

## Pectin Methylesterases: A Review

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### Abstract

Pectin methylesterase (PME) is the first enzyme acting on pectin, a major component of plant cell wall. PME catalyzes reactions according to the double-displacement mechanism. In plants, PMEs can be classified on the basis of presence or absence of the PRO domain in pectin methylesterase into Type 1 and Type II. Type 1 contains one to three PRO domains and two or three introns, type II PMEs are without PRO domain and with five or six introns. Once PMEs are secreted into the cell wall, mature PMEs exhibit three different modes of action: single chain mechanism, multiple chain mechanism and multiple attack mechanism. For bacterial and plant PMEs, single chain and multiple chain mechanism is used and for fungal PMEs only multiple chain mechanism has been proposed. PME activity is regulated by differential expression both spatially and temporally.

**Keywords:** Pectin methylesterase; Demethylestrification; Catalysis; Transacylation; Fruit firming

### Introduction

Pectinolytic enzymes or pectinases are a heterogeneous group of enzymes that hydrolyze the pectic substances present in plants. They include polygalacturonases, pectin lyase, and PME that hydrolyze the glycosidic bonds of pectic substances. Pectin methylesterase (E.C. 3.1.1.11), a methyl ester group hydrolytic enzyme is produced by plants, pathogenic fungi, and bacteria also constantly being used in the wine, juice, and other food industries [1]. In *Arabidopsis* PMEs, it was revealed that PMEs have unique patterns of methylesterification that provide immunity to *Pseudomonas syringae* pv *maculicola* ES4326 (Pma ES4326) [2]. Pectin methylesterase catalyzes reactions according to the double-displacement mechanisms, de-esterification through transferring the C<sub>6</sub> carboxyl groups in the pectin-PME complexes to water molecules altering the degree and pattern of methyl esterification and transacylation through transferring the C<sub>6</sub> carboxyl groups to the hydroxyl groups of another pectin molecules and resulting in the formation of high molecular weight pectins with new non-methoxy ester linkages [3-8]. PME has been purified and characterized from several fruit sources including tomato [9], orange, papaya [10], apple [11], kiwi [12], grapefruit pulp and mandarin orange fruit [13]. PMEs having diverse and well-defined activities could be useful for making “designer pectins” for fulfilling new demands from food industry. It has been reported that PME activity is partly regulated by PME I (Pectin methylesterase inhibitor) [14,15] For example, in flax (*Linum usitatissimum*) Lu PMEs gene family, most highly conserved residues were catalytic residues, while in Lu PME I gene family, cysteine's forming disulphide bridges between helices  $\alpha 2$  and  $\alpha 3$  were found to be conserved residues [16]. The modification of pectin by PMEs is known to modify the quality of plant-based food products. It has been recently confirmed that PME has a definite role in plant defence system by virus induced gene silencing of PME gene in *Nicotiana benthamiana* [17].

### Types of Pectin Methylesterases

Several pectin methylesterase isoforms differing in molecular weight, pI and biochemical activity were detected in all higher plants examined so far as well as in a number of plant pathogenic fungi and bacteria [18]. In a study, a basic PME which is an isoform of B3a found to be encoded by LuPME3 from *Linum usitatissimum* and play great role in flax root development [19]. Pectin methylesterase belongs to class 8 (CE-8) of the carbohydrate esterase [20]. Plant pectin methylesterase

belong to large multigene families. For instance, in *Arabidopsis thaliana*, 66 open reading frames (ORF) have been annotated as putative full-length PMEs. There are 89 PME ORFs in *Populus trichocarpa* [21] whereas this number is substantially lower in *Oryza sativa* with 35 ORFs, probably due to the low level of pectin in their cell wall. Higher plant PMEs are frequently organised in pre-pro-proteins. The PRE domain leading to the export of PMEs to the cell wall is formed by a common type signal peptide (SP) and by a transmembrane domain (TM or signal anchor). Different PMEs possess one, both, or neither of these motifs. Those with neither motif are classified as putatively soluble isoforms. The mature, active part of the protein (PME domain) is preceded by an N-terminal extension (PRO region) that shares similarities with the pectin methylesterase inhibitors. The PRO-regions can vary in length and shows a relatively low level of amino acid identity between isoforms [22]. In *Arabidopsis*, the isoelectric point of the 26 PME domains tends to be basic whereas that of the PRO region is neutral or acidic [23,24]. Similar differences in length and isoelectric point between PRO and PME domains have also been found in several other species [25], and might influence the optimal pH activities of specific isoforms.

Pectin methylesterases can be classified on the basis of presence or absence of the PRO domain into Type I and Type II. In PMEs Type I (500-900 amino acids; 52-105 kDa) there are 1-3 PRO domains and two or three introns (Figure 1) whereas PMEs Type II (250 to 400 amino acids; 27-45 kDa) contain no PRO domain with five or six introns [26,27]. The Type II sequences have a structure close to that of the PMEs identified in phytopathogenic organisms (bacteria, fungi).

### Mode of Action of Mature Pectin Methylesterase

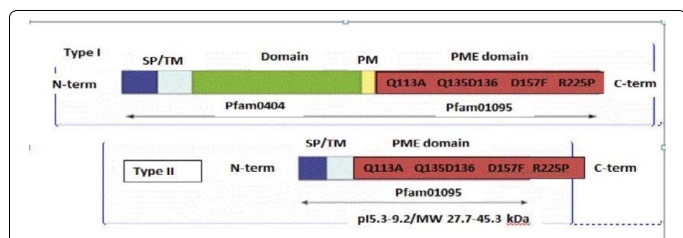
After their secretion into the cell wall, mature PMEs could exhibit three different modes of action (I) a single-chain mechanism where the enzyme converts all substrate sites on the polymeric chain, (II)

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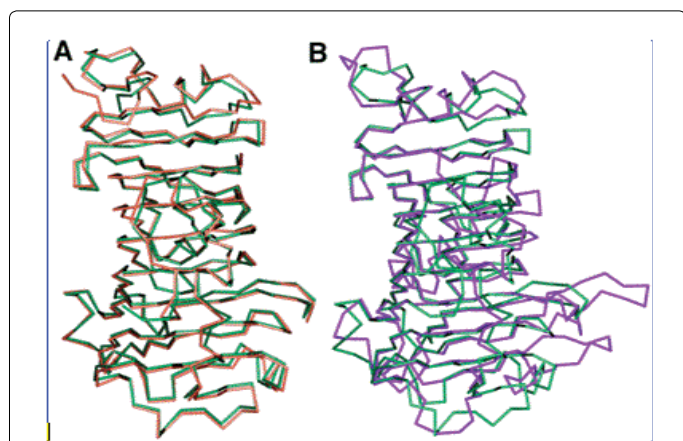
Received February 17, 2015; Accepted May 15, 2015; Published May 20, 2015

Citation: Kohli P, Kalia M, Gupta R (2015) Pectin Methylesterases: A Review. J Bioprocess Biotech 5: 227 doi:[10.4172/2155-9821.1000227](https://doi.org/10.4172/2155-9821.1000227)

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**Figure 1:** Pectin methylsterase(PME) structural motifs. Type I and Type II PMEs possess a conserved PME domain with characteristic, highly conserved amino acid fragments. Type I PMEs possess an N-terminal extension designated the PRO domain and a processing motif (PM) that might be a putative target for subtilisin like proteases. The targeting to the endomembrane system leading to the export of PMEs to the cell wall is mediated either by a signal peptide (SP) or a transmembrane domain (TM or signal anchor). Different PMEs possess one, both, or neither of these motifs.



**Figure 2:** Comparison of the known structures of PMEs. (A) Overlay of the Ca trace of PME from tomato (green) and PME from carrot (orange). Structures are almost completely superimposable. (B) Superimposition of PME from tomato (green) and PME from *E. chrysanthemi* (violet). Although the  $\beta$ -helices are completely superimposable, main differences (showed by arrow) are located in the length of the turns protruding out from the  $\beta$ -helix in proximity of the putative active site cleft.

a multiple-chain mechanism where the enzyme catalyses only one reaction and then dissociates from the substrate and, (III) a multiple-attack mechanism where the enzyme catalyses a number of reaction cycles before the enzyme-polysaccharide complex dissociates. Plant and bacterial PMEs produce products with contiguous regions of galacturonic acid and both a single chain and multiple-attack mechanism have been proposed [28-30]. In contrast, fungal PMEs attack more randomly and a multiple-chain mechanism has been proposed for these enzymes [31-34]. When PMEs act randomly on pectic polymer, the demethylesterification releases protons that promote the action of endopolygalacturonases [33], and contribute to cell wall loosening. When PMEs act linearly on methylated pectin, PMEs give rise to blocks of free carboxyl groups that could interact with  $Ca^{2+}$ , creating the so called junction zones. Because the action of endopolygalacturonases in such a gel is limited, this action pattern of PMEs contributes to cell wall stiffening.

### Regulation of Pectin Methylsterase Activity

Pectin methylsterases in the apoplasm catalyze the demethylesterification of homogalactouronic acids (HGAs), exposing carboxyl residues which can be cross-linked by calcium. These changes affect the rheological properties, porosity, and ionic status of cell

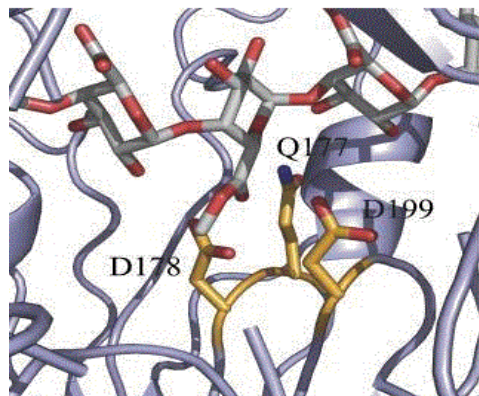
wall. It follows that the tight control of PME activity, both spatially and temporally, occupies a central position in the control of cell wall growth and development. PMEs convert the methoxyl groups on the polygalacturonic acid chain into negatively charged carboxyl groups by releasing both protons and methanol. It is generally believed that most plant PMEs remove methyl esters in a block-wise fashion, creating long contiguous stretches of deesterified pectins [34]. PME activity is regulated by differential expression, multiple PME isoforms have been shown to be expressed in certain tissues at the same time. From the action pattern of the few PME isoforms studied so far, it appears that the intrinsic activity of different isoforms is same but certain substrate specificities and reaction mechanisms require different environmental conditions [34-36]. The distribution of carboxyl units along the pectin backbone and to a lesser extent the methylation degree, are important in controlling PME activity. Free carboxyl groups in the vicinity of the ester linkage increase the affinity of the enzyme for its substrate [37]. Immobilization of PMEs at these anionic sites has either increased or decreased their deesterification rate, possibly due to conformational changes induced by the microenvironment [35]. Different isoforms have different pH optima, and the action pattern of certain isoforms has been shown to be pH dependent [35,36]. Cations are also necessary for PME activity, where they affect the binding of the enzyme to its substrate [38].

### Structure of Pectin Methylsterase

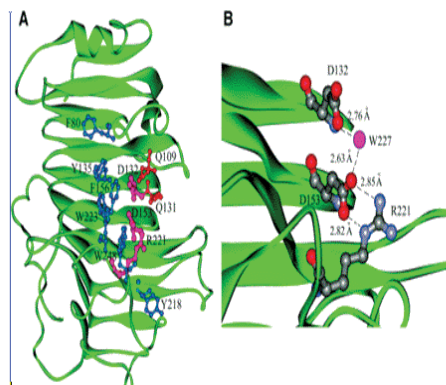
Three dimensional crystallographic structures of PMEs facilitated to elucidate the mechanism of action and substrate specificity of PMEs. Up to now 3 three dimensional structures have been solved: a bacterial PME, from *Erwinia chrysanthemi* [39,40] and two plant PMEs, are from *Daucus carota* and *Solanun lycopersicon* (Figure 2A) [15]. The enzyme folds into a right handed parallel  $\beta$ -helix, first observed in pectate lyase C [41] and typical of pectic enzymes [39].

The three dimensional structures of the carrot and tomato PMEs showed striking similarities, and their structures are superimposable. In addition, both of these plant PMEs showed similar folding topology to PME from *Erwinia chrysanthemi*, although one major difference lies in the length of turns protruding from the  $\beta$ -helix in proximity of the substrate cleft (Figure 2B), in particular, turns that protrude out of the  $\beta$ -helix are much longer in the bacterial enzyme, making its putative active site cleft deeper and narrower than that of plant PMEs.

In tomato PME-1 the  $\beta$ -helix consists of seven complete coils, which have different lengths because the number of amino acids located in the loops connecting the  $\beta$ -strands is variable. Each coil consists of three  $\beta$ -strands that line up to form three extended parallel  $\beta$ -sheets (PB) called PB1, PB2, and PB3. The N-terminal region of PME is composed of a short  $\alpha$ -helix followed by a  $\beta$ -strand that lines up with PB1. The C-terminal region has an extended conformation in which a long tail and four short and distorted  $\alpha$ -helices protrude out of the parallel  $\beta$ -helix flanking PB1. The active site of PME is located on the PB3. Many aromatic residues (Phenylalanine (Phe) 80, Tyrosine (Tyr) 135, Phe 156, Tyr 218, Trp 223, and Trp 248) putatively involved in substrate binding are located in active site pocket. These residues are well conserved in plant PMEs Tyr 135, also the Phe 156 and Trp 223 residues are conserved in PME of *E. chrysanthemi*. The structures of the Michaelis complexes of PME from *Erwinia chrysanthemi* with methylated hexagalacturonates, suggest the following residues are involved in the reaction mechanism: Asp 199, Asp 178 and Gln 177 (Figure 3). Arg 267 is conserved amongst all PMEs, is not a direct participant in catalysis [40].



**Figure 3:** Stereo-view of the active site residues in the Michaelis complex.



**Figure 4:** Close-Up View of the Tomato PME Active Site. (A) Structure of tomato PME in which residues involved in catalysis (violet), in stabilization of the catalytic intermediate (orange), and in substrate binding (blue) are shown in ball and stick representation. (B) Further close-up view representation of amino acid residues and a water molecule (blue ball) putatively involved in catalysis; H-bond pattern is highlighted.

In analogy with the proposed mechanism of action of PME from carrot and from *Erwinia Chrysantemi* [42], a mechanism of catalysis of PME-1 from tomato, has been described in which Asp 153, polarized by the proximity with Arg 221, performs a nucleophilic attack on the carboxymethyl group of the substrate. The tetrahedral anionic intermediate formed is stabilized by the interaction with two conserved Glutamine (Gln) residues (Gln109 and Gln131). Afterwards, asparagine (Asp) 132 likely acts as a proton donor in the cleavage step where methanol is released. The resulting carboxylate group of Asp 132 then behaves as a base and receives a proton from an incoming water molecule (W227), thus restoring the active site of the enzyme (Figure 4).

### Location of Disulfide Bonds in Pectin Methylsterase from Tomato and Comparison with Pectin Methylsterase from Other Sources

Till date, four different types of pectinesterases have been determined in primary structure, the pectinesterase from tomato [43,44] *Aspergillus niger* [45], *Erwinia chrysanthemi* [46] and *Pseudomonas solanacearum* [47,48]. These four enzymes are distantly related, exhibiting a low degree of sequence similarity, with residue identities at the 18-33% level (disregarding an elongated N-terminal overshooting end of the *Pseudomonas* enzyme). Attempts at chemical modifications have shown

the presence of reactive tyrosine residues [44], and overall comparisons suggest relationships between pectinesterases, and polygalacturonases, which are also pectin degrading enzymes [46]. Analysis of tomato pectinesterase by carboxymethylation, with and without reduction, showed that the enzyme has two intrachain disulfide bridges. Analysis of fragments obtained from the native enzyme after digestion with pepsin identified bridges connecting cysteine (Cys) Cys-98 with Cys-125, and Cys-166 with Cys-200. The locations of disulfide bridges in tomato pectinesterase are not identical to those in three distantly related pectinesterases (18-33% residue identities) from microorganisms. However, one half-Cys (i.e.Cys-166) position is conserved in all four enzymes. Sequence comparisons of the overall structures suggest a special importance for three short segments of the entire protein. One segment is at the N-terminal part of the tomato pectinesterase, another in the C-terminal portion near the distal end of the second disulfide loop, and the third segment is located in the central part between the two disulfide bridges. The latter segment, encompassing only 40 residues of the entire protein, appears to highlight a functional site in a middle chain segment.

### Functions of Pectin Methylsterases

#### Ubiquitous enzymes involved in many physiological processes

Several studies have shown a strong correlation between PME activity or PME gene expression and physiological processes such as fruit maturation microsporogenesis and pollen tube growth [47,48], cambial cell differentiation [48,49] seed germination and hypocotyl elongation [35]. Interestingly, two recent studies have shown unequivocally that a PME is a host-cell receptor for the Tobacco Mosaic Virus (TMV) movement protein [50,51]. Thus, the interaction between the virus movement protein and the PME is required for viral cell-to-cell movement through plasmodesmata. One hypothesis proposed is that binding of the TMV movement protein interferes with PME activity, altering the cell wall ion balance and consequently inducing changes in the permeability of the plasmodesmata [51].

#### Pectin methylsterases as a methanol source

In 1998, a close correlation was reported between PME activity and levels of methanol in fruit tissues from both wild-type tomato and a PME antisense mutant, indicating that PME is on the primary biosynthetic pathway for methanol production in tomato fruit [52]. Because methanol oxidation to CO<sub>2</sub> could result in the incorporation of methanol carbon into metabolites via the Calvin-Benson cycle, PMEs could play an appreciable, albeit indirect, role in the photosynthetic metabolism of the plant.

#### Pectin methylsterases in cellular adhesion

During separation of the border cells of the root cap of pea, PME activity increases and is correlated with an increase in the amount of acidic pectin and a decrease in cell wall pH [53]. This study was performed using an antisense transgenic plant transformed with a PME gene (*rcpme1*) obtained by screening a root cDNA library [54]. Analysis of transgenic plants showed that *rcpme1* expression is required for the maintenance of extracellular pH, elongation of the cells within the root tip and for cell wall degradation leading to border cell separation.

#### Pectin methylsterases in stem elongation

Transgenic potato plants were transformed with a *Petunia-inflata* derived cDNA encoding a PME in sense orientation under a constitutive promoter. This showed that the apex of the stem contained less PME



activity than the wild type. Furthermore, during the early stages of development, stems of transgenic plants elongated more rapidly than those of the wild type [55].

### Pectin methylesterase genes influence solid wood properties

Wood is primarily composed of lignin, cellulose, and noncellulosic polysaccharides (including neutral and pectic polysaccharides), all of which interact to form a complex structure that provides the foundation for the massive stature unique to the woody life form known as “trees”. Quantitative genetic studies have revealed that wood properties are under strong additive genetic control in representative species across the *Eucalyptus* genus [56-58]. Associations were identified between two PME genes and a range of solid wood properties in *Erwinia pilularis*. Associations in PME7 were primarily with cellulose, pulp yield, and lignin. In contrast, the associations with PME6 were primarily with shrinkage and collapse. The associations between PME6 and PME7 with multiple wood traits are consistent with the current understanding of wood formation and the biochemical activity of PME [59-62].

### Transacylation properties of pectin methyl esterase

PME from *Aspergillus niger* is found to catalyze the transacylation reaction and forms polymerized pectin as observed by an increase in the particle size of PME-catalyzed pectin solution the transacylation mechanism of plant and microbial is different, and there are at least two kind of PME-catalyzed transacylation [63].

### Methyl Esterification of Pectin Plays a Role during Plant-Pathogen Interactions and Affects Plant Resistance to Diseases

Esterification status of pectin plays a critical role in plant-pathogen interactions. De-esterification of pectin affects the susceptibility of the cell wall barrier to fungal and bacterial CWDEs and influences the structure and functionality of the translocation and spreading of viruses. In addition, de-esterification of pectin influences the functionality of the feeding structures evolved by biotrophs and nematodes to sustain their parasitic growth within the plant tissue. PME activity is also required for the production of defense molecules such as OG elicitors or methanol.

### Industrial Applications of Pectin Methylesterases

Pectinases have been utilized in the commercial sector for wine and fruit juice industry since 1930. Commercial pectinase preparations, account for about 25% of the global food enzymes, are produced generally from fungal sources (*Aspergillus niger*) and used in many applications in food industry without purification. According to the specific objectives of the processes, the enzyme PME is used alone or in combination with other enzymes, mostly with other pectinases. The processes that PME involved are introduced below.

### Clarification of Juices

In fruit juices, pectic substances are the main substances responsible for the cloudiness. Thus, in the production of clear fruit juices the pectin in juices should be degraded by the application of pectinases. The degradation of pectin reduces also the viscosity of fruit juices and eases their filtration and concentration. To degrade pectin, PME is used in combination with other pectinases such as PG and PL. Following the degradation of pectin, the viscosity of fruit juice drops and suspended cloud particles lost their stability and precipitated. In the clarification of fruit juices, the PME enzyme may also be used alone. However, such

a treatment needs the addition of  $\text{CaCl}_2$  to fruit juice. This removes pectin from fruit juice as insoluble calcium pectates and causes the clarification [64-66]. Another application of pectinases in fruit juice industry involves liquefaction of fruit mashes. In such an application, the pectinases are combined with cellulases and hemicellulases for the complete disruption of the cell walls. This method can alternatively be used for the production of fruit juices from tropical fruits (e.g. banana) that cannot be processed with classical methods [67,68]. In all these enzymatic treatments, degradation of pectin facilitates pressing and increase juice yield. Also, the aromatic quality of fruit juices increases and amount of waste material reduces. Also, the presence of enzyme in the final product and alteration of organoleptic properties are inevitable [69]. The use of immobilized commercial pectinase named 'Pectinex Ultra SP-L' was studied for mash treatment of carrot puree [68]. This treatment maintains almost 93% of the enzyme after the fifth treatment. However, in practice the application of pectinases are still conducted by the classical methods. However, their use increases the volume of free-run juice (by breakdown of polysaccharides and solubilization of middle lamella), extraction yield of polyphenols, and color and aromatic compounds [70,71]. Pectin methylesterase from *Datura stramonium* in combination with polygalacturonase increased clarity of orange, apple, pomegranate and pineapple juices by 2.9, 2.6, 2.3, and 3.6 fold respectively [72].

### Firming of Fruits and Vegetables before Processing

Texture, an important quality attribute of fresh and processed fruits and vegetables, is closely related with the structural integrity of the primary cell walls and middle lamella, which are mainly composed of pectic substances. In industry, most of the processes such as blanching, freezing, dehydration, pasteurization and sterilization applied to preserve fruits and vegetables cause irreversible physical damages on cellular tissues. However, the negative effects of processing on texture can be overcome by applying different processes, which involve the use of PME enzyme. For example, the application of PME and  $\text{CaCl}_2$  by vacuum infusion is now used for firming fruits and vegetables [73]. The mechanism of firming in this method is based on the demethylation of naturally occurring pectin in plant tissues by the action of PME and the chelation of the added or natural calcium with the free carboxyl groups generated in pectin molecules. Such a chelation causes the formation of networks among pectin molecules (the egg box model), stabilizes pectin and increases the firmness of plant tissues. Some of the successful applications of enzymatic firming by use of PME involves, firming of strawberries before jam making and freezing [74,75] and firming of apples, strawberries, and raspberries before pasteurization [73]. Besides commercial PMEs, the in situ PME in the product may also be used for firming of fruits and vegetables. In this method, the in situ PME should be activated by low temperature blanching. The free carboxyl groups produced then were cross-linked with divalent ions in the medium or  $\text{CaCl}_2$  added to form a stable network. Firming by this method is suitable to apply for whole or sliced potatoes to be processed to French fries [76].

### Modification of Pectin

Pectin produced from apple and orange peels has widespread application in food industry as gelling, thickening, and stabilizing agents. The degree of esterification of pectin molecule greatly influences its functional properties. The Degree of Esterification (DE) of pectin may range between 0 and 100% and on the basis of DE, pectins are divided into two groups; high methoxylated (HM) pectins with a DE higher than 50% and low methoxylated (LM) pectins with a DE lower

than 50%. Pectin can be modified by PME to obtain the required DE value [77,78]. Pectins with low DE are particularly useful to obtain gels without using sugar and acid.

## Production of Low Sugar Jams and Jellies

Traditionally, HM pectin is used for the preparation of jams and jellies. The gelling mechanism of HM pectin is based on hydrophobic interactions and dehydration at low pH (<4.0). In such gels, the presence of high concentrations of sugar (>60 %) is essential for gelling. In contrast, LM pectin forms gels by ionic interactions in which calcium or other divalent cations interact with free carboxylic acid of two adjacent chains, and give rise to cross-linking of these chains. In such a gelling mechanism, the sugar concentration is not very important. Thus, LM pectins are suitable for the production of low sugar (diabetic) jams and jellies [67].

## Other Applications

In a recent study on Arabidopsis PMEs, revealed that PMEs affect patterns of methylesterification in a way that impart pattern-triggered immunity to *Pseudomonas syringae* pv *maculicola* ES4326 (Pma ES4326) [79]. Oils from coconut germ, sunflower seed, palm kernel, rape seed are industrially extracted with organic solvents. The most commonly used solvent is hexane, which is a potential carcinogen. Thus, alternatively, cell wall degrading enzymes, PME and other pectinases are used in combination to extract oil in different crops by liquefying the structural components of their cell walls. Pectinase preparations (such as Olivex) are also used in olive oil industry to increase the oil extraction output and to improve certain olive oil quality indicators [71,80]. Another application of combinational use of PME, other pectinases and cellulases is the peeling of fruits. Peeling of fruits has traditionally been applied by hand or treatment with steam, boiling water, acid or alkali. But these methods sometimes cause poor product quality (losses of fruit juice or disintegrations at the fruit surface) in delicate fruits. Also, in chemical methods, the disposal of the used peeling solution is a great problem. Therefore, application of pectinases and cellulases by vacuum infusion can be used as an alternative method for peeling of delicate fruits. For example, a commercial preparate (Rohament PC) containing pectinases and cellulases could be applied to remove peels and skins of oranges and to obtain whole fruit segments [81].

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Citation: Kohli P, Kalia M, Gupta R (2015) Pectin Methylsterases: A Review. J Bioprocess Biotech 5: 227 doi:10.4172/2155-9821.1000227

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