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Review Article

Use of Pectinases in Juice Clarification Processes: A Scoping Review

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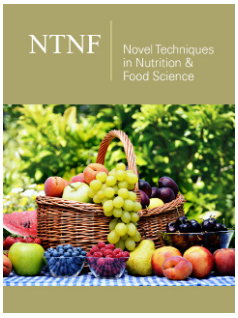

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Abstract

This review aimed to gather articles addressing juice clarification by pectinases of microbial origin. In all, 33 articles were eligible for review, with most of the research coming from Asia, South America and Europe. The microorganisms most used in pectinase production are from the genus Aspergillus sp. and polygalacturonase has been the most widely used type of pectinase in research. As ideal temperatures, most studies range between 40 °C and 60 °C and an acidic pH in the range of 3.5to7.0 is ideal. For clarification, most of the studies were done on apple juice. The result of enzymatic clarification using pectinases was promising and effective in all the studies included in the review. This study showed that pectinases are widely used in the food industries. In addition, the enzymatic clarification is ecologically correct, is highly effective and does not interfere negatively in the product.

Keywords: Pectin; Pectinolytic enzymes; Fungal; Bioprospecting; Microbiology

Introduction

Pectin is an important constituent of the middle lamella and cell wall of plants. They are characterized as heteropolysaccharides with D-galacturonic acid in the main chain linked by α-1,4 glycosidic bonds (Figure 1). In addition, the structure of pectin presents residues of L-rhamnose, arabinose, galactose and xylose and esterification with methyl or acetyl groups [1,2]. Several microorganisms can produce compounds and enzymes capable of degrading pectin and this variety of producible compounds has several applications at the industrial level, especially pectinases produced by filamentous fungi [3,4]. Pectinase is widely used in the fruit juice industry since fruits naturally have a high concentration of pectin. From a commercial perspective, this pectin concentration can cause a turbid appearance in juices. Pectinase will act by degrading the pectin present, leaving a better visual aspect in the product [5-7]. Pulp treatment, fruit juice extraction and clarification are examples of steps where microbial pectinases can be applied as they contribute to viscosity reduction and juice clarity and increase juice yield [8,9]. Clarification using enzymatic treatment is regarded as an alternative to traditional physical-chemical methods and is proving to be an increasingly promising method [8,10]. As they have desirable and advantageous characteristics, industrial enzymes are increasingly in focus, requiring constant research to optimize their production and the discovery of new sources, aiming at cost reduction and process improvement [11]. Given this, there is a need to make enzymes the target of studies, seeking the emergence of new enzyme systems and the discovery of new sources of pectinases. Pectinases are enzymes widely used in paper, food and textile industries for several commercial applications. Because they are effective and cost-effective enzymes, they are increasingly employed in the food industry for clarification in beverages such as fruit juices [12-14]. This study aimed to gather evidence of using pectinases exclusively from microbial sources for the enzymatic clarification of different fruit juices, given the need to discover new sources of pectinases and aim at the emergence of new enzyme systems.

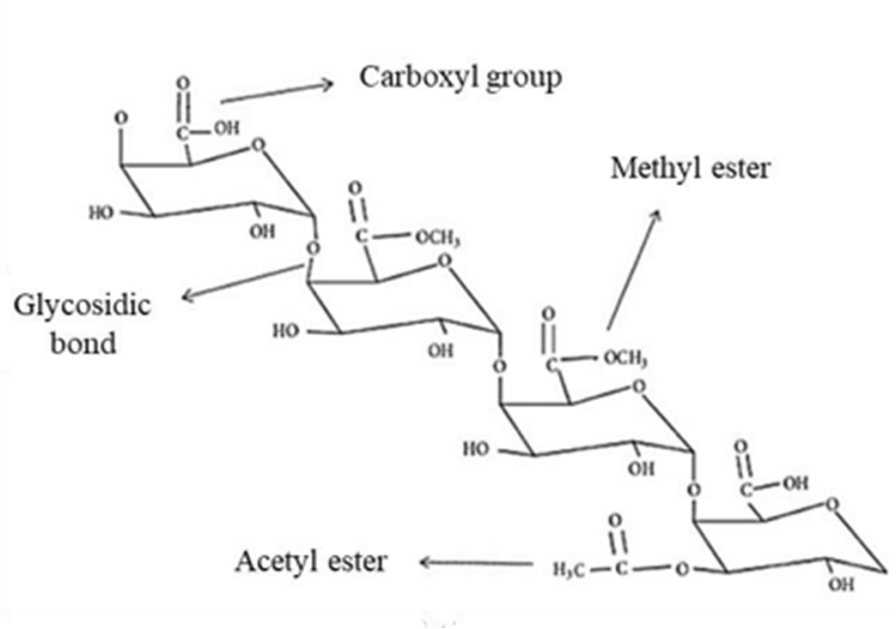

Figure 1: Pectin structure and its functional groups (adapted from Oumer 1 and Cosgrave2).

Methods

This is a scoping review that followed the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and met the guidelines of the Cochrane Handbook for Systematic Reviews of Interventions, The Cochrane Collaboration.

Strategic searches, study selection and data extraction

The systematic review was last updated in March 2022. The manual search was performed by searching for eligible articles in the list of references of the studies included. Articles were searched in ScienceDirect, PubMed and PMC (PubMed Central) databases on the NCBI website using the descriptors "pectinase," "clarification," "beverages," "juice" and "pectin" with the Boolean operator "AND." There was no time limitation; however, there was a language limitation and only articles published in English were included. Only original research articles that used pectinase of microbial origin for juice clarification were included. The excluded articles were as follows: those that used pectinase from sources other than microbial ones; papers that, despite using pectinase, did not use clarification; publications that did not use pectinase in clarification and also studies not published in English and those that were duplicates.

Result

After a systematic search of the databases, 525 publications were identified as potentially eligible. However, duplicates were removed in the pre-selection, resulting in 522 studies (Figure 2). Of these, 395 were excluded for not meeting the inclusion criteria (Table 1). A total of 127 studies remained. After reading the titles and abstracts, 77 articles were selected to be read in full and finally, 33 studies were selected that fit the main focus of the proposal for this study. Appendix 1 shows the excluded studies because they did not meet the current criteria for this systematic review [10,15-45]. Table 1 shows a summary of the results found in the articles selected after full-text reading and their main findings. It is observed in the studies included in the review that the microorganisms that are most prominent in the production of pectinases are from the genus *Aspergillus sp*., more specifically *Aspergillus niger* [23,27,33,34,39,44,45]. Other species of *Aspergillus sp*. Can also be observed in other studies [10,19,26,28,32,35]. India (28%) and China (27%) have the most research papers published in the English language on pectinases in juice clarification, as shown in (Figure 3). The pectinases constitute a complex and heterogeneous enzyme group. Although some of the research included in the review deals with proteases and tannases, the focus of this review was on Pectin Methyl Esterase (PME), Pectin Lyase (PL) and Polygalacturonases (PGs), including their divisions into endo polygalacturonase (endo-G) and Exo-polygalacturonase (Exo-PG). In 24 of the 33 studies included in the review, polygalacturonases were the most commonly used enzymes. Of these 24, four reported using Exo-PGs, seven reported using Endo-PGs and 13 reported using only Pgs. According to Table 1, PLs were used in four studies and SMCs in two. In addition, eight studies reported using only pectinases without specifying their type. As for the types of juices, apple juice was notably the most studied by researchers.

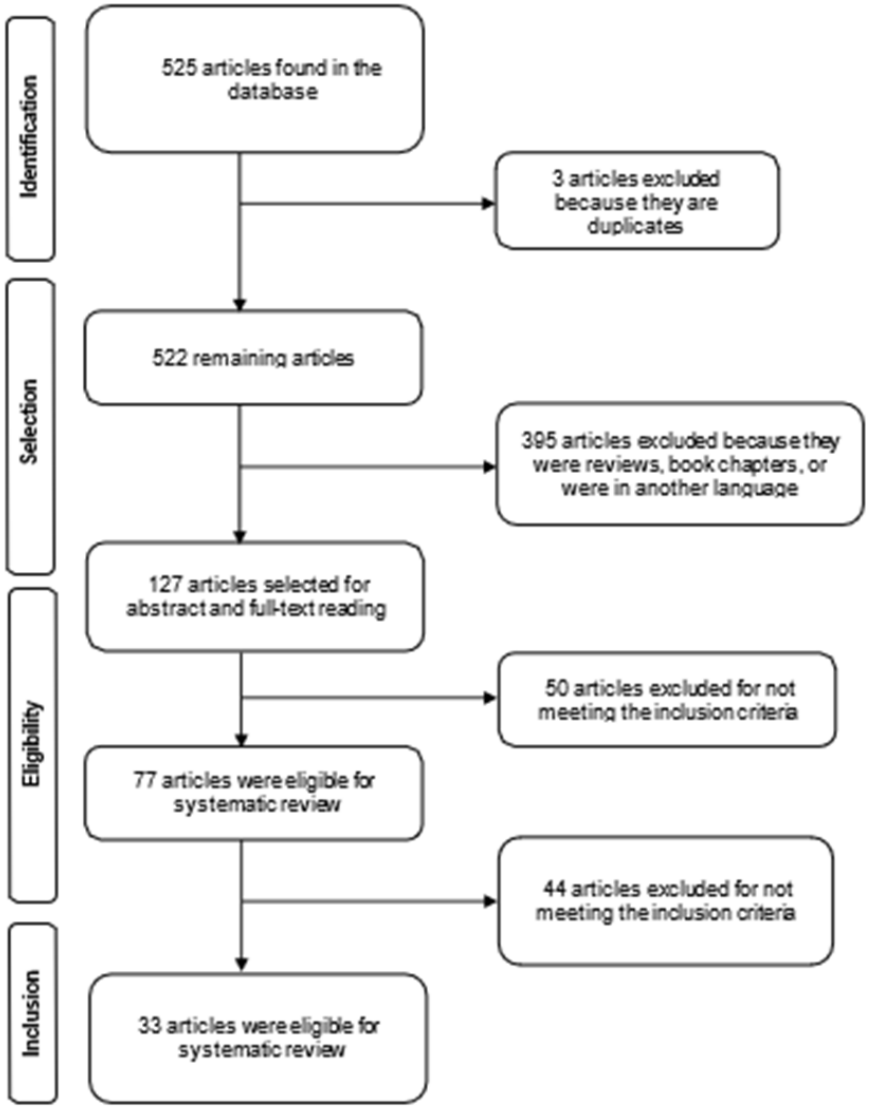

Figure 2: Flowchart of the article selection process (PRISMA).

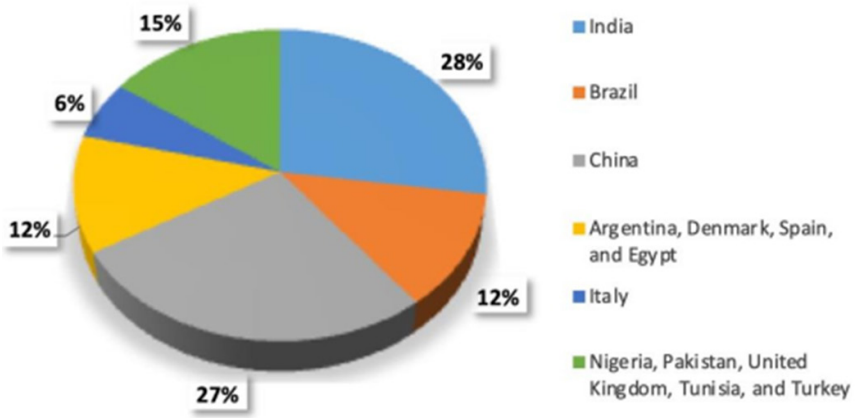

Figure 3: Distribution of studies included in the review by country.

Table 1: Articles included in the scoping review.

Author	Country	Producing Microorganism	Enzyme Type	Optimal Conditions	Juice Type	Activity
Karataş et al. [15]	Turkey	*Sporothrix Schenckii*	Exo-Polygalacturonase (Exo-PG)	Optimum temperature 60 °C and pH 4.0.	Apple juice	Turbidity reduction up to 80%, promising for apple juice clarification.
Koshy et al. [16]	India	*Bacillus tequilensis*	Pectinase	Optimum temperature 40 °C and pH 7.5.	Papaya juice	The pectinase from this species showed a positive effect in clarifying this juice.
Pagnoceli et al. [17]	Brazil	*Penicillium janthinellum*	Exo-polygalacturonase (Exo-PG)	Optimum temperature 50 °C and pH 5.0	Apple, mango and orange juice	Excellent performance in juice clarification, promising enzyme for industrial application
Carli, Meleiro et al. [18]	Brazil	*(Stereum purpureum) Chondroster um purpureum*	Endo-polygalacturonase (Endo-PG)	Optimum temperature 60- 70 °C and pH 4.5	Papaya and Apple Juice	The enzyme proved promising in the clarification of these two juices and remained active throughout the days of study; therefore, it is attractive for industrial application
Irshad et al. [19]	Pakistan	*Aspergillus ornatus*	Polygalacturonase (PG), Pectin Lyase (PL) and Pectinamethylesterase (PME)	Optimum temperature 45 °C to 65 °C and pH between 4.0and 5.0	Apple, mango, peach apricot and juice	There was significant clarification in the juices, showing that the three enzymes were effective.
Sassi et al. [20]	Tunisia	*Penicillium occitanis*	Endo-polygalacturonase (Endo-PG)	Optimum temperature at 35 °C and pH between 6.0 and 7.0.	Pear, banana and citrus juice	Clarification of 60, 28.3 and 34%, respectively, showing greater efficacy in pear and banana juice than in citrus juice.
Tapias et al. [21]	Argentina and Spain	*Streptomyces halstedii*	Polygalacturonase (PG)	Optimum temperature at 50 °C and optimum pH between 7 and 11, acidic pH (3 to 6) inactivates the enzyme.	Plum and grape juice	The juices were efficiently clarified. Besides decreasing turbidity and viscosity, there was an increase in the amount of reducing sugars.
Cheng et al. [22]	China	*Penicillium oxalicum*	Endo- polygalacturonase (Endo-PG)	Optimum temperature from 60 to 70 °C and stable pH from 2.2 to 7.0	Papaya Juice	Interesting enzyme for juice clarification biocatalysis. It can also be considered in the textile and paper industries.
Ajayi et al. [23]	Nigeria	*Aspergillus niger*	Polygalacturonase (PG)	Optimum temperature 27 °C and acid pH	Tomato juice	Appreciable enzymatic activity, effective in clarifying the tomato juice and increasing its yield, possibly because of pectin hydrolysis, releasing the sap.
Pan et al. [24]	China	*Neosartorya fischeri*	Polygalacturonase (PG)	Optimum temperature 65 °C and pH between 3.5 to 6.5	Apple and strawberry juice	Great potential in industrial applications, showing efficiency in clarifying strawberry and apple juices; however, the optimal dosages need further study.
Tu et al. [25]	China	*Achaetomium sp.*	Endo- polygalacturonase (Endo-PG)	Optimum temperature 45 °C and optimum pH 6.0	Papaya Juice	Endo-PG alone reduced the viscosity of papaya juice by 17.6% and clarified it by 59.1%.
Diano et al. [26]	Italy	*Aspergillus sp.*	Pectinase	Optimum temperature 50 °C and pH 4.0	Apple juice	Promising results for industrial applications for apple juice clarification
Saxena et al. [27]	India	*Aspergillus niger*	Polygalacturonase (PG)	Optimum temperature 45 °C and pH 4.8	Apple juice	Within one hour of incubation, the transmittance of the apple juice improved by 55%. Enzyme immobilization can be considered an advantage in industrial terms, lowering the cost and promoting reuse.

Dey et al. [28]	India	*Aspergillus awamori*	Polygalacturonase (PG)	**	Apple juice	Maximum clarification of the juice is reached after two hours of incubation at 50 °C. An increase in reducing sugar content was noted after clarification.
Konda et al. [29]	India	*Saccharomyces cerevisiae*	Polygalacturonase (PG) and Pectin lyase (PL)	Optimum temperature 40 °C and pH between 4.0 and 5.0.	Banana Juice	The pectinase isolated from the microorganism generates maximum clarification compared to commercial pectinase.
Lu et al. [30]	China	*Zygoascus hellenicus*	Exo- polygalacturonase (Exo-PG)Pectinase	Temperatura ótima 60 °C e pH ideal 5,0	Tangerine, Orange, Grapefruit and Apple	Turbidity decreased by 3.51%, 4.36%, 8.04% and 12.2%, respectively. Apple juice had the most promising clarification. Organoleptic characteristics were also improved.
Prajapat et al. [31]	India	*Bacillus subtilis*	Pectinase	Optimal temperature 50 °C and pH 5.0	Blackberry, Apple, Orange and Pineapple Juices	Clarification was effective, observing a reduction in the turbidity of blackberry juice by 46%, apple juice by 86%, orange juice by 78% and pineapple juice by 38%.
De Alencar Guimarães et al. [32]	India	*Aspergillus japonicus*	Polygalacturonase (PG)	Optimal temperature 55 °C, optimal pH 4.0	Palmer And Tommy Mango Juice, White Guava, Banana, Apple, Acerola and Papaya.	Efficient clarification, in Palmer mango juice by 65%, Tommy mango by 42%, Baranca guava by 40%, banana by 11%, apple by 9%, acerola by 2.4% and papaya by 1.7%, showing potential for industrial application.
Pinelo et al. [10]	Brazil	*Aspergillus spp.*	Pectinase and Protease	**	Cherry Juice	The study shows that pectin plays an essential role in both immediate turbidity and cold-storage turbidity. The addition of the combined protease and pectinase can be considered an effective alternative to reduce turbidity.
Deng et al. [33]	Denmark	*Aspergillus niger*	Polygalacturonase	Optimum temperature of 60 °C at pH 3.0 to 4.5.	Apple juice	The light transmittance of the apple juice was improved to 96.8%. The immobilized enzyme showed higher temperature tolerance and a wider pH adaptation range.
Cerreti et al. [34]	China	*Aspergillus niger*	Pectinase and protease	Optimal temperature between 25 and 50 °C	Pomegranate Juice	Successful clarification of pomegranate juice (the ratio of protease: pectinase was 1:2) per 100 g of juice. In addition, this type of clarification treatment caused a substantial decrease in protein and phenol haze formation activity, thus reducing the potential turbidity of the juice.
Kundu et al. [35]	Italy	*Aspergillus awamori*	Polygalacturonase(PG) and Tanase	Optimum temperature 40 °C.	Mixed citrus juice (Assam lemon, Kachai lemon and Pomelo)	Successful de-bittering and clarification of mixed citrus fruit juice using tannase and polygalacturonase. There was a maximum de-fattening of 51.87% and a clarification of 95.69%.
Ázar et al. [36]	India	*Calonectria pteridis*	Polygalacturonase	Optimal temperature of 60 °C and pH of 4.0	Apple juice	Purified polygalacturonase was applied to the clarification of apple juice, resulting in an increase in volume and amount of reducing sugars released and being effective in clarification.

Ismail et al. [37]	Brazil	*Trichoderma viride*	Pectinases	Ideal pH at 7.0 and temperature of 40 °C	Apple, Lemon and Orange Juices	The results presented the low-cost onion peel residue as the main substrate for producing fungal pectinase and its subsequent use in clarifying fruit juices (apple, lemon and orange) with remarkable stability during and after this process, which certainly enhances the fruit juice processing.
Yuan et al. [38]	Egypt	*Penicillium sp.*	Endo- polygalacturonase (Endo-PG)	Optimal temperature of 40 °C and pH of 3.5	Apple juice	Clarification of apple juice by more than 70% when combining pectin lyase and endo- polygalacturonase and it is more efficient than endo-PG alone.
Salim et al. [39]	China	*Aspergillus niger*	Polygalacturonase (PG), Pectin lyase (PL) and Pectin methylesterase (PME)	**	Apple and Orange Juice	A considerable reduction in color and turbidity was observed in all juices. The maximum production of the pectinolytic cocktail complex in the presence of a cheaper substrate in a low concentration makes the enzyme useful for industrial sectors, especially in the juice industry.
Poondla et al. [40]	Pakistan and the United Kingdom	*Saccharomyces cerevisiae*	Polygalacturonase (PG) and Pectin lyase (PL)	Optimal temperature of 30 °C and pH between 4.5 and 6.0	Mango and Orange Juice	Good clarification was observed when mango and orange juices were treated with S. cerevisiae isolate pectinase.
Yang et al. [41]	India	*Bispora sp.*	Endo- polygalacturonase	Optimal temperature of 50 °C and pH of 3.5	Apple juice	Effective in viscosity reduction and clarification, slightly more effective than commercial pectinase compound
Amin et al. [42]	China	*Penicillium janczewskii*	Exo- polygalacturonase (Exo-PG)	Optimal temperature of 45 °C and pH of 6.0	Apple, Mango and Peach Juices	Efficient performance, in addition to good enzymatic stability and great prospects for bio-industrial exploration.
Cheng et al. [43]	Pakistan and China	*Penicillium oxalicum*	Endo- polygalacturonase (Endo-PG)	Optimal temperature of 55 °C and pH of 5.5	Banana, Papaya, Pitaya and Mango Juices	It showed good catalytic activity, reducing viscosity and improving juice yield, in addition to being effective in clarification.
Rai et al. [44]	India	*Aspergillus niger*	Pectinase	Optimal temperature 42 °C and pH around 3.6	Mosambi juice (sweet orange)	A comparative study with various clarification methods. The enzymatic treatment presents clarification in about 62% of the juice. The best result is found in combining enzymatic treatment followed by bentonite adsorption.
Verma et al. [45]	India	*Aspergillus niger*	Pectinase	Optimal temperature 55 °C and pH around 3.5	Apple juice	Successful enzymatic application, effective in juice clarification

(**) No information.

It was cited in 19 out of 33 articles. Fruit juices such as orange, papaya, mango, banana, citrus and peach were also highlighted and the results were promising in all the included studies. The pectinases employed were effective in clarification, removing turbidity and improving the aspect of the studied juices. Most studies find a range of 40 °C to 60 °C as the optimal temperature for enzyme activity; however, there is promising research with temperatures below 40 °C, such as 27 °C, 30 °C and 35 °C as the optimal temperature, [20,23,40] and thermal stability in a range from 25 °C to 50 °C [34]. There are also studies where temperatures above 60 °C are described as the optimum temperatures [2,18]. where the enzyme presents stability ranging from 60 °C to 70 °C and works where 65 °C is the optimum temperature for the enzyme [24]. Most studies report acidic pH as the best for enzyme activity, more specifically in the range of 3.5 to 7.0. This result is probably because most enzymes come from filamentous fungi that commonly produce more acidic enzymes, while bacteria tend to produce more alkaline enzymes. Good pH stability was found in some research: a PG from the bacteria *Streptomyces halstedii* with pH stability of 7 to 11 [21] a PG from the fungus *Neosartorya fischeri* with pH stability in a pH range of 3.5 to 6.5 [24] and an Endo-PG, also from the fungus *Penicillium oxalicum*, with pH stability of 2.2 to 7.0 [22].

Discussion

Pectin, also called pectic substances, is the compound hydrolyzed by pectinases. It is a heteropolysaccharide rich in sugars

such as galacturonic acid and methanol. They have an insoluble part, protopectin, which is hydrolyzed by the protopectin's, which is capable of turning this protopectin into soluble pectin, the pectinaceans [46,47]. Enzymes started to be discovered in the middle of the XIX century. However, only at the beginning of the twentieth century did they start to have an industrial application. The first enzymes applied industrially were the pectinases of fungal origin, with applications focused on wine and fruit juice production. Only years later, enzymes of bacterial origin began to be employed in this sector [12, 48].

Pectinases, or pectinolytic enzymes, are widely distributed in higher plants, modifying pectin during the fruits' natural ripening process. They efficiently hydrolyze pectin polymers, having a more specific action because they are a heterogeneous group composed of Pectin Methyl Esterase (PME), Pectin Lyase (PL) and polygalacturonases (PGs) (Figure 4) [14,22,49,50]. PME is responsible for removing the ester grouping from the pectin structure, producing pectic acid and methanol in the process [51]. The PGs act in the depolymerization process, catalyzing the hydrolytic cleavage of the Poly Galacturonic Acid (PGA) chain in water, splitting the α1-4 glycosidic bond into galacturonic monomers, the most studied group within the pectinases family. PGs are divided into two subgroups: endo-polygalacturonases (Endo-PG), which can hydrolyze PGA randomly, producing trigalacturonic and digalacturonic acids and Exo-polygalacturonases (Exo-PG), which can hydrolyze PGA into Mon galacturonic acid [52,53,54]. PL also acts in the depolymerization of pectin and can break the glycosidic bonds of pectic acid by catalyzing the β-elimination between two esterified galacturonic acid residues [55]. Enzymes capable of modifying or degrading polysaccharides are called active carbohydrate enzymes (CAZymes).

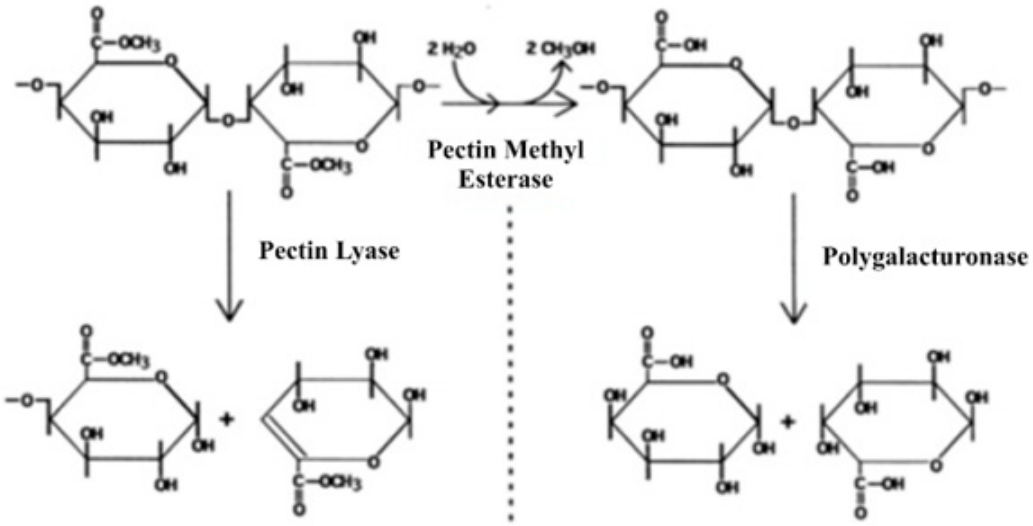

Figure 4: Different types of pectinases and their attack points within the pectin molecule [26].

According to their structural similarity and an amino acid sequence, they are grouped into five families in the Carbohydrate Active Enzyme (CAZymes) database (http://www.cazy.org): enzymes able to hydrolyze glycosidic bonds between carbohydrates; (II) Glycosyl Transferases (GTs): enzymes in charge of the biosynthesis of glycosidic bonds; (III) Polysaccharide Lyases (PLs): enzymes that fragment polysaccharide chains by a β-elimination mechanism; (IV) Carbohydrate Esterases (CEs): enzymes that catalyze the de-esterification of methyl or acetyl esterified polysaccharides and (V) Auxiliary Activities (AAs): Enzymes that degrade lignin and cleave the monooxygen bond of the polysaccharide [56]. Pectinases are part of a complex enzymatic group with the catalytic ability to hydrolyze the pectin glycosidic bonds by reactions of de-esterification (esterases) or depolymerization (hydrolases and lyases) [57]. According to the CAZy database, the deesterifiers belong to family IV, the class of carbohydrate Esterases (CEs), while the depolymerizers belong to families I and III, the class of glycoside Hydrolases (GHs) and polysaccharide Lyases (PLs), respectively. Next, the main classes of pectinases discussed in this review and their respective families are presented.

- GH28 - Polygalacturonase (PG):

I. Endo – polygalacturonase (Endo-PG) (EC 3.2.1.15);

II. Exo - polygalacturonase (Exo-PG) (EC 3.2.1.67);

- CE8 - Pectin methylesterase (PME) (3.1.1.11);
- PL1, -3, -9 - Pectin lyase (PL) (EC 4.2.2.10);

The microbial source is preferable for obtaining pectinases, although these enzymes can be obtained from other sources. Microbial enzymes from filamentous fungi have an enzymatic pH close to many fruit juices, around 3.0 to 6.0, while bacteria produce more alkaline pectinases. Among the advantages of microbial sources, we can mention the wide biodiversity and rapid growth, in addition to the ease of genetic manipulation [12,13,58,59,60,61].

In the food industry, pectinases are applied in several processes, including reducing the viscosity of fruit pulp, causing better extraction and filtration and can also be used for juice clarification because of their action in the degradation of the pectin present in fruits. Filamentous fungi and yeasts are the main producers of acid pectinases, widely employed to clarify fruit juices and wine production [14,62,63]. Fruit juice is naturally cloudy since fruits have a high concentration of pectin that

forms colloids in the juice, causing turbidity, which is not attractive to consumers. Moreover, the traditional juice extraction processes are not very attractive because they consume much energy. Because of this, enzymatic treatment with pectinases is seen as an effective alternative [5,64]. Pectinases are necessary for juice manufacturing because fruits rich in pectin tend to generate juices with higher viscosity and turbidity. Thus, these enzymes are employed in the process, aiming to hydrolyze the polysaccharides into simpler sugars, reducing the viscosity of the juice and giving it a clearer appearance, thus attracting consumers. In

addition, these macromolecules contribute to increasing juice redemption, reducing processing time and stimulating the release of phenolic compounds from fruit peels. Treating fruits with pectinases also aids extraction and further reduces the viscosity and turbidity of juices [6,7,64]. Originally, the suspended particles in the juices would be removed by traditional methods through physical processes such as centrifugation or chemical methods such as the addition of tannic acid. However, these methods can interfere with the final product, which is not advantageous considering that the visual aspect of the product directly impacts consumer choice. In this scenario, enzymatic clarification proves to be increasingly promising for its ability to improve product appearance and its ability to improve product quality and product coloration [65-68].

The disadvantages of traditional methods include the juice's low recovery and being a long and time-consuming process, with some inefficiency. Moreover, the addition of chemicals to aid these processes can cause changes in color, aroma and even flavor of the juice. Therefore, enzymatic clarification becomes preferable to traditional methods because of its high catalytic efficiency, high degree of specificity and low energy consumption [69,30]. Pectinases are responsible for the breakdown of structural polysaccharides in fruit pulps and can reduce the pulp's viscosity, turbidity and consistency. If the fruit has too much pectin, it can cause a low juice yield and pectinases increase the pulp's binding capacity, improving this characteristic and improving the visual aspect [70-72,30]. The enzymatic process seems to be one of the best choices for juice production. In this sense, enzymes, being biocatalysts, have activity, substrate specificity and the ability to work in mild environmental conditions; thus, they are environmentally friendly [73,74].

In addition to improving the pulp yield, the enzymatic treatment can eliminate free radicals and increase the amount of reducing sugars, resulting in a clear juice without opacity [75]. Pectinases are increasingly receiving more attention in the world scenario, impacting several areas because of their actions and applications, as an effective biological catalyst and having an environmentally friendly character. Studies with apple juice are the most abundant [62]. The successful application of enzymes for the depectinization of various fruit juices, including apple, banana, orange, lemon, pineapple, grape, pomegranate, mosambi, mango, papaya and guava, has been reported by some researchers [76,77,15]. conducted a very promising study with Exo-polygalacturonase from *Sporothrix schenckii*, achieving a turbidity reduction of approximately 80% in this type of juice. Similar to Diano et al. [26] with pectinase from Aspergillus sp., Saxena et al. [27] and Deng et al. [26] with *Aspergillus niger* and Ázar et al. [36] with *Calonectria pteridis* using polygalacturonases also achieved promising results in this type of juice. As in these studies, the enzymes used were of fungal origin, the pH was around 4.0 in all works and the temperature ranged from 40 °C to 60 °C, not exceeding this limit. As mentioned before, enzymes of bacterial origin tend to be more alkaline than those of fungal origin. A clear example is demonstrated in the study by Tapias et al. [21], using polygalacturonase from *Streptomyces halstedii* to clarify plum and grape juices.

The authors obtained a good clarification as the enzyme reduced turbidity and viscosity and increased the amount of reducing sugars in the juice. However, the optimal enzyme pH was around 7 to 11. The enzyme was observed to be inactive at acidic pH. Another example of the application of enzymes of bacterial origin is presented in the study by Koshy et al. [16], where pectinase from *Bacillus tequilensis* is used to clarify papaya juice. The enzyme was promising in clarifying this type of juice; however, its optimal pH was 7.5, higher than those found in enzymes of fungal origin. However, although pectinases in the industry are considered a promising method, some drawbacks, such as instability at adverse temperatures and pHs and difficulty in recovery and recyclability, limit their industrial application [36]. The inherent difficulty in enzymatic recovery and recycling reduces operational efficiency. Consequently, there is a growing interest in immobilization techniques to improve enzyme performance in juice processing. Continuous clarification steps, widely employed in industry, are enabled exclusively by enzyme immobilization [78,79]. The application of enzymes with appropriate physicochemical and kinetic properties is essential to preserve product quality and ensure consumer acceptance. Different fruit matrices require distinct enzymatic treatments, for example, grape juice requires the combined application of pectinases and cellulases for the complete elimination of turbidity and viscosity [80]. The instability of the enzyme leads to a certain delay in its industrial advancement since this characteristic in its commercialization, given the multiple pHs and temperatures, limits its resistance and application in commercial processes [81,82]. Therefore, there is a need for research that can optimize the production of these enzymes, aiming to make them more stable [8,78,79].

Hence, thermophilic enzymes are gaining more and more prominence because of their temperature stability during fermentative processes. In addition, research in enzyme immobilization is increasingly gaining space because of cost reduction since it enables enzyme reuse [9,50]. In a study by Sassi et al. [20] using *Penicillium occitanis*, temperatures of 35 °C were used

to clarify pear, banana and citrus juices with endo-polygalacturonase at pH 7.0. The work showed more promising results in pear and banana juices than in citrus, possibly due to the more alkaline pH of the enzyme. Ajayi et al. [23] achieved very successful results in clarifying tomato juice using polygalacturonase from *Aspergillus niger* at a temperature of 27 °C, which is lower than the commonly used temperature. In addition to demonstrating the effectiveness of clarification, the authors also point out an increase in juice yield, possibly due to the pectin hydrolysis. Benuci et al. [70] conducted a promising study, immobilizing pectinases and proteases on chitosan spheres by polydialdehyde starch, which is considered an effective method for clarifying pomegranate juice. However, the study reported that it would have been more appropriate to have chosen another support, such as glass, since the enzyme system is stressed during clarification, requiring resistant support for the process.

Concluding Remarks

Pectin is an essential component of the cell wall of plants, conferring its rigidity and is important in the fruit's development. However, despite suffering degradation by pectinases in the ripening process, it is an aggravating factor in the juice industry for interfering with the visual aspect of the product, often displeasing consumers. Therefore, the clarification process is necessary to improve the quality and visual aspect of the product to please the consumers and the use of enzymes is a viable alternative in this scenario. The enzymatic clarification makes this activity environmentally friendly and ecologically correct and besides its low cost and great effectiveness, it does not interfere negatively with the product. Because of this, we conclude that it is more and more necessary to research new enzyme-producing sources, like filamentous fungi, especially pectinases that are effective for application in the food industry, thus increasing the quality of the products that will be marketed.

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Author Contributions

B.D: drafting, writing, editing; A.C.S: revising; A.M. revising, writing, editing. Furthermore, all the authors have contributed significantly to the conception, planning e interpretation of this work and approval of the manuscript.

Conflict of Interest

The authors have no conflict of interest to declare.

Supporting Information

Table S1. Works excluded from scope review after reading and justifications.

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