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# PECTINASES: STRUCTURE, FUNCTIONS AND BIOTECHNOLOGICAL APPLICATIONS

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**Abstract**

The use of pectinases stands as essential biocatalysts with significant structural diversity, functional versatility, and promising biotechnological applications. Understanding the molecular intricacies of these enzymes and harnessing their potential opens up new avenues for sustainable and eco-friendly solutions in various industrial sectors. Pectinase is one of the developed enzymes of fruit and textile industries. These enzymes break down complex polysaccharides of plant tissues into simpler molecules like galacturonic acids. The role of acidic pectinases in bringing down the cloudiness and bitterness of fruit juice is well established. Recently, there have been a good number of reports on the application of alkaline pectinases in the textile industry for the retting and degumming of fibre crops, production of good quality paper, fermentation of coffee and tea, oil extractions and treatment of pectic water waste. This project discusses a comprehensive overview of the structure, functions of pectinases and biotechnological application. It begins by introducing the enzyme group and its classification, followed by structure and an in-depth exploration of their functions in different sectors, including food, beverage, textile, and biofuel industries. Furthermore, the project discusses the mechanism of pectin degradation and the factors influencing enzyme activity. Lastly, potential future directions and challenges in the field of pectinase research are addressed. The information presented in this project serves as a valuable resource for researchers, scientists, and industrial professionals interested in the applications and potential of pectinases.

**Keywords**

pectinase, galacturonic acids, fermentation, classification, biotechnological

## 1. Introduction

Pectinases are a class of lyases (EC: 3.1. 1.11) which are capable of hydrolysing pectin, and have gained significant importance due to their versatile functions across various industries. Some of the first enzymes employed in homes for domestic fruit and vegetable juice extraction were pectinases. Wines and fruit juices preparation was the first pectinase commercial application observed in 1930, the chemical makeup of plant tissues didn't become evident until the 1960s, so, scientists were able to use a wider variety of enzymes more effectively (Kashyap, *et al*., 2001). Pectinases or pectinolytic enzymes are one of the enzymes of choice of the commercial sector today. Microbial pectinases are said to make almost 25% of all food enzyme sales worldwide (Oumer, 2017). These enzymes primarily work by degrading the lengthy, intricate pectin molecules in fruit pulp, which are present as structural polysaccharides and cause pulp turbidity.

The cell wall is a structural component of plant cells that provides structural support and protection. It consists of various polysaccharides, proteins, and other compounds, with pectin being a major component. A vast and intricate family of polysaccharides known as pectin considerably contributes to the physical characteristics of cell wall of the plant (Smith & Harris, 1999). Pectin plays a critical role in cell adhesion, which is essential for maintaining tissue integrity. Calcium ions form bridges between pectin molecules, which lead to the formation of "egg-box" structures. These cross-links strengthen the cell wall and enhance its resistance to mechanical stresses. Pectin helps growing plant cells expand by enabling the passage of water into the cell wall, which aids in cell elongation and promotes plant growth in general. Pectin's dynamic nature also enables it to respond to various environmental stresses, such as changes in pH, temperature, or ionic concentrations. Pectin provides mechanical strength and flexibility to the cell wall, allowing it to maintain the shape and integrity of the plant tissues; it also acts as a barrier against pathogens by restricting their access to the plant cells and helps in the healing of wounds or damaged tissues. Its ability to form a gel-like matrix and influence cell adhesion and communication make it a key player in maintaining the overall structure and function of plant tissues (Smith & Harris, 1999).Galacturonan (α-D-galacturonic acid) is the fundamental component of pectic compounds. Pectic compounds are divided into protopectin, pectinic acid, pectin, and polygalacturonic acid based on the degree of esterification (Gummadi *et al*., 2007), and these are discussed in this project. Pectinases are enzymes that have the ability to alter the structure and characteristics of pectin which are essential for processes like fruit ripening and the softening of plant tissues (Smith & Harris, 1999). A broad and varied collection of enzymes called pectinases is involved in the breakdown of pectic materials. The occurrence of different versions of these enzymes is likely explained by the variety of pectic compounds found in plant cells. pectinases are categorized according to their substrate and manner of enzymatic reaction. By converting pectic materials into saturated and unsaturated galacturonans, nature uses pectinases to recycle carbon, which is then further

broken down into 5-keto-4-deoxy-uronate,3-phosphoglyceraldehyde and pyruvate. By destroying pectic chemicals found in the cell wall, pectinases from fungi like Fusarium oxysporum, Botrytis cinerea, and Aspergillus flavus are known to have significant part in plant virulence or pathogenicity. During the last phases of fruit ripening, pectinases, particularly polygalacturonase, are known to play an important role in the breakdown of pectin (Gummadi *et al*., 2007). Pectinase is now an important part of juice gotten from fruit as well as having many biotechnological uses. The main aim of this project is to provide an in-depth exploration of the pectinolytic enzymes, their classification, structure, functions of pectinases, and their impact in biotechnology. Pectinases' rising demand is due in part to their use in many biotechnological applications, some of which are covered here.

### *1.1. Pectinases*

A complicated collection of enzymes known as pectinases or pectinolytic enzymes breaks down different pectic compounds (pectin) found in the plant cell-walls central lamella (Anand *et al*., 2020). Pectic polysaccharides of plant tissues are broken down by a large range of enzymes called pectinases into simpler compounds like galacturonic acids. Its activity is influenced by a quite number of variables, such as temperature, pH, the amount of substrate present, and the presence of inhibitors or activators (Bezerra *et al*., 2021). Pectinases have long been employed to boost fruit juice yields and clarity. A variety of pectinolytic enzymes are needed to completely digest pectic compounds because they are a very complicated macromolecule category. Pectinases are widely dispersed enzymes found in bacteria, fungi, and plants. These enzymes differ in their cleavage mechanism and selectivity and can be divided essentially into two groups depending on whether they work on pectin smooth regions or pectin hairy regions (Anand *et al*., 2020).

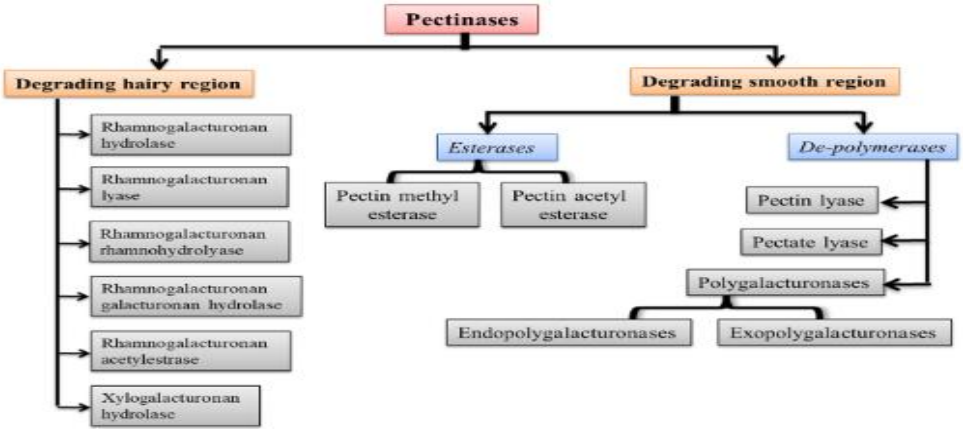


**Fig.1**: Classification of Pectinases due to the specificity of substrate and type of reactions they catalysed (Anand *et al.,* 2020).

### *1.2. Classification of Pectinases (Pectic enzymes)*

Due to the cleavage site, pectinases are classified into three major groups, they are:1. Pectinesterases (PEs) (EC 3.1.1.11)2. Hydrolases comprising of poly-galacturonases (PGs) (EC 3.2.1.15)3. Lyase/trans- eliminates consisting: pectate lyase (PLs) (EC 4.2.2.2) and pectin lyase (PNLs) (EC 4.2.2.10) (Oumer, 2017).1.2.1Pectinesterases (PE) (EC 3.1.1.11): Pectin esterase, also known as Pectin methyl esterase, catalyzes the de-esterification of pectin's methyl group, resulting in the formation of methanol and pectic acid. The enzyme preferentially works on the methyl ester group of a galacturonate unit near to a non-esterified galacturonate unit. It works before the enzymes that need non-esterified substrates, such as pectate lyases and polygalacturonases (Oumer, 2017). It catalyzes the reaction described here: pectin + n H2O = n methanol + pectate (Giovane *et al.*, 2005).

### *1.3. Structure of Pectin esterase*

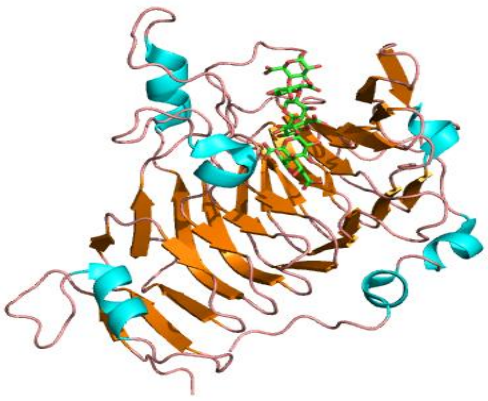

**Fig 2**: Crystal Structure of Pectin esterase (Frieset al., 2007).

### *1.4 Polygalacturonases (PGs) (EC 3.2.1.15):*

These are the pectinolytic enzymes that, when water is added across the oxygen bridge, catalyze the hydrolytic cleavage of the polygalacturonic acid chain (Kashyap *et al.* 2001). Biochemically, polygalacturonases are characterized by several important properties: (Ibbett, *et al.*, 2013). Substrate specificity: Polygalacturonases specifically target polygalacturonic acid, which is the main component of pectin. They cleave the glycosidic bonds between the galacturonic acid residues, leading to the depolymerization of pectin. Optimal pH: The activity of polygalacturonases is influenced by pH. Different polygalacturonases have different pH optima, but generally, they exhibit maximal activity in the acidic to neutral pH range. For example, fungal polygalacturonases typically have an optimal pH around 4-6, while bacterial polygalacturonases have a broader pH range, often optimal around pH 6-7. Temperature sensitivity: The activity of polygalacturonases is also temperature-dependent. Each polygalacturonase enzyme has an ideal temperature at which it performs best. The optimal temperature for most polygalacturonases is between 30 – 60oC. However, there can be variations among different polygalacturonases from various sources. Enzyme kinetics: Polygalacturonases follow typical Michaelis-Menten kinetics, where the rate of enzymatic reaction counts on the substrate concentration. The kinetic parameters, such as the substrate concentration at half-maximal velocity (Km) and the maximum reaction velocity (Vmax), vary depending on the specific polygalacturonase.

Structure of Polygalacturonases

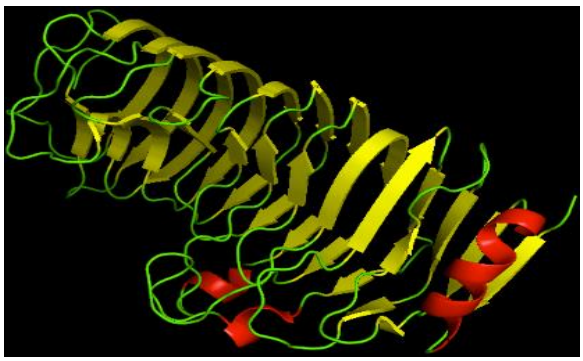

**Fig 3:** Crystal Structure of Polygalacturonase (Cho et al., 2001).

### 1.5 *Pectin lyase (PNLs) (EC 4.2.2.10):*

Pectin lyase cleaves the α-1,4-glycosidic bond between galacturonic acid residues. Through the trans-elimination of glycosidic connections, pectin lyase catalyzes the random cleavage of pectin, particularly high esterified pectin, to produce unsaturated methyl-oligogalacturonates. Ca2+ is not absolutely necessary for pectate lyase, but it and other cations can accelerate it (Oumer, 2017). Biochemically, polygalacturonases are characterized by several important properties: (Liu & Li, 2013).

1. Catalytic Mechanism: Pectin lyase catalyzes the hydrolysis of pectin by employing a two-step mechanism. Initially, a water molecule is activated by the enzyme's catalytic residues, leading to the formation of a covalent glycosyl-enzyme intermediate. Subsequently, the intermediate undergoes a nucleophilic attack by a water molecule, resulting in the regeneration of the enzyme and the release of a product. 2. Substrate Specificity: Pectin lyases exhibit substrate specificity toward various pectin substrates. Their activity is influenced by factors such as the degree of methylation and acetylation of pectin, as well as the presence of neutral sugars in the polysaccharide chain. The variation in substrate specificity among different pectin lyases enables them to target different regions of pectin molecules.

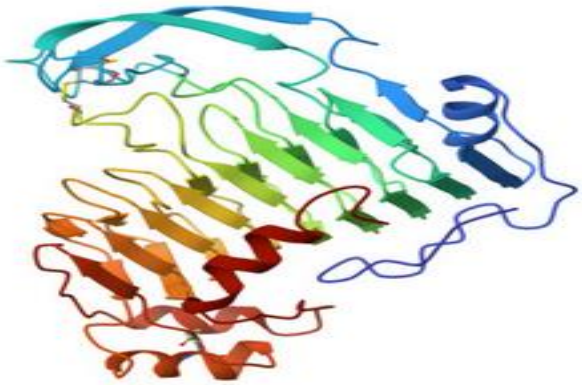

**Fig 4**: Crystal Structure of Pectin lyase (Mayans et al., 1997)

### 1.6 Pectate lyase (PLs) (EC 4.2.2.2):

Pectate lyase uses a β-elimination mechanism to breaks the α-1,4-glycosidic bond in polygalacturonan chains. Pectate lyase preferentially cleaves glycosidic bonds on polygalacturonic acid, resulting in an unsaturated product via a trans-elimination reaction. Pectate lyase has an absolute Ca2+ ions requirement. Hence, it is strongly inhibited by EDTA which is the chelating agents (Oumer, 2017). Biochemically, pectate lyase belongs to the class of lyases, which are enzymes involved in breaking chemical bonds without using water as a co-substrate. It specifically targets pectate, a linear chain of galacturonic acid residues linked by α-1,4-glycosidic bonds, and cleaves it through β-elimination. This results in the formation of unsaturated oligosaccharide products, such as

oligogalacturonides. Pectate lyase enzymes are typically categorized into different isoforms based on their specificities and characteristics, which include: exo-pectate lyases and endo-pectate lyases. Exo-pectate lyases: act on the non-reducing ends of pectinEndo-pectate lyases: cleave the internal glycosidic bonds (Asgher *et al.*, 2016).

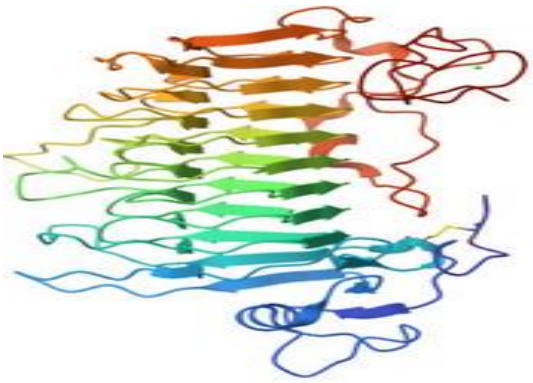

**Fig 5:** Crystal Structure of pectate lyase (Jenkinset al.,2004).

### *1.7 Pectin structure (Pectic substances)*

Chemically speaking, pectic compounds are intricate colloidal acid polysaccharides having a galacturonic acid residue backbone connected by (α1-4) connections, L-rhamnose, arabinose, galactose, and xylose make up the side chains of the pectin molecule. Galacturonic acid's carboxyl groups are neutralized by sodium, potassium, or ammonium ions and partially esterified by methyl groups. The American Chemical Society classified pectic compounds into four categories (Kashyap et al., 2001), as pectic acid, pectinic acid, protopectin, and pectin, also based on the type of modification of the backbone chain (Kashyap et al., 2001).

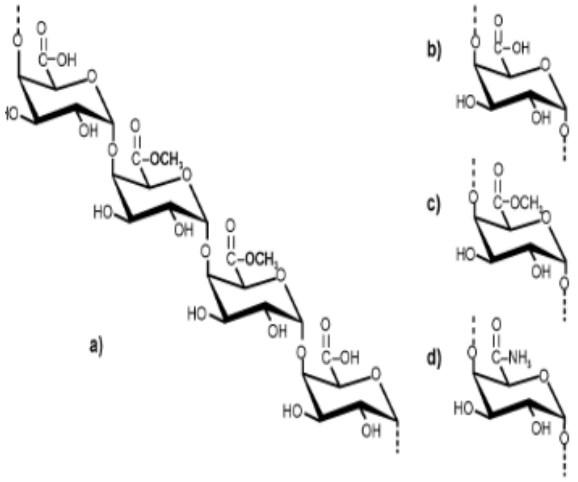


**Fig.6:(**a) A pectin molecule's repetitive portion and functional groups (b) carboxyl(c) ester (d) amide in pectin chain (Kashyap et al., 2001).

### *1.8 Protopectin*

This is a parent pectic material that, when hydrolyzed under certain conditions, produces pectinic acid or pectin. The water-insoluble pectic chemicals present in plant tissues and the source of soluble pectic substances are sometimes referred to as protopectin (Kashyap et al., 2001).

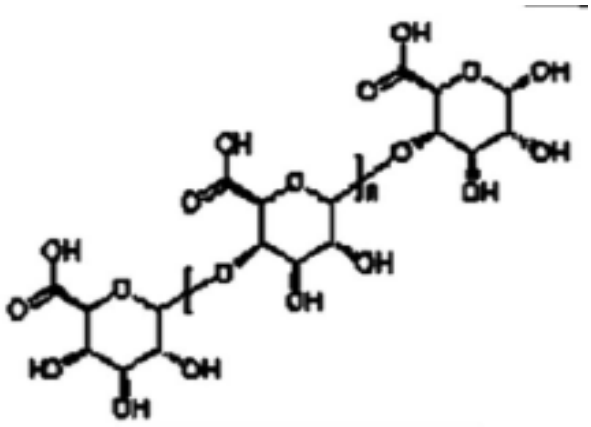

**Fig 7:** Chemical Structure of Protopectin (Satapathy et al., 2020).

### *1.9 Pectic acids*:

Pectic acids are galacturonans that have the fewest methoxyl groups. Pectates are pectic acid's neutral or acidic salts.

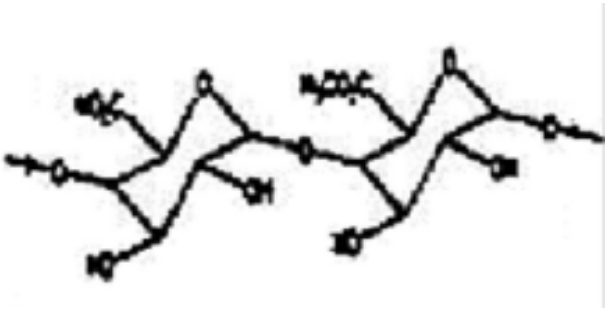

**Fig 8:** Chemical Structure of Pectic Acid (Satapathy et al., 2020).

*1.10 Pectinicacids(polymethylgalacturonate):*

These are galacturonans that include varying numbers of methoxyl groups. Pectinates are pectinic acid salts that are either neutral or acidic (Kashyap, *et al*., 2001). When combined with acid and sugar or, if the methyl concentration is low enough, with specific additional chemicals like calcium salts, pectinic acid alone has the unusual ability to produce a gel.

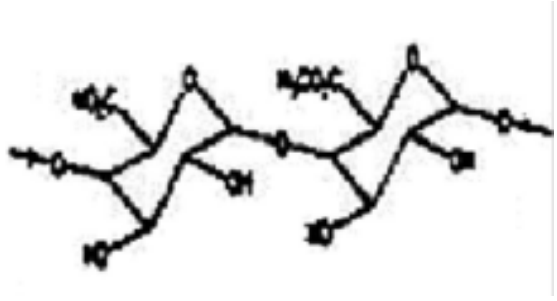

**Fig 9**: Chemical Structure of Pectinic Acid (Satapathy et al., 2020).

Pectin: A general term for a collection of materials with wildly different compositions but primarily pectinic acid. Pectin can interline with other structural polysaccharides and proteins to create insoluble protopectin, which is found in its native form in the cell wall.

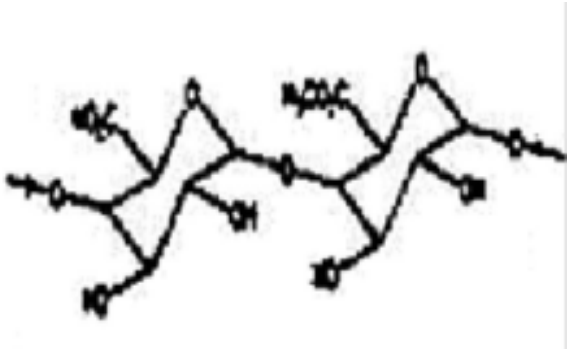

**Fig 10**: Chemical Structure of Pectin (Satapathy et al., 2020).

*1.11 Source of Pectinases (Pectic enzymes)*

Although plants and bacteria can also generate pectinases, the filamentous fungus Aspergillus sp. is the most common source because it produces a complex of pectinolytic enzymes, such as the de-esterifying and chain-splitting enzymes. Oranges and tomatoes both contain those (Pandey *et al*., 2006). Microbial sources of pectinase: are enzymes produced by various microorganisms, including bacteria and fungi. Microbial sources of pectinase have several advantages over traditional plant-based sources, including scalability, cost-effectiveness, and the capacity to manipulate genetically (Smith & Johnson, 2022).1.4.1.1 Bacterial Sources: A number of bacterial strains have been discovered as pectinase makers. Bacillus species, particularly Bacillus subtilis and Bacillus licheniformis, have shown significant pectinase activity. Furthermore, Pseudomonas species, particularly Pseudomonas fluorescens, have been found to release pectinolytic

enzymes. According to research, these bacteria can be grown under regulated circumstances, enabling for industrial-scale enzyme manufacturing (Smith & Johnson, 2022).

*1.12 Fungal Sources*:

Fungi have also emerged as significant sources of pectinase enzymes. Aspergillus strains, such as Aspergillus niger and Aspergillus oryzae, have been widely studied for their pectinolytic capabilities. Other fungal species have been investigated as potential sources of pectinases, including Penicillium spp., Trichoderma spp., and Fusarium spp. These fungi can be grown on a variety of agricultural and industrial wastes, making the process more sustainable and economically effective (Smith & Johnson, 2022).

*1.13 Plant sources of pectinase:*

Citrus fruits, apples, mangoes, papaya, and pineapples are examples of plant-based sources that offer promising reservoirs of industrially valuable enzymes (Doe, 2023).1.5 Function of Pectinases (Pectic enzymes) Pectin esterase largely alters the localized pH of the cell wall, which changes the integrity of the cell wall (Giovane *et al.*, 2005). Their functions in industries are as follows:

Food Industry: Pectinases are essential in the food business because they make it easier to extract juice from fruits, make clarifying and filtration procedures better, and improve the texture and sensory qualities of different food products (Smith et al., 2018). Fruit juice contains colloids, or polysaccharides like pectin and starch, which could result in a fouling problem during the filtration process (Oumer, 2017). Pectinases are used to pre-treat juices to reduce the viscosity of the juice and reduce the amount of pectin, which accelerates the subsequent filtration process. This improves the juice's clarity (Oumer, 2017). Beverage Industry: Pectinases are widely used by the beverage industry to improve filtering techniques, extract juice, and clarify wine and fruit liquids (Gomez *et al.*, 2020). Wine processing industry also recognizes the importance of pectinases where the enzyme can be applied at different stages (Oumer, 2017). Fruit is crushed with the addition of pectinases to increase juice production and expedite anthocyanin released, pectinase treatment settles out suspended particles before or after fermentation. After fermentation, wine is given an enzyme boost to increase its clarity and rate of filtering (Kashyap *et al.*, 2001). Textile Industry: Textile industry uses pectinases to modify and remove pectin-based contaminants from fabrics, such as de-sizing, scouring, and bio-polishing thereby enhancing dye absorption and fabric quality as a result (Couto *et al.*, 2019). Pectinases, in addition with amylases, lipases, hemi-cellulases, and cellulases, have been used in place of toxic caustic soda to safely and sustainably remove sizing agents from cotton. Utilizing specific enzymes, the novel process known as "bio-scouring" purges the fiber of non-cellulosic impurities. For this purpose, pectinases have been used without causing any damage to cellulose (Oumer, 2017). Pharmaceutical Industry: Due to their high fiber content in formulations for lowering blood glucose and cholesterol, pectic substances or direct pectin derived from pectinase treatment through the fermented process from the peels of various fruits

and vegetables are being considered as a fruitful composition in pharmaceutical-based products. Pectin is said by scientists to play an important role in the prevention and treatment of serious illnesses like diabetes and obesity, but how well it does so rely entirely on the kind, molecular weight, and level of esterification of the viscosity (Oumer, 2017). Biofuel Industry: Pectinases contribute to the production of biofuels by facilitating the hydrolysis of pertinacious materials, thus improving the efficiency of biofuel conversion processes (Pandey *et al*., 2019).

## 2. Biotechnological Applications of Pectinases

Biotechnological uses of pectinases in processes has significantly increased recently in the textile and food industries, as well as in the fermentation of wine, tea and coffee, extraction of oil and processing of plant fibers, among other things. (Anand *et al*., 2020). Additionally, pectinases have widespread uses in the fruit and vegetable processing industry as a yield enhancer, colorant, and juice clarifier.

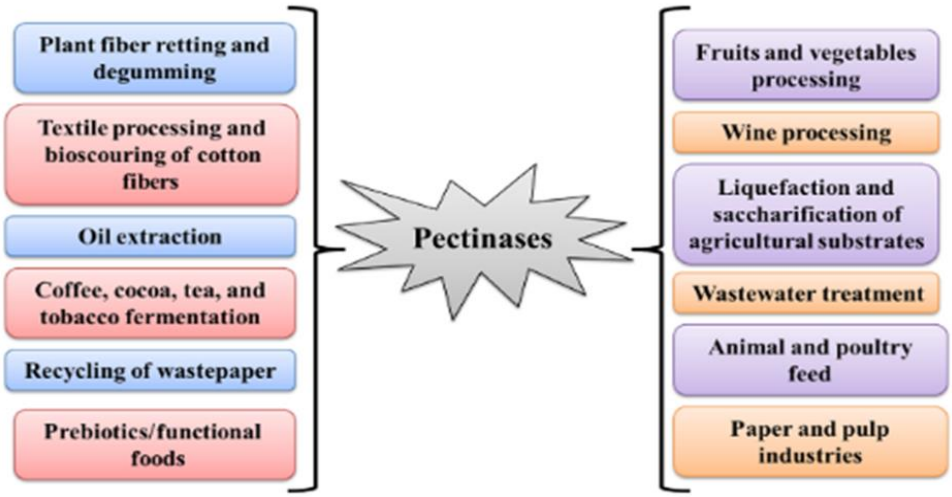


**Fig10**: Different Applications of Pectinases (Anand et al., 2020).

### *2.1 Plant fibre retting and degumming*

Using pectinolytic enzymes, plant fibers such as jute, coir, ramie, cotton, sunn hemp, flax, and hemp are retted and degummed with less harm to the fibers and with greater technical viability. By destroying the pectin found in the middle lamella and main cell wall, pectinases play a crucial part in the retting, maceration, and degumming of these fibers. Pectinases are used to extend the mechanical qualities of fibers including jute, flax, and ramie by raising their tensile strength and brightness (Anand et al., 2020).

## 3. Bio-scouring of Cotton Fibers and processing of textile

The production of textiles is an expanding sector that pollutes the environment heavily by utilizing large amounts of harmful chemicals, electricity, and water. The demand for textile fibers was 93.5 million metric tons in 2016 which is projected above 95 million metric tons in 2020. Due to their harmless makeup and environmentally benign approach, the

use of enzymes in the production of textiles is becoming considerably more popular as demand rises. Instead of using harsh chemicals, bio-scouring is a significant environmentally friendly method for removing some non-cellulosic impurities and making the surface of fibers more hydrophilic. Because pectin degradation removes waxes, many studies have suggested using acidic, neutral and alkaline pectinases as the best enzymes for bio-scouring cotton (Anand et al., 2020).

## 4. Oil extraction

According to Mehanni *et al*. (2017) oil quality over organically processed oil is improved by pectinase treatment during oil extraction in terms of color, intensity, fatty acid content, and peroxide value. Pectinases are alkaline by nature, therefore using them to process oil makes it easier to extract oil in an aqueous phase. As a result, the pectinase-treated extraction method yielded more oil with higher stability and organoleptic (polyphenolic and vitamin E) content. (Kashyap *et al*.,2001).

### *4.1 Tobacco, cocoa, tea and coffee fermentation*

Using pectinases, coffee beans' mucilage coat can be removed. Oumer and Abate (2017) studied how to demucilate coffee using the pectinase enzyme from B. subtilis strain Btk27. In this study, pectinase activity was highest at pH 7.5 and 50oC, and metal ions Mg2+ and Ca2+ increased enzyme activity. Similarly, pectinase enzymes depectinize the white mass of mucilaginous pulp that forms during the fermentation of cocoa seeds, causing it to flow away and speed up the microbial metabolites diffusion into the beans (Ouattara *et al.,* 2008). By breaking down the pectin found in the cell walls of tea leaves, pectinase treatment also helps tea ferment. It also eliminates the foamy property of tea powders, improving the product's quality (Pedrolli *et al*., 2009). Microbes that produce pectinase are also used in the tobacco fermentation. Pectin found in the leave changes during the fermentation process. Pectin methylesterase is prevalent in tobacco leaves. Due to the action of enzymes on pectinic acid in tobacco leaves during the fermentation process, free methanol is produced (Long *et al*., 2017).

### *4.2 Recycling of waste papers*

De-inking is a step in the waste paper recycling from printing presses that removes ink particles from the fiber surface and separates them from the fiber slurry using floating and washing techniques (Lasheva *et al*., 2013). Deinking using enzymes is urgently required because conventional deinking procedures employ a significant number of hazardous chemicals. Due to its great efficacy, ability to save energy, and positive effects on the environment, enzymatic deinking (using pectinases, hemi-cellulases, cellulases, and lignolytic) is widely used (Saxena & Singh, 2017).

*4*

### *4.3 Prebiotics/functional foods*

Pectinase and its derivatives have been noted as prebiotic/functional foods in recent years, and they are employed for their enzymatic synthesis of pectin and pectin-derived oligosaccharides (PDO), which help the host immunological system (Khan et al., 2013). Grapefruit pectins are typically added to children's meals as a stabilizer to improve nutrition and the physical development of young children. It was proposed that pectin and its PDO play a role in sustaining the release of hormones in the gut in

addition to protecting the host against bowel inflammatory illnesses (Tolhurst *et al.*, 2012).

### *4.4. Processing of Fruits and Vegetables*

The processing of pulp, the extraction of fruit juice, and clarifying are necessary for the use of microbial pectinases in the vegetable and fruit business.

Pectinases contribute to reduce the clarification of juice, viscosity and in maceration of vegetables along with decreasing the fermentation time. After being treated with pectinase, the material was easier to press than the control and produced much more fruit and vegetable juices, including carrot and beet juices (Anand *et al.*, 2020). During the preparation of citrus juice, this enzyme also helps to stabilize the liquid and remove turbidity. A combination of pectinases with other enzymes such as amylases, cellulases, and hemi-cellulases is used to clarify juices. Fruit juices have reportedly been treated with pectinases to increase their total sugars, total soluble solids, antioxidant, acidity, and vitamin C content (Sharma *et al*., 2013).

### *4.5 Processing of Wine*

Pectinases are often used in the wine-making process to improve wine quality, facilitate the extraction process that enables filtering, and ultimately increase color and flavor. Compared to conventional procedures, enzyme-treated wines require less time for filtration and are more stable (Anand et al., 2020). According to Armada and colleagues (2010), adding pectinase to red wines enhances their stability, flavor, and structural integrity. Additionally, wines that have undergone enzyme treatment have higher anthocyanin, total phenolic, tannin, and color intensity levels (Sharma et al., 2013). In the production of wine, pectinases are frequently combined with additional enzymes such glucanases, hemicelluloses, and glycosidases.

### *4.6 Saccharification and liquefaction of agricultural substrates*

Pectinases are frequently used to break down pertinacious material in agro-industrial waste that has high pectin content. Apple pomace, citrus peel, cassava pulp, banana waste, potato pulp, and sugar beet pulp are just a few examples of the pectin-rich materials that are processed to make bioethanol or are used as fermentable sugars (Anand *et al*., 2020). For the conversion of polysaccharides (carbohydrates, proteins, and lipids) found in the plant cell wall into simple sugars, a variety of enzymes including pectinase, hemicellulases, and cellulases are used.

### *4.7 Waste-water treatment*

Wastewater from the food processing of fruits and vegetables, which contains a significant amount of pectin, is released. Conventional methods use various physical and chemical processes, such as physical dewatering, coagulation, and hydrolysis by chemicals, followed by methanogenesis. Utilizing pectinase-producing microorganisms during the pre-treatment of wastewaters makes it much easier to degrade pectin components and improves their compatibility for decomposition throughout the activated sludge treatment process (Jayani *et al*., 2005). This eco-friendly treatment is cheap and less time-consuming. The pectinase-producing microorganisms in the activated sludge process break down a substantial amount of pectinaceous

compounds in the effluents from the citrus processing industry (Anand *et al*., 2020).

### *4.8 Paper and pulp industries*

The paper and pulp industries are increasingly using enzymes like pectinases, xylanases, and ligninases as a substitute for chlorine-containing bleaching chemicals, which have major negative environmental effects (Bajpai, 2018). Pectinases aid in the resolution of retention concerns in pulp bleaching by depolymerizing galacturonic acid polymers, lowering the cationic requirement of pectin solutions and the filtrate of the peroxide bleaching (Anand *et al.,* 2020). Additionally, it has been noted that bleached pulp contains a significant amount of pectins, which give paper its yellow hue, and that pectin impairs dewatering during sheet creation because to the high cationic demand. The pectins in the pulp's aqueous phase are broken down by the application of pectinase both alone and in conjunction with other enzymes in the bleached pulp.

### *4.9. Animal and poultry feed*

The world's fastest expanding sector is the animal and poultry feed market, which is significant. In the early 1980s, the use of different enzymes for animal and poultry feed was started. The first report of the use of enzymes in feed diets was the supplementation of β-glucanase into barley-based diets. In general, a group of multienzymes such as pectinases, xylanases, proteinases, amylases, cellulases, and glucanases are utilized to prepare feed enzymes. Also, the use of various enzymes when preparing ruminants' food leads to an improvement in the quality of feeds and increased dietary energy absorption capability (Anand *et al.*,2020). The use of multienzyme, namely pectinases, glucanases, and hemicellulases, improves the digestibility of a mixture of vegetable proteins (sorghum, soy, and canola) in broilers for more than 40 days, according to Petersen (2001). Enzyme usage in feed has improved the weight gains and feed conversion significantly.

### *4.10 Plant Viruses purification*

Before purification, there is a short window for learning more about a virus. To conduct the biological and physicochemical experiments, a pure form of plant viruses is required. Numerous techniques are used for the purification of plant viruses, but in order to determine the virus' actual nature, purification systems might be used. Alkaline pectinases and cellulases can be employed to break down the tissue and release the virus when viruses are connected with phloem in particular situations (Anand *et al.,* 2020).

### *4.11. Isolation of protoplast*

In the realm of biotechnology, protoplasts have a wide range of uses, including genetic modification, membrane-based research, and plant tissue culture. Protoplasts are plant, fungi or bacteria cells devoid of cell wall either enzymatically, chemically or mechanically (Ozojiofor, 2017). It has been mentioned that pectinases are used with a variety of other enzymes, including cellulases and chitinases, to isolate protoplast (Rebello *et al.,* 2017). For Protoplast fusion to occur, the cell walls of the organisms need to be degraded and to achieve this, lytic enzymes such as xylanases, pectinases, proteases and cellulases or macerozymes are

employed for this process to degrade the plant cell walls (Ozojiofor, 2017).According to Parani and Eyini (2011), 3-day-old Pleurotus and P. Flabellatus mycelia were subjected to a 3-hour treatment of cellulases, pectinases, and chitinases in KCl (0.6 M) to act as an osmotic stabilizer and phosphate buffer with a pH of 6.0. This resulted in the release of significant amounts of protoplasts.

## 5. Industrial Application of Pectinases

There are two types of pectinases: acidic and alkaline pectinases based on industrial point of view (Gummadi *et al*., 2007). The acidic pectinases are used extensively in the maceration of plant tissues as well as the extraction and clarification of both sparkling clear juice (apple, pear, grape, and wine) and murky juice (lemon, orange, pineapple, and mango). They are also useful in the isolation of protoplasts (Ozojiofor, 2017). While Alkaline pectinases may be used in the scouring of cotton, the degumming of plant fibers to enhance the quality of the fiber, the fermentation of coffee and tea, the paper industry, and the purification of plant viruses, among other potential uses.

### *5.1 Pectinase application in Genetically Modified (GM) Foods*

Genetic Engineering of Tomato's Pectic Enzymes Fruit's cell wall breakdown as it ripens is a significant factor in determining the texture of fresh fruit. Genetic engineering can alter the cell wall enzymatic activity during ripening, which can affect cell wall polysaccharide metabolism and texture. The ultimate viscosity and processing properties of processed tomato products are influenced by the texture of fresh fruit. The role of particular enzymes may now be assessed in both fresh fruit and processed goods thanks to the development of transgenic tomato lines that modify the expression of one or more genes (Kalamaki *et al.,* 2007).

### *5.2 Single Transgenic Tomato Line*

To slow tomato softening, polygalacturonase (PG) was the initial focus of genetic modification. By expressing an antisense PG transgene under the control of the cauliflower mosaic virus 35S (CaMV35S) promoter, transgenic plants were created in which PG expression was reduced to 0.5 - 1% of wild-type levels. Fruit from plants with wild-type PG levels was normal in ripening. Fruit treated with PG suppressors had a longer fruit storage life than untreated fruit (Kalamaki *et al.,* 2007).

### *5.3 Double Transgenic Tomato Lines*

Fruit's ability to be stored longer was increased. Altering the expression of specific cell wall hydrolases had little to no impact on fruit softening, indicating that a group of cell-wall-modifying enzymes may work together to cause fruit softening during ripening in the PG inhibited fruit. The creation of twin transgenic lines helps researchers better understand how cell wall breakdown during ripening affects the feel of ripe fruit and the physicochemical characteristics of processed tomato products (Kalamaki *et al.,* 2007). By mating two homozygous single transgenic lines, double transgenic lines were created. The force needed to compress the tomato's blossom end by 2 mm was used to determine the texture of the fruit after it was collected at the mature green stage and left to develop at 20oC. At all stages of ripening, fruit from the double suppressed line was firmer than fruit from

controls and single transgenic lines. Fruit that was allowed to ripen on the vine showed the same outcomes. Fruit from the twofold suppressed line was 20% stiffer than controls at the red ripe stage. Using a Bostwick consist meter, the flow characteristics of juice made from control, single transgenic, and double transgenic lines at the mature green/breaker, pink, and red ripe phases were assessed. In all genotypes, average juice viscosity dropped as fruit ripened. Juice from the suppressed PG and double suppressed lines had higher viscosity than control at the red ripe stage (Kalamaki *et al.*, 2007).

## 6. Conclusion

Pectinases can be employed in a variety of industrial processes that require the degradation of pectin. Different kinds of pectinolytic enzymes have been produced using a variety of microorganisms. Microbial pectinases account for 25% of all food and industrial enzyme sales globally, and their market is rapidly expanding. Due to pectinase's huge potential in a variety of industries whenever pectin degradation is required. Pectinases exhibit diverse functions and have proven to be invaluable tools in various industries. This paper provided a comprehensive overview of the functions of pectinases, their classification, structure and their functions. By understanding the potential of pectinases, researchers and industrial professionals can harness their applications to improve processes and develop innovative products. Potential future directions in pectinase research should be focus on enzyme engineering, immobilization techniques, and optimization of enzyme production. Additionally, it should address the challenges faced in maximizing the utilization of pectinases in various industries.

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