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Bio-Refining Fruit Residues: Dual Recovery of Industrial Pectinase and Antioxidant Carotenoids from *Aspergillus niger* and Papaya Waste

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Abstract

This research establishes a sustainable circular bio-economy model for the dual recovery of industrial enzymes and high-value antioxidants from fruit residues. By utilizing orange peels as a substrate, the indigenous fungus *Aspergillus niger* (Isolate A1) was identified as a highly efficient pectinase producer, exhibiting a robust 18.6 mm hydrolysis zone. This laboratory-produced pectinase was subsequently applied to papaya (*Carica papaya* L.) waste to enhance lycopene extraction through enzymatic degradation of the complex cell wall matrix. Optimization trials revealed that a 2% enzyme concentration and a 3-hour incubation period maximized lycopene yield at 15.1 mg/100g, while a ternary solvent system (Hexane:Acetone:Ethanol) achieved a 94% extraction efficiency. Comparative analysis demonstrated that this enzyme-assisted extraction (EAE) method delivers a remarkable 138.7% increase in lycopene recovery over traditional mechanical methods. These findings confirm that integrating microbial bioprocessing with agricultural waste valorization provides an eco-friendly and cost-effective strategy for the functional food and pharmaceutical industries, transforming low-value residues into potent industrial biocatalysts and therapeutic pigments.

Keywords: *Aspergillus niger*, Bio-refining, Carotenoids, (EAE), Lycopene, Papaya waste, Pectinase, Waste valorization.

1. Introduction

Think of pectinases as nature's own "molecular keys" that gently unlock the goodness hidden inside plants. While they're industrially famous for making fruit juices crystal clear and refining textiles, their real magic lies in how they replace harsh, toxic chemicals like calcium carbide used for ripening [1]. By using friendly microbes like *Aspergillus niger* to soften fruit and boost its natural flavor, we're not just making food production more efficient—we're choosing a safer, more sustainable path that respects both the environment and our own health [2].

Pectinases are the heavy hitters of the food world, making up a quarter of the entire global enzyme market because of how efficiently they break down plant structures [3, 4]. While plants produce them naturally, we mostly rely on helpful microbes like *Aspergillus niger* to do the heavy lifting because they are incredibly productive and cost-effective [5]. These enzymes are the secret behind crystal-clear juices, smooth wines, and even the effortless peeling of fruit. By turning simple orange peels into a powerhouse for enzyme production and using smart math to maximize every drop, this process proves we can create high-quality food products while being much kinder to the planet [6, 7].

Carotenoids are nature's colorful bodyguards, acting as essential pigments that protect our health by neutralizing harmful free radicals and reducing oxidative stress [9]. Since our bodies cannot produce them, we rely on our diet to provide these potent antioxidants, which are crucial in defending against modern "civilization diseases" like cancer and heart disease [10, 11]. By keeping our internal chemistry in balance, these nutrients serve as a vital, natural shield against chronic illness and cellular damage.

Lycopene is the vibrant red pigment that gives fruits like papaya and tomatoes their color, but it serves as much more than just a natural dye [12]. As a powerful antioxidant, it acts like a cellular shield, neutralizing harmful free radicals to protect our hearts from cholesterol buildup and artery disease [13]. It has even been linked to a lower risk of certain cancers and vision loss, making it a vital nutrient for long-term wellness. By focusing on extracting lycopene from papaya, this study aims to unlock new ways to harness these protective benefits for human health [14].

Papaya (*Carica papaya* L.) is a global tropical staple, with India leading production, yet it faces a staggering waste problem—up to 70% of some varieties are lost after harvest due to their short shelf life [15, 16]. In regions like Spain, nearly half of the crop is discarded, representing a significant environmental burden and economic loss. By repurposing

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these leftover pulps and peels to extract health-boosting carotenoids, we can transform agricultural waste into a valuable resource [17, 18]. This approach not only shrinks the industry's environmental footprint but also turns discarded fruit into high-value ingredients for the health and wellness market [19].

2. Materials and Methods

i). Isolation and Screening of the Producer Organism: Indigenous fungi were naturally cultured from orange peels and isolated onto Potato Dextrose Agar to identify active strains. A pure colony of *Aspergillus niger* was then carefully selected and sub-cultured onto agar slants to serve as a consistent source for enzyme production.

ii). Morphological Identification: The isolated strain was microscopically confirmed as *Aspergillus niger* by identifying its signature carbon-black spores and characteristic conidial structures through Lactophenol Cotton Blue staining.

iii). Qualitative Assay (Pectin Utilization): The isolate was inoculated onto pectin agar and flooded with iodine after incubation to detect "zones of clearance," which visually confirmed the secretion of extracellular pectinase.

iv). Submerged Fermentation and Enzyme Production: A standardized spore suspension was inoculated into a production medium and fermented under controlled conditions for 3 to 5 days to maximize the secretion of pectinase enzymes.

v). Extraction and Partial Purification: Following incubation, the broth was centrifuged to separate the fungal biomass from the clear supernatant, which was then collected as the crude enzyme extract for analysis.

vi). Quantitative Enzyme Analysis: Pectinolytic activity was quantified by using the DNSA method to spectrophotometrically measure the color intensity produced by reducing sugars released from the pectin substrate.

vii). Production of Lycopene using pectinase enzyme: Lycopene was recovered through a multi-stage process of substrate pretreatment, enzymatic hydrolysis, and solvent extraction.

viii). Substrate Preparation: Fresh papaya was cleaned, deseeded, and ground into a concentrated pulp to create a uniform substrate for enzymatic treatment.

ix). Enzymatic Treatment: Papaya pulp was incubated in a pH 5.0 buffer with pectinase for 4–6 hours to degrade cell walls and maximize lycopene release.

x). Enzymatic Extraction of Lycopene: Crude enzyme was added to papaya pulp to degrade cell walls, followed by solvent extraction and separation to recover the concentrated lycopene phase.

xi). Phase Separation and Concentration: The mixture was partitioned in a separating funnel to collect the lycopene-rich organic layer, which was then evaporated to yield the purified pigment residue.

3. Results

i). Primary Screening of Fungal Isolates for Pectinase Activity

Isolate A1 (*Aspergillus niger*) emerged as the top performer, producing a robust 18.6 mm hydrolysis zone on pectin agar. By efficiently degrading the structural pectin in papaya cell walls, this isolate was selected as the optimal catalyst for maximizing lycopene recovery.

Table 1: Primary screening of fungal isolates for pectinase activity

Fungal Isolate	Incubation Time (h)	Clear Zone Diameter (mm)	Relative Activity
Isolate A1 (*Aspergillus* sp.)	48	18.6 ± 0.4	High
Isolate A2	48	12.1 ± 0.3	Moderate
Isolate A3	48	7.8 ± 0.2	Low

ii). Effect of Enzymatic Treatment on Lycopene Yield

Crude pectinase from *Aspergillus* effectively liquefied the papaya pulp, reducing slurry viscosity to facilitate superior solvent penetration. Lycopene recovery peaked at 14.2 mg/100g after 4 hours, marking the point of total intracellular pigment release.

Table 2: Lycopene extraction yield from papaya pulp using pectinase enzyme

Extraction Method	Incubation Time (h)	Lycopene Yield (mg/100g)	Colour Intensity
Control (Solvent Only)	4	5.4 ± 0.3	Light Orange
Pectinase Assisted	2	10.8 ± 0.5	Bright Orange-Red
Pectinase Assisted	4	14.2 ± 0.6	Deep Reddish-Orange

iii). Effect of Pectinase Concentration on Lycopene Extraction

Lycopene recovery peaked at 14.2 mg/100g using a 2% pectinase concentration, identifying the optimal dosage for papaya pulp. Increasing the enzyme load to 4% yielded no further gains, indicating substrate saturation at the 2% threshold.

Table 3: Effect of pectinase concentration on lycopene extraction

Pectinase concentration (% v/v)	Lycopene yield (mg/100g)	Mean ± SD (n=3)
1	9.2	9.2 ± 0.4
2	14.2	14.2 ± 0.6
3	14.5	14.5 ± 0.5
4	14.3	14.3 ± 0.4

iv). Effect of Incubation Time on Lycopene Yield from Papaya Pulp

Lycopene yield peaked at 15.1 mg/100g after 3 hours, successfully balancing matrix breakdown with pigment stability. Extending incubation to 5 hours triggered oxidative degradation, causing yield to decline as the unsaturated molecules reacted to light and heat.

Table 4: Effect of Incubation Time on Lycopene Yield (Pectinase-Assisted)

Incubation Time (h)	Lycopene Yield (mg/100g)	Mean ± SD
1	7.5	7.5 ± 0.3
2	12.8	12.8 ± 0.5
3	15.1	15.1 ± 0.4
5	13.8	13.8 ± 0.6

v). Comparison of Solvent Systems and Extraction Efficiency

The ternary solvent system reached 94% efficiency, leveraging a polar/non-polar balance to outperform single solvents. By displacing water and capturing hydrophobic lycopene, this mixture maximized recovery compared to Ethyl Acetate (74%).

Table 5: Solvent System Efficiency for Papaya Lycopene Extraction

Solvent System	Lycopene Yield (mg/100g)	Extraction Efficiency (%)
Hexane	8.2	65%
Ethyl Acetate	10.4	74%
Hexane:Acetone:Ethanol	15.4	94%

vi). Comparison of Mechanical and Enzyme-Assisted Extraction Methods

Pectinase treatment more than doubled lycopene recovery, delivering a massive 138.7% increase over mechanical methods. Enzymatic degradation of the pectin matrix is confirmed as the critical factor for unlocking maximum pigment from papaya.

Table 6: Comparison of Mechanical and Pectinase-Assisted Extraction

Extraction Method	Lycopene Yield (mg/100g)	Mean ± SD (n=3)	Increase in Yield (%)
Mechanical extraction	6.2	6.2 ± 0.4	—
Enzyme-assisted (Pectinase)	14.8	14.8 ± 0.5	138.7%

4. Discussion

- The study indicates a circular bio-economy approach by repurposing agricultural waste, spotlight upon orange peels, to produce high-value pectinase from *Aspergillus niger*, which is then utilized to enhance the recovery of lycopene from papaya fruit residues. The production of pectinase using agro-waste substrates is evidance based previous literature supports these aspects, which best part its industrial relevance and economically viable [3, 12, 14]. Isolate A1 emerged as a highly efficient producer, exhibiting significant pectinolytic activity that facilitated the breakdown of the complex pectin-cellulose matrix in papaya cell walls. This enzymatic degradation is consistent with previous findings where microbial pectinases effectively hydrolyze plant cell wall components, enhancing the release of intracellular compounds [7, 11].
- Further, lycopene extraction is highly sensitive to processing parameters, identifying a 2% enzyme concentration as the saturation point where the substrate is fully utilized. Similar optimization trends have been reported in enzyme production and activity studies, where substrate-enzyme interactions reach a plateau beyond optimal concentrations [2, 6]. Furthermore, the research highlighted a critical balance between yield and stability; while the peak yield of 15.1 mg/100 g was achieved at 3 hours, extended incubation triggered oxidative degradation of lycopene molecules. This observation aligns with earlier reports indicating that prolonged exposure to enzymatic and environmental conditions may degrade sensitive carotenoids [18].
- Next to it the results were shown that (Hexane:Acetone:Ethanol) achieved a 94% extraction efficiency by effectively separating hydrophobic carotenoids from the aqueous phase. Previous studies demonstrating that mixed solvent systems significantly improve carotenoid extraction efficiency due to enhanced solubility and mass transfer [18]. The superiority of enzyme-assisted extraction over conventional methods further supports latest research, which reports improved extraction yields through enzymatic cell wall disruption [1, 4].
- Overall, the integration of microbial enzyme production with waste valorization represents a sustainable and eco-friendly bioprocessing strategy. This approach aligns with current trends in green biotechnology, where enzymatic technologies are increasingly employed to convert low-value agricultural residues into high-value bioactive compounds [15, 17]. The findings reinforce the potential of enzyme-assisted extraction as an efficient tool for industrial applications in food, pharmaceutical, and nutraceutical sectors.

5. Conclusion

The research confirms that enzyme-assisted extraction (EAE) using pectinase from *Aspergillus niger* serves as a vastly superior alternative to traditional mechanical methods, yielding a remarkable 138.7% increase in lycopene recovery. By strategically integrating the microbial synthesis of enzymes with the valorization of papaya residues, the study addresses the dual industrial challenges of environmental waste management and the rising demand for natural, bioactive pigments. This dual-recovery model functions as a sustainable biorefinery, where pectinases facilitate the breakdown of complex cell wall matrices to unlock trapped antioxidants, providing an eco-friendly and cost-effective blueprint for the functional food and pharmaceutical sectors. Ultimately, this approach transforms low-value agricultural "waste" into high-value industrial biocatalysts and life-saving antioxidants, embodying the core principles of a circular bio-economy.

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