Adaptation of a Spectrophotometric Assay for Pectinmethylesterase to a Kinetic Microplate Reader

RANDALL G. CAMERON, BÉLA S. BUSLIG, and PHILIP E. SHAW

ABSTRACT

A continuous spectrophotometric assay for pectinmethylesterase (PME) has been adapted to a kinetic microplate reader. Linear standard curves could be constructed from 0 to 50 nMol galacturonic acid and remained linear for 15 min. Activity estimates from commercial orange peel PME were accurate from 0.009 to 0.015 units. Comparison of microplates with differing protein binding efficiencies indicated that low and medium binding plates showed significantly higher activity levels than high binding plates. The utility of the assay was demonstrated on two commercial mixtures of pectolytic enzymes and two commercial PMEs. The assay is extremely rapid; 24 samples replicated three times each could be performed in less than 1 hr. Additionally, it requires very small volumes of substrate (final volume per well was 200 μL) and sample (less than 10 μL per well).

Key Words: spectrophotometry, pectinmethylesterase, citrus, fruit, kinetic microplate

INTRODUCTION

PECTINMETHYLESTERASE (PME) is an important component in many commercially available mixtures of pectolytic enzymes (Rombouts and Pilnik, 1978; Baldwin and Pressey, 1989). These pectolytic mixtures are used to clarify fruit juices, treat pulp to increase juice release or oil yield, macerate and liquefy fruits and vegetables (Rombouts and Pilnik, 1978) and in other specialized applications such as protoplast generation (Baldwin and Pressey, 1989). In the citrus juice industry PME is responsible for the quality defects of juice cloud-loss and concentrate gelation in fresh and under-pasteurized products (Wenzel et al., 1951; Versteeg et al. 1980). PME also is hypothesized to function in fruit softening although its role is unclear (Tucker et al., 1982). Both Tucker et al. (1982) and Koch and Nevins (1990) suggested that PME may influence ripening by providing a suitable substrate (demethylated pectin) for polygalacturonase activity.

Additionally, PME has several important biological functions. PME has been hypothesized to be a key enzyme in the regulation of cell-wall synthesis and extension (Ricard and Noat, 1986; Moustacas et al., 1986; Nari et al., 1986). A role for PME in pathogenicity has been advanced by Boccara and Chatain (1989). They reported that mutants of *Erwinia chrysanthemi*, the causative agent in soft rot disease, lacking a functional PME gene were noninvasive.

Due to the technological and biological importance of PME it is highly desirable to be able to rapidly and accurately estimate PME activity in food related products and plant or fungal lysates. Ideally, it would be advantageous to do so with a minimal amount of sample, on a statistically adequate number of replicates and in a brief period of time. To accommodate these three criteria we have adapted a continuous spectrophotometric assay for PME (Hagerman and Austin, 1986) to a kinetic microplate reader. Recently, several other assays have been adapted to microplate readers (Fox and Robyt, 1991; Plantner, 1991). The major advantages of the kinetic microplate reader are: (1) speed, (2) ease of sample replication, (3) small volumes of sample and substrate, (4) high sensitivity, (5) potential for automation, and (6) availability of computer software for data manipulation and reporting.

Our objective was to compare activity estimates of commercially available preparations of PME obtained with 96-well microplates possessing different protein binding efficiencies. We also demonstrated the accuracy and applicability of the microplate assay by measuring PME activity in commercial pectolytic enzyme mixtures.

Authors Cameron and Shaw are with the USDA-ARS, South Atlantic Area, Citrus and Subtropical Products Laboratory, P.O. Box 1909, Winter Haven, FL 33883-1909. Author Buslig is with the Florida Department of Citrus, P.O. Box 1909, Winter Haven, FL 33883-1909.

MATERIALS & METHODS

Chemicals

Pectolyase (PL), pectinase (Pase), orange peel pectinmethylesterase (OPPME), tomato pectinmethylesterase (TPME), sodium chloride, galacturonic acid monohydrate (GA) and bromothymol blue (BTB) were purchased from Sigma Chemical Co. Pectin (68-73% esterification, "high" and "medium" degree of polymerization) was provided by H.P. Bulmer Ltd.

Equipment

All colorimetric assays and standard curve determinations were made with a Dynatech MR5000 Kinetic Microplate Reader equipped with a plate shaker and temperature controller. Plates (Costar) of non-binding, medium-binding and high protein binding efficiency were tested.

Colorimetric assay

Stock solutions. Standards and sample assays were prepared essentially as described by Hagerman and Austin (1986). A modification was the use of a final concentration of 0.00125% BTB (w/v) instead of 0.0005% BTB (w/v). The concentration of BTB was increased to give higher optical densities. Samples and standards were assayed at a final volume of 200 μl per microplate well. Stock solutions used were 0.025% BTB (w/v) in 3mM potassium phosphate buffer ($KH_2PO_4 - K_2HPO_4 \cdot 3H_2O$, pH 7.5), 0.5% pectin (w/v, containing 100 mM NaCl and 0.01% NaN_3, w/v) and 2.5 mM GA.

Standard curve. To determine the concentration range of GA that would yield a linear standard curve a microplate containing increasing GA concentrations was tested. The optical density at 620 nm for a solution (0.00125% BTB, 0.375% pectin, 75mM NaCl, 0.01% NaN_3, pH 7.5) containing from 0 to 220 nMol GA· mL^{-1} (0, 10, 20. . .220 nMol· mL^{-1}) was recorded. Each concentration was replicated three times and the plate was read every 30 sec for 30 min at 30°C. The plate was shaken for 5 sec immediately before each reading.

Reaction mix preparation and assay procedure. For convenience the reaction mix was prepared in individual tubes (cluster tubes, Costar) that were arrayed in an 8 × 12 matrix at the same dimensions as the wells of the microplate. This allowed multichannel pipettors to be used for liquid transfer. These tubes contained 1.2 mL. The reaction mix was prepared to give a final concentration of 0.375% pectin, 75mM NaCl, 0.0075% NaN_3 and 0.00125% BTB (see Table 1 for volumes) when 190 μL was transferred to a microplate well containing 10 μL of sample. Pectin solution and water were adjusted to pH 7.50 - 7.52 just prior to use. The sequence of events for an assay were: (1) adjust pH of water to 7.50 - 7.52; (2) add water to cluster tubes for standard curve and samples; (3) add GA to standard curve tubes (0, 5, 20, 35, 50, 65 and 80 nMol GA per mL; (4) add 50 μL BTB

Table 1—Amounts of stock solutions and sample per ml or microplate well

Sample vol. ·mL^{-1} (µL)	Stock H_2O	Vol. ·mL^{-1} BTB (µL)	Pectin	Sample Vol. ·well^{-1} (µL)	Reaction mix ·well^{-1} (µL)	Final vol. ·well^{-1} (µL)
5	195	50	750	1	199	200
25	175	50	750	5	195	200
50	150	50	750	10	190	200
100	100	50	750	20	180	200
200	0	50	750	40	160	200

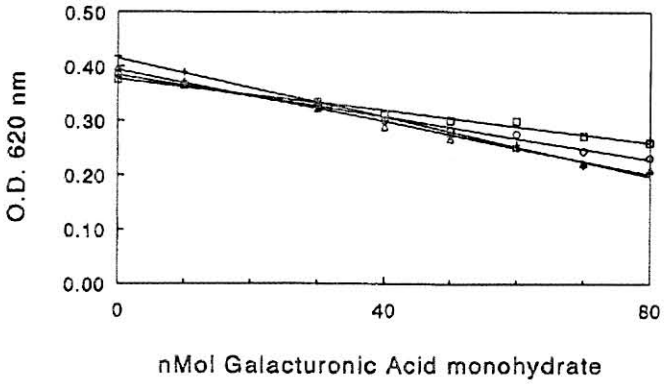

Fig. 1—Linear fits for 0 to 80 nMol GA and their change in absorbance at 620 nM through time. + = 0 min, Δ = 5 min, ○ = 15 min, □ = 30 min.

stock to each tube; (5) add sample to individual wells on microplate; (6) adjust pectin stock solution to pH 7.50 - 7.52; (7) add 750 µL pectin stock to each cluster tube; (8) mix by capping cluster tubes and inverting 2-3 times; and (9) transfer to the microplate with a multichannel pipettor 200 µL of each standard (in triplicate) and then a volume of the reaction mix equal to 200 µL minus sample-volume-per-well was pipetted into the appropriate sample wells. If different amounts of sample were being assayed on one plate the reaction mix was added to the wells containing the smallest sample volume first. After transferring the standards and adding reaction mix to all wells, the plate was placed in the microplate reader and the assay was started. An assay consisting of blanks (3 replicates), standards (7 × 3 replicates each) and samples (24 × 3 replicates each) could be prepared in 30 min. Typically a plate was assayed for 5 min with readings taken every 10 sec during the assay. The plate was shaken for 5 sec immediately before each reading. Assays were performed at 30°C. One unit of enzyme was defined as the amount required to release 1 µMol of acid equivalents · min^{-1} under these conditions. Preliminary assays were performed to determine appropriate sample volumes and dilutions to obtain linear activity curves.

Activity estimation. To estimate enzyme activity, standard curves for the first reading of a plate and the reading at 1 min, 3 min or 5 min (depending on activity levels) were used to estimate the amount of acid equivalents present in the sample at that time. The difference between the amount present in the first and last reading was considered to be equivalent to the amount of H^+ released by enzyme catalysis. The appropriate factors for duration of the assay, amount of sample and sample dilution were then used to estimate PME activity in the sample.

Comparison of microplates. Microplates of different protein binding efficiencies were compared by adding equal amounts and activity of TPME to individual wells and plotting the change in O.D. 620 nm with time. Statistical analysis of results from plates with different binding efficiencies were performed using the applicable modules of MSTAT-C (Michigan State University, 1988) and the PROC-REG module of SAS (SAS® Institute Inc., 1988).

Titrimetric assay

Activity of OPPME was estimated by continuous titration against 20 mM NaOH after adding 2.5, 5 or 10 µL of enzyme to 5 mL of 0.5% pectin (w/v) in 150 mM NaCl which had been adjusted to pH 7.0 or 7.5 with NaOH. All assays were run for 5 min at 30°C. The amount of NaOH required to maintain the set pH was used to calculate

Table 2—Correlation coefficient (r) of absorbance change through time

Time (min)	r
0	−0.997149
5	−0.996521
10	−0.994151
15	−0.990756
20	−0.988862
25	−0.984617
30	−0.980215

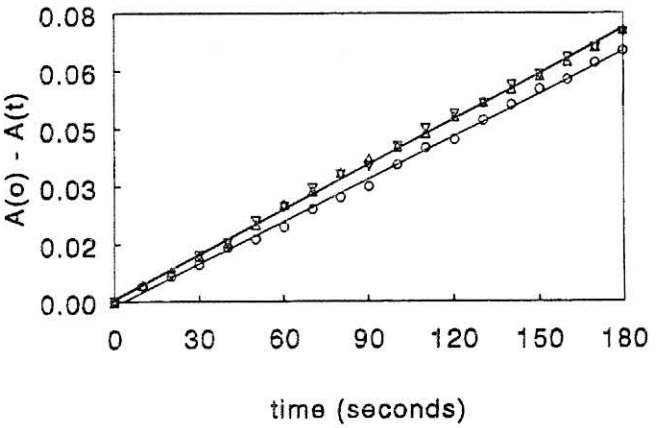

Fig. 2—Graphical comparison of reaction rates (3µL PME) on microplates of different protein binding efficiencies. Δ = non-binding, ▽ = medium binding, ○ = high binding.

enzyme activity. A unit of PME was defined as that amount which yields 1 µMol acid equivalents·min^{-1} under these conditions.

RESULTS & DISCUSSION

Standard curve determination

A linear standard curve could be obtained between 0 and 80 nMol GA when a final concentration of 0.00125% BTB was employed. However, concentrations above 50 nMol occasionally deviated from linearity. For this reason standards of 0, 5, 20, 35, and 50 nMol GA were used to estimate activity. Over 20 assays were performed and the standard curve was always linear between 0 and 50 nMol GA. Linear standard curves could not be obtained consistently with a concentration of 0.0005% BTB (data not shown). Both at low and high concentrations of GA the standard curve optical densities changed with time (Fig. 1). With low concentrations (0, 5, 20 nMol) the optical density decreased with time while it increased for higher concentrations of GA. The correlation coefficient (r) for this optical density change at each time point remained greater than −0.99 when calculated at 5 min intervals over a 15 min period. The r value decreased to −0.980215 after a 30 min period (Table 2). However, since all assays were complete by 5 min, decay of the standard curve did not affect activity estimates. The change in optical density of the standards was most probably related to a slight pH change related to an increase in dissolved CO_2 brought about by shaking of the microplate.

Comparison of microplates with different protein binding capacities

Graphical analysis of the data indicated differences between plates (Fig. 2) with the high binding efficiency plate showing a lower activity estimate up to 5 min. Statistical comparison of the regression lines confirmed that such differences were real with the high binding efficiency plate differing significantly from the low and medium binding efficiency plates at most levels of enzyme used (Table 3). The reasons for this effect due to binding efficiency are not clear. Graphical results

Table 1—Amounts of stock solutions and sample per ml or microplate well

Sample vol. ·mL^{-1} (µL)	Stock H_2O	Vol. ·mL^{-1} BTB (µL)	Pectin	Sample Vol. ·well^{-1} (µL)	Reaction mix ·well^{-1} (µL)	Final vol. ·well^{-1} (µL)
5	195	50	750	1	199	200
25	175	50	750	5	195	200
50	150	50	750	10	190	200
100	100	50	750	20	180	200
200	0	50	750	40	160	200

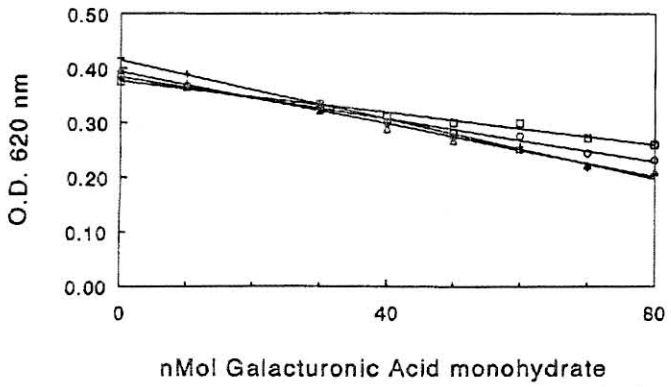

Fig. 1—Linear fits for 0 to 80 nMol GA and their change in absorbance at 620 nM through time. + = 0 min, Δ = 5 min, ○ = 15 min, □ = 30 min.

stock to each tube; (5) add sample to individual wells on microplate; (6) adjust pectin stock solution to pH 7.50 - 7.52; (7) add 750 µL pectin stock to each cluster tube; (8) mix by capping cluster tubes and inverting 2-3 times; and (9) transfer to the microplate with a multichannel pipettor 200 µL of each standard (in triplicate) and then a volume of the reaction mix equal to 200 µL minus sample-volume-per-well was pipetted into the appropriate sample wells. If different amounts of sample were being assayed on one plate the reaction mix was added to the wells containing the smallest sample volume first. After transferring the standards and adding reaction mix to all wells, the plate was placed in the microplate reader and the assay was started. An assay consisting of blanks (3 replicates), standards (7 × 3 replicates each) and samples (24 × 3 replicates each) could be prepared in 30 min. Typically a plate was assayed for 5 min with readings taken every 10 sec during the assay. The plate was shaken for 5 sec immediately before each reading. Assays were performed at 30°C. One unit of enzyme was defined as the amount required to release 1 µMol of acid equivalents · min^{-1} under these conditions. Preliminary assays were performed to determine appropriate sample volumes and dilutions to obtain linear activity curves.

Activity estimation. To estimate enzyme activity, standard curves for the first reading of a plate and the reading at 1 min, 3 min or 5 min (depending on activity levels) were used to estimate the amount of acid equivalents present in the sample at that time. The difference between the amount present in the first and last reading was considered to be equivalent to the amount of H^+ released by enzyme catalysis. The appropriate factors for duration of the assay, amount of sample and sample dilution were then used to estimate PME activity in the sample.

Comparison of microplates. Microplates of different protein binding efficiencies were compared by adding equal amounts and activity of TPME to individual wells and plotting the change in O.D. 620 nm with time. Statistical analysis of results from plates with different binding efficiencies were performed using the applicable modules of MSTAT-C (Michigan State University, 1988) and the PROC-REG module of SAS (SAS® Institute Inc., 1988).

Titrimetric assay

Activity of OPPME was estimated by continuous titration against 20 mM NaOH after adding 2.5, 5 or 10 µL of enzyme to 5 mL of 0.5% pectin (w/v) in 150 mM NaCl which had been adjusted to pH 7.0 or 7.5 with NaOH. All assays were run for 5 min at 30°C. The amount of NaOH required to maintain the set pH was used to calculate

Table 2—Correlation coefficient (r) of absorbance change through time

Time (min)	r
0	−0.997149
5	−0.996521
10	−0.994151
15	−0.990756
20	−0.988862
25	−0.984617
30	−0.980215

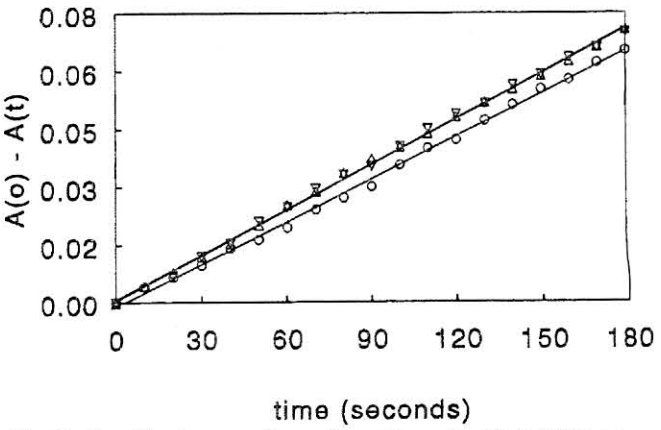

Fig. 2—Graphical comparison of reaction rates (3µL PME) on microplates of different protein binding efficiencies. Δ = non-binding, ▽ = medium binding, ○ = high binding.

enzyme activity. A unit of PME was defined as that amount which yields 1 µMol acid equivalents·min^{-1} under these conditions.

RESULTS & DISCUSSION

Standard curve determination

A linear standard curve could be obtained between 0 and 80 nMol GA when a final concentration of 0.00125% BTB was employed. However, concentrations above 50 nMol occasionally deviated from linearity. For this reason standards of 0, 5, 20, 35, and 50 nMol GA were used to estimate activity. Over 20 assays were performed and the standard curve was always linear between 0 and 50 nMol GA. Linear standard curves could not be obtained consistently with a concentration of 0.0005% BTB (data not shown). Both at low and high concentrations of GA the standard curve optical densities changed with time (Fig. 1). With low concentrations (0, 5, 20 nMol) the optical density decreased with time while it increased for higher concentrations of GA. The correlation coefficient (r) for this optical density change at each time point remained greater than −0.99 when calculated at 5 min intervals over a 15 min period. The r value decreased to −0.980215 after a 30 min period (Table 2). However, since all assays were complete by 5 min, decay of the standard curve did not affect activity estimates. The change in optical density of the standards was most probably related to a slight pH change related to an increase in dissolved CO_2 brought about by shaking of the microplate.

Comparison of microplates with different protein binding capacities

Graphical analysis of the data indicated differences between plates (Fig. 2) with the high binding efficiency plate showing a lower activity estimate up to 5 min. Statistical comparison of the regression lines confirmed that such differences were real with the high binding efficiency plate differing significantly from the low and medium binding efficiency plates at most levels of enzyme used (Table 3). The reasons for this effect due to binding efficiency are not clear. Graphical results

Adaptation of a Spectrophotometric Assay for Pectinmethylesterase to a Kinetic Microplate Reader

RANDALL G. CAMERON, BÉLA S. BUSLIG, and PHILIP E. SHAW

ABSTRACT

A continuous spectrophotometric assay for pectinmethylesterase (PME) has been adapted to a kinetic microplate reader. Linear standard curves could be constructed from 0 to 50 nMol galacturonic acid and remained linear for 15 min. Activity estimates from commercial orange peel PME were accurate from 0.009 to 0.015 units. Comparison of microplates with differing protein binding efficiencies indicated that low and medium binding plates showed significantly higher activity levels than high binding plates. The utility of the assay was demonstrated on two commercial mixtures of pectolytic enzymes and two commercial PMEs. The assay is extremely rapid; 24 samples replicated three times each could be performed in less than 1 hr. Additionally, it requires very small volumes of substrate (final volume per well was 200 μL) and sample (less than 10 μL per well).

Key Words: spectrophotometry, pectinmethylesterase, citrus, fruit, kinetic microplate

INTRODUCTION

PECTINMETHYLESTERASE (PME) is an important component in many commercially available mixtures of pectolytic enzymes (Rombouts and Pilnik, 1978; Baldwin and Pressey, 1989). These pectolytic mixtures are used to clarify fruit juices, treat pulp to increase juice release or oil yield, macerate and liquefy fruits and vegetables (Rombouts and Pilnik, 1978) and in other specialized applications such as protoplast generation (Baldwin and Pressey, 1989). In the citrus juice industry PME is responsible for the quality defects of juice cloud-loss and concentrate gelation in fresh and under-pasteurized products (Wenzel et al., 1951; Versteeg et al. 1980). PME also is hypothesized to function in fruit softening although its role is unclear (Tucker et al., 1982). Both Tucker et al. (1982) and Koch and Nevins (1990) suggested that PME may influence ripening by providing a suitable substrate (demethylated pectin) for polygalacturonase activity.

Additionally, PME has several important biological functions. PME has been hypothesized to be a key enzyme in the regulation of cell-wall synthesis and extension (Ricard and Noat, 1986; Moustacas et al., 1986; Nari et al., 1986). A role for PME in pathogenicity has been advanced by Boccara and Chatain (1989). They reported that mutants of *Erwinia chrysanthemi*, the causative agent in soft rot disease, lacking a functional PME gene were noninvasive.

Due to the technological and biological importance of PME it is highly desirable to be able to rapidly and accurately estimate PME activity in food related products and plant or fungal lysates. Ideally, it would be advantageous to do so with a minimal amount of sample, on a statistically adequate number of replicates and in a brief period of time. To accommodate these three criteria we have adapted a continuous spectrophotometric assay for PME (Hagerman and Austin, 1986) to a kinetic microplate reader. Recently, several other assays have been adapted to microplate readers (Fox and Robyt, 1991; Plantner, 1991). The major advantages of the kinetic microplate reader are: (1) speed, (2) ease of sample replication, (3) small volumes of sample and substrate, (4) high sensitivity, (5) potential for automation, and (6) availability of computer software for data manipulation and reporting.

Our objective was to compare activity estimates of commercially available preparations of PME obtained with 96-well microplates possessing different protein binding efficiencies. We also demonstrated the accuracy and applicability of the microplate assay by measuring PME activity in commercial pectolytic enzyme mixtures.

Authors Cameron and Shaw are with the USDA-ARS, South Atlantic Area, Citrus and Subtropical Products Laboratory, P.O. Box 1909, Winter Haven, FL 33883-1909. Author Buslig is with the Florida Department of Citrus, P.O. Box 1909, Winter Haven, FL 33883-1909.

MATERIALS & METHODS

Chemicals

Pectolyase (PL), pectinase (Pase), orange peel pectinmethylesterase (OPPME), tomato pectinmethylesterase (TPME), sodium chloride, galacturonic acid monohydrate (GA) and bromothymol blue (BTB) were purchased from Sigma Chemical Co. Pectin (68-73% esterification, "high" and "medium" degree of polymerization) was provided by H.P. Bulmer Ltd.

Equipment

All colorimetric assays and standard curve determinations were made with a Dynatech MR5000 Kinetic Microplate Reader equipped with a plate shaker and temperature controller. Plates (Costar) of non-binding, medium-binding and high protein binding efficiency were tested.

Colorimetric assay

Stock solutions. Standards and sample assays were prepared essentially as described by Hagerman and Austin (1986). A modification was the use of a final concentration of 0.00125% BTB (w/v) instead of 0.0005% BTB (w/v). The concentration of BTB was increased to give higher optical densities. Samples and standards were assayed at a final volume of 200 μl per microplate well. Stock solutions used were 0.025% BTB (w/v) in 3mM potassium phosphate buffer ($KH_2PO_4 - K_2HPO_4 \cdot 3H_2O$, pH 7.5), 0.5% pectin (w/v, containing 100 mM NaCl and 0.01% NaN_3, w/v) and 2.5 mM GA.

Standard curve. To determine the concentration range of GA that would yield a linear standard curve a microplate containing increasing GA concentrations was tested. The optical density at 620 nm for a solution (0.00125% BTB, 0.375% pectin, 75mM NaCl, 0.01% NaN_3, pH 7.5) containing from 0 to 220 nMol GA· mL^{-1} (0, 10, 20. . .220 nMol· mL^{-1}) was recorded. Each concentration was replicated three times and the plate was read every 30 sec for 30 min at 30°C. The plate was shaken for 5 sec immediately before each reading.

Reaction mix preparation and assay procedure. For convenience the reaction mix was prepared in individual tubes (cluster tubes, Costar) that were arrayed in an 8 × 12 matrix at the same dimensions as the wells of the microplate. This allowed multichannel pipettors to be used for liquid transfer. These tubes contained 1.2 mL. The reaction mix was prepared to give a final concentration of 0.375% pectin, 75mM NaCl, 0.0075% NaN_3 and 0.00125% BTB (see Table 1 for volumes) when 190 μL was transferred to a microplate well containing 10 μL of sample. Pectin solution and water were adjusted to pH 7.50 - 7.52 just prior to use. The sequence of events for an assay were: (1) adjust pH of water to 7.50 - 7.52; (2) add water to cluster tubes for standard curve and samples; (3) add GA to standard curve tubes (0, 5, 20, 35, 50, 65 and 80 nMol GA per mL; (4) add 50 μL BTB

Table 3—Activity estimates (3 min) as affected by plate binding efficiency[a]

Amount of TPME µL	Activity (µEq acid · min^{-1}·10^3)		
	Nonbinding	Medium binding	High binding
1	3.633a	3.718a	2.772a
2	6.243ac	6.174a	4.796bc
3	8.552a	7.436ab	6.509b
4	10.216a	9.958a	7.754b
5	11.238a	10.921ab	9.249b
6	13.773a	11.618a	10.619b
7	14.076a	13.776ab	11.989b
8	15.968a	16.066a	14.294a

[a] Activity values on the same line denoted with different letters differ significantly at the 5% level.

Table 4—Activity estimates from assaying known amounts of OPPME; r = 0.99986 for 0.009, 0.012 and 0.015 units

Added activity (units)	Estimated activity (units)
0.006	0.00294 ± 0.00022 (n = 16)
0.009	0.00898 ± 0.00037 (n = 32)
0.012	0.01205 ± 0.00056 (n = 32)
0.015	0.01495 ± 0.00042 (n = 16)

indicated that relatively small differences existed between low and medium binding plates, and greater between high and medium or low. However statistical calculations could discriminate between low and medium binding plates with about the same frequency as between low and high binding plates. Analysis of variance of each enzyme level indicated significant differences in all but the lowest and highest enzyme concentrations. Applying individual comparisons at each level showed that in most cases the low and medium binding capacity plates were significantly different from the high binding plate (Table 3). The anomalous results observed were probably due to variations in individual standard errors.

Accuracy and applicability of activity estimates

Titrimetric assays of OPPME were conducted to determine the accuracy of the manufacturers reported activity for the commercial enzyme preparation. The manufacturer reported that it contained 295 units · mL^{-1}. Titrimetric estimates were in close agreement giving a value of 288.6 ± 11.21 units · mL^{-1} (n = 16). Colorimetric assay of 0.006, 0.009, 0.012, and 0.015 units of OPPME indicated that the microplate assay was accurate between 0.009 and 0.015 units of enzyme (Table 4). Higher concentrations could be assayed but were limited by the rapidity of the change in optical density. The activity estimate obtained by assaying 0.006 units was only half the predicted value (0.0029 units). The reason for loss of accuracy at very low enzyme levels is unknown but may be due to the low buffering capacity of the pH indicator and pectin. Also, an increase in dissolved CO_2 concentration from plate shaking may contribute.

To assay PL and Pase, solutions of 0.005 U µL^{-1} (PL) and 0.0012 U· µL^{-1} (Pase) were prepared. Units of PL and Pase were calculated on the basis of the liberation of galacturonic acid from polygalacturonic acid. PME assays indicated that both enzyme preparations contained low levels of PME activity (Pase = 2.959 × 10^{-4} ± 0.084 × 10^{-4} µEq acid · min^{-1} · µL^{-1}, n = 36; PL = 8.126 × 10^{-4} ± 0.281 × 10^{-4} µEq acid min^{-1} · µL^{-1}, n = 36). These values are equivalent to 0.163 units of PME per unit of PL and 0.245 units PME per unit Pase. After chromatography with Q-Sepharose Baldwin and Pressey (1989) reported values equal to 0.086 units of PME per unit PL. The discrepancy between the values we found and those calculated from data in Baldwin and Pressey (1989) possibly were due to batch-to-batch variations in PL (E.A. Baldwin, personal communication).

CONCLUSIONS

A COLORIMETRIC ASSAY for PME activity has been adapted successfully to a kinetic microplate reader. The method was accurate and applicable to commercial PMEs. The sensitivity of the assay was slightly improved. As little as 0.009 units of PME could be measured accurately. Statistical analysis of measured activity levels showed significant differences between low and medium vs. high protein binding efficiency plates, with high binding plates showing lower activity estimates. The assay requires very small volumes of substrate and enzyme. The microplate format allows extensive replication making statistical treatment possible. Adaptation of the colorimetric PME assay to a kinetic microplate reader saves time. Once familiar with the procedure, 24 samples could be assayed, with each sample replicated three times, in less than 1 hr.

REFERENCES

Baldwin, E.A. and Pressey, R. 1989. Pectic enzymes in pectolyase. Separation, characterization, and induction of ethylene in fruits. Plant Physiol. 90: 191.
Boccara, M. and Chatain, V. 1989. Regulation and role in pathogenicity of *Erwinia chrysanthemi* 3937 pectin methylesterase. J. Bacteriol. 171: 4085.
Fox, J.D. and Robyt, J.F. 1991. Miniaturization of three carbohydrate analyses using a microsample plate reader. Anal. Biochem. 195: 93.
Hagerman, A.E. and Austin, P.J. 1986. Continuous spectrophotometric assay for plant pectin methyl esterase. J. Agric. Food Chem. 34: 440.
Koch, J.L. and Nevins, D.J. 1990. The tomato fruit cell wall. II. Polyuronide metabolism in a nonsoftening tomato mutant. Plant Physiol. 92: 642.
Michigan State University. 1989. MSTAT-C. A microcomputer program for the design, management and analysis of agronomic research experiments. Michigan State Univ., East Lansing, MI.
Moustacas, A.M., Nari, J., Diamantidis, G., Noat, G., Crasnier, M., Borel, M., and Ricard, J. 1986. Electrostatic effects and the dynamics of enzyme reactions at the surface of plant cells. 2. The role of pectin methyl esterase in modulation of electrostatic effects in soybean cell walls. Eur. J. Biochem. 155: 191.
Nari, J., Noat, G., Diamantidis, G., Woudstra, M., and Ricard, J. 1986. Electrostatic effects and the dynamics of enzyme reactions at the surface of plant cells. 3. Interplay between limited cell-wall autolysis, pectin methyl esterase activity and electrostatic effects in soybean cell walls. Eur. J. Biochem. 155: 129.
Plantner, J.J. 1991. A microassay for proteolytic activity. Anal. Biochem. 195: 129.
Ricard, J. and Noat, G. 1986. Electrostatic effects and the dynamics of enzyme reactions at the surface of plant cells. 1. A theory of the ionic control of a complex multi-enzyme system. Eur. J. Biochem. 155: 183.
Rombouts, F.M. and Pilnik, W. 1978. Enzymes in fruit and vegetable juice technology. Process Biochemistry 13: 9.
SAS Institute Inc. 1987. *SAS/STAT™ Guide for Personal Computers, Version 6 Edition*. SAS Institute Inc., Cary, NC.
Tucker, G.A., Robertson, N.G., and Grierson, D. 1982. Purification and changes in activities of tomato pectinesterase isoenzymes. J. Sci. Food Agric. 33: 396.
Versteeg, C., Rombouts, F.M., Spaansen, C.H., and Pilnik, W. 1980. Thermostability and orange juice cloud destabilizing properties of multiple pectinesterases from orange. J. Food Sci. 45: 969.
Wenzel, F.W., Moore, E.L., Rouse, A.H., and Atkins, C.D. 1951. Gelation and clarification in concentrated citrus juices. I. Introduction and present status. Food Technol. 5: 454.
Ms received 12/3/91; revised 3/4/92; accepted 3/24/92.

Mention of a trademark or proprietary product is for identification only and does not imply a warranty or guarantee of the product by the U.S. Department of Agriculture over other products which may also be suitable.