.1075
	0.12	120	24	72	0.12	0.054

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Juice type	Enzyme (%)	mg/100 mL	mg/20 mL	Units	Concentration	Clarity (Abs 540 nm)
Grape juice	Control	–	–	–	–	0.282
	0.04	40	8	24	0.04	0.2075
	0.08	80	16	48	0.08	0.1585
	0.12	120	24	72	0.12	0.1045
	0.16	160	32	96	0.16	0.058
Pomegranate juice	Control	–	–	–	–	0.359
	0.1	100	20	60	0.1	0.265
	0.2	200	40	120	0.2	0.154
	0.3	300	60	180	0.3	0.077
	0.4	400	80	240	0.4	0.038

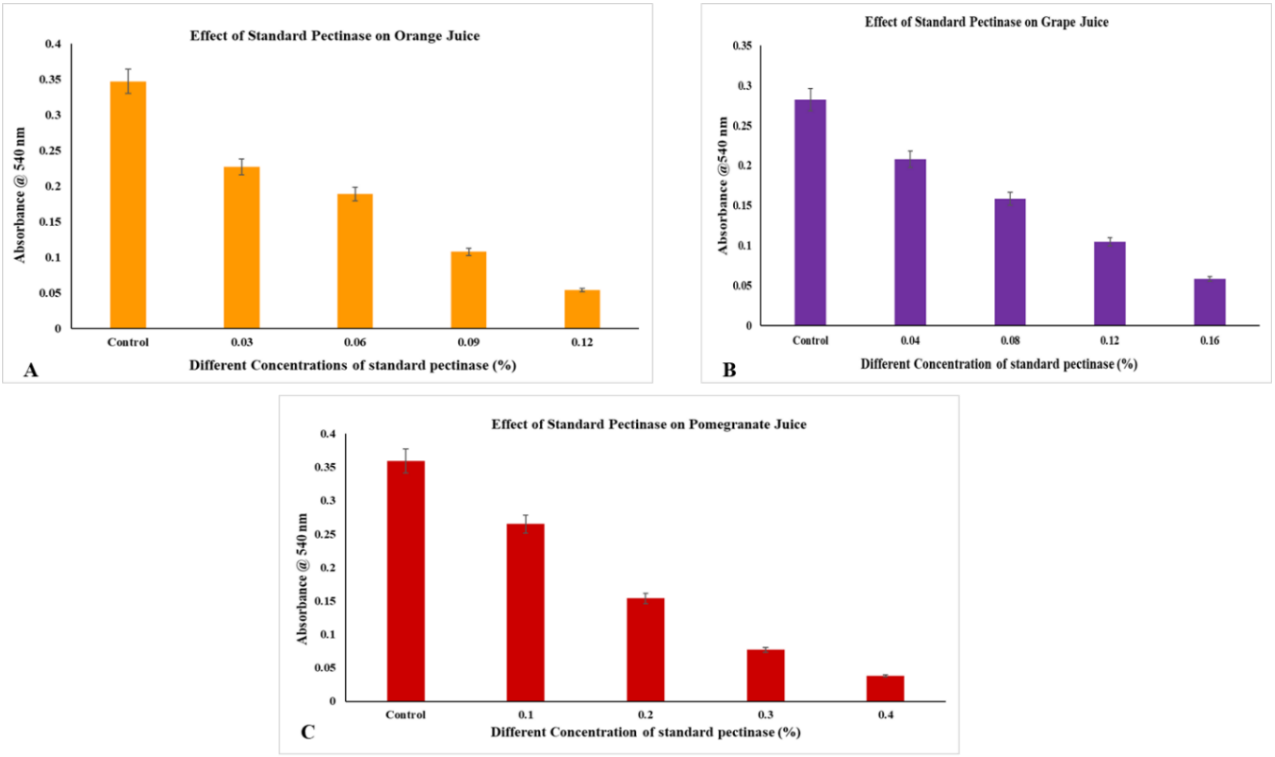

Figure 4. Effect of standard pectinase concentration on the absorbance. Effect of different concentrations of standard *A. niger*-derived pectinase on juice clarification in (A) orange juice, (B) grape juice, and (C) pomegranate juice.

3.5 Comparison of clarification efficiency: pectinase vs standard pectinase

Orange juice

In orange juice, partially purified pectinase derived from *B. mori* showed a non-linear response to enzyme concentration. Absorbance decreased compared with the control, confirming improved clarification; however, the lowest absorbance was observed at 0.5%, indicating maximum clarification at an intermediate enzyme concentration. The slightly higher absorbance at 0.75% and 1% may indicate over-treatment, possibly due to the release of soluble colloids contributing to residual turbidity.

In contrast, standard pectinase derived from *A. niger* produced a consistent concentration-dependent reduction in absorbance, with maximum clarification achieved at 0.12%, a lower enzyme concentration than that required for the partially purified enzyme. Both enzyme preparations clarified orange juice, although the standard pectinase achieved maximum clarification at a lower concentration.

Grape juice

For grape juice, the partially purified enzyme produced a steady decrease in absorbance with increasing enzyme concentration, with the lowest absorbance at 1%, indicating progressive clarification without evidence of over-treatment. Standard pectinase also showed a concentration-dependent decrease in absorbance, achieving maximum clarification at 0.16%. Thus, both enzyme preparations followed a similar clarification trend in grape juice, although the concentration ranges required for maximum clarification differed.

Pomegranate juice

Pomegranate juice treated with partially purified pectinase showed a gradual decrease in absorbance, with maximum clarification observed at 1% enzyme concentration. Standard pectinase produced a marked and continuous reduction in absorbance, with the lowest value at 0.4% enzyme concentration. Although this concentration was higher than that required for orange and grape juices, it was lower than the concentration used for maximum clarification with the partially purified enzyme.

Across all three fruit juices, both partially purified pectinase and standard pectinase effectively enhanced juice clarity by reducing absorbance at 540 nm. Standard enzymes generally show high catalytic efficiency because of refined purification and optimized production processes, whereas partially purified enzymes may retain sufficient activity for industrial use at lower preparation cost (Patidar *et al.*, 2018). The ability of partially purified enzymes to achieve effective clarification at higher concentrations has also been reported in applications where cost efficiency is important (Rebello *et al.*, 2017; Praveen and Suneetha, 2014). Advances in microbial enzyme production and protein engineering have further improved industrial pectinase stability and substrate specificity. These findings highlight the importance of optimizing enzyme source and concentration for each fruit juice.

3.6 Immobilized pectinase activity and application in orange juice clarification

Pectinase was successfully immobilized in calcium alginate beads, with uniform bead formation observed (50 ± 3 beads per batch) and an average wet weight of 60 ± 1.2 mg (n = 3). The immobilized enzyme retained substantial catalytic activity, with activity increasing with enzyme loading. Standard pectinase exhibited activities of 2.24 ± 0.02 U/bead at 5 mg and 4.30 ± 0.02 U/bead at 10 mg, while the test enzyme showed activities of 2.89 ± 0.03 U/bead at 50 mg and 4.93 ± 0.01 U/bead at 100 mg, indicating retention of enzymatic function after entrapment (Table 5).

Application of immobilized pectinase in orange juice clarification demonstrated a reduction in turbidity compared with both control and alginate-only control samples. Absorbance at 540 nm was highest in untreated control juice (approximately 1.0), followed closely by the alginate control, confirming that the matrix alone had no clarifying effect. Enzyme-treated samples showed lower absorbance values, with the lowest value observed for standard pectinase at 10 mg (approximately 0.55), followed by test enzyme at 100 mg (approximately 0.62), standard enzyme at 5 mg (approximately 0.85), and test enzyme at 50 mg (approximately 0.82). These results indicate that immobilized pectinase retained clarification ability, with higher enzyme loading resulting in improved performance (Figure 5).

The retention of activity may be attributed to mild calcium alginate gelation conditions that preserve enzyme conformation, while the porous bead structure permits substrate diffusion and limits enzyme leakage. Similar immobilization systems have been reported to improve enzyme stability and reusability in industrial applications (Bogra *et al.*, 2013). Immobilized pectinases

can retain substantial activity over multiple cycles and exhibit improved operational stability compared with free enzymes (Mohammadi *et al.*, 2020). Polymer-based immobilization systems may also improve storage stability and reuse efficiency in juice processing (Soozanipour *et al.*, 2019). The activity values obtained in this study are consistent with reports that immobilized enzymes retain catalytic efficiency while providing advantages such as easy separation and reuse (Oliveira *et al.*, 2025).

Immobilized pectinase has been reported to improve clarification of pineapple and barberry juices by enhancing clarity, reducing viscosity, and improving filtration efficiency (Hosseini *et al.*, 2021).

Table 5. Pectinase activity of embedded enzymes

Sample	Activity (U/bead)
std-5 mg	2.24 ± 0.02
std-10 mg	4.30 ± 0.02
T-50 mg	2.89 ± 0.03
T-100 mg	4.93 ± 0.01

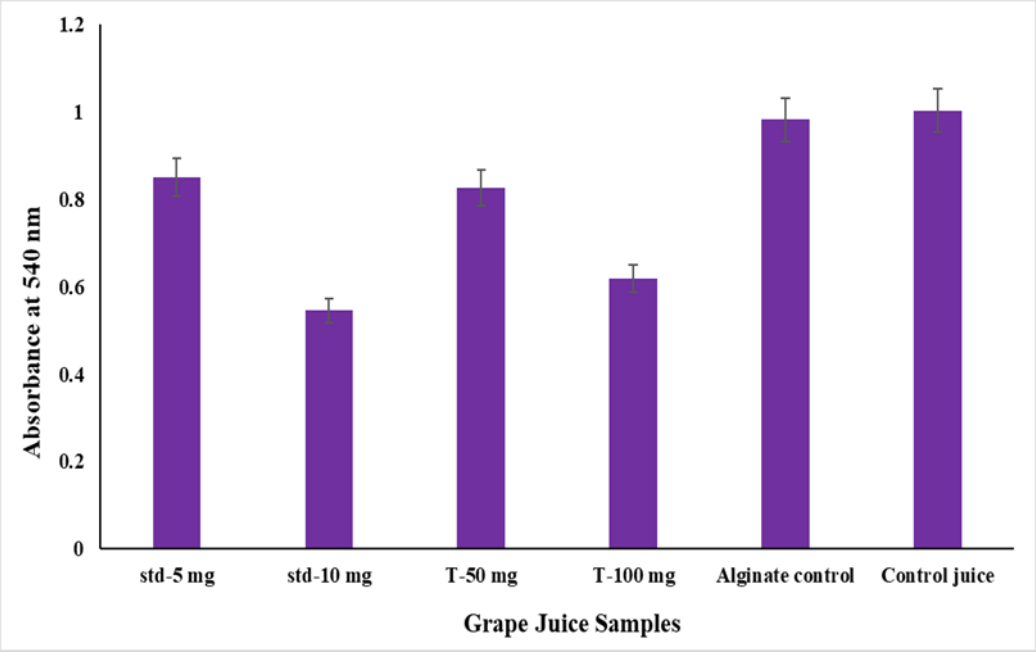

Figure 5. Effect of enzyme-embedded alginate beads on orange juice clarification.

3.7 Effect of yeast and pectinase on colour intensity

The present study showed that baker's yeast and pectinase treatment improved the colour intensity of the prepared wine samples. Colour intensity was calculated as the sum of absorbance values at 420 nm, 520 nm, and 620 nm. The control sample, without enzyme treatment, recorded a colour intensity of 3.424. Treatment with baker's yeast alone (Group 1) slightly increased the colour intensity to 3.469, possibly due to fermentation-related changes that promoted pigment extraction and stabilization. Yeast-associated pectinolytic activity has also been reported to influence compound extraction and wine characteristics.

The addition of pectinase along with baker's yeast further increased colour intensity. Group 2, treated with baker's yeast and 100 mg/kg pectinase, showed a value of 3.4705, whereas Group 3, treated with baker's yeast and 200 mg/kg pectinase, showed

the highest colour intensity of 3.51 (Figure 7). The gradual increase with increasing pectinase concentration suggests improved extraction of colour compounds from grape tissues.

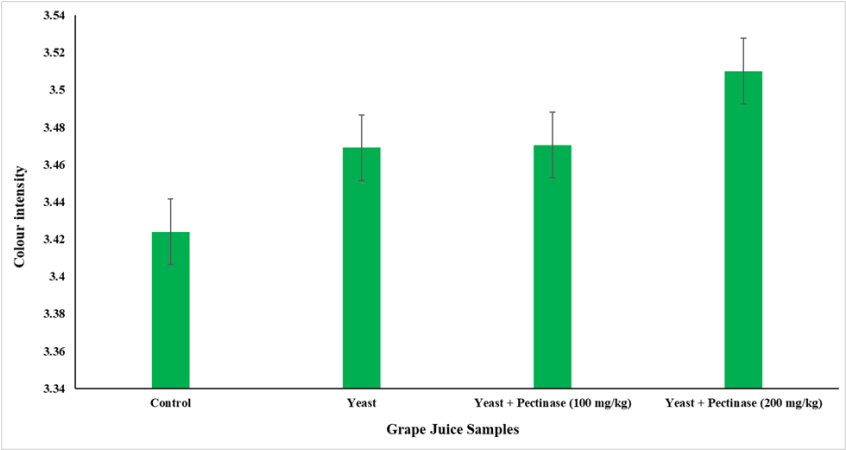

Figure 7. Effect of baker's yeast and pectinase on colour intensity of juice samples, expressed as total colour intensity calculated from the sum of absorbance values at 420 nm, 520 nm, and 620 nm (A420 + A520 + A620).

Pectinase breaks down pectin and other cell-wall polysaccharides, facilitating the release of anthocyanins, tannins, and other phenolic compounds responsible for wine colour. Zhang *et al.* (2024) reported that pectinase-assisted cell-wall hydrolysis enhanced anthocyanin and pigment extraction during wine processing. Similarly, Bichescu *et al.* (2012) reported improved colour intensity and hue values in pectinase-treated wines due to enhanced extraction of anthocyanins and polyphenols.

The present findings are also supported by studies in other fruit beverages. Pectinase treatment improved anthocyanin retention and clarity in red dragon fruit beverages (Pham *et al.*, 2024), enhanced red colour while reducing browning in chokeberry juice (Lachowicz *et al.*, 2018), and reduced turbidity and haze formation in pomegranate juice without affecting anthocyanin composition.

Overall, the combined use of baker's yeast and pectinase improved colour development in the prepared wine. The highest colour intensity observed in Group 3 indicates that 200 mg/kg pectinase was more effective than 100 mg/kg under the present conditions. These results agree with reports that pectinase and yeast treatment can improve colour expression, clarification, and overall quality of fruit-based beverages (Nighojkar *et al.*, 2019; Chinelate *et al.*, 2025). (figure 6)

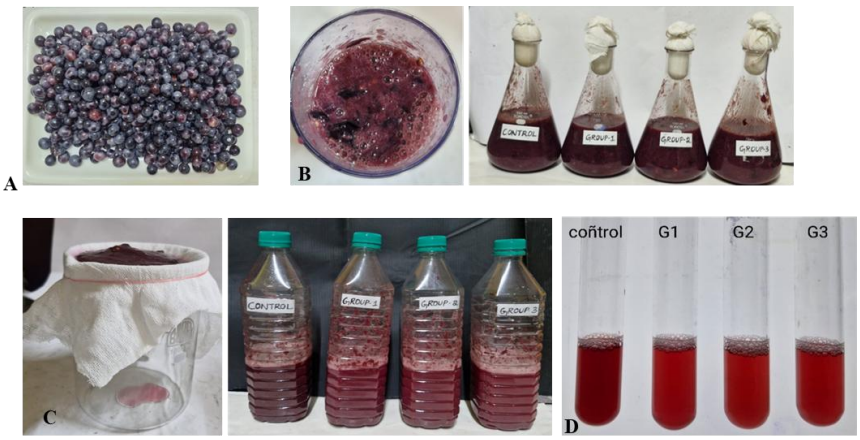

Figure 6. Effect of yeast and pectinase treatment on grape wine fermentation and colour development. (A) Fresh grapes used for wine preparation. (B) Maceration of grapes and transfer into fermentation flasks. (C) Removal of pomace after fermentation and bottling of wine. (D) Collection of clarified supernatants for analysis of colour intensity. Treatments included: Control (no additives), G1 (baker's yeast 200 g/kg), G2 (baker's yeast 200 g/kg + pectinase 100 mg/kg), and G3 (baker's yeast 200 g/kg + pectinase 200 mg/kg).

3.8 Effect of yeast and pectinase on percentage colour composition

The percentage distribution of Yellow (Y%), Red (R%), and Blue (B%) colour components, calculated from absorbance values at A420, A520, and A620, respectively, is presented in Figure 8. The control sample showed a higher yellow component (44.79%), followed by red (38.57%) and blue (16.65%), representing the natural colour composition of the untreated juice. Treatment with baker's yeast alone (Group 1) reduced the yellow and blue components to 42.43% and 14.13%, respectively, while increasing the red component to 43.44%. This suggests that yeast treatment may promote the release or stabilization of red pigments.

The addition of pectinase along with baker's yeast caused a progressive increase in red colour contribution. Group 2, treated with 100 mg/kg pectinase, showed red, yellow, and blue values of 49.83%, 37.21%, and 12.95%, respectively. Group 3, treated with 200 mg/kg pectinase, showed the highest red contribution (53.90%), with reduced yellow (36.58%) and blue (9.52%) components. The increased red colour percentage at higher pectinase concentration indicates improved extraction and solubilization of red pigments, likely anthocyanins, through degradation of pectic substances and cell-wall polysaccharides.

Pectinase is commonly used in fruit juice processing to improve clarification, pigment extraction, and colour stability. In red dragon fruit beverages, pectinase improved juice clarity and anthocyanin retention, thereby helping to maintain red colour characteristics (Pham *et al.*, 2024). Similarly, pectinolytic enzyme treatment produced an intense and stable red colour in chokeberry juice without browning (Lachowicz *et al.*, 2018). In blackberry wine, pectinase enhanced the extraction of colour substances, likely due to increased release of anthocyanin pigments (Zobundžija, 2016). However, enzyme treatment effects may vary depending on fruit type, pectinase concentration, and treatment duration. In raspberry juice, pectinase treatment reduced total anthocyanin content under certain conditions, indicating possible pigment degradation during excessive enzymatic treatment (Jiang *et al.*, 2007). In contrast, the present study showed a continuous increase in red colour percentage from 38.57% in the control to 53.90% in Group 3, indicating that the selected pectinase concentrations were favourable for colour enhancement.

Overall, baker's yeast combined with increasing pectinase concentration shifted the juice colour profile from yellow and blue towards red dominance. The baker's yeast and 200 mg/kg pectinase treatment produced the highest red colour contribution and may therefore be considered the most effective treatment for improving colour expression and visual quality of the fruit juice.

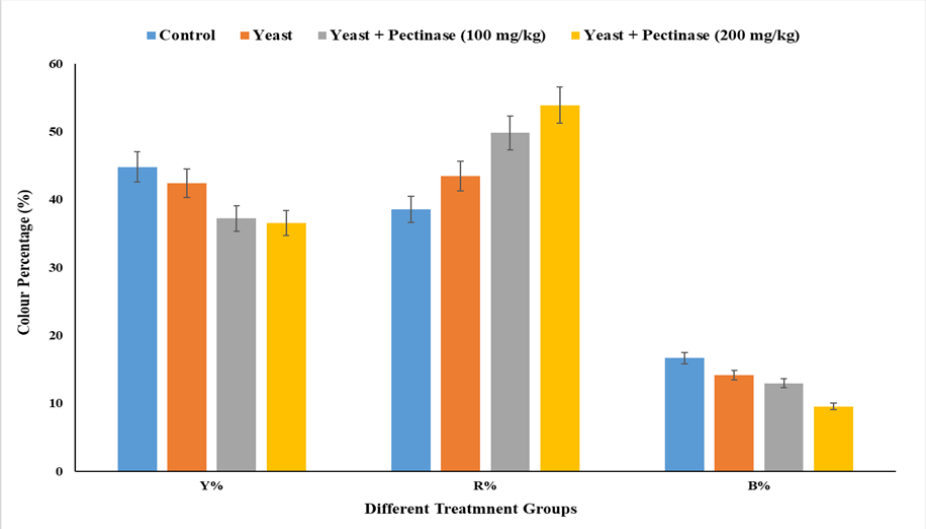

Figure 8. Percentage distribution of colour components in grape juice samples (based on absorbance at 420 nm, 520 nm, and 620 nm; Y% = Yellow, R% = Red, B% = Blue).

3.9 Effect of yeast and pectinase on hue (A420/A520)

Hue, expressed as the A420/A520 ratio, was used to evaluate the balance between yellow–brown and red pigments in the samples. The control showed the highest hue value (1.16), indicating a greater contribution of yellow–brown pigments relative

to red pigments. Treatment with baker's yeast alone reduced the hue value to 0.98, suggesting an increase in red colour expression or a reduction in yellow–brown pigment contribution.

A further reduction in hue was observed when pectinase was combined with baker's yeast. Group 2, treated with 100 mg/kg pectinase, showed a hue value of 0.75, while Group 3 treated with 200 mg/kg pectinase recorded the lowest value of 0.68. The lower hue value in Group 3 indicates a shift towards red-dominant colouration, which agrees with the higher red percentage and colour intensity observed in this treatment (Figure 9).

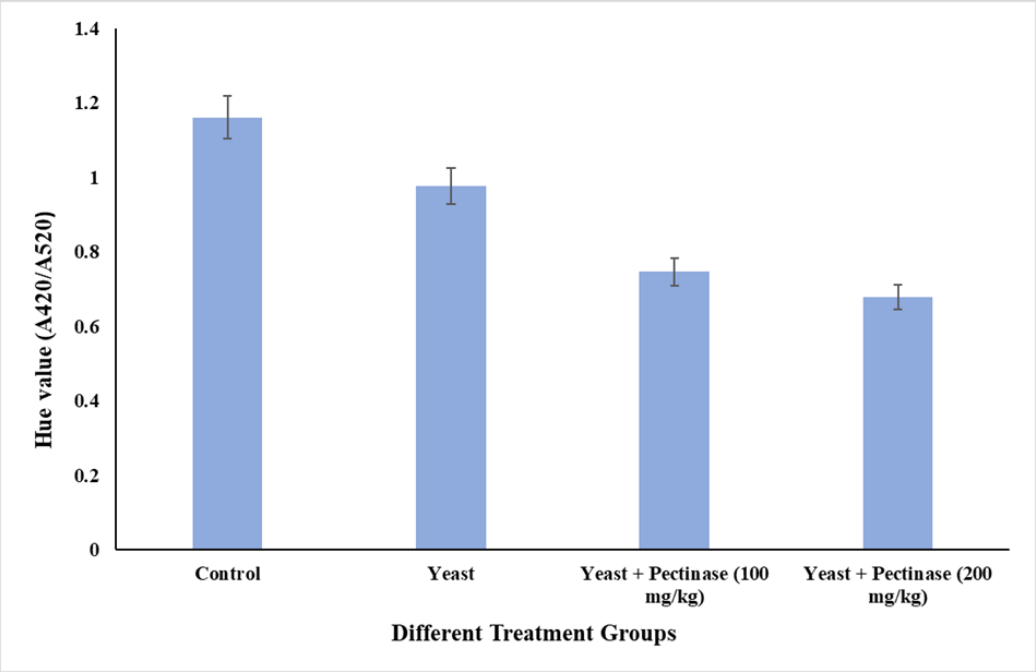

Figure 9. Effect of yeast and pectinase treatment on hue value (A420/A520) of wine samples.

Pectinase degrades pectic substances in fruit cell walls, facilitating the release of anthocyanins and other polyphenolic pigments responsible for red colouration (Nighojkar *et al.*, 2019). In grape juice and wine processing, pectinase treatment has been reported to improve colour quality, clarity, and dark-red colour expression, which is generally associated with lower A420/A520 values. Similarly, pectinolytic enzymes produced an intense and stable red colour in chokeberry juice without browning during storage (Lachowicz *et al.*, 2018).

Overall, the progressive decrease in hue from 1.16 in the control to 0.68 in Group 3 confirms that increasing pectinase concentration in the presence of baker's yeast shifted the colour profile towards a more intense red hue. This enzyme-assisted improvement in colour is important for producing visually appealing fruit juices and wines with enhanced sensory quality (Charumathi and Suchitra, 2024).

3.10 Total phenol content

The total phenolic content showed a clear reduction in all treated groups compared with the control (Figure 10). The control sample, without enzyme treatment, recorded the highest TP content of 5.1076 mg/mL. Treatment with baker's yeast alone (Group 1) reduced the TP content to 3.6076 mg/mL. Similarly, Group 2, treated with baker's yeast and 100 mg/kg pectinase, recorded 3.6250 mg/mL, while Group 3, containing baker's yeast and 200 mg/kg pectinase, showed the lowest TP content of 3.5320 mg/mL. Overall, the treatments resulted in an approximately 29–31% reduction in measurable total phenolic content compared with the control.

The reduction in phenolic content may be related to interactions between phenolic compounds and yeast cells, oxidation during treatment, or adsorption of phenolics onto yeast cell walls. Although pectinase can promote the release of bound phenolic compounds by degrading pectic substances, its effect depends strongly on fruit type, enzyme concentration, processing duration, and juice composition. In the present study, increasing pectinase concentration from 100 to 200 mg/kg did not

increase phenolic release, indicating that the applied treatment conditions did not favour the retention or extraction of measurable phenolic compounds.

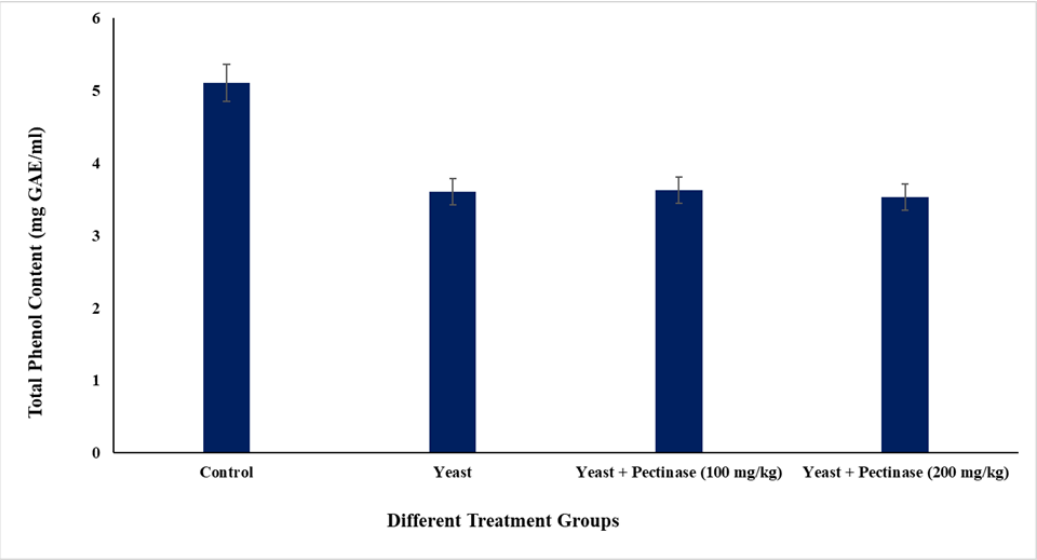

Figure 10. Total phenolic content of prepared wine samples under different treatments.

4. CONCLUSION

The present study demonstrates that pectinase derived from *Bombyx mori L.* gut microbiota is an effective biocatalyst for improving fruit juice clarification, enhancing juice yield, and modifying quality attributes in orange, grape, and pomegranate juices. Enzyme treatment resulted in a clear, concentration-dependent improvement in juice extraction efficiency, with maximum yields of 21.5 mL (orange), 17.5 mL (grape), and 19.5 mL (pomegranate) compared with their respective controls. Concurrently, turbidity was significantly reduced, confirming effective hydrolysis of pectic substances responsible for juice cloudiness.

Immobilization of pectinase in calcium alginate beads successfully retained enzymatic activity and demonstrated effective clarification of orange juice, with performance improving with increasing enzyme loading. Although immobilized systems showed slightly lower efficiency than free commercial enzyme, they offer advantages in stability, reusability, and process control, supporting their applicability in continuous processing systems. In wine fermentation, pectinase treatment enhanced colour intensity, improved pigment distribution, and reduced hue values, indicating enhanced extraction of anthocyanins and improved visual quality. However, a reduction in total phenolic content was observed in yeast-treated systems, suggesting phenolic transformation, binding, or degradation during fermentation rather than simple release.

Overall, the findings confirm that pectinase significantly improves juice processing efficiency and product quality, with performance strongly influenced by enzyme source, concentration, and immobilization strategy. The study highlights the potential of microbiota-derived pectinase as a sustainable and economically viable alternative for industrial juice clarification and related food processing applications. Future studies should focus on full-scale purification of the *Bombyx mori*-derived pectinase, its stability under industrial processing conditions, and pilot-scale validation of the immobilized enzyme system.

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AUTHOR CONTRIBUTIONS

Kishore Kumar Basavesha: methodology, investigation, writing—original draft.

Seema Jayananda Patel: conceptualization, supervision.

Koushik Bandlu Raghuram: formal analysis.

Gajendrakumar Pujar: methodology.

Ganesh G Tilve: review of the draft.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

Ethics approval: Not required, as the study did not involve human or animal subjects.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

REFERENCES

[1] Anand, G., Yadav, S., Gupta, R., Yadav, D., 2020. Pectinases: from microbes to industries, in: Chowdhary, P., Raj, A., Verma, D., Akhter, Y. (Eds.), Microorganisms for Sustainable Environment and Health. Elsevier, Amsterdam, pp. 287–313. https://doi.org/10.1016/B978-0-12-819001-2.00014-0

[2] Ashkan, Z., Zahirinejad, S., Hemmati, R., Dinari, A., 2023. Alginate as support material in enzyme immobilization, in: Alginate Biomaterial. Springer, pp. 327–360.

[3] Attique, S.A., ul Ain, Q., Hussain, N., Bilal, M., Iqbal, H.M.N., 2023. Enzyme immobilization approaches, in: Biocatalyst Immobilization: Foundations and Applications. Elsevier, pp. 37–54.

[4] Banerjee, S., Maiti, T.K., Roy, R.N., 2022. Enzyme producing insect gut microbes: an unexplored biotechnological aspect. Crit. Rev. Biotechnol. 42(3), 384–402. https://doi.org/10.1080/07388551.2021.1942777

[5] Bélafi-Bakó, K., Nemestóthy, N., 2017. The role of biocatalysis and membrane techniques in processing high-pectin content food stuffs and wastes. Academic Press, pp. 277–292. https://doi.org/10.1016/B978-0-12-811372-1.00009-9

[6] Bichescu, C., Bahrim, G., Stănciuc, N., Râpeanu, G., 2012. Colour enhancement of Fetească neagră wines by using pectolytic enzymes during maceration. Ann. Univ. Dunărea de Jos Galaţi Fasc. VI Food Technol. 36(1), 18–25.

[7] Bogra, P., Kumar, A., Kuhar, K., Panwar, S., Singh, R., 2013. Immobilization of tomato (Lycopersicon esculentum) pectinmethylesterase in calcium alginate beads and its application in fruit juice clarification. Biotechnol. Lett. 35(11), 1895–1900. https://doi.org/10.1007/s10529-013-1278-3

[8] Charumathi, G., Suchitra, M.R., 2024. Nutritional and biological applications of pectinases: a comprehensive overview. SBV J. Basic Clin. Appl. Health Sci. https://doi.org/10.4103/sbvj.sbvj_1_24

[9] Chinelate, G.C.B., da Silva, E.B., de Almeida Araújo, L., Albuquerque, K.G.S., 2025. Biotechnology: industry, studies, and applications. Troppo – Editora & Eventos & Treinamentos. https://doi.org/10.29327/5586690

[10] Dhanjal, D.S., Chopra, R.S., Chopra, C., 2020. Metagenomics and enzymes: the novelty perspective. Springer, Singapore, pp. 109–131. https://doi.org/10.1007/978-981-15-6529-8_7

[11] Dreher, M., 2018. Whole fruits and fruit fiber emerging health effects. Nutrients 10(12), 1833. https://doi.org/10.3390/nu10121833

[12] Egwim, E.C., Ogudoro, A.C., Folashade, G., 2013. The effect of pectinase on the yield and organoleptic evaluation of juice and wine from banana and paw-paw. Ann. Food Sci. Technol. 14(2), 206–214.

[13] Guo, K., 2024. Changes in the main physicochemical properties and electrochemical fingerprints in the production of sea buckthorn juice by pectinase treatment. Molecules 29. https://doi.org/10.3390/molecules29051035

[14] Gupta, D.M.G., Modi, G., 2023. Polymer-based strategies for enzyme immobilization: a comprehensive review. Tuijin Jishu/J. Propuls. Technol. 44, 792–802.

[15] Hmar, B.Z., Mishra, S., Pradhan, R.C., 2017. Design, fabrication, and testing of a pulper for Kendu (Diospyros melanoxylon Roxb.). J. Food Process Eng. 41(1). https://doi.org/10.1111/jfpe.12642

[16] Hosseini, S.S., Khodaiyan, F., Mousavi, S.M., Azimi, S.Z., 2021. Continuous clarification of barberry juice with pectinase immobilised by oxidized polysaccharides. Food Technol. Biotechnol. 59(2), 174–184. https://doi.org/10.17113/ftb.59.02.21.6976

[17] Jiang, J., Paterson, A., Piggott, J.R., 2007. Effects of pectolytic enzyme treatments on anthocyanins in raspberry juice. Int. J. Food Sci. Technol. 25(5), 596–600. https://doi.org/10.1111/j.1365-2621.1990.tb01120.x
[18] Kashyap, D.R., Vohra, P.K., Chopra, S., Tewari, R., 2001. Applications of pectinases in the commercial sector: a review. Bioresour. Technol. 77(3), 215–227.
[19] Kishore Kumar, B., Patel, S.J., Kumar, G.P., 2026. Isolation and characterization of pectinase-producing yeast from the gut microbiota of silkworms (*Bombyx mori* L). J. Appl. Nat. Sci. 18(2), 979–984. https://doi.org/10.31018/jans.v18i2.7546
[20] Lachowicz, S., Oszmiański, J., Kolniak-Ostek, J., 2018. Influence of different pectinolytic enzymes on bioactive compound content, antioxidant potency, colour and turbidity of chokeberry juice. Eur. Food Res. Technol. 244(11), 1907–1920. https://doi.org/10.1007/s00217-018-3103-7
[21] Mantovani, C.F., Geimba, M.P., Brandelli, A., 2005. Enzymatic clarification of fruit juices by fungal pectin lyase. Food Biotechnol. 19(3), 173–181. https://doi.org/10.1080/08905430500316284
[22] Mohammadi, M., Mohammadi, M., Rezaei Mokarram, R., Shahvalizadeh, R., Sarabandi, K., Lim, L-T., Hamishehkar, H., 2020. Immobilization and stabilization of pectinase on an activated montmorillonite support and its application in pineapple juice clarification. Food Biosci. 36, 100625. https://doi.org/10.1016/j.fbio.2020.100625
[23] Nachal, N., Pegu, K., Arya, S.S., 2023. Enhancement of physicochemical stability and reduction in enzyme and microbial activity of apple juice by hydrodynamic cavitation processing. J. Agric. Food Res. 100797. https://doi.org/10.1016/j.jafr.2023.100797
[24] Nighojkar, A., Patidar, M.K., Nighojkar, S., 2019. Pectinases: production and applications for fruit juice beverages. Woodhead Publishing, pp. 235–273. https://doi.org/10.1016/B978-0-12-815259-1.00008-2
[25] Nisha, M.K., 2016. Efficacy of purified pectinase obtained from Paecilomyces variotii in extraction and clarification of juice from grapes and pomegranate fruits. Int. J. Pharma Bio Sci. 7. https://doi.org/10.22376/ijpbs.2016.7.4.b479-484
[26] Oliveira, S.C., Araújo, S.M., Dencheva, N.V., Denchev, Z.Z., 2025. Polyamide microparticles with immobilized enological pectinase as efficient biocatalysts for wine clarification: the role of the polymer support. Molecules 30(1), 114. https://doi.org/10.3390/molecules30010114
[27] Panda, G., Vivek, K., Mishra, S., Pradhan, R.C., 2021. Characterization and optimization of process parameters for enzyme assisted extraction of Kendu (Diospyros melanoxylon Roxb.) fruit juice. Int. J. Fruit Sci. 21(1), 299–311. https://doi.org/10.1080/15538362.2021.1873220
[28] Pandey, C., Sharma, P., Gupta, N., 2022. Engineering to enhance thermostability of xylanase: for the new era of biotechnology. J. Appl. Biol. Biotechnol. 11(2), 1–14. https://doi.org/10.7324/JABB.2023.110204
[29] Patel, V.B., Chatterjee, S., Dhoble, A.S., 2022. A review on pectinase properties, application in juice clarification, and membranes as immobilization support. J. Food Sci. 87(8), 3338–3354. https://doi.org/10.1111/1750-3841.16233
[30] Patidar, M.K., Nighojkar, S., Kumar, A., Nighojkar, A., 2018. Pectinolytic enzymes-solid state fermentation, assay methods and applications in fruit juice industries: a review. 8(4), 199. https://doi.org/10.1007/s13205-018-1220-4
[31] Pham, B.A., Vu, N.D., Phan, P.H., Huynh, B.L., Long, T.B., Pham, V.T., 2024. Pectinase-driven optimization of pectin hydrolysis for enhanced clarity, anthocyanin retention, and consumer appeal in red dragon fruit mint flavored beverage. J. Food Process Preserv. 2024(1). https://doi.org/10.1155/jfpp/9273464
[32] Pinelo, M., Zeuner, B., Meyer, A.S., 2010. Juice clarification by protease and pectinase treatments indicates new roles of pectin and protein in cherry juice turbidity. Food Bioprod. Process. 88(2–3), 259–265.
[33] Praveen, K.G., Suneetha, V., 2014. Natural culinary fruit peels as a potential substrate for pectinolytic enzyme. Int. J. Drug Dev. Res. 6(3), 109–118.
[34] Rebello, S., Anju, M., Aneesh, E.M., Sindhu, R., Binod, P., Pandey, A., 2017. Recent advancements in the production and application of microbial pectinases: an overview. Rev. Environ. Sci. Biotechnol. 16(3), 381–394. https://doi.org/10.1007/s11157-017-9437-y
[35] Rehman, H., Baloch, A.H., Nawaz, M.A., 2021. Pectinase: immobilization and applications. A review. Trends Pept. Protein Sci. 6, 1–16 (e1). https://doi.org/10.22037/tpps.v6i.33871
[36] Satapathy, S., Rout, J.R., Kerry, R.G., Thatoi, H., Sahoo, S.L., *et al.*, 2020. Biochemical prospects of various microbial pectinase and pectin: an approachable concept in pharmaceutical bioprocessing. Front. Nutr. 7, 117.
[37] Sharma, K., 2024. Silkworm gut microflora as a source: biotechnological applications. Asian J. Biol. 20(9), 30–46. https://doi.org/10.9734/ajob/2024/v20i9436

[38] Shrestha, S., Rahman, M.S., Rahman, M.S., Qin, W., 2021. New insights in pectinase production development and industrial applications. Appl. Microbiol. Biotechnol. 105, 9069–9087. https://doi.org/10.1007/s00253-021-11705-0

[39] Sikodia, N., Battan, B., Chahal, S., Sharma, J., 2024. Efficient extraction and clarification of fruit juices using concurrently produced xylano-pectinolytic enzymes. J. Microbiol. Biotechnol. Food Sci. 13(5), e10260. https://doi.org/10.55251/jmbfs.10260

[40] Singh, B., Mal, G., Gautam, S., Mukesh, M., 2019. Insect gut—a treasure of microbes and microbial enzymes. Springer, Cham, pp. 51–58. https://doi.org/10.1007/978-3-030-21309-1_5

[41] Soozanipour, A., Taheri-Kafrani, A., Barkhori, M., Nasrollahzadeh, M., 2019. Preparation of a stable and robust nanobiocatalyst by efficiently immobilizing of pectinase onto cyanuric chloride-functionalized chitosan grafted magnetic nanoparticles. 536, 261–270. https://doi.org/10.1016/j.jcis.2018.10.053

[42] Zhang, C., Liu, H., Zhang, B., *et al.*, 2024. Effect of different pectinase treatments on the quality of Hutai No. 8 wine. China Brew. 43(5), 68–74. https://doi.org/10.11882/j.issn.0254-5071.2024.05.011

[43] Zhang, C., Wang, Y., Lv, Y., Yang, X., Wei, X.Y., 2023. Influence of pectin domains and protein on the viscosity and gelation properties of alkali-extracted pectin from green tea residue. Food Chem. 430, 137039. https://doi.org/10.1016/j.foodchem.2023.137039

[44] Zobundžija, D., 2016. Effect of additives on the colour and aroma compounds of blackberry wines. Master's tab thesis, Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek.