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Functional characterization of the pectin's degrading enzyme pectin: A Review Paper

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Abstract:

The enzyme families known as pectinases are responsible for breaking down plant tissues' pectin into simpler compounds like galacturonic acids. They are linked to other polymers and carbohydrates as chain molecules having a rhamnogalacturonan backbone. These pectinases are widely used in the wine and fruit juice industries. It is used in the fruit juice business for clarifying, which results in a decrease in viscosity and the generation of clear juice. Enzymatic pulp liquefaction increases juice production, while maceration of structured tissue into a suspension of intact cells helps generate pulpy meals. To meet the rising demand around the world, microbial pectinase has found its way into more and more industries. Microorganisms, specifically bacteria, fungi, and yeast, are the primary producers of pectinase, utilizing inexpensive agro-industrial waste as substrates to facilitate its manufacture. Based on their specificity and cleavage method, these enzymes may be roughly classified into two types that operate on the "smooth" or "hairy" parts of the pectin. Pectinase is one of the most common enzymes present in fungi, bacteria, and plants. The pectinolytic enzymes and their substrates are discussed in this review, along with the pectin-enzyme's functional characterization.

Key Words: Pectinase, Pectin, Pectinolytic microorganisms

1. Introduction:

Small quantities of molecules called enzymes are found in living things' cells, and they have the ability to accelerate chemical processes without changing themselves after the reaction. The food, chemical, and pharmaceutical industries greatly benefit from enzymes due to their many benefits over chemical catalysts, such as their tunable activity, high catalytic efficiency, and high specificity [1]. The primary source of industrial enzyme requirements is microorganisms. Because of their rapid growth, short lifespan, and simplicity of genetic alteration, microorganisms are used in industry to synthesize enzymes. A few competing firms provide, standardize, and commercialize microbial enzymes. Among these enzymes of great industrial significance are pectinases. These enzymes find extensive usage in many important industries, including those dealing with food, textiles, beverages, pulp and paper, and biofuels [2]. Plant infections target cells and facilitate their entry and dissemination throughout the host tissue by producing a range of enzymes that degrade cell walls. These phytopathogenic enzymes include cellulases, pectinases, and proteases. Based on their mechanism of action and substrate selectivity, pectinases are a class of enzymes that are further subdivided into various subgroups. Examples of these include lyases, hydrolases, and methyl deesterases. The use of renewable resources, especially agricultural and forest leftovers, whose main constituents include cellulose, starch, lignin, xylan, and pectin, has drawn attention globally due to rising energy needs. The food sector uses pectinolytic enzymes extensively to produce wine and juice [3]. Enzymes known as pectinases target pectin and depolymerize it through de-esterification processes, hydrolysis, and trans elimination. This breaks down the ester link that holds the pectin's carboxyl and methyl groups together. Higher plants' cell walls contain a type of complex polysaccharide called pectin, which is the cementing agent for the cellulose network and is affected by these enzymes. Pectinases account for 10% of all industrial enzymes produced globally, and demand for them is increasing every day [4]. Pectinases are divided into external and intracellular categories based on how they secrete. An extracellular enzyme is released (secreted) into the surrounding media from the cell. Extracellular enzymes often hydrolyze large substrate molecules into smaller ones that can be more easily transported inside the cell membrane, in contrast to intracellular enzymes that work inside the confines of the cell. Membrane proteins continue to be connected to the cell membrane in some manner. Based on how they target the galacturonan component of pectin molecules, intracellular and extracellular pectinases are categorized [5]. Both solid-state fermentation (SSF)

and submerged fermentation can create pectinases. Submerged fermentation refers to the process of cultivating microbes in a liquid broth. It produces a lot of effluents, needs a lot of water, and must be constantly stirred. Enzyme production in SSF often does not necessitate aseptic conditions [6]. It involves aerobic conditions, the absence or near absence of free water, and microbial growth on or inside solid substrate particles [3]. The manufacture and characterization of pectinase, the pectinolytic enzymes, and their substrates, and the industrial use of these enzymes are all covered in this study.

2. Pectin

Acrylates of carboxylic groups with methanol form pectin, a polymeric material. It may be separated into two areas: the "hairy region" and the "smooth region" (Fig. 1). Depending on where it comes from, esterification's vary in degree. The bulk of it is composed of three structurally well-characterized polysaccharide motifs: homogalacturonan (HGA), rhamnogalacturonan I (RG I), and rhamnogalacturonan II (RG II) [7]. Enzymes that break down cell walls have the ability to significantly alter the architecture of the network made up of these three polysaccharides. With a-1,4-linked residues of D-galacturonic acid, homogalactolacturonan is the backbone chain of the pectin molecule. This is susceptible to O-6 methylation. The primary cell walls of certain plants have the more complex RG II structure, whereas the heavily branched region with numerous side chains of a-1,2-linked L-rahmnopyranose residues is home to RG I [8]. Instead of being a structural polymer, it is thought to function as a signal molecule in the formation of plant cell walls [9]. Side chains such as arabinans, Galatians, or arabinogalactans, which are connected to rhamnose by b-(1,4) connections, branch the pectin molecule at the rhamnogalacturonan portion. The galactose units in the major side chains are coupled by b-(1–4) connections, whereas the arabinose units are area-(1–5) linked. In addition to these neutral sugars, xylopyranose, D-glucopyranose, and L-fucopyranose may be found in the side chains of pectins; on the other hand, RG II includes D-apiose, 2-O-methyl-D-xylose, and 2-Omethyl-L-fucose. There was also evidence of acetylation in the homogalacturonan region, which is consistent with the C2 and C3 acetylation sites observed in RG I galacturonic acid residues. The composition of pectins can vary [10]. Most molecules are made up of blocks of "smooth" and "hairy" sections that include residues of D-galactolacturonic acid. In solution, the molecule is stretched and curled (worm-like) with a great deal of flexibility rather than taking on a straight shape. Pectins' "hairy" areas are significantly more pliable and may include pendant arabinogalactans. Because of their charge, the carboxylate groups in pectins have a tendency to enlarge their structure unless they connect through divalent cationic bridging, which ensures a significant negative charge in most situations [8]. These carboxylic acid groups undergo methylation to produce their methyl esters, which occupy a comparable amount of space but are

significantly more hydrophobic and, as a result, have a distinct impact on the surrounding water's structure. The degree of esterification, typically about 70%, determines the characteristics of pectins. Since there has been significant variation in the makeup of pectins from various sources, Therefore, the pectin employed for the research should also affect the kinetic properties of pectin lyases, such as Km and kcat.

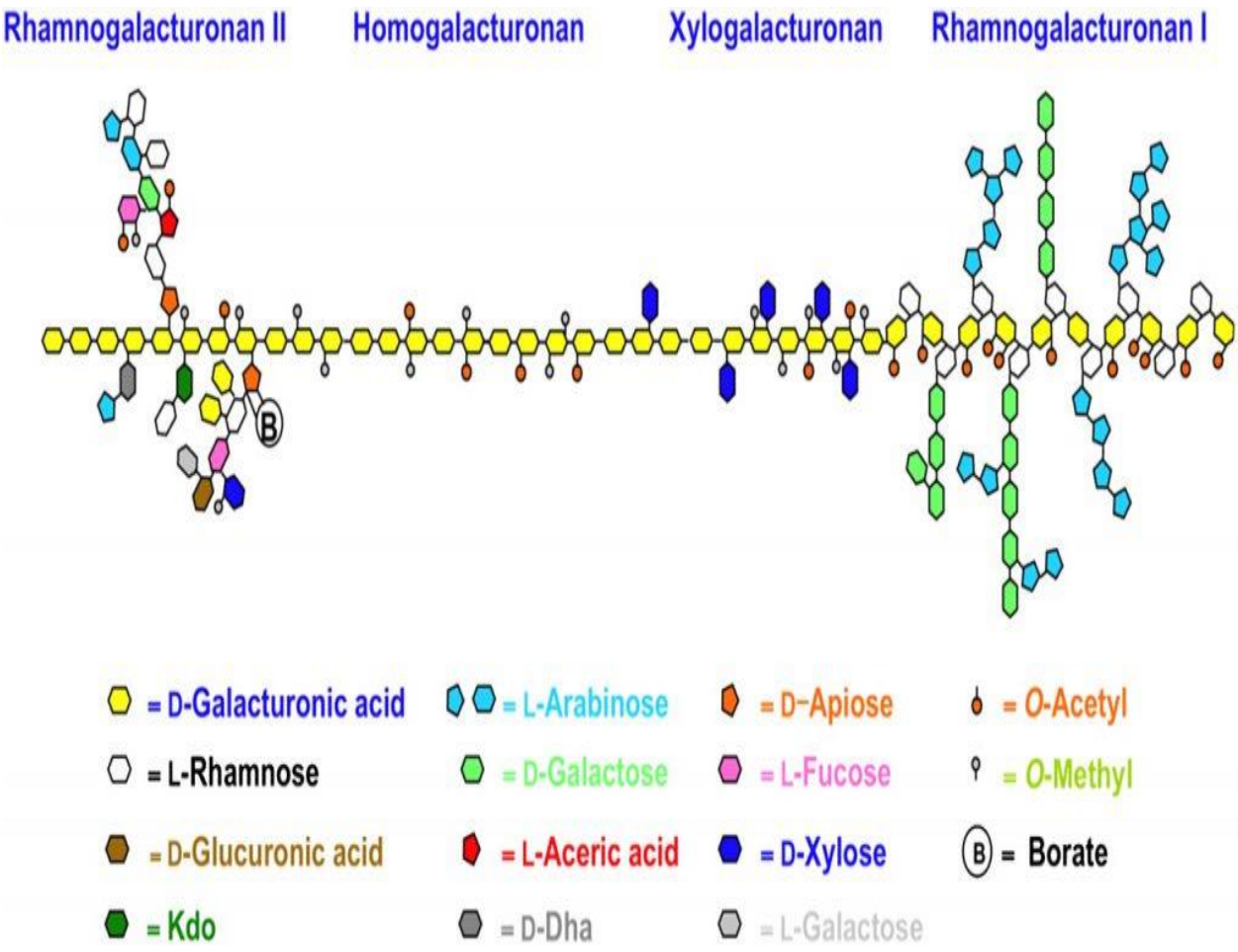

Fig. 1. Structure of pectin [8]

3. Pectinase Enzyme

A complex collection of enzymes known as pectinase breaks down pectic materials into galacturonates. Both plants and bacteria manufacture pectinase [11]. Pectic compounds are high molecular-weight glycosidic macromolecules. Between 30 and 50 percent of dicotyledonous plants' cell walls are made of pectic polysaccharides [12]. Pectin hydrolases, lyases, and esterases with varying specificities make up the majority of the pectinase superfamily of enzymes that act on polymers [13].

4. Pectic Substratec

Plants at a higher molecular level include pectic compounds, which are complex glycosidic polymers. In the main cell wall, they join the walls of neighboring immature cells and make up the middle lamellae, a thin layer of extracellular sticky. In order to keep plant tissues together and structurally sound, they are crucial [14]. To differing degrees, D-galacturonic acid is found in each of the three main pectic polysaccharide families. According to the American Chemical Society, pectic acid, protopectin, pectin, and pectin acid are the four main types of pectic compounds used as substrates in the manufacture of pectinase. The primary criterion for categorizing those pectic compounds was their water solubility, as shown in Table 1 [15]. Fruit and vegetable cell walls and middle lamella contain pectin, one of the most complicated substrates (heteropolysaccharides) [16]. Table 2 lists the fruits and vegetables that contain pectins, including apples, citrus fruits, and beets.

Table 1: The classification and characterization of pectic compounds.

S/No	Type of pectin	Description	Source
1	Pectic acid	Galacturonans are soluble, lack a methoxyl group, and have an acidic normal salt known as pectate.	Oumer [14]
2	Pectinic acid	When the right circumstances are met, different concentrations of methoxyl can combine with sugar to produce a gel.	Nawaz et al., [16]
3	Protopectin	Pectic compounds, which are mostly found in unripe fruit, are hydrophobic, and protopectinase breaks them down.	Nawaz et al. [16]
4	Pectin	Methanol is used to esterify 75% of the galacturonate units' carboxyl groups.	Oumer [14]

Table 2: Various fruits and vegetables' pectin percentages [19]

S/No	Fruit/vegetable	Tissue	Pectin substance (%)
1	Peaches	Fresh	0.1–0.9
2	Banana	Fresh	0.7–1.2
3	Lemon	Fresh	0.63
4	Strawberries	Fresh	0.6–0.7
5	Carrots	Dry matter	6.9–18.6
6	Peas	Fresh	0.9–1.4
7	Avocado peel	Dry matter	3.4–5.2
8	Orange pulp	Dry matter	1.4–2.8
9	Tomatoes	Dry matter	2.4–4.6
10	Potatoes	Dry matter	1.8–3.3
11	Apple	Fresh	0.6–1.6
12	Sugar beet pulp	Dry matter	10–30

4.1. The Classification of Pectinase

Higher plants and microorganisms mostly contain pectic compounds, which are hydrolyzed by a class of mixed enzymes called pectinases [17]. The formation of pectin-containing compounds by microbes and plants is catalyzed by a group of enzymes called pectinases. The majority of commercial enzymes are obtained from fungal cultures [18]. Pectinase affects the abscission process, illness, senescence, fruit ripening, cell wall metabolism, and cell proliferation. Since the 1970s, pectinases have been produced commercially from various microorganisms, primarily fungi [19]. The International Union of Biochemistry and the Enzyme Commission both classify pectinase enzymes as hydrolases [20]. Fig. (2) shows the mode of action of the most studied pectinases.

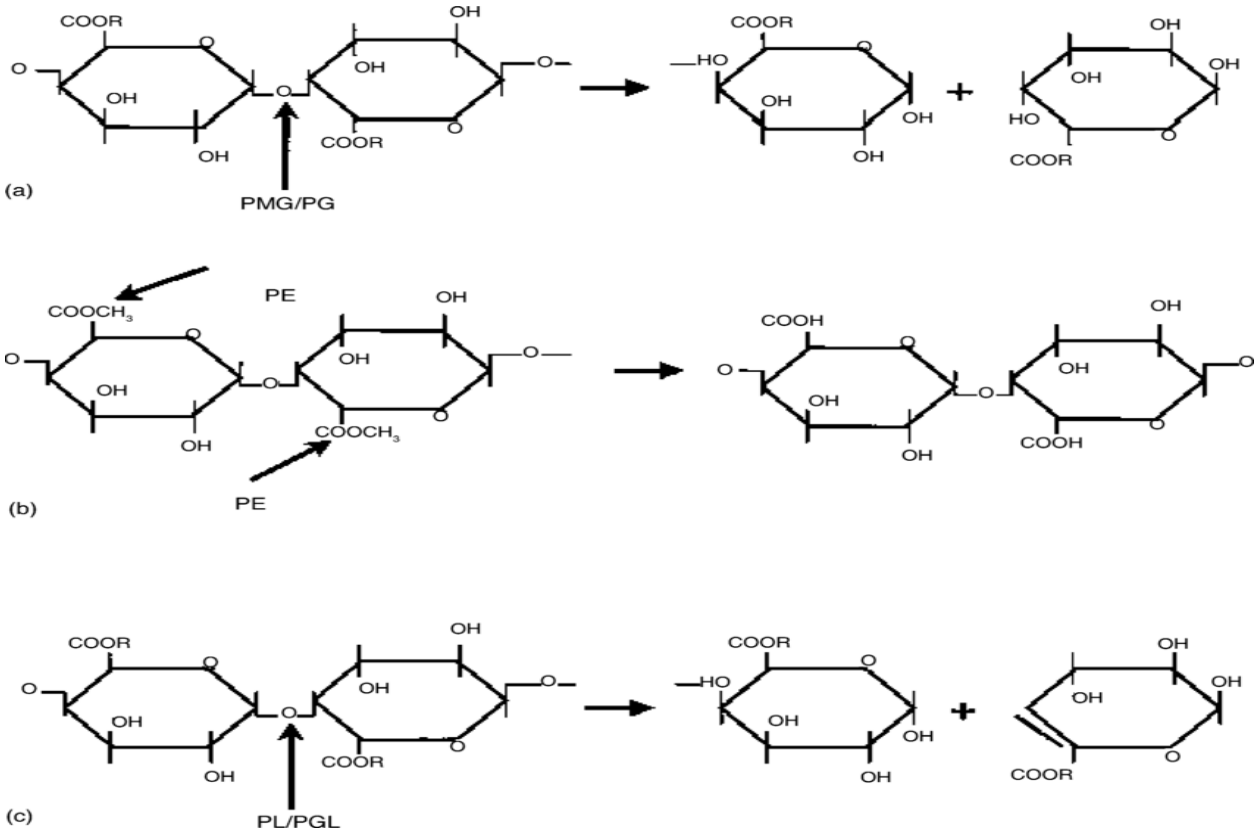

Fig. (2). Mode of action of pectinases: (a) R = H for PG and CH3 for PMG; (b) PE; and (c) R = H for PGL and CH3 for PL. The arrow indicates the place where the pectinase reacts with the pectic substances. PMG, polymethylgalacturonases; PG, polygalacturonases (EC3.2.1.15); PE, pectinesterase (EC 3.1.1.11); PL, pectin lyase (EC-4.2.2.10) [20].

4.1.1. Protopectinase

Protopectin is the insoluble part of pectic compounds that needs extra solvents to decompose, as was previously shown in Table 1. The insoluble protopectin present in unripe fruits is broken down by a kind of pectinase known as protopectinase, which yields highly polymerized soluble pectin as a byproduct. This enzyme is also known as pectinosinase [21]. Protopectinase has been shown to convert insoluble protopectin into highly polymerized soluble pectin. Using

the carbazole sulfuric acid method, one can evaluate the amount of pectin-related material produced by protopectin and determine its activity [22].

4.1.2. Pectin Methylesterases

Figure 1 shows that pectin contains several functional groups, one of which is the methoxyl ester. This methoxyl group is removed from pectic substances by pectin methylesterases, which ultimately results in the production of methanol and pectic acid. Pectin methylesterase, a carboxylic acid esterase that is a member of the hydrolase group of enzymes, is also referred to as pectin pectylhydrolase, pectinesterase, pectin demethoxylase, pectase, and pectolipase [23]. While pectin methylesterase from fungi eliminates methyl groups in a random fashion using a multichain system, pectin methylesterase from plants moves through molecules in a single, solitary process, either at the non-reducing end or close to a free carboxyl group [24]. The formation of the negatively charged Pectate polymer and methanol shown in Figure 2 [25] is caused by pectin methylesterases de-esterifying the methyl ester link at the α1-4 D galacturonosyl subunit when water is added. Oumer [14] claims that the action of pectin methylesterase in pectic compounds finally produced pectate.

4.1.3. Polygalacturonase

One of the pectinase classes, polygalacturonase is also known as depolymerase due to its role in the depolymerization process. When water is available, the polygalacturonic acid chain is hydrolytically cleaved by pectinolytic enzymes called polygalacturonases [26]. Enzyme activity causes galacturonic monomers to separate along their alpha 1-4 glycosidic link. According to research by Patidar et al. [27], polygalacturonases may be divided into two categories. Endo-polygalacturonase and exo-polygalacturonase are two examples. Oligogalacturonic acids are released when polygalacturonic acids are hydrolyzed by endopolygalacturonase. Mono-galacturonate is produced when exo-polygalacturonase hydrolyzes pectic acids. The pectinolytic-depolymerase enzymes known as polygalacturonases hydrolyze polygalacturonic acid chains at the 1-4 α glycosidic linkage site, causing the water molecule to be induced in Figure 3. The family of pectinase enzymes, which includes polygalacturonases, is the subject of the most research because of its functional, technological, and biological significance in the food processing industry as well as in the relationship between fungi and plants [21].

4.1.4. Pectin Lyases (PL)

The conversion of pectin, preferably high-esterified pectin, into unsaturated methyloligogalacturonates occurs when pectin lyase facilitates the random breakage of pectin through the elimination of glycosidic connections. Although Ca2+ is not absolutely necessary for PLs, it and other cations can induce them

[6]. Up to this point, every pectin lyase that has been discovered is an endo-PL (EC 4.2.2.10) [28]. The process by which pectin lyase A, found in Aspergillus niger, converts methyloligogalacturonates into mono-, di-, tri-, and tetragalacturonates and unsaturated di-, tri-, and tetragalacturonates was studied by Van Alebeek and colleagues [29]. In the reaction products, unsaturated monogalacturonates were not found. The polysaccharides lyase family1 includes both lyase groups. Bacterial lyases are more active in alkaline media, whereas fungal lyases exhibit maximum activity in acidic and neutral media. The complete hydrolysis of the pectin substrate still requires enzymes that cut the rhamnogalacturonan chain [30].

5. Enzyme Purification for Pectinolysis

Enzyme activity examination of the crude extract did not reveal either a single enzyme acting alone or a network of enzymes collaborating to degrade substrates. The separation of enzymatic complex components involved in substrate breakdown, optimal activity conditions, and regulation of enzyme synthesis can be achieved by the characterization of pure enzymes, making this area of study crucial. By 470-fold purification using three steps: acetone precipitation, Sepharose Q chromatography, and Sephacryl S-100 chromatography, Contreas-Esquivel and Voget [31] were able to recover 8.6% of the original activity by removing PGI from an Aspergillus kawakii culture extract. Sp-Sepharose ion exchange chromatography and Sephadex G-75 gel filtration were used to extract the PG from Thermoascus aurantiacus. The process led to a recovery rate of 24.6% and a specific activity increase of 21-fold [32]. An enzyme called pectin lyase and exopolygalacturonase were produced by the bacterium Acrophialophora nainiana. The enzyme was purified 9.37 times. The enzyme recovery process involved three stages: filtration on Sephacryl S-100 gel, ion exchange on DEAE-Sepharose, and further filtering on Sephadex G-50 gel. The total enzyme level was 60.6%. To extract the pectin lyase from Bacillus sp. DT7, Kashyap, and associates [34] developed a purification technique. Following an ammonium sulfate precipitation step, the enzyme was subjected to column chromatography using DEAE-Sephacel and Sephadex G-150. With the help of three processes—ammonium sulphate fraction, DEAE-Cellulose ion exchange, and Sephadex G-100 gel filtration—the pectin lyase produced by Aspergillus flavus was refined for 58 successive rounds. The original activity recovered at 10.3% [35]. Based on their analysis using hydrophobic and ion exchange column chromatography, Semenova and colleagues [30] determined that Aspergillus japonicus produces five distinct pectinases: PGI, PGII, PEI, PEII, and PL. Lydicus staphylococcus type Ultrafiltration followed by column chromatography on CM-Cellulose and Sephadex G-100 enhanced the specific activity of purified polygalacturonase by 57.1 times while increasing the yield to 54.9%. [36]. As

demonstrated, pectinolytic enzymes have been effectively purified using standard chromatography procedures.

6. Methods of Pectinase Enzyme Production

Both solid substrate fermentation (SSF) and submerged fermentation (SmF) are methods used in the industrial production of microbial enzymes. Typically, batch or fed-batch methods are used in stirred tank reactors with aerobic conditions to produce SmF enzymes. The use of SmF methods in enzyme manufacturing is more unfeasible in most poor nations' situations because of high energy and capital expenditures, as well as the infrastructure needed for large-scale production. The process of submerged fermentation involves growing bacteria on liquid broth; it produces a lot of wastewater and calls for large amounts of water and constant stirringSSF combines microbial growth and product formation on or inside particles of a solid substrate under aerobic circumstances, in the absence or near absence of free water, and frequently does not need aseptic conditions for enzyme synthesis [37]. Improving the cultural conditions was crucial for the commercial production of pectinolytic enzymes since it resulted in increased extracellular enzyme synthesis in liquid culture using low-cost carbon sources [38].

6.1. Submerged fermentation (SmF)

Two advantages of submerged fermentations (SmF) include improved choices for process control and analysis, and the basis for designing studies to increase fermentation yield by employing an optimal medium [39].Some positive aspects of enzymatic hydrolysis are its high specificity, lack of harsh chemical addition, softer process conditions, and the absence of growth inhibiting substances. Large-scale production of pectinolytic enzymes is carried out by Aspergillus [36] and Penicillium [37] species.

6.2. Solid state fermentation (SSF)

The growth of microorganisms on solid surfaces devoid of free water is the fundamental idea behind solid-state fermentation (SSF). It has an amazing ability to produce enzymes [38, 39]. Research on solid-state fermentation as an enzyme production technique is expanding because of its potential advantages over submerged fermentations, which include simplicity, high productivity, and concentrated output. This change, which lowers the viscosity of the medium, makes agitation easier and makes it possible to use standard bioreactors. Fig(3) [40].

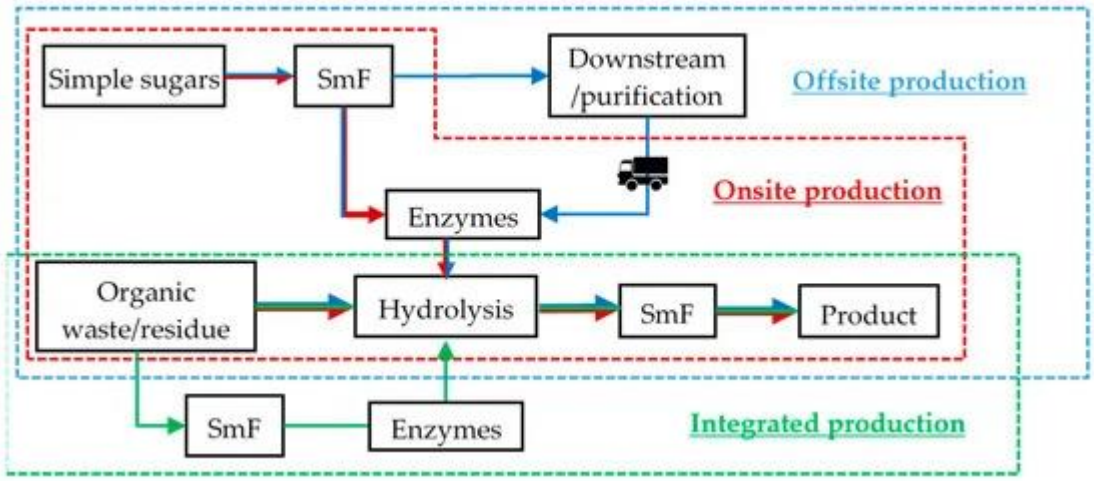

Fig. (3). Diagram illustrating the typical offshore (blue box and lines), onsite (red box and lines), and integrated (green box and lines) manufacturing of enzymes as explained by Johnson

7. Substrates Used for Pectinas Production

In addition to the lack of harmful or inhibiting components, medium carbon, nitrogen, inorganic ions, and growth stimulants must be present. In addition to a carbon supply, nitrogen growth factor medium for submerged fermentation needs a lot of water. Materials of mostly plant origin, such as starchy materials including grains, rice, maize, roots, tubers, and legumes, as well as cellulose lignin, proteins, and lipid materials, are the most commonly employed substrate for solid-state fermentation for the generation of pectinase [41]. The most common substrates for SSF used to produce pectinase are byproducts of agriculture and food processing, including grape stalks, cassava, sugar beet pulp, citrus peel, corn cob, banana peel, sawdust, and fruit pomace (apple pomace) (Figure 3) [42].

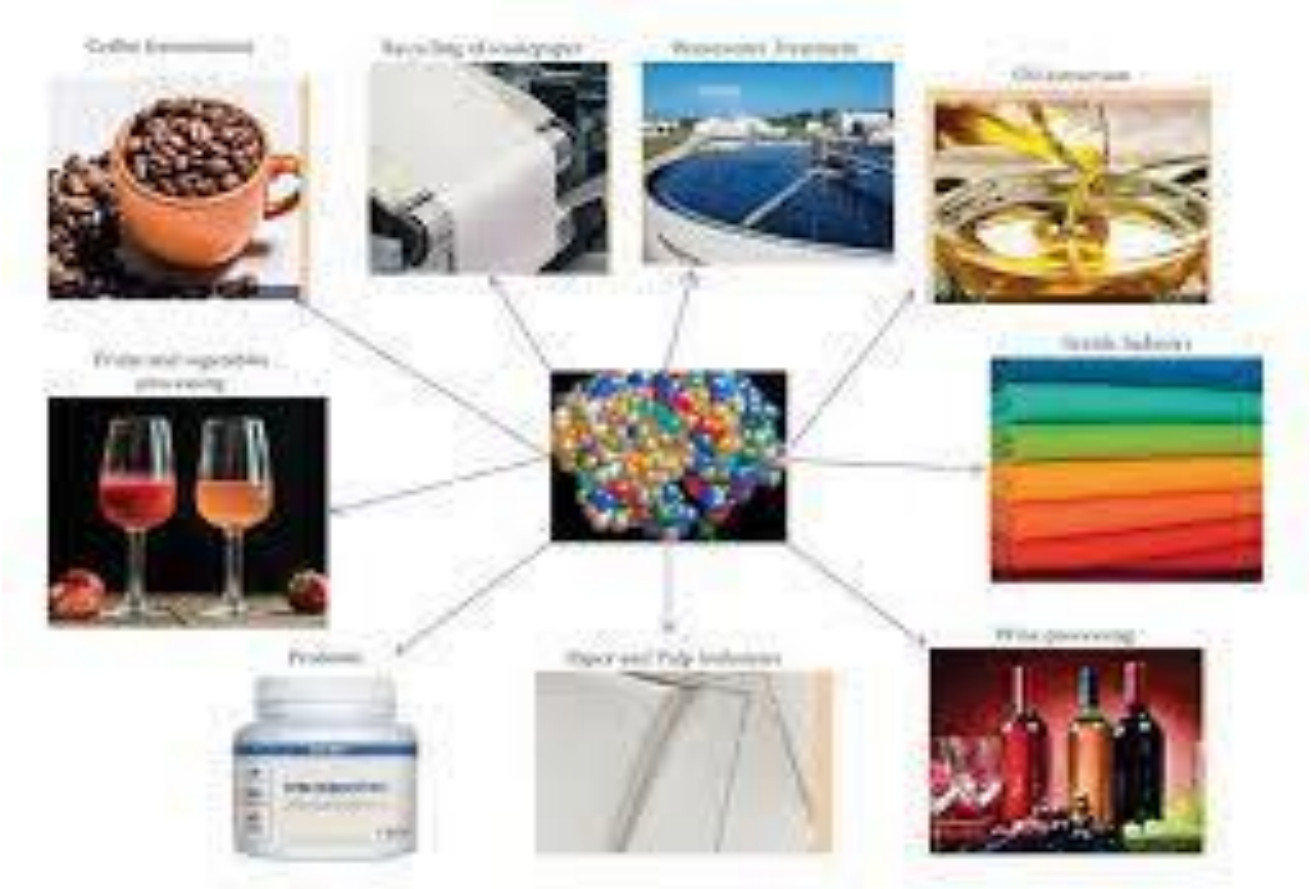

Figure 4: Various applications of pectinases [26].

8. A factor influencing the production of pectinase.

Numerous factors influence the development of microbial pectinase.

8.1. Impact of pH on the Production of Pectinase.

Many researches have shown how pH affects the synthesis of pectinase. The synthesis of pectinase by Bacillus species was examined by Torimiro and Okonji [54]. Their study attempted to determine the ideal pH range for pectinase synthesis, which was between 4 and 10. However, the highest concentration of pectinase was found at pH 7. Bacillus species MFW7 used cassava as a substrate to produce and optimize pectinase. The production of pectinase was optimized across a wide range of pH values, including 3.5, 4.5, 5.5, 6.5, 7.5, 8.5, and 9.5. According to Kumar et al., the highest pectinase activities were found at pH 6.5. Fig. (5) [42]. Pectinase derived from Chryseobacterium indologenes strain SD was found in another study. The maximum amount of pectinase was produced at pH 7.5, according to research by [43], but this production was optimized using several pH ranges of 5–9 at 0.5 intervals. Using Bacillus sphaericus, the synthesis of polygalacturonase was optimized over a variety of pH values. At 0.6 intervals,

the pH ranges were 4.4, 5, 5.6, 6.2, 6.8, and 7.4. According to [40], the greatest polygalacturonase activity were achieved at 6.8 from that pH fluctuation.

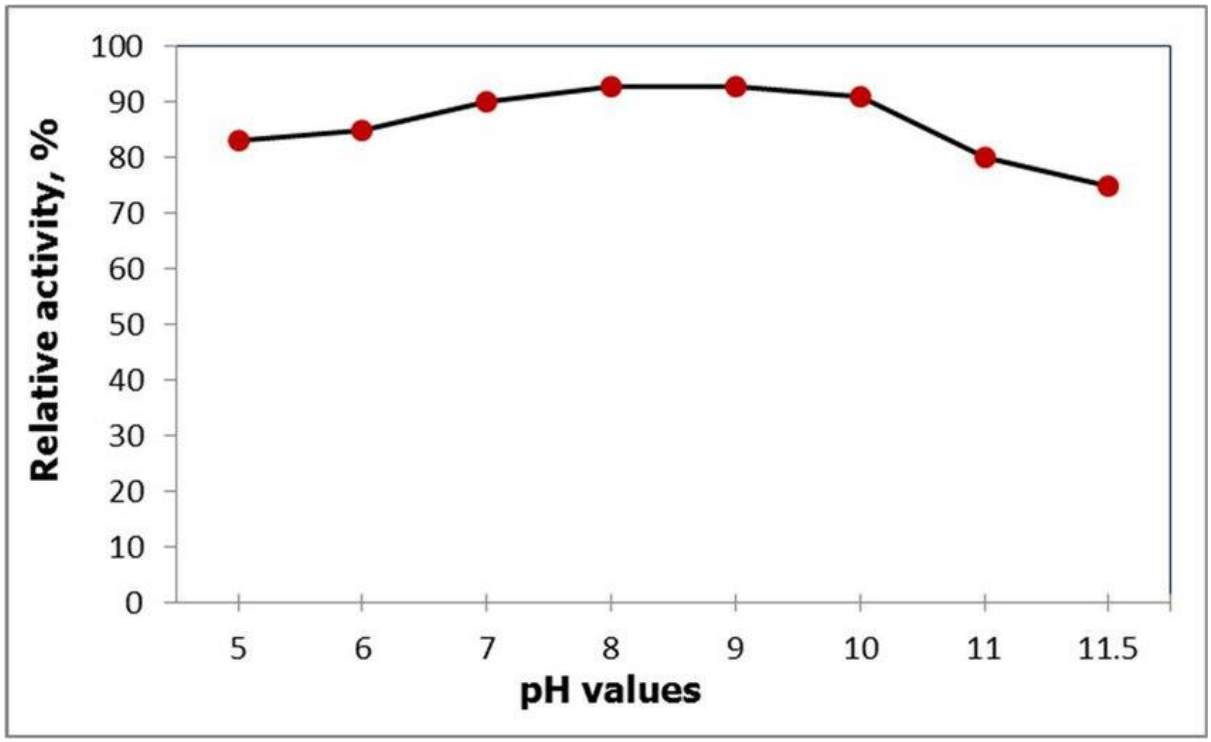

Fig. (5). Effect of pH on pectinase activity

8.2. the Effect of Temperature.

In varying temperature ranges, different studies found that different bacterial species produced the most pectinase. over intervals of five degrees Celsius, the polygalacturonase was generated over a range of temperatures from 25 to 50 degrees Celsius. Bacillus sphaericus generated the most polygalacturonase at 30°C from this temperature [44]. Enterobacter tabaci NR1466677 was the one that generated the most polygalacturonase. This investigation was conducted between 20°C and 45°C. 35°C was shown to be the ideal temperature for this enzyme's synthesis [45]. the highest amount of pectinase that Erwinia species FW2 can generate at different temperatures. 20 to 65°C was the temperature range. [41] found that the highest pectinase production was recorded at 37°C among those temperatures. A recently discovered strain of Brevibacillus borstelensis (P35) produces the alkaline pectin lyase. The temperature range in which this alkaline pectinase was generated was 20–100°C. According to Demir et al., the highest concentration of this pectin lyase was recorded at 60°C . The newly obtained Bacillus subtilis strain also developed an extracellular pectinase, and according to [38], the greatest overall activity of pectinase from Bacillus subtilis cultured in media containing pectin as a carbon source occurred at 37°C. Bacillus licheniformis strain GD2 is a pectinolytic bacterium that produces polygalacturonase, one of its components. Three temperature ranges between 45°C and 65°C were used to manufacture polygalacturonase, with 45°C showing the highest level of polygalacturonase activity. Another study demonstrates that

polygalacturonase may be produced by Erwinia species. The greatest quantity of polygalacturonase activity has been shown in studies conducted at various temperature ranges, ranging from 20 to 45°C. [46] showed that Erwinia carotovora MTCC1428 exhibited the highest polygalacturonase activity at 35°C.

8.3. the Effect of Fermentation Times.

The maximum amount of pectinase that various bacteria may produce fluctuates periodically. Erwinia carotovora MTCC1428 produced the highest amount of polygalacturonase activity at the end of 72 hours of fermentation time in liquid state fermentation conditions [33], while Bacillus species MFW7 produced a significant amount of pectinase after 96 hours of incubation in fermentation medium, as reported by [19]. Using Bacillus species, the maximum polygalacturonase activity were detected after 120 hours of fermentation. Chryseobacterium indologenes strain was identified as the other bacterial isolate, K6. After 72 hours of incubation, this isolate generates the most extracellular pectinase [28]. The Cocci species also generate the alkaline pectinase. After 72 hours of fermentation, this species generated the most pectinase [44]. Erwinia species FW2 and Bacillus species FW5 were used to manufacture the most pectinase possible. According to. [17], the two distinct species generated the most pectinase during 96 hours of fermentation.

8.4. the Effect of Substrate Concentration.

The synthesis of pectinase was impacted by variations in pectin concentration. At 0.8% pectin concentration, the maximum pectinase activity was detected. As [44] examined, the greatest activity of pectinase was achieved by choosing several concentration ranges (0.1–1%) in 0.1% intervals of pectin concentration. According to other research, pectinase activity rose with increasing pectin concentration until it reached its optimal concentration, at which point it declined. According to. [47], the highest level of pectinase activity was seen at 0.5% pectin concentration and then declined after this concentration, out of the different pectin concentration ranges of 0.1%, 0.2%, 0.5%, 1%, and 1.5%. One component of pectinase, polygalacturonase, has excellent activity at several citrus pectin concentrations, including 0.25%, 0.5%, 0.75%, 1%, 1.25%, and 1.5%. According to. [23], the maximum level of polygalacturonase activity was seen at 1.25% of the citrus pectin concentration, and the activities of polygalacturonase declined beyond that optimal pectin concentration. Enterobacter aerogenes NBO2 may manufacture polygalacturonase using glucose, sucrose, galactose, and soluble starch as a carbon source. Various carbon source concentrations (0.5%, 1%, 1.5%, 2%, 2.5%, and 3% w/v) were used to synthesize this enzyme. Fig. (6) According to research by. [47], the highest concentration of polygalacturonase was found at 1% of each carbon source out of those different concentrations. Using Aspergillus Niger as a substrate, sugar beet showed the greatest Exo and Endo pectinase

activity of 0.79 U/ml and 0.01 U/ml, respectively . The highest pectinase activity of 350.28 and 478.25 Uml-1 protein was reported by. [48].

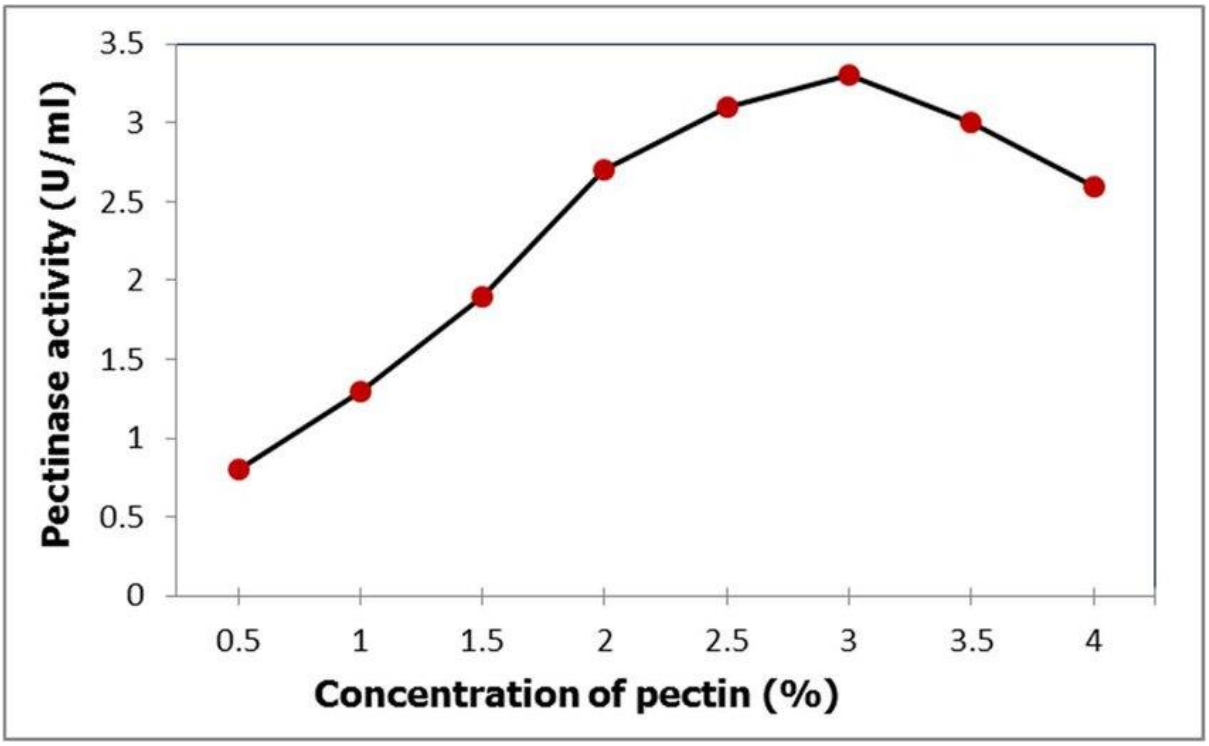

Fig. (6). Effect of pectin concentration on activity of pectinase

9. Industrial and food Applications of Microbial Pectinases

Studies on pectinase applications are being conducted worldwide in order to maximize the enzymes' fixed activity. Pectinase's broad use is ascribed to its rising demand worldwide. Depending on the physical circumstances available, different pectinolytic enzyme applications are used. [40] Many traditional industrial processes have long made use of pectinases, including those that deal with textiles, plant fibers, coffee, tea, oil extraction, and the degrading of pectinaceous compounds in industrial effluent. Figure. (7)

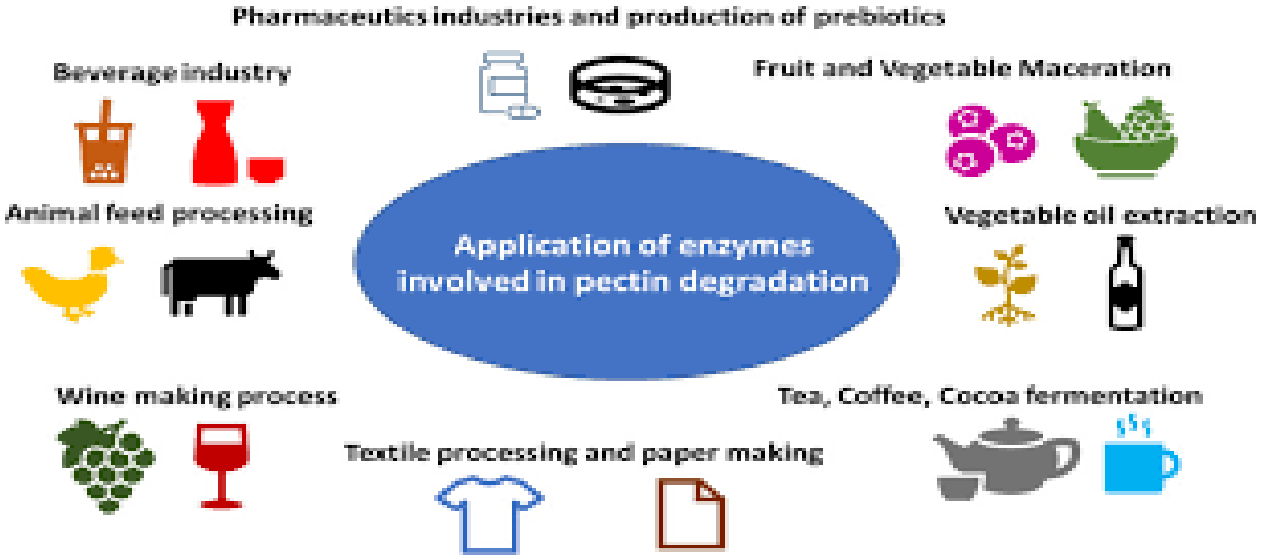

Figure. (7). Industrial and food Applications of Microbial Pectinases [20].

9.1. Fruits and Vegetable Processing

The fruit and vegetable industry makes extensive use of microbial pectinases for tasks such as clarifying, pulp treatment, and fruit juice extraction. Vegetable maceration, juice clarity, and viscosity reduction are all facilitated by pectinases, which also shorten fermentation times [41]. The fruit and vegetable juice industry makes heavy use of pectinase. There are a variety of juices made by these businesses that selectively hydrolyze middle lamella polysaccharides; these include hazy juices, sparkling clear juices, and unicellular products. The goal is to keep plant cells intact [43]. When making fruit juice, most businesses use the pectinolytic enzyme, a relatively new kind of enzyme. Fruit juice is inherently cloudy because it contains the polysaccharide pectin [20]. Pectin is naturally present in high concentrations in all fruits. Due to the production of colloid in the juice caused by this high pectin content, processing clear fruit juices becomes problematic. Another issue in the market is the occurrence of cloudiness in fruit juices. The conventional methods of fruit juice extraction are also quite energy-intensive and unappealing. Consequently, pectinase is an essential enzyme for making and refining fruit juice [22]. The results of treating fruit and vegetable mash with an enzymatic solution were pulp with favorable pressing characteristics and high juice extraction [24]. To decrease viscosity and prevent pectin-protein flocculation, the fruit juice business uses pectinase prior to clarifying. Pectinase has been extensively studied for its potential to increase permeation flux in several filtration processes, including microfiltration, ultrafiltration, and reverse osmosis when added to fruit juice [18,19]. According to Ajayi et al., pectinase enzymes were extracted from damaged apples and utilized as a basis to clear apple juice extracted from different apple varieties using varying concentrations of pectinase. For the purpose of clarifying the juice, we compared the amounts of water and commercial pectinase [27]. By breaking down the pectin content, pectinase improves the pulp's pressing ability, lowers

the viscosity of fruit juice, and breaks down the jelly structure during the clarifying process, increasing fruit juice yields. Pectinase enzymes play a key part in starch production by refining vegetable fibers. This is done in a variety of industrial processes, including curing coffee, cocoa, and tobacco, canning orange segments, and extracting sugar from date fruits [35]. By minimizing impaired vision and breaking down pectic structures, pectinases enhance the generation of fruit juice [42]. Phenolic molecules are encouraged to be released from the fruit peel when pectinases are used to prepare fruit juice [29].

9.2. Coffee and Tea Fermentation

When making tea for drinking, instant tea powder is a crucial step in the fermentation process. Because it is manufactured from leaves, this instant tea powder has a significant amount of pectin. Because of the high concentration of pectin, when tea is prepared with this powder, foam development appears on the drink. Pectinases, such as Polygalacturonase, are employed in the tea-making process to break down the pectins that cause instant tea granules to froth. This improves the tea's quality, causes color changes, and makes it more valuable in the marketplace [44]. Pectinase is also used in the coffee fermentation process. The hard coatings that surround the internal components of coffee beans are called mucilage. Furthermore, it is unpleasant to utilize the thick, gelatinous mucilage to make coffee. Alkaline pectinase is used in the process to remove the mucilage covering before using the coffee bean [20,34]. In order to boost tea fermentation and froth-producing properties and to remove the mucilage layer from the beans, pectinolytic bacteria are utilized to ferment coffee [21]. The mucilaginous covering on coffee beans can be extracted using alkaline pectinases, which have a history of use in tea fermentation [17] (Figure 7). Prevent the granules of instant tea from foaming.

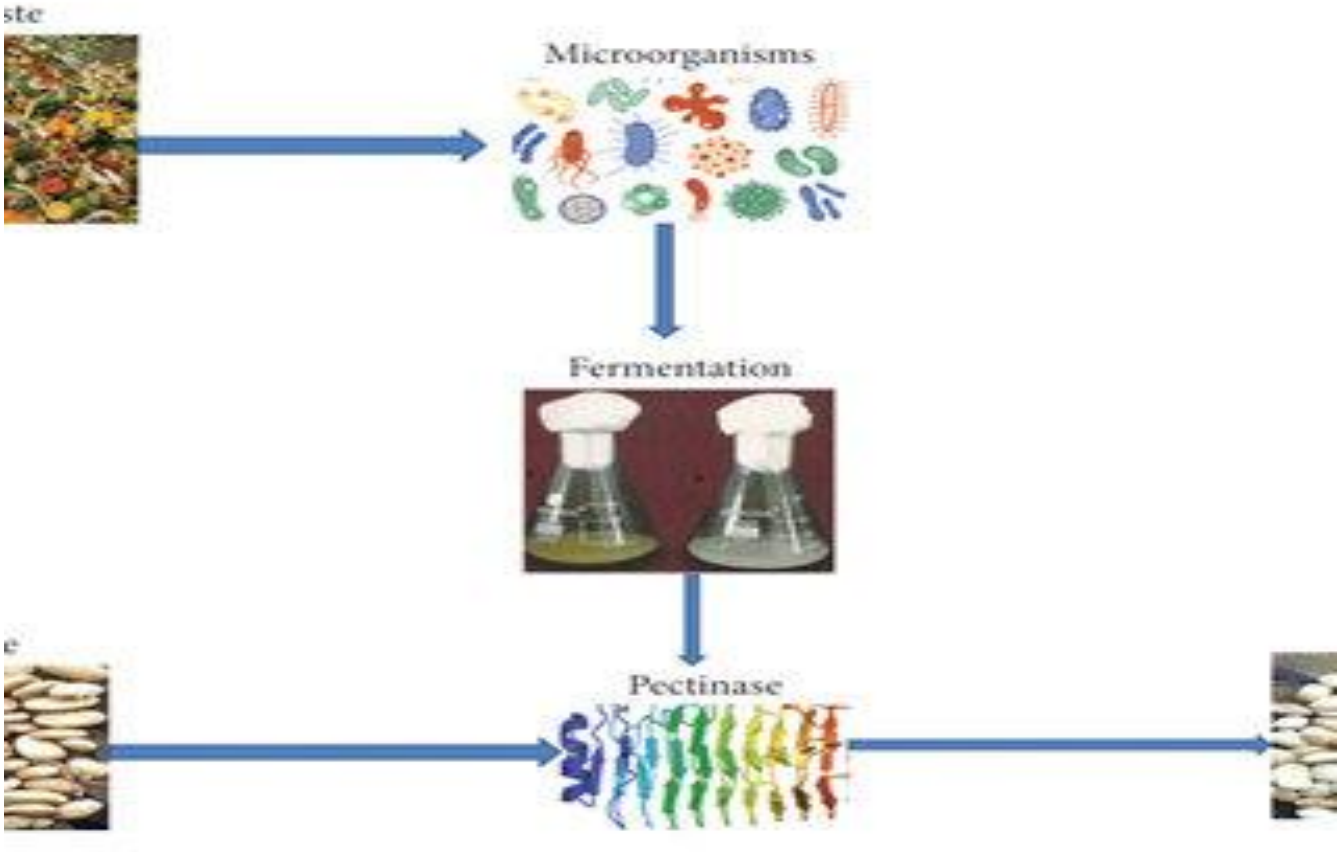

Figure (8): Pectinase for deglutinating coffee beans [20].

9.3. Wine Processing

The main functions of pectinolytic enzymes in winemaking are to improve filtering, increase juice output, enhance taste and color, and help in extraction [48]. Pectinases are used in winemaking to enhance color and flavor, speed up maceration, boost juice extraction yield, and speed up filtration. Pectinases were used to macerate the fruits before inoculating alcoholic fermentation. The quality of the wine is enhanced by this technique [49]. Fruit crushing with pectic enzymes increases the volume of free-flowing juice and shortens the pressing time in winemaking. In addition to enhancing the color and stability of red wines, it helps filter and clarify juice [19].

9.4. Oil Extraction.

For the purpose of extracting oil from a variety of sources, including flaxseed, olives, dates, and so on, pectinase and other cell wall degrading enzymes (CWDE) have been extensively investigated [50]. Pectinases can be used to extract citrus oils, including lemon oil, since they interfere with pectin's emulsifying qualities, which stop oils from being extracted from citrus peel extracts [14, 51].

9.5. Textile Processing.

Pectinase has been used in the textile industry to remove sizing agents from cotton, replacing the use of harsh chemicals, in conjunction with other enzymes such amylase, lipase, cellulase, and hemicellulase [52]. To obtain effective whiteness and absorbency of the textile fabric, cotton has been bioscourged using several combinations of enzymes, including cellulose with pectinase and cellulose with pectinase and protease [36]. By using enzymes like pectinases in combination with amylases, lipases, cellulases, and other hemicellulolytic enzymes to eliminate sizing agents, the textile industry has reduced its use of harsh chemicals and the amount of waste chemicals it releases into the environment, improving worker safety and the quality of the fabric [53]

9.6. Recycling of Waste Paper.

Environmental risks are created by chemical deinking; however, enzymatic deinking minimizes pollution risks, energy consumption, disposal problems, and enhances performance. During the deinking process, a group of enzymes (pectinases, hemicellulases, cellulases, and ligninolytic enzymes) is used. Enzymes alter the bonding properties of ink and fiber, resulting in the removal of ink from the surface of the fibers during washing [54].

9.7. Wastewater Treatment.

Numerous methods, such as physical dewatering, spray irrigation, chemical coagulation, direct activated sludge treatment, and chemical hydrolysis followed by methane fermentation, have been studied for the treatment of wastewater from citrus processing enterprises. Due to the pectic compounds' chemical resistance, the lengthy treatment times, the high treatment costs, and the complexity of the process steps, these procedures are inefficient [55]. Wastewater is a byproduct of the vegetable food manufacturing industry. Since such veggies are naturally high in pectic compounds, their wastes include them. Pectinolytic enzyme pretreatment of these wastewaters makes it easier to remove pectinaceous material and prepares it for activated sludge treatment breakdown [56]. The vegetable food processing sector releases pectin as a by-product along with wastewaters. Pectinolytic enzymes improve the removal of pectinaceous material from these wastewaters and make them suitable for activated sludge treatment breakdown [21]. Enzymes called pectinases are used to extract pectin from wastewater prior to treatment. Utilizing pectinolytic organisms to treat activated sludge is a time-saving, economical, and ecologically advantageous procedure [57].

9.8. Prebiotics/Functional Foods.

A fermented meal known as a prebiotic enables certain alterations in the composition and/or activity of the gut microbiome to strengthen the host immune

system [58]. In the current generation of prebiotics, pectin and pectin-derived oligosaccharides (PDO) are showing great promise. Intestinal bacteria have been shown to ferment methylated pectin, producing health-promoting short-chain fatty acids (SCFA) such butyrate, propionate, and acetate [59]. Pectinase is utilized to increase the antioxidant capacity of food and to create nutraceuticals and functional food ingredients [38].

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Conflicts of Interest

There is no conflict of interest, according to the authors.

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