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PECTINASE: A COMPREHENSIVE REVIEW

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ABSTRACT

Pectinase, an enzyme pivotal for breaking down pectin, a complex polysaccharide inherent in plant cell walls, has undergone significant advancements since its initial discovery in the 19th century. Originally utilized primarily for fruit juice extraction, its utility has since expanded across a spectrum of industries encompassing food and beverage, textiles, pharmaceuticals, and oil extraction. This review comprehensively outlines the historical evolution of pectinase, encompassing its classification, microbial origins, fermentation methodologies, and the impact of various factors on its production. Furthermore, it delves into the projected growth trajectory of the global pectinase market, shedding light on key market players and emerging trends. Additionally, the review explores the manifold applications of pectinase, ranging from the clarification of fruit juices to its role in pharmaceutical drug delivery systems. Moreover, it delineates diverse strategies aimed at augmenting enzyme production, including genetic manipulation, optimization of cultivation parameters, and refining downstream processing techniques. Furthermore, the review identifies burgeoning areas of research and future possibilities, such as investigating the prebiotic attributes of pectin-derived oligosaccharides and the feasibility of large-scale commercial production. In sum, this comprehensive review offers valuable insights into both the present status and prospective avenues of pectinase research and utilization.

Keywords: Pectin, Pectinase, Fermentation, Sources of microbes, Market values

1. Brief history of pectinase development:

In the late 18th century, enzymatic processes began to spread. At the beginning of the 19th century, two chemists who worked in a sugar factory both were discovered a kind of sprouted barley extract that could gelatinize starch into a sweet and transparent liquid. Also, 19th-century Louis Pasteur used the term fermentation to yeast that converts sugar into alcohol [1]. In 1984, the first model of enzyme catalysis was described by Emil Fischer which was called the lock and key model. The enzymatic process occurs under certain energetic conditions where the substrate is converted into a product.

Pectin is an acidic polysaccharide and its basic structure repeats α -1,4 linked D-galacturonic acid, which is present in the primary cell wall, a middle layer of plants [2]. The biological function of pectin substances is to provide mechanical strength to parts of a plant, protect cells and maintain the aqueous phase in cells by absorbing water from various environmental factors [3]. Pectinase (polygalacturonase) is an enzyme that breaks down pectin, a polysaccharide. This may be the most simple and accurate definition of pectinase, but in practice, it covers a myriad of enzymatic species.

The forecasted period between 2023 and 2030 is expected to witness significant growth in the Global Pectinase market. Currently, in 2022, the market is exhibiting a steady growth trajectory. With key players implementing various strategic initiatives, the market is poised for further expansion in the coming years. The global Pectinase market, estimated at USD 363.4 million in 2022 due to the COVID-19 pandemic, is anticipated to reach a readjusted size of USD 450.3 million by 2028, exhibiting a CAGR of 3.6Percent during the forecast period from 2022 to 2028. In light of the economic fluctuations resulting from this health crisis, Protopectinases, which accounted for Percent of the Pectinase global market in 2021, are projected to reach a value of USD million by 2028, growing at a revised Percent CAGR from 2022 to 2028. The Food and Juice segment is expected to experience a Percent CAGR throughout the forecast period. Novozymes, DuPont (IFF), and DSM stand out as the top 3 companies in the global pectinase market, collectively representing about 60Percent of the total market share. Geographically, Europe commands approximately 40Percent of the global market share, followed by China with about 20Percent.

Among the different types, the Pectinesterase segment holds the largest share at about 30Percent. In terms of application, the food and juice industry segment accounts for over 50Percent of the global share. The research report delves into various factors influencing the market's growth, including trends, restraints, and drivers. It provides insights into segments and applications that could potentially impact the market in the future, based on current trends and historic milestones. The report also analyzes production volumes globally and by region from 2017 to 2028, along with pricing analysis based on different parameters. Evaluation of restraints offers strategic insights for navigating market challenges and capitalizing on emerging opportunities. Additionally, expert opinions are integrated to enhance understanding of market dynamics.

Pectinase finds widespread use in food processing, particularly in the production of beverages such as wine, fruit juice, and vegetable drinks. Its primary mode of action involves the decomposition of pectin into oligogalacturonic acid and galacturonic acid. In brewing processes for vegetable juice, fruit juice, and wine, pectinase is utilized to enhance juice yield.

This review will attempt to capture some of this work and highlight the potential of pectinase, as well as the great future it still holds.

2. **Classification and microbial resource of**

pectinase: The classification of pectinase which is involved rhamnogalacturonan hydrolase (RG hydrolase), rhamnogalacturonan lyase, rhamnogalacturonan rhamnohyrolse, rhamnogalacturonan galactohydrolase. Pectin methylesterase, pectin acetyl esterase, pectin lyase, pectate lyase, polygalacturonases. Enzymes involved in the decomposition of the “hemograturonan” can be practically divided into two classes: esterase and depolymerizes. **Esterases:** This group of enzymes is essentially esterase that remove methoxy and acetyl residues from pectin that produce polygalacturonic acid. **Pectin methyl esterase:** It forms methanol with acid pectin to form methoxy pectin group and classified into carbohydrate Esterase family 8 [4]. **Pectin acetyl esterase:** It hydrolyzes the acetyl ester of pectin acid and acetate. They are divided into 12 and 13 carbohydrate families.

Depolymerase: Homogalacturonan has usually bound through α -1,4 linkages the

transport mechanisms of polygalacturonase or pectin.

Polygalacturonases: Hydrolysis of polygalacturonase catalyzes the α -1,4 -a glycosidic bond of polygalacturonic acid from D- galacturonase. Microbial source of pectinase produced included *A.nominus*, *B.paracheniformis* , *A.kawachii* [5] [6]. **Pectate lyase:** It is preferably cleaving glycosidic bond across the PGA during the extrusion to form an unsaturated product component and also requires complete ca^{+2} ion and strongly inhibited by a chelating agent

such as EDTA [7]. Microorganism is production of pectinase such as *B. amyloliquefaciens* S6 [8], *Massilia eurypsychrophila* [9], *B. subtilis* 168 [10] . **Pectin lyase:** It breaks down pectin by an elution mechanism to form 4,5-unsaturated oligogalacturonic acid. It is also preferred in the juice filter industry as it does not produce toxic methanol and evaluated from molecular biology. Microbial sources involved the *Bacillus cereus*, *Aspergillus Niger*, *Fusarium lateritum* [11] [12].

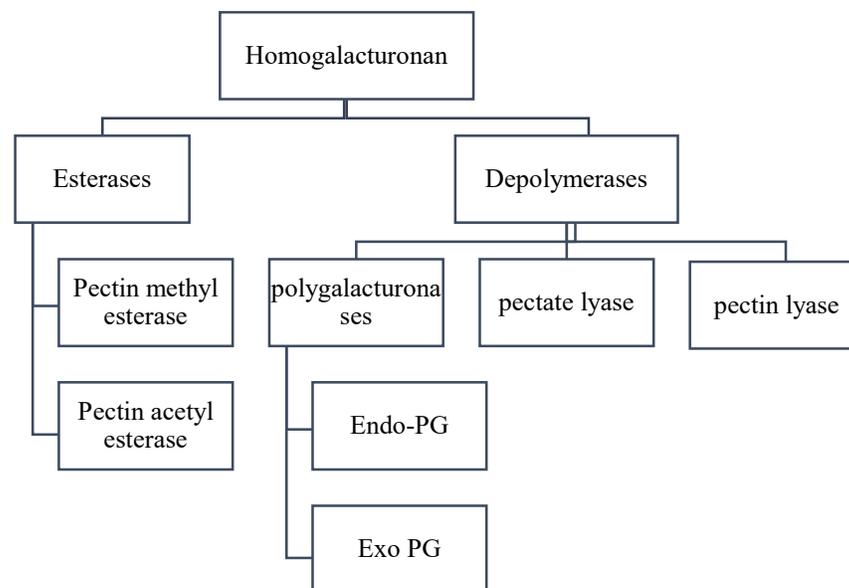


Figure 1: classification of Homogalacturonan Pectin)

Sources of microorganisms: Microbes that decompose fruit and vegetable waste are often capable of producing pectinase.

This waste material provides a natural habitat for pectinase-producing microbes.

Table 1: Pectinase producing microbes and sources			
Microbes	Sources	Species name	References
Bacteria	Dump yards of vegetable wastes	<i>Bacillus circulans</i>	[13]
		<i>Chryseobacterium indologenes Strain SD</i>	[14]
	Cassava waste heap	<i>Bacillus licheniformis</i>	[15]
	Rotten vegetable		[16]
	Fruit waste	<i>Pseudomonas plecoglossicida strain, Klebsiella pneumoniae strain VD, Stenotrophomonas maltophilia strain 13635L</i>	[17]
	Agricultural crop wastes	<i>Bacillus subtilis</i>	[18]
	Fruit and vegetable market waste soil		[19]
Decomposed mango industrial waste	<i>Enterobacter sp. PSTB-1</i>	[20]	
Fungi	Banana peels	<i>Aspergillus terreus NCF 4269.10</i> <i>Aspergillus niger</i>	[21] [22]
	Olive paste and olives	<i>Aspergillus fumigatus</i>	[23]
	Sugar beet pulp	<i>Penicillium citrinum</i>	[24]
Yeast	Citrus fruit peels	<i>Wickerhamomyces anomalus</i>	[25]
	During coffee fermentation	<i>Pichia kudriavzevii</i>	[26]
		<i>Saccharomycopsis fibuligera</i>	
		<i>Papiliotrema flavescens</i>	

3. Several approaches of fermentation modes:

3.1. Submerge fermentation system:

This is the conventional method of making enzymes using a liquid broth containing a high proportion of nutrients and oxygen needed for microbial growth. This can be done in batch or continuous working method with aeration and agitation system provided for the mixing of the media. This method is very effective for bacteria and some fungi species. It has been found using that different substrate and produces high enzymatic activity [27].

3.2. Solid-state Fermentation: SSF method uses a natural habitat for

fungi accumulation and low humidity fermentation. This process occurs with or without free water and used in the pectinase industry for decades. Here is no need to add substrate constantly. Used SSF filter for alkaline production of exogenous polygalacturonate from *Bacillus subtilis* and increased manufacturing process. While using solid-state fermentation with high activities of enzyme production [27].

3.3. An alternative process for increased enzyme production:

Many researchers work on enhancement the yield of pectinase production, stability, and storage in a long term, reduce plant contamination

by immobilization system. Several industries are experimenting with the production of these pectin enzymes and stabilization whole cells by dynamic immobilization using various methods such as binding, absorption, and deposition [28]. A comparative study between the thermodynamic and kinetic properties of free and inert pectinase in alginate beads was reported by [29]. **Genetic Engineering:** Modify the genetic makeup of host organisms (such as bacteria, yeast, or fungi) to overexpress the gene encoding the desired enzyme. This can be achieved through techniques like gene cloning, gene knockout, or gene insertion. **Optimization of Culture Conditions:** Adjust various parameters of the fermentation process, such as temperature, pH, nutrient composition, oxygen levels, and agitation speed, to create optimal conditions for enzyme production [30]. **Use of Inducers and Promoters:** Incorporate inducible promoters into the host organism's genome, which can be activated by specific inducers to initiate enzyme production. This allows for tight

control over enzyme expression. **Fed-Batch and Continuous Culture Systems:** Implement fed-batch or continuous culture techniques to maintain optimal nutrient levels throughout the fermentation process, thus prolonging the production phase and increasing overall enzyme yield. **Co-Cultivation and Mixed Culture Systems:** Cultivate multiple microorganisms together in a symbiotic or synergistic relationship, where each organism contributes to the overall enzyme production process. This can enhance substrate utilization and metabolic pathways, leading to increased enzyme yields. **Immobilization of Enzymes:** Immobilize enzymes onto solid supports or within matrices to improve stability and longevity, allowing for repeated use and continuous enzyme production. **Metabolic Engineering:** Modify metabolic pathways within the host organism to redirect cellular resources towards enzyme synthesis. This can involve altering precursor availability, enzyme kinetics, or regulatory mechanisms to enhance enzyme production. **Optimization of**

Downstream Processing: Streamline purification and recovery processes to minimize losses and maximize enzyme yield. This can involve the use of advanced separation techniques, such as chromatography, filtration, or precipitation. **Strain Development:** Screen and select high-producing strains through mutagenesis, directed evolution, or rational design approaches to generate improved enzyme-producing microorganisms [31]. **Use of Alternative Host Systems:** Explore non-conventional host systems, such as plant cells, insect cells, or mammalian cells, for enzyme production, particularly for enzymes that may require post-translational modifications or specialized folding pathways. By employing these alternative processes, researchers and industrial practitioners can effectively enhance enzyme production yields, leading to increased efficiency and cost-effectiveness in various biotechnological applications. **Effect of different parameters on Enzyme Process:** Enzymatic action is determined by many factors such as enzyme volume, substrate

concentration, temperature and ions also used in several modes may be high pressure, low water action, ionizing radiation. In addition to other protein functions, enzymes have an amino acid chain attached to the same structure that can be broken down and converted into a variety of shapes, the different amino acids being attached to other side. Mainly industrial management is mutual water and alkaline pH treatment. An improved yield was observed to maintain pH at 5 and temperature at 40°C in *Chryseobacterium indologenes* reported the production of 0.564U/ml [32]. Pectinase production and most of the pectinolytic fungi have their incubation time between 3 to 6 days. Achieved maximum pectinase activity is at 38.88U / mg when using *Aspergillus fumigatus* strain formulation for 6 days. The Ge zang. was investigates the production of alkaline pectinase using tobacco stalk waste with the newly isolated strain *Bacillus tequilensis* CAS-MEI-2-33, optimizing fermentation conditions to achieve a pectinase activity of 1370 U/mL under optimal conditions, and studying enzymatic properties and

potential applications. The enzyme from *Penicillium citrinum* exhibited activity over a broad pH range, with optimal activity at pH 6.0. The main findings of the study are: the highest specific activity of pectinase was achieved through ethanol precipitation, the degradation products of pectin were identified as glucose, saccharose, and fructose, and ethanol precipitation was the most effective method for obtaining a high yield of pectinase. Specific activity of pectinase enzyme: 217.44 U/mg; Total activity of pectinase enzyme: 95.15 U/mL/min.

4. Applications of Pectinase: The diverse applications of pectinase on a global scale have generated a demand for their distribution. Various sectors where pectinase finds utility are explored in the following sections.

4.1. Food and beverage industry:

Pectinase, a group of enzymes that degrade pectin, plays a crucial role in the food and beverage industry. It is widely used in the production of clear and concentrated fruit juices, where it aids in pressing, clarification, and liquefaction. Pectinase also finds applications in the extraction,

clarification, and stabilization of fruit juices, as well as in coffee, cocoa, and tea fermentation, and in the preparation of jams and jellies. Furthermore, it is involved in the softening process in the pickling industry and has potential applications in bioenergy production. Despite its importance, the production of pectinase faces challenges in terms of yield and productivity, which has led to the exploration of microbial sources and the use of genetic engineering approaches to enhance production.

4.2. Bio scouring of cotton fibers and textile processing:

Clothing processing is a growing industry that uses many toxic chemicals to the environment, water, energy sources that cause high environmental pollution. Bio sourcing is a unique environmentally friendly procedure that makes fiber surfaces more hydrophilic with the help of chemical treatments rather than specific enzyme [33]. Many researchers have identified acidic, alkaline, and neutral that it is most suitable for pectin contaminations and used to remove wax. Treatment with pectin improves

the physical properties of the clothing, such as whitening tensile strength and tearing tradition in clothing with alkaline scratch. Enzymes, such as pectinases, are effective in removing non-cellulosic impurities from cotton fabrics, enhancing their absorbency and strength, and improving dyeing and finishing processes. This approach significantly reduces the demand for water, energy, and chemicals, leading to lower costs and environmental impact.

4.3. Clarification of fruits and vegetable processing: Pectinase helps reduce viscosity, clarity and softness of vegetables as well as reduces fermentation time. This enzyme also plays a role in removing turbidity and stabilizing the juice during citrus juice processing. The production of exopolygalacturonase produced by *Bacillus subtilis* was observed in carrot juice extraction with a yield of 13.3% using commercial enzyme [34]. Acidic polygalacturonase enzyme produced by the *Aspergillus niger* showed wide potential application in the clarification of orange juice which resulted from 27%

increase in transmittance observed in the clarified juice [35].

4.4. Pharmaceutical industry: Pectin, a biopolymer, has been explored for its potential in drug delivery systems. Studies have shown that pectin-based systems can be effective in controlled drug release, particularly in the colon. The use of pectin in various oral drug delivery platforms, including controlled release, gastro-retentive, and mucoadhesive systems, has also been investigated. Since pectinase has high substrate specificity even number under various conditions, it can act on different pectin substrates and controlled drug release into drug delivery system. When optimized and evaluated a drug delivery system containing pectin and bacterial degradation of pectin present in the compression layer was investigated [36].

4.5. Oil extraction: Pectinase and cellulase are the most favorable enzymes for degrading the cell wall in oil seeds to loosen oil sacs embedded in the seed structures. Increase in oil yield by 20 %. The utilization of pectinase and other cell wall degrading enzymes for the extraction

of oil from different sources such as flaxseed, olives, dates. In industrial petroleum preparation of oil is commonly used with cell wall degrading enzyme [37-40]. The application of crude pectinase gave an optimum oil recovery of 44% with an enzyme dosage of 1.20 %. Oil extraction rate was 93.26% and yield of edible vegetable oil was 88.09%. The processing of pectinase during oil extraction improves the quality of oil compared to organic oil in terms of color intensity, fatty acid content, and peroxide content.

5. Conclusion and upcoming evaluation:

All researcher so far in pectinolytic enzyme engineering have two different purposes. First is a need for industrial stability, second is a potential market for new low cost and inexpensive pectinase to reduce the commercial cost of existing fungal pectinase. Further pectinase about the improvement methodologies and the process of downstream should be made for future studies to improve the strain that could produce a high enzyme that is economically viable. DNA technology will continue to develop exciting new enzyme molecule that can be utilized in various process in food production to

reduce environmental risks, reduce energy consumption, improve product properties to industrial condition. The future vision of pectin-based drug transporters is low molecular weight homogeneous oligosaccharides that provide targeted delivery to the site rapid release of substance contain drugs. This development will result in a wide range of diseases, including various tumors that can affect the transport system which contains natural and modified biopolymers. Other research studies will focus on the evaluation of pectin about their physical-chemical properties.

For further research on the industrial applications of alkaline pectinase produced using tobacco stalks and suggests exploring the potential of the newly isolated strain for biorefining processes and waste utilization. The potential future research could focus on further exploring the prebiotic properties of pectic oligosaccharides, investigating their specific mechanisms of action on gut microbiota, conducting clinical trials to assess their impact on human health, and exploring the development of pectin-derived oligosaccharide-based drugs for cancer and other clinical applications. Additionally, future research could delve

into the structural variations of pectic oligosaccharides and their biological effects, aiming to optimize their functionality for specific health benefits. Exploring the feasibility of large-scale production and commercialization of pectinase for industrial applications.

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