7 Microbial Pectinases and Their Applications

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Abstract

In nature, microorganisms have been endowed with vast potentials. They produce an array of enzymes, which have been exploited commercially over the years. Among them, pectinolytic enzymes are of great significance and are one of the most important enzymes of the commercial sector. Microbial pectinases can be produced from bacteria, actinomycetes, yeasts and fungi. Pectinases are being used in several conventional industrial processes, such as fruit juice extraction and clarification, textile processing, tea and coffee fermentation, recovery of vegetable oils, retting and degumming of plant fibres and paper making. This chapter is aimed at reviewing the various types of pectinases and their biotechnological applications.

Keywords

Pectinase • Fruit juice extraction and clarification • Degumming • Retting • Pectinase genes

Introduction

Recent years have seen a great increase in industrial applications of pectinases owing to their significant biotechnological potential. Pectinases are a heterogenous group of related enzymes that hydrolyse the pectic substances present in the plant cell wall (Whitaker 1990). In nature, a wide variety of microorganisms, e.g. bacteria, yeasts, actinomycetes and filamentous fungi have been reported to produce pectinases (Kapoor et al. 2001; Hoondal et al. 2002; Kuhad et al. 2004; Jayani et al. 2005; Torres et al. 2006; Gupta et al. 2008; Murad and Azzaz 2011; Maleki et al. 2011). The pectinases have been used more commonly in the extraction and clarification of fruit juices and in wine production (Ribiero et al. 2010; Prathyusha and Suneetha 2011; Sandri et al. 2011), while they are also of great significance in other industrial applications as well, such as textile processing, degumming and retting of plant fibres, paper making and coffee and tea fermentations (Kashyap et al. 2001; Hoondal et al. 2002; Jayani et al. 2005; Aggarwal et al. 2008). At present there are several companies worldwide for producing

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Table 7.1 Some commercial pectinases

Supplier	Location	Brand name
C.H. Boehringer Sohn	Ingelheim, Germany	Panzym
Ciba-Geigy, A.G.	Basel, Switzerland	Ultrazyme
Kikkoman Shoyu, Co.	Tokyo, Japan	Sclase
Schweizerische Ferment, A.G.	Basel, Switzerland	Pectinex
Wallerstein, Co.	Des Plaines, USA	Klerzyme
Rohm, GmbH	Darmstadt, Germany	Pectinol, Rohament
Grinsteelvaeket	Aarthus, Denmark	Pectolase
Societe Rapidase, S.A	Seclin, France	Rapidase
Clarizyme Wallerstein, Co.	Des Plaines, USA	Klerzyme
Biocon Pvt. Ltd.	Bangalore, India	Pectinase
Novozyme	Denmark	Pectinex Mash
Lyven	France	Ly Peclyve PR
Danisco	Denmark	MaxLiq
AB Enzymes	Finland	Rohapect® MA Plus

Table 7.2 Composition of pectin in different fruits and vegetables (fresh weight)

Fruit	Pectic substance (%)	Fruit	Pectic substance (%)
Apple	0.5–1.6	Blackcurrant	0.8–1.1
Banana	0.7–1.2	Cranberry	0.8–1.2
Peaches	0.1–0.9	Grape	0.1–0.5
Strawberries	0.6–0.7	Lemon	1.8–2.2
Cherries	0.2–0.5	Carrot	1.2–1.5
Peas	0.9–1.4	Mango	0.3–0.5
Apricot	0.8–1.0	Pineapple	0.04–0.1
Oranges	0.5–3.5	Plum	0.7–0.9
Citrus peel	25–30	Raspberry	0.4–0.6
Blackberry	0.7–0.9		

commercial pectinases to be used in various industrial applications (Table 7.1).

Pectic Substances: Substrate of Pectinase

Pectic substances are glycosidic macromolecules of high molecular weight, which form the largest component of the middle lamella of higher plants (Alkorta et al. 1998; Kashyap et al. 2001; Jayani et al. 2005; Almeida et al. 2005; Pedrolli et al. 2009), accounting approximately 0.1–3.0% of the fresh weight of plants (Table 7.2). Chemically, they are a complex colloidal acidic polysaccharides composed of galacturonic acid residues linked by α-1,4-glycosidic bonds (Fig. 7.1), partially esterified by methyl ester and partially or completely neutralised by one or more basic ions such as sodium, potassium or ammonium (Limberg et al. 2000; Kuhad et al. 2004).

American Chemical Society classified pectic substances into four main types as follows (Alkorta et al. 1998):

Protopectin: It is the water-insoluble pectic substance present in intact plant tissue. Protopectin on restricted hydrolysis yields pectin or pectic acids.

Pectic acid: It is the soluble polymer of galacturonans that contains negligible amount of methoxyl groups. Normal or acid salts of pectic acid are called pectates.

Pectinic acids: It is the polygalacturonan chain that contains up to 75% methylated galacturonate units. Normal or acid salts of pectinic acid are referred to as pectinates.

Pectin (*polymethylgalacturonate*): It is polymeric material in which, at least, 75% of the carboxyl groups of the galacturonate units are esterified with methanol. It confers rigidity on cell wall when it is bound to cellulose in the cell wall.

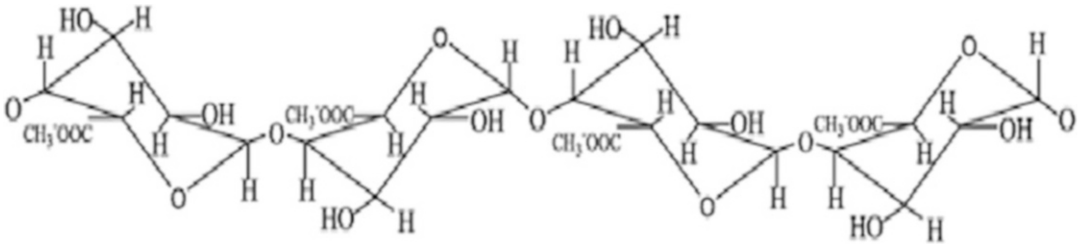

Fig. 7.1 Primary structure of pectic substances (Rexova-Benkova and Markovic 1976)

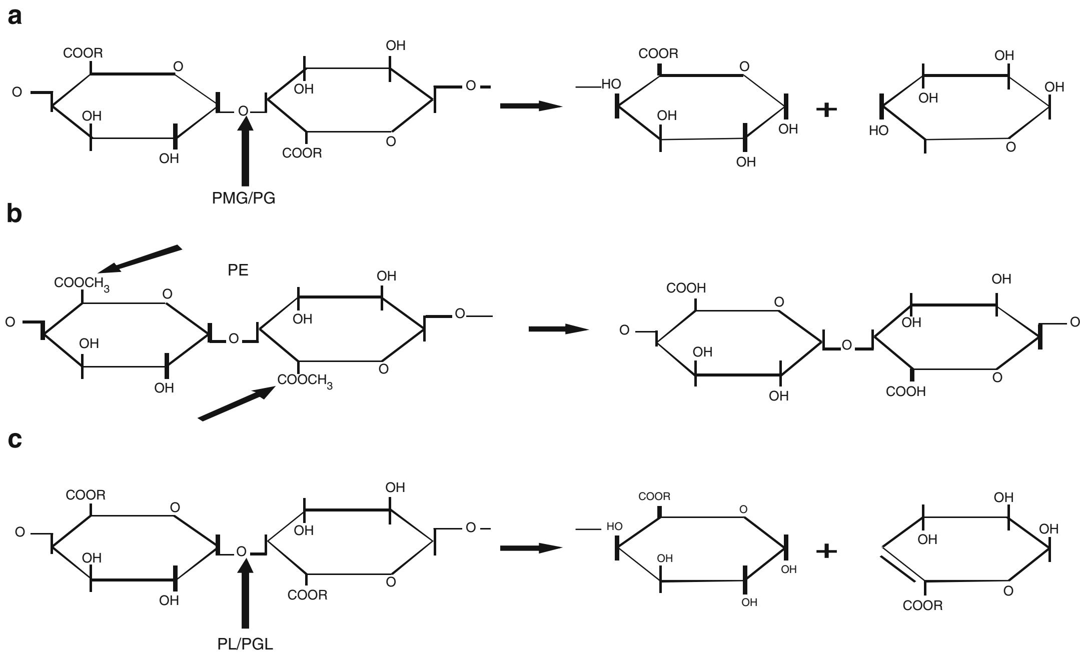

Fig. 7.2 Different types of pectinases and their mode of action on pectic substances (**a**) R=H for PG and CH_3 for PMGL (**b**) PE and (**c**) R=H for PGL and CH_3 for PL. The *arrow* indicates the place where the pectinases reacts with pectic substances. *PMGl* polymethylgalacturonate lyase, *PG* polygalacturonase, *PE* pectinesterase, *PL* pectin lyase (Pedrolli et al. 2009)

Pectinolytic Enzymes

Pectic substances are ubiquitous in the plant kingdom, and their efficient utilisation could enhance the economic competitiveness of bioconversion processes intended to compete with conventional industrial processes (Kapoor et al. 2001; Kapoor and Kuhad 2002). Therefore, nowadays a significant interest in the degradation of pectic substances with pectinases has been generated. Pectinases based on their mode of action (Fig. 7.2) can be divided into three broad groups:

(a) *Protopectinases*: Protopectinases or pectinosinases (PPase) are the enzymes that catalyse the solubilisation of protopectin to produce highly polymerised soluble pectin (Brinton et al. 1927). On the basis of their reaction mechanism, protopectinases are classified into two types, i.e. A type and B type. A type protopectinase reacts with the inner site, i.e. the polygalacturonic acid region of protopectin, whereas the B type protopectinase reacts on the outer site, i.e. on the polysaccharide chains that may connect the polygalacturonic acid chain and cell wall constituents (Sakai et al. 1993).

(b) *Depolymerising enzymes* which break α-1, 4, linkages in the principal pectin chain such as:

(i) *Polygalacturonase*: Polygalacturonases (PGases) are the pectinolytic enzymes that catalyse the hydrolytic cleavage of the polygalacturonic acid chain with the introduction of water across the oxygen bridge. The PGases involved in the hydrolysis of pectic substances are of two types, i.e. endo-polygalacturonases and exo-polygalacturonases. Endo-PG also known as poly(1,4-α-D-galacturonide) glycanohydrolase catalyses random hydrolysis of α–1,4-glycosidic linkages in the pectic acid, while exo-PG also known as poly(1,4-α-D-galacturonide) galacturonohydrolases catalyses hydrolysis in a sequential fashion of α-1,4-lycosidic linkages on pectic acid (Kashyap et al. 2001).

(ii) *Polymethylgalacturonases*: Polymethylgalacturonases (PMGL) catalyses the hydrolytic cleavage of α-1,4-glycosidic bonds in pectin backbone, preferentially highly esterified pectin-forming 6-methyl-D-galacturonate (Jayani et al. 2005; Pedrolli et al. 2009).

(iii) *Lyases*: Lyases or transeliminases are the enzymes that perform non-hydrolytic breakdown of pectates or pectinates by a transeliminative split of the pectic polymer (Sakai et al. 1993; Kashyap et al. 2001; Jayani et al. 2005; Pedrolli et al. 2009). They are classified as endo-pectate transeliminase (pectate lyase, PGL) and endo-pectin transeliminase (pectin lyase, PL). Pectate lyase cleaves glycosidic linkages preferentially on polygalacturonic acid forming unsaturated product through transelimination reaction. Pectate lyases are classified as endo-PGL that acts towards substrate in a random way and exo-PGL that catalyse the substrate cleavage from nonreducing end. Pectin lyase (PL) catalyses the random cleavage of pectin, preferentially high-esterified pectin, producing unsaturated methyloligogalacturonates through transelimination of glycosidic linkages

(c) *Demethoxylating* enzymes such as *pectinesterase*: Pectinesterase (PE) often referred to as pectin methylesterase, pectase, pectin methoxylase, pectin demethoxylase and pectolipase is a carboxylic acid esterase and belongs to the hydrolase group of enzymes (Whitaker 1990). It catalyses the de-esterification of methyl ester linkages of galacturonan backbone of pectic substances to release acidic pectins and methanol (Cosgrove 1997; Kashyap et al. 2001; Malvessi and Silveira 2004; Jayani et al. 2005; Pedrolli et al. 2009).

The action of polygalacturonase, pectin methyl esterase and pectin lyase leads to extensive degradation of middle lamella and cell wall pectin, and this property of pectinases makes them useful in various industrial applications (Ribiero et al. 2010).

Properties of Pectinases

During the last few decades, pectinases from various microorganisms have been studied extensively for their use in various industries. Various properties of pectinases, viz. molecular weight, pH and temperature optima and stability have been summarised in Table 7.3.

Applications of Pectinases

Acidic pectinases are mainly used for the extraction and clarification of fruit juices and wines. Alkaline pectinases are employed for the processing and degumming of plant fibres, in paper making, etc. (Gupta et al. 2008). Some of the industrial applications of pectinases have been discussed as follows:

Fruit Juice Industries

The juices from a wide variety of fruits such as apple, pear, plum, mango, banana, grape, apricot, orange, guava, papaya, strawberry, raspberry and blackberry can be extracted to produce natural beverages. However, the most important

Table 7.3 Characterisation of some microbial pectinases

Pectinase producer	Nature	Molecular weight (kDa)	pI	Optimum temperature (°C)	Optimum pH	References
P. italicum	PMGL	22	8.6	40	6.0–7.0	Alana et al. (1990)
B. macerans	PGL	35	10.3	60	9.0	Miyazaki (1991)
A. japonicas	PMGL	–	7.7	55	6.0	Dinnella et al. (1994)
A. carbonarius	Endo-PG	61 (PG I)	–	55	4.0	Devi and Rao (1996)
		42 (PG II)	–	50	4.1	
		47 (PG III)	–	55	4.3	
Pythium splendens	PMGL	23	8.0	–	8.0	Chen et al. (1998)
Bacillus sp.	PGL	38	–	69	11.0	Singh et al. (1999)
Bacillus sp. KSM-P7	PGL(Pel 7)	33	10.5	60–65	10.5	Kobayashi et al. (1999)
S. cerevisiae	Endo-PG	39	–	45	5.5	Ganivors et al. (2000)
Bacillus sp. DT7	PL	106	–	60	8.0	Kashyap et al. (2000)
A. japonicas	PE	46(PE I)	3.8	–	4.0–5.5	Hasunuma et al. (2003)
		47(PE II)	3.8	–	4.0–5.5	
A. japonicas	Endo-PG	38 (PG I)	5.6	30	4.0–5.5	Semenova et al. (2003)
	Endo-PG	38 (PG II)	3.3	30	4.0–5.5	
A. Japonicas	PL	50	3.8	40–50	4.5–5.5	Semenova et al. (2003)
A. japonicas	Pectin esterase I	46	3.8	40–50	4.5–5.5	Semenova et al. (2003)
	Pectin esterase II	47	3.8	40–50	4.5–5.5	
Mucor flavus	Endo-PGL	40	8.3	45	3.5–5.5	Gadre et al. (2003)
F. moniliforme	PGL	–	–	–	8.5	Dixit et al. (2004)
A. kawachii	Endo-PG	60	–	37	4.0	Esquvel and Voget (2004)
R. oryzae	Endo-PG	31	–	45	4.5	Saito et al. (2004)
B. macerans	PGL	–	–	63–67	8.0–8.5	Morozova et al. (2006)
B. pumilus	PGL	37.3	8.5	70	8.5	Klug-Santner et al. (2006)
B. subtilis	PGL(Pel C)	42	7.8	65	10.0	Soriano et al. (2006)
A. flavus		–	–	50	8.0	Yadav et al. (2008)
Streptomyces lydicus	Exo-PG	43		50	6	Jacob et al. (2008)
A. giganteus	Exo-PG	–	–	55	6.0	Pedrolli et al. (2008)
A. giganteus	PL	–	–	50	8.5	Pedrolli et al. (2008)
P. citrinum	PL	31	–	50	6.5	Rasheedha Banu et al. (2010)
B. pumilus	PL	25	–	60	6	Nadaroglu et al. (2010)
B. subtilis RCK	Exo-PG	54	9.1	60	10.5	Gupta et al. (2008)
A. niger URM4645	Endo-PG	–	–	50	5	Maciel et al. (2011)
	PL			50	5	
	Exo-PG			40	7	

characteristic affecting the extraction of juice is the fruit cell wall, which is a complex structure of interwoven polymers composed of bundles of crystalline cellulose microfibres embedded in an aqueous gel of hemicellulose and pectin (Alkorta et al. 1998).

As a result, application of pectinases, cellulases and hemicellulases (collectively called macerating enzymes) has been observed useful in the extraction and clarification of fruit and vegetable juices (Kaur and Satyanarayana 2004; Unejo and Pastore 2007; Ribiero et al. 2010; Prathyusha and Suneetha 2011). Pectinases degrade pectin and other high molecular weight components in the cell wall, resulting in increased juice yield and decreased viscosity, thus giving a crystalline appearance to the final product with reduction in filtration time up to 50%

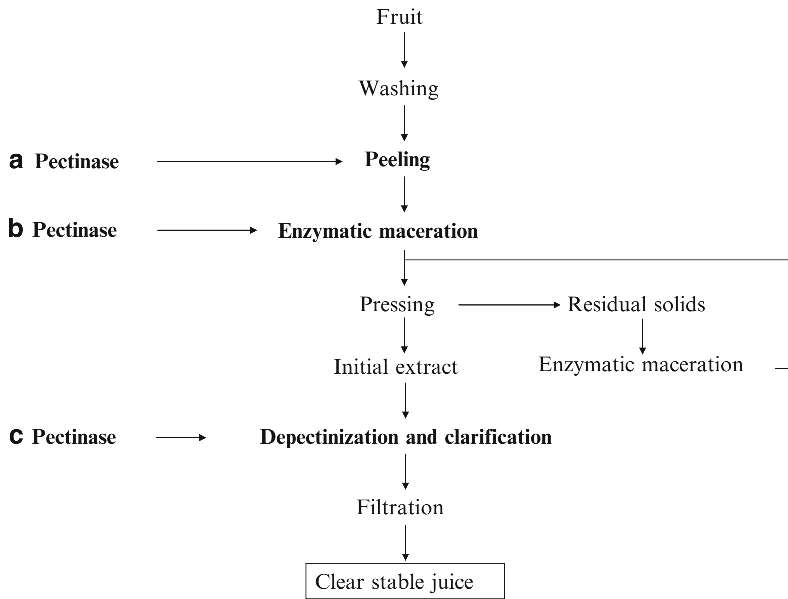

Fig. 7.3 Figure illustrating various steps in enzymatic processing of fruit juice

(Sandri et al. 2011; Maleki et al. 2011). Figure 7.3 depicts various steps of fruit juice processing with pectinases, which are as follows:

A. Peeling

Peeling is the first step in the processing of fruits and vegetables for juice extraction. Conventional methods of peeling (chemical, mechanical, steam and freeze peeling) cause high peeling losses and damage the flesh, affecting the quality of the fruit. Enzymatic peeling is thus suggested a more recent alternative (Pagan et al. 2006). The main advantages of enzymatic peeling are good quality of the final product, as well as reduced heat treatment and industrial waste.

It is an advantageous fact that the process does not require extensively harsh treatments (high temperatures), and as a result, the peeled fruits retain their structural integrity and fresh fruit properties. Pectin, cellulose and hemicellulose are the polysaccharides responsible for the adherence of the peel to the fruit. Therefore, treating the fruit with the corresponding enzymes provides the peeling of the fruit. Toker and Bayindirli (2003) used commercial enzymes (concentrated enzyme with pectinase, hemicellulase and cellulase activities) for enzymatic peeling of apricots, nectarines and peaches (stone fruits) and observed that enzymatic peeling was successful at moderately high temperatures concluding that the enzymatic peeling could thus be an alternative to mechanical or chemical peeling of stone fruits. Similarly, Pagan and co-workers (2006) optimised the conditions of temperature and concentration of the enzymatic preparation (polygalacturonases, hemicellulases and arabinases) for peeling of oranges and concluded that by treating the fruit with pectinase concentration of 5.0 ml/30 g peel for 28 min at 40.4°C produced the maximum weight loss, indicating a good peeling efficiency. In yet another report, Pretel and group (2007a, b) observed that peeling of different varieties of orange can be achieved with pectinases at 40°C.

B. Enzymatic Maceration

In the process of extracting juice, pectinases can be used to obtain a higher yield of sugar and soluble solids, resulting in a higher juice yield (Ribiero et al. 2010). In the production of white grape and red grape juice, pectinases have an important role in depectinisation to increase juice yield with the highest extraction of the

pigment that is naturally present in the grape. In general, more than 95% of the soluble solids from the fruit can be extracted by enzymatic treatment that would otherwise cause haze. By reducing fruit mash viscosity and improving solid/liquid separation, pectinases also increase colour extraction and juice volume (Rashmi et al. 2008). Moreover, the enzymatic maceration also results in subtle but generally beneficial changes in fruit juice flavour. Demir and co-workers (2001) observed that pectinase treatment of carrot puree decreased its viscosity from 90 to 6.5 poise, while the dry matter content and the total yield were found to be increased due to polysaccharide degradation. In the production of white grape and red grape juice, pectinases have an important role in depectinisation to increase juice yield. Significant improvements were obtained in the extraction, colour intensity and stability of wine treated with the recombinant yeast strains expressing pectate lyase and polygalacturonase genes by Louw and group (2006). Dzogbefia and Djokoto (2006) treated 200 g of papaya mash with different doses of the pectic enzyme extract and observed a rapid increase in flow rate of the free-run juice. Interestingly, Oliviera and co-authors (2006) observed that apple juice processing by enzymatic liquefaction yielded 83.5% sugars with 16.5% of pomace, while processing by pressing resulted in a yield of 64.5% sugars with 35.5% of pomace, thereby demonstrating that the enzymatic liquefaction process presents practical advantage over the process of extraction by pressing by minimising the production of pomace, the main factor that accounts for the high water retention in the conventional extraction systems, thereby providing a clean technology for apple juice processing. In another report, Lieu and Le (2010) observed that enzymatic treatment after sonication treatment of grape mash increased extraction yield by 7.3% and shortened the treatment time four times. Similarly, there are also several reports on the enhanced recovery and clarification of juices from dates, peach, plum, apricot and pineapple (Abbes et al. 2011; Joshi et al. 2011; Tran and Lee 2011).

C. Clarification

One of the major problems encountered in the preparation of fruit juices and wine is the cloudiness which is primarily due to the presence of pectins. Several authors have successfully used pectinolytic enzymes for clarification of fruit juices and wine. Singh and Gupta (2003) clarified apple juice using pectinolytic enzyme and gelatin. Lee and co-workers (2006) optimised conditions for enzymatic clarification of banana juice and achieved better clarity (decreased viscosity and turbidity) in the enzyme-treated juice. Sin and group (2006) used pectinase enzyme for clarification of sapodilla juice and recommended 0.1% enzyme concentration at 40°C for 120 min optimum for efficient clarification. In another report, Rai and co-workers (2007) studied the effect of various pretreatment methods on the clarification of mosambi juice and observed that maximum permeate flux during ultrafiltration was observed with enzymatic treatment followed by adsorption using bentonite. Similarly, Kareem and Adebowale (2007) clarified orange juice using crude fungal pectinase and obtained 51% reduction in the viscosity of the treated juice with a yield of 97% compared to the 73% yield in the untreated juice. Liew Abdullah and group (2007) established the optimum conditions for the enzymatic treatment of carambola fruit juice and reported that 0.1% enzyme concentration at 30°C for 20 min gave the highest clarity of the juice compared to the control. Cheirslip and Umsakul (2008) achieved fourfold higher clarity in the enzyme-treated banana wine compared with the control. Pinelo and co-authors (2010) concluded that pectin contributes to turbidity development during cold storage of cherry juice. Thus, the use of pectinases will help in increasing clarity of cherry juice. Vijayanand and group (2010) optimised clarification of litchi pulp with different doses of pectinase. The enzyme facilitated removal of insoluble solids and increased juice extraction. Nakkeeran and co-authors (2011) used polygalacturonase produced from *Aspergillus carbonarius* for the extraction and clarification of apple juice and achieved better yields and clarity (decreased viscosity) in enzymatically treated juice than the untreated juice. While, Yuan and co-workers

(2011) showed that the addition of endo-PG 1 from *Penicillium* sp. CGMCC 1669 and a commercial pectate lyase increased the efficiency of juice clarification reducing the intrinsic viscosity of apple juice by 33.1% (Yuan et al. 2011). In another report, Diaz and group (2011) used exo-polygalacturonase for clarification of grape must and achieved decrease in turbidity by 97.5%. A significant improvement in colour and clarity scores of plum, peach, pear and apricot juices was achieved using pectinase from *A. niger* (Joshi et al. 2011). Sandri and co-workers (2011) used fungal pectinases for clarification of apple, butia palm fruit, blueberry and grape juices and obtained greater clarification in enzyme-treated juice than in untreated juice.

Degumming of Plant Fibre

The removal of heavily coated, noncellulosic gummy material from the cellulosic part of plant fibres is called degumming and is necessary prior to the utilisation of fibres for textile making (Said et al. 1991). In a classical degumming process, this gum is removed by treatment of decorticated fibres with hot alkaline solution with or without application of pressure (Cao et al. 1992). In addition to the high consumption of energy, this process also results in serious environmental pollution. Biotechnological degumming using pectinases presents an ecofriendly and economic alternative to the conventional chemical process (Jayani et al. 2005). The use of pectinases in industrial processes is usually linked to a reduced consumption of energy as well as chemicals and thus beneficial for the environment (Demarche et al. 2011). Several authors have successfully used pectinolytic enzymes for degumming of plant fibres. Sharma and Satyanarayana (2006) applied pectinase produced from *B. pumilus* dcsr1 for treatment of ramie fibres and observed that the enzyme selectively degraded only the noncellulosic gummy material of the fibre, making the enzyme choice for degumming process. Jacob and co-workers (2008) treated handstripped sun-dried fibres with crude pectinase obtained from *Streptomyces lydicus* and observed that the fibre cells were intact in the control, while the cells were separated in the enzyme-treated sample when observed under scanning electron microscope. Sharma and group (2011) used pectinase from *Pseudozyma* sp. for degumming of flax fibres and achieved 4471 ± 19.5 μg g^{-1} dry fibre galacturonic acid with maximum weight loss of 11 ± 1.2% after 12 h of incubation.

A combined microbial and enzymatic process can also be used to reduce the consumption of energy and chemicals and achieve a better degumming (Deshpande and Gurucharanam 1985). Kapoor and co-workers (2001) observed that neither of the two treatments (chemical and enzymatic) alone is sufficient for an efficient degumming process. When chemically treated fibres were subsequently treated with polygalacturonase from *Bacillus* sp. MG-cp-2, a complete removal of gummy material was observed in ramie and sunn hemp fibre. The reason to this effect may be that when the fibre was treated with chemical, it would have caused greater porosity, softness, swelling and separation of the fibre, thus rendering it more accessible to enzymatic attack. Similarly, Kashyap and co-authors (2001) used combined (chemical and enzymatic) treatment to degum buel bast fibres and observed that the chemically treated buel fibres when subsequently treated with optimised (400 U/ml) doses of crude pectinase resulted in the release of maximum amount of galacturonic acid (575 μmol/g dry fibres) and a decrease in dry weight (43%) of the fibres. Basu and group (2009) also used combined degumming (enzymatic and chemical) process with *B. pumilus* strain DKS1 to degum ramie bast fibres on a large scale (400 kg) and achieved more than 20.81% increase of single fibre tenacity.

Retting of Plant Fibres

Retting is a fermentation process in which certain bacteria and fungi decompose the pectin of the bark and release fibre to be used for linen making in textile industry. Commercially, retting is done by one of the two basic forms (anaerobic retting

and dew retting). Anaerobic retting or water retting is achieved by submerging straw sheaves in water pits, in concrete tanks or in running freshwater. However, the process produces environmentally unacceptable fermentation waste (Sharma and Van Sumere 1992) and was therefore discontinued in western countries. While in dew retting (an aerobic process), plant straw is thinly spread on the ground and exposed to the action of the fungi and aerobic bacteria for 2–10 weeks. However, there are also several major disadvantages of dew retting such as dependence on geographical regions, coarser and lower-quality fibre, less consistency in fibre characteristics and occupation of agricultural fields for several weeks (Van Sumere 1992). Dew retting further results in a heavily contaminated fibre that is particularly disadvantageous in cotton textile mills. Enzyme retting was thus evaluated as a replacement for traditional microbial retting methods. Enzymatic retting is faster, readily controlled and produces fewer odours. Van Sumere and Sharma (1991) evaluated Flaxzyme, a commercial enzyme mixture from Novo Nordisk (Denmark) which consists of pectinases, hemicellulases and cellulases, at a concentration of 3 g/l for enzyme retting and produced fibre fineness, strength, colour and waxiness comparable to the best water-retted fibre. Akin and co-workers (2001) used Viscozyme L, a pectinase-rich commercial enzyme product, and ethylenediaminetetraacetic acid (EDTA) for treating Ariane fibre flax and North Dakota seed flax straw residue and produced the best test yarns. Evans and group (2002) compared enzyme retting of flax fibres with the water-retted fibre and achieved 62% increase in enzyme-retted fibre yield, while fibre strength did not differ between the two treatments. Akin and co-authors (2007) optimised enzyme retting of flax with pectate lyase and observed that enzymatic treatment at for 1 h at 55°C followed by treatment with EDTA for 24 h provided the best fibres. Saleem and co-workers (2008) observed that treating bast fibres with pectinases also improves mechanical characteristics of reinforced polypropylene in terms of decreased tensile strength and reduced cross-sectional area. Alix et al. (2012) compared mechanical properties of pectate lyase treated green flax fibres with dew-retted fibres and observed improvement in the properties of enzyme-treated fibres. Thus, apart from being environment-friendly, enzymatic method also improves the properties of the retted fibre.

Bioscouring of Cotton Fibres

Pectinases have been used in conjunction with amylases, lipases, cellulases and hemicellulases to remove sizing agents from cotton in a safe and ecofriendly manner, replacing toxic caustic soda used for the purpose earlier (Hoondal et al. 2002; Sawada and Ueda 2001). An additional asset of this process is that besides being energy conservative and more environmental friendly, pectinolytic enzymes do not affect the cellulose backbone, thus drastically limiting fibre damage. Klug-Santner and co-workers (2006) reported up to 80% of pectin removal from the outer layer of cotton by a purified endo-pectate lyase from *B. pumilus* BK2. Wang and co-authors (2007) optimised bioscouring condition of cotton-knitted fabrics with an alkaline pectinase from *B. subtilis* WSHB04-02 by using response surface methodology and achieved a desired pectin removal percentage companied with adequate wettability. In another report, Aggarwal and group (2008) employed cutinase and pectinase for cotton bioscouring and reported that the treatment is effective in the degradation of cotton waxes and pectin, allowing the design and introduction of a competitive innovative enzymatic scouring process. Hebeish and co-workers (2009) investigated conditions for effective bioscouring of cotton-based fabrics and observed that the bioscoured substrates exhibit fabrics performances comparable to the conventional alkali scouring. Recently, Abdel-Halim and co-authors (2010) carried out bioscouring of cotton fabrics with combinations of different pectinase preparations and different surfactants followed by emulsification posttreatment and achieved higher hydrophilicity along with smooth and clear fibre surface in the treated fibre when visualised under scanning electron microscopy.

Paper Making

Pectinases depolymerise polymers of galacturonic acids, subsequently reducing the cationic demand of pectin solutions and the filtrate resulting from the peroxide bleaching, thereby solving retention problems in pulp bleaching (Reid and Ricard 2000; Viikari et al. 2001). During paper making, it has also been found that bleached pulp contains a substantial amount of undesired pectins. By incorporating pectinase in the bleached or alkali-treated pulp, such harmful pectins in the aqueous phase of the pulp are degraded. Ahlawat and co-workers (2007) investigated the suitability of pectinase produced from *B. subtilis* SS in pulp bleaching for paper making and showed an increase in brightness (4.3%), whiteness (14.8%) and florescence (65.3%) and reduction in kappa number (15%), permanganate number (6.1%) and chemical oxygen demand in the treated pulp. In another study, the enzymatic prebleaching of kraft pulp with xylano-pectinolytic enzymes from the same bacterial isolate resulted in 25% reduction in active chlorine consumption in subsequent bleaching stages without any decrease in brightness along with improvement in pulp properties (Kaur et al. 2010).

Extraction of Vegetable Oils

Canola, coconut, sunflower seed, palm and olive oil are traditionally produced by extraction with organic solvents, most commonly hexane that is a potential carcinogen. Addition of cell wall degrading enzymes also improves the oil quality in terms of increased phenolic compounds with high antioxidant activities (Vierhuis et al. 2001). Cell wall degrading ability of pectinolytic enzymes also allows their use for vegetable oil extraction in aqueous process (Kashyap et al. 2001). The mild conditions during the process ensure the stability of the extracted components, resulting in better oil quality in terms of oxidative stability parameters. As the process takes place in water, degumming of the extracted oil is unnecessary since the phospholipids are retained in the residual solid phase (Latif et al. 2008). Moreau and co-workers (2004) achieved corn oil yields of about 35% using commercial pectinase as against 27% in hexane-extracted sample, while the chemical composition of oil obtained by both the treatments was similar. Latif and group (2008) concluded that an aqueous enzyme-assisted extraction process for canola oil has the potential to be an environment-friendly alternative to solvent extraction and has the added benefit that it can simultaneously recover high-quality protein for human consumption.

Functional Foods

Pectin and pectic polysaccharides are emerging as bioactive food ingredients. Grapefruit pectin used industrially as a stabiliser and as a supplement to baby food improves nutrition and physical development of children. These oligogalacturonides and their breakdown products by pectinolytic enzymes are classified as 'probiotics' because they are not hydrolysed in the upper gastrointestinal tract and can be used as health promoters in human and animal nutrition, stimulating selective growth of species of resident bacteria in the intestinal lap (Lang and Dornenburg 2000).

Coffee and Tea Fermentation

Fermentation of tea and coffee with pectinases accelerates the fermentation process. Enzymatic treatment removes mucilagenous coat from coffee beans and destroys the foam-forming property of instant tea powders by destroying pectins, thus improving the quality of the final product (Jayani et al. 2005; Pedrolli et al. 2009). According to Angayarkanni and co-workers (2002), adding pectinases in association with cellulases and hemicellulases to the tea-leaf fermenting bath raises the tea quality index by 5%. Masaud and Jespersen (2006) concluded that pectinolytic enzymes of yeast are involved in the degradation of pectin during coffee fermentation.

Improvement in the Extraction of Cassava Starch

Cassava pulp, the solid residue produced after extraction of starch, contains a significant proportion of starch granules (68%, dry basis) and fibre (27%, dry basis). The high fibre content probably reduces the extraction of remaining starch keeping the beads together and stuck in a fibrous network. This network can be disrupted by enzymatic methods, based on the application of a mixture of pectinases and cellulases that destroy the structural integrity of the matrix responsible for trapping of beads, exposing and releasing the starch (Sriroth et al. 2000).

Improvement in Antioxidant Property of Juices

Treatment with pectinases also increase the phenolic and antioxidant content of fruit juices whose potential benefits for human health have been recognised in recent years. The addition of commercial pectolytic enzymes is a common practice in winemaking to increase phenolic content of wines, especially anthocyanins. These enzymes may also improve the stability, taste and structure of red wines, because not only anthocyanins are released from the skins but also tannins bound to cell walls may be extracted because of enzymatic action. Landbo and Meyer (2004) reported that the yield, anthocyanin level, level of total phenolics as well as clarity of black currant juice were improved by using pectinolytic enzymes. Similarly, Kelebek and co-workers (2007) observed that the wines produced by pectinase treatment were higher in total phenolics, tannins and colour intensity and concluded that the increase in colour intensity may be due to an increase in polymeric anthocyanin content and/or due to co-pigmentation effects caused by the enhanced extraction of other phenolic fractions by pectinase treatment. In another report, Markowski and co-authors (2009) applied two commercial pectinolytic enzymes in apple juice processing and found an increase in the content of phenolic compounds. Armada and co-workers (2010) observed that the wines obtained from the enzymatic maceration showed highest content of varietal compounds and other desirable compounds such as ethyl esters or phenylethyl acetate.

Cloning of Pectinase Genes

In the light of economic benefits and biotechnological applications of pectinolytic enzymes, screening and designing of new pectinases with higher efficiency and specificity via genetic manipulations has become a major focus of researchers. Use of recombinant DNA technology facilitates the economic production of large quantities of pure pectinases and the engineering of tailor-made enzymes for specific applications. Many microbial pectinase genes have been cloned over the past years and still the research is focused in this area. A few of them have been listed in Table 7.4.

The advantages of use of recombinant DNA technology in pectinase production are listed as follows:

(a) *Increased Yield of Enzyme*: There are several reports where mutagenesis and protoplast fusion had shown significant improvement in the yield of pectinase production. Cao and co-workers (1992) identified two mutants of alkalophilic *Bacillus* sp. NTT33 resistant to catabolic repression, producing polygalacturonase activities up to 82.4% higher than wild type. Po16, a *P. occitanis* mutant presented improved cellulase and pectinase production as compared to wild type strain (Jain et al. 1990). A prototrophic hybrid was developed after protoplast fusion of auxotrophic mutants of *Aspergillus* sp. CH-Y-1043 and *A. flaviceps* ATCC 16795, which showed 15-fold increase in endo-PGase production than the wild strain of *Aspergillus* sp. Similarly, interspecific protoplast fusion of *A. niger* and *A. carbonaria* resulted in hybrids with higher production of both endo- and exo-pectinases (sixfold higher than the wild type strain) and improved growth on SSF with wheat bran as a sole source of nutrients (Kavitha and Kumar 2000). Antier and group (1993) isolated pectinase

Table 7.4 Cloning and expression of pectinase from various microorganisms

Parent strain	Gene cloned	Host for cloning	Vector for cloning	Vector for expression	Reference
A. oryzae KBN616	*Pg*	*Escherichia coli* MV1184	pUC18	–	Kitamoto et al. (1993)
A. aculeatus	*Rhg A*	*E. coli* BB4	pBluescript (SK+)	λEMBL4	Suykerbuyk et al. (1995)
A. flavus	*Pec A, Pec B*	*E. coli* LE392	EMBL3	pCFC80	Whitehead et al. (1995)
P. janthinellum	*Pga*	–	pSTA14	pSRI-019	Ishida et al. (1997)
Pseudomonas syringae pv. Lachrymus 859	*Pel s*	*P. syringae* BUVS 1	pCPP34 pCPP$&	–	Bauer and Collmer (1997)
A. niger	*PgaE*	–	pUC18, pPROM-H	pGW635	Parenicova et al. (1998)
S. cerevisiae IM1-8b	*Pgu1*	*E. coli*	pBluescript (SK+)	pBEJ16, pYES2	Blanco et al. (1998)
Botrytis cinerea	*Endo-PG*	*E. coli* DH5α	pBluescript (SK/KS)	λEMBL3	Wubben et al. (1999)
A. tubingensis	*XghA*	–	–	pCVlacK	Vlugt-Bergmans et al. (2000)
S. cerevisiae	*Pgl*	–	–	–	Ganivors et al. (2000)
A. niger	*Pg*	*E. coli* DH5α	–	pGW635	Pagès et al. (2000)
Bacillus sp. *Strain KSM-p15*	*Pectate lyase*	*E. coli* HB101	pHSG398	–	Ogawa et al. (2000)
Bacillus sp.	*PehK*	–	pHV300PLK	pHYPEHK	Sawada et al. (2001)
Fusarium circinatum	*Fcpg*	–	PGEMT	–	Chimwamurombe et al. (2001)
Bacterium strain ANT/505	*Pectate lyase*	*E. coli* BL21	pUC 18	pRSET – A	Truong et al. (2001)
Geotrichum candidum Ap2	*Ap2pg1*	*E. coli* DH5α	pBluescript (SK+)	–	Nakamura et al. (2002)
Treponema pectinovorum	*PelA*	*E. coli* DH5α	pBluescript II (SK+)	pQE30	Walker and Ryan (2003)
Bacillus alcalophilus NTT 33	*Pectate lyase*	*E. coli*	pBluescript ks(t) II		Zhai et al. (2003)
B. subtilis 168	*YuvA*	*E. coli* 5 K	pJF1184E	pET 28a	Soriano et al. (2006)
P. occitanis	*PnaI*	*E. coli* TOP1OF	pUC II	–	Lahiani et al. (2008)
A nidulans	*Pectate lyase*	*E. coli*	pMD18-T	pet 28(a)	Zhao et al. (2007)
B. subtilis	*Pectate lyase*	*E. coli*	pPIC9K	pPIC9K	Zhuge et al. 2008
P.griscoroseum	*PG*	*E. coli*	–	pAN52pgg2	Teixeira et al. (2011)

hyperproducing UV mutants of *A. niger* by looking for a deoxyglucose resistance phenotype. Sieiro and co-workers (2003) reported that the recombinant endopolygalacturonase produced by heterologous expression of the *Saccharomyces* PGU1 gene in *Schizosaccharomyces pombe* had higher Km value, thermostability and pH stability than native enzyme and was more efficient in reducing the viscosity of polygalacturonic acid.

(b) *Production of Pure Enzymes*: Large varieties of industrial applications of the pectinases require action of a single pectic enzyme of high purity or that of several enzymes in the appropriate proportions (Ceci and Lozano 1998). Accordingly, it would be of considerable use to produce the different pectic enzyme individually, and in large quantities, so that they can be mixed as required. Pure pectin lyases are suitable for reducing viscosity of cloud stable fruit juices rather than conventional enzymes which contain interfering enzyme activities such as pectinesterase, which leads to production of methanol. Using the pure enzyme avoids this problem. In certain instances the use of enzymes becomes feasible only when they are pure like in production of low esters pectins using pure pectinesterase.

Single component retting enzyme, endopolygalacturonase such as one from *Rhizopus oryzae*, has been used effectively by Zhang and co-workers (2005) for retting flex fibres.

(c) *Evolutionary Links*: On the basis of cloning of polygalacturonase genes and other proteomic tools, Schiott and co-workers (2010) suggested that the pectinolytic enzymes from fungal symbionts of *Acromyrmex echinatior* leaf-cutting ants represent secondarily evolved adaptations that are convergent to those normally found in phytopathogens.

Conclusion

There are a number of industrial processes to which pectinases can be applied to improve the quality and yield of final products. Thus, it is important to investigate the production conditions and physicochemical characteristics of new pectinolytic enzymes for specific industrial process. Screening a large number of microorganisms for high active enzymes combined with protein engineering, direct evolution and metagenome approaches can lead to more efficient and stable enzymes. Moreover, research on the development of novel bioreactors to carry out efficient hydrolysis of pectic substrates by pectinases has not received much attention. In order to develop novel bioreactors, an understanding of the stability characteristics of pectinases is required. This poses a challenging task for protein and biochemical engineers. In the near future, biochemical techniques and innovative experiments in cellular and molecular biology could offer real breakthrough in pectinase research.

References

Abbes F, Bouaziz MA, Blecker C, Masmoudi M, Attia H, Besbes S (2011) Date syrup: effect of hydrolytic enzymes (pectinase/cellulase) on physicochemical characteristics, sensory and functional properties. LWT- Food Sci Technol 44:1827–1834

Abdel-Halim ES, Konczewicz W, Zimniewska M, Al-Deyab SS, El-Newehy MH (2010) Enhancing hydrophilicity of bioscoured flax fabric by emulsification post-treatment. Carbohydr Polym 82:195–201

Abdullah Liew AG, Sulaiman NM, Aroua MK, Megat MJ, Megat Mohd Noor MJ (2007) Response surface optimization of conditions for clarification of carambola fruit juice using a commercial enzyme. J Food Eng 81:65–71

Aggarwal PB, Nierstrasz VA, Bouwhuis GH, Warmoeskerken MMCG (2008) Cutinase and pectinase in cotton bioscouring: an innovative and fast bioscouring process. Biocatal Biotransform 26:412–421

Ahlawat S, Battan B, Dhiman SS, Sharma J, Mandhan RP (2007) Production of thermostable pectinase and Xylanase for their potential application in bleaching of kraft pulp. J Ind Microbiol Biotechnol 34:763–770

Akin DE, Foulk JA, Dodd RB, McAlister DD (2001) Enzyme-retting of flax and characterization of processed fibers. J Biotechnol 89:193–203

Akin DE, Condon B, Sohn M, Foulk JA, Dodd RB, Rigsby L (2007) Optimization for enzyme-retting of flax with pectate lyase. Ind Crop Prod 25:136–146

Alana A, Alkorta I, Domínguez JB, Llama MJ, Serra JL (1990) Pectin lyase activity in a *Penicillium italicum* strain. Appl Environ Microbiol 56:3755–3759

Alix S, Lebrun L, Marais S, Philippe E, Bourmaud A, Baley C, Morvan C (2012) Pectinase treatment on technical fibres of flax: effects on water sorption and mechanical properties. Carbohydr Polym 87:177–185

Alkorta I, Garbisu C, Liama MJ, Serra JL (1998) Industrial applications of pectic enzymes: a review. Process Biochem 33:21–28

Almeida C, Brányik T, Moradas-Ferreira P, Teixeira J (2005) Use of two different carriers in a packed bed reactor for endopolygalacturonase production by a yeast strain. Process Biochem 40:1937–1942

Angayarkanni J, Palaniswamy M, Murugesan S, Swaminathan K (2002) Improvement of tea leaves fermentation with *Aspergillus* spp. Pectinase J Biosci Bioeng 94:299–303

Antier P, Minjares A, Roussos S, Raimbault M, Gustavo V-G (1993) Pectinase hyper-producing mutants of Aspergillus niger C28B25 for solid-state fermentation of coffee pulp. Enzyme Microb Technol 15:254–260

Armada L, Fernnadez E, Falque E (2010) Influence of several enzymatic treatments in aromatic composition of white wines. LWT- Food Sci Technol 43:1517–1525

Basu S, Saha MN, Chattopadhyay D, Chakarbati K (2009) Large scale degumming of ramie fibre using a newly isolated Bacillus pumilus DKS1 with high pectate lyase activity. J Ind Microbiol Biotechnol 36:239–245

Bauer DW, Collmer A (1997) Molecular cloning, characterization, and mutagenesis of a *pel* Gene from *Pseudomonas syringae* pv. *lachrymans* encoding a Member of the *Erwinia chrysanthemi* PelADE family of pectate lyases. Mol Plant Microbe Interact 10:369–379

Blanco P, Sieiro C, Reboredo NM, Villa TG (1998) Cloning, molecular characterization, and expression of an endo- polygalacturonase-encoding gene from *Saccharomyces cerevisiae* IM1-8b. FEMS Microbiol Lett 164:249–255

Brinton CS, Dore WH, Wichmann HJ, Willaman JJ, Wilson CP (1927) Definitions written by the committee on nomenclature of pectin of the agriculture food division. J Am Chem Soc 49:38–40

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 Microb Technol 14:1013–1016

Ceci L, Lozano J (1998) Determination of enzymatic activities of commercial pectinases for the clarification of apple juice. Food Chem 61:237–241

Cheirslip B, Unsakul K (2008) Processing of banana based wine product using pectinase and α-amylase. J Food Process Eng 31:78–90

Chen WC, Hsieh HJ, Tseng TC (1998) Purification and characterization of a pectin lyase from Pythium splendens infected cucumber fruits. Bot Bull Acad Sin 39:181–186

Chimwamurombe PM, Brenda D, Wingfield AMB, Wingfield MJ (2001) Cloning and sequence analysis of the endopolygalacturonase gene from the pitch canker fungus, *Fusarium circinatum*. Curr Microbiol 42:350–352

Cosgrove DJ (1997) Assembly and enlargement of the primary cell wall in plants. Annu Rev Cell Biol 13:171–201

Demarche P, Junghanns C, Nair RR, Agathos SN (2011) Harnessing the power of enzymes for environmental stewardship. Biotechnol Adv 30(5):933–953. http://dx.or/10.1016/j.biotechadv.2011.05.013

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. J Food Eng 47:275–280

Deshpande KS, Gurucharanam K (1985) Degumming of ramie fibres: role of cell wall degrading enzymes of *Aspergillus versicolor*. Ind J Bot 8:79–81

Devi NA, Rao AGA (1996) Fractionation, purification and preliminary characterization of polygalacturonases produced by *Aspergillus carbonarius*. Enzyme Microb Technol 18:59–65

Diaz AB, Bolivar J, de ory I, Caro I, Blandino A (2011) Applicability of enzymatic extracts obtained by solid-state fermentation on grape pomace and orange peels mixtures in must clarification. LWT- Food Sci Technol 44:840–846

Dinnella C, Lanzarini G, Stagni A (1994) Immobilization of an endopectin lyase on g-alumina: study of factors influencing the biocatalytic matrix stability. J Chem Technol Biotechnol 59:237–241

Dixit VS, Kumar AR, Pant A, Khan MI (2004) Low molecular mass pectate lyase from *Fusarium moniliforme*: similar modes of chemical and thermal denaturation. Biochem Biophys Res Commun 315:477–484

Dzogbefia VP, Djokoto DK (2006) Combined effects of enzyme dosage and retention time on papaya juice extraction with the aid of pectic enzymes-a preliminary report. J Food Biochem 30:117–122

Esquivel JC, Voget CE (2004) Purification and partial characterization of an acidic polygalacturonase from *Aspergillus kawachii*. J Biotechnol 110:21–28

Evans JD, Akin DE, Foulk JA (2002) Flax-retting by polygalacturonase-containing enzyme mixtures and effects on fiber properties. J Biotechnol 97:223–231

Gadre RV, Driessche GV, Beeumen JV, Bhat MK (2003) Purification, characterization and mode of action of an endo-polygalacturonase from the psychrophilic fungus *Mucor flavus*. Enzyme Microb Technol 32:321–330

Ganivors A, Nedjaoum N, Gognies S, Muzart M, Nedjma M, Belarbi A (2000) Purification and characterization of acidic endo polygalacturonase encoded by the PGL-1 gene from *Saccharomyces cerevisiae*. FEMS Microbiol Lett 183:131–135

Gupta S, Kapoor M, Sharma KK, Nair LM, Kuhad RC (2008) Production and recovery of an alkaline exo-polygalacturonase from Bacillus subtilis RCK under solid-state fermentation using statistical approach. Bioresour Technol 99:937–945

Hasunuma T, Fukusaki EI, Kobayashi A (2003) Methanol production is enhanced by expression of an *Aspergillus niger* pectin methylesterase in tobacco cells. J Biotechnol 106:45–52

Hebeish A, Hashem M, Shaker N, Ramadan M, El-Sadek B, Hady MA (2009) New development for combined bioscouring and bleaching of cotton based fabrics. Carbohydr Polym 78:961–972

Hoondal GS, Tiwari RP, Tiwari R, Dahiya N, Beg QK (2002) Microbial alkaline pectinase and their applications: a review. Appl Microbiol Biotechnol 59: 409–418

Ishida Y, Kakibuchi K, Hirao Y, Izumori K (1997) Cloning and characterization of a polygalacturonase-encoding gene from *Penicillium janthinellum*. J Ferment Bioeng 84:257–260

Jacob N, Poorna AC, Prema P (2008) Purification and partial characterization of polygalacturonase from *Streptomyces lydicus*. Bioresour Technol 99:6697–6701

Jain S, Duron H, Tiraby G (1990) Production of extracellular pectinase enzymes by a mutant (PO16) of Penicillium occitanis. Appl Microbiol Biotechnol 34:308–312

Jayani RS, Saxena S, Gupta R (2005) Microbial pectinolytic enzymes a review. Process Biochem 40:2931–2949

Joshi V, Parmar M, Rana N (2011) Purification, characterization of pectinase produced from apple pomace and its evaluation in the fruit juice extraction and clarification. Indian J Nat Prod Resour 2(2):189–197

Kapoor M, Kuhad RC (2002) Improved polygalacturonase production from Bacillus sp. MG-cp-2 under submerged (SmF) and solid state (SSF) fermentation. Lett Appl Microbiol 34:317–322

Kapoor M, Beg QK, Bhushan B, Singh K, Dadhich KS, Hoondal GS (2001) Application of an alkaline and thermostable polygalacturonase from Bacillus sp, MG-cp-2 in degumming of ramie (*Boehmeria nivea*) and sun hemp (Crotalaria juncea) bast fibres. Process Biochem 36:803–807

Kareem SO, Adebowale AA (2007) Clarification of orange juice by crude fungal pectinase from citrus peel. Niger Food J 25(1)

Kashyap DR, Chandra S, Kaul A, Tewari R (2000) Production, purification and characterization of pectinase from a *Bacillus* sp. DT7. World J Microbiol Biotechnol 16:277–282

Kashyap DR, Vohra PK, Chopra S, Tiwari R (2001) Applications of pectinases in the commercial sector: a review. Bioresour Technol 77:215–227

Kaur G, Satyanarayana T (2004) Production of extracellular pectinolytic, cellulolytic and xylanolytic enzymes by thermophilic mould Sporotrichum thermophile *Apinis* in solid-state fermentation. Indian J Biotechnol 3:552–557

Kaur A, Mahajan R, Singh A, Garg G, Sharma J (2010) Application of cellulase-free xylano-pectinolytic enzymes from the same bacterial isolate in biobleaching of kraft pulp. Bioresour Technol 101:9150–9155

Kavitha R, Kumar-Umesh S (2000) Genetic improvement of *Aspergillus carbonarius* for pectinase overproduction during solid-state growth. Biotechnol Bioeng 67(1):121–125

Kelebek H, Canbas A, Carbaroglu A, Selli S (2007) Improvement of anthocyanin content in the cv. Okuzgozu wines by using pectinolytic enzymes. Food Chem 105(1):334–339

Kitamoto N, Kimura T, Kito Y, Ohmiya K, Tsukagoshi N (1993) Structural features of polygalacturonase gene cloned from *Aspergillus oryzae* KBN616. FEMS Microbiol Lett 111:37–41

Klug-Santner BG, Schnitzhofer W, Vrsanská M (2006) Purification and characterization of a new bioscouring pectate lyase from *Bacillus pumilus* BK2. J Biotechnol 121:390–401

Kobayashi T, Hatada Y, Higaki N (1999) Enzymatic properties and deduced amino acid sequence of a high-alkaline pectate lyase from an alkaliphilic *Bacillus* isolate. Biochim Biophys Acta 1427:145–154

Kuhad RC, Kapoor M, Rustagi R (2004) Enhanced production of alkaline pectinase from *Streptomyces* sp. RCK-SC by whole cell immobilization and solid-state cultivation. World J Microbiol Biotechnol 20:257–263

Lahiani HT, Ayadi M, Hadj-Taieb N, Ali MB, Gargouri A (2008) Genomic organisation of a polygalacturonase gene from a hyperpectinolytic mutant strain of *Penicillium occitanis*. FEMS Microbiol Lett 281:23–29

Landbo A-K, Meyer AS (2004) Effects of different enzymatic maceration treatments on enhancement of anthocyanins and other phenolics in black currant juice. Innov Food Sci Emerg Technol 5:503–513

Lang C, Dornenburg H (2000) Perspectives in the biological function and the technological application of polygalacturonases. Appl Microbiol Biotechnol 53:366–375

Latif S, Diosady LL, Anwar F (2008) Enzyme-assisted aqueous extraction of oil and protein from canola (Brassica napus L.) seeds. Eur J Lipid Sci Technol 110:887–892

Lee WC, Yusof S, Hamid NSA, Bahrain BS (2006) Optimizing conditions for enzymatic clarification of banana juice using response surface methodology (RSM). J Food Eng 73:55–63

Lieu LN, Le VVM (2010) Application of ultrasound in grape mash treatment in juice processing. Ultrason Sonochem 17:273–279

Limberg G, Korner R, Buchholt HC, Christensen TM, Roepstorff P, Mikkelsen JD (2000) Analysis of different de-esterification mechanisms for pectin by enzymatic fingerprinting using endopectin lyase and endopolygalacturonase II from *A. niger*. Carbohydr Res 327:293–307

Louw C, LaGrange D, Pretorius IS, VonRosenburg P (2006) The effect of polysaccharide-degrading wine yeast transformants on the efficiency of wine processing and wine flavour. J Biotechnol 125:447–461

Maciel MHC, Herculano PN, Porto TS, Teixeira MFS, Moreira KA, Souza-Molta CM (2011) Production and characterization of pectinases from forage palm by *Aspergillus niger* URM4645. Afr J Biotechnol 10(13):2469–2475

Maleki MH, Tajick Ghanbary MA, Ranjbar G, Asgharzadeh A, Lotfi A (2011) Screening of some zygomycetes strain for pectinase activity. J Microbiol Biotechnol Res 1:1–7

Malvessi E, Silveira MM (2004) Influence of medium composition and pH on the production of polygalacturonases by *Aspergillus oryzae*. Braz Arch Biol Technol 47:693–702

Markowski J, Mieszczakowska M, Pocharski W (2009) Effect of apple cultivar and enzyme treatment on phenolic compounds content during clear apple juice production. Int J Food Sci Technol 44:1002–1010

Masoud W, Jespersen L (2006) Pectin degrading enzymes in yeasts involved in fermentation of coffea arabica in East Africa. Int J Food Microbiol 110:291–296

Miyazaki Y (1991) Purification and characterization of an endo-pectate lyase from *Bacillus macerans*. Agric Biol Chem 55:23–30

Moreau RA, Johnston DB, Powell MJ, Hicks KB (2004) A comparison of commercial enzymes for the aqueous enzymatic extraction of corn oil from corn germ. JAOCS 81(11):1071–1075

Morozova VV, Semenova MV, Salanovich TN (2006) Application of neutral-alkaline pectate lyase to cotton fabric boil off. Appl Biochem Microbiol 42:603–608

Murad HA, Azzaz HH (2011) Microbial pectinases and ruminant nutrition. Res J Microbiol 6:246–269

Nadaroglu H, Taskin E, Adiguzel A, Medine G, Demir N (2010) Production of a novel pectin lyase from *Bacillus pumilus* (P9), purification and characterization and fruit juice application. Rom Biotechnol Lett 15:5167–5176

Nakamura M, Hisashi IWA, Kei ARAP (2002) Cloning and characterization of a polygalacturonase gene *Ap2pgl* from *Geotrichum candidum* citrus race Ap2 pathogenic to apple fruit. J Gen Plant Pathol 68:333–337

Nakkeerran E, Kumar-Umesh S, Subramaniam R (2011) *Aspergillus carbonarius* polygalacturonases purified by integrated membrane process and affinity precipitation for apple juice production. Bioresour Technol 102:3293–3297

Ogawa A, Sawada K, Saito K, Hakamada Y, Sumitomo N, Hatada Y, Kobayashi T, Ito S (2000) A new high-alkaline and high-molecular-weight pectate lyase from a *Bacillus* isolate: enzymatic properties and cloning of the gene for the enzyme. Biosci Biotechnol Biochem 64:1133–1141

Oliveira KF, Malavolta L, Souza CS, Vionte EJ, Laluca C (2006) Pectinolytic activity secreted by yeasts isolated from fermented citrus molasses. J Appl Microbiol 100:633–640

Pagan A, Conde J, Ibarz A, Pagan J (2006) Orange peel degradation and enzyme recovery in the enzymatic peeling process. Int J Food Sci Technol 41:113–120

Pagès S, Heijne WHM, Kester HCM, Visser J, Benen JAE (2000) Subsite mapping of *Aspergillus niger* endopolygalacturonase II by site-directed mutagenesis. J Biol Chem 275:29348–29353

Parenicová L, Benen JAE, Kester HCM, Visser J (1998) *pga*E encodes a fourth member of the endopolygalacturonase gene family from *Aspergillus niger*. Eur J Biochem 251:72–80

Pedrolli DB, Gomes E, Monti R, Carmona EC (2008) Studies on productivity and characterisation of polygalacturonase from Aspergillus giganteus submerged culture using citrus pectin and orange waste. Appl Biochem Biotechnol 144(2):191–200

Pedrolli DB, Monteiro AC, Gomes E, Carmona CE (2009) Pectin and pectinases: production, characterization and industrial application of microbial pectinolytic enzymes. Open Biotechnol J 3:9–18

Pinelo M, Zeuner B, Meyer AS (2010) Juice clarification by protease and pectinase treatments indicate new roles of pectin and protein in cherry juice turbidity. Food Bioprod Process 88:259–265

Prathyusha K, Suneetha V (2011) Bacterial pectinases and their potent biotechnological application in fruit processing/juice production industry: a review. J Phytol 3:16–19

Pretel MT, Botella MA, Amoros A, Zapata PJ, Serreno M (2007a) Optimization of vacuum infusion and incubation time for enzymatic peeling of Thomson and Mollar oranges. LWT- Food Sci Technol 40:12–22

Pretel MT, Botella MA, Amoro A, Zapata PJ, Serrano M (2007b) Optimization of vacuum infusion and incubation time for enzymatic peeling of Thompson and Mollar oranges. LWT 40:12–20

Rai P, Majumdar GC, DasGupta S, De S (2007) Effect of various pre-treatment methods on permeate flux and quality during ultrafiltration of mosambi juice. J Food Eng 78:561–568

Rasheedha Banu A, Kalpana Devi M, Gnanaprabhal GR, Pradeep BV, Palaniswamy M (2010) Production and characterization of pectinase enzyme from *Penicillium chrysogenum*. Indian J Sci Technol 3:377–381

Rashmi R, Siddalinga K, Sneha G, Shabana S, Syama A, Radhik V (2008) Partial purification and biochemical characterization of extracellular pectinase from *Aspergillus niger* isolated from groundnut seeds. J Appl Biosci 9:378–384

Reid I, Ricard M (2000) Pectinase in papermaking: solving retention problems in mechanical pulps bleached with hydrogen peroxide. Enzyme Microb Technol 26:115–123

Rexova-Benkova L, Markovie O (1976) Pectic enzymes. Adv Carbohydr Chem 33:323–385

Ribiero DS, Henrique SMB, Oliviera LS, Macedo GA, Fleuri LF (2010) Enzymes in juice processing: a review. Int J Food Sci Technol 45:635–641

Said S, Fonseca MJV, Siessere V (1991) Pectinase production by Penicillium frequentans. World J Microbiol Biotechnol 7:607–608

Saito K, Takakuwa N, Oda Y (2004) Purification of the extracellular pectinolytic enzyme from the fungus *Rhizopus oryzae* NBRC 4707. Microbiol Res 159:83–86

Sakai T, Sakamoto T, Hallaert E, Vandamme EJ (1993) Pectin, pectinase and protopectinase: production, properties and applications. Adv Appl Microbiol 39:213–294

Saleem Z, Rennebaun H, Pudel F, Grimm E (2008) Treating bast fibres with pectinase improves mechanical characteristics of reinforced thermoplastic composites. Compos Sci Technol 68:471–476

Sandri IG, Fontana RC, Barfknecht DM, Silveira MM (2011) Clarification of fruit juices by fungal pectinases. LWT- Food Sci Technol 44:2217–2222

Sawada K, Ueda M (2001) Enzyme processing of textiles in reverse micellar solution. J Biotechnol 89:263–269

Sawada K, Suzumatsu A, Kobayashi T, Ito S (2001) Molecular cloning and sequencing of the gene encoding an exopolygalacturonase of a *Bacillus* isolate and properties of its recombinant enzyme. Biochimica et Biophysica Acta 1568:162–170
Schiott M, Rogowska-Wrzesinska A, Roepstroff P, Boomsma JJ (2010) Leaf-cutting ant fungi produce cell wall degrading pectinase complexes reminiscent of phytopathogenic fungi. BMC Biol 8:156
Semenova MV, Grishutin SG, Gusakov AV, Okunev ON, Sinitsyu AP (2003) Isolation and properties of pectinases from the fungus *Aspergillus japonicus*. Biochemistry 68:559–569
Sharma DC, Satyanarayana T (2006) A marked enhancement in the production of a highly alkaline and thermostable pectinase by *Bacillus pumilus* dcrl in submerged fermentation by using statistical methods. Bioresour Technol 97:727–733. J Food Eng 73:313–319
Sharma HSS, Van Sumere CF (1992) Enzyme treatment of flax. Genet Eng Biotechnol 12:19–23
Sharma S, Mandhan RP, Sharma J (2011) *Pseudozyma* sp. SPJ: an economic and eco-friendly approach for degumming of flax fibres. World J Microbiol Biotechnol 27:2697–2701
Sieiro C, Poza M, Vilanora M, Villa TG (2003) Heterologous expression of the *Saccharomyces cerevisiae* PGU1 gene in Schizosaccharomyces pombe yields an enzyme with more desirable properties for the food industry. Appl Environ Microbiol 69:1861–1865
Sin HN, Yusof S, Sheikh Abdul Hamid N, Rahman RA (2006) Optimization of enzymatic clarification of sapodilla juice using response surface methodology. J Food Eng 73:313–319
Singh S, Gupta R (2003) Apple juice clarification using fungal pectinolytic enzyme and gelatin. Indian J Biotechnol 3:573–576
Singh SA, Plattner H, Diekmann H (1999) Exo polygalacturonate lyase from a thermophilic *Bacillus* sp. Enzyme Microb Technol 25:420–425
Soriano M, Diaz P, Pastor FIJ (2006) Pectinolytic systems of two aerobic sporogenous bacterial strains with high activity on pectin. Curr Microbiol 50:114–118
Sriroth K, Chollakup R, Chotineeranat S, Piyachomkwan K, Oates CG (2000) Processing of Cassava waste for improved biomass utilization. Bioresour Technol 71:63–69
Suykerbuyk ME, Schaap PJ, Stam H, Musters W, Visser J (1995) Cloning, sequence and expression of the gene coding for rhamnogalacturonase of *Aspergillus aculeatus*; a novel pectinolytic enzyme. Appl Microbiol Biotechnol 43:861–870
Teixeira JA, Goncalves DB, de Queiroz MV, de Araujo EF (2011) Improved pectinase production in *Penicillium griseoroseum* recombinant strains. J Appl Microbiol. doi:10.1111/j.1365-2672.2011.05099
Toker I, Bayindirli A (2003) Enzymatic peeling of apricots, nectarines and peaches. LWT 36:215–221
Torres EF, Sepulveda TV, Gonzalez GV (2006) Production of hydrolytic depolymerising pectinases. Food Technol Biotechnol 44:221–227
Tran PPT, Le VVM (2011) Effects of ultrasound on catalytic efficiency of pectinase preparation during the treatment of pineapple mash in juice processing. Int Food Res J 18:347–354
Truong LV, Tuye H, Helmke E, Binh LT, 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 5:35–44
Unejo M, Pastore GM (2007) Pectinases: industrial applications and perspectives. Quim Nova 30:388–394
Van Sumere CF (1992) Retting of flax with special reference to enzyme retting. In: Sharma HSS, Van Sumere CF (eds) The biology and processing of flax. M. Publications, Belfast, pp 157–198
Van Sumere CF, Sharma HSS (1991) Analyses of fine flax fibre produced by enzymatic retting. Asp Appl Biol 28:15–20
Vierhuis E, Servili M, Baldioli M, Schols HA, Voragen AGJ, Montedoro GF (2001) Effect of enzyme treatment during mechanical extraction of olive oil on phenolic compounds and polysaccharides. J Agric Food Chem 49:1218–1223
Viikari L, Tenkanen M, Suurnakki A (2001) Biotechnology in the pulp and industry. In: Rehm HJ (ed) Biotechnology, vol 2. Wiley VCH, Chichester, pp 523–546
Vijyanand P, Kulkarni SG, Prathibha GV (2010) Effect of pectinase treatment and concentration of litchi juice on quality characteristics of litchi juice. J Food Sci Technol 47:235–239
Vlugt-Bergmans C, Vander JB, Meeuwsen PJA, Voragen AGJ (2000) Endo-xylogalacturonan hydrolase, a novel pectinolytic enzyme. Appl Environ Microbiol 33:36–41
Walker SG, Ryan ME (2003) Cloning and expression of a pectate lyase from the oral spirochete *Treponema pectinovorum* ATCC 33768. FEMS Microbiol Lett 226:385–390
Wang Q, Fan X-R, Hua Z-Z, Chen J (2007) Optimizing bioscouring condition of cotton knitted fabrics with an alkaline pectinase from *Bacillus subtilis* WSHB04-02 by using response surface methodology. Biochem Eng J 34:107–113
Whitaker JR (1990) Microbial pectinolytic enzymes. Microb Enzyme Biotechnol 2:133–176
Whitehead MP, Shieh MT, Cleveland TE, Cary JW, Dean RA (1995) Isolation and characterization of polygalacturonase genes (*pecA* and *pecB*) from *Aspergillus flavus*. Appl Environ Microbiol 61:3316–3322
Wubben JP, Mulder W, Ten Have A, Van Kan JA, Visser J (1999) Cloning and partial characterization of endopolygalacturonase genes from *Botrytis cinerea*. Appl Environ Microbiol 65:1596–1602
Yadav S, Yadav PK, Yadav D, Deo K, Yadav S (2008) Purification and characterization of an alkaline pectin lyase from *Aspergillus flavus*. Process Biochem 43:547–552
Yuan P, Meng K, Huang H, Shi P, Luo H, Yang P, Yao B (2011) A novel acidic and low-temperature-active

endo-polygalacturonase from Penicillium sp. CGMCC 1669 with potential for application in apple juice clarification. Food Chem 129:1369–1375

Zhai C, Cao J, Wang Y (2003) Cloning and expression of a pectate lyase gene from *Bacillus alcalophilus* NTT33. Enzyme Microb Technol 33(2):173–178

Zhang J, Henriksson H, Szabo IJ, Henriksson G, Johansson G (2005) The active component in the flax-retting system of the zygomycete Rhizopus oryzae sp. is a family 28 polygalacturonase. J Ind Microbiol Biotechnol 32:431–438

Zhao Q, Yuan S, Zhang Y, Zhu H, Dai C, Yang F, Fengmin H (2007) Expression, purification and characterization of pectate lyase A from *Aspergillus nidulans* in *Escherichia coli*. World J Microbiol Biotechnol 23(8):1057–1064

Zhuge B, Du GC, Shen W, Zhuge J, Chen J (2008) Expression of a *Bacillus subtilis* pectate lyase gene in *Pichia pastoris*. Biochem Eng J 40:92–98