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Microbial Pectic Enzymes in the Food and Wine Industry

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1. Introduction

Pectins are polysaccharides ubiquitous in the plant kingdom and constitute the major component of plant cell walls. The pectinases are a group of related enzymes capable of degrading pectin. Therefore, this group of enzymes have been used for decades in the food and winemaking industry for the processing of fruit juices (Mohnen, 2008; Prade et al., 1999; Ribeiro et al., 2010).

The pectinases are synthesized by plants and microorganisms, the latter being used for industrial production. Microorganisms are used to produce many enzymes of industrial interest in processes relatively inexpensive and environmentally friendly. Moreover, enzymatic catalysis is preferred over other chemical methods since it is more specific, less aggressive and generates less toxicity (Hoondal et al., 2002; Lara-Márquez et al., 2011).

Advances in biotechnology, especially in the fields of molecular biology and microbial genetics, have led to major advances in enzyme technology and have allowed, in many cases, the development of new producing strains and microbial enzymes. The production of pectinases may also benefit from these technologies.

This article reviews the characteristics of pectic substances, the types and mode of action of enzymes which degrade them and the main applications of commercial preparations of microbial pectinases in the food and winemaking industry, followed by a review of new microorganisms and pectolytic enzymes, evaluating new approaches to their production, marketing and use.

2. Pectic substances

Pectic substances are polysaccharides of high molecular weight, with a negative charge, appearing mostly in the middle lamella and the primary cell wall of higher plants, found in the form of calcium pectate and magnesium pectate. They are formed by a central chain containing a variable amount although in high proportion of galacturonic acid residues linked through α -(1-4) glycosidic bonds partially esterified with methyl groups (Fig. 1).

This molecule is known as pectin, while the demethylated molecule is known as polygalacturonic acid or pectic acid. Several L-rhamnopyranosyl residues may be attached to the main chain through its C-1 and C-2 atoms. In addition, galacturonate residue may be

acetylated at the C-2 and C-3 positions, and side chains of residues of neutral sugars may be linked to the galacturonic acid or to the C-4 of the rhamnose residue in the main chain (Caffall & Mohnen, 2009; Mohnen, 2008; Pilnik & Voragen, 1970; Rombouts & Pilnik, 1980).

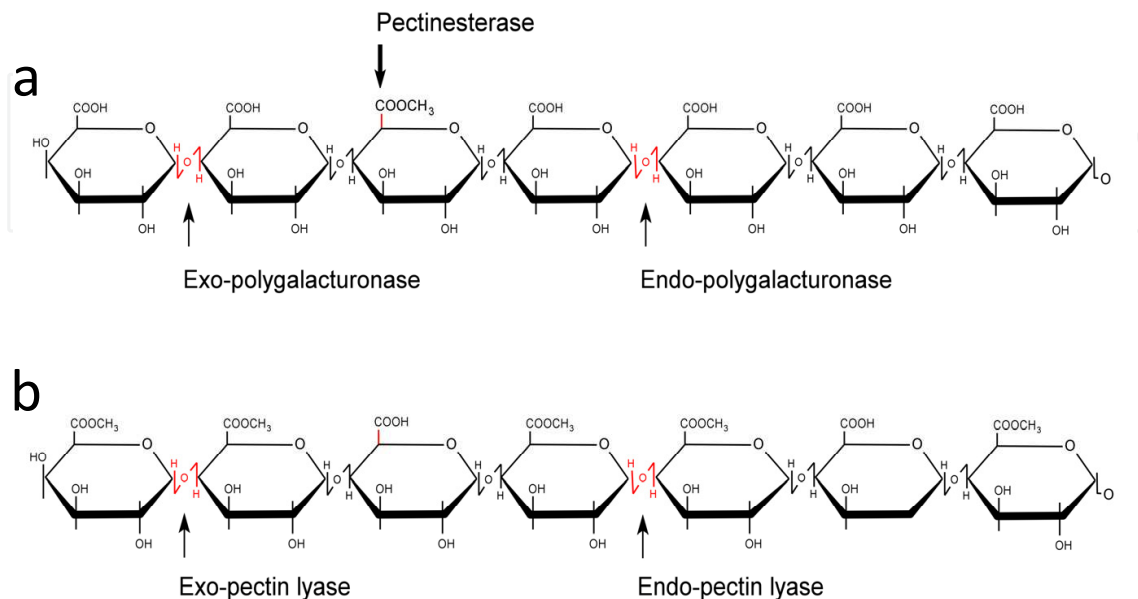


Fig. 1. Structure (main chain) of low (a) and high (b) methylated pectic substances and site of action of enzymes involved in their degradation

The generic name of pectic substances is used for referring to four types of molecules: protopectin (pectic substance in intact tissue), pectinic acids (polygalacturonan containing >0.75% methylated galacturonate units), pectic acids (polygalacturonan that contains negligible amount of methoxyl groups), and pectins (pectinic acid with at least 75% methylated galacturonate units). Protopectines are insoluble in water, while the rest are wholly or partially soluble in water (Alkorta et al., 1998; Kertesz, 1951).

Pectic substances represent between 0.5-4% of fresh weight plant material (Jayani et al., 2005; Sakai et al., 1993). In addition to their role as cementing and lubricating agents in the cell walls of higher plants, they are responsible for the texture of fruits and vegetables during growth, maturation and their storage (Alkorta et al., 1998; Caffall & Mohnen, 2009). Furthermore, pectic substances are involved in the interaction between plant hosts and their pathogens (Collmer & Keen, 1986; Prade et al., 1999).

Pectins have numerous and important applications in the food and pharmaceutical industries. In the food sector, it is primarily used as a gelling agent, replacing sugars and/or fats in low-calorie food and as nutritional fiber (Panchev et al., 1988; Sakai et al., 1993; Thakur et al., 1997). The pharmaceutical industry offers them as preparations to reduce cholesterol or to act as a lubricant in the intestines thus promoting normal peristaltic movement without causing irritation. In addition, these polysaccharides are used as drug delivery systems, which can also reduce the toxicity of these and make their activity longer lasting without altering their therapeutic effects (Morris et al., 2010; Pilnik & Voragen, 1970; Schols et al., 2009; Thakur et al., 1997).

3. Pectolytic enzymes

The enzymes which hydrolyze pectic substances are known as pectic enzymes, pectinases or pectinolytic enzymes (Blanco et al., 1999). Based on its mode of action and substrate preference these enzymes are classified into three types:

- I. Protopectinases, which solubilize protopectin forming soluble pectin
- II. Esterases (pectin methyl esterases and pectin acetyl esterases), which eliminate methoxyl and acetyl residues from pectin giving rise to polygalacturonic acid
- III. Depolymerases, which break the glycosidic α -(1-4) bonds between galacturonic residues via:
 1. Hydrolysis (polygalacturonases)
 2. Transelimination (pectin lyases and pectate lyases)

Also, the latter enzymes are subdivided into endo- if its pattern of action is random or exo- if its pattern of action is at the terminal end (Fogarty & Kelly, 1983; Rexova-Bencova & Markovic, 1976; Sakai, 1992; Whitaker, 1990). The detailed classification of these enzymes, their mode of action and final product are shown in Table 1 and in Fig. 1.

Enzyme	EC N ^o	Main substrate	Mode of action	Product
Esterases				
Pectin methyl esterase	3.1.1.11	Pectin	Hydrolysis	Pectic acid + methanol
Pectin acetyl esterase	3.1.1.6	Pectin	Hydrolysis	Pectic acid + methanol
Depolymerases				
<i>Hydrolases</i>				
Protopectinases		Protopectin	Hydrolysis	Pectin
Endopolygalacturonase	3.2.1.1.5	Pectic acid	Hydrolysis	Oligogalacturonates
Exopolygalacturonase	3.2.1.6.7	Pectic acid	Hydrolysis	Monogalacturonates
<i>Lyases</i>				
Endopectate lyase	4.2.2.2	Pectic acid	Transelimination	Unsaturated oligogalacturonates
Exopectate lyase	4.2.2.9	Pectic acid	Transelimination	Unsaturated oligogalacturonates
Endopectinlyase	4.2.2.10	Pectin	Transelimination	Unsaturated methyl-oligogalacturonates

Table 1. Pectolytic enzymes classified according to its mode of action

4. Pectic enzymes in nature: Microbial pectinases

Pectic enzymes are widely distributed in nature and are produced by bacteria, yeast, fungi (Fig. 2A) and plants. (Lang & Dornenburg, 2000; Whitaker, 1990). In plants, pectic enzymes are very important since they play a role in elongation and cellular growth as well as in fruit ripening (Sakai, 1992; Ward & Moo-Young, 1989; Whitaker, 1990). Pectolytic activity of microorganisms plays a significant role, firstly, in the pathogenesis of plants since these enzymes are the first to attack the tissue (Collmer & Keen, 1986; Whitaker, 1990). In addition, they are also involved in the process of symbiosis and the decay of vegetable residues (Hoondal et al., 2002; Lang & Dornenburg, 2000).

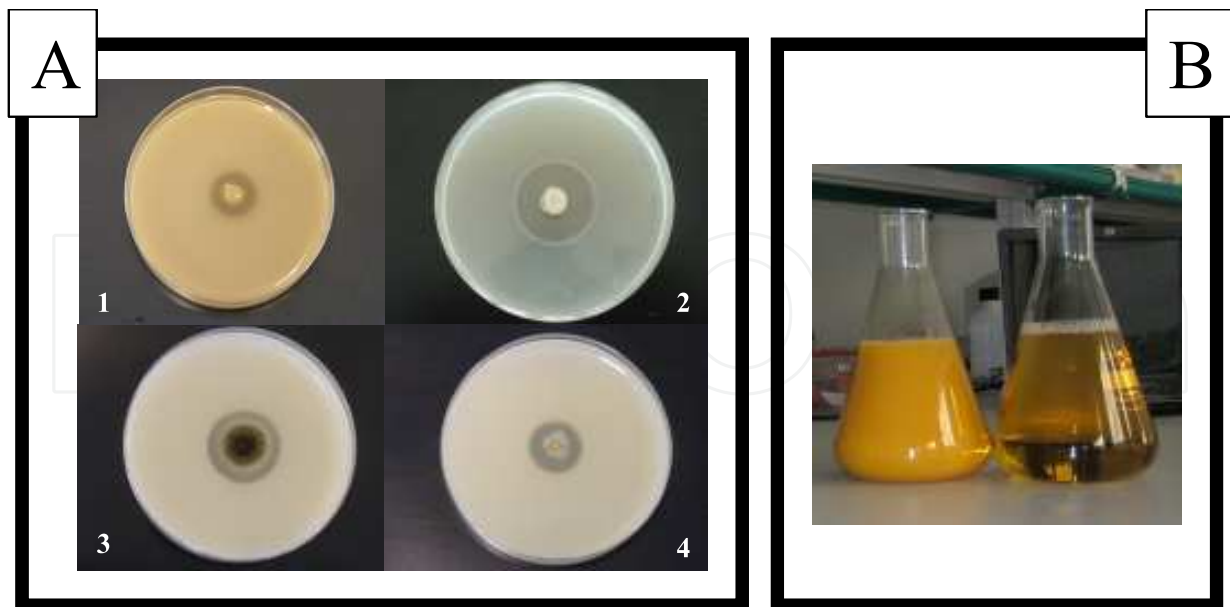


Fig. 2. A: Pectic enzymes produced by different microorganisms growing on minimal medium supplemented with polygalacturonic acid and detected by a clear halo around the colonies. 1, *Xanthomonas campestris* CECT 97; 2, *Kluyveromyces marxianus* CECT 1043; 3, *Aspergillus niger* CECT 2088; 4, *Botryotinia fuckeliana* CECT 20518. B: Cloudy (peach) and clear (apple) juices. CECT: Spanish Type Culture Collection

The microbial world has shown to be very heterogeneous in its ability to synthesize different types of pectolytic enzymes with different mechanisms of action and biochemical properties (Favela-Torres et al., 2005; Gummadi & Panda, 2003). Pectic enzymes are produced by both prokaryotic microorganisms, which primarily synthesize alkaline pectinases, and by eukaryotic microorganisms, mostly fungi that synthesize acid pectinases (Hoondal et al., 2002; Jayani et al., 2005; Kashyap et al., 2001). Furthermore, the production of these enzymes has also been described in yeast (Alimardani-Theuil et al., 2011; Blanco et al., 1999).

There are many studies that have been conducted related to the characterization of different microbial pectic enzymes concerning their mechanisms of action and biochemical properties. The optimal pHs that these enzymes may act range between 3.5-11, while the optimal temperatures vary between 40-75 °C (Gummadi & Panda, 2003; Kashyap et al., 2001). Table 2 shows an example of the diversity of pectic enzymes produced by microorganisms. Given the features of the substrate on which they act and the effect that is required of them, acidic and depolymerizing pectinases are of great interest for the food industry although some applications such as the extraction of oils requires the alkaline ones (Hoondal et al., 2002).

5. Pectinases in the food industry

5.1 Extraction of fruit and vegetable juices

The main industrial application of pectinases is the extraction and clarification of fruit and vegetable juices. Most of the microbial pectinases produced by the industry are dedicated to this purpose. Pectins are responsible for the turbidity and consistency of the juice causing an

increase in their viscosity which hinders its clarification, filtration and concentration (Alkorta et al., 1998). The degradation of pectic substances in mashed fruit purees is achieved through the addition of pectolytic enzymes resulting in an increase in juice yield and its clarification as well as a decrease in viscosity. Treatment with pectinases also provides filtering of the product (de Gregorio et al., 2002; Fernandez-Gonzalez et al., 2004; Ribeiro et al., 2010; Sarioglu et al., 2001; Souza et al., 2003).

Microorganism	Enzyme	Optimal pH	Optimal temperature (°C)	Reference
Bacteria				
<i>Bacillus</i> sp NT-33	Polygalacturonase	10.5	75	Cao et al., 1992
<i>Bacillus</i> sp DT7	Pectin lyase	8	60	Kashyap et al., 2000
Fungi				
<i>Aspergillus ficuum</i>	Pectin lyase	5	50	Yadav et al., 2008
<i>Penicillium frequentans</i>	Endopolygalacturonase	3.5-5	50	Boirin et al., 1996
<i>Sclerotium rolfii</i>	Endopolygalacturonase	3.5	55	Chane & Shewal, 1995
<i>Penicillium paxilli</i>	Pectin lyase	5	35	Szajer & Szajer, 1982
Yeasts				
<i>Saccharomyces cerevisiae</i>	Endopolygalacturonase	5.5	45	Blanco et al., 1994
<i>Kluyveromyces marxianus</i>	Endopolygalacturonase	4.5	55	Serrat et al., 2002

Table 2. Biochemical properties of some pectinases

The fruit and vegetable juice industry uses mainly acidic pectinases of fungal origin, principally from *Aspergillus* spp. Commercial preparations are mixtures of polygalacturonases, pectate lyases and pectin esterases. Pectate lyases can act on the esterified pectin while the polygalacturonases act on the desesterified pectin thus it might require previous action of the pectin esterases. Pectic enzymes treatments vary depending on the type of juice (Fig. 2B):

5.1.1 Clear juices (i.e., apple, pear, grape)

In this type of product, pectolytic enzymes are added to increase the yield in juice during the pressing and for removing matter in suspension. In the case of apple juice, the most commonly used enzymes are those that can depolymerize the highly esterified apple pectin. Apple juice can be obtained through a two-step process consisting of a first treatment of the crushed apple mush with pectinases to obtain the premium juice followed by pomace liquefaction treatment made with a mixture of different pectinases and cellulases for the complete extraction of the juice (Will et al., 2000).

After washing and crushing, the apples are pressed to obtain the juice. Pectic enzymes are used to facilitate the pressing and juice extraction and to assist in the separation of a flocculant precipitate by means of sedimentation, filtration or centrifugation. If a cloudy product is required, the juice is pasteurised after pressing to inactivate residual enzymes. Centrifugation removes the large-size remains leaving small particles in suspension.

However, if a clear juice is required, these suspended particles have to be withdrawn. In order to do this a treatment with mixtures of commercial enzymes is carried out containing pectinases, cellulases and hemicellulases. Finally, the fluid is centrifuged to obtain the clear juice (Grassin & Fauquembergue, 1996; Kashyap et al., 2001). Although it has been noted that the proper clarification of apple juice requires mixtures of polygalacturonase and pectin methyl esterase (Yamaski et al., 1964), subsequent studies have shown that it can be clarified by only using a pure pectin lyase (Ishii & Vokotsuka, 1973).

5.1.2 Cloudy juices (i.e., citrus, tomato, nectars)

In the case of orange juice, where natural pectin esterases are present, pectin is only partially methylated. Polygalacturonases are the pectic enzymes which are most commonly used and of great interest for this type of fruit juice.

In the process of orange juice extraction, pectinases can be added at the end of the pulp wash extraction to reduce viscosity or, preferably, at the end of the first finisher. This leads to higher yield in juice, a better extraction of soluble solids and to a lower viscosity. The action of these enzymes just reduces the viscosity without attacking the insoluble pectin that maintains the stability of the cloud. Enzyme preparations should lack or have the least possible amount of pectin methyl esterases to avoid the clarification of the product. It has been suggested that the best enzyme might be pure pectin lyase (Kashyap et al., 2001; Rebeck, 1990).

5.2 Maceration products of plant tissues

The enzymatic maceration of plant tissues allows the transformation of these organized tissues in suspensions of intact cells that constitute the pulpy products that are used as a basis for preparing juices, nectars, baby food and some dairy products such as yoghurts. Enzyme preparations for this purpose contain cellulases, hemicellulases and pectic enzymes which should only act on the middle lamella of the plant tissue (Kashyap et al., 2001).

5.3 Extraction of vegetable oil

Vegetable oils of olive, sunflower, coconut, palm or canola are obtained by extraction with organic solvents such as hexane. The use of pectolytic enzymes, in this case preferably alkaline, allows the extraction of vegetable oils in an aqueous process. Enzyme preparations based on cellulases, hemicellulases and pectinases have been used successfully in the extraction of olive oil. The enzyme treatment not only improves oil yield and stability but also increases polyphenols and vitamin E content enhancing its organoleptic quality (Hoondal et al., 2002; Iconomou et al., 2010; Kashyap et al., 2001; West, 1996).

5.4 Coffee, cocoa and tea fermentation

Traditionally, fermentation of coffee is made with pectolytic microorganisms in order to remove the layer of mucilage from the coffee beans. With the same purpose, commercial enzyme preparations containing pectinase is sprayed onto the beans to ferment. A cheaper alternative is to use, with the same purpose, the filtrate of inoculated fermentations. The enzyme treatment significantly reduces the fermentation time (Amorim & Amorim, 1977; Kashyap et al., 2001; Serrat et al., 2002; Silva et al., 2000). Cocoa fermentation is essential to develop the chocolate flavour. This fermentation is carried out by a succession of different microorganisms, some of them pectolytic. Pectic enzymes allow the degradation of the cocoa

pulp and are indispensable for the fermentation process and the good quality of fermented beans (Ouattara et al., 2010; Schwan & Wheals, 2004). Similarly, treatment of tea leaves with pectic enzymes of fungal origin (at a dose adjusted to avoid damaging the leaf), facilitates and accelerates the fermentation (Carr, 1985; Kashyap et al., 2001).

6. Pectinases in the wine industry

Wine is the result of the fermentation of grape juice. Pectinases are the most important enzymes used by the winemaking industry although commercial preparations may contain other enzymatic activities such as hemicellulases, glucanases and glycosidases (Rombouts & Pilnik, 1980).

Pectic enzymes are synthesized naturally by the plant and are present in the grape. However, they have low activity during the wine producing process (Ducasse et al., 2011). Microbial pectolytic enzymes especially of fungal origin are resistant to the conditions of fermentation and can be used to facilitate processes, improve quality and diversify products. So far, commercial enzymes are produced all from fungi, mainly of the genus *Aspergillus*. Although all enzymes are produced by *Aspergillus*, pectinase preparations currently available for the wine market are very different. Both the type of activities as well as their concentration in the preparations depend on the strain of *Aspergillus* used, the fermentation conditions for production, the nature of the fermented substrate and the degree of partial purification.

Various research studies have shown that the addition of pectolytic enzymes leads to increased levels of methanol in wine (Revilla & González-SanJosé, 1998; Servili et al., 1992) due to the activity of pectin methyl esterase. Methanol is toxic and its maximum concentration in wine is regulated. Therefore, pectin methyl esterase activity should be at low concentrations in commercial mixtures.

The functions of pectic enzymes in the winemaking process are to support the extraction process, maximize juice yield, facilitate filtration and intensify the flavour and colour.

6.1 Extraction, clarification and filtration

The addition of pectinase to the must reduces its viscosity and causes the grouping of suspended particles in larger aggregates that can be removed by sedimentation. If the enzymes are added to the pulp before pressing, must yield increases, facilitating the pressing and enhancing the colour. Macerating enzyme preparations for this purpose contain pectinases as well as cellulases (Ribereau-Gayon et al., 2006). A high level of polygalacturonase is very effective for clarification but may require the prior action of pectin lyase activity. For this reason the enzyme preparations with a high content of pectin lyase are desirable when very fast racking is required to prevent any problems related to must oxidation, development of endogenous microbiota and nutrient loss. In addition, wines made with pectic-enzyme-treated grape must significantly reduce filtration times (Blanco et al., 1997; Blunt, 2000).

6.2 Intensification and stabilization of colour

In the case of musts obtained from red grapes, the degradation of cell walls in the skin of the grapes through pectolytic enzyme treatment results in an increased release of phenolic

compounds responsible for colour (Busse-Valverde et al., 2011; Pinelo et al., 2006). Early work related to the use of pectinases to enhance the colour of the wines were a bit confusing because Ough et al. (1975) were able to intensify the colour of wine by the enzyme treatment, but Wightman et al. (1997) found that some enzyme preparations containing pectic enzymes reduced red wine colour. Subsequent studies performed with two different enzyme preparations (Watson et al., 1999) confirmed that both allow to produce wines with higher concentrations of anthocyanins and total phenols and have a higher colour intensity. Similarly, in 1994 the Australian Wine Research Institute conducted a study with different commercial preparations of pectic enzymes and in all cases concluded that its use leads to faster and better colour extraction during maceration, pressing and fermentation as well as improve the clarification of the product (van Rensburg & Pretorius, 2000).

6.3 Boosting aroma and flavour

The aromatic profile of wines consists of two components: the varietal aromas characteristic of the variety of grape used and the aromas originated by the yeast during fermentation (Piñeiro et al., 2006; Vilanova & Sieiro, 2006). In many cases the grape variety used completely determines the aroma of the wine, especially in young wines. The volatile compounds of grapes include monoterpenes, C13 nor-isoprenoides, benzene derivatives and aliphatic alcohols. The aromatic components of the grape may appear as free forms, which contribute directly to its scent or bound forms, of greater concentration, to sugars and nonvolatile. Nonodorous glycoside flavour precursors accumulate in the grape especially in the skin during the ripening process (Bayonove, 1993; Williams et al., 1989; Winterhalter & Skouroumounis, 1997). The hydrolysis of these precursors via beta-glucosidases releases the olfactory active aglycones (Günata et al., 1988; Williams et al., 1989). Pectic enzymes help break down the cell walls of the grapes and thus to extract the aromatic precursors. The addition of pectic enzymes during the extraction or fermentation of the must results in an increase in aromatic precursors susceptible to being attacked by beta-glucosidases from the must, those produced by yeasts and bacteria during fermentation or those which are included in commercial enzyme preparations, thereby enhancing the aroma of wines (Comitini et al., 2011; du Toit et al., 2011; Gómez-Plaza et al., 2000; Pinelo et al., 2006).

7. Biotechnology production of microbial pectic enzymes

As discussed until now, there are numerous applications of microbial pectic enzymes in food and wine-making industry. Not surprisingly, the sales volume of these enzymes represents 25% of the enzymes that are commercialized in the food and alcoholic beverages industry. These many applications require one or more types of pectinases that must act in very different condition according to the process in which they are involved. For example, while in the extraction and clarification of fruit juice enzymes can be used at temperatures between 45-95 °C (Kashyap et al., 2001), the wine industry employs temperatures below 15-10 °C (Gómez-Plaza et al., 2000). Although most food applications pectinases have to act in a medium acid, in others, such as oil extraction, they should perform in a medium alkaline (Hoondal et al., 2002).

So far, commercial pectic enzymes are prepared only from cultures of filamentous fungi, mainly of the genus *Aspergillus*. In fact, in the European Union, the OIV (Organisation

Internationale de la Vigne et du Vin) regulates the origin of these enzymes for the wine industry. Commercial products contain mainly a mix of polygalacturonase, pectin lyase and pectin methyl esterase. The use of different strains of *Aspergillus* and modification of substrates and culture conditions can lead to mixtures enriched in one type of enzyme. Companies, including AEB, Gist-Brocades, Novo Nordisk and Lallemand, offer different products with pectic enzymes in different proportions to suit the needs of each process.

However, these commercial pectic preparations are not always optimal for each process, and its use is not without side effects and controversy. Thus, while pectin methyl esterase activity present in the samples may be necessary for the action of polygalacturonase in the case of pectin with a high degree of esterification, its action may lead to an undesirable increase of methanol in the products (Vilanova et al., 2000). Moreover, although the pectic enzymes are the most abundant in commercial mixtures, they can also contain other undesirable activities, such as in the case of making wine, polyphenoloxidases or cinnamyl esterases (Mantovani et al., 2005; van Rensburg & Pretorius, 2000).

At present especially in recent years, the accumulated knowledge of new microbial pectolytic enzymes as well as methodological and technological advances can address the production and use of these enzymes with a different approach. The diversity of applications and conditions in which these enzymes must work also demand a large number of different enzymes capable of acting in such conditions. Even more interesting would be having more robust, broad-spectrum enzymes which allow for a more versatile use in different applications.

Considering the variety of enzymes, traditionally the majority of studies refer to the pectinases of *Erwinia* and *Bacillus* within the bacteria and various fungi especially *Aspergillus* (de Vries and Visser, 2001; de Vries et al., 2002; Gummadi & Panda, 2003; Hoondal et al., 2002; Jayani et al., 2005; Yadav et al., 2009) although there has been a major advance in the description and characterization of pectic enzymes produced by yeast in the last 15 years (Alimardani-Theuil et al., 2011; Blanco et al., 1999; Rodríguez-Gómez & Serrat, 2008). In recent years there has been also a growing interest in studying pectic enzymes with very interesting properties from the point of view of their application. These include thermostable pectinases (Kar & Ray, 2011; Swain & Ray, 2010) or pectinases with optimal activity at low temperatures (Cabeza et al., 2011; Merin et al., 2011; Nakawagua et al., 2004; Padma et al., 2011).

The possibility of producing different types of pectolytic enzymes separately and for later preparation of their mixtures in the proper proportions would allow to provide more suitable commercial preparations for each application and lacking undesirable activities.

In this sense, microorganisms such as some strains of yeast *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* which produce only one type of enzyme (Blanco et al., 1999; Serrat et al., 2002) or that constitutively synthesize it (Serrat et al., 2002) are of great interest. Similarly, obtaining constitutive mutants from producing strains allow the optimization of production and contribute to making cost-effective production processes (Favela-Torres et al., 2006; Trigui-Lahiani et al., 2008). Furthermore, these strains can be used to produce pectinases that accumulate together with other metabolites in the culture broth, which contributes favourable to the overall economy of the process (Serrat et al., 2011). Heterologous expression of enzymes in prokaryotic or eukaryotic systems is a technique of great interest for the production of a single type of enzyme. Table 3 shows some of the many pectic enzymes, both from bacteria, yeast and fungi, which are rightly expressed in

Escherichia coli and different species of yeast. Although *E. coli* is unable to carry out post-translational modifications of proteins, fungal pectolytic enzymes expressed in these bacteria are active (Wang et al., 2011). However, one of the most interesting strategies seems to be the expression of pectinase genes in yeast, particularly in *Pichia pastoris*, in which very high levels of constitutive expression has been achieved (Sieiro et al., 2009). In some cases changes in glycosylation patterns conducted by yeast did not affect the activity and characteristics of recombinant pectinases (Sieiro et al., 2009), while other changes do occur with respect to the characteristics of the native protein, which even lead to enzymes with interesting properties for certain applications (Lang & Looman, 1995; Sieiro et al., 2003).

Microorganism gene origin	Type of enzyme	Host strain	Reference
<i>Xanthomonas campestris</i>	Pectate lyase	<i>E. coli</i>	Xiao et al., 2008
<i>Streptomyces coelicolor</i>	Polygalacturonase	<i>E. coli</i>	Xiao et al., 2008
<i>Pseudoalteromonas haloplanktis</i> ANT/505	Pectate lyase	<i>E. coli</i>	Truong et al., 2001
<i>Thermotoga maritima</i>	Exo-polygalacturonase	<i>E. coli</i>	Parisot et al., 2003
<i>Burkholderia capacia</i>	Endo-polygalacturonase	<i>E. coli</i>	Massa et al., 2007
<i>Phytophthora capsici</i>	Pectate lyase	<i>E. coli</i>	Wang et al., 2011
<i>Erwinia chrysantemy</i>	Pectate lyase	<i>S. cerevisiae</i>	Laing & Pretorius, 1993
<i>Aspergillus niger</i> RH5344	Polygalacturonase	<i>S. cerevisiae</i>	Lang & Looman, 1995
<i>Aspergillus aculeatus</i>	Pectin methyl esterase	<i>S. cerevisiae</i>	Christgau et al., 1996
<i>S. cerevisiae</i> IM1-8b	Endo-polygalacturonase	<i>S. cerevisiae</i>	Blanco et al., 1998
<i>S. cerevisiae</i> IM1-8b	Endo-polygalacturonase	<i>Schizosaccharomyces pombe</i>	Sieiro et al., 2003
<i>K. marxianus</i> CECT1043	Endo-polygalacturonase	<i>P. pastoris</i>	Sieiro et al., 2009
<i>Bispora sp.</i> MEY-1	Endo-polygalacturonase	<i>P. pastoris</i>	Yang et al., 2011

Table 3. Microbial pectic enzymes expressed in different host strains

8. Conclusions

The enzymes that degrade the pectic substances play an essential role in the food and winemaking industries because they are used to degrade the pectins that interfere with the

extraction and clarification of fruit juices and oils as well as being important in the fermentation of coffee, cocoa and tea. Also, in the wine industry they play an important role by contributing to the release of the molecules responsible for aroma and colour, two of the major components that characterize a wine.

Traditionally, this industry uses different mixtures of pectolytic enzymes derived from fungi cultures, mainly of the genus *Aspergillus*, not always completely adequate for the processes they must carry out because of the type and concentration of different enzyme activities that they contain, not without undesirable effects due to other non-pectic enzymes that may be present in the mixtures.

The exploration of microbial biodiversity has allowed, especially in recent years, to identify and characterize new pectic-enzyme-producing microorganisms with different biochemical characteristics, some potentially very interesting from the point of view of their application. Also, it has been technically possible, on the one hand, to select wild strains and constitutive mutants that produce a single enzyme, and, on the other hand, the heterologous expression in bacteria and yeast of numerous genes which encode pectic enzymes, obtaining producing strains of interest.

All this opens the possibility of producing different pectic enzymes individually and preparing commercial mixtures of these, adapted to each process. Research focused on protein engineering in order to obtain pectic enzymes more robust and versatile as well as the optimization of production processes with new strains are necessary for the successful completion of this new approach for the production and use of microbial pectinases.

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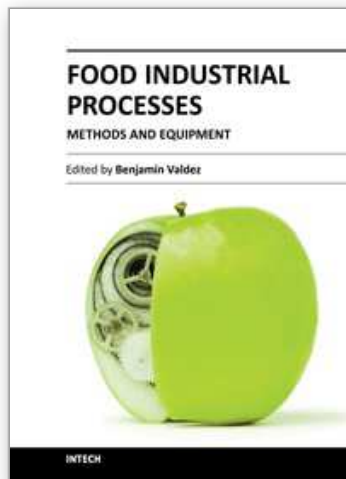
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The global food industry has the largest number of demanding and knowledgeable consumers: the world population of seven billion inhabitants, since every person eats! This population requires food products that fulfill the high quality standards established by the food industry organizations. Food shortages threaten human health and are aggravated by the disastrous, extreme climatic events such as floods, droughts, fires, storms connected to climate change, global warming and greenhouse gas emissions that modify the environment and, consequently, the production of foods in the agriculture and husbandry sectors. This collection of articles is a timely contribution to issues relating to the food industry. They were selected for use as a primer, an investigation guide and documentation based on modern, scientific and technical references. This volume is therefore appropriate for use by university researchers and practicing food developers and producers. The control of food processing and production is not only discussed in scientific terms; engineering, economic and financial aspects are also considered for the advantage of food industry managers.

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