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Efficacy of *Bacillus Velenzensis-Derived* Pectinase in Olive Oil Extraction and Cotton Bioscouring

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KEYWORDS

Bacillus velenzensis, pectinase, olive oil extraction, cotton bioscouring, enzymatic treatment, FTIR

ABSTRACT:

Aim: Pectinases are industrially significant enzymes used in eco-friendly processing across food and textile sectors. The present study aimed to evaluate the efficiency of pectinase produced by Bacillus velezensis in enhancing olive oil extraction and in cotton bioscouring. The study also examined whether enzymatic treatment could serve as a sustainable alternative to conventional chemical methods.

Methodology: The enzyme was purified (15.2-fold) and applied at optimized concentrations for both applications. Olive oil extraction efficiency was assessed through oil yield, turbidity, phenolic content, and draining time. Cotton bioscouring performance was evaluated by fabric weight loss and confirmed using FTIR spectral analysis.

Results: Pectinase treatment increased olive oil yield from 7.18% to 9.21%, reduced turbidity to 35.5% of the control, and shortened draining time from 15 s to 11 s, with a slight increase in phenolic content. In textile processing, enzymatic bioscouring caused a higher fabric weight reduction (24.65%) than alkaline scouring (18.01%). FTIR analysis confirmed effective removal of non-cellulosic impurities.

Interpretation: The findings demonstrate that the pectinase is effective in improving oil recovery and fabric scouring efficiency. Enzymatic processing offers a greener and sustainable alternative to conventional chemical methods. This approach supports environmentally friendly industrial practices in both food and textile sectors.

1. Introduction

Pectinases are a diverse group of enzymes that catalyze the hydrolysis of pectin, a complex polysaccharide present in the primary cell walls of plants (Anand et al., 2020; John et al., 2020). These enzymes are commercially important because of their catalytic efficiency, substrate specificity, and environmental compatibility (Amin et al., 2019; Garg et al., 2016). The demand for pectinases has increased globally due to their wide applications across several industrial sectors, including food and beverage processing, textile manufacturing, and biofuel production. In the food industry, pectinases are employed in fruit juice clarification, wine production, and oil extraction, where they degrade pectic substances and facilitate the release of trapped cellular components (Amin et al., 2019). Their industrial relevance has extended to processes such as coffee and tea fermentation, wastewater treatment, and biofuel generation, primarily due to their high specificity and reduced energy requirements compared to conventional chemical methods (Khan et al., 2025).

Pectinases are also utilized in cotton bioscouring, which is an environmentally sustainable alternative to conventional alkaline scouring. Enzymatic bioscouring effectively removes non-cellulosic components such as pectins and waxes from raw cotton fibres, improving hydrophilicity, absorbency, and overall fabric quality, while minimizing chemical usage and

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wastewater generation (Bristi et al., 2019; Colombi et al., 2021; Diab et al., 2023). In contrast, traditional scouring employs strong alkalis that result in fabric damage and environmental pollution (Calafell & Garriga, 2004; Luo et al., 2024).

In our previous investigation, a pectinase-producing strain of *Bacillus velenzensis* was isolated and characterized. The enzyme was further purified 15.2-fold through a multistep purification procedure.

The present study was designed to evaluate the performance of the purified pectinase in oil extraction and cotton bioscouring, with emphasis on its operational parameters and potential applications in industrial bioprocessing.

2. Materials And Methods

2.1. Production & purification of pectinase

Bacillus velenzensis-derived pectinase was produced using production media (10 g/L yeast extract, 5.5 g/L pectin, and 10 g/L glucose (pH 7)), and the resulting crude enzyme was purified using multistep purification steps culminating in gel filtration. The purified pectinase was then used for further application study

2.2. Pectinase effect on oil extraction

Fresh olives were collected and washed. Olive (10 g) was ground for 30 s in a paste containing pectinase (20 U/ml/g of olive paste). A heat-inactivated pectinase sample was used as the control. After grinding, the paste was centrifuged at 3000 g for 10 min to separate the oil. The extracted oil was then tested for its physicochemical properties, namely turbidity, draining time, and oil yield in the presence and absence of pectinase during extraction.

Oil turbidity was calculated by measuring the absorbance at 830 nm, followed by determining the relative turbidity.

$$Relative\ turbidity\ (\%) = \frac{Absorbanceof\ sample}{Absorbance\ of\ control} X\ 100$$

The percentage of oil yield was measured in terms of the amount of oil yielded per unit weight of olive oil used.

$$\%\ of\ oil\ yield\ \left(\frac{w}{w}\right) = \frac{Weight\ of\ extrcated\ oil\ (g)}{Weight\ of\ oilve\ oil\ paste\ (g)} \times 100$$

The draining time was measured by determining the time taken for the differentially extracted oils to elute 1 mL from a glass pipette calibrated to 25 °C. This was repeated multiple times, and the average time was calculated.

The quality of the olive oil was also estimated in terms of the volume of phenolic compounds present in the extracted oil. Olive oil (350 µL) was mixed with 250 µL of a water–methanol (60:40) mixture to extract the phenolic compounds. The extracted phenolic compounds present in olive oil were incubated with 40 µL of the extract with 0.11% Folin–Ciocalteu (Merck) for 8 min in the dark, followed by the addition of 300 µL of 20% w/v Na_2CO_3 solution and incubation in the dark for 2 hrs. After incubation, the absorbance of the samples was measured at 760 nm. A standard graph with increasing concentrations of gallic acid (0.50-500 µg/ml) was also performed (Ortiz et al., 2017), and the amount of phenolic present was estimated in terms of micrograms of gallic acid per gram of olive oil.

2.3. Effect of pectinase on cotton scouring

Cotton fabric (100% woven) purchased from Coimbatore, India, was desized by boiling in hot water for 15 min, followed by washing in distilled water, and the weight of the cotton pieces was measured. For bioscouring, 50 U/mL of purified pectinase dissolved in acetate buffer pH 4.0 was incubated at 40 °C for 10 h with continuous shaking, washed to remove the enzyme, dried, and weighed. For the alkaline treatment method, cotton pieces were incubated in solution (1% sodium hydroxide, 0.2 % triton X 100) at 90 ºC for 45 min, following which they were washed in cold distilled water and drying at 45 ºC. The initial and final weights of the pieces were measured to determine the percentage of weight loss (Rajendran *et al*., 2011).

$$Percentage\ of\ weight\ loss = \frac{Inital\ weight - final\ weight}{Initial\ weight} \times 100$$

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2.4. FTIR analysis of cotton

A 5 mm × 5 mm section of the fabric was placed onto a potassium bromide (KBr) pellet for FTIR analysis. Spectral measurements were performed using a PerkinElmer Spectrum Two FTIR spectrometer. The instrument was calibrated prior to analysis, and baseline correction was applied to eliminate background interference. Spectra were recorded from 4000–400 cm^{-1} range with 4 cm^{-1} resolution. The spectra were analysed to identify the functional groups present in the fabric extracts.

3. Results and Discussion

3.1. Effect of pectinase on olive oil extraction

The ability of the purified pectinase to improve olive oil extraction was evaluated by measuring various parameters, like the yield, draining time, clarity of the extracted oil, and amount of phenol present in the extracted oil in the presence of pectinase (Figure 1a). The addition of pectinase during the extraction process improved the overall yield of olive oil by 9.21% when compared to 7.18 % in the case of the control. Furthermore, the relative oil turbidity in the presence of pectinase was reduced to 35 % compared with that of the control (100 %). There was also a reduced draining time of 11 s compared with 15 s, as observed in the control-treated sample. These observations indicate that pectinase has a positive impact on the yield, draining time, and clarity of oil during the extraction process. Further the amount of phenolic present in the differently treated oil was evaluated in terms of gallic acid using standard curve (Figure 1b). A slight increase in the total phenolic (133.45 µg GAE/g) was observed in the pectinase-treated group, compared to that in the control (132.540 µg GAE/g).

Pectinase was found to increase the olive oil yield to 9.21 % when compared to the 7.18 % in untreated samples. Similarly, an improvement in yield was observed when *Bacillus licheniformis-derived* enzymatic solution containing pectinase improved the yield from 3.79g/100g of olives to 4.12g/100 g of olives, which was more efficient than commercially available enzyme (Mortabit et al., 2014). On a similar front, pretreatment of olive pastes with a combination of pectinase and cellulase was shown to improve the overall yield of olive oil by 4.53 % (Huang et al., 2022).

Moreover, the presence of pectinase resulted in a reduction of oil turbidity to 35%, as opposed to the control, which was maintained at 100%. Furthermore, a marginal increase in total phenolic content (133.45 µg GAE/g) was observed in the pectinase-treated group relative to the control (132.540 µg GAE/g). The application of commercially available pectinase and cellulase has been demonstrated to enhance oil recovery by 11% and improve clarity. Also, a total phenolic content of 165 mg/kg was observed in comparison to control samples (Sharma et al., 2014), which is like our observations. Viscozyme, an enzyme combination containing pectinase, was shown to significantly improve oil yield and total phenolic content by more than 2-fold when applied to olive paste of the *Frantoio* cultivar (Chih et al., 2012).

3.2. Pectinase as a bioscouring agent

Cotton scouring is an important process to enhance the quality of cotton by removing impurities. The effectiveness of the purified pectinase as a bioscouring agent was assessed by incubating the enzyme with cotton samples and measuring the subsequent weight loss (Figure 2). Compared with the untreated control group, the cotton samples treated with pectinase exhibited a 24.65% reduction in weight. In contrast, treatment with alkaline reagents resulted in 18.01% weight reduction. These results indicate that pectinase is more efficient in improving cotton quality than alkaline treatment. Further, the effect of bioscouring and alkaline treatment on cotton was evaluated using FTIR analysis. In the FTIR spectrum of the enzyme-treated fabric (Figure 3c), a noticeable reduction in transmittance was observed at wavenumbers 3333.18 cm^{-1} (N–H stretch), 2899.06 cm^{-1} (C–H stretch), 1334.47 cm^{-1} (C–N stretch), and 1053.22 cm^{-1} (C–O stretch), relative to the control (Figure 3a) and the fabric treated with alkaline solution (Figure 3b). This decrease implies an increase in absorbance, indicating a higher concentration of functional groups or alterations in the bond characteristics resulting from enzymatic action. These structural changes are likely associated with the breakdown of non-cellulosic components and the development of new molecular interactions, contributing to the improved softness and higher weight reduction (24.65%) observed in bioscoured cotton. Conversely, the chemically scoured fabric showed smaller weight loss (18.01%) and fewer spectral changes,

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suggesting a less pronounced effect. Thus, pectinase treatment appears to be more efficient at enhancing cotton surface properties than conventional alkaline scouring.

Additionally, purified pectinase was evaluated for its efficacy in cotton scouring, revealing a significant impact by reducing the fabric weight by 24.65%, in contrast to an 18.01% reduction observed with alkaline treatment. Additionally, a marked decrease in transmittance at wavelengths corresponding to N-H, C-H, C-N, and C-O stretches was noted in bioscoured cotton compared to control or alkaline-treated samples, suggesting enhanced scouring efficiency with pectinase. Marine *Bacillus subtilis-derived* pectinase was shown to have a significant impact on weight reduction during the bioscouring of cotton under optimised conditions (10% enzyme dissolved in 20 mM phosphate buffer at pH 7.0 was incubated at 60 °C for 2 hrs (Joshi et al., 2013).

3.3. FTIR analysis of cotton

Furthermore, Fourier-transform infrared (FTIR) analysis revealed enhanced absorption at approximately 1637 cm^{-1} in both alkaline and pectinase-scoured fabrics, which was attributed to the protonation of ionised carboxylate groups. Alkaline scouring exhibited broader peaks (3310–3350 cm^{-1}, 2850 cm^{-1}, 1427–1029 cm^{-1}), indicating non-specific component removal, whereas pectinase selectively removed pectin (Joshi *et al*., 2013). Similarly, pectinase derived from *Fusarium* spp. resulted in a 0.89 % reduction in fabric weight. Furthermore, its application led to a decrease in the peak at approximately 1730 cm^{-1}, indicating a reduction in impurities (Rajendiran et al., 2011).

4. Conclusion

Pectinase was shown to improve olive oil extraction efficiently. Significant improvements in yield, reduction in turbidity, and lower oil-draining time were observed upon the application of pectinase in the extraction process. The phenolic content of 133.45 µg GAE/g was also observed in oil extracted in the presence of pectinase. Similarly, pectinase aided in the reduction of fabric weight better than the alkaline method. The better removal of non-cellulosic impurities was also demonstrated by FTIR analysis. These observations highlight the potential application of B. *velenzensis*-derived pectinase in industrial processes.

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Author's declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Authors' Contributions

All authors contributed significantly to this study.

Conflict of Interest

The authors declare that there is no conflict of interest.

Research Content

This manuscript contains original research that has not been published previously and is not under consideration for publication elsewhere. All data presented are authentic and were generated as part of this study.

Ethical Approval

This study did not involve human participants or vertebrate animals. Therefore, ethical approval was not required.

Data Availability

None

Consent to Publish

All authors have read and approved the final manuscript and consent to its publication.

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Table 1: Effect of pectinase on the extraction of olive oil

Properties	**Control**	**Test**
Total Phenols (µg GAE/g)	132.54	133.45
Relative oil turbidity %	100	35.5
% yield (w/w)	7.18	9.21
Oil draining time (s)	15	11

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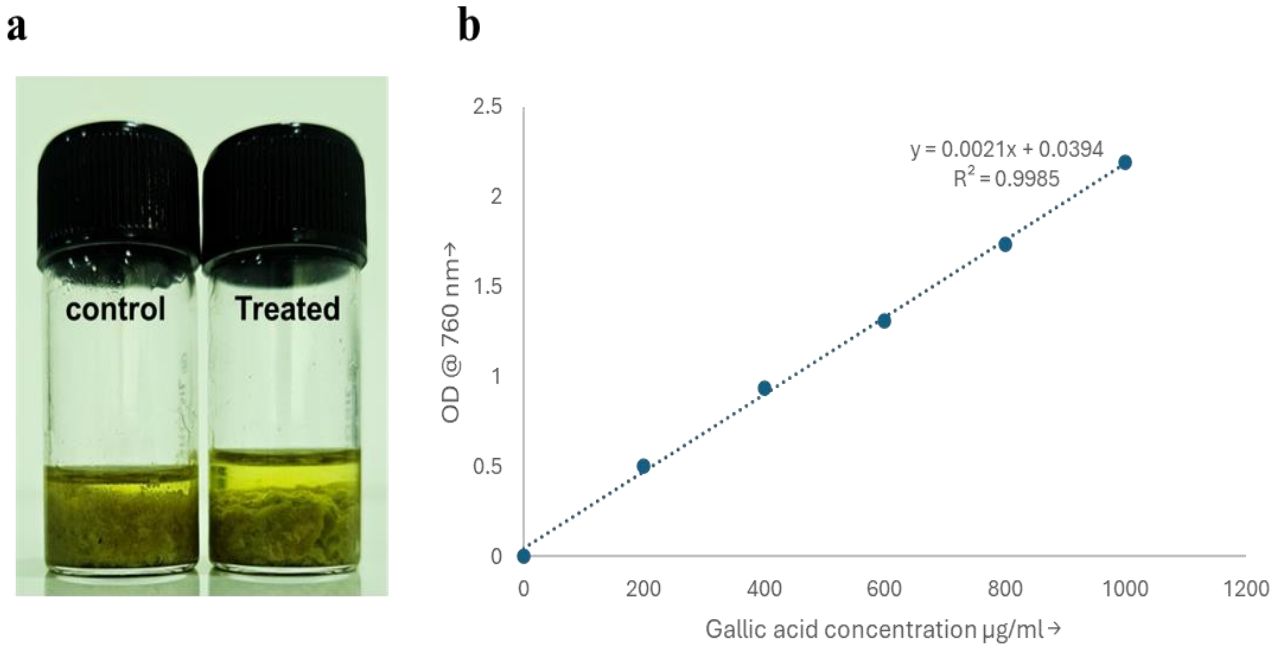

Figure 1: Effect of pectinase on olive oil extraction

a) Olive oil extracted using pectinase (treated) compared to control. b) Standard gallic acid graph

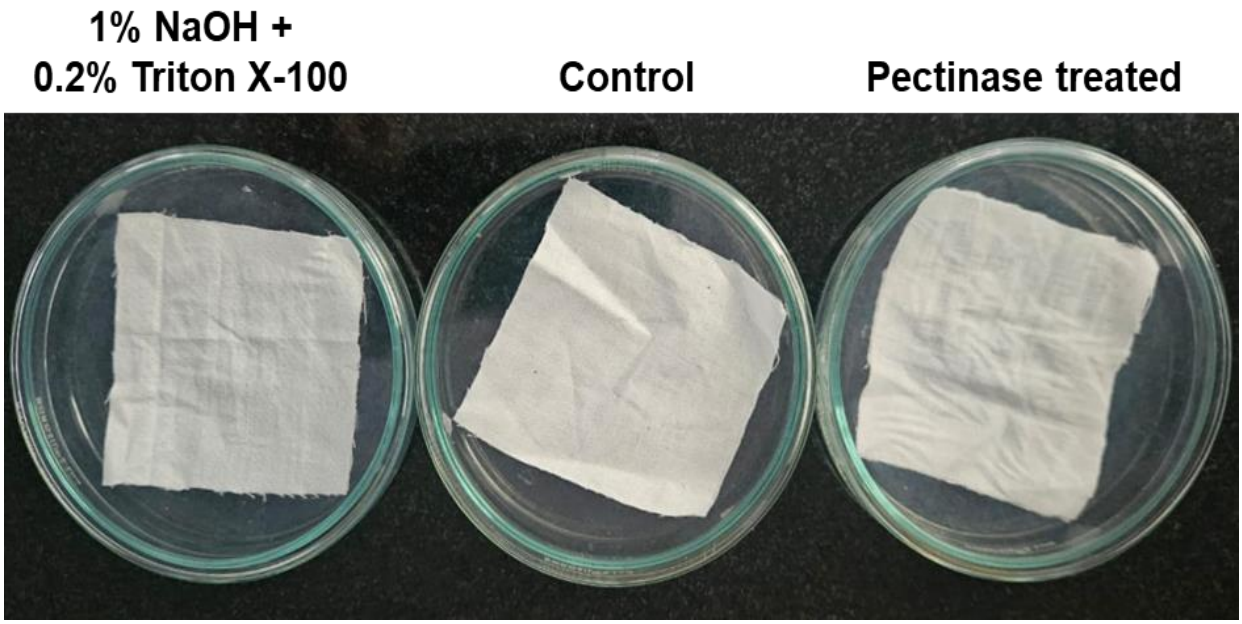

Figure 2: Effect of alkaline and pectinase treatment on cotton scouring

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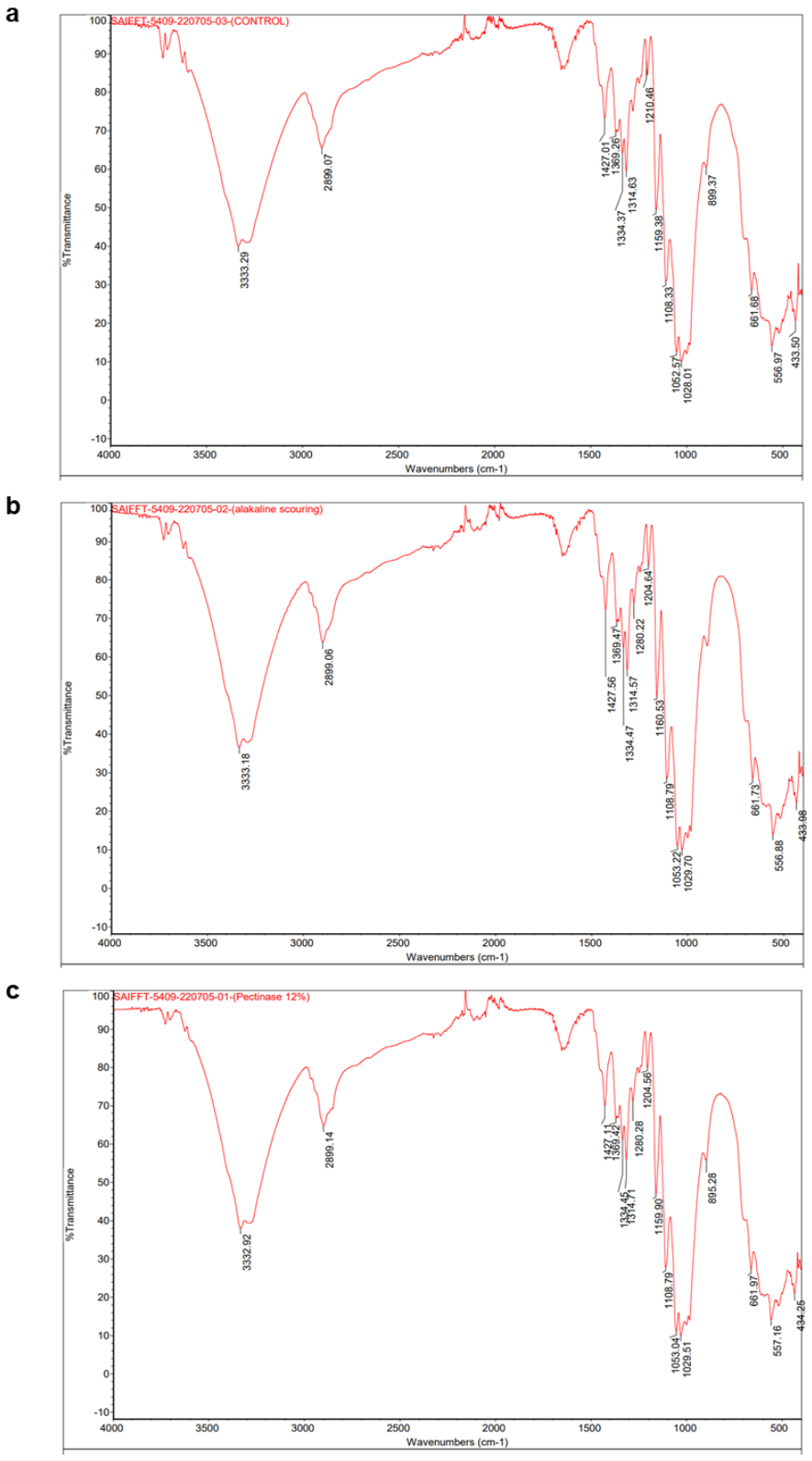

Figure 3: FTIR analysis of treated cotton a) Control; b) alkaline treatment; c) pectinase treated