Pectin Methylesterase of *Datura* species, purification, and characterization from *Datura stramonium* and its application

Sameer Dixit¹, Santosh Kumar Upadhyay², Harpal Singh¹, Bindu Pandey¹, Krishnappa Chandrashekar¹, and Praveen Chandra Verma¹,*

¹CSIR-National Botanical Research Institute; Council of Scientific and Industrial Research; Rana Pratap Marg; Lucknow, UP India; ²Department of Biotechnology; National Agri-Food Biotechnology Institute; Ministry of Science and Technology; Mohali, Punjab, India

**Keywords:** clarification, *Datura*, enzyme kinetics, pectin methylesterase, industrial application

Pectin methylesterases (PME; EC 3.1.1.11) involved in de-esterification of pectin and have applicability in food, textiles, wines, pulp, and paper industries. In the present study, we compared PME activity of different parts of 3 *Datura* species and found that fruit coat showed maximum PME activity followed by leaf and seed. PME from leaves of *D. stramonium* (DsPME) was purified and characterized. DsPME showed optimum activity at 60 °C and pH 9 in the presence of 0.3 M NaCl. DsPME was stable at 70 °C and retained more than 40% activity after 60 min of incubation. However, enzyme activity completely abolished at 80 after 5 min of incubation. It follows Michaelis-Menten enzyme kinetics. Km and Vmax with citrus pectin were 0.008 mg/ml and 16.96 µmol/min, respectively. DsPME in combination with polygalactouronase (PGA) increased the clarity of orange, apple, pomegranate and pineapple juices by 2.9, 2.6, 2.3, and 3.6-fold, respectively in comparison to PGA alone. Due to very high de-esterification activity, easy denaturation and significant efficacy in incrementing clarification of fruit juice makes DsPME useful for industrial application.

**Introduction**

Pectinases are heterogeneous group of enzyme complex involved in pectin hydrolysis. It is composed of pectinesterases, polygalacturonases, and pectin lyases. Pectinesterases de-esterify pectins and release methanol and acidic pectin, which are further completely degraded by polygalacturonases (PGA). Pectin lyases depolymerise pectin by β-elimination mechanism without involving other enzymes.¹ Pectin methylesterases (PME) are involved in de-esterification of methyl-esterified pectin, which cannot be recognized by PGAs and pectate lyases.² Therefore, PMEs play an important role in degradation of such pectins.

PMEs are ubiquitous, reported from plants, fungi, and bacteria.³ They play important role in cell wall expansion, softening of plant tissues during maturation and storage, and decomposition of plant waste materials.³ They are involved in fruit ripening,⁴ senescence and abscission,⁵ cell wall maturation and regeneration,⁶ cell growth,⁷ salinity stress,⁸ the defense-related mechanism,⁹ and others.¹⁰ Methanol produced during PME activity plays a vital role in plants, such as protection of photosynthetic machinery from photo inhibition, stimulating the growth of C3 plants,¹¹ signaling between plants and defense against herbivores.¹²,¹³

Among pectinases, PMEs have attracted a lot of attention in recent years due to its critical role in physiology of plant and its application in food processing industry. They play a major role in fruit juice and vegetable oil extraction, fruit juice clarification, fermentation of tea and coffee, bleaching of paper, degumming of plant fibers, treatment of waste water and others.¹¹,¹² Several sources such as tomato,¹³ papaya,¹⁴ peach,¹⁵ tomato,¹⁶ apple,¹⁷ and orange,¹⁸ have been explored for isolation of efficient PME and its utilization in several industrial applications. Bharti et al., (2011) analyzed solanaceous plants viz., *Capsicum annuum* L. (Chilli), *Solanum lycopersicum* L. (tomato), and *Datura stramonium* for methanol content and found a higher level of methanol emission in *Datura* compared with others.¹⁹ This might be due to either high expression level of PME or highly active PME.

The present study aims to compare PME activity from different parts of 3 *Datura* species, which would be purified from the selected plant tissues (i.e., leaves of *D. stramonium*). Purified PME to be characterized for salt, temperature, pH optima, heat stability, denaturation, and industrial application in fruit juice clarification.

**Results**

**Extraction of total soluble protein**

Total soluble protein (TSP) was isolated from leaves, seeds, and fruit coat of all 3 species (*Datura metel* [Dm], *Datura inoxia*

Correspondence to: Praveen Chandra Verma; Email: praveencverma@nbri.res.in
Submitted: 07/03/2013; Revised: 07/08/2013; Accepted: 07/09/2013
Citation: Dixit S, Upadhyay S, Singh H, Pandey B, Chandrashekar K, Verma P. Pectin Methylesterase of *Datura* species, purification, and characterization from *Datura stramonium* and its application. Plant Signal Behav 2013; 8:e25681; http://dx.doi.org/10.4161/psb.25681
We could isolate adequate amount of protein from leaves and seeds but not from fruit coat (Table 1).

**Comparison of PME activity**

Specific activity of PME was calculated in leaves, seed, and fruit coat of 3 species of *Datura*. Fruit coat showed maximum activity followed by leaves and seed in each plant. Specific activities 17.2, 26.3, and 21.3 units/mg was observed in fruit coat of *Datura metel* (Dm), *Datura inoxia* (Di), and *Datura stramonium* (Ds), respectively. However, seeds showed least activity in all the 3 species. PME isolated from leaves of Dm, Di, and Ds showed specific activity 9.7, 8.6, and 15.0 units/mg, respectively. On the other hand fruit coat of Di and the seeds of Ds showed maximum and minimum activity respectively (Fig. 1). Concentration of TSP isolated from Dm leaves was higher in comparison to others, but the specific activity of PME in Ds leaves was 1.5 fold higher than Dm leaves. Ds leaves were available in sufficient amount, therefore it was selected for the purification of PME.

**Purification of PME**

TSP was first precipitated with ammonium sulfate, then fractionated by anion exchange chromatography, which significantly enriched the PME activity in some eluted fractions (D9-D15) (Fig. 2A). These fractions were analyzed on SDS-PAGE and showing similar band pattern (Fig. 2B). Fraction D15 showed maximum PME activity, which was enriched approximated 14-fold (Fig. 2A; Table 2). It was further purified by size exclusion chromatography and eluted fractions were analyzed for PME activity. Fraction showing highest PME activity was enriched up to 25 fold (Table 2). SDS-PAGE analysis showed >95% homogeneity of this fraction (Fig. 2C). PME activity was also confirmed by in-gel assay (Fig. 2C). Both SDS-PAGE and in-gel band corresponded to ~33 kDa.

**Temperature optima**

Purified DsPME was used for the analysis of temperature optima for activity. The activity of PME was increases on increasing temperature. The maximum activity of DsPME was observed at 60 °C after that activity decreased sharply up to almost zero at 90 °C (Fig. 3A).

### Table 1. Total soluble protein isolated from leaves, seeds and fruit coats of *Datura metel*, *Datura inoxia* and *Datura stramonium* calculated by Bradford method

| Plants       | Tissue part | Total soluble Protein (mg/ml) |
|--------------|-------------|-------------------------------|
| *D. stramonium* | Fruit Coat  | 0.7348 ± 0.03                 |
|              | Seed        | 2.9175 ± 0.57                 |
|              | Leaf        | 1.3190 ± 0.60                 |
| *D. inoxia*  | Fruit Coat  | 0.6570 ± 0.06                 |
|              | Seed        | 2.7893 ± 0.48                 |
|              | Leaf        | 2.0905 ± 0.71                 |
| *D. metel*   | Fruit Coat  | 0.7930 ± 0.05                 |
|              | Seed        | 3.0119 ± 0.21                 |
|              | Leaf        | 3.0175 ± 0.63                 |

![Figure 1. PME specific activity in leaves, seeds, and fruit coats of *Datura metel*, *Datura inoxia*, and *Datura stramonium*. Figure shows highest activity in fruit coats followed by leaves and then in seeds of all 3 species.](image-url)

![Figure 2A. Purification of PME by anion exchange chromatography.](image-url)

![Figure 2B. SDS-PAGE analysis of purified PME.](image-url)

![Figure 2C. In-gel assay of purified PME.](image-url)
The activity of DsPME was present at all tested pH (3–11), but high activity was observed between pH 7–10. We could not see statistically significant difference in the activity from pH 7–10, but the maximum activity was observed at pH 9 (Fig. 3B).

**Heat stability and Denaturation**

DsPME was stable at 60 °C without compromising on its activity. It retained more than 90% activity at 60 °C for 60 min. At 70 °C, enzyme lost 46 and 61% activity in 30 and 60 min, respectively. Activity was completely abolished at 80 °C after 5 min of incubation (Fig. 4).

**Effect of monovalent ions**

Significant effect of Na\(^+\) and K\(^+\) ion was observed on DsPME activity. The optimum activity was achieved at 0.3 M concentration of NaCl, which later on decreases sharply. In case of KCl, almost equal activity was present from 0.15 M to 0.3 M. It showed that a low concentration of K\(^+\) ion could also support...
PME activity. However, total enzyme activity was higher in NaCl (5.3 U) than KCl (3.5 U) at optimum ion concentrations. It showed that PME works efficiently in the presence of Na\(^+\) (Fig. 3C and D).

Calculation of Km and Vmax

Purified DsPME was used for enzyme kinetics study. DsPME followed the Michaelis-Menten enzyme kinetics. Activity increased with increase in substrate concentration and reached to saturation. Km and Vmax of enzyme were 0.008 mg/ml and 16.96 µmol/min (Fig. 5).

Clarification of fruit juices by DsPME

DsPME in combination with PGA showed significant increase in clearing all 4 tested juices (orange, apple, pineapple, and pomegranate). Combined activity of DsPME and PGA on pineapple juice showed maximum clarification (3.6 fold) as compared with the PGA alone. However, combined activity of DsPME and PGA on orange, apple, and pomegranate juices was 2.9, 2.6, and 2.3 fold, respectively in comparison to PGA alone (Fig. 6). Results suggested that DsPME helps in pectin degradation, which is useful in clarification of fruit juices. Further DsPME increased degradation of pectin in combination with PGA.

Discussion

In the present study, TSP was isolated from leaves, seeds, and fruit coat of 3 different species of Datura and specific activity of PME was estimated. Fruit coat showed highest PME activity followed by leaves then seeds. Earlier, Laats et al., (1997) analyzed the expression of PME in pod, endosperm, and seed hulls of green beans (Phaseolus vulgaris), and reported 20 times higher activity in seed hulls as compared with pods.\(^{23}\) PME activity in guava fruits increases with maturation.\(^{24}\) High PME activity in tomato fruits has also been reported as compared with leaves that increases with increase in maturity of fruits.\(^{18}\) These results showed that expression of PME is always higher in fruits of plants in comparison to other plant parts. We also observed highest

---

**Figure 3.** (A) Temperature optima of DsPME: Figure shows optimum activity at 60 °C. (B) pH optima of DsPME: Figure shows optimum activity at pH 9. (C) Effect of Na\(^+\): Figure shows optimum activity at 0.3 M NaCl. (D) Effect of K\(^+\): Figure shows almost equal activity from 0.15 M to 0.3 M.
PME activity in fruit coat followed by leaves and seeds. This might be due to low accumulation or accumulation of modified (inactive/ less active) PME in Datura seeds. Further, PME is a highly regulated enzyme, generally involved in cell elongation and cell separation etc. Seed is a storage organ and does not require cell elongation or separation or other activity during the storage. Therefore, all the enzymes and proteins could be present in dormant stage in seed till the commencement of germination. This might also be the reason of lower PME activity in seeds.

Specific activity of PME was highest in fruit coat, but the protein quantity was very low as availability of fruit coat was limiting. Therefore, we used plant leaves for purification of PME. Specific activity of PME in Ds leaves was higher in comparison to others. Therefore, Ds leaves were selected for purification of PME. To reduce the contamination of pigments and secondary metabolites (which may interfere during chromatography) in TSP, it was precipitated with 80% ammonium sulfate. Protein pellet was solubilized in TrisCl (pH 8) and dialyzed overnight in 10 kDa dialysis membrane to reduce salt and other remaining low molecular weight contaminants. Supernatant was loaded on Q sepharose anion exchange column and eluted fraction showed ~14 fold enriched PME activity in selected fractions. Specific PME activity was further enriched by ~25 fold after size exclusion chromatography. About 20- to 30-fold enrichment in specific activities after purification has also been reported in case of orange and green beans. Purified DsPME corresponded to ~33 kDa on SDS-PAGE and in-gel activity assay. PME of similar size has been reported from different plants.

Purified DsPME was characterized for temperature optima, pH optima, salt requirements, thermo stability, and enzyme kinetics. DsPME showed optimum activity at 60 °C. Previously reported PME from banana and papaya showed optimum activity at 63 and 70 °C, respectively. However, PME with very high optimum temperature (90 °C) has also been reported.

Plant PMEs showed maximum activity at basic pH ranging from 7.5 to 9.0. DsPME was also worked efficiently at pH ranging from 7 to 10 with optimum activity at pH 9. pH 8.0 is reported as optimal for peach PME.

DsPME showed maximum activity in the presence of 0.3 M of NaCl. The activity of PME increased on increasing the concentration of monovalent ions because they mainly interact with substrate rather than PME, but activity decreased sharply above optimum salt concentration. It is reported that the carboxylate group just neighboring to the ester bond is required for interaction of enzyme to pectin. It is possible that very high concentrations of monovalent ions interact with carboxylate group and interfere in enzyme binding. This might be the reason for decline in activity above optimum concentration of monovalent ions.

Thermal stability studies of DsPME showed that it was stable at 70 °C with more than 40% activity; however it lost complete activity at 80 °C. Similar results have been reported in case of orange PME. However PMEs with very high thermal stability are also reported. Acerola and guava fruit PME are reported to be stable at more than 90 °C. The inactivation time required for industrial application should be equal to 1 min at 90 °C. In this regard, DsPME might be more useful for industrial application because of its high activity and easy inactivation.

Enzyme kinetics studies showed that Km value of DsPME was very low. This indicates that it had very high affinity for the substrate. The might also be due to the citrus pectin as substrate in the present study as citrus pectin is reported as best substrate.
for PME activity due to the high degree of esterification. Km value of DsPME was lower than *Lycopersicon esculentum* and orange PME when used same substrate. However, we could not compare Km value of other studies because it depends on source of substrate, reaction temperature, salt concentration, pH of reactions and other different parameters.

Role of PMEs is reported in fruit juice clarification. Purified DsPME was used in clearing of juice from 4 different fruits (orange, apple, pineapple, and pomegranate) in combination with PGA. It is reported that PME enhances pectin degradation process and helps in complete degradation of pectin in combination with PGA. DsPME significantly enhanced the clarification of all 4 tested juices in combination with PGA. Results showed that it can also be utilized in juice industries. Significant increase in color, total soluble solids, titrable acidity and total sugar in the enzymatic extracted juices are also reported. Effect of PME on clarification of juices is also observed, PME increases the recovery of juice from different fruits. Juices normally present inside the pulp of fruit and enclosed by vacuole or cell wall, in which pectin act as major cementing agent. PME de-esterifies pectin into methanol and galactouronic acid and makes pectin more susceptible for degradation by pectinases. It results in loosening of cell wall, which in turn helps in release of sap/juice very easily.

### Plant material

Three *Datura* species (D. stramonium (Ds), D.inoxia (Di) and D. metel (Dm)) were collected from field of National Botanical Research Institute (80° 59'E, 26° 55'N) and Central Institute of Medicinal and Aromatic Plants (80° 58′56″E, 26°53′40″N), Lucknow, India. Leaves and fruits were sorted, cleaned, and used for isolation of total soluble protein (TSP) and PME activity analysis.

### Total soluble protein extraction

Total soluble protein was extracted from 3 different parts (leaves, fruit coat, and seeds) of plants. Plant tissue (1 g) was powdered in liquid nitrogen and homogenized in 4 ml PBS (pH 7.5) at 4°C. Homogenate was centrifuged (12,000 ‘g, 15 min) and supernatant was collected in fresh tube. The total protein was precipitated using 80% ammonium sulfate as previously described method by Scopes RK, 1982, and centrifuged (12,000 ‘g, 15 min, 4°C). Pellet was solubilized in Tris-Cl (pH 8, 20mM) and dialyzed over night in same buffer to remove ammonium sulfate.

### Activity guided purification of PME from *Datura stramonium* leaves

Supernatant was filtered through 0.22 µm filters and loaded on Q sepharose-FF anion exchange column (GE Healthcare) pre-equilibrated in buffer (20mM Tris-Cl, pH 8). Column was washed till OD$_{280}$ becomes less than 0.0002. Bound proteins were eluted by a linear gradient of 1M NaCl and fractions were collected at fix volume (1 ml) intervals. Each fraction was analyzed for PME activity by gel diffusion assay. Fractions showing PME activity were analyzed on SDS-PAGE. Fractions with high activity and similar protein profile were pooled and used for further purification. Pooled sample was further fractionated by size exclusion chromatography (Superdex-200 column, GE Healthcare) in 20 mM Tris-Cl (pH 8.0) containing 150 mM NaCl. Eluted fractions were again analyzed for PME activity by gel diffusion assay. Fraction showing maximum activity was further analyzed by in-gel assay. Sample was mixed with loading dye (without DTT) and separated on 12% SDS-PAGE in duplicate without heat denaturation. One was stained with coomassie brilliant blue G and another was used for in-gel enzyme assay. Gel was washed in 2.5% TritonX100 for 5 min to remove SDS followed by PBS, and then incubated with 0.125% citrus pectin solution (prepared in PBS, pH 7.5) at 30°C for 45 min. Gel was rinsed in PBS and stained with 0.05% ruthenium red.

### Table 2. Enrichment in Pectin methylesterase activity after different steps of purification

| Purification steps       | Activity (units/ml) | Protein (mg/ml) | Specific Activity (U/mg) | Purification Factor |
|--------------------------|--------------------|----------------|--------------------------|--------------------|
| Crude                    | 250                | 2.41           | 103.7                    | 1.00               |
| Ammonium Sulfate ppt.    | 380                | 3.43           | 110.7                    | 1.06               |
| Ion exchange             | 290                | 0.44           | 1517.0                   | 14.00              |
| Gel filtration           | 178                | 0.06           | 2617.0                   | 25.23              |
Protein quantification
Protein quantity was determined by 3 different methods: 1) analyzing absorbance at 280 nm in nano-drop spectrophotometer; 2) Bradford method; and 3) densitometry on SDS-PAGE. Bovine serum albumin was used as standard in all methods.

PME activity assay
Activity of PME was calculated by titration assay and gel diffusion assay. In titration assay, activity was determined by measuring the amount of free carboxyl groups of substrate in the reaction. Reaction mixture (30 ml) was composed of 0.125% citrus–pectin solution, 0.15 M NaCl and 0.2 ml enzyme, and pH adjusted to 8. Enzyme activity was performed at 30 °C for 45 min and stopped by incubating at 100 °C for 10 min. It was titrated against 0.1 M NaOH. Reaction mixture without enzyme was taken as control. PME activity was calculated using following formula.

\[
\text{PME (units/ml)} = \frac{\text{[NaOH (Reaction)-NaOH (Blank) in ml] (Molarity of NaOH)}}{1000} 
\]

\[
\text{PME} = \frac{\text{One unit of PME was defined as the amount of enzyme, which releases 1 µmol of carboxyl groups/min.}}{\text{(Time)(ml Sample)}} 
\]

\[
\text{PME (units/ml)} = \frac{\text{[NaOH (Reaction)-NaOH (Blank) in ml] (Molarity of NaOH)}}{1000} 
\]

Gel diffusion assay was performed in 2% agarose gel containing 0.125% pectin. Sterile filter paper discs were placed on the gel. Enzyme was poured on discs and allowed to diffuse through the gel at 30 °C for 12 h; gel bed was washed with PBS and stained with 0.05% ruthenium red. Diameter of stained circle on gel bed corresponds to the PME activity. Larger the diameter on gel bed, the higher the PME activity.

Temperature optima
To determine the temperature optima of enzyme, reaction mixture was incubated at different temperatures (30, 40, 50, 60, 70, 80, and 90 °C) for 45 min and stopped by incubating at 100 °C for 10 min, then used for titration assay. Reaction mixture without enzyme was taken as control.

Thermo-stability and denaturation
Enzyme was incubated at various temperatures for different time periods. Residual activity was analyzed by gel diffusion assay and calculated by given formula:

\[
\text{(Dc-Ds)} 
\]

\[
\% \text{Residual activity} = 100 \times \text{100} 
\]

\[
\text{Dc} = \text{Diameter in control sample} 
\]

\[
\text{Ds} = \text{Diameter of heated sample} 
\]

pH Optima
PME activity at different pH was analyzed by gel diffusion assay because we could not perform titration at different pH. Gel of different pH (3–11) was prepared and enzyme reaction was performed as described above. Diameter of circle in each gel corresponds to the PME activity at different pH.

Effect of monovalent ions
The effect of monovalent ions on the activity of PME was calculated by titration assay. The reaction was performed with different concentration (0.1, 0.15, 0.2, 0.3, 0.4, and 0.5) of NaCl and KCl. A reaction without enzyme was also performed with each reaction, served as a control.

Enzyme kinetics
Enzyme reaction was performed with substrate (citrus pectin, Sigma) concentrations (S) ranging from 0.125 to 10.0 mg/ml at pH 7.0 and 30 °C and reaction velocity (V) calculated by titration assay. Data was analyzed by Sigma Plot 10.0, and Michaelis-Menten constant (Km) and maximum velocity (Vmax) of purified DsPME was calculated.

Clarification of fruit juices by DsPME
Study was performed in combination with polygalactouronase (PGA). Fresh juice was extracted from apple, pineapple, orange, and pomegranate, and filtered. DsPME (20 units) in combination with commercial PGA was mixed with each juice (15 ml) and incubated at 50 °C for 8 h. Juice without any enzyme and with PGA alone was used as control. Clarity in juices was analyzed as earlier described.

Statistical analysis
All the experiments were performed in triplicates and the average was calculated. The data obtained from the studies were analyzed using linear and nonlinear regression on Sigma Plot 10.0.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
Authors are thankful to Council of Scientific and Industrial Research for funding in the form of EMPOWER project, and CSIR-National Botanical Research Institute for lab facility. SKU and HS are thankful to CSIR for Senior Research Fellowship.

Supplemental Materials
Supplemental materials may be found here:
http://www.landesbioscience.com/journals/psb/article/25681/
References
1. Jayani RS, Saxena S, Gupta R. Microbial pectinolytic enzymes: A review. Process Biochem 2005; 40:2931-44.
2. Benen JAE, Voragen AGJ, Visser J. Pectic enzymes in J. Agric Food Chem 2003; 51:791-5; PMID:12083891.
3. Christgau S, Kofod LV, Halkier T, Andersen LN, Hockauf M, Dittrich K, et al. Pectin methyl esterase from Aspergillus aculeatus: expression cloning in yeast and characterization of the recombinant enzyme. Biochem J 1996; 319:705-12; PMID:8920970.
4. Hagerman AE, Austin PJ. Continuous spectrometric assay for plant pectin methyl esterase. J Agric Food Chem 1986; 34:440-4; http://dx.doi.org/10.1021/jf00069a015.
5. Fischer RL, Bennett AB. Role of cell wall hydrolyases in fruit ripening. Annu Rev Plant Physiol 1991; 42:677-703; http://dx.doi.org/10.1146/annurev.ph.42.090191.003331.
6. Liners F, Van Cutsem P. Distribution of pectic polysaccharides throughout walls of suspension-cultured carrot cells. Protoplasma 1992; 170:10-21; http://dx.doi.org/10.1007/BF01384453.
7. Sheu EA, Gibeaut DM, Carpita NC. Structural analysis of the cell walls regenerated by carrot protoplasts. Planta 1989; 179:293-308; http://dx.doi.org/10.1007/BF00391074.
8. Nari J, Noat G, Ricard J. Pectin methylesterase, metal ions and plant cell-wall extension. Hydrolysis of pectin by plant cell-wall pectin methyl esterase. Biochem J 1991; 279:343-50; PMID:1675486.
9. Viglione L, Balestriere C, Giovane A. Isolation and characterization of pectin methyltransferase from apple fruit. J Food Sci 1989; 54:635-53; http://dx.doi.org/10.1111/j.1365-2621.1989.tb04674.x.
10. Castaldo D, Quagliuolo L, Servillo L, Balestrieri G, Balaban M, Arreola A, Peplow JF. Purification and characterization of a papaya (Carica papaya L) pectin methyltransferase isolated from a commercial papain preparation. Food Chem 1992; 42:181-9; http://dx.doi.org/10.1016/S0927-6510(00)00043-X.
11. Javert H, Wicker L. Partial purification and characterization of pectin methyltransferase. J Food Biochem 1991; 15:241-52; http://dx.doi.org/10.1111/j.1745-4519.1991.tb00159.x.
12. de Assis SA, Martins ABG, Guaglianoni DG, de Faria Oliveira OM. Partial purification and characterization of pectin methyltransferase from acerola (Malpighia glabra L.). J Agric Food Chem 2002; 50:4103-7; http://dx.doi.org/10.1021/jf011247y.
13. Bharti SK, Bhatia A, Tewari SK, Sidhu OP, Roy R. Application of HR-MAS NMR spectroscopy for studying chemotype variations of Withania somnifera (L.) Dunal. Magn Reson Chem 2011; 49:659-67; PMID:21915899; http://dx.doi.org/10.1002/mrc.2817.
14. Pellas J, Rastrelli C, Mellecorizzi EJ. New insights into pectin methyltransferase structure and function. Trends Plant Sci 2007; 12:267-77; PMID:17499907; http://dx.doi.org/10.1016/j.tplants.2007.04.001.
15. Laats MM, Grosdenis F, Recourt K, Alftins G, Voragen J, Wichers HJ. Partial Purification and Characterization of Pectin Methyltransferase from Green Beans (Phaseolus vulgaris L.). J Agric Food Chem 1997; 45:567-72; http://dx.doi.org/10.1021/jf960341p.
16. Carvalho AB, De Assis SA, Leite KMSC, Bach EE, Oliveira OM. Pectin methyl esterase activity and ascorbic acid content from guava fruit, cv. Prediserta, in different phases of development. Int J Food Sci Nutr 2009; 60:255-65; PMID:18608560; http://dx.doi.org/10.1080/09637480901752244.
17. Bayer IT, Halitschke R, Kessler A, Schittko U. Purification and characterization of pectin methyltransferase from orange (Citrus sinensis) cv. Pera. J Food Biochem 2005; 29:367-80; http://dx.doi.org/10.1111/j.1745-4519.2005.00056.x.
18. Do Amaral SH, De Assis SA, De Oliveira OM. Partial purification and characterization of pectin methyltransferase from orange (Citrus sinensis) cv. Pera. J Food Biochem 2005; 29:367-80; http://dx.doi.org/10.1111/j.1745-4519.2005.00056.x.
19. Lu Nguyen BL, Loey AV, Vandeven I, Hendrick IM. Purification, characterization, thermal, and high-pressure inactivation of pectin methyltransferase from bananas (cv. Cavendish). J Biotechnol Bioeng 2002; 78:683-91; http://dx.doi.org/10.1002/bit.10249.
20. Vasu P, Savary BJ, Cameron RG. Purification and characterization of a pectin (Papaya pectin) methyltransferase isolated from a commercial papain preparation. Food Chem 2012; 133:366-72; http://dx.doi.org/10.1016/j.foodchem.2012.01.042.
21. Arbabshir SM, Asli BA, Jumainah AH, Jamilah B. Purification and properties of pectinesterase from sorousp (Annona muri-cata) pulp. Food Chem 1997; 59:33-40; http://dx.doi.org/10.1016/S0308-8146(96)00043-X.
22. Scopes RK. Protein Purification: Principles and Practice; Third Edition; Springer, New York, 1982; 346-348.
23. Kertesz ZL. Pectic enzymes the determination of pectinmethyllyase activity. J Biochem 1937; 12:589-98.
24. Downie B, Dirk LM, Hadfield KA, Wilkins TA, Bennett AB, Bradford KJ. A gel diffusion assay for quantification of pectin methyllyase activity. Anal Biochem 1998; 264:149-57; PMID:9866676; http://dx.doi.org/10.1006/abio.1998.2847.
25. Balaban MO, Arreola AG, Marshall M, Peplow A, Wei CI, Cornell J. Inactivation of pectinesterase in orange juice by supercritical carbon dioxide. J Food Sci 1991; 56:743-6; http://dx.doi.org/10.1111/j.1745-4519.1991.tb05372.x.
