Chapter

Fungal Pectinases in Food Technology

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Abstract

Pectins contribute to the firmness of plant tissues and confer rigidity to cell walls, protecting the plant from droughts and withering. Fungi have been endowed with an array of extracellular pectolytic enzymes that provide them valuable tools for infecting their host. Pectolytic enzymes are broadly referred to as "pectinases" because they act upon pectin and pectic substances. Pectinases are the most frequently and widely used enzymes in food processing and winemaking. Currently, pectinases are applied in the food industry either to create new products or to improve physicochemical and organoleptic characteristics of conventional products, as well as to increase the yield. This review aims at casting some light on the classification and biochemical aspects of pectinases, especially those of interest in winemaking and food industries. Additionally, it gives a comprehensive summary of current applications of fungal pectinases in the field of food and beverage technology.

Keywords: fungal pectinases, pectic substances, polygalacturonase genes, pectin lyase genes, vegetable food processing, winemaking

1. Introduction

Enzymes are important tools used in the field of food technology, where enzymatic reactions are preferred rather than chemical methods due to the high affinity of enzymes for their substrates, specificity, and that they generate less toxicity. Nowadays, all manufactured food products are prepared using some enzyme at some stage of the production of the food or its ingredients. Different types of enzymes are produced at a commercial scale, which are categorised according to the substrate they act upon. Proteases, lipases, cellulases, and pectinases are the most prevalent of the trade enzymes. Enzymes can also receive different names according to the type of reaction that they catalyse. Thus, lytic enzymes and oxidoreductases are commercialised to be used in the food industry to create new products, or to improve the physicochemical characteristics of conventional products, to enhance their organoleptic properties, and to increase the yield. Among them, pectolytic enzymes, which are broadly referred to as "pectinases", act upon pectin and pectic substances. They are the most frequently and broadly used enzymes in the field of food processing [1] and animal feed. They are also used in paper production and textile industry to degrade the pectic substances that cover

Company	Web	Brand name	Location
AB Enzymes	http://www.abenzymes.com/	Rohament, Rohapect	Darmstadt, Germany
Advanced Enzyme Technologies Ltd.	http://www.advancedenzymes.com/	SebMash, Seb Enzymes, Seb Enzymes	Chino (CA), USA
AEB (International) Ltd.	http://www.aeb-group.com/	Endozym, Pectizym, Pectocel	Stuttgart, Germany
Apollo Scientific Ltd	https://store.apolloscientific.co.uk/	Macerozyme	Stockport, UK
Biocatalysts Ltd.	http://www.biocatalysts.com/	Pectinase	Cardiff, UK
DSM	http://www.dsm.com/	Rapidase, Crystalzyme, Klerzyme	Delft, The Netherlands
DuPont™ Danisco®	http://www.danisco.com/	Danisco xylanase, Diazyme, Laminex®MaxFlow	Copenhagen, Denmark
Erbslöh Geisenheim AG	http://www.erbsloeh.com/	Fructozym®, Trenolin®	Geisenheim, Germany
Esseco Group	http://www.essecogroup.com/	EnartisZym	San Martino, Italy
Habio	http://en.habio.net/	Food grade xylanases	Sichuan, China
Hubei Hongxin Ruiyu Fine Chemical Co. Ltd.	http://www.hbhxry.com	Pectase	Shanghai, China
Kanto Chemical Co. Inc.	https://www.kanto.co.jp/english/	Polygalacturonase	Chuo-ku, Japan
Laffort	http://www.laffort.com/en	Lafase, Lafazym, Optizym	Bordeaux, France
Lallemand Inc.	http://www.lallemand.com/	Lallzyme	Montreal, Canada
Leveking Enzymes	http://www.levekingenzymes.com/	Xylanase	Shenzhen, China
Maps Enzymes Ltd.	http://www.mapsenzymes.com/	Xylanase, Beta glucanase	Ahmedabad, India
Megazyme International	https://www.megazyme.com/	Pectate lyase, Pectinases	Wicklow, Ireland
Novartis	https://www.novartis.com/	Natuzyme	Basel, Switzerland
Novozymes A/S	http://www.novozymes.com/en/	Novozymes, Pectinex, Ultrazym	Bagsvaerd, Denmark
Shandong Longda Bio-products Co. Ltd.	http://www.longda-enzyme.com/abouten.html	Acid Pectinase, cellulase	Shandong Province, China
Sunson Industry Group Co. Ltd.	http://www.chinaenzymes.com/	Sunson® PEC-pectinase	Yinchuan, China
Tokio Chemical Industry Co. Ltd	https://www.tcichemicals.com/JP/en/	Pectinase from A. niger	Saitama, Japan
Wallerstein Co.	https://www.centerchem.com/products/	Klerzyme	Colorado, USA
Yakult Pharmaceutical Industry Co. Ltd.	http://www.yakult.co.jp/english/	Macerozyme, Pectinase	Tokyo, Japan

Table 1.
Abbreviated list of suppliers of commercial pectinases commonly used in food and beverage industry.

cellulose fibres. Moreover, novel biomedical and drug delivery applications of pectic substances [2] have extended the use of pectinases in the pharmaceutical industry as promising tools to obtain the desired macromolecular product. Commercial enzymes can be obtained from animal tissues, plants, and microorganisms. However, microbial pectolytic enzymes represent about 25% of the total commercial enzymes that are used in food processing [1]. A list of companies that produce commercial pectinases for the food, juice and beverage industries is given in **Table 1**.

There are many attempts to produce pectolytic enzymes on a large scale using different strains of bacteria and fungi. However, *Aspergillus* spp. especially, *Aspergillus niger*, is still the most traditionally used fungus for the production of more than 30 commercial pectolytic enzymes, which are used in the food sector [3] due to its safety status GRAS (Generally Recognized as Safe), according to the USA Food and Drug Administration (FDA) [4].

Although the pectinase market is exposed to fluctuations in the global market the value of industrial pectinase sales in 2019 amounted $30.04 million [5]. The enzyme market is expected to rise further in the near future. Similarly to food enzymes, feed enzymes are gaining increasing importance as they improve the health and performance of livestock. Consequently, development of enzyme production by optimizing fermentation and technique parameters, generation of new strains with a high production via molecular biology and microbial genetics, are current goals of scientists [1].

2. Pectinase substrates

The name "pectinase" indicates that it is an enzyme whose substrate is "pectin." This name is derived from the Greek word "πηκτικός" pēktikós, which means congeal or solidify. Pectin is the descriptive name given to a diverse group of the compounds that are responsible for gel formation. They are normally extracted from fruits and processed to be used, particularly in jams and jellies. Chemically, pectin is an extensive and heterogeneous group of polysaccharides of high molecular weights, whose backbone structure contains galacturonic acid as the main unit. The free carboxylic group of galacturonic acid may be stabilized by divalent ions. Consequently, pectin is generally found in the form of calcium and magnesium pectates. Pectin in native form is present in the primary plant cell wall of dicotyledonous and some monocotyledonous plants [6] as well as in the middle lamella [7]. It may be interlinked with other macromolecules to form insoluble protopectin [8]. The structure and composition of pectic macromolecules depend on the plant source, and some of them may be complex branched heteropolysaccharides containing more than 17 different glycosyl residues, as described in detail in other chapters of this book.

Pectic substances represent about 0.5–4.0% of the weight of fresh plants [9] and maybe expanded to reach 10–30% of the total weight in some plants like turnips, pineapple, tomato pulp, and citrus peels [10]. They contribute to the firmness and structure of plant tissues; they confer rigidity on cell walls [11] and protect the plant from droughts, withering, hazardous microorganisms, and an array of other plant pathogens.

Pectic substances could be taken as indicators of maturity and ripening of vegetable and fruits during growing as well as evolution of texture through storage. Nutritionally, insoluble pectic substances of fruits and vegetables are a significant part of the dietary fibre, which provides the beneficial effect of protecting consumers from chronic diseases, especially diabetes and colorectal cancer [12, 13].

As for the soluble pectic substances, which are normally extracted from agro-industrial by-products, such as citrus and apple peels are traditionally used as gelling and/or thickening substances in the field of food processing and of other industries.

The main unit in the chemical structure of the pectin and pectic substances is α-D-galacturonic acid which is linked by (1 → 4) bonds. The side chains of the pectic macromolecule may include even 17 different types of monosaccharides, of which α-L-arabinofuranose, α-D-galactopyranose, α-L-rhamnopyranose, and β-L-xylofuranose are the most abundant units. As a matter of fact, pectic substances include two different high molecular weight fractions of polysaccharides: homo- and hetero-polysaccharides, and thus, according to their monosaccharide composition and structure, pectic substances can be classified following a simplified system into the following main groups: homogalacturonans, rhamnogalacturonans type I, rhamnogalacturonans type II, xylogalacturonans, and other heterogalacturonans.

Homogalacturonan. The homopolysaccharide homogalacturonan (**HG**) is a linear chain of (1 → 4) linked α-D-galactopyranosyluronic acid (*Galp*A) residues in which some of the carboxyl groups are either methyl esterified or O-acetylated at C3 or C2 depending on the plant of pectin source. As homogalacturonans are linear polysaccharide chains, they could be described as "smooth" regions of pectic macromolecules.

Rhamnogalacturonan-I. The heteropolysaccharide group named rhamnogalacturonan-I **(RG-I)** includes pectic substances with a backbone structure consisting of the repeating disaccharide [(1 → 4)-α-D-*Galp*A-(1 → 2)-α-L- *Rhap*-(1→)] where α-L-*Rhap* stands for α-L-rhamnopyranose. The backbone of *Galp*A residues may be O-acetylated on C-2 and/or C-3 [14]. There is no conclusive chemical evidence that the *Galp*A residues are methyl esterified, however, an enriched RG-I-like wall fraction from flax has been reported to contain methyl esters [15].

Rhamnogalacturonan-II. Rhamnogalacturonan-II (**RG-II**) is a group of non-soluble pectic polysaccharides found normally in plant cell walls that are solubilised by treating the cell wall with endopolygalacturonases. They have molecular weights ranged between 5 and 10 kDa. The backbone of RG-II contains at least eight repeating units of 1 → 4-linked α-D-*Galp*A residues and it is substituted with highly complex side chains that contain at least 12 different glycosyl residues [16]. Two structurally distinct disaccharides (chains C and D) are attached to C-3 of the backbone and two structurally distinct oligosaccharides (chains A and B) are attached to C-2 of the backbone (**Figure 1**).

Depending on the plant source and the method of isolation, between 20 and 80% of the *Rhap* residues are substituted at C-4 with neutral and acidic oligosaccharides. The oligosaccharides contain linear and branched α-L-arabinofuranose and β-D-galactopyranose residues. Some of these side chains may be terminated with α-L-fructopyranose, β-D-glucuronic acid, and 4-O-methyl β-D-glucuronic acid residues [18, 19].

Other heterogalacturonans. Among heterogalacturonans xylogalacturonans (**XG**) have been gaining more attention and found mainly in fruit pectins [20]. They have a homogalacturonan backbone with frequent single xylose residues linked β-(1 → 3) to about half of the galacturonic acid residues.

Rhamnogalacturonans I and II and xylogalacturonans are branched-chain polysaccharides, and some authors describe them as "hairy" regions of pectic macromolecules [2].

As mentioned above, this classification is made according to the monosaccharide composition and structure of the pectic substances. Nevertheless, pectic substances can be also grouped into four categories according to their molecular weight, water-solubility, and degree of methoxylation of their carboxylic acid groups, namely:

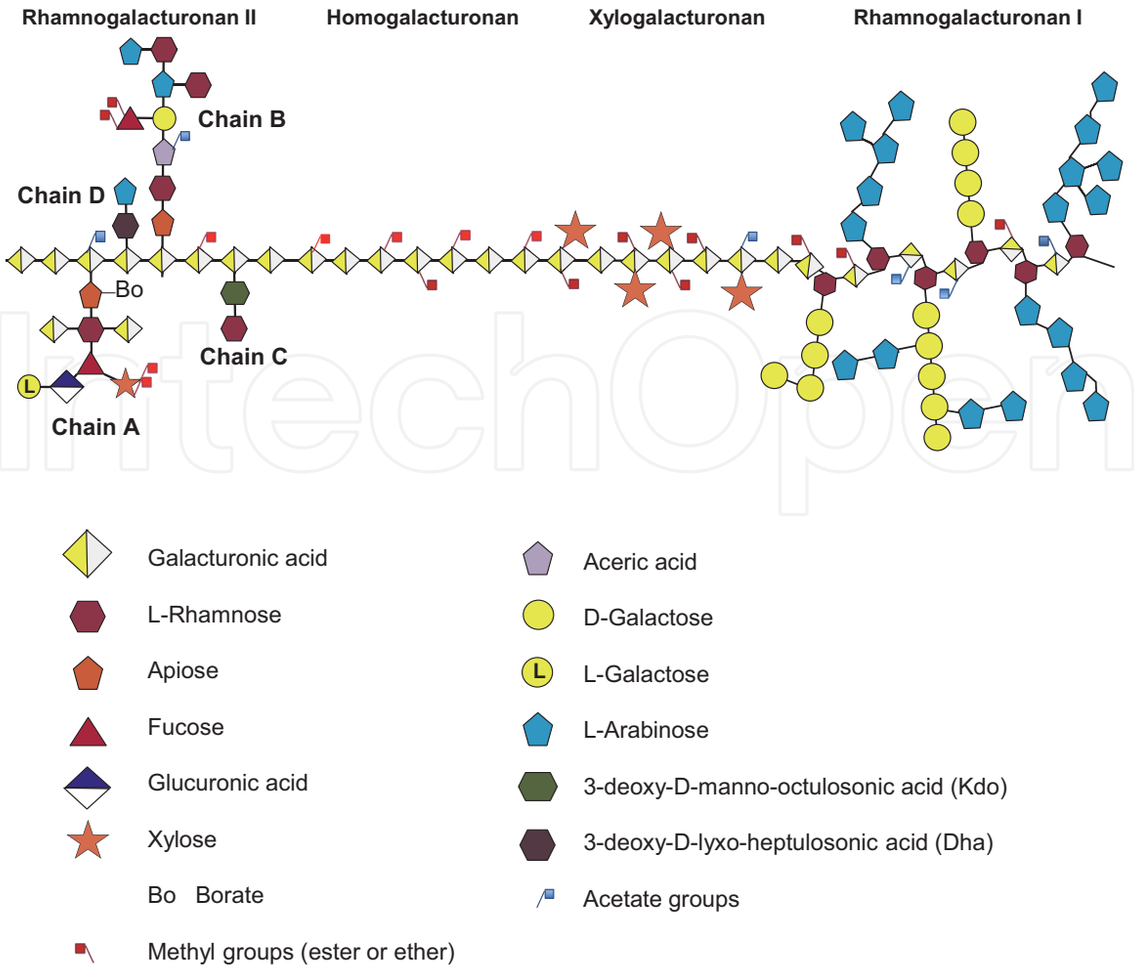

Figure 1.
Model structure of pectin as methyl ester polygalacturonic acid branched at RGI, RG-II, and XG (modified from [17]).

pectic acid, pectinic acid, pectin, and protopectin [8]. Whereas protopectin is water-insoluble, the other three are either totally or partially soluble in water.

Protopectin is the parent form of pectic substances and upon restricted hydrolysis yields pectin, pectinic, and pectic acids, as well as other derivatives. It is the term used to describe the native water-insoluble pectic substances found in the plant cell wall and middle lamella from which soluble pectic substances are produced.

Pectic acid is a product of the hydrolysis of pectin it is mainly polygalacturonic acid in the form of a linear polymer of the repeating unit of galacturonic acid, which contains negligible amounts of methoxyl groups. Free carboxylic groups could be partially or completely neutralised by sodium, potassium, or ammonium ions. Salts of pectic acid are called pectates.

Pectin represents the main carbohydrate component of the primary cell wall and middle lamella which accounts for about one-third of the total cell wall material [21]. As mentioned above, the main unit of pectin is galacturonic acid esterified at its carboxylic group with methanol, and the methoxylation ratio of galacturonic acid residues fluctuates between 60 and 90%.

Pectinic acid is the intermediate in methyl ester content between pectic acid and pectin ($0 \leq$ methoxylation ratio $< 60\%$). Pectinates is a common name of pectinic acid salts.

The molecular weights of pectic substances range from 25 to 360 kDa and their degree of methoxylation fluctuates depending on the plant source and method of extraction. Free hydroxyl groups of galacturonic acid are partly or completely

neutralised by sodium, potassium, or ammonium ions, and some of the hydroxyl groups on C2 and C3 may be acetylated [22].

3. Pectolytic enzymes and their classification

Pectolytic enzymes constitute a wide group of enzyme activities that are responsible for the degradation of the pectic substances summarised in the previous section. During fruit ripening, endogenous pectolytic enzymes act upon insoluble pectic substances and turn them into soluble pectic substances. As a result, the surrounding cell wall loses its grip and firmness, and consequently the plant tissue softens. Analogously, plant pathogens attack their host by secreting many different pectolytic enzymes in addition to cellulases and proteases [23]. This means that pectolytic enzymes exist in different forms depending on the source, the substrates they act on, and the products to be split from the substrate. Generally, pectolytic enzymes are divided into two groups: depolymerizing enzymes and esterases (also named saponifying enzymes) [24]. Depolymerizing enzymes can cleave the α-(1 → 4) glycosidic bonds in the backbone of the pectin chain, and in this group polygalacturonase (**PG**), pectin lyase (**PNL**), and pectate lyase (**PL**) are included. While pectin methyl esterases (**PE**) (commonly named "pectin esterases") and pectin acetyl esterase (**PAE**) break down ester linkages splitting methoxyl or acetyl groups, liberating the carboxylic groups of pectin polygalacturonic acid residues.

Classification of the plethora of pectolytic activities and their correct naming can be achieved following the consensus recommendations of the International Union of Biochemistry and Molecular Biology [25] and thus, pectolytic enzymes can be grouped as follows:

I. **Glycosidases** (*EC 3.2.1*) that hydrolyse O-glyosidic bonds, among which **polygalacturonases** are the main enzymes acting on pectic substances (**Figure 2**). These enzymes catalyse the hydrolysis of α-(1 → 4) glycosidic linkages in galacturonans. There are three types of these enzymes:

   a. *Endo*-polygalacturonase (***endo*-PG** *EC 3.2.1.15*) catalyses random hydrolysis of α-(1 → 4) glycosidic linkages in pectates and other galacturonans. Other names of this enzyme: polygalacturonase, *endo*-galacturonase, pectin-depolymerase, pectinase; pectolase, pectin-hydrolase, and *endo*-polymethylgalacturonase (*endo*-PMG).

   b. *Exo*-polygalacturonase (***exo*-PG** *EC 3.2.1.67*) catalyses the hydrolysis in a sequential cleavage of the α-(1 → 4) glycosidic linkage of the

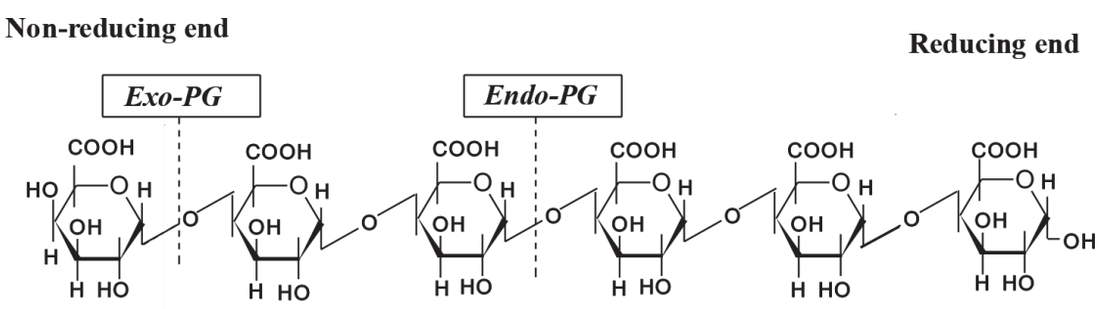

Figure 2.
Sites of hydrolysis of α-(1 → 4) glycosidic linkages catalysed by polygalacturonase enzymes.

non-reducing end of pectates and other galacturonan chains. Other names: galacturan 1,4-α-galacturonidase, poly [(1 → 4)-α-D-galacturonide] galacturonohydrolase, poly(galacturonate) hydrolase, *exo*-D-galacturonase, poly (1,4-α-D-galacturonide) galacturonohydrolase, and *exo*-polymethylgalacturonase (*exo*-PMG).

c. *Exo*-polygalacturonan-digalacturono hydrolase (*EC 3.2.1.82*) is a very specific activity that catalyses the hydrolysis of pectic acid from the non-reducing end, releasing digalacturonate. Other names of this enzyme: poly [(1 → 4)-α-D-galactosiduronate] digalacturonohydrolase, exopolygalacturonosidase, and others.

d. There are some other glycosidases in the *EC 3.2.1* group that can hydrolyse O- glycosidic bonds in rhamnogalacturonans and other heteropolysaccharides, such as β-galactosidase (*EC 3.2.1.23*), non-reducing end α-L-arabinofuranosidase (*EC 3.2.1.55*), non-reducing end β-L-arabinopyranosidase (*EC 3.2.1.88*), arabinogalactan *endo*-β-1,4-galactanase (*EC 3.2.1.89*), arabinan *endo*-1,5-α-L-arabinanase (*EC 3.2.1.99*), rhamnogalacturonan hydrolase (*EC 3.2.1.171*), xylan 1,3-β-xylosidase (*EC 3.2.1.72*), rhamnogalacturonan galacturonohydrolase (*EC 3.2.1.173*), and rhamnogalacturonan rhamnohydrolase (*EC 3.2.1.174)*. These glycosidases cooperate in degrading side chains, but their role is not as relevant as that of polygalacturonases for the degradation of pectic molecules.

II. **Polygalacturonan lyases** are a group of enzymes that cleave α-(1 → 4) glycosidic linkages by trans-elimination or ß-elimination (*EC 4.2.2*) to give oligosaccharides with an unsaturated bond between C4 and C5 at their non-reducing ends (**Figure 3**). There are several subclasses for these enzymes:

a. Pectin lyases **(PNL** *EC 4.2.2.10***)** catalyse the eliminative cleavage of α-(1 → 4) glycosidic linkages in pectins. Other names: pectin trans-eliminase, *endo*-pectin lyase, polymethylgalacturonic transeliminase, pectin methyltranseliminase, pectolyase, polymethoxygalacturonide lyase, and polymethylgalacturonate lyases (PMGL). They prefer to act upon highly esterified pectins without the prior action of other enzymes [26] and demethylation of pectins progressively slows their activity. Two activities can be included under this denomination: *endo*-pectin lyase, which comprises most of the studied pectin lyases (*endo*-PNL = *endo*-PMGL) and *exo*-pectin lyase (*exo*-PNL = *exo*-PMGL), which includes scarcely reported enzymes [27].

b. Pectate *endo*-lyases (***endo*-PL** *EC 4.2.2.2*) catalyse the cleavage of α -(1 → 4) glycosidic linkages in pectic acid and pectates. They show specificity for pectates in their anion form over methyl esterified pectins. Other names: *endo*-pectate lyase; polygalacturonic transeliminase; pectic acid transeliminase; polygalacturonate lyase; pectic lyase; α-1,4-D-*endo* polygalacturonic acid lyase, and others. This enzyme type is usually employed for de-gumming natural fibres in the paper and textile industries [28]. It is also used for the preparation of fruit and vegetable maceration products and agriculture wastewater treatment [9].

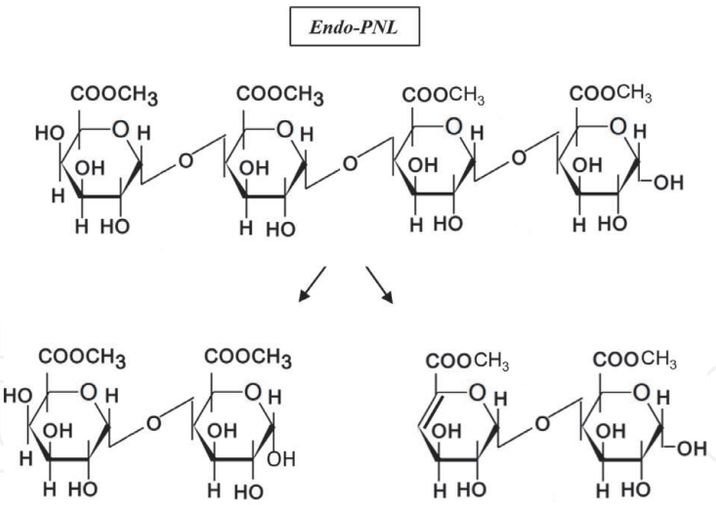

Figure 3.
Endo-*pectin lyase catalyzed reaction*

c. Pectate *exo*-lyases (disaccharide-lyase *EC 4.2.2.9*; trisaccharide-lyase *EC 4.2.2.22*) that release di- and tri-saccharides upon cleavage of pectic acid and pectates.

d. Oligogalacturonide lyase (*EC 4.2.2.6*) cleaves a digalacturonate derivate to render two oxidised monosaccharides.

e. Other polysaccharide lyases that cleave rhamnogalacturonans (rhamnogalacturonan-*endo* lyase *EC 4.2.2.23* and rhamnogalacturonan-exo lyase *EC 4.2.2.24*) (IUBMB, 2021).

III. **Esterases** that hydrolyse carboxylic ester linkages (*EC 3.1.1*) in galacturonans include some subclasses:

a. Pectin esterase **(PE** or PME *EC 3.1.1.11***)** catalyses the hydrolysis of the ester linkage between the methoxyl group and the carboxylic group of galacturonic acid residues in the pectin or pectinic acid backbone, releasing methanol (**Figure 4**). Other names: pectin methylesterase (PME); pectin demethoxylase; pectin methoxylase; pectase and pectinesterase.

The presence of calcium ions maintains fruit firmness by binding to free negatively charged carboxylic acid groups of the pectin molecules that are not methoxylated, forming what is defined as the "egg-box" model with a structure of calcium ion cross-bridges between pectin chains [29]. In this regard, the activity of the pectin esterase can play a role in fruit texture [30].

b. Pectin acetyl esterase (**PAE** *EC 3.1.1.6*) splits the acetyl group from pectin. The enzyme acts preferentially on the ester linkage of a galacturonate unit next to a non-esterified galacturonate unit [8].

c. Other carboxyl-esterases, like rhamnogalacturonan acetylesterase (*EC 3.1.1.86*) that hydrolyses acetyl groups in type I rhamnogalacturonans.

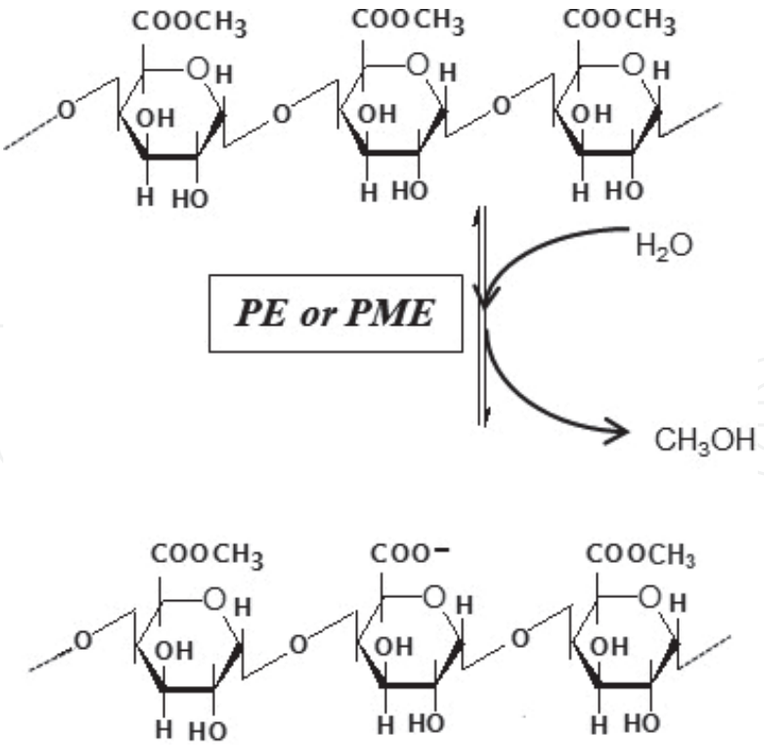

Figure 4.
Pectin esterase (EC 3.1.1.11) catalysed hydrolysis of the methoxy group of pectin to yield methanol.

IV. **Protopectinases.** A mixture of some of the previous enzymes besides other polysaccharidases, such as cellulase or hemicellulase, and protease, can act on the water-insoluble protopectin aggregates, turning them into highly water-soluble pectic substances [9]. This heterogeneous group of enzymes that act on protopectin aggregates is commonly known as protopectinases.

4. Fungal pectolytic enzymes

Fungi secrete pectolytic enzymes into their growth medium in combination with some other polysaccharide-degrading enzymes, like cellulase, hemicellulase, amylase, and other extracellular secreted enzymes, such as proteases. All these enzymes play an important role in infecting host cells by filamentous fungi. From the biotechnological point of view, extracellular enzymes are easier to obtain than intracellular enzymes, as secreted enzymes are recovered in the culture broth supernatant and simultaneously separated from the remaining cellular biomass of the producer organism. These reasons make filamentous fungi excellent candidates for enzyme production. Nevertheless, in order to use fungal enzymes in the industrial sector, stability and biochemical characteristics of such enzymes produced under various growing conditions must be studied. Purification of an enzyme is needed to estimate its biochemical properties and specificity. On the other hand, the numerous steps that are usually required for the complete purification of an enzyme, consume a long time, largely increase the economic cost and resources. In addition, the purification process could have negative effects on the enzyme activity. Consequently, the balance between technical and economic requirements is mandatory for the industrial production of enzymes.

There are different methods and techniques to separate and isolate pectolytic enzymes from crude extracts. These methods are diverse in their efficiency

and resolution. Precipitation of the enzyme from crude extracts using natural salts (e.g., ammonium sulphate) or organic solvent (e.g., ethanol) followed by column chromatography is a satisfactory procedure to get commercial purified enzymes [10].

One of the early attempts to purify pectolytic enzymes was that of two *exo*-PG isolated from crude extracts of *A. niger* after DEAE-cellulose chromatography, using 0.2 M sodium acetate buffer at pH 4.6 as the eluting solvent. The specific activity of both enzymes was increased 209- and 205-fold with 8.6–1% recovery, respectively [31]. PG from *Rhizopus stolonifer* was also separated by ethanol precipitation followed by CM-Sepharose 6B ion-exchange chromatography and the eluate was further purified to reach 10-fold by gel filtration onto Sephadex G-100 [32]. PG and PNL from *Aureobasidium pullulans* LV10 were separated by CM-Sepharose 6B followed by DEAE-cellulose chromatography and gel filtration on Sephadex G-100 [33]. *Endo*-PG from Rohament P, a commercial pectolytic enzyme from *A. niger*, was isolated and separated into three isoenzymes by preparative isoelectric focusing onto Bio-gel P-60 [34]. Here it should be noted that multiple purification steps will elevate the enzyme price, thereby, researchers in the field of food processing should pay great attention to make the purification method easy, fast, and inexpensive while increasing as much as possible the enzyme activity.

Immobilised metal ion affinity polysulphone hollow-fibre membranes with a high capacity for protein adsorption were successfully used to separate PNL and PE from a commercial pectolytic preparation [35]. A rapid and simple method to separate pectinases, including PE and PG, from potato enzyme preparations using perfusion chromatography (Poros HS), was introduced by Savary [36]. This method was an economical purification strategy for PE and PG enzymes from crude extract at a commercial scale. Regarding cold-active pectinases isolated from psychrotolerant yeasts, few activities have been characterized that showed pectolytic activity at low temperatures, reaching down to 5°C [37].

The term "pectinase" is widely preferred in the industrial context, and a wide range of pectinase-producing fungi and procedures for recovering and purifying the enzyme can be found in literature and have been successfully applied for industrial purposes [1, 3, 38]. In addition, the production of recombinant pectinases by genetically manipulated fungi has gained the attraction of researchers and biotechnologists [3, 39]. Nevertheless, as mentioned above, the GRAS status awarded by the FDA to the enzyme producer organism is a relevant characteristic when enzyme production is intended for the food industry. **Table 2** shows fungal producers of pectinolytic enzymes that are currently included in the inventory of GRAS notices, being *Aspergillus* the most repeated genus in the GRAS inventory, and *A. niger* the species with the highest number of notices, as mentioned above. With regard to the use of enzymes in foods in the European Union, it is subject to the legislation of its member states, and currently, the European Food Safety Authority (EFSA) is in the process of evaluating the safety of more than 300 food enzymes, whose applications were submitted for approval to be included in a future EU list of authorised food enzymes [40].

5. Pectolytic enzymes from fungi and yeast with GRAS status

Tables 3 and **4** show polygalacturonases (*EC* 3.2.1.15) and pectin lyases (*EC* 4.2.2.10) from fungal and yeast species that possess the GRAS status and that can be currently found at the NCBI Protein Database. **Table 3** shows the

	Species
Filamentous fungi	
	Aspergillus niger
	Aspergillus oryzae
	Aspergillus tubingensis
	Candida rugosa
	Candida cylindracea
	Disporotrichum dimorphosporum
	Humicola insolens
	Leptographium procerum
	Penicillium camemberti
	Penicillium chrysogenum
	Penicillium funiculosum
	Rhizopus oryzae
	Trichoderma harzianum
	Trichoderma reesei
Yeast	
	Kluyveromyces lactis
	Kluyveromyces marxianus
	Saccharomyces cerevisiae

Table 2.
Filamentous fungi and yeast species producers of pectinolytic enzymes that are included in the GRAS inventory of the FDA.

exo-polygalacturonase encoding genes found in fungi: pgxA, pgxB, and pgxC, and those that encode *endo*-polygalacturonases, which are quite numerous: pgaA, pgaB, pgaC, pgaI, and pgaII; whereas only the pgU1 gene was found in GRAS yeast strains. All the polygalacturonases encoded by these genes belong to the glycosyl hydrolases family 28.

The genes that encode pectin lyases found in GRAS fungi are quite numerous as well and pectin lyases from *Aspergillus* strains have been characterised according to their substrate degradation profile [41]. **Table 4** shows the pectin lyase encoding genes that have been sequenced from GRAS fungi: pel1, pel2, pelA, pelB, pelC, pelD, pelE, and pelF. All the encoded proteins belong to the polysaccharide lyase family 6. It is worth mentioning that no pectin lyase has been described in GRAS yeast.

The protein structure of the *endo*-polygalacturonase II of *A. niger* was determined by crystallographic techniques [42] and its sequence is 60% identical to the *endo*-polygalacturonase I. The 1.70 Å resolution crystal structure of *endo*-polygalacturonase I is shown in **Figure 5**. It is folded into a right-handed parallel beta helical structure comprising 10 complete turns. This structure includes a narrow substrate-binding cleft, in which the Arg96 residue, previously shown to be critical for the enzyme activity, was shown to interact with the polygalacturonic acid units of the backbone of its substrate [43].

The protein structure of the pectin lyase A and pectin lyase B of *A. niger* were as well resolved in the 90s [44, 45]. *A. niger* pectin lyases shown in **Table 4** share

Species	Strain	Protein ID	Protein length (aas)	Protein name (gene name)
Aspergillus niger	ZJ5A	AQT01640.1	362	endo-polygalacturonase
Aspergillus niger	CBS 101883	XP_025459364.1	370	endo-polygalacturonase A (pgaA)
Aspergillus niger	ATCC 9029	sp|Q9P4W3.1	362	endo-polygalacturonase B (pgaB)
Aspergillus niger	CBS 101883	XP_025460991.1	362	endo-polygalacturonase B (pgaB)
Aspergillus niger	CBS 513.88	XP_001399628.1	362	endo-polygalacturonase C
Aspergillus niger	CBS 513.88	XP_001390812.1	384	endo-polygalacturonase C
Aspergillus niger	CBS 101883	XP_025455246.1	378	endo-polygalacturonase C
Aspergillus niger	CBS 101883	XP_025455528.1	368	endo-polygalacturonase I (pga1)
Aspergillus niger	N400	CAA41693.1	368	endo-polygalacturonase I (pga1)
Aspergillus niger	RH 5344	P0CU55.1	362	endo-polygalacturonase II (pgaII)
Aspergillus niger	An15c0180	CAK42510.1	362	endo-polygalacturonase II (pgaII)
Aspergillus niger	CBS 513.88	CAK42510.1	362	endo-polygalacturonase II (pgaII)
Aspergillus niger	CBS 101883	XP_025453406.1	362	endo-polygalacturonase II (pgaII)
Aspergillus niger	CBS 101883	PYH57102.1	434	exo-polygalacturonase A (pgxA)
Aspergillus niger	CBS 513.88	ABD61563.1	434	exo-polygalacturonase A (pgxA)
Aspergillus niger	CBS 513.88	ABD61564.1	438	exo-polygalacturonase B (pgxB)
Aspergillus niger	CBS 513.88	ABD61565.1	440	exo-polygalacturonase C (pgxC)
Aspergillus niger	CBS 513.88	ABD61562.1	435	exo-polygalacturonase X
Aspergillus oryzae	PO	AHA43015.1	367	endo-polygalacturonase
Aspergillus oryzae	3042	EIT75145.1	367	endo-polygalacturonase C
Aspergillus oryzae	100-8	KDE81298.1	367	endo-polygalacturonase C
Aspergillus oryzae	KBN616	BAA03244.2	363	polygalacturonase
Aspergillus tubingensis	NW756	CAA41695.1	362	endo-polygalacturonase II (pgaII)
Kluyveromyces marxianus	DMKU3-1042	XP_022674029.1	394	endo-polygalacturonase (pgu1)
Penicillium chrysogenum	P2niaD18	KZN90643.1	369	polygalacturonase
Rhizopus oryzae	NRRL 29086	ACA48699.1	383	polygalacturonase
Rhizopus oryzae	YM9901"	BAD67423.1	383	polygalacturonase
Saccharomyces cerevisiae	S288C	NP_012687.3	361	endo-polygalacturonase (pgu1)
Saccharomyces cerevisiae	YJM693	AJR55394.1	361	endo-polygalacturonase (pgu1)
Saccharomyces cerevisiae	YJM1208	AJR66759.1	376	endo-polygalacturonase (pgu1)
Trichoderma harzianum	T6776	KKO99256.1	457	endo-polygalacturonase
Trichoderma reesei	QM6a	XP_006966948.1	401	polygalacturonase
Trichoderma reesei	QM6a	XP_006969524.1	401	polygalacturonase

Table 3.
Polygalacturonases (EC 3.2.1.15) included in the NCBI Protein Database that were obtained from fungal and yeast GRAS species.

Species	Strain	Gene name	Protein ID	Protein length (aas)	Protein name
Aspergillus niger	FDAARGOS_311	pel1	TPR10550.1	215	pectin lyase 1
Aspergillus niger	SC323	pelA	AKA88528.1	379	pectin lyase A
Aspergillus niger	CBS 513.88	pelA	CAK48529.1	379	pectin lyase A
Aspergillus niger	N400	pelA	CAA43130.1	379	pectin lyase A
Aspergillus niger	EIM-6	pelA	AFJ80127.1	370	pectin lyase A
Aspergillus niger	EIM-7	pelA	AFJ80126.1	371	pectin lyase A
Aspergillus niger	ZJ5	pelA	ALB05716.1	379	pectin lyase A
Aspergillus niger	CBS 513.89	pelB	CAK37997.1	379	pectin lyase B
Aspergillus niger	CBS 101883	pelB	PYH55243.1	379	pectin lyase B
Aspergillus niger	ATCC13496	pelB	RDH19663.1	379	pectin lyase B
Aspergillus niger	CBS 101883	pelB	XP_025453298.1	379	pectin lyase B
Aspergillus niger	N400	pelB	CAA46521.1	378	pectin lyase B
Aspergillus niger	N400	pelC	AAW03313.1	378	pectin lyase C
Aspergillus niger	ZJ5	pelC	AIX03726.1	475	pectin lyase C
Aspergillus niger	CBS 513.88	pelD	XP_001402523.3	373	Pectin lyase D
Aspergillus niger	CBS 513.88	pelD	CAK47350.1	373	pectin lyase D
Aspergillus niger	N756	pelD	AAA32701.1	373	pectin lyase D
Aspergillus niger	MTCC:404	pelD	AIE38009.1	373	pectin lyase D
Aspergillus niger	CBS 120.49	pelE	ACE00421.1	370	pectin lyase E
Aspergillus niger	An76	pelE	GAQ35327.1	382	pectin lyase E
Aspergillus niger	An76	pelF	GAQ35503.1	379	pectin lyase F
Aspergillus niger	An76	pelF	GAQ40247.1	475	pectin lyase F
Aspergillus niger	CBS 513.89	pelF	XP_001389926.1	379	pectin lyase F
Aspergillus niger	CBS 120.49	rglA	CAD36194.1	499	rhamnogalacturonan lyase A
Aspergillus oryzae	KBN616	pel1	BAB82467.1	381	pectin lyase 1
Aspergillus oryzae	KBN616	pel 2	BAB82468.1	375	pectin lyase 2
Aspergillus oryzae	RIB40	pel2	Q2TXS4.1	375	pectin lyase 2
Aspergillus oryzae	100–8	pelF	KDE86143.1	381	pectin lyase F
Aspergillus tubingensis	WU-2223 L	pelB	XP_035354767.1	379	pectin lyase B
Aspergillus tubingensis	WU-2223 L	pelD	XP_035361751.1 GFN20947.1	373	pectin lyase D
Humicola insolens	Y1		QEI10431.1	248	pectate lyase
Penicillium camemberti	FM013		CRL23631.1	241	pectate lyase

Table 4.
Pectin lyases (EC 4.4.4.10) included in the NCBI protein database that were obtained from fungal and yeast GRAS species.

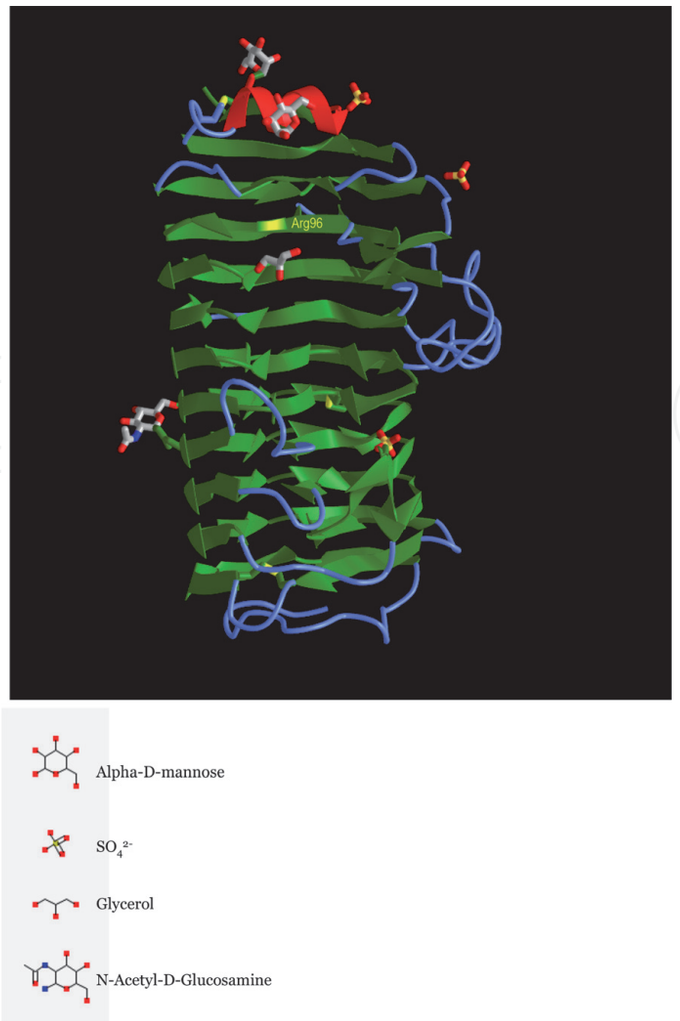

Figure 5.
PDB Image of Endo-*polygalacturonase I from A. niger at 1.7 Å resolution [43]. Available from: https://www.ncbi.nlm.nih.gov/Structure/pdb/1NHC. Code of colours: Beta strands in green, loops in blue, alpha hélix in red.*

46–65% amino acid sequence identity. The 1.70 Å resolution crystal structure of pectin lyase B is shown in **Figure 6** [45] and it shows a parallel beta helical structure, where residues Asp154, Arg176, and Arg236 were expected to play a role in the catalysis [44]. In contrast to the previously shown structure of *endo*-polygalacturonase, the pectin lyase structure shows a number of loops of various sizes and conformations that protrude from the central helix, which bind oligosaccharides and probably confer function to the enzyme [45].

6. Applications of acidic pectinases in food technology

Pectolytic enzymes can be grouped into two categories according to their optimum pH values. The first one includes acidic pectinases that have optimum activity at pH 3.0–5.5 and 30–50°C. This group of enzymes is generally secreted by fungi, especially *Aspergillus sp*., and rarely secreted by bacteria. The second group is alkaline pectinases, which are typically bacterial pectinases and rarely secreted by fungi. The optimum pH values for this group of enzymes fluctuate from 8.0 to 10.5, while optimum temperatures are ranged from 45 to 75°C [46].

In the field of food technology, acidic pectinases are routinely used to increase the proportion of juice extraction by mechanical pressing or crushing [9, 47, 48], to increase filtration efficiency and clarification of fruit juice. These are mandatory operations for fruits and vegetables rich in pectic substances such as citrus and

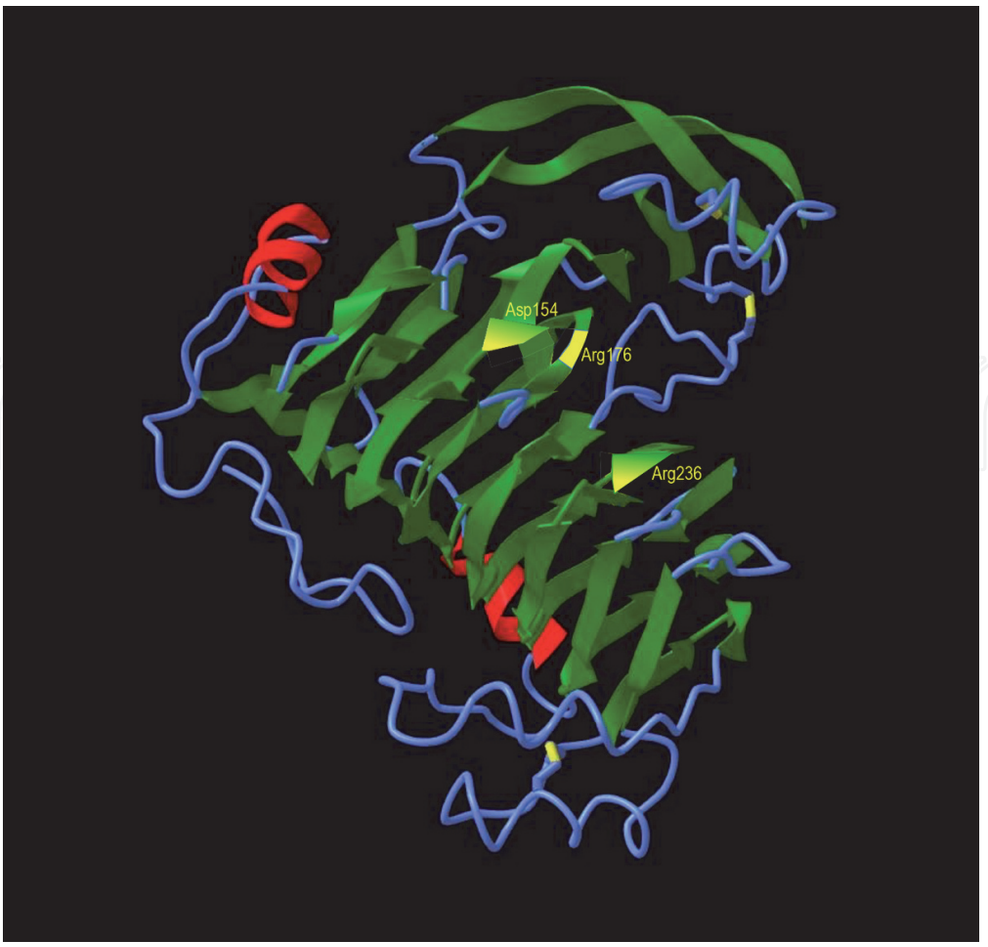

Figure 6.
PDB Image of Pectin Lyase B from A. niger at 1.7 Å resolution [45] Available from: https://www.ncbi.nlm.nih.gov/Structure/pdb/1QCX. Code of colours: Beta strands in green, loops in blue, alpha hélix in red.

tomato, as well as for liquefaction. Specialised pectolytic enzymes that act only upon the middle lamella (macerating enzymes) can be used to prepare products focused on preserving the integrity and shape of the plant cells. These enzymes keep the pulp juice with the taste and flavour of the original fruit. Pectinases can also be used in protoplast fusion technology [49]. Therefore, a wide variety of objectives that require the addition of pectolytic enzymes have been reported according to the purpose of the technological process.

7. Extraction of vegetable and fruit juice

In the course of juice preparation from fresh fruits with mechanical crushing or pressing, a soluble proportion of pectic substances (water-soluble pectin) is released in the liquid phase leading to an increase of the juice viscosity. However, insoluble pectic substances remain bound to hemicellulose and cellulose fibrils by means of side chains [50]. Water is retained with pulp particles leading to hinder the flow of cell sap, which remains bound to the pulp in the form of a jellified mass. Consequently, the juice yield is low. Also, raw press juice contains insoluble pectin particles (cloudy particles) that carry surface negative charges, which can coat positively charged surface proteins forming particles that give unpleasant mouth-feeling and off-tastes for many consumers. A mixture of pectolytic enzymes with cellulase could be useful to degrade both pectin and cellulose of cell walls and middle lamella by breaking down the pectin chain and other attached polysaccharides to their mono- and oligo-monomers. As a result, a crystal-clear juice with a

good appearance, low viscosity, high stability, appropriate mouth-feeling, and taste characteristics will be obtained, in addition to the benefit of an increase in the yield. Pectinase treatments are usually performed to prepare juice from fruits containing a high percentage of pectic substances, such as apple, pear, berries, citrus, and banana [51]. A mixture of pectinases can also decrease the filtration time up to 50% [52]. However, in some cases, it is necessary to use a mixture of pectinases, cellulases, arabinases, and xylanases to increase the fruit pressing efficiency for juice extraction [53]. In this respect, Josh *et al.* [54] found that a partially purified pectinase, produced by *A. niger* in solid-state fermentation of apple pomace, could increase juice extraction from 52 to 78% in plum, 38 to 63% in peach, 60 to 72% in pear, and 50 to 80% in apricot. The advantages of pectinase addition are: increase of colour, titrable acidity, total sugars in the extracted juices, decrease of pH, Brix/acid ratio, and relative viscosity. Biochemical properties of some microbial pectinases, including those produced by bacteria, can be found in a former review [39].

Mixtures of pectinases, cellulases, and hemicellulases can be used to assist in the extraction of edible oils, thus to increase the extraction efficiency of vegetable oils. The mixture of enzymes that hydrolyze complex polysaccharides of the cell walls of oilseeds and oily fruits, liquefies the structure of cell walls, leading to the release of the sap of cells including oil and fat-soluble active molecules (e.g., α- and β-carotenes, sterols, and vitamin D) that prevent oxidation of oils.

8. Liquefaction and stabilisation of juice

From the chemical point of view, liquefaction is the process of converting a substance from its solid or gas phase into a liquid phase. As for food technology, liquefaction means the process of turning insoluble macroparticles of tissues into smaller and soluble particles via degrading enzymes [55]. Vacuum infusion of pectinases [56] has a commercial application to soften the peel of citrus fruits.

Nectars are cloudy fruit juices mixed with syrup and citric acid to produce a ready-to-drink beverage. A serious defect of nectars is the precipitation of the cloudy particles in the bottom of the container, forming a gel and leaving a clear supernatant layer, which consumers decline. The addition of *exo*-pectinases could

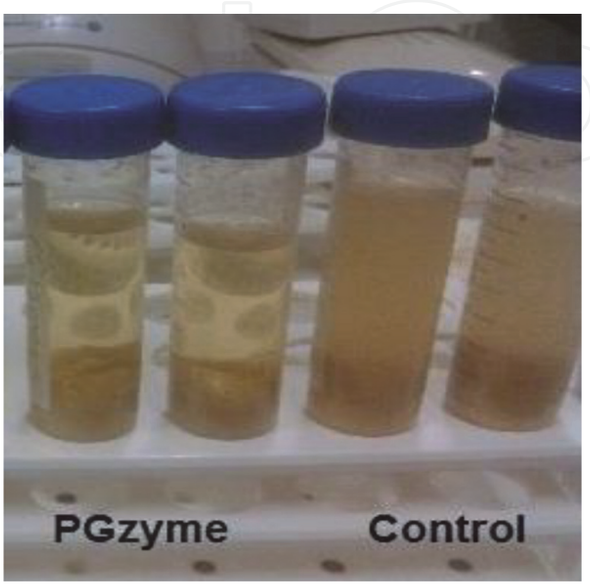

Figure 7.
Effect on banana juice of the pectinase extract PGzyme produced by Aspergillus sojae (ATCC 20235) on Banana juice as clarification and stabilization enzyme [57].

improve the stability of cloudy particles and render stable homogeneous nectar. Commercial enzymes that have high PG and PNL activities combined with cellulases and hemicellulases are used to decrease the viscosity and to keep cloud stability. In this respect, a crude extract that contained a mixture of pectinases (exo-PG, endo-PG and PNL) produced by *Aspergillus sojae* ATCC 20235, which was named PGzyme, was successfully employed to clarify a cloudy banana juice. As a result, the cloudy juice was converted into a crystal stable translucent drink (**Figure 7**) [57].

9. Food texture

Maceration is a process by which an organised tissue is transformed into a suspension of intact cells, resulting in pulpy products (soft texture), which are used as a base material for pulpy juices nectar, baby foods, and ingredients for dairy products, such as puddings and yogurts [39]. Enzymatic degradation of pectin after a mild mechanical treatment often improves the properties of the final product. An appropriate treatment with pectinase will transform the mechanically disrupted tissue into a suspension of intact cells. In this regard, PG is the best pectolytic enzyme to be used for this purpose [10].

The phenomenon of hard-to-cook (HTC) legumes was found to be dependent upon the presence of water-insoluble pectic substances in the middle lamella that keep the firmenss of the legume tissues. HTC dry broad beans (*Vicia faba* L.) possess a hard middle lamella, and some early studies [58] showed that when they were soaked in a citrate buffer pH 4.5 containing 0.1% of a commercial pectolytic preparation (Rohament P) at 45°C for 12 hours before cooking, the enzyme did not affect the grain wetting coefficient. Moreover, large amounts of galacturonic acid and reducing sugars were leached out into the soaking medium. As a result, the cooking period was reduced and texture of cooked beans (assessed as Kramer's shear force) was severely soften by the presence of the enzyme preparation in the soaking water. Accordingly, it was speculated that the activity of the pectolytic preparation was acting upon insoluble protopectin, leaching out galacturonic acid and sugars in the soaking water, and leading to a loss of tissue cohesion [7, 58]. In this regard, results from later experiments on HTC broad beans soaked in an aqueous solution of a crude extract of pectinase activity produced by *A. sojae* (ATCC 20235) showed that the soaking time was reduced by half and the cooking time of beans was reduced to one sixth with respect to control experiments without the pectinase treatment. Results showed that the insoluble pectic substances (protopectin) of the middle lamella of the bean shell and cotyledons were degraded by the pectinase activity, causing the loss of tissue coherence and leading to a soft texture of the cooked food (**Figure 8**) [59].

10. Enzymes for grape juice and wine production

Grape (*Vitis vinifera*) production can be found on every continent except Antarctica because the vine plant is able to adapt to a wide range of environments, and because of the increasing importance of fermentation-based industries in the world economy. The grape berry has three major types of structures (**Figure 9**): pulp (80–85% of total weight), skin (7–11%), and seed (2–6%) [60], with the sheer bulk of juice being derived from the pulp, and remarkably, aromas and red grape juice colour is derived from the skin. Enological enzymes can be used at several stages in juice and wine processing and their application in the wine industry started in the 70s [61]. The main stages for winemaking are: a) pre-fermentation processing, b)

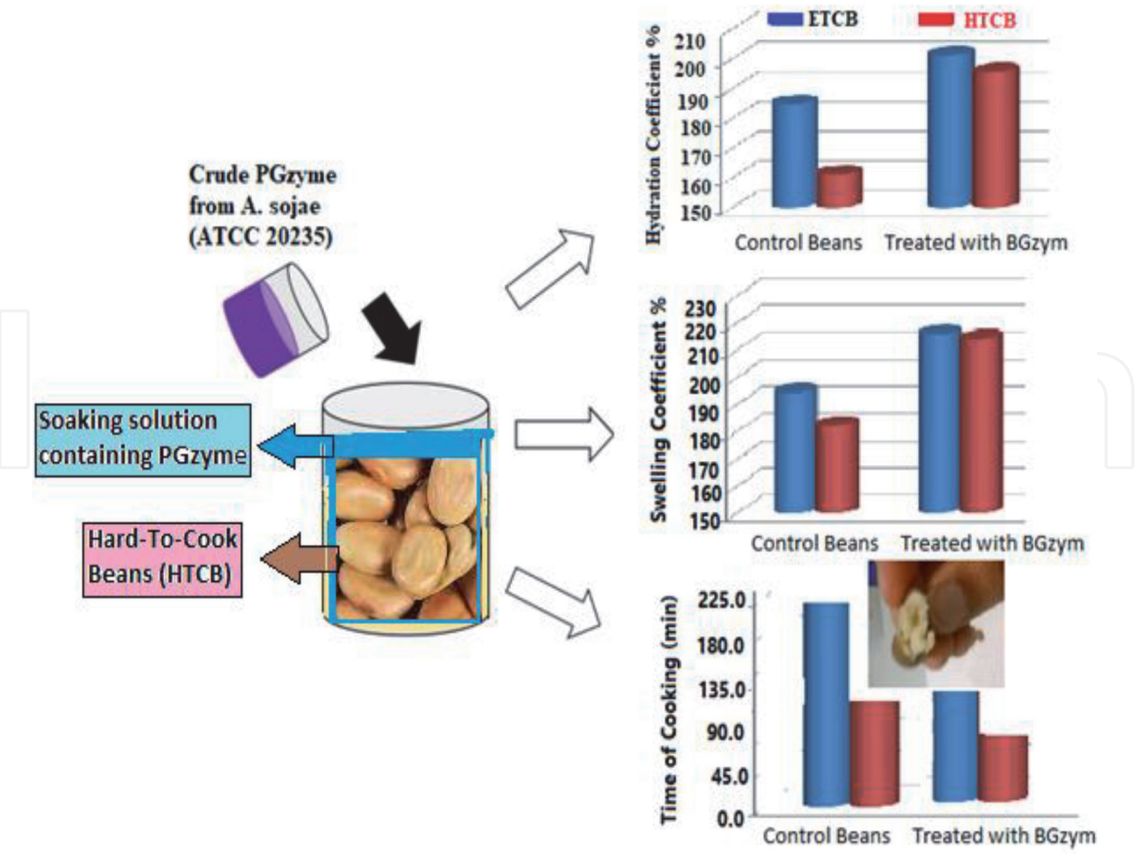

Figure 8.
Effect of 10 U PGzyme /ml at 40°C on the Hard-to-cook beans (HTCB) during soaking and cooking time [59].

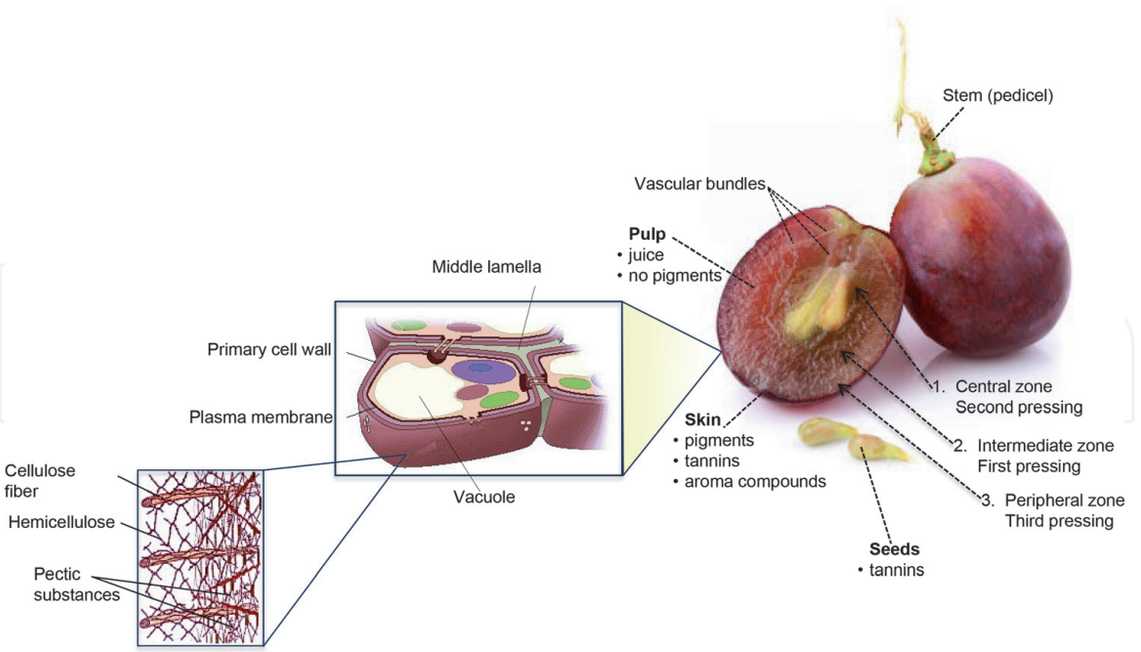

Figure 9.
Grape berry diagram and tissue organisation.

fermentation, c) post-fermentation processing, and ageing. Enzymes can be used in the first and third stages to improve: juice extraction, clarification, filtration, colloidal stability (by preventing haze from forming later in processing), aroma extraction, and in the case of red grapes, to improve colour extraction.

Commercial pectolytic enzyme preparations commercialised for grape juice and wine production are actually complex mixtures of several enzymes that degrade the cell walls and middle lamella of grape tissues, as well as polysaccharide colloids of the produced grape juices. The main enzyme activity of commercial preparations is polygalacturonase (*exo*- and *endo*-PG) accompanied with pectin lyase (PNL) [62]. In addition, these commercial preparations contain other enzymes that act in synergy with the former activities, among which, cellulases, hemicellulases, and other glycosidases (α-L-rhamnosidase, α-L-arabinofuranosidase, β-D-glucosidase) potentiate the lytic effect of these commercial enzyme preparations. When grape berries are infected by the mould *Botrytis cinerea* (grey rot or "pourriture grise") it is recommended to use specific pectolytic preparations that also contain *exo* β-(1 → 3) glucanases and β-(1 → 6) glucanases that degrade the β-D-glucan macromolecules produced by the mould and responsible for grape juice and wine spoilage [63]. Currently, commercial pectolytic enzyme preparations for enological use come from fungi belonging to the *Aspergillus* [37] and *Trichoderma* [61] genera that possess the status of GRAS organisms. Moreover, the International Organisation of Vine and Wine (OIV) requires manufacturers to inform their customers if the enzymes supplied have been produced from genetically modified organisms (GMOs). This information must be given either on the product label or on the technical documentation (resolution OIV-OENO 485–2012) and currently, both consumers and winemakers are not in favour of the GMO origin.

The endogenous pectolytic activities of grape cells and those of the wine fermenting yeasts [64] also participate in the whole lytic process of grape tissues. It is worth noting that only a low percentage (about 11.5%) of indigenous fungi strains associated with grapes and wine have been reported to be able to produce significant amounts of extracellular pectinases under enological conditions. Those strains belonged to *Aureobasidium pullulans, Filobasidium capsuligenum* [65] and to the yeast species included in **Table 2**. Under winemaking conditions, ethanol and sulphurous anhydride can be present at concentrations reaching 15% (vol/vol) and 120 mg L^{-1} respectively, which are quite restrictive conditions for grape and yeast pectinase activities [66] and to a lower extent, also for pectinase activities produced by filamentous fungi. These organisms have evolved to infect plant cells, they are well adapted to overcome the plant cell barriers and to degrade vegetable cell walls and pectic substances, as demonstrated by the number of pectinase encoding genes identified in fungi.

11. Grape processing and juice extraction

Destemming and crushing of grape berries are the first mandatory procedures for grape juice extraction, and simultaneously maceration starts as grape skins are torn into smaller pieces. At this stage, commercial pectolytic enzyme preparations can be used to degrade the polysaccharides of cell walls and middle lamella, thus facilitate juice release, liberation of polyphenols (pigments, tannins) and aroma molecular precursors that are located at the skin cells [67]. Pectolytic enzymes used in enology are selected preparations that are active at the acidic pH of grape juices (pH 3.0–4.0) under enological conditions and within wide limits of temperature [68], although at lower temperatures (15–5°C) the pectolytic activity decreases [37]. Pectolytic enzymes allow better extraction of the juice, but they also allow better extraction of the components of skins and occasionally of seeds. As shown in **Figure 9**, the pulp intermediate zone is easily released and crushed, whereas the peripheral zone, which includes skin cells, is the most difficult to extract and needs extra pressure to release its components. If grapes are not ripe enough, extraction of vegetal and astringent compounds from skins and seeds will depreciate the juice

quality. In this case, the fruit should be processed without the addition of pectolytic enzymes and avoiding maceration procedures, and consequently, the yield will considerably decrease, but the obtained juice will be low in bitterness and astringency. Thus, we see that enzyme addition at this stage should be wisely used to improve grape varietal characteristics and give character to the juice and wine. Normally, the more the damage is done to the skin and internal berry cell walls, the greater the release of berry components. Consequently, both aromas and flavours increase.

In the case of red-wine making, red grape skins are allowed to be present during the alcoholic fermentation and the polyphenols responsible for the red colour and sensory properties such as astringency or mouth-filling, which include anthocyanins and tannins among others, are further extracted as the ethanol produced during the fermentation facilitates their release from skin cells [69]. In the case of white and rosé wines, grape musts are submitted to pressing avoiding further contact with skins and seeds.

12. Grape juice clarification and filtration

In fact, white grape juice clarification was one of the first applications of enzymes in enology. After pressing, the grape juice is turbid as it contains numerous particles that confer a negative trait. Clarification can be achieved by gravity spontaneous setting, but in most occasions, it should be induced and facilitated by the addition of commercial pectolytic preparations [70, 71]. In all cases, the addition of pectolytic enzymes accelerates the clarification process; its technological efficacy is easily determined by pilot experiments measuring juice turbidity, and the methodology to be employed can be optimised for each specific type of elaboration. Further clarification after fermentation will facilitate the subsequent filtration of the resulting wine. Filtration is a key procedure to obtain a premium wine, and it is also most delicate because at this stage the colloidal matrix of the wine is very complex. The aim of adding pectolytic preparations at this stage is to increase wine filterability by degrading pectins and a variety of colloids (other polysaccharides, protein aggregates, glucans from *Botrytis cinerea*, yeast mannoproteins) responsible for clogging filtration membranes. Enzyme addition allows filtering larger wine volumes before filter clogging, and the enzyme cost is compensated for by the reduction in filtering costs and time reduction. Moreover, filtration is a process that all wines and most grape juices should undergo before bottling.

13. Extraction of polyphenols and aroma compounds: improvement of sensorial characteristics

The objective of adding commercial pectolytic preparations during red grape maceration stages is to improve the extraction of polyphenols and aroma molecules from the intracellular content of grape skin cells. The consequence of this higher yield of extraction is obtaining more structured wines, higher colour intensity, and wine aroma complexity [72]. Moreover, the resulting wine will be more easily clarified and filtered as well as the juice yield will be substantially increased. It is important to note that among the secondary activities present in commercial enological enzyme preparations, cinnamoyl esterase activities should be eliminated because they hydrolyse ester bonds of hydroxycinnamic acids, which are present in red grape skins [73], and release the free phenolic acids. These are substrates for the cinnamate decarboxylase of some *Saccharomyces cerevisiae* strains and of the wine spoiling yeast *Brettanomyces*. Cinnamate decarboxylase transforms the free phenolic

acids into volatile phenols that confer off-odours described as medicinal odour, leather or smoky to white wines, and barnyard or horsey odours to red wines [74]. Therefore, it should be made sure that no traces of cinnamoyl esterase activities are present in enzyme preparations for enological use. In addition, the pectin methylesterase activity (PE) should be negligible in the commercial enzyme preparations because no methanol should be generated when they are added [75].

The commercial enzyme preparations may also potentiate wine aroma expression by releasing volatile molecules that in their glycosylated form are not volatile, and consequently odourless. Primary or varietal aromas of wine are those whose origin are molecules of the grape cells, and they provide the characteristic aromatic profile of the grape variety used to elaborate the wine. Most of those volatile molecules appear in the grape cell as bound forms to sugars, becoming non-volatile and odourless. Glycosidic enzymes hydrolyse the linkage to the sugar residue and release the odorous molecule [76]. In addition, pectinolytic activities collaborate to break down cell walls and thus, liberate the aromatic volatile molecules.

It is important to point out that glycosidic enzymes used in enology should possess high specificity for their substrate, and β-glucosidases that hydrolyse anthocyanin glucosides should not be present in the commercial enzyme preparation because they provoke dreadful consequences of colour loss in red wines.

Finally, it should be underlined that enzymes can only extract what is already in the vegetable cells and tissues, and a good quality starting material is a requirement to obtain processed foods and beverages of premium quality. Pectinases can help to speed-up processes of extraction, maceration, clarification, filtration, and colloidal stabilisation of the processed product and thus they enhance product quality and improve the efficiency of the process, nevertheless when they are not properly used, they can spoil the final product. Additionally, pectinases can be used to upgrade food industry by-products to obtain value-added products and to produce animal feedstock. In this regard, pectinases have become a current trend in the feed industry and their use opens new perspectives for further research in the production of fungal pectinases.

Acknowledgements

The authors acknowledge the support from EU project PGSYS-Exchange Project FP7-People-2012-IRSES-269211.

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