

ISSN: 2584-1491 | www.iircj.org Volume-3 | Issue-4 | April-2025 | Page 492-508

# PECTINASES IN MODERN BIOTECHNOLOGY: ITS INDUSTRIAL APPLICATIONS AND FUTURE HORIZONS

Tanisha Mitra<sup>1</sup>, Priyambada Singh<sup>1</sup> <sup>1</sup>Department of Microbiology, Kalinga University, Raipur(492101), Chhattisgarh, India Correspondence: <u>tanimitra123@gmail.com</u>

#### ABSTRACT

The bioactive molecules known as enzymes control a wide range of chemical reactions in living tissues. Pectinases, the enzyme responsible for breaking down Pectin (Water-Soluble Carbohydrate present in plant cell walls and tissues), serve as an extensive enzyme for industrial use. Widely employed in industries such as textile processing, fruit juice clarification, pulp & paper industry, and waste treatment, these enzymes have gained prominence for their eco-friendly and energy-efficient catalytic properties. With advances in microbial biotechnology, the large-scale production of pectinases—particularly from fungi like *Aspergillus niger* and bacteria such as *Bacillus subtilis*—has become increasingly efficient through submerged and solid-state fermentation strategies. This review lays emphasis into the types, mechanisms, microbial sources, structural insights, and current industrial uses of pectinases such as sustainable bioprocessing, role in biorefineries, etc. It also highlights recent developments and emerging trends in recombinant enzyme technology and proposes future directions for research and commercial application.

Keywords: Biocatalysts, Microbial Enzymes, Industrial Biotechnology, Enzyme Engineering

#### 1. Introduction

Pectinases, also known as pectinolytic enzymes, are a crucial class of enzymes that break down pectin, a complex heteropolysaccharide that is normally found in the primary cell walls and middle lamella of higher plants. These enzymes catalyse the hydrolysis, trans-elimination, or deesterification of pectin, leading to its depolymerization. Given their efficacy, pectinases are now extensively utilized across diverse industries, including fruit juice clarification, textile processing, plant fiber degumming, and wastewater treatment (Jayani et al., 2005; Hoondal et al., 2002). Pectin is notably abundant in fruits and vegetables, where it plays a crucial structural role in maintaining tissue firmness and plant rigidity (Cosgrove, 1997). The enzymatic breakdown of this biopolymer by pectinases facilitates various industrial applications by enhancing product quality, increasing yields, and reducing environmental impacts. Compared to chemical methods, enzymatic processes offer advantages such as substrate specificity, lower energy consumption, and reduced production of harmful by-products, aligning well with sustainable and eco-friendly processing standards (Alkorta et al., 1998; Sharma et al., 2013).

Because of their ability to produce high-yield enzymes and their resistance to a variety of fermentation conditions, bacterial strains like Bacillus subtilis and fungal strains like

ISSN: 2584-1491 | www.iircj.org Volume-3 | Issue-4 | April-2025 | Page 492-508

Aspergillus niger are popular microbiological sources of pectinases (Garg et al., 2016; Yu et al., 2017). These microorganisms produce a diversity of pectinolytic enzymes— pectin lyases (PLs), pectin methylesterases (PMEs) & polygalacturonases (PGs)—each with distinct action modes and industrial utility. Initially introduced in the early 20th century for clarifying fruit juices (Garg et al., 2016), pectinases have since emerged as critical tools in modern bioprocessing. Recent developments in protein engineering and recombinant DNA technology have produced more stable and efficient pectinase varieties appropriate for harsh industrial settings (Sharma et al., 2013). This review comprehensively explores the history, classification, sources, and structural mechanisms of pectinases, emphasizing their industrial relevance and highlighting future trends that could redefine enzymatic applications in green biotechnology.

#### 2. History of Pectinase Research

The first step in creating pectinases was comprehending the structure of pectic compounds and how pectolytic enzymes break them down. Later, the microbial pectinase production came to light for several decades. The exploration of pectin-degrading enzymes dates back to the early 20th century when Kertesz (1930) first described microbial degradation of pectic substances during studies on fruit juice clarification. This marked the beginning of industrial application of pectinases, with early recognition of their potential in the food processing industry.

Over time, interest expanded beyond fruit clarification as researchers delved into the enzymatic breakdown of pectin and its role in plant tissue softening and microbial virulence. Early advances came from studies on fungi such as *Aspergillus niger*, which is still one of the most commonly used organisms because of its high extracellular enzyme yield and generally recognized as safe (GRAS) status, provided early advancements (Pariza & Foster, 1983). In the late 20th century, bacterial sources like *Erwinia chrysanthemi* and *Bacillus subtilis* became subjects of intensive research due to their ability to produce thermostable and alkaline pectinases, suitable for non-food industrial applications (Gummadi & Panda, 2003; Naidu & Panda, 1998).

More recently, advances in metagenomics and environmental screening have led to the discovery of novel pectinase-producing microbes from extreme environments. Strains like *Paenibacillus sp., Streptomyces sp.*, and thermophilic *Geobacillus spp.* have demonstrated promising pectinolytic activity under harsh industrial conditions (Haile & Ayele, 2022; Ghaffar et al., 2022). Genomic and proteomic tools are now being used to engineer these enzymes for enhanced performance, such as improved thermostability and broad pH tolerance. Recombinant DNA (rDNA) technology has also facilitated the production of pectinase genes in heterologous hosts like *Escherichia coli* and *Pichia pastoris*, facilitating easier purification and modification (Patidar et al., 2021). These advancements are opening the door to more environmentally friendly and cost-effective production systems in the future.

Volume-3 | Issue-4 | April-2025 | Page 492-508

# **3. Structure of Pectinases**

The three-dimensional architectures of pectinases provide insights into the molecular foundation of enzyme function and the significance of individual amino acids within the active sites (Mayans et al., 1997; Jensen et al., 2010). These structural details elucidate the subtle distinctions in substrate specificity and catalytic performance among various pectinolytic enzymes. The first pectinase crystal structure to be solved was Erwinia chrysanthemi pectate lyase C (PelC) (Yoder et al., 1993), which showed a distinctive parallel  $\beta$ -helix fold. This same structure has since been observed in other pectate lyases including *Bacillus subtilis* Pel, PelA, PelE, and Pel9A (Pickersgill et al., 1994; Jenkins et al., 2001).

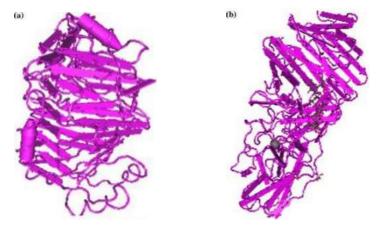
Pectinase enzymes typically have a complex structure that enables them to break down pectin. A common structural feature in many pectinases is the parallel  $\beta$ -helix, a right-handed cylindrical structure composed of parallel  $\beta$ -strands. With seven to nine helical turns, this parallel  $\beta$ -helix creates a prism-like shape that contributes to three parallel  $\beta$ -sheets (PB1, PB2, PB3). These sheets define the substrate-binding cleft, located between PB1 and surrounding loop regions. The cleft is essential for catalysis, where the substrate interacts and is cleaved. The detailed mechanisms of substrate binding and catalysis in this cleft have been studied using site-directed mutagenesis, molecular modelling, and X-ray crystallography, which have revealed critical insights into enzyme specificity and efficiency (Kita et al., 1996; Boz, 2021). The significance of particular residual amino acid present in the active site has been determined through recent research, highlighting their role in substrate recognition and the catalytic process. Furthermore, advancements in structural research have improved our comprehension of how pectinase enzymes are tailored for their industrial applications, including in food processing and biofuel production (Sánchez et al., 2022; Sun et al., 2023).

*Figure 1* illustrates this structural diversity: (a) shows the structure of pectin lyase A from *Aspergillus aculeatus*, highlighting the  $\beta$ -helix architecture and substrate-binding groove; (b) displays the rhamnogalacturonan lyase from the same organism, which has a similar core fold but different surface loops, enabling it to recognize more complex pectin domains. These structural insights clarify how different enzymes from the same family adapt to specific substrates, underscoring their industrial and biological versatility.

Despite this progress, no representative structure for polymethylgalacturonase (PMG) has been solved to date (Sharma et al., 2013).



ISSN: 2584-1491 | www.iircj.org Volume-3 | Issue-4 | April-2025 | Page 492-508

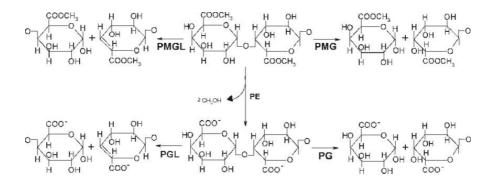


**Fig. 1:** *Aspergillus aculeatus* K 150a substrate complex's (a) Pectin Lyase A (PNLA) and (b) Rhamnogalacturonan Lyase (RG-Lyase) three-dimensional structure

#### 4. Mode of Action of Pectinases

"Various microorganisms-including bacteria, actinomycetes, fungi, and yeasts-have been recognized for their ability to generate pectin-degrading enzymes, collectively referred to as microbial pectinases. Among these, extensively studied types include lyases, pectin esterases, polygalacturonases, and to some extent, proteases. Polygalacturonases (PGs), the most prevalent class of pectinases, act as catalysts in the hydrolysis of polygalacturonic acid chains by incorporating water molecules, as illustrated schematically in relevant literature (Alkorta et al., 1998). Another important enzyme, protopectinase, acts specifically on protopectin to convert it into soluble pectin. In contrast, lyases use a trans-elimination process to cleave the galacturonic acid polymer. In contrast, pectin esterases catalyse the breakdown of methyl ester bonds in the pectin backbone, leading to the release of methanol and other pectic compounds (Sharma et al., 2011). Pectinases play a crucial role in the fruit and vegetable processing industry, where they enhance juice extraction and improve clarity (Sharma et al., 2011). These enzymes are categorised based on how they affect the galacturonan backbone and mainly affect the pectic components of plant cell walls. Pectin depolymerases function by breaking glycosidic bonds between methyl-esterified galacturonic acid residues, whereas pectin methyl esterases remove methyl groups, converting high-methoxyl pectin into low-methoxyl pectin or pectic acid. Polygalacturonases cleave glycosidic bonds adjacent to unesterified carboxylic groups via hydrolysis, while pectate lyases utilize a  $\beta$ -elimination mechanism. Pectin lyases (PLs) and endo-polygalacturonases (endo-PGs) randomly break the pectin chain. In contrast, exopectin lyases generate unsaturated dimeric compounds from the reducing end, while exopolygalacturonases cleave monomers or dimers from the non-reducing end. When pectin methyl esterases work in conjunction with polygalacturonases or endo-pectin lyases, they effectively degrade highly methylated pectin substrates (Sharma N. et al., 2013).

ISSN: 2584-1491 | www.iircj.org Volume-3 | Issue-4 | April-2025 | Page 492-508



**Fig. 2:** The enzymatic action of PMGL, PMG, PGL, and PG on the pectin molecule. PMGL (polymethylgalacturonate lyase), PMG (polymethyl galacturonase), PGL (Pectinase Glycosyl Hydrolase), and PG (polygalacturonase) are the enzymes involved in pectin degradation. (Adapted from Garg et al., 2016)

#### 5. Types of Pectinases

Pectin, a complex polysaccharide found in plant cell walls, is broken down by a class of enzymes known as pectinases. Based on how they function and the substrate they target, these enzymes are divided into a number of types. Pectinesterases (PE), also known as pectinmethyl hydrolases, primarily target the methyl ester group of galacturonate units, facilitating the de-esterification of pectin's methoxyl group to yield pectic acid (Cosgrove, 1997). Polygalacturonases (PGs) degrade polygalacturonic acid, causing the cleavage of glycosidic linkages and depolymerizing pectin (Cosgrove, 1997). Pectin lyases (PLs) break down the 1,4-glycosidic bonds connecting galacturonic acid units, producing unsaturated oligogalacturonides, which are commonly used in industries like fruit juice production for extraction and clarifying processes (Schols & Voragen, enhancing 1996). Rhamnogalacturonases (RGase) specifically hydrolyse the  $\alpha$ -1,4-glycosidic linkage between rhamnose and galacturonic acid in pectin, releasing smaller pectin fragments (Schols & Voragen, 1996). Pectinases are further divided into three groups according to how they function: endo-liquefying or depolymerizing enzymes, such as endopolygalacturonases (endo-PGs) and endo-pectate lyases (endo-PLs), which break down internal glycosidic linkages randomly through hydrolysis (Tapre & Jain, 2014); exo- or saccharifying enzymes, including exo-polygalacturonases (exo-PGs) and exo-pectate lyases (exo-PLs), which react on non-reducing ends to gradually release monomeric units (Tapre & Jain, 2014); and trans-eliminating enzymes, such as pectin lyases (PLs) and certain pectate lyases (PLs), which produce unsaturated oligogalacturonides through a transelimination process, distinct from hydrolysis (Tapre & Jain, 2014). These enzymes are crucial in various industrial applications, including food processing, where they aid in juice extraction, clarification, and other processes that require the breakdown of pectin (Sánchez et al., 2022; Boz, 2021).

ISSN: 2584-1491 | www.iircj.org

Volume-3 | Issue-4 | April-2025 | Page 492-508

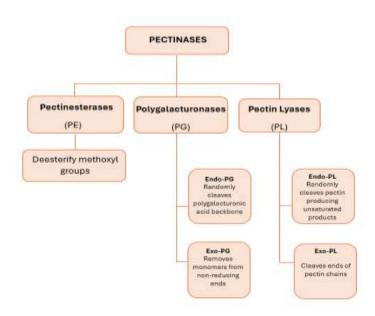


Fig. 3: Types of pectinases (created using MS Word)

#### 6. Microbial Sources of Pectinases

Various kinds of animals, including bacteria, fungus, yeasts, insects, nematodes, protozoa, and plants produce pectinolytic enzymes naturally (Garg et al., 2016). The most typical food source for microorganisms that produce pectinase is decomposing plant matter, which is produced in large quantities by saprophytic fungi (Gummadi et al., 2003). Multiple studies on microbial enzymes have highlighted the development of various pectinase forms, each exhibiting distinct molecular masses and kinetic properties. Below mentioned (Table 1.) is the list of some of the microbial sources of pectinases and their source of isolation.

S.no.	Sources	Microbial Isolates	References
Bacteria			
1.	Agro-waste	Bacillus subtilis	Torimiro & Okonji, 2013
2.	Soil	Bacillus sp. (ZGL14)	Yu et al., 2017
3.	National center for agricultural utilization research (USA)	Bacillus pumilus	Tepe et al., 2014
4.	Market solid waste	Bacillus cereus	Namasivayam et al., 2011
5.	Hot spring	Bacillus halodurans (M29)	Mei et al., 2013

SamagraCS Publication House

13.

Fermented soybean

			5
6.	Soil sample	Bacillus mojavensis 14	Ghazala et al., 2015
7.	Pig intestine	Lachnospira pectinoschiza	Cornick et al., 1994
8.	Soil	Pseudomonas solanacearum	Schell et al., 1994
9.	Algerian raw milk	Lactobacillus lactis subsp. cremoris	Karam et al., 1995
10.	Saintpaulia ionantha	E. chrysanthemi 3604	Laurent et al., 2000
11.	Industrial wastewater	Paenibacillus polymyxa	Khan et al., 2022
12.	Soil from compost yard	Geobacillus thermodenitrificans	Ghaffar et al., 2022

Bacillus velezensis

Sharma et al., 2023

Innovation and Integrative Research Center Journal

ISSN: 2584-1491 | www.iircj.org

Fungi			
1.	Rotten lemons	Aspergillus niger	Maldonado et al., 1994
2.	Brazilian soil samples	Penicillum sp.	Jayani et al., 2005
3.	Fruit market	Rhizomucor sp.	Siddiqui et al., 2013
4.	Soil	Rhizopus sp.	Jayani et al., 2005
5.	Agrowaste samples	Trichoderma sp.	Okafor et al., 2010
6.	Champagne wines	Saccharomyces cerevisiae	Gainvors et al., 1994
7.	The DSM collection and the Institut für Gärungsgewerbe und Biotechnologie in Berlin	Candida sp.	Call et al., 1984
8.	Professor Sidney Crow, Department of Biology, Georgia State University, Georgia, USA	Pichia sp.	Moharib et al., 2000
9.	Patagonian lake Toncek	Rhodotorula sp.	Libkind et al., 2004
10.	Infected vegetable	Aureo basidium sp.	Parini et al., 1988
11.	Agricultural waste (banana peel)	Aspergillus tubingensis	Yadav et al., 2021



ISSN: 2584-1491 | www.iircj.org

Volume-3 | Issue-4 | April-2025 | Page 492-508

12.	Rotten pineapple	Penicillium citrinum	Bhatti et al., 2020
13.	Grape pomace	Trichoderma viride	Singh et al., 2019

Plants			
1.	Malaysian Agricultural Research and Development Institute (MARDI), Serdang	Carica papaya	Fayyaz et al., 1995; Innocenzo et al., 2001
2.	Various Plants	Lycopersicum esculentum	Warrilow et al., 1994
3.	Local supermarkets	Prunus malus	Macdonald and Evans, 1996
4.	Citrus world, lake Wales, FL	Vitis vinifera	Corredig et al., 2000
5.	Valencia	Citrus sp.	Arias and Burns, 2002
6.	Plantation in Coatlán del Rio	Pouteria sapota	Arenas-Ocampo et al., 2003
7.	Several trees	Malphigia glabra L.	Assis et al., 2004
8.	Wild fig	Ficus carica	Ahmed et al., 2020
9.	Guava	Psidium guajava	Rani et al., 2021
Yeast			
1.	Apple pomace	Pichia kudriavzevii	Tsegaye et al., 2021

# 7. Applications of Pectinases

According to Kertesz's early report from 1930, pectinases were first used commercially to clarify apple juice. Since then, their role has expanded significantly, and today, pectinases are indispensable in various traditional and emerging industrial processes. Their effectiveness has been established across various applications, including plant fiber processing, textile production, tea and coffee fermentation, oil extraction, wastewater treatment, and even virus purification (Jayani et al., 2005; Garg et al., 2016). In the last few years, the application of microbial pectinases has expanded to include sustainable bioprocessing, such as enzymatic retting and eco-friendly bio-scouring in the textile sector (Sharma et al., 2023). Their use in fruit juice clarification and pulp treatment continues to evolve, with modern enzyme blends tailored for specific fruits and pH conditions,

-Innovation Innovation and Integrative Research Center Journal

ISSN: 2584-1491 | www.iircj.org

Volume-3 | Issue-4 | April-2025 | Page 492-508

improving yield and processing time (Haile & Ayele, 2022). Furthermore, current research emphasizes their integration in circular bioeconomy models, where agro-industrial waste can serve as both a substrate and a pectinase production medium (Singh et al., 2021). These enzymes are also being explored for their role in novel biotechnological systems, such as biorefineries and smart enzyme formulations, for enhanced productivity and reduced environmental impact (Ghaffar et al., 2022).

#### 7.1. Fruit Juice Extraction

The most prevalent industrial use of pectinases involves the extraction and purification of fruit juices. To make fruit juices clearer, a combination of pectinases and amylases is utilized, reducing filtration time by 50% (Blanco et al., 1999). Modern formulations of pectinolytic enzymes are tailored to specific fruit matrices, enhancing yield and maintaining nutritional quality (Ortiz et al., 2017; Haile & Ayele, 2022). Pectin methylesterase and calcium infusion in citrus fruits have been shown to enhance texture and firmness during processing, offering commercial advantages in canned or pickled products (Baker & Wicker, 1996; Mehanni et al., 2017).

# 7.2. Bio-Scouring of Cotton Fibres & Textile Processing

Within the textile industry sector, pectinases have replaced caustic soda for bioscouring—a pretreatment process that removes pectin, waxes, and non-cellulosic impurities from cotton. This ecofriendly enzymatic method reduces pollution, water use, and energy consumption compared to traditional alkaline treatments (Hoondal et al., 2000). Recent research has optimized microbial pectinase blends for better fabric softness and dyeability while preserving fiber integrity (Sharma et al., 2023; Patidar et al., 2021).

d Integrative Research Center Journal

# 7.3. Degumming of Plant Bast Fibres

Outside of the xylem, phloem, or pericycle, the soft, flexible fibres called bast fibres, form in clusters. Bast fibres include Sun hemp and Ramie. Before the fibres are used to make textiles, the gum inside of them needs to be removed. The chemical degumming procedure is poisonous, non-biodegradable, and polluting. For the aforementioned issue, biotechnological degumming with pectinases and xylanases offers a cost-effective and environmentally beneficial solution (Kapoor et al., 2001). Recent advances have demonstrated the scalability of this process using solid-state fermentation techniques (Almeida et al., 2023).

# 7.4. Retting of Plant Fibres

Retting is the process of separating bast fibres from the woody core by removing pectic substances. Pectinase-mediated retting provides a controlled and faster alternative to traditional microbial retting, which is slow and environmentally challenging. Enzymatic retting has shown to improve fiber fineness, reduce microbial contamination, and shorten processing time (Hoondal et al., 2002; Sharma et al., 2023). This approach is particularly profitable to flax, jute, and hemp processing industries seeking greener solutions. This method is being increasingly adopted by sustainable textile producers worldwide.

# 7.5. Tea & Coffee Fermentation

During coffee processing, pectinases are used to remove the mucilage layer that coats the beans of coffee, a crucial step in the wet fermentation process. Enzymatic treatment accelerates this mucilage breakdown, ensuring uniform fermentation and enhancing bean quality (Carr, 1985). Similarly, in tea production, pectinases help in reducing foam-forming components in instant tea powders by degrading pectins. This results in improved solubility and clarity of tea extracts, especially in ready-to-drink products. More recently, fungal-derived pectinases have been employed in specialty tea and coffee production due to their enhanced activity at varied temperatures and pH levels (Bhatti et al., 2020; Ghaffar et al., 2022).

# 7.6. Paper Pulp Industry

In the paper pulp industry, pectinases help in the removal of pectic substances that interfere with fiber bonding and water drainage. Pectins increase the cationic demand and reduce paper strength by binding with metal ions and retention aids. Pectinases such as polygalacturonases and pectate lyases depolymerize polygalacturonic acid chains, thereby reducing the cationic demand and improving the performance of retention agents (Reid & Ricard, 2000). This enzymatic treatment enhances water removal during papermaking and reduces the need for chemical additives, making the process more eco-efficient (Viikari et al., 2001). Novel applications include their use in peroxide bleaching processes for improved pulp brightness and lower chemical usage (Nguyen et al., 2020; Viikari et al., 2001).

# 7.7. Plant Viruses Purification

Pectinases aid in the isolation of virus particles trapped within pectin-rich plant tissues in plant virology. When used alongside cellulases, these enzymes facilitate the gentle maceration of tissue, yielding high-purity viral preparations ideal for vaccine development and diagnostics (Salazar & Jayasinghe, 1999; Nguyen et al., 2020).

# 7.8. Wastewater Treatment

Pectin-rich wastewater from citrus processing and vegetable canning industries presents significant challenges for conventional treatment systems due to the resistance of pectic substances to microbial degradation. These substances increase chemical oxygen demand (COD) and reduce treatment efficiency. Pectinases, especially alkaline endopectate lyases produced by alkalophilic *Bacillus* species, are used to pretreat this wastewater by degrading the pectic polysaccharides into simpler, biodegradable compounds (Tanabe et al., 1987). This enzymatic pretreatment significantly enhances the performance of downstream

ISSN: 2584-1491 | www.iircj.org Volume-3 | Issue-4 | April-2025 | Page 492-508

biological processes, such as activated sludge systems, by reducing the organic load and improving microbial assimilation. Studies have also shown that this method reduces sludge volume and accelerates processing time (Hoondal et al., 2002). Traditional chemical treatments, which are frequently expensive and harmful to the environment, can be replaced with an environmentally friendly enzymatic method. More recent approaches utilize engineered microbial consortia and enzyme cocktails to improve treatment efficacy in agro-industrial wastewater systems (Singh et al., 2021).

# 7.9. Oil Extraction

In the extraction of edible oils from fruits like olives, dates, and flaxseed, pectinases play a key role in breaking down cell wall polysaccharides, facilitating the release of intracellular oils. Enzymatic oil extraction leads to higher yields, reduced extraction time, and improved oil quality with lower peroxide and free fatty acid content (Mehanni et al., 2017; Ortiz et al., 2017). Compared to conventional solvent extraction, enzymatic methods are safer and environmentally friendly. Current studies are optimizing process parameters using statistical tools and machine learning to maximize enzymatic oil extraction efficiency (Almeida et al., 2023).

# 7.10. Enhancement of Red Wines' Chromaticity and Stability

Pectinases are added during red wine production to degrade pectin in grape skins, which facilitates the release of anthocyanins and tannins—key compounds responsible for color, aroma, and mouthfeel. Enzymatic maceration improves wine color intensity, turbidity reduction, and filterability (Revilla & González-San José, 2003). Wines treated with pectinolytic enzymes also show better long-term stability and sensory quality compared to untreated controls. Their role in stabilizing color compounds during aging is being further optimized using recombinant enzymes with higher activity at wine fermentation conditions (Ghaffar et al., 2022).

# 7.11. Paper Making

Pectinases have found valuable application in the paper industry, particularly in addressing the challenges posed by pectic substances during the paper-making process. Paper production involves forming sheets from a suspension of fibres, fines, and fillers such as clay and calcium carbonate. Effective drainage is essential, and this process relies on retention aids—usually cationic polymers—to prevent the loss of fine particles through the filter fabrics (Horn & Linhart, 1996). However, bleaching with alkaline peroxide releases pectins and polygalacturonic acids, which interfere with these retention systems due to their high cationic demand (Holmbom, 1991). The degree of polymerization influences this demand: long-chain polygalacturonates interact more strongly with cationic agents, whereas shorter fragments have negligible effects (Thornton et al., 1994). Pectinases depolymerize these acidic polysaccharides, thereby reducing cationic demand and improving both retention and drainage efficiency (Reid & Ricard, 2000). In addition to improving pulp

ISSN: 2584-1491 | www.iircj.org

Volume-3 | Issue-4 | April-2025 | Page 492-508

quality, this enzymatic method makes the process of producing paper more economical and environmentally friendly.

#### 8. Conclusion

Pectinases enhance both yield and quality in various industrial processes and are recognized as essential enzymes within the sector. Ongoing research continues to unveil novel applications for pectinases, which have become crucial biocatalysts across diverse industries due to their specificity, eco-friendly nature, and operational efficiency. Their use has notably improved yield, product quality, and sustainability in processes ranging from juice clarification and fiber retting to oil extraction and textile processing. Looking forward, the future of pectinases lies in harnessing advanced protein engineering and recombinant DNA technologies to develop more robust and substrate-specific enzyme variants. Genetically modified microbial hosts such as *Pichia pastoris* and *Escherichia coli* are increasingly being explored for heterologous expression of engineered pectinase genes to enhance stability and reduce production costs (Patidar et al., 2021; Haile & Ayele, 2022).

Pectinases' incorporation into biorefinery models —particularly in lignocellulosic biomass valorisation—holds promise for more sustainable production of biofuels, organic acids, and bioplastics. Moreover, their potential use in biomedical applications, including targeted drug delivery and cancer therapy, is gaining attention due to their ability to degrade extracellular matrices (Nguyen et al., 2020). With the advancement of computational tools, artificial intelligence (AI), and machine learning, enzyme modelling and directed evolution are being significantly accelerated, offering new avenues for the design of tailor-made pectinases with desirable industrial traits (Almeida et al., 2023). Continued exploration of microbial diversity, coupled with omics technologies, will further expand the enzyme repertoire available for industrial bioprocessing. With increasing demand for greener technologies, pectinases are poised to become a cornerstone of sustainable and intelligent biomanufacturing.

#### 9. References

- 1. Ahmed, N., Hassan, S., & Ali, R. (2020). Extraction and characterization of pectinase from wild fig (*Ficus carica*). *Biocatalysis and Agricultural Biotechnology*, 25, 101617.
- 2. Akita M, Suzuki A, Kobayashi T, Ito S, Yamane T (2000) Crystallization and preliminary X-ray analysis of high-alkaline pectate lyase. Acta Crystallogr D 56:749–750
- 3. Alkorta I, Garbisu G, Llama MJ, Serra JL (1998) Industrial applications of pectic enzymes: a review. Process Biochem 1:21–28 Rev Environ Sci Biotechnol.
- 4. Almeida, J. R. M., Ferreira, M. A., & Martins, C. O. (2023). AI-assisted design of pectinases for industrial applications. Enzyme Engineering Journal, 12(1), 55–66.
- 5. Almeida, J. R. M., Ferreira, M. A., & Martins, C. O. (2023). AI-assisted design of pectinases for industrial applications. *Enzyme Engineering Journal*, 12(1), 55–66.
- Arenas-Ocampo ML, Evangelista-Lozano S, Arana-Errasquin R, Jiménez-Aparicio A, Dávila Ortíz G (2003) Softening and biochemical changes of sapote mamey fruit (*Pouteria sapota*) at different development and ripening stages. J Food Chem 27:91–95

-Innovation Innovation and Integrative Research Center Journal

ISSN: 2584-1491 | www.iircj.org

- 7. Arias C. R., Burns J. K., Friedrich L. M., Goodrich R. M., & Parish M. E. (2002). Yeast species associated with orange juice: evaluation of different identification methods. *Applied and environmental microbiology*, 68(4), 1955-1961.
- Assis SA, Fernandes P, Ferreira BS, Trevisan HC, Cabral JMS, Oliveira OMMF (2004) Screening of supports for the immobilization of pectin-methylesterase from acerola (*Malpighia glabra* L.). J Chem Technol Biotechnol 79:277–280
- 9. Baker R. A., & Wicker L. (1996). Current and potential applications of enzyme infusion in the food industry. *Trends in Food Science & Technology*, 7(9), 279-284.
- Bhatti, H. N., Asgher, M., Abbas, A., & Nawaz, R. (2020). Production of pectinase from *Penicillium citrinum* using pineapple waste. *Preparative Biochemistry & Biotechnology*, 50(4), 381–389.
- 11. Blanco P., Sieiro C., & Villa T. G. (1999). Production of pectic enzymes in yeasts. *FEMS Microbiology Letters*, 175(1), 1-9.
- 12. Boz, O. (2021). Advances in pectinase applications: Industrial and environmental perspectives. International Journal of Biological Macromolecules, 168, 1037-1049. https://doi.org/10.1016/j.ijbiomac.2020.12.216
- 13. Call H. P., Harding M., & Emeis C. C. (1985). Screening for pectinolytic Candida yeasts: optimization and characterization of the enzymes. *Journal of Food Biochemistry*, 9(3), 193-210.
- 14. Carr, J. G. (1985). Tea, coffee and cocoa. In B. J. B. Wood (Ed.), *Microbiology of Fermented Foods* (Vol. 2). Elsevier.
- 15. Cornick N. A., Jensen N. S., Stahl D. A., Hartman P. A., & Allison M. J. (1994). Lachnospira pectinoschiza sp. nov., an anaerobic pectinophile from the pig intestine. *International Journal of Systematic and Evolutionary Microbiology*, 44(1), 87-93.
- 16. Corredig M, Kerr W, Wicker L (2000) Separation of thermostable pectinmethylesterase from marsh grapefruit pulp. J Agric Food Chem 48:4918–4923.
- 17. Cosgrove D. J. (1997). Assembly and enlargement of the primary cell wall in plants. Annual Review of Cell and Developmental Biology 13:171–201
- D'INNOCENZO, M. A. R. I. S. A., & Lajolo F. M. (2001). Effect of gamma irradiation on softening changes and enzyme activities during ripening of papaya fruit. *Journal of Food Biochemistry*, 25(5), 425-438.
- 19. Fayyaz A., Asbi B. A., Ghazali H. M., Man Y. C., & Jinap S. (1995). Kinetics of papaya pectinesterase. *Food Chemistry*, 53(2), 129-135.
- Gainvors A., Frezier V., Lemaresquier H., Lequart C., Aigle M., & Belarbi A. (1994). Detection of polygalacturonase, pectin-lyase and pectin-esterase activities in a Saccharomyces cerevisiae strain. *Yeast*, 10(10), 1311-1319.
- 21. Garg G., Singh A., Kaur A. *et al.* (2016) Microbial pectinases: an ecofriendly tool of nature for industries. *3 Biotech* 6, 47.
- Ghaffar, M., Naseem, Z., Nawaz, M. A., & Akhtar, K. (2022). Characterization and application of a novel pectinase from thermophilic *Geobacillus* species for industrial use. *Biotechnology Reports*, 33, e00758.
- 23. Ghazala I., Bouallegue A., Haddar A., & Ellouz-Chaabouni S. (2019). Characterization and production optimization of biosurfactants by Bacillus mojavensis I4 with biotechnological potential for microbial enhanced oil recovery. *Biodegradation*, *30*, 235-245.
- 24. Haile S., & Ayele A. (2022). Pectinase from microorganisms and its industrial applications. *The Scientific World Journal*, 2022.

- Innovation Innovation and Integrative Research Center Journal

ISSN: 2584-1491 | www.iircj.org

- 25. Haile, S., & Ayele, A. (2022). Pectinase from microorganisms and its industrial applications. The Scientific World Journal, 2022, 1–12.
- 26. Haile, S., & Ayele, A. (2022). Pectinase from microorganisms and its industrial applications. *The Scientific World Journal*, 2022, 1–12.
- 27. Holmbom B. (1991). Chemical changes in peroxide bleaching of mechanical pulps. *Das Papier*, 45, 16-22.
- 28. Hoondal G., Tiwari R., Tewari R., Dahiya N. B. Q. K., & Beg Q. (2002). Microbial alkaline pectinases and their industrial applications: a review. *Applied microbiology and biotechnology*, *59*, 409-418.
- 29. Horn D, Linhart F (1996) Retention aids. In: Roberts JC (ed) Paper chemistry. Blackie Academic and Professional, London, pp 64–82
- 30. Jayani, R. S., Saxena, S., & Gupta, R. (2005). Microbial pectinolytic enzymes: a review. *Process Biochemistry*, 40(9), 2931-2944.
- Jenkins J, Mayans O, Smith D, Worboys K, Pickersgill RW (2001) Three-dimensional structure of Erwinia chrysanthemi pectin methylesterase reveals a novel esterase active site. J Mol Biol 305:951–960
- 32. Jensen MH, Otten H, Christensen U, Borchert TV, Christensen LL, Larsen S, Leggio LL (2010) Structural and biochemical studies elucidate the mechanism of rhamnogalacturonan lyase from *Aspergillus aculeatus*. J Mol Biol 404:100
- 33. Kapoor, M., Beg, Q. K., Bhushan, B., Singh, K., Dadhich, K. S., & Hoondal, G. S. (2001). Application of an alkaline and thermostable polygalacturonase from Bacillus sp. MG-cp-2 in degumming of ramie (Boehmeria nivea) and sunn hemp (Crotalaria juncea) bast fibres. *Process Biochemistry*, 36(8-9), 803-807.
- 34. Kapoor, M., Beg, Q. K., Bhushan, B., Singh, K., Dadhich, K. S., & Hoondal, G. S. (2001). Application of an alkaline and thermostable polygalacturonase from *Bacillus sp.* MG-cp-2 in degumming of ramie and sunn hemp bast fibres. *Process Biochemistry*, 36(8–9), 803–807.
- 35. Karam N. E., & Belarbi A. (1995). Detection of polygalacturonases and pectin esterases in lactic acid bacteria. *World Journal of Microbiology and Biotechnology*, *11*, 559-563.
- 36. Khan, F., Ali, N., & Siddiqui, M. F. (2022). Production of pectinase by *Paenibacillus polymyxa* isolated from industrial wastewater. *Biocatalysis and Agricultural Biotechnology*, 40, 102278.
- 37. Kita N, Boyd CM, Garrett MR, Jurnak F, Keen NT (1996) Differential effect of site-directed mutations in PelC of pectate lyase activity, plant tissue maceration, and elicitor activity. J Biol Chem 271:26529–26535
- 38. Kulkarni N. G., Kar J. R., & Singhal R. S. (2017). Extraction of flaxseed oil: a comparative study of three-phase partitioning and supercritical carbon dioxide using response surface methodology. *Food and Bioprocess Technology*, *10*, 940-948.
- Laurent F, Kotoujansky A, Bertheau Y (2000) Overproduction in *Escherichia coli* of the pectin methylesterase A from *Erwinia chrysanthemi* 3937: one-step purification, biochemical characterization, and production of polyclonal antibodies. Can J Microbiol 46:474–480
- 40. Libkind D, Pérez P, Sommaruga R, Diéguez MC, Ferraro M, Brizzio S, Zagarese H, Van Broock M (2004) Constitutive and UV-inducible synthesis of photoprotective compounds (carotenoids and mycosporines) by freshwater yeasts. Photochem Photobiol Sci 3:281–286
- 41. Lietzke SE, Keen NT, Yoder MD, Jurnak F (1994) The three-dimensional structure of pectate lyase E, a plant virulence factor from *Erwinia chrysanthemi*. Plant Physiol 106:849–862
- 42. Macdonald HC, Evans R (1996) Purification and properties of apple pectinesterase. J Sci Food Agric 70:321–326

ISSN: 2584-1491 | www.iircj.org

- 43. Maldonado M. C., Strasser de Saad A. M., & Callieri D. (1994). Purification and characterization of pectinesterase produced by a strain of Aspergillus niger. *Current Microbiology*, 28, 193-196.
- 44. Mayans O, Scott M, Connerton I, Gravesen T, Benen J, Visser J, Pickersgill R, Jenkins J (1997) Two crystal structures of pectin lyase A from *Aspergillus* reveal a pH driven conformational change and striking divergence in the substrate binding clefts of pectin and pectate lyases. Structure 5:677– 689
- 45. Mehanni A. E. S., El-Reffaei W. H. M., Melo A., Casal S., & Ferreira I. M. (2017). Enzymatic extraction of oil from Balanites Aegyptiaca (Desert Date) kernel and comparison with solvent extracted oil. *Journal of food biochemistry*, *41*(2), e12270.
- 46. Mei Y., Chen Y., Zhai R., & Liu Y. (2013) Cloning, purification and biochemical properties of a thermostable pectinase from *Bacillus halodurans* M29. J Mol Catal B Enzym, 94: 77-81.
- 47. Moharib S. A., El-Sayed S. T., & Jwanny E. W. (2000). Evaluation of enzymes produced from yeast. *Food/Nahrung*, 44(1), 47-51.
- 48. Naidu G. S. N., & Panda T. (1998). Production of pectolytic enzymes-a review. *Bioprocess Engineering*, 19, 355-361.
- 49. Namasivayam E., Mariappan K., Jiji A., Kumar M., & Richard L. (2011). Production of extracellular pectinase by Bacillus cereus isolated from market solid waste. *Journal of Bioanalysis & Biomedicine*, *3*.
- 50. Nguyen, T. L., Kim, J. H., & Lee, M. Y. (2020). Pectinase in biomedical applications: A review of therapeutic potential and delivery systems. Journal of Biomaterials Applications, 35(2), 174–186.
- 51. Nguyen, T. L., Kim, J. H., & Lee, M. Y. (2020). Pectinase in biomedical applications: A review of therapeutic potential and delivery systems. *Journal of Biomaterials Applications*, 35(2), 174–186.
- Okafor U. A., Okochi V. I., Chinedu S. N., Ebuehi O. A. T., & Onygeme-Okerenta B. M. (2010). Pectinolytic activity of wild-type filamentous fungi fermented on agro-wastes. *African Journal of Microbiology Research*, 4(24), 2729-2734.
- Ortiz G. E., Ponce-Mora M. C., Noseda D. G., Cazabat G., Saravalli C., López M. C., ... & Albertó E. O. (2017). Pectinase production by *Aspergillus giganteus* in solid-state fermentation: optimization, scale-up, biochemical characterization and its application in olive-oil extraction. *Journal of Industrial Microbiology and Biotechnology*, 44(2), 197-211.
- 54. Parini C., Fortina M. G., & Manachini P. L. (1988). Properties of two pectin lyases produced by Aureobasidium *pullulans* LV 10. *Journal of Applied Microbiology*, 65(6), 477-481.
- 55. Pariza M. W., & Foster E. M. (1983). Determining the safety of enzymes used in food processing. *Journal of Food Protection*, 46(5), 453-468.
- 56. Patidar, M. K., Nighojkar, S., Kumar, A., & Nighojkar, A. (2021). Recombinant production of alkaline pectinase and its application in cotton scouring. Biotechnology Letters, 43(1), 125–134.
- 57. Patidar, M. K., Nighojkar, S., Kumar, A., & Nighojkar, A. (2021). Recombinant production of alkaline pectinase and its application in cotton scouring. *Biotechnology Letters*, 43(1), 125–134.
- 58. Pickersgill R, Jenkins J, Harris G, Nasser W, Robert-Baudouy J (1994) The structure of *Bacillus subtilis* pectate lyase in complex with calcium. Nat Struct Biol 1:717–723
- 59. Rani, R., Sharma, K., & Sharma, V. (2021). Plant-based production of pectinase from *Psidium* guajava peel. *International Journal of Environmental Science and Technology*, 18(6), 1393–1402.
- 60. Reid I., & Ricard M. (2000). Pectinase in papermaking: solving retention problems in mechanical pulps bleached with hydrogen peroxide. *Enzyme and Microbial Technology*, 26(2-4), 115-123.
- Revilla I., & González-SanJosé M. L. (2003). Compositional changes during the storage of red wines treated with pectolytic enzymes: low molecular-weight phenols and flavan-3-ol derivative levels. *Food Chemistry*, 80(2), 205-214.

ISSN: 2584-1491 | www.iircj.org

- 62. Salazar L., & Jayasinghe U. (1999). Fundamentals of purification of plant viruses. *Techniques in plant, virology, CIP., Training Manual, JO, Virus Purification, International Potato Centre, Peru,* 1-10.
- 63. Sánchez, C., et al. (2022). New insights into pectinase applications in food and environmental sectors. Food Bioprocessing and Technology, 15(8), 1287-1302. https://doi.org/10.1007/s11947-022-02717-2
- 64. Sathyanarayana N. Gummadi, T. Panda (2003). Purification and biochemical properties of microbial pectinases—a review. , 38(7), 987–996.
- 65. Schell M. A., Denny T. P., & Huang J. (1994). Extracellular virulence factors of Pseudomonas solanacearum: role in disease and regulation of expression. In *Molecular mechanisms of bacterial virulence* (pp. 311-324). Dordrecht: Springer Netherlands.
- 66. Schols H. A., & Voragen A. G. J. (1996). Complex pectins: structure elucidation using enzymes. In *Progress in biotechnology* (Vol. 14, pp. 3-19). Elsevier.
- 67. Schols, H. A., & Voragen, A. G. J. (1996). Pectin and pectinases: Part of the pectin chemistry. Food Hydrocolloids, 10(4), 319-324. https://doi.org/10.1016/S0268-005X(96)80026-5
- 68. Sharma N., Rathore M. & Sharma M. Microbial pectinase: sources, characterization and applications. *Rev Environ Sci Biotechnol* 12, 45–60 (2013).
- 69. Sharma NR, Sasankan A, Singh A, Soni G (2011) Production of polygalacturonase and pectin methyl esterase from agrowaste by using various isolates of Aspergillus niger. Insight Microbiol 1:1–7
- 70. Sharma, A., Meena, R. S., & Rathore, R. (2023). Isolation and optimization of pectinase-producing *Bacillus velezensis* from fermented food. *Journal of Microbial Biotechnology*, 49(2), 201–210.
- 71. Siddiqui M. A., Pande V., & Arif M. (2013). Polygalacturonase production from Rhizomucor pusillus isolated from fruit markets of Uttar Pradesh. *Afr J Microbiol Res*, 7(3), 252-259.
- 72. Singh, R., Kaur, M., & Dhillon, G. S. (2019). Biovalorization of grape pomace using *Trichoderma viride* for pectinase production. *Waste and Biomass Valorization*, 10(8), 2273–2283.
- 73. Sun, Y., et al. (2023). Structural insights into pectinase enzymes: Implications for biocatalysis. *Biochimica et Biophysica Acta (BBA) - General Subjects, 1867*(1), 19-29. https://doi.org/10.1016/j.bbagen.2022.129988
- 74. Tanabe H., Kobayashi Y., & Akamatsu I. (1986). Pretreatment of Pectic wastewater from orange canning by soft-rot Erwinia corotovora. *Journal of Fermentation Technology*, 64(3), 265-268.
- 75. Tanabe H., Yoshihara K., Tamura K., Kobayashi Y., Akamatsu I., Niyomwan N., & Footrakul P. (1987). Pretreatment of pectic wastewater from orange canning process by an alkalophilic Bacillus sp. *Journal of Fermentation Technology*, *65*(2), 243-246.
- 76. Tapre A. R., & Jain R. K. (2014). Pectinases: Enzymes for fruit processing industry. *International Food Research Journal*, 21(2), 447.
- 77. Tepe O., & Dursun A. Y. (2014). Exo-pectinase production by Bacillus pumilus using different agricultural wastes and optimizing of medium components using response surface methodology. *Environmental Science and Pollution Research*, 21, 9911-9920.
- Thomas LM, Doan CN, Oliver RL, Yoder MD (2002) Structure of pectate lyase A: comparison to other isoforms. Acta Crystallogr D 58:1008–1015
- 79. Thornton J., Ekman R., Holmbom B., & Örså F. (1994). Polysaccharides dissolved from Norway spruce in thermomechanical pulping and peroxide bleaching. *Journal of Wood Chemistry and Technology*, *14*(2), 159-175.
- 80. Torimiro N., & Okonji R. E. (2013). A comparative study of pectinolytic enzyme production by Bacillus species. *African Journal of Biotechnology*, *12*(46), 6498-6503.

ISSN: 2584-1491 | www.iircj.org

- 81. Tsegaye, Z., Kebede, T., & Yismaw, S. (2021). Production of pectinase enzyme by *Pichia kudriavzevii* from apple pomace. *African Journal of Biotechnology*, 20(14), 607–614.
- 82. Viikari L., Tenkanen M., & Suurnäkki A. (2001). Biotechnology in the pulp and paper industry. *Biotechnology: Special processes, 10,* 523-546.
- 83. Warrilow AGS, Turner RJ, Jones MG (1994) A novel form of pectinesterase in tomato. Phytochemistry 35:862–872
- 84. Yadav, D., & Nandal, P. (2021). Production and optimization of pectinase from *Aspergillus tubingensis* using banana peel waste. *Environmental Technology & Innovation*, 23, 101691.
- 85. Yoder MD, Keen NT, Jurnak F (1993) New domain motif: structure of pectate lyase C, a secreted plant virulence factor. Science 260:1503–1507
- Yu P., Zhang Y., & Gu D. (2017) Production optimization of a heat-tolerant alkaline pectinase from Bacillus subtilis ZGL14 and its purification and characterization. *Bioengineered*, 8(5), 613-623.

