11

Microbial Pectic Enzymes in the Food and Wine Industry

Carmen Sieiro[1], Belén García-Fraga[1], Jacobo López-Seijas[1], Abigaíl F. da Silva[1] and Tomás G. Villa[2]
[1]*Department of Functional Biology and Health Sciences, University of Vigo*
[2]*Department of Microbiology and Parasitology, University of Santiago de Compostela*
Spain

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).

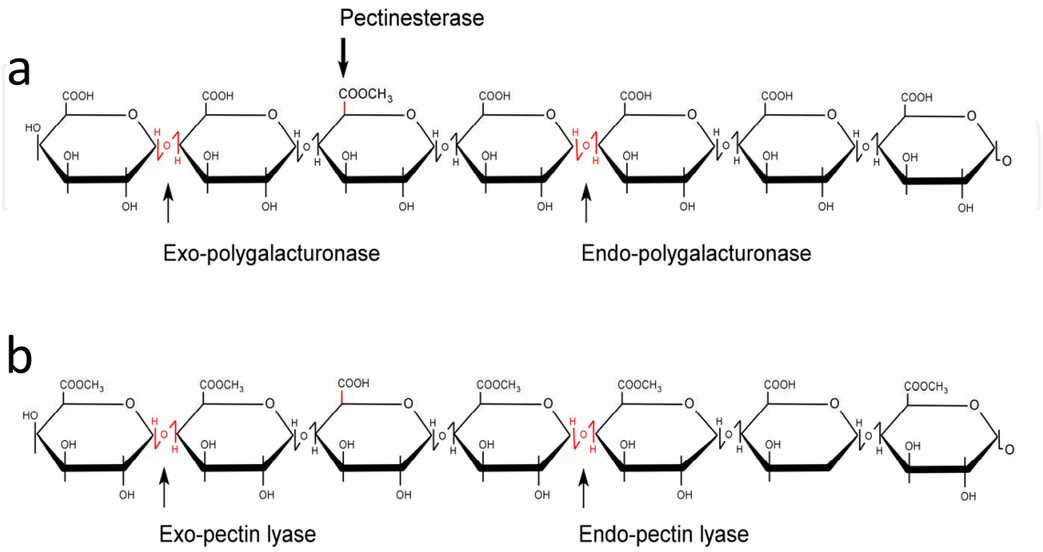


Fig. 1. Structure (main chain) of low (a) and high (b) methylated pectic substances and site of action of enzymes involved in their degradation

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 & Markovie, 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 Nº	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				
Hydrolases				
Protopectinases		Protopectin	Hydrolysis	Pectin
Endopolygalacturonase	3.2.1.1.5	Pectic acid	Hydrolysis	Oligogalacturonates
Exopolygalacturonase	3.2.1.6.7	Pectic acid	Hydrolysis	Monogalacturonates
Lyases				
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).

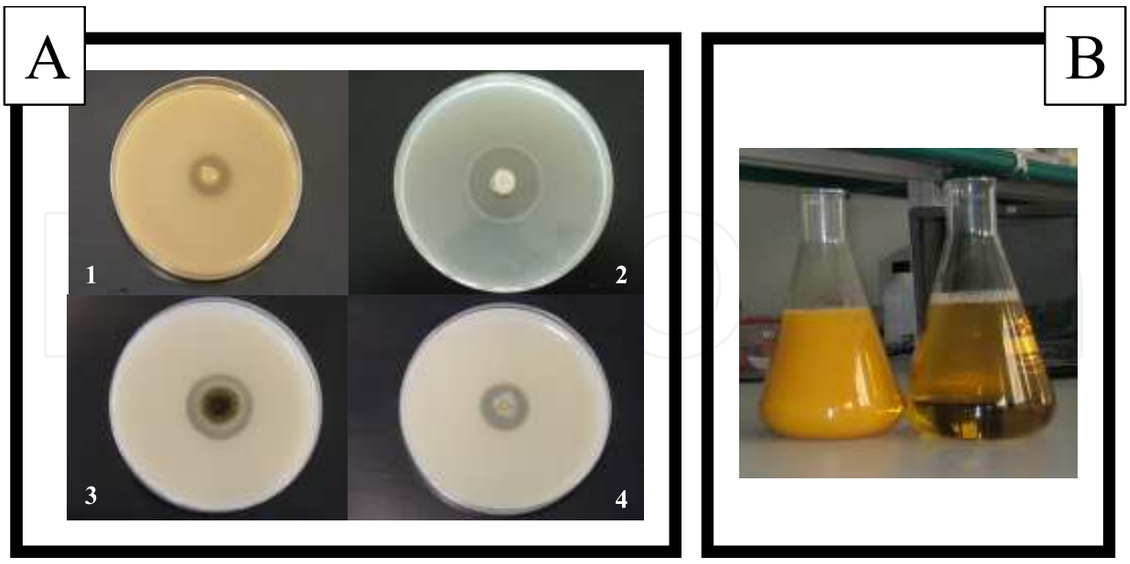


Fig. 2. A: Pectic enzymes produced by different microorganisms growing on minimal medium supplemented with polygalacturonic acid and detected by a clear halo around the colonies. 1, *Xanthomonas campestris* CECT 97; 2, *Kluyveromyces marxianus* CECT 1043; 3, *Aspergillus niger* CECT 2088; 4, *Botryotinia 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 micro-organisms. 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				
Bacillus sp NT-33	Polygalacturonase	10.5	75	Cao et al., 1992
Bacillus sp DT7	Pectin lyase	8	60	Kashyap et al., 2000
Fungi				
Aspergillus ficuum	Pectin lyase	5	50	Yadav et al., 2008
Penicillium frequentans	Endopolygalacturonase	3.5-5	50	Boirin et al., 1996
Sclerotium rolfsii	Endopolygalacturonase	3.5	55	Chane & Shewal, 1995
Penicillium paxilli	Pectin lyase	5	35	Szajer & Szajer, 1982
Yeasts				
Saccharomyces cerevisiae	Endopolygalacturonase	5.5	45	Blanco et al., 1994
Kluyveromyces marxianus	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 enhanzing 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 reduces 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 ºC (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-Gámez & 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**
Xanthomonas campestris	Pectate lyase Polygalacturonase	*E. coli*	Xiao et al., 2008
Streptomyces coelicolor	Polygalacturonase	*E. coli*	Xiao et al., 2008
Pseudoalteromonas haloplanktis ANT/505	Pectate lyase	*E. coli*	Truong et al., 2001
Thermotoga maritima	Exo-polygalacturonase	*E. coli*	Parisot et al., 2003
Burkholderia capacia	Endo-polygalacturonase	*E. coli*	Massa et al., 2007
Phytophthora capsici	Pectate lyase	*E. coli*	Wang et al., 2011
Erwinia chrysantemy	Pectate lyase Polygalacturonase	*S. cerevisiae*	Laing & Pretorius, 1993
Aspergillus niger RH5344	Polygalacturonase	*S. cerevisiae*	Lang & Looman, 1995
Aspergillus aculeatus	Pectin methyl esterase	*S. cerevisiae*	Christgau et al., 1996
S. cerevisiae IM1-8b	Endo-polygalacturonase	*S. cerevisiae*	Blanco et al., 1998
S. cerevisiae IM1-8b	Endo-polygalacturonase	*Schizosaccharomyces pombe*	Sieiro et al., 2003
K. marxianus CECT1043	Endo-polygalacturonase	*P. pastoris*	Sieiro et al., 2009
Bispora sp. MEY-1	Endo-polygalacturonase	*P. pastoris*	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.

9. References

Alimardani-Theuil, P., Gainvors-Claise, A. & Duchiron, F. (2011). Yeasts: An attractive source of pectinases-From gene expression to potential applications: A review. *Process Biochemistry,* Vol.46, pp. 1525-1537

Alkorta, I., Garbisu, C., Llama, M.J. & Serra, J.L. (1998). Industrial applications of pectic enzymes: a review. *Process Biochemistry*, Vol.33, pp. 21-28

Amorim, H.V. & Amorim, V.L. (1977). Coffee enzyme and coffee quality. In: *Enzymes in food and beverage processing*, R.L. Ori & A.J. st Angelo, (Eds.), pp. 27-56, American Chemical Society, ISBN: 084120375X, Indiana, USA

Bayanove, C. (1993). Les composés terpeniques. In : *Les acquisitions récentes en chromatographie du vin. Applications á l´ analyse sensorielle des vins*, B. Doneche, (Ed.), pp. 99-119, ISBN: 2877773647, Paris, France

Blanco, P., Sieiro, C. & Villa, T.G. (1999). Production of pectic enzymes in yeasts. *FEMS Microbiology Letters*, Vol.175, pp. 1-9

Blanco, P., Sieiro, C., Díaz, A. & Villa, T.G. (1994). Production and partial characterization of an endopolygalacturonase from *Saccharomyces cerevisiae*. *Canadian Journal of Microbiology*, Vol.40, pp. 974-977

Blanco, P., Sieiro, C., Díaz, A., Reboredo, N.M. & Villa, T.G. (1997). Grape juice biodegradation by polygalacturonases from *Saccharomyces cerevisiae*. *International Biodeterioration and Biodegradation,* Vol.40, pp. 115-118

Blanco, P., Sieiro, C., Reboredo, N.M. & Villa, T.G. (1998). Cloning, molecular characterization, and expression of an endo-polygalacturonase-encoding gene

from *Saccharomyces cerevisiae* IM1-8b. *FEMS Microbiology Letters,* Vol.164, pp. 249-255

Blunt, M.K. (2000). Cellulases and related enzymes in biotechnology. *Biotechnology Advances,* Vol.18, pp. 355-383

Borin, M.D.F., Said, S. & Fonseca, M.J.V. (1996). Purification and biochemical characterization of an extracelular endopolygalacturonase fron *Penicillium frequentans*. *Journal of Agricultural and Food Chemistry*, Vol.44, pp. 1616-1620

Busse-Valverde, N., Gómez-Plaza, E., López-Roca, J.M., Gil-Muñoz, R. & Bautista-Ortín, A.B. (2011). The extraction of anthocyanins and proanthocyanidins from grapes to wine during fermentative maceration is affected by the enological technique. *Journal of Agricultural and Food Chemistry,* Vol.59, pp. 5450-5455

Cabeza, M.S., Baca, F.L., Puntes, E.M., Loto, F., Baigori, M.D. & Morata, V.I. (2011). Selection of psychrotolerant microorganisms producing cold-active pectinases for biotechnological processes at low temperature. *Food Technology and Biotechnology,* Vol.49, pp. 187-195

Caffall, K.H. & Mohnen, D. (2009). The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. *Carbohydrate Research*, Vol.344, pp. 1879-1900

Cao, J., Zheng, L. & Chen, S. (1992). Screening of pectinase producer from alkalophilic bacteria and study on its potential application in degumming of ramie. *Enzyme and Microbial Technology*, Vol.14, pp. 1013-1016

Carr, J.G. (1985). Tea, coffee and cocoa. In: *Microbiology of fermented foods*, B.J.B. Wood, (Ed.), pp. 133-154, Elsevier Applied Science, ISBN: 0751402168, London, UK

Chane, P.S. & Shewal, J.G. (1995). Pectinase production by *Sclerotium rolfsii.* Effects of culture conditions. *Folia Microbiologica*, Vol.40, pp. 111-117

Christgau, S., Kofod, L.V., Halkier, T., Andersen, L.N., Hockauf, M., Dorreich, K., Dalboge, H. & Kauppinen, S. (1996). Pectin methyl esterase from *Aspergillus aculeatus*: Expression, cloning in yeasts and characterization of the recombinant enzyme. *Biochemical Journal,* Vol.319, pp. 705-712

Collmer, A. & Keen, N.T. (1986). The role of pectic enzymes in plant pathogenesis. *Annual Review of Phytopathology*, Vol.24, pp. 383-409

Comitini, F., Gobbi, M., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I. & Ciani, M. (2011). Selected non-*Saccharomyces* wine yeasts in controlled multistarter fermentations with *Saccharomyces cerevisiae*. *Food Microbiology,* Vol.28, pp. 873-882

de Gregorio, A., Mandalani, G., Arena, N., Nucita, F., Tripodo, M.M. & lo Curto, R.B. (2002). SCP and crude pectinase production by slurry-state fermentation of lemon pulps. *Bioresource Technology*, Vol.83, *pp. 89-94*

de Vries, R.P. & Visser, J. (2001). *Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides. *Microbiology and Molecular Biology Reviews,* Vol.65, pp. 497-522

de Vries, R.P., Jansen, J., Aguilar, G., Parenicova, L., Joosten, V., Wulfert, F., Benen, J.A. & Visser, J. (2002). Expression profiling of pectinolytic genes from *Aspergillus niger*. *FEBS Letters,* Vol.530, pp. 41-47

Demir, N., Acar, J., Sarioglu, K., Mutlu, M. (2001). The use of commercial pectinase in fruit juice industry. Part 3: Immobilized pectinase for mash treatment. *Journal of Food Engineering*, Vol.47, pp. 275-280

du Toit, M., Engelbrecht, L., Lerm, E. & Krieger-Weber, S. (2011). *Lactobacillus*: the next generation of malolactic fermentation starter cultures-an overview. *Food and Bioprocess Technology,* Vol.4, pp. 876-906

Ducasse, M.A., Williams, P., Canal-Llauveres, R.M., Mazerollest, G., Cheynier, V. & Doco, T. (2011). Effect of macerating enzymes on the oligosaccharide profiles of Merlot red wines. *Journal of Agricultural and Food Chemistry*, Vol.59, pp. 6558-6567

Favela-Torres, E., Aguilar, C.N., Contreras-Esquivel, J.C. & Viniegra-González, G. (2005). Pectinases. In*: Enzyme Technology*. A. Pandey, C. Webb, C.R. Soccol & C. Larroche, (Eds.), pp. 265-287, Asiatech Publishers Inc., ISBN: 8187680121, New Delhi, India

Favela-Torres, E., Volke-Sepúlveda, T. & Viniegra-González, G. (2006). Production of hydrolytic depolymerizing pectinases. *Food Technology and Biotechnology*, Vol.44, pp. 221-227

Fernández-González, M., Úbeda, J.F., Vasudevan, T.G., Otero, R.R.C. & Briones, A.I. (2004). Evaluation of polygalacturonase activity in *Saccharomyces cerevisiae* wine strains. *FEMS Microbiology Letters*, Vol.237, pp. 261-267

Fogarty, W.M. & Kelly, C.T. (1983). Pectic enzymes. In: *Microbial enzymes and biotechnology*, W.M. Fogarty, (Ed.), pp. 131-182, Applied Science Publishers, ISBN: 0853341850, London, England

Gómez-Plaza, E., Gil-Muñoz, R., López-Roca, J.M. & Martínez, A. (2000). Colour and phenolic compounds of a young red wine, influence of wine-making techniques, storage temperature, and length of storage time. *Journal of Agricultural and Food Chemistry,* Vol.48, pp. 736- 741

Grassin, C. & Fauquembergue, P. (1996). Fruit Juices. In: *Industrial Enzymology*. T. Godfrey & S. West, (Eds.), pp. 225-264. Industrial enzymology, second ed. Stockholm Press, ISBN: 0935859381, New York, USA

Gummadi, S.N. & Panda, T. (2003). Purification and biochemical properties of microbial pectinases: a review. *Process Biochemistry*, Vol.38, pp. 987-996

Günata, Y.Z., Bitteur, S., Brillouet, J.M., Bayonove, C. & Cordonnier, R. (1988). Sequential enzymatic hydrolysis of potentially aromatic glycosides from grapes. *Carbohydrate Research,* Vol.184, pp. 139-149

Hoondal, G.S., Tiwari, R.P., Tewari, R., Dahiya, N. & Beg, Q.K. (2002). Microbial alkaline pectinases and their industrial applications: a review. *Applied Microbiology and Biotechnology,* Vol.59, pp. 409-418

Iconomou, D., Arapoglou, D. & Israilides, C. (2010). Improvement of phenolic antioxidants and quality characteristics of virgin olive oil with the addition of enzymes and nitrogen during olive paste processing. *Grasas y Aceites*, Vol.61, pp. 303-3011

Ishii, S. & Yokotsuka, T. (1973). Susceptibility of fruit juices to enzymatic clarification by pectin lyase and its relation to pectin in fruit juice. *Journal of Agricultural and Food Chemistry,* Vol.21, pp. 269-272

Jayani, R.S. Saxena, S. & Gupta, R. (2005). Microbial pectinolytic enzymes: a review. *Process Biochemistry,* Vol.40, pp. 2931-2944

Kar, S. & Ray, R.C. (2011). Purification, characterization and application of thermostable exo-polygalacturonase from *Streptomyces erumpens* MTCC 7317. *Journal of Food Biochemistry,* Vol.35, pp. 133-147

Kashyap, D.R., Chandra, S., Kaul, A. & Tewari, R. (2000). Production, purification and characterization of pectinase from *Bacillus* sp. DT7. *World journal of Microbiology and Biotechnology*, Vol.16, pp. 277-282

Kashyap, D.R., Vohra, P.K., Chopra S. & Tewari, R. (2001). Applications of pectinases in the commercial sector: a review. *Bioresource Technology*, Vol.77, pp. 215-227

Kertesz, Z.I. (1951). *The pectic substances*. Interscience Publishers, ISBN: 0471377538, New York, USA

Laing, E. & Pretorius, I.S. (1993). Co-expression of an *Erwinia chrysantemy* pectate lyase-encoding gene (*pelE*) and an *E. carotovora* polygalacturonase-encoding gene (*peh1*) in *Saccharomyces cerevisiae*. *Applied Microbiology and Biotechnology,* Vol.39, pp. 181-188

Lang, C. & Dörnenburg, H. (2000). Perspectives in the biological function and the technological application of polygalacturonases. *Applied Microbiology and Biotechnology*, Vol.53, pp. 366-375

Lang, C. & Looman, A. C. (1995). Efficient expression and secretion of *Aspergillus niger* RH5344 polygalacturonase in *Saccharomyces cerevisiae*. *Applied Microbiology and Biotechnology,* Vol.44, pp. 147-156

Lara-Márquez, A., Zavala-Páramo, M.G., López-Romero, E. & Camacho, H.C. (2011). Biotechnological potential of pectinolytic complexes of fungi. *Biotechnology Letters*, Vol.33, pp. 859-868

Mantovani, C.F., Geimba, M.P. & Brandelli, A. (2005). Enzymatic clarification of fruit juices by fungal pectin lyase. *Food Biotechnology,* Vol.19, pp. 173-181

Massa, C., Degrassi, G., Devescovi, G., Venturi, V. & Lamda, D. (2007). Isolation, heterologous expression and characterization of an endo-polygalacturonase produced by the phytopathogen *Burkholderia capacia*. *Protein Expression and Purification,* Vol.54, pp. 300-308

Merin, M.G., Mendoza, L.M., Farias, M.E., & de Ambrosini, V.I.M. (2011). Isolation and selection of yeasts from wine grape ecosystem secreting cold-active pectinolytic activity. *International Journal of Food Microbiology,* Vol.147, pp. 144-148

Mohnen, D. (2008). Pectin structure and biosynthesis. *Current Opinion in Plant Biology*, Vol.11, pp. 266-277

Morris, G., Kök, S., Harding, S. & Adams, G. (2010). Polysaccharide drug delivery systems based on pectin and chitosan. *Biotechnology & Genetic Engineering Reviews*, Vol.27, pp. 257-284

Nakawawa, T., Nagaoka, T., Taniguchi, S., Milyaji, T. & Tomizuka, N. (2004). Isolation and characterization of psychrophylic yeasts producing cold-adapted pectinolytic enzymes. *Letters in Applied Microbiology,* Vol.38, pp. 383-387

Ouattara, H.G., Reberchon, S., Niamke, S.L. & Nasser, W. (2010). Biochemical properties of pectate lyases produced by three different *Bacillus* strains isolated from fermenting cocoa beans and characterization of their cloned genes. *Applied and Environmental Microbiology*, Vol.76, pp. 5214-5220

Ough, C.S., Noble, A.C., & Temple, D. (1975). Pectic enzyme effects on red grapes. *American Journal of Enology and Viticulture*, Vol.26, pp. 195-200

Padma, P.M., Anuradha, K. & Reddy, G. (2011). Pectinolytic yeasts isolates for cold-active polygalacturonase production. *Innovative Food Science & Emerging Technologies,* Vol.12, pp. 178-181

Panchev, I.N., Kirtchev, N.A., Kratchanov, C.G. & Proichev, T. (1988). On the molecular weight of pectic substances and its relation to their gel strengths. *Carbohydrate Polymers*, Vol.8, pp. 257-269

Parisot, J., Laglois, V., Skanyan, V. & Rabiller, C. (2003). Cloning, expression and characterization of a thermostable exopolygalacturonase from *Thermotoga maritima*. *Carbohydrate Research,* Vol.338, pp. 1333-1337

Pilnik, W. & Voragen, A.G.J. (1970). Pectic substances and other uronides. In: *The biochemistry of fruits and their products*, A. C. Hulme, (Ed.), Vol.1, pp. 53-87, Academic Press, ISBN: 0123612012, London, England

Piñeiro, Z., Natera, R., Castro, R., Palma, M., Puertas, B. & Barroso, C.G. (2006). Characterization of volatile fraction of monovarietal wines: Influence of winemaking practices. *Analytica Chimica Acta,* Vol.563, pp. 165-172

Pinelo, M., Arnous, A. & Meyer, A.S. (2006). Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. *Trends in Food Science & Technology,* Vol.17, pp. 579-590

Prade, R.A., Zhan, D., Ayouby, P. & Mort, A.J. (1999). Pectins, pectinases and plant-microbe interactions. *Biotechnology & Genetic Engineering Reviews*, Vol.16, pp. 361-391

Rebeck, H. (1990). Processing of citrus juices. In: *Production and packaging of non-carbohydrate fruit juices and fruit beberages*, D. Hick, (Ed.), Van Nosrand Reihold, New York, USA

Revilla, I. & González-SanJosé, M.L. (1998). Methanol release during fermentation of red grapes treated with pectolytic enzymes. *Food Chemistry*, Vol.63, pp. 307-312

Rexová-Benková, L. & Marcovic, O. (1976). Pectic enzymes. *Advances in Carbohydrate Chemistry*, Vol.33, pp. 323-385

Ribeiro, D.S., Henrique, S.M.B., Oliveira, L.S., Macedo, G.A. & Fleuri, L.F. (2010). Enzymes in juice processing: a review. *Interntional Journal of Food Science and Technology*, Vol.45, pp. 635-641

Ribereau-Gayon, P., Dubourdieu, D., Doneche, B. & Lonvaud, A. (2006). *Handbook of Enology, Vol. I. The Microbiology of Wine and Vinifications* (2nd edition), John Wiley & Sons, ltd., ISBN: 0470010347, New York, USA

Rodríguez-Gámez, O. & Serrat, M. (2008). Poligalacturonasas de levaduras: un producto biotecnológico de grandes potencialidades. *Tecnología Química,* Vol.28, pp. 80-90

Rombouts, F.M. & Pilnik, W.L. (1980). Pectic enzymes. In: *Economic microbiology: microbial enzymes and bioconversions*, A. H. Rose, (Ed.), Vol.5, pp. 227-282. Academic Press, ISBN: 0125965559, London, England

Sakai, T. (1992). Degradation of pectins. In: *Microbial degradation of natural products*, G. Winkelmann, (Ed.), pp. 57-81, VCH, ISBN: 3527283544, Weinheim & New York, Germany & USA

Sakai, T., Sakamoto, T., Hallaert, J. & Vandamme, E.J. (1993). Pectin, pectinase and protopectinase: production, properties and applications. *Advances in Applied Microbiology*, Vol.39, pp. 213-294

Sarioglu, K., Demir, N., Acar, J. & Mutlu, M. (2001). The use of commercial pectinase in the fruit juice industry, part 2: Determination of the kinetic behaviour of immobilized commercial pectinase. *Journal of Food Engineering*, Vol.47, pp. 271-274

Schols, H.A., Visser, R.G.F. & Voragen, A.G.J. (2009). Pectin and Pectinases. Wagenigen Academic Publishers, ISBN 978-90-8686-677-9, Wagenigen, Netherlands

Schwan, R.F. & Wheals, A.E. (2004). The microbiology of cocoa fermentation and its role in chocolate quality. *Critical Reviews in Food Science and Nutrition*, Vol.44, pp. 205-221

Serrat, M., Bermúdez, R.C. & Villa, T.G. (2002). Production, purification and characterization of a polygalacturonase from a new strain of *Kluyveromyces marxianus* isolated from coffee wet-processing wastewater. *Applied Biochemistry and Biotechnology*, Vol.97, pp. 193-208

Serrat, M., Rodríguez, O., Camacho, M., Vallejo, J.A., Ajeitos, J.M. & Villa, T.G. (2011). Influence of nutritional and environmental factors on ethanol and endopolygalacturonase co-production by *Kluyveromyces marxianus* CCEBI 2011. *International Microbiology*, Vol.14, pp. 41-49

Servili, M., Begliomini, A.L., Montedoro, G., Petruccioli, M. & Federici, F. (1992). Utilisation of a yeast pectinase in olive oil extraction and red wine making processes. *Journal of the Science of Food and Agriculture*, Vol.58, pp. 253-260

Sieiro, C., Poza, M., Vilanova, M. & Villa, T.G. (2003). Heterologous expression of the *Saccharomyces cerevisiae PGU1* gene in *Schizosaccharomyces pombe* yields an enzyme with more desirable properties for the food industry. *Applied and Environmental Microbiology,* Vol.69, pp. 1861-1865

Sieiro, C., Sestelo, A.B.F. & Villa, T.G. (2009). Cloning, characterization, and functional analysis of the *EPG1-2* gene: a new allele coding for an endopolygalacturonase in *Kluyveromyces marxianus*. *Journal of Agricultural and Food Chemistry,* Vol.57, pp. 8921-8926

Silva, C.F., Schwan, R.F., Dias, E.S. & Wheals, A.E. (2000). Microbial diversity during maturation and natural processing of coffee cherries of *Coffea arabica* in Brazil. *International Journal of Food Microbiology,* Vol.60, pp. 251-260

Souza, J.V.B., Silva, E.S., Maia, M.L.S. & Teixeira, M.F.S. (2003). Screening of fungal strains for pectinolytic activity: endopolygalacturonase production by *Peacilomyces clavisporus* 2A.UMIDA.1. *Process Biochemistry*, Vol.39, pp. 455-458

Swain, M.R. & Ray, R.C. (2010). Production, characterization and application of a thermostable exo-polygalacturonase by *Bacillus subtilis* CM5. *Food Biotechnology*, Vol.24, pp. 37-50

Szajer, I. & Szajer, C. (1982). Pectin lyase of *Penicillium paxilli*. *Biotechnology Letters,* Vol.4, pp. 549- 552

Thakur, B.R., Singh, R.K. & Handa, A.K. (1997). Chemistry and uses of pectin: a review. *Critical Reviews in Food Science and Nutrition*, Vol.37, pp. 47-73

Trigui-Lahiani, H., Ayadi, M., Hadj-Taieb, N., Ben Ali, M. & Gargouri, A. (2008). Genomic organization of a polygalacturonase gene from a hyperpectinolytic mutant strain of *Penicillium ocitanis*. *FEMS Microbiology Letters,* Vol.281, pp. 23-29

Truong, L.V., Tuyen, H., Helmke, E., Binh, L.T. & Schweder, T. (2001). Cloning of two pectate lyase genes from the marine Antarctic bacterium *Pseudoalteromonas haloplanktis* strain ANT/505 and characterization of the enzymes. *Extremophiles,* Vol.5, pp. 35-44

van Rensburg, P. & Pretorius, I.S. (2000). Enzymes in winemaking: Harnessing natural catalysts for efficient biotransformation: a review. *South African Journal of Enology and Viticulture,* Vol.21, pp. 52-73

Vilanova, M. & Sieiro, C. (2006). Determination of free and bound terpene compounds in Albariño wine. *Journal of Food Composition and Analysis,* Vol.19, pp. 694-697

Vilanova, M., Blanco, P., Cortés, S., Castro, M., Villa, T.G. & Sieiro, C. (2000). Use of a *PGU1* recombinant *Saccharomyces cerevisiae* strain in oenological fermentations. *Journal of Applied Microbiology,* Vol.89, pp. 876-883

Wang, H.Z., Fu, L. & Zhang, X.G. (2011). Comparison of expression, purification and characterization of a new pectate lyse from *Phytophthora capsici* using two different methods. *BMC Biotechnology,* (DOI: 10. 1186/1472-6750-11-32)

Ward, O.P. & Moo-Young, M. (1989). Enzymatic degradation of cell wall and related plant polysaccharides. *Critical Reviews in Biotechnology*, Vol.8, pp. 237-274

Watson, B., Goldberg, N., Chen, H.P. & McDaniel, M. (1999). Fermentation processing effects on colour, phenolic profiles, and sensory character of Oregon Pinot noir wines. In: *Proceedings of the 12th International Oenological Symposium*, Montreal, Canada

West, S. (1996). Olive and other oils. In: *Industrial enzymology* (2nd edition), T. Godfrey & S. West, (Eds.), pp. 293-300, Stockholm Press, ISBN: 0333594649, New York, USA

Whitaker, J.R. (1990). Microbial pectinolytic enzymes. *Microbial enzymes and biotechnology* (2nd edition), W.M. Fogarty & C.T. Kelly, (Eds.), pp. 133-176, Elsevier Science Ltd., ISBN: 1851664866, London, England

Wightman, J.D., Price, S.F., Watson, B.T. & Wrolstad, P.E. (1977). Some effects of processing enzymes on anthocyamino and phenolics in Pinot noir and Cabernet Sauvignon wines. *American Journal of Enology and Viticulture*, Vol.48, pp. 39-48

Will, F., Bauckhage, K. & Dietrich, H. (2000). Apple pomace liquefaction with pectinases and cellulases: analytical data of the corresponding juices. *European Food Research and Technology*, Vol.211, pp. 291-297

Williams, P.J., Sefton, M.A. & Wilson, B. (1989). Non volatile conjugated of secondary metabolites as precursors of varietal grape flavor components. In: *Flavor chemistry: trends and developments,* R. Teranishi, R.G. Buttery & F. Shaihidi, (Eds.), pp. 35-48, ISBN: 0841215707, Washington D.C., USA

Winterhalter, P. & Skouroumounis, G.K. (1997). Glycoconjugated aroma compounds: occurrence, role and biotechnological transformation. In: *Biotechnology of aroma compounds. Advances in biochemical engineering/biotechnology,* T. Scheper, (Ed.), pp. 74-105, Springer, ISBN: 3540614826, Berlin, Germany

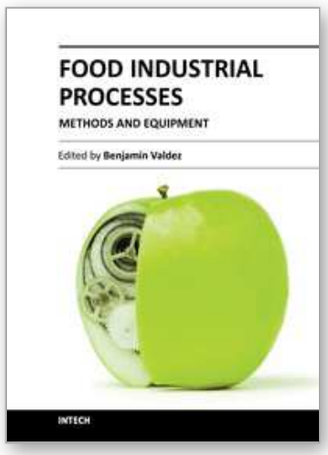
Xiao, Z., Boyd, J., Grosse, S., Beauchemin, M., Coupe, E. & Lau P.C.K. (2008). Mining *Xanthomonas* and *Streptomyces* genomes for new pectinase-encoding sequences and their heterologous expression in *Escherichia coli*. *Applied Microbiology and Biotechnology,* Vol.78, pp. 973-981

Yadav, S., Yadav, P., Yadav, D. & Yadav, K. (2008). Purification and characterization of an acidic pectin lyase produced by *Aspergillus ficuum* strain MTCC 7591 suitable for clarification of fruit juices. *Annals of Microbiology*, Vol.58, pp. 61-65

Yadav, S., Yadav, P.K., Yadav, D. & Yadav, K.D.S. (2009). Pectin Lyase: A review. *Process Biochemistry*, Vol.44, pp. 1-10

Yamaski, M., Yasui, T. & Arima, K. (1964). Pectic enzymes in the clarification of apple juices. Part I. Study on the clarification reaction in a simplified model. *Agricultural and Biological Chemistry,* Vol.28, pp. 779-787

Yang, Y., Luo, H.I., Li, J.A., Wang, K., Cheng, H.P., Bai, Y.G., Yuan, T.Z., Fan, Y.L. & Yao, B. (2011). Cloning, expression and characterization of an acidic endopolygalacturonase from *Bispora* sp. MEY-1 and its potential application in juice clarification. *Process Biochemistry,* Vol.46, pp. 272-277



Food Industrial Processes - Methods and Equipment
Edited by Dr. Benjamin Valdez

ISBN 978-953-307-905-9
Hard cover, 418 pages
Publisher InTech
Published online 22, February, 2012
Published in print edition February, 2012

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.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Carmen Sieiro, Belén García-Fraga, Jacobo López-Seijas, Abigaîl F. da Silva and Tomás G. Villa (2012). Microbial Pectic Enzymes in the Food and Wine Industry, Food Industrial Processes - Methods and Equipment, Dr. Benjamin Valdez (Ed.), ISBN: 978-953-307-905-9, InTech, Available from: http://www.intechopen.com/books/food-industrial-processes-methods-and-equipment/microbial-pectic-enzymes-in-the-food-and-wine-industry

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen