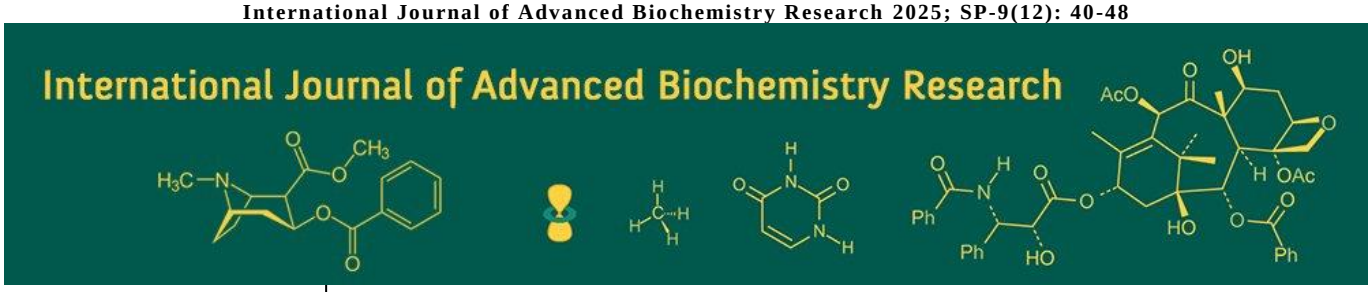

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Effect of enzyme combinations and temperature condition on production of white pepper (*Piper nigrum* L.)

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Abstract
The present study investigated the combined effects of microbial enzyme mixtures and temperature on optimizing white pepper (*Piper nigrum* L.) production. Fully matured and ripened berries of five varieties (Jeerakamunda, Panniyur-1, SV-18, SV-11 and IISR Thevam) along with a composite of all the five varieties, were subjected to enzyme combination treatments (pectinase, cellulase and acid protease at 300 U g^{-1}) and single-enzyme treatments (600 U g^{-1}) at elevated temperature (40 °C). The parameters evaluated included duration for complete peeling, recovery percentage, colour, bulk density and piperine content. Among the enzyme combinations, acid protease + pectinase at pH 5.0 showed superior efficiency in completing decortication within 1 day in Jeerakamunda, Panniyur-1 and IISR Thevam, compared with 10 days in controls. The highest recovery (42.75%) and bright white colour were achieved under these conditions. Temperature elevation to 40 °C reduced peeling time to 1day but occasionally caused uneven colouration and higher moisture levels compared to control. The highest recovery under temperature treatment (43.90%) occurred in Panniyur-1 treated with acid protease, while the maximum piperine content (1.27%) was recorded in pectinase-treated berries at pH 5.0. The results demonstrate that treatments with combination of enzymes at room temperature significantly enhance pericarp removal, product appearance, while reducing retting duration and offering a sustainable alternative to conventional processing for white pepper production.

Keywords: White pepper, enzyme synergy, pectinase, acid protease, temperature

Introduction
White pepper (*Piper nigrum* L.) is a valued spice known for its mild flavour and light colour, obtained by removing the pericarp of fully ripened pepper berries. Conventional retting of the berries in water for 10-14 days is time-consuming, unhygienic and environmentally problematic owing to foul odour and high effluent load (Rabinovich *et al.*, 2004) [6]. To overcome these drawbacks, enzyme-assisted retting has emerged as an efficient and eco-friendly alternative (Rosnah and Chan, 2014) [7].

The hydrolytic enzymes such as pectinase, cellulase and protease can effectively degrade cell-wall polysaccharides and protein matrices, resulting in faster pericarp loosening and improved product quality (Mustafa *et al.*, 2015) [5]. However, the efficacy of single enzymes often remains limited because of the complex structural composition of the pepper pericarp, which contains pectin, cellulose, hemicellulose and lignin. The combined use of complementary enzymes enhances substrate accessibility and accelerates hydrolysis through synergistic interactions (Zhang *et al.*, 2015) [10]. Enzyme synergy has been successfully applied in food and fibre industries to improve yield and surface quality, yet its application in white pepper processing remains insufficiently explored.

Temperature is another critical factor influencing enzyme kinetics and substrate solubilization. Moderate heating generally enhances reaction rates and permeability of the pericarp tissues. Optimizing both enzyme combinations and temperature is therefore essential to achieve efficient, uniform and high-quality decortication within minimal time.

The present investigation was undertaken to evaluate the effect of enzyme combinations and temperature treatments on the efficiency of pericarp removal in white pepper. The study aimed to identify suitable enzyme mixtures, determine optimal operating temperature and assess their influence on processing time, recovery, colour and piperine content across five commercial cultivars of *Piper nigrum* L.

The outcome is expected to establish a scientifically validated, farmer-friendly and sustainable enzymatic protocol for large-scale white pepper production.

Materials and Methods

Fully matured and ripened berries of five pepper varieties *viz.*, Jeerakamunda, Panniyur-1, SV-18, SV-11 and IISR Thevam, along with a composite sample comprising equal proportions of all five, were collected from farmers in Sirsi, Karnataka. The samples were cleaned and stored at -20 °C in the Department of Post-Harvest Technology, Central Laboratory, College of Horticulture, Bagalkot. Commercial enzymes were obtained from Enzyme Bioscience, Surat, with activities of neutral cellulase (43,500 U g^{-1}), neutral protease (55,000 U g^{-1}), acid protease (59,000 U g^{-1}) and pectinase (33,400 U g^{-1}). Enzyme activities were standardized according to treatment requirements.

All experiments were conducted in the Department of Plantation, Spices, Medicinal and Aromatic Crops, College of Horticulture, UHS Bagalkot, following a two-factor Factorial Completely Randomized Design (FCRD) with pepper varieties (Factor A) and enzyme treatments (Factor B), including a conventional retting control maintained for each variety.

Combination of enzyme treatment (300 U g^{-1})

Fully matured and ripened pepper berries of each variety (20 g per treatment) were subjected to enzymatic retting using combinations of neutral cellulase + pectinase at pH 7.4 and acid protease + pectinase at pH 5.0. Each enzyme was standardized to an activity of 300 U g^{-1} in 400 mL sodium phosphate buffer and 50 mL of the enzyme combination solution was used per treatment. Retting was performed at room temperature (25°C), to reduce microbial contamination, buffer solutions were sterilized by boiling and the pepper berries were surface-treated with 70% ethanol for 3 minutes prior to enzyme immersion in the buffer solution. For the control treatment, berries were soaked in 50 mL of water, which was replaced daily until the pericarp softened. The loosened outer skin was then manually removed by gentle rubbing, followed by thorough washing and sun-drying for 2-4 days in accordance with conventional white pepper processing practices.

Preparation of Sodium phosphate buffer

0.1 M Sodium phosphate buffers for pH 5.0, 7.4 and 8.5 were prepared using $NaH_2PO_4.2H_2O$ and Na_2HPO_4. For pH 7.4, 2.901 g $NaH_2PO_4.2H_2O$ and 11.555 g Na_2HPO_4 were dissolved in 1 L of distilled water; for pH 5.0, 15.387 g $NaH_2PO_4.2H_2O$ and 0.193 g Na_2HPO_4 and for pH 8.5, 0.271 g $NaH_2PO_4.2H_2O$ and 13.949 g Na_2HPO_4. The pH of each solution was checked and adjusted using NaOH or H_3PO_4 as required.

Preparation of enzyme solutions

The enzyme combination was standardized to an activity of 300 U g^{-1}, wherein 2.758 g of neutral cellulase and 3.593 g of pectinase were dissolved in 400 mL of buffer solution at pH 7.4. Similarly, the acid protease + pectinase combination was prepared by dissolving 2.033 g of acid protease and 3.593 g of pectinase in buffer (pH 5.0). Peeling progress was periodically monitored until complete pericarp removal was achieved.

Temperature-assisted enzyme treatment (600 U g^{-1} at 40 °C)

In this experiment, enzyme activity was increased to 600 U g^{-1} and treatments were conducted at a controlled temperature of 40°C using a laboratory water bath. Four commercial individual enzymes (neutral cellulase, neutral protease, acid protease and pectinase) were evaluated individually at three pH levels (5.0, 7.4 and 8.5).

Preparation of enzyme solutions

Enzyme solutions were prepared to achieve an activity of 600 U/g. For pH 7.4, 5.517 g of neutral cellulase, 4.363 g of neutral protease and 7.185 g of pectinase were added per 400 mL buffer. For pH 5.0, 4.067 g of acid protease and 7.185 g of pectinase were added, while for pH 8.5, 7.185 g of pectinase was used. Enzymes were initially dissolved in 300 mL of buffer and the final volume was adjusted to 400 mL to account for the volume increase after enzyme addition. Subsequently, 20 g of pepper berries from each variety were immersed in 50 mL of the enzyme solution and incubated in a water bath maintained at 40°C. The progression of pericarp softening was monitored daily until complete peeling was achieved. The buffer solutions were not sterilized with ethanol or heat, as the elevated temperature minimized microbial contamination. The duration for complete peeling was recorded based on visible pericarp removal.

Number of days for complete peeling

The time required for complete pericarp removal from matured and ripened pepper berries was recorded and expressed in days. Peeling was considered complete when most berries exhibited fully exposed white surfaces and the presence of detached pericarp flakes at the bottom of the flask confirmed full decortication.

Percentage recovery of white pepper

$$\text{Percentage recovery of white pepper} = \frac{\text{Final weight of the sample}}{\text{Initial weight of the sample}} \times 100$$

The recovery percentage of white pepper was determined by comparing the sample weight before and after processing, enabling a direct berry-to-berry evaluation of yield efficiency. The percentage recovery was calculated using the formula.

Colour variation in processed berries

The surface colour of the processed white pepper berries was visually evaluated and graded based on appearance as follows: '-' for black, '+' for brown, '++' for dirty white, '+++' for half white and '++++' for bright white, following the method described by Vinod *et al.* (2013) [8].

Moisture content (%)

The moisture content of the berries was determined using a digital moisture meter and the readings were automatically recorded. The results were expressed as a percentage on a fresh weight basis.

Bulk density (g L^{-1})

For bulk density measurement, dried white pepper berries were filled to the brim of a 25 mL measuring cylinder under

standard laboratory conditions, and the weight was recorded. Bulk density was calculated and expressed in grams per litre (g L^{-1}) after appropriate volume adjustment.

Piperine Analysis (RP-HPLC)

For detailed quality assessment, Panniyur-1 samples treated with pectinase (600 U g^{-1}) at pH 5.0 and 7.4 at room temperature, along with the control, were analyzed for piperine content using Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC). Piperine content in *Piper nigrum* oleoresin was quantified using Reverse-Phase High-Performance Liquid Chromatography (Kurangi (2020) [4]. The analysis was performed on a C18 column (250 mm × 4 mm, 5 µm) using a mobile phase of acetonitrile, water and acetic acid (60:39.5:0.5, v/v/v) under a gradient elution program for 30 minutes. The flow rate was maintained at 1.0 mL min^{-1}, with UV detection at 340 nm, a 10-minute run time and an injection volume of 20 µL. A standard piperine solution (97% purity) was prepared by dissolving 50 mg of piperine in 100 mL of solvent to obtain the stock solution, followed by serial dilution for calibration. Sample solutions were prepared by dissolving 101.23 mg of oleoresin extract in 25 mL of solvent and diluted appropriately to achieve concentrations within the linear detection range. Both standard and sample solutions were filtered through 0.45 µm membrane filters prior to injection. Piperine percentage was determined from chromatographic peak areas of the sampleand standard using the following equation:

$$\text{Piperine content (\%)} = \frac{A_{sp}}{A_s} \times \frac{C_s \times P}{C_{sp}} \times \frac{1}{\text{Dilution factor}} \times 100$$

Where,
Asp = Peak area of the sample
As = Peak area of the standard
Cs = Concentration of standard (mg/mL)
Csp = Concentration of sample (mg/mL)
P = Purity of standard (0.97)

Results and Discussion

Combination of enzyme treatment (300 U g^{-1})

Number of days for complete peeling

The shortest peeling duration (1day) was observed in Jeerakamunda (T_2), Panniyur-1 (T_5) and *IISR Thevam* (T_{14}) under the combined treatment of acid protease + pectinase at pH 5.0 (Fig. 1), demonstrating the efficiency of synergistic enzymatic hydrolysis for rapid pericarp removal. Slightly longer durations (1.2-1.5 days) were recorded in treatments such as T_1, T_{13}, and T_{17}. The longest duration among enzyme treatments (3.5 days) occurred in SV-18 (T_7, neutral cellulase + pectinase at pH 7.4), indicating varietal resistance to enzyme penetration. Control samples required up to 10 days for complete peeling due to slow microbial degradation during water retting, corroborating trends observed in earlier experiments.

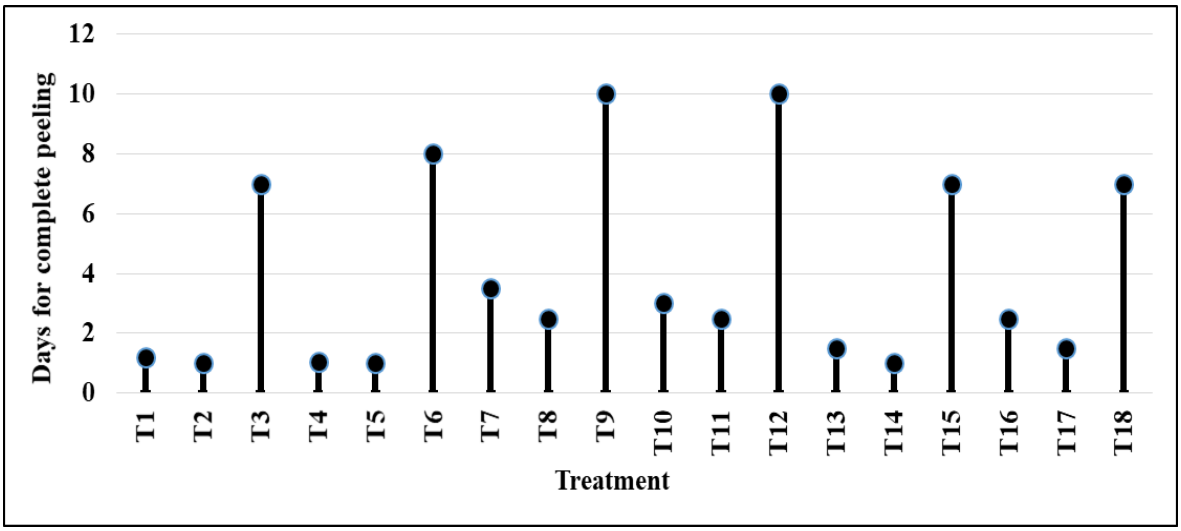


Fig 1: Days for complete peeling for white pepper production after treatment with combination of enzymes at 300 U/g

Where, T_1: Jeerakamunda berries retted with neutral cellulase + pectinase at pH 7.4; T_2: Jeerakamunda berries retted with acid protease + pectinase at pH 5; T_3: Jeerakamunda berries retted with water (control); T_4: Panniyur-1 berries retted with neutral cellulase + pectinase at pH 7.4; T_5: Panniyur-1 berries retted with acid protease + pectinase at pH 5; T_6: Panniyur-1 berries retted with water (control); T_7: SV 18 berries retted with neutral cellulase + pectinase at pH 7.4; T_8: SV 18 berries retted with acid protease + pectinase at pH 5; T_9: SV-18 berries retted with water (control); T_{10}: SV 11 berries retted with neutral cellulase + pectinase at pH 7.4; T_{11}: SV 11 berries retted with acid protease + pectinase at pH 5; T_{12}: SV-11 berries retted with water (control); T_{13}: IISR Thevam berries retted with neutral cellulase + pectinase at pH 7.4; T_{14}: IISR Thevam berries retted with acid protease + pectinase at pH 5; T_{15}: IISR Thevam berries retted with water (control); T_{16}: Composite varieties berries retted with neutral cellulase + pectinase at pH 7.4; T_{17}: Composite varieties berries retted with acid protease + pectinase at pH 5; T_{18}: Composite varieties berries retted with water (control).

Percentage Recovery of White Pepper

White pepper recovery improved markedly under enzyme combinations compared with controls. The highest recovery (42.75%) was obtained in Jeerakamunda (T_1, neutral cellulase + pectinase at pH 7.4), followed by T_5, T_2, T_{13}, T_{14} and T_{15} (Table 1). This enhancement can be attributed to the synergistic degradation of cellulose and pectin, which facilitated efficient pericarp separation and minimized seed loss. Mustafa *et al.* (2015) [5] reported that pectinase effectively weakens the middle lamella, improving yield,

while Rosnah and Chan, 2014 [7] observed that combined enzyme action promotes uniform peeling and reduces seed breakage. In contrast, the lowest recovery was observed in the control (T_9, SV-18 with water), likely due to incomplete and uneven pericarp removal during prolonged microbial retting, consistent with Aziz *et al.* (2018) [2] who associated conventional retting with poor yield efficiency.

Colour Variation

Bright white (++++) berries, considered the most desirable grade, were recorded in twelve treatments (T_2, T_3, T_5, T_6, T_7, T_9, T_{10}, T_{12}, T_{14}, T_{15}, T_{16} and T_{18}), including some controls. Half-white (+++) berries were seen in T_1, T_4, T_8, T_{11}, T_{13} and T_{17} (Table 1). The superior colour observed under pectinase-based combinations, particularly at acidic pH, can be attributed to efficient pericarp removal with minimal browning of the seed surface. Similar findings were reported by Aziz *et al.* (2018) [2], who emphasized that enzymatic retting under controlled pH yields brighter and more uniform white pepper than microbial retting, which often produces dull or uneven colour.

Moisture Content

The lowest moisture content (8.73%) was observed in T_{17} (composite varieties with acid protease + pectinase at pH 5.0), whereas the highest moisture content (10.70-10.71%) occurred in control treatments T_3 (Jeerakamunda) and T_{15} (IISR Thevam) (Table 2). Elevated moisture in control samples likely resulted from prolonged soaking and incomplete pericarp removal, which increased water retention. Conversely, enzymatic retting, especially with acid protease + pectinase, enhanced pericarp degradation, allowing quicker drying and lower residual moisture. These results align with Zhang *et al.* (2015) [10], who reported that enzyme-assisted retting accelerates drying efficiency by reducing bound water in plant tissues.

Bulk Density

Significant differences in bulk density were recorded among treatments (Table 2). The highest bulk density (585.35 g L^{-1}) was observed in T_1 (Jeerakamunda with neutral cellulase + pectinase at pH 7.4), while the lowest (536.61 g L^{-1}) occurred in the control T_{18} (composite varieties with water). Higher bulk density under enzymatic retting reflects more uniform pericarp removal, improved seed compactness and reduced inter-seed voids. In contrast, lower values in controls were associated with incomplete pericarp removal, uneven seed surface and higher moisture retention. Treatments showing greater recovery and heavier berry weight also exhibited correspondingly higher bulk density, consistent with Rosnah and Chan, 2014 [7], who linked enzymatic retting with enhanced physical quality traits of white pepper.

Table 1: Recovery percentage and colour of berries after combination of enzyme treatment at 300 U/g

S. No.	Treatment	Variety	Enzyme	pH	Recovery percentage (%)	Colour variation
1	T_1	Jeerakamunda	Neutral cellulase+Pectinase	7.4	42.75	+++
2	T_2		Acidic protease+Pectinase	5.0	42.35	++++
3	T_3		Control		40.25	++++
4	T_4	Panniyur-1	Neutral cellulase+Pectinase	7.4	39.55	+++
5	T_5		Acidic protease+Pectinase	5.0	42.55	++++
6	T_6		Control		40.05	++++
7	T_7	SV-18	Neutral cellulase+Pectinase	7.4	38.40	++++
8	T_8		Acidic protease+Pectinase	5.0	37.50	+++
9	T_9		Control		31.50	++++
10	T_{10}	SV-11	Neutral cellulase+Pectinase	7.4	32.50	++++
11	T_{11}		Acidic protease+Pectinase	5.0	34.35	+++
12	T_{12}		Control		33.15	++++
13	T_{13}	IISR Thevam	Neutral cellulase+Pectinase	7.4	41.25	+++
14	T_{14}		Acidic protease+Pectinase	5.0	40.75	++++
15	T_{15}		Control		41.05	++++
16	T_{16}	Composite varieties	Neutral cellulase+Pectinase	7.4	35.05	++++
17	T_{17}		Acidic protease+Pectinase	5.0	39.15	+++
18	T_{18}		Control		38.75	++++
	Mean				38.38	
	S.Em± (AxB)				1.15	
	C. D. @ 1% (AxB)				3.45	

Where,

'++' for dirty white coloured berries; '+++' for half white coloured berries; '++++' for bright white coloured berries

Table 2: Moisture content and bulk density of the berries after combination of enzyme treatment at 300 U/g

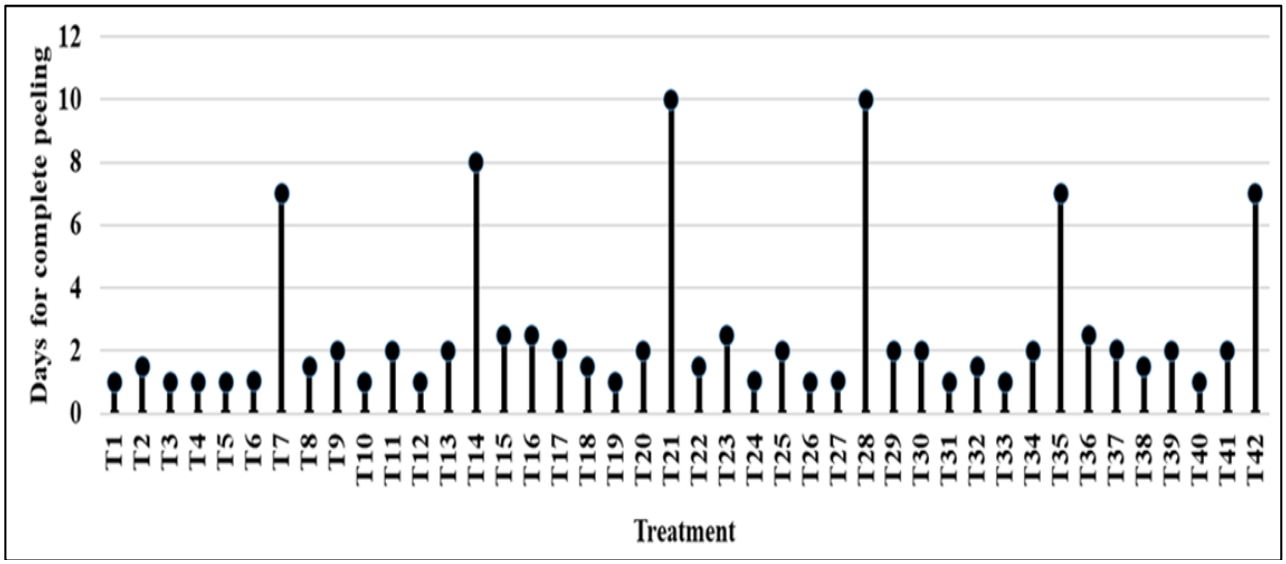
S. No.	Treatment	Variety	Enzyme	pH	Moisture content (%)	Bulk density (g/L)
1	T_1	Jeerakamunda	Neutral cellulase+Pectinase	7.4	10.30	585.35
2	T_2		Acidic protease+Pectinase	5.0	9.83	578.80
3	T_3		Control		10.71	573.60
4	T_4	Panniyur-1	Neutral cellulase+Pectinase	7.4	10.14	561.54
5	T_5		Acidic protease+Pectinase	5.0	9.33	552.62
6	T_6		Control		10.21	565.31
7	T_7	SV-18	Neutral cellulase+Pectinase	7.4	9.15	552.33
8	T_8		Acidic protease+Pectinase	5.0	8.82	547.35
9	T_9		Control		9.72	561.50

10	T_{10}	SV-11	Neutral cellulase+Pectinase	7.4	9.90	581.24
11	T_{11}		Acidic protease+Pectinase	5.0	9.61	575.81
12	T_{12}		Control		10.40	575.52
13	T_{13}	IISR Thevam	Neutral cellulase+Pectinase	7.4	10.54	575.43
14	T_{14}		Acidic protease+Pectinase	5.0	9.80	570.10
15	T_{15}		Control		10.70	543.31
16	T_{16}	Composite varieties	Neutral cellulase+Pectinase	7.4	9.41	549.50
17	T_{17}		Acidic protease+Pectinase	5.0	8.73	545.66
18	T_{18}		Control		9.50	536.61
	Mean				9.82	562.86
	S.Em± (AxB)				0.03	0.16
	C. D. @ 1%(AxB)				0.09	0.48

Temperature-assisted enzyme treatment (600 U g^{-1} at 40 °C)

Number of Days for Complete Peeling

The minimum peeling duration (1 day) was recorded in eleven treatments (T_1, T_3, T_4, T_5, T_{10}, T_{12}, T_{19}, T_{26}, T_{31}, T_{33}, T_{40}), followed by T_6, T_{24} and T_{27} (1.05 days). The longest duration (2.5 days) among enzyme treatments occurred in T_{15} (SV-18 with neutral cellulase at pH 7.4), T_{16} (SV-18 with neutral protease at pH 7.4), T_{23} (SV-11 with neutral protease at pH 7.4) and T_{36} (composite varieties with neutral cellulase at pH 7.4). The controls showed the longest peeling time (10 days) in T_9 (SV-18) and T_{12} (SV-11) due to microbial retting (Fig. 2). Rapid pericarp removal within one day in most pectinase-based treatments reflected the synergistic effect of enzyme activity and temperature, where heat facilitated tissue softening and enzyme penetration and pectinase and protease degraded the middle lamella and protein linkages. Longer peeling times in SV-18 and SV-11 under neutral conditions indicated varietal resistance and reduced enzyme efficiency.



Where, T_1: Jeerakamunda berries retted with neutral cellulase at pH 7.4, T_2: Jeerakamunda berries retted with neutral protease at pH 7.4, T_3: Jeerakamunda berries retted with acid protease at pH 5, T_4: Jeerakamunda berries retted with pectinase at pH 7.4, T_5: Jeerakamunda berries retted with pectinase at pH 5, T_6: Jeerakamunda berries retted with pectinase at pH 8.5, T_7: Jeerakamunda berries retted with water (control), T_8: Panniyur-1 berries retted with neutral cellulase at pH 7.4, T_9: Panniyur-1 berries retted with acid protease at pH 5, T_{10}: Panniyur-1 berries retted with pectinase at pH 7.4, T_{11}: Panniyur-1 berries retted with pectinase at pH 5, T_{12}: Panniyur-1 berries retted with pectinase at pH 8.5, T_{13}: Panniyur-1 berries retted with water (control), T_{14}: SV18 berries retted with neutral cellulase at pH 7.4, T_{15}: SV18 berries retted with neutral protease at pH 7.4, T_{16}: SV18 berries retted with acid protease at pH 5, T_{17}: SV18 berries retted with pectinase at pH 7.4, T_{18}: SV18 berries retted with pectinase at pH 5, T_{19}: SV18 berries retted with pectinase at pH 8.5, T20: SV18 berries retted with water (control), T21: SV11 berries retted with neutral cellulase at pH 7.4, T_{22}: SV11 berries retted with neutral protease at pH 7.4, T_{23}: SV11 berries retted with acid protease at pH 5, T_{24}: SV11 berries retted with pectinase at pH 7.4, T_{25}: SV11 berries retted with pectinase at pH 5, T_{26}: SV11 berries retted with pectinase at pH 8.5, T_{27}: SV11 berries retted with water (control), T_{28}: IISR Thevam berries retted with neutral cellulase at pH 7.4, T_{29}: IISR Thevam berries retted with neutral protease at pH 7.4, T_{30}: IISR Thevam berries retted with acid protease at pH 5, T_{31}: IISR Thevam berries retted with pectinase at pH 7.4, T_{32}: IISR Thevam berries retted with pectinase at pH 5, T_{33}: IISR Thevam berries retted with pectinase at pH 8.5, T_{34}: IISR Thevam berries retted with water (control), T_{35}: Composite varieties berries retted with neutral cellulase at pH 7.4, T_{36}: Composite varieties berries retted with neutral protease at pH 5, T_{37}: Composite varieties berries retted with acid protease at pH 7.4, T_{38}: Composite varieties berries retted with pectinase at pH 7.4, T_{39}: Composite varieties berries retted with pectinase at pH 5, T_{40}: Composite varieties berries retted with pectinase at pH 8.5, T_{41}: Composite varieties berries retted with water (control)

Fig 2: Days for complete peeling for white pepper production after treatment with enzymes at 600 U/g and 40 °C

Percentage recovery of white pepper

Percentage recovery showed limited variation among enzyme + temperature treatments but differed significantly across varieties. The highest recovery (43.90%) was observed in T_{10} (Panniyur-1 retted with acid protease at pH 5.0), while the lowest (29.55%) occurred in T_{26} (SV-11 retted with pectinase at pH 5.0), (Table 3). The superior recovery in Panniyur-1 was linked to its genotypic

adaptability and pericarp composition, which favour proteolytic degradation under acidic conditions. Lower recovery in SV-11 was likely due to varietal cell-wall rigidity and partial enzyme inactivation at higher temperature. These findings indicate that moderate temperature aids enzymatic hydrolysis in certain varieties but may also compromise enzyme stability. Recovery was positively associated with berry weight, supporting the interdependence of physical parameters and process efficiency.

Table 3: Recovery percentage and colour of the berries after treatment with enzymes at 600 U/g and 40 °C

S. No.	Treatment	Variety	Enzyme	pH	Recovery percentage (%)	Colour variation
1	T_1	Jeerakamunda	Neutral cellulase	7.4	38.85	+++
2	T_2		Neutral protease	7.4	40.30	++
3	T_3		Acid protease	5.0	42.05	+++
4	T_4		Pectinase	7.4	41.40	+++
5	T_5		Pectinase	5.0	41.35	+++
6	T_6		Pectinase	8.5	39.40	++
7	T_7		Control		40.25	++++
8	T_8	Panniyur-1	Neutral cellulase	7.4	40.05	+++
9	T_9		Neutral protease	7.4	41.25	+++
10	T_{10}		Acid protease	5.0	43.90	+++
11	T_{11}		Pectinase	7.4	38.95	+++
12	T_{12}		Pectinase	5.0	43.35	++++
13	T_{13}		Pectinase	8.5	40.65	++
14	T_{14}		Control		40.05	++++
15	T_{15}	SV-18	Neutral cellulase	7.4	35.55	++++
16	T_{16}		Neutral protease	7.4	32.35	++
17	T_{17}		Acid protease	5.0	33.65	++++
18	T_{18}		Pectinase	7.4	30.60	+++
19	T_{19}		Pectinase	5.0	33.15	++++
20	T_{20}		Pectinase	8.5	32.50	+++
21	T_{21}		Control		31.50	++++
22	T_{22}	SV-11	Neutral cellulase	7.4	31.15	+++
23	T_{23}		Neutral protease	7.4	30.05	+++
24	T_{24}		Acid protease	5.0	30.60	+++
25	T_{25}		Pectinase	7.4	30.60	+++
26	T_{26}		Pectinase	5.0	29.55	+++
27	T_{27}		Pectinase	8.5	30.50	+++
28	T_{28}		Control		33.15	++++
29	T_{29}	IISR Thevam	Neutral cellulase	7.4	38.45	+++
30	T_{30}		Neutral protease	7.4	38.70	++
31	T_{31}		Acid protease	5.0	39.90	+++
32	T_{32}		Pectinase	7.4	38.15	++
33	T_{33}		Pectinase	5.0	39.20	+++
34	T_{34}		Pectinase	8.5	37.65	++
35	T_{35}		Control		41.05	++++
36	T_{36}	Composite varieties	Neutral cellulase	7.4	36.85	++
37	T_{37}		Neutral protease	7.4	35.80	++
38	T_{38}		Acid protease	5.0	35.70	++
39	T_{39}		Pectinase	7.4	37.45	++
40	T_{40}		Pectinase	5.0	35.55	+++
41	T_{41}		Pectinase	8.5	38.00	++
42	T_{42}		Control		38.75	++++
	Mean				36.85	
	S.Em±(AxB)				1.11	
	C. D. @ 1% (AxB)				NS	

NS: Non-significant; where, '++' for dirty white coloured berries; '+++' for half white coloured berries; '++++' for bright white coloured berries

Colour Variation

Bright white (++++) berries were observed in ten treatments (T_7, T_{12}, T_{15}, T_{17}, T_{19}, T_{21}, T_{28}, T_{35}, T_{42}, T_{18}, including controls). Enhanced whiteness in treatments T_{12}, T_{15}, T_{17} and T_{19} resulted from selective hydrolysis of pectic substances and minimal oxidation of phenolics during enzymatic retting at acidic pH (Table 3). Conversely, treatments at pH 7.4 and 8.5 yielded half-white (+++) and dirty-white (++) berries due to incomplete pericarp removal and localized over-hydrolysis at 40 °C, which released phenolic pigments that oxidized and redeposited on the berry surface, producing dull coloration. Rosnah and Chan, 2014 [7] reported that enzymatic peeling minimizes colour deterioration compared with water retting. These results reinforce that enzyme type and pH are key factors for achieving uniform, bright white pepper that meets commercial quality standards.

Moisture Content

Moisture content varied among enzyme + temperature treatments. The lowest (5.48%) occurred in T_{12} (Panniyur-1 retted with pectinase at pH 5.0 under ambient conditions), while the highest (6.92%) was recorded in T_{40} (composite varieties retted with pectinase at pH 5.0 at 40 °C) (Table 4). Overall, temperature-assisted enzymatic retting produced slightly higher moisture levels than ambient treatments, likely due to heat-induced softening of the pericarp,

enhancing water absorption and retention. Rapid enzymatic action under elevated temperature may also limit water loss during drying. These findings align with Rosnah and Chan, 2014 [7], who observed that temperature-assisted enzymatic retting marginally increases moisture without compromising quality.

Bulk Density

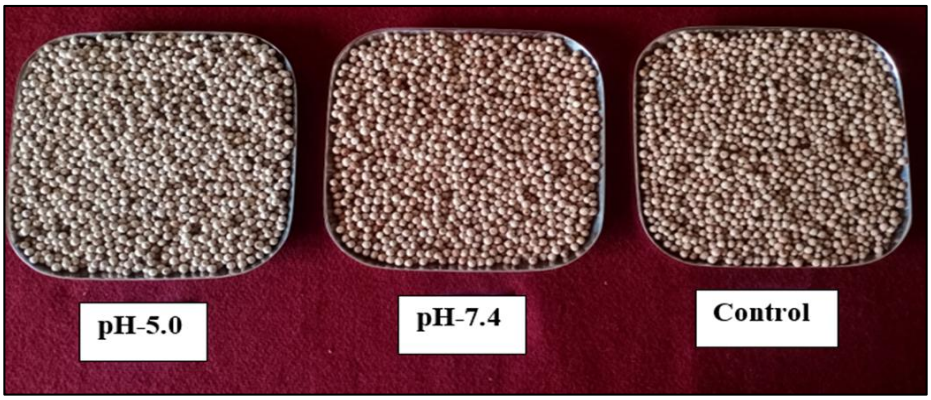
Bulk density values corresponded closely with berry weight and size. The highest (576.55 g L^{-1}) was recorded in T_{10} (Panniyur-1 retted with acid protease at pH 5.0), which also exhibited the heaviest berries (8.78 g). The lowest (536.61 g L^{-1}) was found in T_{42} (composite varieties retted with water) (Table 4). Higher bulk density in enzymatic treatments indicates more uniform pericarp removal and denser packing, while lower values in control and mixed-variety samples reflect irregular seed shape, incomplete decortication, and varietal composition dominated by lighter SV-18 berries. Vinod *et al.* (2013) [8] also reported that bulk density correlates with berry weight and varietal characteristics, regardless of the peeling method, underscoring that enzymatic retting primarily improves uniformity rather than intrinsic density traits.

Piperine content

The piperine content in Panniyur-1 berries was highest under pectinase treatment at pH 5.0 (1.27%) (Fig. 4), followed by the control (0.52%) and lowest at pH 7.4 (0.31%) (Table 5). Superior retention at acidic pH may be attributed to more effective pericarp degradation, which facilitated clean separation of the outer layers while protecting kernel constituents from leaching and oxidation. Wood *et al.* (1988) reported that piperine is highly sensitive to processing environments, emphasizing the need for optimized conditions to minimize degradation. Similarly, Ashari *et al.* (2014) and Zhang *et al.* (2015) [10] demonstrated that enzymatic retting under controlled acidic conditions preserves key biochemical constituents, including piperine, compared with conventional water retting. Minor variations among treatments may also result from post-harvest drying and handling differences that influence compound stability.

Table 4: Moisture content and bulk density of the berries after treatment with enzymes at 600 U/g and 40ºC

S. No.	Treatment	Variety	Enzyme	pH	Moisture content (%)	Bulk density (g/L)
1	T_1	Jeerakamunda	Neutral cellulase	7.4	11.41	569.52
2	T_2		Neutral protease	7.4	10.90	569.30
3	T_3		Acid protease	5.0	10.52	565.36
4	T_4		Pectinase	7.4	10.85	568.71
5	T_5		Pectinase	5.0	10.40	564.84
6	T_6		Pectinase	8.5	10.83	565.00
7	T_7		Control		10.71	573.60
8	T_8	Panniyur-1	Neutral cellulase	7.4	10.90	563.71
9	T_9		Neutral protease	7.4	10.55	562.82
10	T_{10}		Acid protease	5.0	10.00	576.55
11	T_{11}		Pectinase	7.4	10.31	562.51
12	T_{12}		Pectinase	5.0	9.92	576.10
13	T_{13}		Pectinase	8.5	10.10	562.02
14	T_{14}		Control		10.21	565.31
15	T_{15}	SV-18	Neutral cellulase	7.4	10.30	544.71
16	T_{16}		Neutral protease	7.4	9.84	543.56
17	T_{17}		Acid protease	5.0	9.51	542.40
18	T_{18}		Pectinase	7.4	9.90	543.24
19	T_{19}		Pectinase	5.0	9.11	542.00
20	T_{20}		Pectinase	8.5	9.83	542.32
21	T_{21}		Control		9.72	561.50
22	T_{22}	SV-11	Neutral cellulase	7.4	10.92	562.35
23	T_{23}		Neutral protease	7.4	10.65	561.70
24	T_{24}		Acid protease	5.0	10.20	574.00
25	T_{25}		Pectinase	7.4	10.44	575.32
26	T_{26}		Pectinase	5.0	10.20	573.25
27	T_{27}		Pectinase	8.5	10.51	574.33
28	T_{28}		Control		10.40	575.52
29	T_{29}	IISR Thevam	Neutral cellulase	7.4	11.04	566.55
30	T_{30}		Neutral protease	7.4	10.62	565.83
31	T_{31}		Acid protease	5.0	9.91	564.51
32	T_{32}		Pectinase	7.4	10.33	565.14
33	T_{33}		Pectinase	5.0	9.50	564.93
34	T_{34}		Pectinase	8.5	10.35	565.35
35	T_{35}		Control		10.70	543.31
36	T_{36}	Composite varieties	Neutral cellulase	7.4	9.82	548.94
37	T_{37}		Neutral protease	7.4	9.50	548.50
38	T_{38}		Acid protease	5.0	9.41	546.63
39	T_{39}		Pectinase	7.4	9.52	546.23
40	T_{40}		Pectinase	5.0	8.90	545.82
41	T_{41}		Pectinase	8.5	9.35	546.70
42	T_{42}		Control		9.50	536.61
	Mean				10.18	559.91
	S.Em± (AxB)				0.03	0.16
	C. D. @ 1% (AxB)				0.08	0.48



White pepper production from Panniyur-1 using pectinase (600U/g) at pH 5.0, pH 7.4 and Control

Table 5: Piperine content of Panniyur-1 berries after treatment with pectinase (600U/g) at pH 5.0 and 7.4

S. No.	Enzyme	Sample description	Results% (w/w)
1	Pectinase at pH 5.0	Orange coloured semi solid in an eppendorf tube	1.27
2	Pectinase at pH 7.4		0.31
3	Control	Light orange coloured semi solid in an eppendorf tube	0.52

Chromatographic validation through RP-HPLC confirmed the retention trends, with a standard piperine peak at 6.423 min (97% purity) (Fig. 3). The treatment at pH 5.0 exhibited the highest peak area (1.14 × 10^6 units), indicating greater piperine concentration, whereas the lowest peak area (2.79 × 10^5 units) was recorded at pH 7.4. These results reaffirm that acidic enzymatic retting promotes better piperine preservation, while neutral conditions accelerate degradation, consistent with the findings of Wood *et al.* (1988) and Zhang *et al.* (2015) [10].

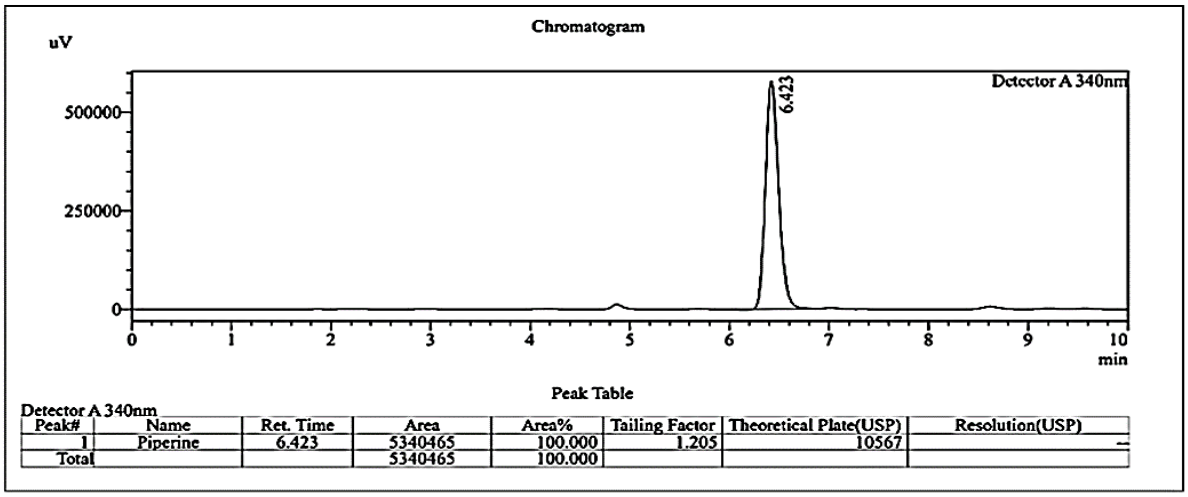


Fig. 3: Chromatogram of isolated piperine from Standard sample

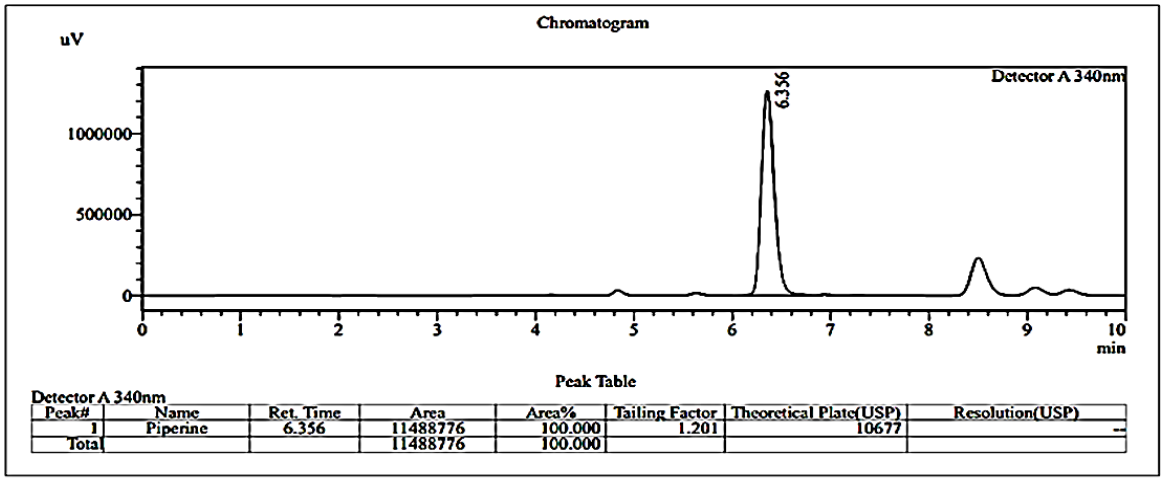


Fig. 4: Chromatogram of isolated piperine in Panniyur-1 after treatment with pectinase (600 U/g) at pH 5.0

Conclusion

Combination of enzymes offers a superior and sustainable alternative to conventional water retting for white pepper production. The combination of acid protease + pectinase at pH 5.0 proved most effective across varieties, achieving complete pericarp removal within 1day while maintaining desirable recovery, berry weight, moisture and bulk density characteristics. Temperature-assisted enzymatic treatments at 40 °C reduced peeling duration to one day but resulted in slightly inferior colour attributes, nonetheless, acid protease at pH 5.0 and pectinase at pH 5.0 under thermal conditions exhibited superior recovery and early peeling, respectively. Overall, the optimized enzymatic combinations demonstrated strong potential for large-scale, eco-friendly white pepper processing, offering improved efficiency, product quality and reduced environmental impact compared to traditional retting methods.

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Reference

1. Ashari MF, Ibrahim MD, Husaini A, Zulkharnain A. Accelerated production of white pepper using integrated mechanical and enzymatic solutions in an automated machine. Key Eng Mater. 2014;572:304-7.
2. Aziz NS, Sofian-Seng NS, Mohd RNS, Lim SJ, Mustapha WA. A review on conventional and biotechnological approaches in white pepper production. J Sci Food Agric. 2018;99(6):2665-76.
3. Bhat DS, Hegde L, Narayanpur VB, Hegde NK, Shet RM, Rajashekhara E. Studies on improvement in black pepper (*Piper nigrum*) through selection, hybridization and mutation breeding. [PhD thesis]. Bagalkot (India): Univ. Hort. Sci.; 2023.
4. Kurangi B, Jalalpure S. A validated stability-indicating RP-HPLC method for piperine estimation in black pepper, marketed formulation and nanoparticles. Indian J Pharm Educ Res. 2020;54(3):677-86.
5. Mustafa DSB A, Zulkharnian AB, Husaini AAS BA. Enzymatic retting of *Piper nigrum* L. using commercial Pectinase (Peelzyme). J Chem Pharma Sci. 2015;8(2):360-364.
6. Rabinovich ML, Bolobova AV, Vasilchenko LG. Fungal decomposition of natural aromatic structures and xenobiotics: a review. Appl Biochem Microbiol. 2004;40:1-17.
7. Rosnah S, Chan SC. Enzymatic retting of green pepper berries for white pepper production. Int Food Res J. 2014;21(1):237-45.
8. Vinod V, Kumar A, Zachariah TJ. Isolation, characterization and identification of pericarp-degrading bacteria for the production of off-odour-free white pepper from fresh berries of *Piper nigrum* L. J Appl Microbiol. 2013;116:890-902.
9. Wood AB, Barrow ML, James DJ. Piperine determination in pepper (*Piper nigrum* L.) and its oleoresins: a reversed phase high-performance liquid chromatographic method. Flavour Fragr J. 1988;3:55-64.
10. Zhang R, Feng J, Dou Z, Xie H, He A. Optimization of the conditions for pepper soaking peeling by enzymatic method. Indian J Biochem. 2015;9(6):244-51.