FABRICATION OF IMMOBILIZED MULTI-ENZYME SYSTEM IN GLUTARALDEHYDE ACTIVATED GELATIN BLOCKS TARGETING THE QUALITY AND YIELD OF APPLE JUICE

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ABSTRACT

Driven by rising consumer demand for nutritionally beneficial and high-quality fruit juices, the present study employed enzyme technology to improve the yield and quality of apple juice. Pectinase and Cellulase were produced from locally isolated *Bacillus* sp. AA-04 and *Bacillus* sp. SM-07 and partially purified using salt fractionation technique. Maximum activity of Pectinase was found at 5 minutes, 50 °C, and pH 7.5. Cellulase required 10 minutes, 55 °C, and pH 8.0 for its optimal activity. The V_{max} and K_m were 497 U/mL and 0.115 mg/mL for Pectinase, whereas, Cellulase exhibited V_{max} 370.4 U/mL and Km 0.1177 mg/mL. Apple pulp pretreated with soluble Pectinase and Cellulase at 40 °C for 45 minutes resulted in 57.89 % increase in juice yield. Multi-enzyme immobilized gel blocks fabricated from 30 % gelatin (w/v) and 20% (v/v) glutaraldehyde were used for juice clarification. The reduction in the viscosity and turbidity of apple juice were observed by 35% and 55% respectively as compared to raw juice, at 50 °C after 45 minutes of treatment. The multi-enzyme gel blocks were reused for 6 operational cycles of juice clarification.

Keywords: Pectinase; Cellulase; juice; clarification; gelatin; immobilization.

1. Introduction

Beverage industry constitutes the largest contribution in industrial zone of Pakistan. According to the market report published on Juice processing enzymes market size, share and growth by Business Research Insights in 2025, the global juices processing enzymes market is projected to expand at a CAGR of 6.18% over 2026–2035, with an estimated size of USD 0.73 billion in 2026 and expected growth to USD 1.23 billion by 2035 (Business Research Insights, 2025). Rise in preferences of healthy ingredients will steer the juice processing industry and increase in consumption of processed juices will propel the market growth for juice processing enzymes including, pectinase, cellulase, amylase and protease. But juice filtration from the fruits and its stable clarification remains a considerable task for juice producing industries in order to improve the quality and yield of juices in a cost effective manner. The juices consist of soluble sugars, organic acids, pigments, polysaccharides, along with small amount of proteins (Lozano, 2006). The presence of colloidal polysaccharides like pectin, cellulose, lignin, and starch, along with proteins, significantly impacts the final juice quality, causing undesirable haze and leading to the thickening of juice during storage (Kumar, 2015). In the fruit juice industry, traditional methods of clarifying juices involve mechanical or thermal processes (Anuradha *et al.*, 2016). However, due to the intricate structure of fruit polysaccharides, extracting juice from this highly viscous jellified pulp becomes challenging, resulting in decreased juice yield. For this purpose, an alternative solution for optimal extraction of fruit juice and its clarification is proposed in the present work; that is an enzymatic approach. The enzymatic treatment of fruit pulp plays a very important role in the removal of undesirable turbidity as it removes the insoluble polymers. Pectin and cellulose are major structural polysaccharides that can be hydrolyzed by pectinase and cellulase, respectively. Their enzymatic degradation reduces the structural complexity of fruit pulp, thereby facilitating juice filtration and contributing to an overall improvement in juice yield (Chauhan *et al.*, 2015; Jayasekara and Renuka, 2019). Pectinase refers to a mixture of enzymes including pectolyase, pectinmethylesterase and polygalacturonase. It attacks on pectin which is a structural polysaccharide with a backbone of galacturonic acid residues linked by α-(1-4) linkages, present in fruits and vegetables (Haile *et al.*, 2022). Cellulase enzymes are mainly produced by bacteria and fungi, and they efficiently

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break down cellulose into glucose units through the coordinated actions of different enzymes, including endo-β-1,4 glucanase, cellobiohydrolase, and β-D-glucosidase (Islam and Roy, 2008).

Among variety of different industrial applications, pectinase and Cellulase find great commercial utilization in food and beverage industry that constitutes largest contribution in industrial zone of Pakistan (Anand *et al*., 2020). Despite the appreciable catalytic property of enzymes, their utilization in free form presents some problems including low operational stability, high production or import cost and difficult product recovery. Immobilization technology introduce new insights into the commercial viability of enzymatic methods (Scheibel *et al*., 2024). Among different methods of immobilization, enclosing enzyme within hydrogels provides better operational stability and reusability of enzymes in easiest manner (Costa *et al*., 2023). Enzyme hydrogels typically consist of a hydrophilic polymer matrix, such as gelatin, alginate, chitosan, or polyethylene glycol (PEG), which provides a biocompatible and water-swollen scaffold. Enzymes are immobilized within the matrix through physical entrapment or chemical crosslinking, allowing them to remain active and functional within the hydrogel (Haima *et al*., 2021; Suzuki *et al*., 2022). Therefore, the present study was designed to isolate and characterize potential pectinase- and cellulase-producing *Bacillus* species from rotten fruit peels. The research further aimed to evaluate the synergistic application of these enzymes through co-immobilization to enhance the efficiency of the conventional juice filtration and clarification process. By integrating pectinase and cellulase activities, the study seeks to improve juice yield, clarity, and processing efficiency, thereby offering a cost-effective and industrially viable enzymatic approach for fruit juice processing.

2. MATERIALS AND METHODS

The strains *Bacillus* sp. AA-04 (Pectinase) and *Bacillus* sp. SM-07 (Cellulase) were isolated and identified in research laboratory of Biochemistry department, Jinnah University for Women, Karachi, Pakistan. Citrus Pectin, Carboxymethyl cellulose, $(NH_4)_2SO_4$ and other fermentation medium components were purchased from Bio-Lab. Glutaraldehyde and gelatin were purchased from Merck (Germany) for preparation of hydrogel blocks.

An automatic autoclave machine (China), High speed refrigerated centrifuge (Hettich, Germany), Spectrophotometer (BMS 1100, Canada), Incubators (Memmert, Germany), Ostwald's Viscometer and turbidimeter (HI 93703, Romania) were also used in the study.

2.1 Isolation of Pectinase and Cellulase producing Bacteria

In order to isolate Pectinase and Cellulase producing bacteria, mixed fruit peels were buried in the rhizosphere area in Jinnah University garden for one week. After one week, soil was collected from the site where the peel waste was dumped and placed in sterilized falcon tubes. 1 g of soil sample was then weighed and added in 100 mL sterilized deionized water taken in Erlenmeyer flask. Then the flask was incubated at 37 ^{0}C for five days. After incubation, 1 mL of soil suspension was taken in 9 mL sterilized deionized water and serially diluted. The dilutions 10^{-3}, 10^{-4} and 10^{-5} were poured on agar plates containing carboxy-methyl cellulose (CMC) and pectin separately. The plates were then incubated at 37 ˚C for 24 hours. After incubation, mixed cultures were obtained on the plates.

For the selection of the strain capable of degrading pectin and carboxy-methyl cellulose, colonies were picked from the respective plates and streaked on pectin-agar plates and CMC-agar plates respectively. These plates were incubated at 37 ˚C for 24 h. After incubation, plates were flooded with iodine solution to observe the zone of clearance. The strains which showed the biggest hydrolytic zone as compared to others were selected. The selected strains were repeatedly streaked on a nutrient-agar plate and then incubated at 37 ˚C for 24 h. Pure cultures were preserved at 4˚C.

2.2 Production of Pectinase and Cellulase

The culture media for pectinase and cellulase production from *Bacillus* sp. AA-04 and *Bacillus* sp. SM-07, isolated from garden soil were prepared by adding different components as shown in Table 1; pH of the medium was kept at 7.0 before sterilization.

For the production of pectinase and cellulase, rapidly growing overnight cultures of *Bacillus* sp. were inoculated in their respective 10 mL inocula and incubated at 37 ˚C for 24 h. The inocula were transferred to 90 mL media and incubated again at 37 ˚C for 24 h. By using refrigerated centrifuge machine, the crude enzyme or cell free filtrate (CFF) was obtained by centrifugation of fermented medium at 10,000 rpm for 10 min at 0 ˚C.

2.3 Partial Purification of Enzymes

Partial purification of crude pectinase and cellulase was performed by using salt precipitation technique. Ammonium sulfate was used as a salt to precipitate the enzyme protein by developing a concentration gradient from 20% to 80% (w/v).

2.4 Assay Procedure for Pectinase and Cellulase

Pectinase and Cellulase activities were determined by estimating the amount of reducing sugars released from the substrates citrus pectin and carboxymethyl cellulose using 40 mg/mL galacturonic acid and 40 mg/mL glucose respectively by DNSA (3,5- Dinitrosalicylic acid) method (Jain *et al*. 2020). The amount of enzyme required to release 1.0 µmol of reducing sugar from the substrate within one minute under optimal conditions, is referred to as 1 UNIT.

2.5 Characterization of Partially Purified Pectinase and Cellulase

The Michaelis-Menten constants, K_m and V_{max} have been calculated by measuring the pectinase and cellulase activities in presence of different concentrations of their substrates citrus pectin and carboxymethyl cellulose, respectively ranging from 0.25 – 3 g%. The enzyme activities of pectinase and cellulase were monitored at different time intervals, including 2, 5, 10, 15, and 20 minutes of incubation with their substrates. The optimum temperature for each enzyme was determined by performing enzyme assay across a temperature range of 40 to 70 ˚C, with increments of 5 ˚C. Similarly, to determine the optimum pH for maximum enzyme activity, enzymatic reactions were performed at pH 6, 7, 7.5, 8, and 9.

2.6 Development of Bi-functional Cross-linked Catalytic Hydrogel Blocks

Gelatin was dissolved in deionized water in boiling water bath. When the temperature of the solution dropped to 45 ˚C, crude pectinase and cellulase were added simultaneously and gently stirred for 30 s to form homogenous solution. The enzyme-gelatin mixture was immediately poured into petri-plate and glutaraldehyde was added, mixed gently and allowed for solidification at 37 ˚C for 1 h. After solidification, the cross-linked enzyme hydrogel containing pectinase and cellulase was cut into small blocks of equivalent size of 2.0 x 2.0 sq mm. The hydrogel blocks were stored in 50 mM phosphate buffer of pH 7.0 at 4 ˚C for the treatment of apple juice.

2.6.1 Optimization of Gelatin concentration for hydrogel formation

Gelatin was used in different concentrations ranging from 5 – 40 g% for hydrogel formation.

2.6.2 Optimization of Glutaraldehyde concentration for hydrogel formation

Glutaraldehyde was also added in different concentration from 10 – 30% (v/v) to obtain the optimum pore size for maximum retention of enzyme in hydrogel blocks.

2.6.3 Determination of Immobilization Efficiency:

$$\% \text{ Immobilization efficiency} = \frac{\textbf{Enzyme activity in immobilized gel blocks}}{\textbf{Soluble enzyme activity}} \times \mathbf{100} \qquad (1)$$

2.7 Treatment of Apple Pulp with Soluble Enzymes (Pectinase and Cellulase) and Optimization

Four apples of equal weight were taken, washed and sterilized with 0.05 g% potassium meta-bisulfate. After washing and sterilization, apples were cut in to small pieces and homogenized using homogenizer machine in four beakers separately, labeled as control, T_1, T_2, and T_3 respectively. Soluble Cellulase and Pectinase were added in each test beakers in the ratio of 1:1 (v/v) and incubated at different temperatures ranging from 40 ˚C to 60 ˚C for different time intervals (15 minutes to 60 min). For blank (untreated), same volume of deionized water was added in place of enzymes. Apple Juice was extracted from enzyme treated apple pulp through centrifugation at 10,000 rpm for 10 min. After centrifugation, supernatant was collected and measured for volume in Blank, T_1, T_2, and T_3 using graduated cylinders. All experiments were performed in triplicate, and the results are presented as mean ± standard error (SE).

2.8 Treatment of Apple Juice with Bi-functional Catalytic-Hydrogel Blocks (Immobilized Enzyme)

Apple juice was treated with multi-enzymes immobilized hydrogel blocks ranging from 0.5 g% to 6 g% at different temperatures ranging from 40 ˚C to 60 ˚C for different time intervals (15 min to 60 min). After incubation, viscosity and turbidity were measured. Each experimental condition was performed in triplicate, and the results are expressed as mean ± standard error (SE).

2.9 Operational Stability or Reusability of Hydrogel Blocks

After each enzyme-substrate reaction, the enzyme-hydrogel blocks (containing Pectinase and Cellulase) were removed from the reaction mixture, washed with deionized water and reused for the next run of enzyme-substrate reaction. The same process was repeated till the hydrogel blocks showed no activity. Reusability of enzyme-hydrogel blocks was analyzed in terms of viscosity and turbidity of apple juice after each reaction cycle.

2.10 Measurement of Viscosity and Turbidity

Turbidity of both treated and untreated juice was measured by using turbidimeter. In this method, turbidimeter was first calibrated with 10 FTU solution i.e. deionized water. After calibration, sample was taken in cuvette and placed in turbidimeter. The result was appeared after 20 seconds in FTU.

Viscosity was measured by using Ostwald's Viscometer. For viscosity measurements in juice, The Ostwald's Viscometer was washed with distilled water and dried. The viscometer was then filled with 10 mL of juice and flow was noted until the liquid moved down from mark A to B. The same was repeated for all tubes.

Viscosity of the liquid (NL) = (TL / TW) (DL / DW). NW **(2)**

Where;

- TL = Time of flow of liquid
- TW = Time of flow of water
- DL = Density of liquid
- DW = Density of liquid
- NW = Viscosity of the water

3. RESULTS AND DISCUSSION

3.1 Isolation of Pectinolytic and Cellulolytic Bacteria from Soil

In this study, serially diluted soil samples were screened for pectinolytic and cellulolytic bacteria based on clear hydrolysis zones on pectin and carboxymethyl cellulose plates. The P2 colony exhibited a hydrolysis zone diameter of 2.3 cm, larger than P1 (1.5 cm) and P3 (1.8 cm), leading to its selection as the pectinase producer (Fig. 1a). This strain was identified as *Bacillus* sp. AA-04 through colony morphology and biochemical tests. Similarly, the C4 colony, selected for cellulase production (Fig. 1b), was identified as Bacillus sp. SM-07. Microorganisms serve as efficient bioreactors for large-scale production and commercialization of microbial enzymes. Various microbial species with pectinolytic activity have been reported including *Aspergillus* sp., *Erwinia* sp., *Bacillus* sp., and *Penicillium* sp. being commonly utilized for commercial production purposes (Satpathy *et al*., 2023). Co-production of xylanase, cellulase, and pectinase have also been reported through solid-state fermentation by *Aspergillus* sp. (Sosa-Martínez *et al*., 2024).

3.2 Partial purification and Characterization of Pectinase and Cellulase

Partial purification of enzyme protein is essential for subsequent characterization and analysis, as well as for industrial applications, where stability and high enzyme concentrations are required for efficient processes. In the present study, Pectinase and Cellulase from *Bacillus* sp. AA-04 and *Bacillus* sp. SM-07 respectively were partially purified by using the gradient ammonium sulfate precipitation technique. The cell free filtrate was subjected to precipitation with different concentration of ammonium sulfate ranging from 30 to 80% (w/v). It was observed that the precipitation of pectinase and cellulase increased as the concentration of ammonium sulfate was raised from 30% to 60%. However, enzyme activity declined beyond 60 % saturation. Maximum precipitation of pectinase was observed at 40% $(NH_4)_2SO_4$ (Riaz *et al*., 2023a) with the specific activity of 298 U/mg and fold purification of 4.175 as compared to crude enzyme. Similarly, cellulase was maximally precipitated at 60% $(NH_4)_2SO_4$ (Riaz *et al*., 2023b) with the specific activity of 206 U/mg and fold purification of 6.24 (Table 2).

The Michaelis-Menten constants V_{max} and K_m provide important insights into the enzymatic properties of pectinase and cellulase, which are essential for their use in industrial applications. For pectinase, V_{max} was found to be 497 U/ml and K_m was 0.115 mg/ml (Fig. 2), while for cellulase, V_{max} was 370.4 U/ml and K_m was 0.1177 mg/ml (Fig. 3). Pectin exhibited optimal activity after 5 minutes of incubation with substrate (citrus pectin), whereas; Cellulase was found maximally active after 10 minutes of incubation with carboxymethyl cellulose (Fig. 4).

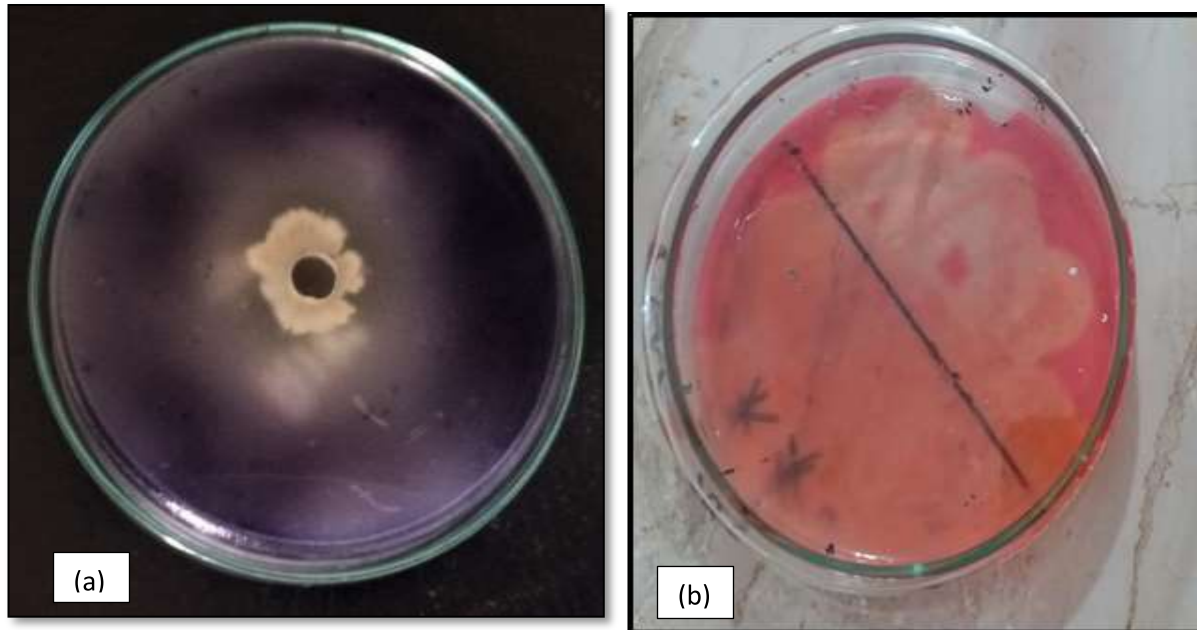

Fig. 1. Primary screening of enzyme-producing *Bacillus* species isolated from rotten fruit peels: **(a)** qualitative screening of pectinase activity by *Bacillus* sp. AA-04, showing a clear hydrolysis zone on pectin-containing agar medium; **(b)** qualitative screening of cellulase activity by *Bacillus* sp. SM-07, indicated by a clear zone of cellulose degradation on carboxymethyl cellulose (CMC) agar medium.

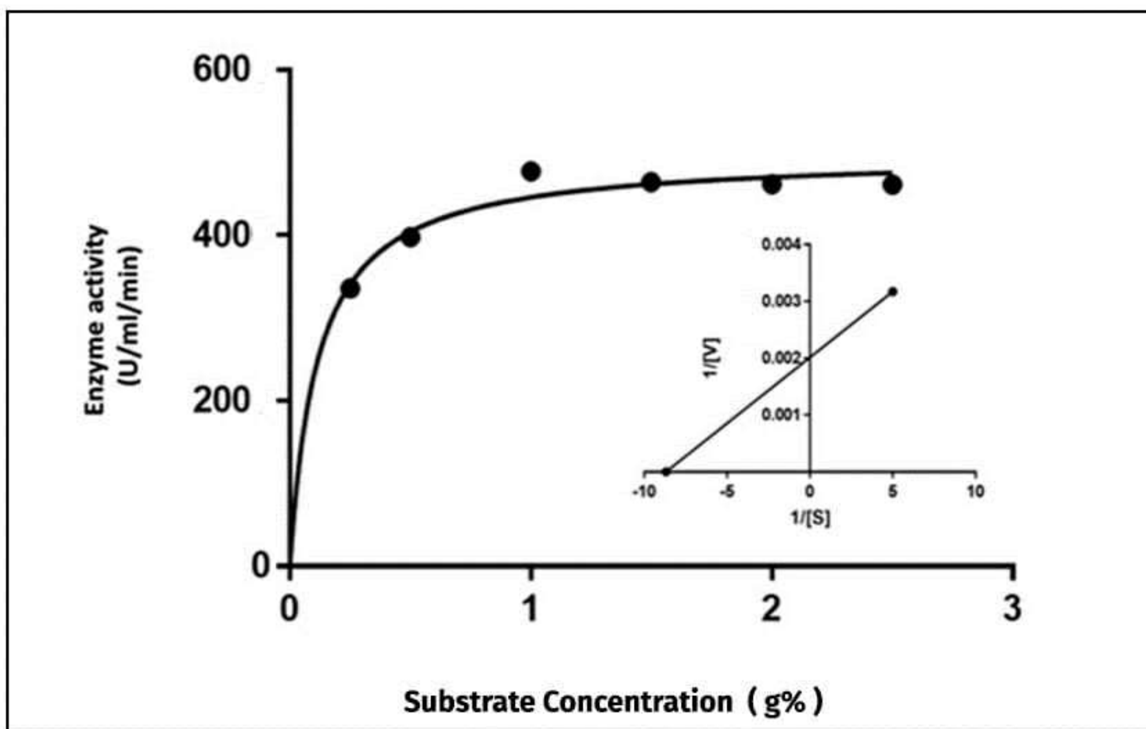

Fig. 2. Michaelis-Menten and Lineweaver-Burk plot of Pectinase produced from *Bacillus* sp. AA-04.

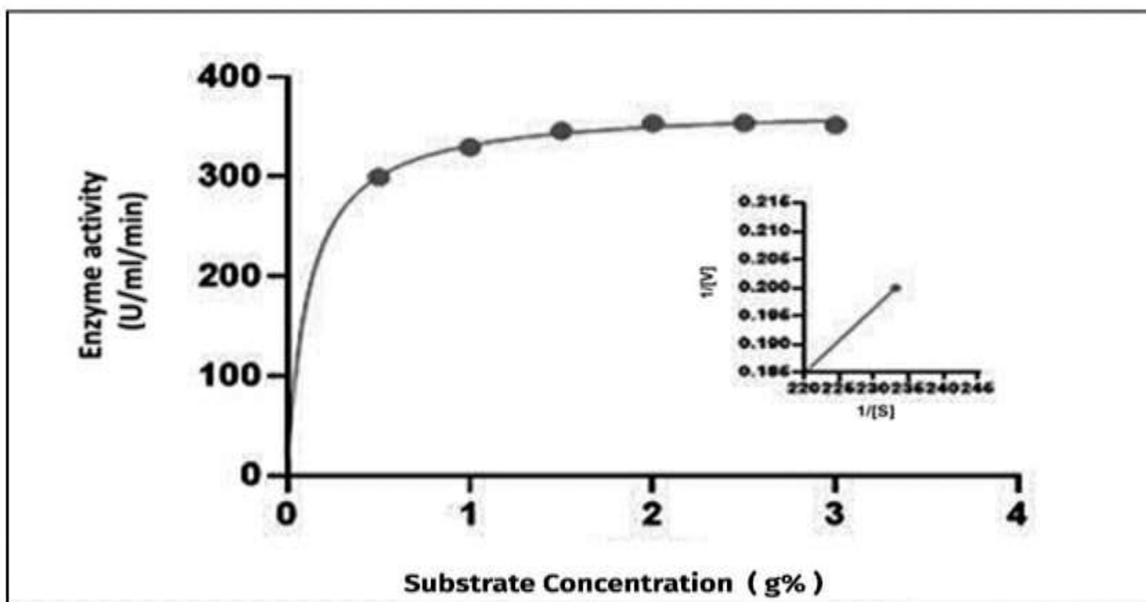

Fig. 3. Michaelis-Menten and Lineweaver-Burk plot of Cellulase produced from *Bacillus* sp. SM-07.

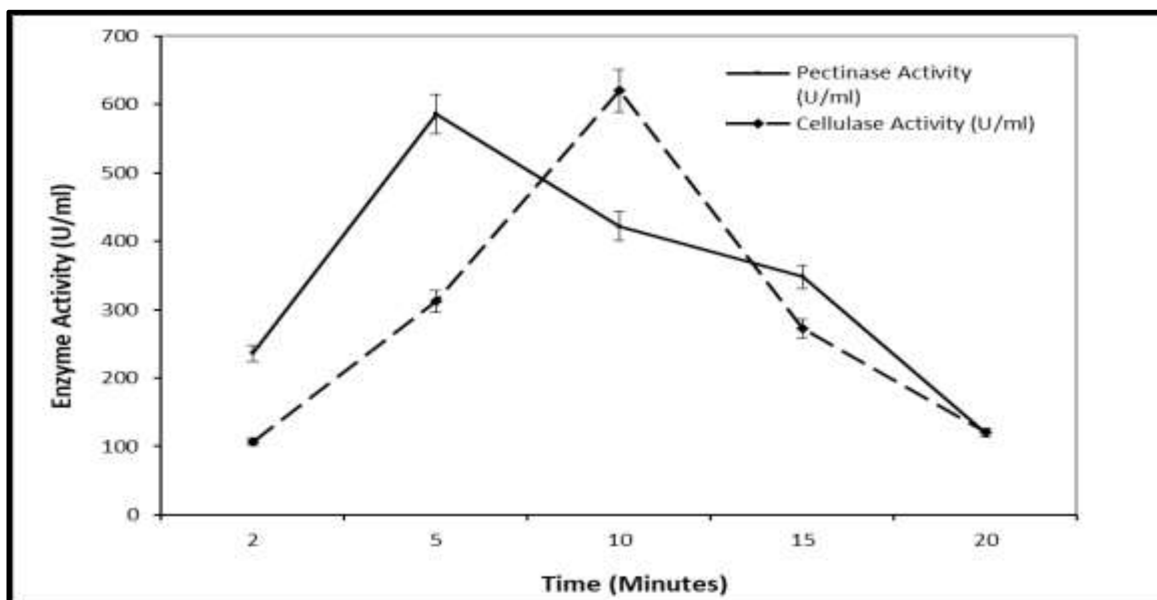

Fig. 4. Effect of reaction time with substrates (citrus pectin and carboxymethyl cellulose) on activity of Pectinase and Cellulase, respectively.

Maintaining the right pH and temperature conditions ensures maximum enzyme activity, leading to higher quality juice with fewer particulates and improved clarity. The characterization of pectinase revealed that the enzyme exhibited optimal activity at pH 7.5, but its activity decreased significantly when the pH shifted slightly to a more alkaline condition, from 7.5 to 8. This attribute of being more active in an acidic environment enhances its importance for the fruit juice industries. In contrast, ellulase demonstrated optimal activity at pH 8 (Fig. 5). Both pectinase and cellulase were found to be thermostable, with their optimal activities occurring at 50 ˚C and 55 ˚C, respectively, indicating that they can function effectively at moderately high temperatures, which is beneficial for speeding up the clarification process and minimizing microbial growth during processing (Fig. 6).

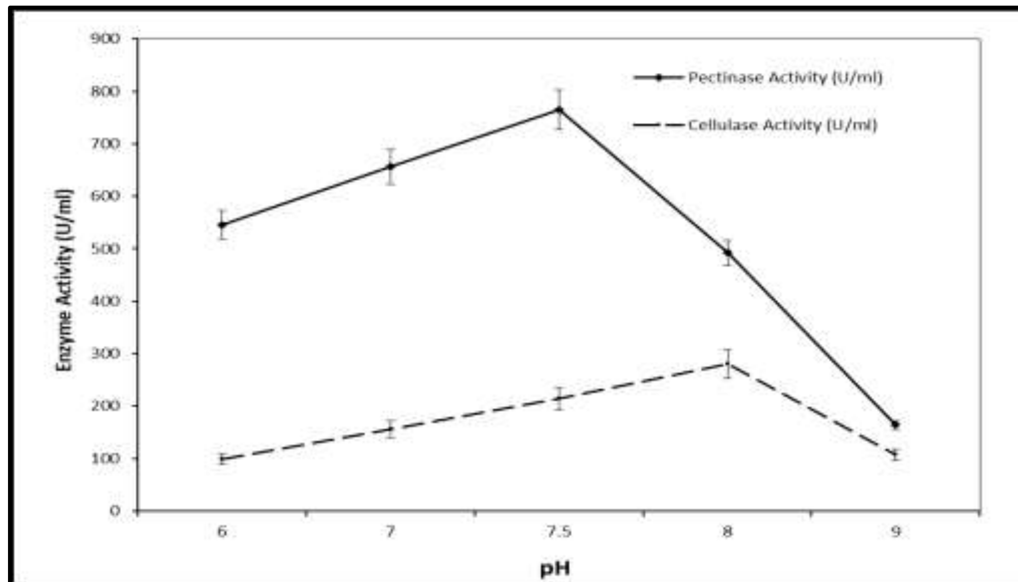

Fig. 5. Effect of reaction pH on activity of Pectinase and Cellulase.

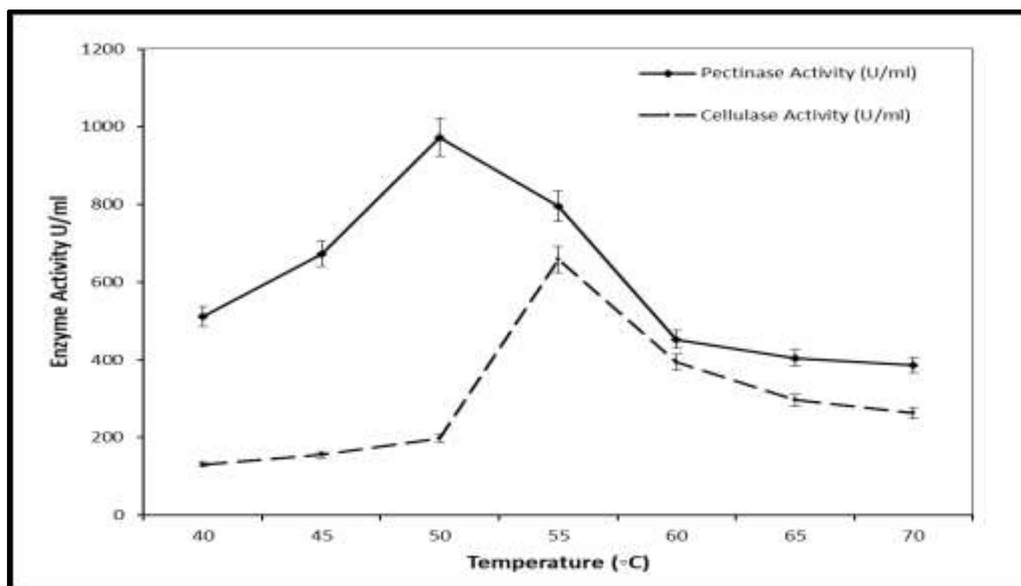

Fig. 6. Effect of reaction temperature on activity of Pectinase and Cellulase.

3.3 Optimization of Gelatin and Glutaraldehyde concentration for enzyme hydrogel formation

For the fabrication of mechanically stable and catalytically efficient bi-functional enzyme hydrogel blocks, 30% gelatin (w/v) and 20% (v/v) glutaraldehyde was found optimal. Gelatin, a natural polymer, forms the backbone of enzyme hydrogels, while glutaraldehyde serves as a crosslinking agent. The concentration of both components directly influences the degree of crosslinking within the hydrogel matrix. Optimization ensures that the crosslinking density is sufficient to provide mechanical stability to the hydrogel while maintaining the desired porosity and swelling behavior. Following optimization, immobilization efficiency of pectinase and cellulase were found to be 83.57 and 78.81% respectively (Table 3).

3.4 Treatment optimization of Apple Pulp with Soluble Multi-Enzyme System with Respect to Time and Temperature

High juice yield is an important goal for juice manufacturer. The juice yield serves as a crucial indicator in assessing the suitability of fruit for juice processing. Economically, a lower juice yield raises production expenses, prompting

manufacturers to routinely assess fruit juice yield before processing. In the present work, we noticed 19 mL juice extraction from 107 gm apple pulp from control (untreated pulp) whereas, juice yield was increased up to 21 % i.e. 23 mL juice was collected after 15 minutes treatment with enzyme mix consisting of pectinase and cellulase in the ratio of 1:1 (v/v) at 40 ˚C. The juice yield was further increased to 57.89 % with 30 mL juice collection after 45 minutes of incubation under same conditions (Table 4). Padma *et al*. (2017) demonstrated the maximum yield of apple juice was obtained with enzyme concentration 1 U/ml at 50 ˚C for 30 minutes. Guava samples treated with pectinase exhibited a greater juice yield compared to those without pectinase treatment. It has also been reported that following pectinase treatment, the FR guava cultivar demonstrated the highest juice yield, which was 15.17% higher than its untreated counterpart (Chen *et al*., 2023). Whereas Srivastava and Sudhir (2013) reported that the juice yield increased from pulp by enzymatic treatment in apple juice ranges from 70-80%.

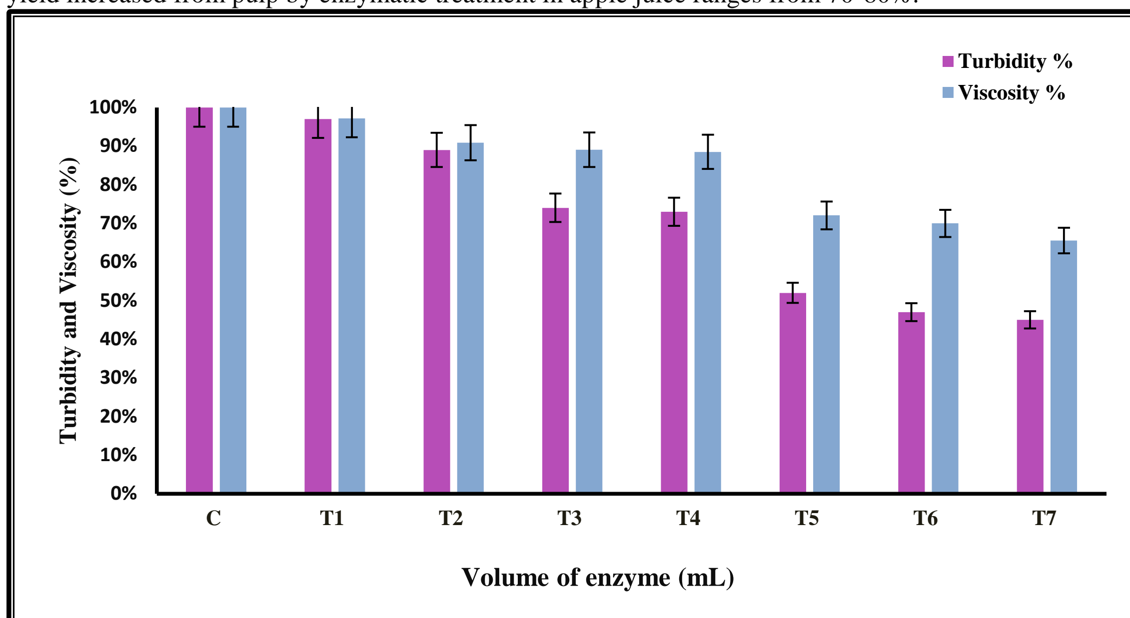

Fig. 7. Effect of different concentrations of immobilized multi-enzyme system (pectinase and cellulase in a 1:1 v/v ratio) on the viscosity and turbidity of apple juice. Enzyme treatments were carried out at 50 °C for 45 min. C represents the untreated control, while T1–T7 correspond to immobilized enzyme concentrations of 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 g%, respectively.

3.5 Effect of different concentrations of Bi-functional Catalytic-Hydrogel Blocks on Viscosity and Turbidity of Apple Juice

In the present study, the effect of multi-enzyme system on turbidity and viscosity of apple juice were also investigated The continuous decline of turbidity and viscosity was observed with increasing enzyme concentration because of the hydrolysis of pectin and cellulose which are responsible for causing high viscosity and turbidity. The viscosity and turbidity were reduced by 35% and 55% respectively, by treatment with multi-enzyme hydrogel blocks (6 g%) at 50 ˚C after 45 minutes (Fig. 7) as compared to untreated control. According to the physical and chemical characteristics, each fruit contains difference degrees of natural turbidity in their juices due to the presence of insoluble cell fragments that originate from its pulpy tissue (Abdullah *et al*., 2007).

Kharazmi and Asghar (2023) proposed co-immobilization of Pectinase and xylanase onto functionalized iron oxide nanoparticles. The co-immobilized enzymes exhibited enhanced stability against temperature and pH variations compared to their free forms, retaining significant activity through multiple cycles. When applied to pineapple juice, the nanobiocatalyst achieved a substantial 53% reduction in turbidity after 120 minutes.

The study proposed by Ezeh *et al*. (2023) focused on *Yarrowia phangngaensis* and its pectinase-producing potential using agro-waste substrates. Optimization of culture conditions significantly boosted pectinase production, with banana peels proving most effective among various substrates. The optimized pectinase effectively clarified apple juice, achieving a 39.33% reduction in turbidity and 59.84% improvement in clarity after treatment. Kothari *et al.* (2013) worked on apple juice clarification with soluble multi-enzyme system (Cellulase and Pectinase) and showed 50% juice clarification but, after 4 hours incubation time with Cellulase and Pectinase.

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3.6 Operational Stability or Reusability of Immobilized Multi-Enzyme System in the Treatment of Apple Juice

Operational stability or reusability of immobilized enzyme contributes to the main factor of immobilization which reduces the cost of enzyme and provides many advantages that increases its applicability in industry as compared to soluble enzyme. Reusability of immobilized enzyme systems helps maintain consistent product quality batch after batch. Consistency is particularly important in the food and beverage industry, where consumers expect uniform taste, color, and texture in products like apple juice (Bié *et al*., 2022).

In the present study, the reusability of multi-enzyme system was noticed up to six cycles in accordance of viscosity and turbidity. It was observed that the same batch of immobilized multi-enzyme system reduced 23% viscosity and 33% turbidity after 6 cycles of repeated use (Fig. 8). The study performed by Hassan *et al*. (2020) focused on enhancing the efficiency and cost-effectiveness of enzymatic treatments for apple juice clarification by immobilizing pectinase and xylanase from M. hiemalis onto genipin-activated alginate beads. The immobilized enzymes maintained suitability for up to 5 process cycles, retaining around 45-49% of their initial activity. Whereas, Singh *et al*. (2024) immobilized pectinase, cellulase and amylase on calcium alginate and found 23.8%, 24.4%, and 36.5% residual enzyme activity respectively after 5 cycles of juice clarification.

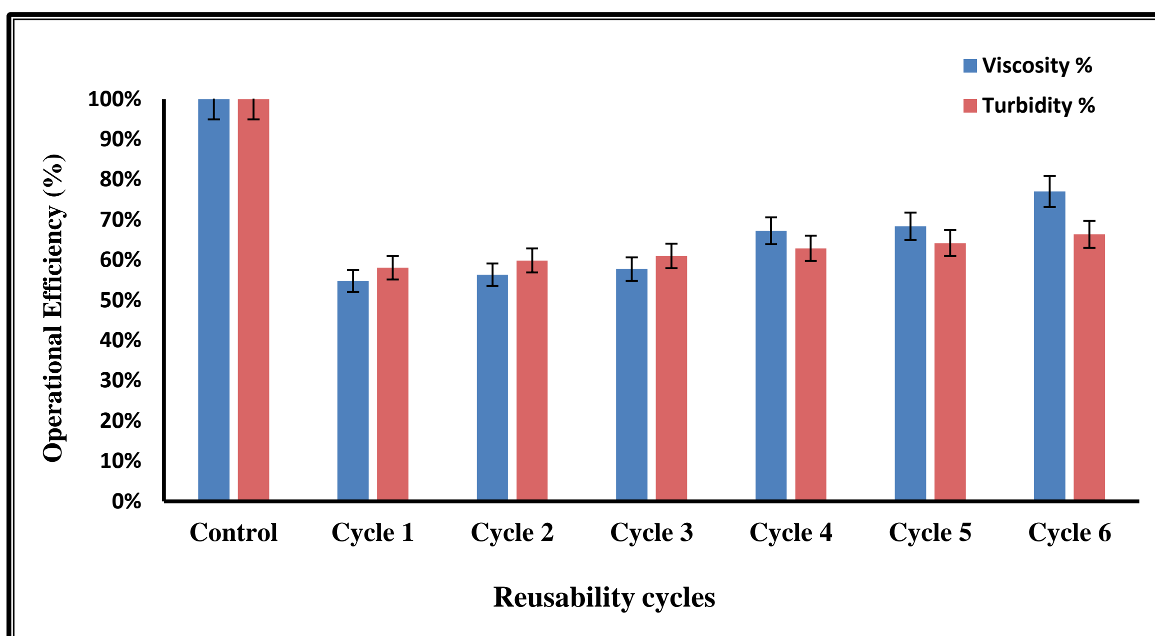

Fig. 8. Operational stability and reusability of the immobilized multi-enzyme system during repeated cycles of apple juice treatment.

Table 1. Media composition for Cellulase and Pectinase Production through Submerged Fermentation from *Bacillus* sp. AA-04 and *Bacillus* sp. SM-07 respectively.

Media Components for Pectinase Production	Media Components for Cellulase Production
Pectin 1.0 g %	Carboxymethyl cellulose 2.0 g %
Yeast extract 0.3 g %	Yeast extract 0.5 g %
Potassium nitrate 0.2 g %	Sodium chloride 0.005 g %
Potassium di hydrogen phosphate 0.2 g %	Potassium di hydrogen phosphate 0.1 g %
Dipotassium hydrogen phosphate 0.2 g %	Magnesium sulfate 0.04 g %

Table 2. Partial Purification of Pectinase and Cellulase produced from *Bacillus* sp. AA-04 and *Bacillus* sp. SM-07, respectively.

Purification Step	Enzyme Activity (U/mL)		Total Protein (mg/mL)		Specific Activity (U/mg)		Fold Purification	
	Pectinase	Cellulase	Pectinase	Cellulase	Pectinase	Cellulase	Pectinase	Cellulase
Crude Enzyme	167	75	2	2.3	71	33	1	1
$(NH_4)_2SO_4$ Enzyme Precipitates	224	132	0.91	1.02	245	131	3.43	3.96
Desalted Enzyme	295	208	0.99	1.01	298	206	4.175	6.24

Table 3. Immobilization Efficiency of Pectinase and Cellulase Co-immobilized in Gelatin Hydrogel Blocks.

Specifications	**Co-immobilized Enzymes**	
Weight of the gel blocks	5.45 g	
Volume of enzyme mix (1:1) trapped in gel blocks	5.0 mL	
Volume of enzyme trapped	1.09 mL/g	
	Pectinase	**Cellulase**
Units of enzyme before entrapment	274 U/mL/min	203 U/mL/min
Units of enzyme after entrapment	229 U/g/min	160 U/g/min
Immobilization efficiency	83.57 %	78.8 %

Table 4. Effect of Enzymatic treatment time (minutes) on Apple's Juice Yield

	Concentration (v/w)		**Time (minutes)**	**Temperature (°C)**	**Juice Yield (mL)**	**Increase in Juice Yield (%)**
	Enzyme Mix 1:1 (mL)	**Apple (g)**				
Control	-	107	15	40	19	-
T1	2	107	15	40	23	21.05
				50	26	36.8
				60	26	36.8
T2	2	107	30	40	26	36.84
				50	28	47.36
				60	27	42.10
T3	**2**	**107**	**45**	**40**	**30**	**57.89**
				50	27	42.10
				60	26	36.8
T4	2	107	60	40	29	52.62
				50	25	31.57
				60	23	21.04

4. Conclusion

Pectinase and cellulase were locally produced from *Bacillus* AA-04 and *Bacillus* SM-07, respectively, using rotten fruit peels as a low cost substrate. This strategy eliminated the dependence on imported commercial enzymes, thereby significantly reducing processing costs. The enzymatic method improved apple juice extraction, significantly increasing juice yield compared to conventional mechanical methods. Juice quality was also enhanced, with reduced viscosity and turbidity. Additionally, using an immobilized enzyme system proved cost-effective and

durable, maintaining operational stability for up to six successive cycles. These findings highlight the practical applicability of immobilized pectinase-cellulase systems in industrial juice processing, offering a cost-effective, sustainable, and scalable approach for producing high-quality clarified juices.

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Disclosure Statement

The authors report there are no competing interests to declare.

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