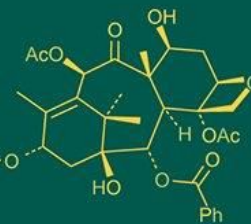
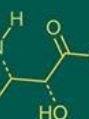
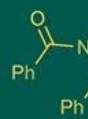


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Valorisation of organic waste through bioenzyme production: A physico-chemical and biochemical characterisation study

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Abstract

The present study includes the preparation of bioenzyme from peels of citrus and banana, leaves of neem and remnants of turmeric using a fermentation ratio of 1:3:10 (organic waste: jaggery: water). After the fermentation period, the prepared bioenzymes were analysed for key physicochemical parameters. Physical parameters such as pH, odour and total dissolved solids (TDS) to assess the fermentation quality and stability of the product. In addition, the phytochemical composition was determined by estimating alkaloids (mg/g AE), flavonoids (mg/g QE), phenols (mg/g GAE), and saponins (mg/g SE), which are known for their bioactive properties. Furthermore, the enzymatic profile of the bioenzymes was evaluated through enzyme assays to detect the presence of lipase, protease, amylase and cellulase which play an important role in the degradation of organic materials during fermentation. Understanding these properties is fundamental to evaluating the functional efficiency, stability and environmental applicability of bioenzyme-based products, which have emerged as promising alternatives to conventional chemical inputs in agricultural and environmental remediation contexts.

Keywords: Bioenzyme, fermentation, phytochemicals, enzyme activity, organic waste, sustainability

1. Introduction

In recent years growing interest in sustainable and eco-friendly technologies has encouraged the exploration of biological alternatives derived from natural resources. Bioenzymes are biologically derived catalytic compounds produced through the natural decomposition and fermentation of organic substances. Unlike synthetic chemical catalysts, organic bioenzymes are eco-friendly, biodegradable and rich in diverse enzymatic constituents. The present study investigates organic bioenzymes preparation that involves collecting organic waste from kitchens, vegetable markets and juice shops, followed by shredding to reduce particle size and then the shredded waste is mixed with jaggery and water in a 1:3:10 ratio and fermented in airtight plastic containers with screw caps to allow controlled gas release. Understanding the physicochemical properties of bioenzymes is essential for evaluating their composition, stability and potential applications. The characterization of physicochemical parameters such as alkaloids, flavonoids, phenols and saponins is important for understanding the biochemical environment of the fermented product and the stability of enzymatic activity. Phenols and flavonoids are known for their strong antioxidant and antimicrobial properties, helping to inhibit microbial growth and enhance the stability of the fermented product. Alkaloids possess antimicrobial and bioactive characteristics that can interfere with microbial metabolism, while saponins exhibit natural surfactant properties that aid in the breakdown and emulsification of organic substances. Therefore, analysing these constituents helps in evaluating the antimicrobial potential, biochemical activity and practical applications of bioenzymes, while also providing scientific insight into their role in the valorization and sustainable utilization of organic waste materials. This study aims to provide a standardized understanding of how organic fermentation products can be utilized as effective, low-cost, and environmentally benign antimicrobial barriers in various domestic and professional settings. Furthermore, the production of bioenzymes represents an effective strategy for the utilization of organic waste materials.

The escalating global challenge of organic waste accumulation necessitates innovative strategies for sustainable management with bioenzyme production emerging as a promising valorisation pathway. This approach not only mitigates environmental pollution associated with waste disposal but also generates value-added products with diverse industrial applications. The production of these bioenzymes from organic waste represents a significant contribution to a circular economy, transforming waste into valuable resources. This study evaluates properties of bioenzyme as a multifunctional cleanser while emphasizing its role in organic waste valorization and mitigation of greenhouse gas emissions associated with landfill disposal.

2. Materials and Methods

2.1 Preparation of Bioenzymes

For the preparation of bioenzymes fruit peels of citrus and banana were collected from vegetable markets and juice shops, neem leaves were collected from near by area and remnants of turmeric was collected from an experimental field of Department of Vegetable Science of Dr. YSP UHF, Nauni, Solan (HP), India. The organic waste were further divided into smaller pieces in order to expand the surface area of the reaction. The bioenzyme mixture was prepared using a 1:3:10 ratio of jaggery, organic waste and water, respectively. Accordingly, jaggery (10 g), peels (30 g) and water (100 mL) were added to an airtight plastic container and mixed thoroughly. Yeast (*Saccharomyces cerevisiae*) was also added to the mixture in order to reduce the fermentation time. Gases were produced inside the container during fermentation; the lid was opened every day for a minute during the first week and then closed again to release the pressure that had built up. After a few days, the gases significantly decreased, so the venting interval was gradually increased. Following a three-month fermentation period, filtration was done to remove undigested or solid debris from the liquid portion in order to improve the characteristics of the bioenzyme and it was kept in an airtight jar.



Fig 1: Bio-enzymes after the completion of a three-month anaerobic fermentation period

2.2. Physico-chemical properties of Bioenzymes

The physical characteristics of bio-enzymes prepared from citrus, banana, turmeric and neem organic waste like colour, odour, pH, turbidity and total dissolved solids (TDS) were studied by sight, smell, pH meter, Turbidity meter and TDS meter which provide preliminary information about the nature and quality of the fermented extracts.

Enzymatic activity like cellulase, protease, lipase and amylase was assessed in order to understand bioenzymes enzymatic composition and biodegradation potential.

2.2.1 Cellulase enzyme: 1% Carboxy Methyl Cellulose sodium salt was prepared and 20 mL sample was poured per plate into sterile Petri dishes. 0.3% Congo red solution was added to the enzyme plate as an indicator. Flooding with 1N NaCl as a destaining solution was done. Muruganandam *et al* (2022) ^[8].

2.2.2 Protease enzyme: Protease agar (1.5%) was prepared with 1% (w/v) Casein as substrate. The plates were then incubated at 37 °C for 24-48 hours. Plates were stained with methylene blue and zone of clearance was observed. Proteinase K was used as positive control (0.03gm/5ml). Samriti *et al* (2019) ^[13].

2.2.3 Amylase enzyme: 1% starch was prepared. Wells of 4mm diameter were made, and 50µl of sample enzyme was inoculated into each well. Flooding with iodine solution was done. The hydrolysis of starch was indicated by the appearance of clear zones surrounding the wells against deep blue brown. Neupane and Khadka (2019) ^[12].

2.2.4 Lipase enzyme: Nutrient Agar 50ml with 1% Tween 20 was prepared. 350µl of sample was poured into the wells. The plates were kept for incubation at 37 °C for 24 hours. Lipase activity will be seen by the presence of precipitation around the wells. Pabba *et al* (2024) ^[2].

Biochemical composition of the prepared bioenzymes were done, indicating the presence and quantity of alkaloids, flavonoids, phenols and saponins:

2.2.5 Alkaloid (mg of AE/g): The plant extract of 1ml was denatured in DMSO and then 1 ml of 2N HCl was added and filtered. This solution was transferred to a separation funnel and added 5 ml of bromocresol green solution and 5 ml of phosphate buffer. The mixture was shaken well with and diluted to the volume with chloroform. The absorbance of mixture was measured in 470 nm (Lahare *et al.* 2021) ^[11].

2.2.6 Flavonoid (mg/QE/g): 10 mg quercetin made up to 10 ml with methanol in a volumetric flask. From the above solution (1mg/ml), 1 ml was pipetted out and made up to 10 ml with methanol to get 100 mcg/ml quercetin standard solution (stock solution). From the stock solution, solutions of concentration 25, 50, 75, 100, 125 and 150 mcg/ml were prepared. To each of these 4 ml water was added followed by 0.3 ml of 5% sodium nitrite. After 5 minutes 0.3 ml of 10% aluminium chloride solution and at the 6th minute 2 ml of 1M sodium hydroxide was added. The total volume was made up to 10 ml with distilled water. A blank was prepared without addition of aluminium chloride solution. The absorbance was measured against the blank at 510 nm using UV-Visible spectrophotometer. (Kavitha and Indira 2016) ^[16].

2.2.7 Phenols (mg of GAE/g): 1 ml of extract was added to 9 ml of distilled water to which 1 ml of Folin-Ciocalteu phenol reagent was added and the mixture was shaken well. Then after 5 minutes 10 ml of 7% sodium carbonate solution was added to the mixture. The standard solutions of gallic acid were prepared in concentrations range of (20, 40, 40, 60, 80 and 100) µg/ml. The mixture was incubated for 90 minutes at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Ultraviolet UV-Visible

spectrophotometer. Total phenol content was expressed as mg of GAE/g of extract (Lahare *et al.* 2021)^[11].

2.2.8 Saponins (mg of SE/g): 20 g of extract and 100 ml of 20% ethanol was added into conical flask. The sample was heated for 4 hours at 55 °C. The mixture was then filtered and the residue re-extracted with another 200 ml of 20% ethyl alcohol. The combined extracts were reduced to 40 ml over a water bath at about 90 °C. The concentrate was then transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added to the extract and vigorously shaken. The aqueous layer was recovered while the diethyl ether layer was discarded and the purification process was repeated. 60 ml of n-butanol was added and the combined n-butanol extracts was washed twice with 10 ml of 5% sodium chloride. The remaining solution was then heated in a water bath and after evaporation; the samples were dried in the oven to a constant weight and values were expressed as mg/g of extract (Roghini and Vijayalakshmi 2018)^[14].

3. Result & Discussion

3.1 Physical parameters of bio-enzymes

Table 1 presents the physical characteristics of bio-enzymes prepared from citrus, banana, turmeric and neem organic waste. These parameters, including colour, odour, pH and total dissolved solids (TDS) provide preliminary information about the nature and quality of the fermented extracts.

Table 1: Physical parameters of bio-enzymes

Parameters	Bioenzymes			
	Citrus	Banana	Turmeric	Neem
Colour	Light Brown	Light yellow	Bright yellow to golden yellow	Green to yellowish-green
Odour	Citrusy	Sweet	Alcoholic	Slightly bitter
pH	3.87	5.6	4.78	4.21
TDS (mg/l)	1680	2488	1264	1104

The citrus bio-enzyme exhibited a light brown colour, which can be attributed to the presence of phenolic compounds and tannins naturally occurring in citrus peels. The turmeric bio-enzyme initially showed a light yellow colour due to the presence of curcuminoid pigments, which gradually turned brown as fermentation progressed. The banana bio-enzyme appeared light yellow, which is associated with the high carbohydrate, natural sugars and phenolic content of banana peels. In contrast, the neem bio-enzyme displayed a light green colour, likely due to the presence of phenolic compounds and limonoids inherent to neem leaves.

As per the data shown in Table 1 the citrus bio-enzyme exhibited a characteristic citrusy aroma and banana bio-enzyme produced a mildly sweet odour, likely due to the fermentation of natural sugars present in banana peels. The turmeric bio-enzyme exhibited an alcoholic odour, which may be associated with active microbial fermentation and ethanol formation, a characteristic commonly reported in bio-enzymes derived from carbohydrate-rich substrates and the neem bio-enzyme emitted a slightly bitter odour, possibly due to the presence of bitter limonoids and sulfur-containing compounds. The pH analysis of the bio-enzymes (Table 1) revealed that all formulations were acidic to mildly acidic in nature, primarily due to the accumulation of organic acids during microbial fermentation. Citrus bio-

enzyme exhibited the lowest pH (3.87) indicating higher acidity. The study showed that banana bio-enzyme recorded the highest TDS value of 2488 mg/l, indicating a greater release of soluble sugars, organic acids, and minerals. Citrus bio-enzyme exhibited a moderate TDS of value of 1680 mg/l, likely due to the presence of soluble organic acids and phenolic compounds released from citrus peels during fermentation. Turmeric bio-enzyme showed a comparatively lower TDS value (1264 mg/l) reflecting moderate dissolution of curcuminoids and other fermentation by-products. Neem bio-enzyme recorded the lowest TDS value (1104 mg/l), which may be associated with lower soluble sugar content and the predominance of secondary metabolites such as limonoids and phenolics.

3.2 Biochemical parameters of bioenzymes

Table 2 shows the biochemical composition of the prepared bio-enzymes, indicating the presence and quantity of alkaloids, flavonoids, phenols and saponins. These constituents help to explain the potential antimicrobial and treatment efficiency of the bioenzymes.

Table 2: Biochemical parameters of bioenzymes

Bioenzyme	Alkaloids (mg/g AE)	Flavonoids (mg/g QE)	Phenols (mg/g GAE)	Saponins (mg/g SE)
Citrus	0.8	18.3	20.3	7.1
Banana	0.5	14.7	28.9	6.8
Turmeric	1.5	24.6	32.0	6.5
Neem	2.8	12.0	18.0	10.2

Where-

AE: Atropine equivalents; QE: Quercetin equivalents; GAE: Gallic acid equivalents; SE: Saponin equivalents

The alkaloid content of the different bioenzymes was estimated and expressed as atropine equivalents (AE), as presented in Table 2. Among the tested bio-enzymes, neem bio-enzyme exhibited the highest alkaloid content (2.8 mg AE/g), followed by turmeric (1.5 mg AE/g), citrus (0.8 mg AE/g), and banana bio-enzyme (0.5 mg AE/g). This indicates that neem bio-enzyme is a comparatively richer source of alkaloids than the other bio-enzymes studied.

The flavonoid content of the bio-enzymes, expressed as quercetin equivalents (QE), varied notably among the samples. Turmeric bio-enzyme showed the highest flavonoid content (24.6 mg QE/g), closely followed by Citrus (18.3 mg QE/g), banana (14.7 mg QE/g), and neem bio-enzyme (12 mg QE/g). The elevated flavonoid concentration in citrus bio-enzyme highlights its strong antioxidant potential. The saponin content of the bio-enzymes, expressed as saponin equivalents (SE) It was found to be highest in neem bio-enzyme (10.2 mg SE/g), followed by citrus (7.1 mg SE/g), banana (6.8 mg SE/g) and turmeric bio-enzyme (6.5 mg SE/g). Citrus and banana bio-enzymes showed moderate saponin levels, possibly due to the presence of glycosidic compounds in fruit peels, while turmeric bio-enzyme exhibited comparatively lower but appreciable saponin content.

3.2 Enzymatic activity of bioenzymes

Table 3 shows the presence of various enzymes in bioenzymes in order to understand bioenzymes enzymatic composition and biodegradation potential.

Table 3: Enzymatic activity of bioenzymes:

Bioenzyme	Enzyme Activity			
	Cellulase	Lipase	Protease	Amylase
Citrus	+ ve	+ ve	+ ve	+ ve
Banana	+ ve	+ ve	+ ve	+ ve
Turmeric	+ ve	+ ve	+ ve	+ ve
Neem	+ ve	+ ve	+ ve	+ ve

From Table 3 it is observed that cellulase, protease, lipase and amylase were present in all bioenzymes. These enzymatic properties indicate broader industrial relevance beyond antimicrobial applications. For instance, proteases have proven uses in food processing and pharmaceutical formulations, while cellulase and lipases are widely employed in waste degradation and detergent industries.

4. Conclusion

The present study was undertaken to investigate the physicochemical properties of bioenzyme with particular emphasis on the 1:3:10 formulation ratio. Understanding these properties is fundamental to evaluating the functional efficiency, stability and environmental applicability of bioenzyme-based products, which have emerged as promising alternatives to conventional chemical inputs in agricultural and environmental remediation contexts. The research further suggests that scaling these fermentation processes could significantly decrease reliance on hazardous disinfectants, thereby advancing the implementation of zero-waste frameworks in household and industrial cleaning sectors. Future research should prioritise the standardisation of fermentation protocols to ensure consistent phytochemical profiles and to further explore the synergistic effects of varying organic feedstocks on long-term antimicrobial stability. Moreover, examining the regulatory and economic implications of industrial-scale production remains a critical step for bridging the gap between laboratory-scale feasibility and widespread market adoption. Furthermore, the exploration of enzyme immobilisation techniques could enhance the operational stability and reusability of these bio-catalysts, facilitating their deployment in complex industrial environments.

References

- Lubis N, Damayanti R, Wardani S. Antimicrobial activity of eco-enzymes with various dilutions as a natural disinfectant. *J Penelit Pendidik IPA*. 2025;11(5):506-513. doi:10.29303/jppipa.v11i5.11052
- Pabba M, Veena G, Patil AM, Krishna S, Sau N. Exploring fruit peels for ecofriendly bio-enzymes: synthesis, properties and sustainable applications. *Food Sci Nutr Technol*. 2024;9:1-10. doi:10.23880/fsnt-16000338
- Sinha U, Kumari S, Shakil S, Singh S. Wastewater treatment using bio-enzymes extracted from fruit peels. *J Young Sci*. 2024;14:24-30.
- Salah AN, Alwabsi HA. An overview of the antimicrobial activity of some microbial enzymes. *Am J Biochem Biotechnol*. 2024;20(2):140-150. doi:10.3844/ajbbsp.2024.140.150
- Kapadi PS, Desai VH, Mallya S, Sardesai Y. Estimation of antimicrobial efficacy of bio-enzyme extracts. *Indian J Appl Microbiol*. 2022;24(1):21-31. doi:10.46798/ijam.2021.v24i01.3
- Lakra P, Saina SK, Saini A. Synthesis, physio-chemical analysis and applications of bio-enzymes based on fruit and vegetable peels. *J Emerg Technol Innov Res*. 2022;9(9):670-680.
- Rungta S, Ojha A, Mishra SK. Study of physico-chemical properties of bio-enzymes produced from organic household waste and their application. *Discovery*. 2022;58:228-234.
- Muruganandam AR, Sivagurunathan AK, Senthilkumar S, Venkatasubramanian S, Veerabhuvaneshwari V. Preliminary analysis and applications of bio-enzymes from fermented vegetable and fruit peel wastes. *Indian J Nat Sci*. 2022;13(73):1-8.
- Kumar A, Sadhya HK, Ahmad E, Dulawat S. Application of bio-enzyme in wastewater (greywater) treatment. *Int Res J Eng Technol*. 2020;7(5):2886-2890.
- Mavani HAK, Tew IM, Wong L, Yew HZ, Mahyuddin A, Ghazali RA, *et al*. Antimicrobial efficacy of fruit peel eco-enzyme against *Enterococcus faecalis*: an *in vitro* study. *Int J Environ Res Public Health*. 2020;17(14):1-12. doi:10.3390/ijerph17145107
- Lahare RP, Yadav HS, Bisen YK, Dashahre AK. Estimation of total phenol, flavonoid, tannin and alkaloid content in different extracts of *Catharanthus roseus*. *Sch Bull*. 2021;7(1):1-6.
- Neupane K, Khadka R. Production of garbage enzyme from fruit and vegetable wastes and evaluation of enzymatic and antimicrobial efficacy. *Tribhuvan Univ J Microbiol*. 2019;6(1):113-118. doi:10.3126/tujm.v6i0.26594
- Samriti, Sarabhai S, Arya A. Garbage enzyme: compositional analysis of kitchen waste ferments. *Pharma Innov J*. 2019;8(4):1193-1197.
- Roghini R, Vijayalakshmi K. Phytochemical screening and quantitative analysis of flavonoids and minerals in ethanolic extract of *Citrus paradisi*. *Int J Pharm Sci Res*. 2018;11:4859-4864.
- Saramanda G, Kaparapu J. Antimicrobial activity of fermented citrus fruit peel extract. *J Eng Res Appl*. 2017;7(11):25-28.
- Kavitha C, Indira G. Quantitative estimation of total phenolic, flavonoids, tannin and chlorophyll content of *Strobilanthes kunthiana*. *J Med Plants Stud*. 2016;4(4):282-286.