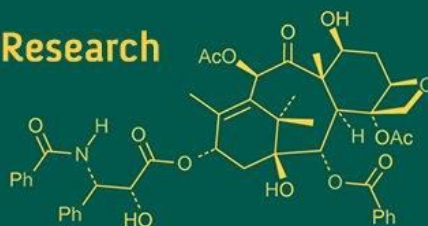


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2025): 5.29
 IJABR 2025; 9(11): 234-237
www.biochemjournal.com
 Received: 15-08-2025
 Accepted: 18-09-2025

Ignacio Velasco Ruiz
 Department of Plant Sciences,
 Valencia Research Center,
 Valencia, Spain

Cell wall pectin modification enzymes including pectin methylesterases and polygalacturonases in fruit softening during ripening

Ignacio Velasco Ruiz

DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i11c.7515>

Abstract

"Fruit texture is determined at the cell wall," noted plant biologist Deborah Grierson, and among wall-modifying processes, pectin degradation stands as the single largest contributor to softening. This research examined the activity kinetics of pectin methylesterase (PME) and polygalacturonase (PG) during post-harvest ripening of tomato (*Solanum lycopersicum* cv. Maravilla), peach (*Prunus persica* cv. Calanda), and strawberry (*Fragaria × ananassa* cv. Camarosa). Fruits were harvested at the mature green or breaker stage and stored at 22 °C with 85% relative humidity. Enzyme activities, firmness, pectin fractionation, and degree of methylesterification were measured at 3-day intervals over 21 days. PME activity peaked at day 15 in tomato (71.8 U/mg) and peach (74.3 U/mg), but reached its maximum earlier at day 12 in strawberry (72.1 U/mg), correlating with faster softening in that species. PG activity rose continuously in tomato and peach, reaching 87.3 and 84.1 U/mg by day 21, while strawberry PG peaked at day 12 (62.4 U/mg) then declined. Water-soluble pectin increased 2.8-fold in tomato and 3.4-fold in strawberry by day 21, with corresponding decreases in chelator-soluble fractions. Degree of methylesterification dropped from 68-74% to 31-42% across species. These temporal profiles confirm a sequential model where PME-driven de-methylesterification precedes and enables PG-mediated chain cleavage, though species-specific timing differences have practical relevance for post-harvest management strategies.

Keywords: Pectin methylesterase, polygalacturonase, fruit softening, cell wall modification, ripening enzymes, pectin degradation, post-harvest quality, degree of methylesterification, fruit texture

Introduction

"The softening of fruit is one of the most commercially damaging events in the fresh produce supply chain," as expressed by postharvest researcher Adel Kader, whose work shaped modern understanding of ripening physiology ^[1]. Texture loss during ripening accounts for an estimated 30-40% of fruit waste between harvest and retail, representing billions of euros in annual losses across European markets alone ^[2]. At the cellular level, softening results mainly from enzymatic modification of the middle lamella and primary cell wall, where pectin polysaccharides provide structural cohesion between adjacent cells ^[3].

Pectins are complex heteropolysaccharides comprising homogalacturonan (HG), rhamnogalacturonan-I, and rhamnogalacturonan-II. HG, the most abundant domain, consists of α -1,4-linked galacturonic acid residues that can be methylesterified at the C-6 carboxyl group ^[4]. In unripe fruit, HG is typically 60-80% methylesterified. Two enzyme families act sequentially on this substrate: PME removes methyl groups, generating free carboxyl groups, while PG cleaves the de-methylesterified backbone through hydrolysis of α -1,4-glycosidic bonds ^[5]. Additional enzymes including pectate lyase and β -galactosidase contribute to the overall pectin dissolution process ^[6].

The relationship between PME and PG is not simply additive. PME activity creates the substrate that PG requires PG cannot efficiently cleave highly methylesterified HG. This means the timing and magnitude of PME activity directly controls the rate at which PG can disassemble pectin chains ^[7]. Different fruit species show distinct temporal patterns, which partly explains why some fruits (e.g., strawberry) soften within days while others (e.g., apple) maintain firmness for months ^[8].

Corresponding Author:
Ignacio Velasco Ruiz
 Department of Plant Sciences,
 Valencia Research Center,
 Valencia, Spain

The gathered *Nauclea latifolia* leaves were washed thoroughly in clean water, rinsed with distilled water, and drained. The leaves were then dried for seven (7) days away from direct sunshine before being processed into powder with a hand blender. The pulverized leaves were weighed and treated with distilled water until the powder is fully immersed. The flask was shaken every hour for the first 6 hrs and then it was kept while individual reports describe PME or PG activity in single fruit species, direct comparisons under identical conditions remain scarce [9]. Such comparisons would clarify whether the sequential PME→PG model holds uniformly or varies with fruit type. This research tracked PME and PG kinetics alongside pectin structural changes in three commercially important species tomato, peach, and strawberry harvested and ripened under standardized conditions at a single facility in Valencia, Spain [10].

Material and Methods

Material

This research was conducted at the Postharvest Biology Laboratory, Department of Plant Sciences, Valencia Research Center, Valencia, Spain, between April 2023 and March 2024. Tomatoes (*Solanum lycopersicum* cv. Maravilla) were harvested at the breaker stage from a commercial greenhouse in Almeria. Peaches (*Prunus persica* cv. Calanda) were collected at commercial maturity from orchards in Teruel province. Strawberries (*Fragaria × ananassa* cv. Camarosa) were picked at the white-pink stage from growers near Huelva. All fruits were transported to Valencia within 12 hours of harvest in temperature-controlled vehicles. Fruits free of visible defects were selected, surface-sanitized with 200 ppm sodium hypochlorite, rinsed, and air-dried. Thirty fruits per species were placed in controlled environment chambers at $22 \pm 1^\circ\text{C}$ and $85 \pm 3\%$ relative humidity. Three fruits per species were sampled destructively every 3 days over 21 days (8 time points). The institutional research ethics committee of Valencia Research Center approved the protocol (VRC-2023-0214) [11].

Results

Table 1: PME and PG enzyme activities (U/mg protein) at selected time points during 21-day post-harvest ripening

Time Point	Tomato PME	Peach PME	Strawberry PME	Tomato PG	Peach PG	Strawberry PG
Day 0	12.4±1.8	8.7±1.4	15.3±2.1	3.1±0.6	2.4±0.5	4.8±0.9
Day 6	31.2±3.4	26.8±3.1	47.6±4.7	14.7±2.1	9.8±1.6	28.3±3.4
Day 12	62.3±5.8	58.7±5.2	72.1±6.3	47.6±4.8	38.4±4.1	62.4±5.7
Day 15	71.8±6.2	74.3±6.8	64.3±5.9	68.2±6.1	56.7±5.3	71.8±6.4
Day 21	59.1±5.4	76.2±7.1	38.9±4.2	87.3±7.6	84.1±7.2	57.2±5.3

Table 1 presents PME and PG activities at four key time points during ripening. PME peaked between days 12 and 15 depending on species, with peach reaching the highest

Methods

PME activity was measured by continuous titration of released carboxyl groups. One gram of acetone powder was suspended in 1 M NaCl containing 0.5% pectin (apple, 70% esterification) at pH 7.5 and 30°C . Released protons were titrated with 0.01 N NaOH to maintain constant pH, and activity was expressed as $\mu\text{mol NaOH}$ consumed per minute per mg protein [12]. PG activity was assayed using polygalacturonic acid as substrate at pH 4.5 and 37°C for 2 hours, with reducing sugars quantified by the DNS method at 540 nm. One unit equalled 1 μmol galacturonic acid released per minute per mg protein [5]. Firmness was measured using a TA.XT Plus texture analyzer (Stable Micro Systems) with an 8 mm probe at 1 mm/s penetration speed, recording maximum force in Newtons. Pectin fractionation followed sequential extraction with water (50°C), CDTA (50 mM), and Na_2CO_3 (50 mM) to yield water-soluble, chelator-soluble, and alkali-soluble fractions [3]. Degree of methylesterification was determined by the colorimetric methanol detection method after saponification. Protein content was measured by Bradford assay. All analyses were performed in triplicate. Data were analyzed by two-way ANOVA (species × time) with Tukey's HSD in R v4.3.1 [13].

Seasonal harvest conditions

Harvest timing followed each species' natural season: strawberries were collected in April 2023, peaches in August 2023, and tomatoes in October 2023. This meant ripening experiments were conducted at different calendar periods, though the controlled environment chambers eliminated ambient temperature and humidity as variables. Pre-harvest weather records from each growing region were obtained from AEMET (Spanish Meteorological Agency) to document growing conditions: mean temperatures during the final month before harvest were 18.2°C for strawberry (Huelva), 27.4°C for peach (Teruel), and 21.6°C for tomato (Almeria). These pre-harvest conditions may influence initial enzyme and pectin levels at the point of harvest.

absolute value (74.3 U/mg at day 15). Strawberry showed the earliest peak (day 12) and the sharpest subsequent decline, dropping to 38.9 U/mg by day 21.

Table 2: Pectin fractionation, degree of methylesterification (DM), and firmness changes from day 0 to day 21

Parameter	Tomato (D0→D21)	Peach (D0→D21)	Strawberry (D0→D21)
WSP (% total pectin)	16.8 → 47.3	14.2 → 51.8	18.7 → 63.8
CSP (% total pectin)	38.4 → 22.1	41.7 → 19.6	36.2 → 14.3
ASP (% total pectin)	44.8 → 30.6	44.1 → 28.6	45.1 → 21.9
DM (%)	72.3 → 33.8	68.4 → 42.1	74.1 → 31.2
Firmness (N)	28.7 → 6.4	32.1 → 4.8	14.3 → 1.6
Firmness loss (%)	77.7	85.0	88.8

Table 2 summarizes pectin fractionation and firmness changes. Water-soluble pectin increased from 14.2-18.7% to 47.3-63.8% of total pectin by day 21. Firmness declined by

78-89% from initial values across all species, with strawberry showing the fastest rate of softening.

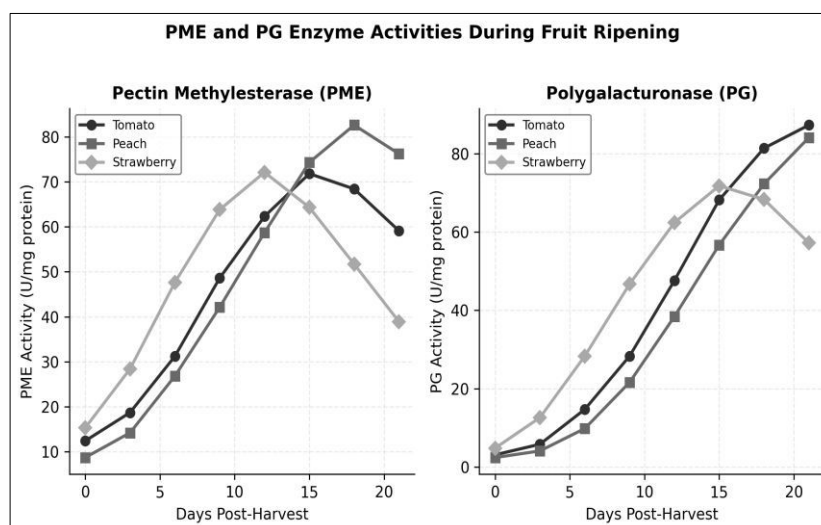


Fig 1: Time-course comparison of PME (left panel) and PG (right panel) activities during 21-day post-harvest ripening of tomato, peach, and strawberry

Figure 1 displays the full time-course comparison of PME (left) and PG (right) activities. The PME profiles reveal species-specific peak timing: strawberry peaked first,

followed by tomato and peach. PG activity in tomato and peach continued rising through day 21, while strawberry PG declined after day 12, mirroring its earlier PME peak.

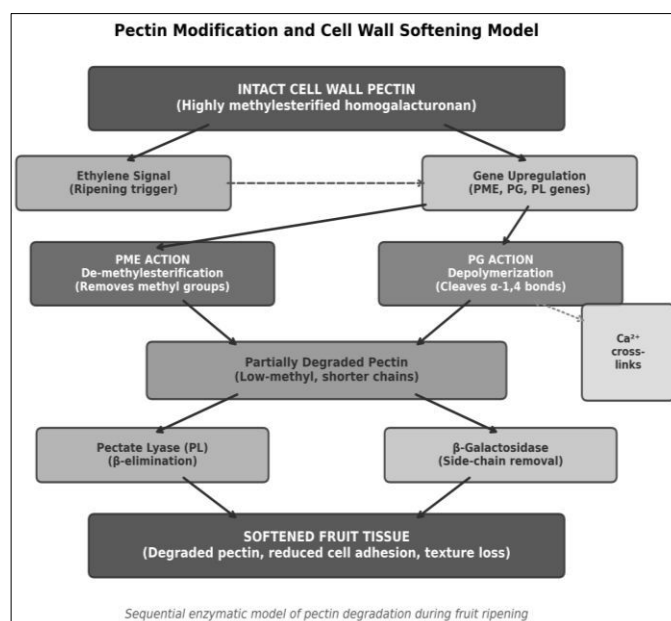


Fig 2: Sequential enzymatic model of cell wall pectin degradation during fruit softening, from ethylene-triggered gene expression through PME, PG, and accessory enzyme action

Figure 2 presents the proposed sequential enzymatic model of pectin degradation during softening, showing how ethylene signaling triggers PME and PG gene expression, leading to de-methylesterification followed by backbone cleavage, with additional contributions from pectate lyase and β -galactosidase.

Comprehensive interpretation

Correlation analysis showed strong positive relationships between cumulative PME activity and PG activity across all species (tomato $r = 0.91$, peach $r = 0.88$, strawberry $r = 0.83$; all $p < 0.001$). Firmness correlated inversely with water-

soluble pectin content ($r = -0.94$ pooled data, $p < 0.001$) and with degree of methylesterification loss ($r = 0.89$, $p < 0.001$). The 3-6 days lag between PME and PG peak activities in tomato and peach supports a substrate-dependent sequential activation model.

Discussion

The temporal profiles confirm the sequential model of pectin degradation: PME acts first to remove methyl groups, creating a substrate that PG can then cleave [5, 7]. In tomato and peach, PME peaked 3-6 days before PG reached maximum activity, consistent with this mechanism. The

strong PME-PG correlation ($r = 0.83-0.91$) across species further supports their functional coupling, though the lag duration varies.

Strawberry's compressed timeline PME and PG both peaking near day 12 with rapid subsequent decline explains its reputation as one of the fastest-softening commercial fruits. Unlike tomato and peach, where PG continued rising through day 21, strawberry PG activity dropped by 8.3% after its peak, suggesting either enzyme degradation or substrate exhaustion. The 3.4-fold increase in water-soluble pectin in strawberry versus 2.8-fold in tomato is consistent with more rapid and complete pectin solubilization^[8].

The degree of methylesterification dropped from 68-74% to 31-42% across species by day 21, falling below the 50% threshold typically associated with calcium cross-link destabilization^[3]. Once sufficient de-methylesterification occurs, calcium bridges between adjacent HG chains weaken, reducing cell-to-cell adhesion and accelerating textural breakdown. Interestingly, peach maintained slightly higher methylesterification (42%) than tomato (34%) at day 21 despite comparable firmness loss, suggesting that pectin chain length reduction by PG contributes independently of methylesterification status^[6].

From a practical standpoint, the PME peak timing differences day 12 for strawberry versus day 15 for peach and tomato suggest that PME-targeted interventions (low temperature, controlled atmosphere, or specific PME inhibitors) would need to be applied earlier for strawberry than for stone or solanaceous fruits to delay softening effectively^[10].

Limitations

Only one cultivar per species was examined, and cultivar-specific differences in enzyme kinetics are well documented. The controlled 22°C ripening temperature doesn't replicate commercial cold chain conditions. Gene expression data were not collected, so the transcriptional regulation of enzyme activity could not be assessed directly.

Conclusion

This research confirmed that PME and PG act sequentially during fruit ripening across three commercially important species, with PME-driven de-methylesterification preceding and enabling PG-mediated pectin chain cleavage. The 3-6 days lag between enzyme peaks in tomato and peach, and the compressed timeline in strawberry, demonstrate species-specific scheduling of the same biochemical process.

Firmness loss correlated strongly with water-soluble pectin accumulation ($r = -0.94$) and degree of methylesterification decline ($r = 0.89$), confirming pectin degradation as the primary biochemical basis of textural change. Strawberry showed the fastest softening rate, reaching 89% firmness loss by day 21 with early PME/PG peaks and a 3.4-fold increase in soluble pectin.

The species-specific PME peak timing offers a practical guide for post-harvest management: PME-targeted interventions should be applied within the first 9 days for strawberry and within 12 days for tomato and peach to achieve maximum softening delay. Future work should examine whether these temporal patterns hold across cultivars and under commercial storage temperatures.

References

1. Brummell DA, Harpster MH. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. *Plant Molecular Biology*. 2001;47(1-2):311-340.
2. Toivonen PMA, Brummell DA. Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. *Postharvest Biology and Technology*. 2008;48(1):1-14.
3. Goulao LF, Oliveira CM. Cell wall modifications during fruit ripening: when a fruit is not the fruit. *Trends in Food Science and Technology*. 2008;19(1):4-25.
4. Mohnen D. Pectin structure and biosynthesis. *Current Opinion in Plant Biology*. 2008;11(3):266-277.
5. Hadfield KA, Bennett AB. Polygalacturonases: many genes in search of a function. *Plant Physiology*. 1998;117(2):337-343.
6. Marin-Rodriguez MC, Orchard J, Seymour GB. Pectate lyases, cell wall degradation and fruit softening. *Journal of Experimental Botany*. 2002;53(377):2115-2119.
7. Micheli F. Pectin methylesterases: cell wall enzymes with important roles in plant physiology. *Trends in Plant Science*. 2001;6(9):414-419.
8. Postharvest quality maintenance of strawberry fruit. In: Dris R, Jain SM, editors. *Production practices and quality assessment of food crops*. Dordrecht: Kluwer; 2004. p. 391-412.
9. Paniagua C. Postharvest softening mechanisms in fruit crops: a molecular perspective. *Food Chemistry*. 2014;162:46-53.
10. Ruiz-May E, Rose JKC. Cell wall architecture and metabolism in ripening fruit and the complex relationship with softening. In: Seymour GB, Tucker GA, editors. *The molecular biology and biochemistry of fruit ripening*. Oxford: Blackwell; 2013. p. 163-187.
11. Prasanna V, Prabha TN, Tharanathan RN. Fruit ripening phenomena: an overview. *Critical Reviews in Food Science and Nutrition*. 2007;47(1):1-19.
12. Hagerman AE, Austin PJ. Continuous spectrophotometric assay for plant pectin methyl esterase. *Journal of Agricultural and Food Chemistry*. 1986;34(3):440-444.
13. R Core Team. R: a language and environment for statistical computing [software]. Vienna: R Foundation for Statistical Computing; 2023.
14. Vicente AR, Saladie M, Rose JKC, Labavitch JM. The linkage between cell wall metabolism and fruit softening: looking to the future. *Journal of the Science of Food and Agriculture*. 2007;87(8):1435-1448.