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Properties and total initial activities of pectin methyl esterase and polygalacturonase in some Egyptian fruits and vegetables

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Abstract

The presence of residual endogenous quality-related fruit and vegetable enzymes in either raw or processed fruit or vegetable products may cause loss of quality during processing or storage. The content and properties of such enzyme in these raw sources vary widely with type, species, and variety of the sources and the nature of the environment in which they grew. This study was carried out to determine the optimum conditions (pH and temperature) and total initial activities of two quality-related hydrolysis enzymes: pectin methyl esterase (PME) and polygalacturonase (PG) in three fruit varieties, mango (*Mangifera indica* var. Zebda), banana (*Musa cavendishii* var. Enana) peel and pulp, olive (*Olea europaea* var. Picual) and three vegetables, green beans (*Phaseolus vulgaris* var. Littel Marvel), tomatoes (*Lycopersicon esculentum* var. Marmand) and cucumber (*Cucumis sativus* var. Ria) grown in Egypt. The PME and PG activities were detected in the six examined homogenates. The optimum pH and temperature values for high enzyme activities were varied according to the type of fruits and vegetables. The highest amount of PME was found in tomato extract (0.037 unit mg^{-1} protein of the sample) and the lowest amount was for mango (0.008 unit mg^{-1} protein). However, the green beans extract had the lowest content of PG whereas the highest content was for cucumber.

Keywords: Fruits, Vegetables, Pectin methyl esterase, Polygalacturonase

1. Introduction

Food texture is a major determinant characteristic of consumer acceptance and preference for foods and beverages [1]. Furthermore, texture is the basic characteristic of fruits and vegetables that is most directly related to product losses in the industry and one of the main reasons for consumer rejection [2]. The postharvest losses of fruits and vegetables in the developing countries account for almost 50% of the production because of excessive softening. The softening that accompanies ripening of fruits exacerbates damage during shipping and handling process. It plays a major role in determining the cost factor, because it has a direct impact on palatability, consumer acceptability, shelf-life, and postharvest disease and pathogen resistance [3]. Texture of plant foods can be attributed to the structure and the chemical composition of the cell wall, the middle lamella and to the turgor generated within living cells by osmosis [4]. In General, reduction in fruit firmness due to softening is accompanied by increased expression of cell wall–degrading enzymes acting up on proteins and carbohydrates [5]. In Payasi *et al. 2009* [6] found that softening is a developmentally programmed ripening process, associated with biochemical changes in cell wall fractions involving hydrolytic processes resulting in breakdown of cell-wall polymers such as cellulose, hemicelluloses and pectin etc.

Various hydrolytic reactions are brought about by polygalacturonase, methyl esterase, pectate lyase, rhamnogalacturonase, cellulase and β-galactosidase etc.

Pectinases are widely distributed in higher plants and microorganisms. Pectic substance is the generic name used for the compounds that are acted upon by the pectinolytic enzymes [7].

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Many pectic enzymes can act on pectic materials causing changes in vegetables and fruits during ripening, harvest, storage, processing and posterior commercialization [8]. Valuable information about the initial activities of endogenous enzymes in Egyptian fruits and vegetables is still lacking. So, this study aimed to evaluate the initial activities and the optimum conditions (pH and temperature) of two hydrolysis enzymes; pectin methyl esterase (PME) and polygalacturonase (PG), the most quality related enzymes in three fruits (banana, mango and olive) and three vegetables (green beans, tomatoes and cucumber) grown in Egypt.

2. Materials and Methods

2.1. Raw materials.

Mango (*Mangifera indica* var. Zebda), banana (*Musa cavendishii* var. Enana), and olive (*Olea europaea* var. Picual) fruits, green beans (*Phaseolus vulgaris* var. Littel Marvel), cucumber (*Cucumis sativus* var. Ria) and tomatoes (*Lycopersicon esculentum* var. Marmand), all at ripe stage were got from Ismailia Governorate local market, Egypt.

2.2. Chemicals and reagents.

All chemicals used in extraction and determination of the enzyme activities were obtained from Sigma–Aldrich (St. Louis, Missouri, USA), while solvents (analytical grade) were from Scharlab (Scharlab Chemie s.a., Barcelona, Spain).

2.3. Preparation of pectin methyl esterase extracts (PME).

PME was extracted according to Yemenicioğlu and Cemeroğlu [9] method. Sliced samples (400-500 g) were homogenized with one liter of deionized water (4 ºC) in a Warring blender for 5 min. The homogenate was filtrated through a five layers of cheese cloth and the remaining residue was washed with four portions of 500 ml of deionized water (4 ºC). The resulting gel-like residue was suspended in 500 ml of 1N NaCl and stirred continuously for 24 h at 4 ºC. This slurry was then filtered through a Buchner funnel containing six layers of cheese cloth. The filtrate was immediately assayed for PME activity.

2.4. Preparation of Polygalacturonase extracts (PG).

The PG extract was prepared according to Abu-Goukh and Bashir [10] method. Samples were washed and diced into 1 cm cubes. Two hundred grams were homogenized in an equal volume of 100 mM sodium acetate buffer (pH 6.0) containing 0.2% sodium dithionite ($Na_2S_2O_4$) and 1% polyvinyl-pyrrolidone (PVPP) for 1 min using a blender. The homogenate was centrifuged at 12,000 xg for 20 min. The residues were suspended in an equal volume of 1M sodium acetate buffer containing 6% NaCl (pH 6.0). The pH of the suspension was adjusted to 6.0, using drops of NaOH (2N). The sample was kept at 4 ºC with continuous stirring for 4 h and then centrifuged. The supernatant was filtered twice using Whatman paper No. 1. The filtrate was dialyzed against distilled water for 48 h with four changes. All operations were carried out in an ice bath. This dialyzed sample constituted the enzyme extract.

2.5. Protein determination.

Protein content of the studied samples was determined spectrophotometrically as described by *Janairo et al*. [11] using Biuret reagent test. For the test sample, 1 ml of the sample extract was added to 4.5 ml of Biuret reagent. The solutions were shaken using a vortex. The color of the solution was observed to be light blue and the absorbance of the protein samples at 545 nm was determined using the UV-VIS spectrophotometer (6505 UV/ VIS, Jenway LTD, Felsted, Dunmow, UK). A calibration curve (R^2=9998) of standard protein solution (bovine albumin, 0.0 – 2.0 mg/ ml) was prepared and tested under similar conditions.

2.6. The pH and temperature optima of the examined enzymes.

***The pH optima*:** The optimum pH for PME and PG activities was determined in the pH ranged from3.5-9.5 using acetate (4.5 - 5.5), phosphate (6.0 - 8.0) and boric-acid-borax (8.5-9.5) buffers adjusted with 0.1M NaOH or 0.1M HCl, using the standard reaction mixture.

***The temperature optima*:** The effect of temperature on the activity of studied enzymes was tested by heating the standard reactions (buffer and substrate) to the appropriate temperatures before addition of the enzyme extract. The activity was assayed at various reaction temperatures controlled by a circulation water bath. The temperature was varied over the range of 10 – 60 ºC. The mixtures of buffer and substrate solution were incubated at the different temperatures. Once temperature equilibrium was reached, the enzyme extract was added to the mixture and the activity was

determined as rapidly as possible at constant temperature.

2.7. Determination of enzyme activities.

***Pectin methyl esterase assay*:** The PME activity was estimated according to the method described by *Alonso et al*. [12] with some modifications. The reaction mixture consisted of 0.5 ml of PME extract and 20 ml of 0.5% citrus pectin solution in 0.1 N NaCl. The pH of the mixture was immediately adjusted to 7.5 by adding 0.1N NaOH and maintained at this value for 5 min by titrating slowly with 0.01 N NaOH. The titration was carried out at 30 ºC and the activity was expressed as unit (U). One unit of PME activity was defined as the amount of enzyme capable of catalyzing the consumption of 1mM of base/ 5 min under assay conditions.

***Polygalacturonase assay*:** The PG activity was examined using the method of *Gross* [13]. Activity of PG was measured spectrophotometrically based on the formation of reducing groups from polygalacturonic acid and quantifying these with 2-cyanoacetamide. The reaction mixture (400 µl) containing 0.2% polygalacturonic acid (350 µl) and enzyme sample (50 µl) were incubated at 40 ºC for 10 min. The reaction was stopped by the addition of 2 ml of cold 10 mM borate buffer, pH 9.0 and 0.4 ml 1% cyanoacetamide. The samples were mixed, immersed in a water bath at 100 ºC for 10 min and cooled immediately in ice. After equilibration at room temperature, absorbance was measured at 276 nm and 22 ºC using spectrophotometer (6505 UV/ VIS, Jenway LTD, Felsted, Dunmow, UK). Blanks were prepared in the same way but using 50 µl of 40 mM Na-acetate buffer (pH 4.4). The amount of reducing groups formed was calculated using a standard curve of mono-D-galacturonic acid, assuming that the concentration of this acid is proportional to the concentration of reducing groups. The PG units were reported in mM mono-D-galacturonic acid per minute at 40 ºC. One unit of enzyme activity was expressed by 1.0 µmoles of galacturonic acid produced per min using galacturonic acid as a substrate.

2.8. Statistical analysis.

All experiments were done in triplicates. Data were expressed as means ± standard deviation (SD) and the coefficient of determinations (R^2) were calculated by SPSS (version 17.0 SPSS Inc., Chicago, IL, USA) program.

3. Results and Discussions

3.1. The optimum pH of the PME and PG activities.

The changes in ionization of prototropic groups in the active site of enzymes at lower acid or higher alkali pH values may prevent proper conformation of the active site, binding of substrates and/or catalysis of the reaction [14,15]. In addition, irreversible denaturation of the protein and/or reduction in stability of the substrate as a function of pH could also influence the catalytic activity of enzymes [16,17]. The effect of pH on activity of the PME enzymes was determined using buffer solutions at pH 5.5 - 9.5 (Figure, 1 A and B). The mango (M) PME showed the highest activity at pH 7.5 (Figure 1-A). Labib *et al.* [18] and Rayan [19] found that the mango PME showed maximum activity at pH 7.5. Banana peel (B:pe) PME also illustrated optimum pH at 7.5 whereas, banana pulp (B:pu) PME showed maximum activity at pH 6.5 (Figure 1-A).

Ly Nguyen et al. [20] studied the effect of pH on banana PME (cv. Cavendish) activity in a pH range of 6.0 to 8.0 using apple pectin solution at 3.5 mg/ ml of 0.117 M NaCl buffer and found that the purified banana PME activity increased to an optimum pH at 7.0. Tomatoes (T), cucumber (C) and Olive (O) PMEs (Figure 1-B) exhibited maximum activities at pH 7.5. These results are in agreement to those obtained by [8,9]. Finally, green beans (G) PME had optimum pH at 6.5 under determination conditions. Anthon and Barrett [21] reported that the maximum activity for G-PME was at pH 7.5.

The effect of pH in the range of 3.5 - 7.5 on the PG activity in the mentioned fruits and vegetables was considered (Figure 2 A and B). While PGs extracted from M, B:pe, B:pu, T and O exhibited maximum activities at pH 4.5. The PG of C and G displayed maximum activities at pH 5.0 (Figure 2-B). As in literature, PG showed maximal activity in the acidic medium at pH optima in the range of 4.0-5.5 for different fruits and vegetables [19, 22, 23, 24, 25, 26, 27].

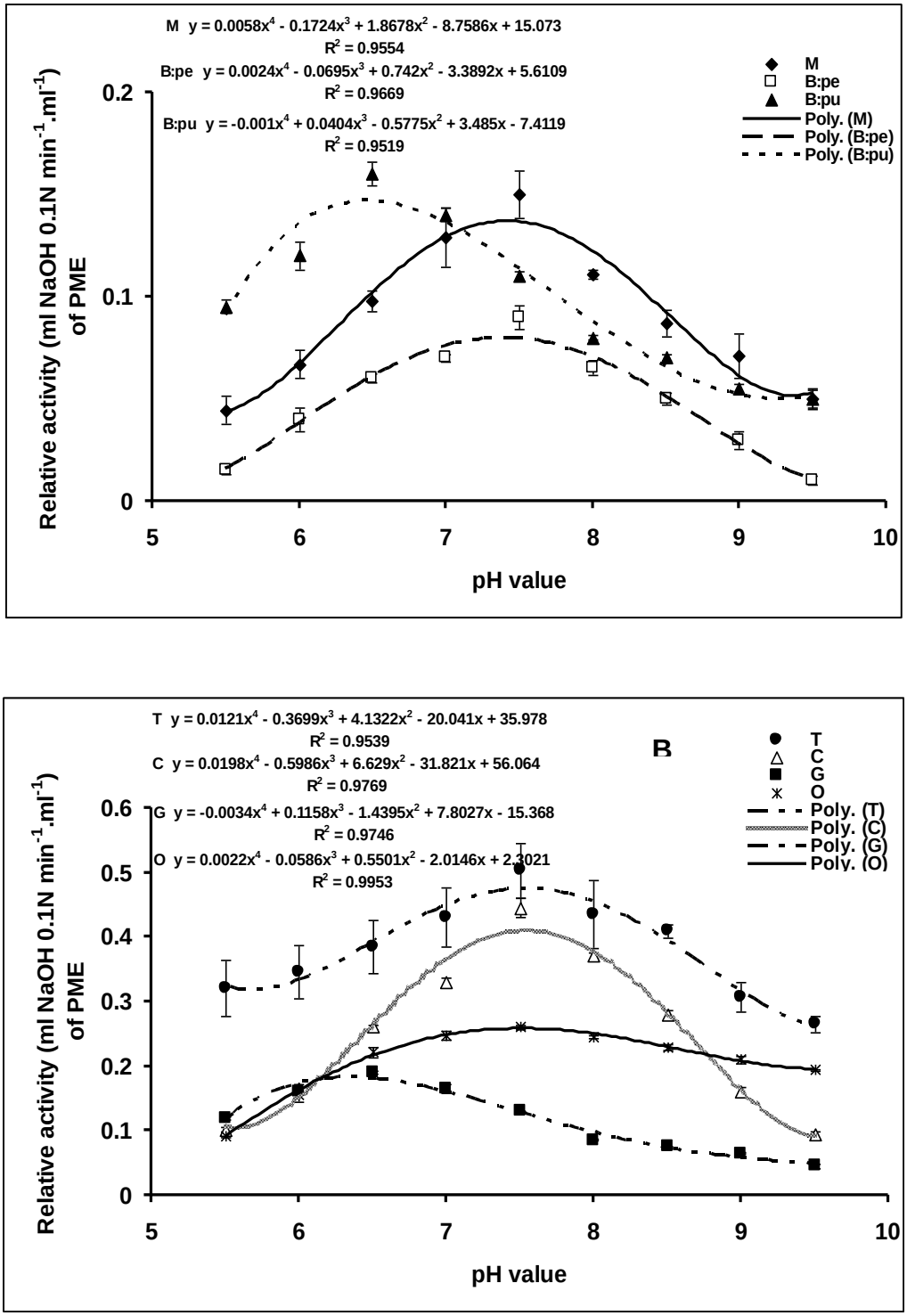
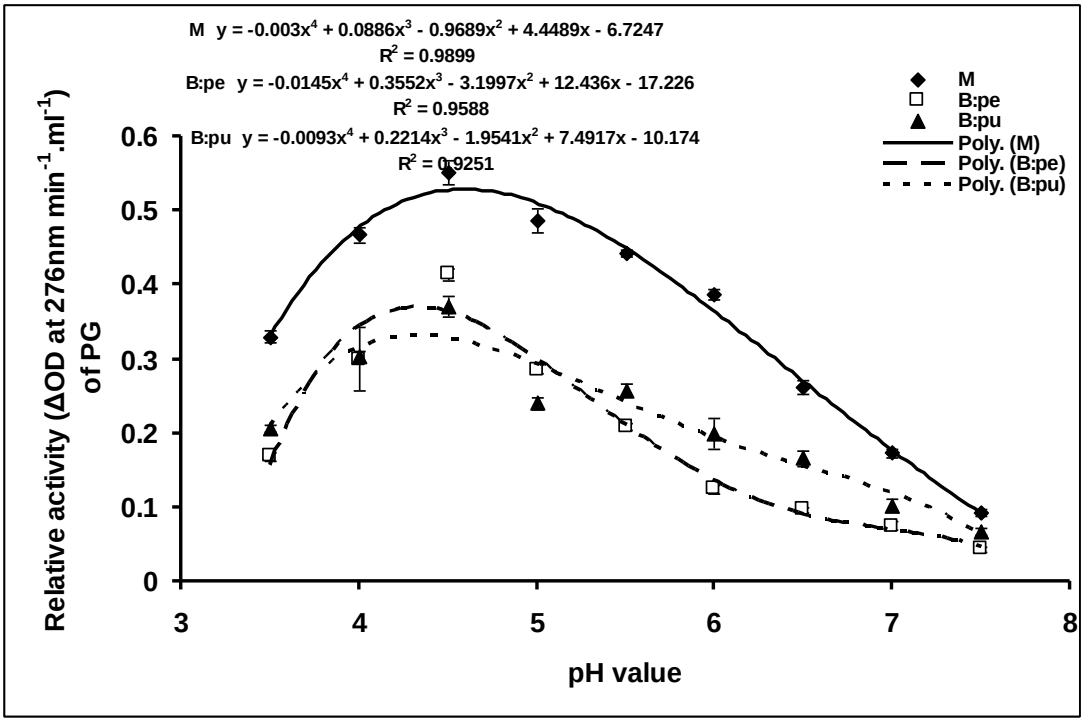


Figure 1. pH profiles of PME activity for investigated samples in 0.1N NaCl solution (pH 5.5 – 9.5). The reaction medium containing 500 mg/ 20 ml pectin and 0.5 ml of enzyme extract. The activities calculated in the form of relative activity of enzyme at the optimum temperature
M, mango; B:pe, banana peel; B:pu, banana pulp; T, tomatoes; C, cucumber; G, green beans; O, olive extracts



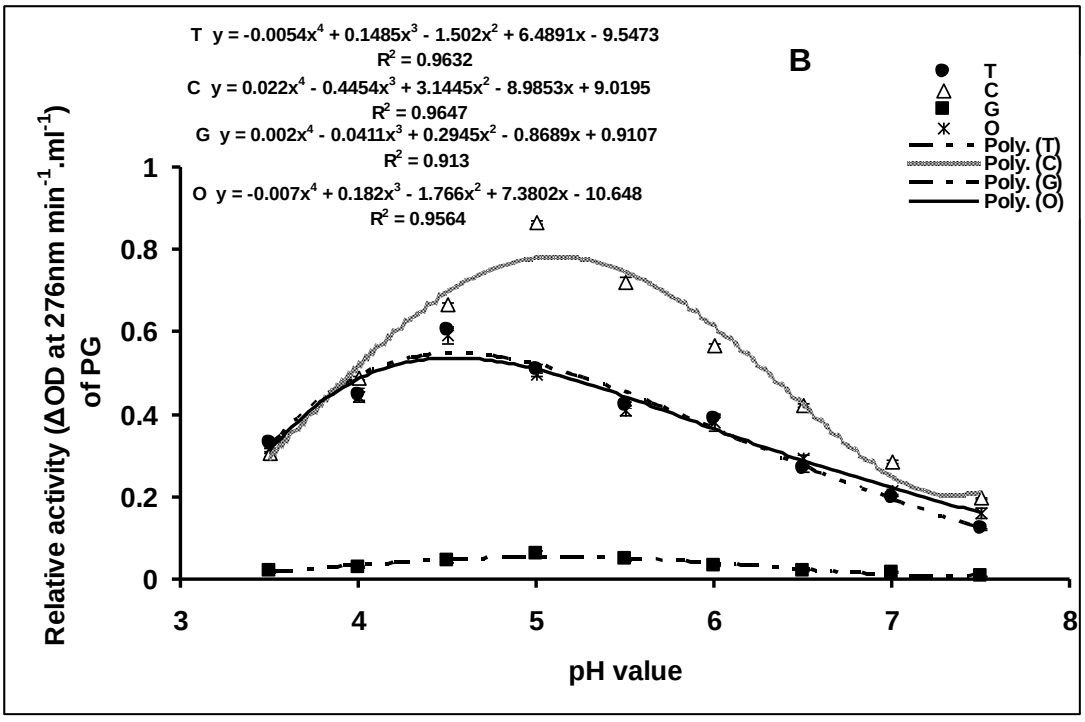


Figure 2. pH profiles of PG activity for investigated samples in 10mM acetate buffer (3.5 - 5.5) and 10mM phosphate buffer (6 – 7.5). The reaction medium containing 0.35ml of 3.28mM polygalacturonic acid and 0.05ml of enzyme extract. The activities calculated in the form of relative activity of enzyme at the optimum temperature
M, mango; B:pe, banana peel; B:pu, banana pulp; T, tomatoes; C, cucumber; G, green beans; O, olive extracts

3.2. The optimum temperature of the PME and PG activities.

Temperature is another important factor that significantly influences the catalytic activity of enzymes. It is well known that a decrease in the kinetic energy of the reactant molecules at low temperatures corresponds to a slower reaction [28]. In addition, integrity of the delicate three-dimensional structure of the enzyme molecule is subjected to disruption and denaturation at high temperatures [15]. The effect of temperature on PME activity in the investigated fruits and vegetables is illustrated in Figure (3 A and B). Mango PME displayed maximal activity at 40 ºC (Figure 3-A). *Labib et al*. [18] and *Rayan* [19] found that the optimum temperature of PME extracted from mango was 50 ºC. Generally optimum temperature of an enzyme is affected like other properties by the plant source and the substrate used in the assay. The B:pe and B:pu-PME had optimum temperature at 30 and 25 ºC, respectively (Figure 3-A). Higher maximal activity value for banana pulp PME was reported at 35 ºC [20]. Figure (3-B) showed the effect of temperature on T-PME and C-PME activity. The two enzymes were found to be most active between 25 and 35 ºC with the optimum temperature at 30 ºC. Giner *et al*. [8] reported an optimum temperature at 25±1 ºC for tomatoes PME. *Yemenicioğlu and Cemeroğlu* [9] determined the optimum temperature of ionically (IPME) and tightly bound PME (TPME) activities extracted from cucumber. They found that the optimum temperatures for the two enzymes (for a 5-min titration period) were 65.0 ºC and 60.0 ºC, respectively. At last, Figure (3-B) demonstrates that PME enzyme showed optimum temperature for maximum activity at 25 and 30 ºC from green beans and olive fruits, respectively. Anthon and Barrett [21] reported that the PME activity from green beans was constant between 30 to 60 ºC.

Regarding polygalacturonase activity, the optimum temperature of M-PG was at 30 ºC (Figure 4-A). Similar results were obtained by [18,19]. However, *Prasanna et al*. [25] estimated the optimum temperature for three PG isozymes isolated from mango, which was around 40 ºC for all the three isoforms. For B:pu-PG and B:pe-PG, the maximum activity was at 40 ºC (Figure 4-A). This result resembled to that obtained by *Pathak et al*. [24]. They found that the optimum temperature of banana PG pulp was 40 ºC. The T-PG enzyme (Figure 4-B) showed optimum temperature at 40 ºC. These results are in agreement with those obtained by *Rodrigo et al*. [26]. They observed that the crude tomatoes PG had maximum activity at temperature ranged from 25 – 55 ºC. Also, the same diagram displays that C-PG reached its highest activity at 30 ºC followed by a sharp decline at temperature above it. Further, Figure (4-B) indicates that, G-PG had maximum activity at 40 ºC, while O-PG showed optimum temperature at 30 ºC. Optimal values of temperature 30 ºC with pectin as a substrate have also been reported [27].

3.3. Total initial activities of the PME and PG enzymes.

The PME activities were calculated for the six sources (Table 1). From these values it is clear that the tomatoes (T) extract had the biggest initial PME activity (0.037 unit mg^{-1} protein) followed by cucumber (0.033 unit mg^{-1} protein). *Gaffe et al.* [29] found that the PME specific activity continued to increase during tomato fruits development until the mature green stage of fruit development before declining slightly during fruit ripening. The tomato ripe fruits had a specific PME activity about 170 mmole/ min/ g protein. The mango extract had the lowest PME activity (0.008 unit mg^{-1} protein) compared with other extracts (Table 1). Banjongsinsiri [33] mentioned that PME from mango showed low activity compared with many fruits and vegetables. Also, *Kermani et al.* [30] showed that the mango puree had lower PME activity (12 unit/ g puree) than that for tomatoes (85 unit/ g).

Synopsis of PG activities in the investigated fruits and vegetables is introduced in Table (2). The highest amount of PG was found in cucumber (C) extract (0.576 unit mg^{-1} protein), then tomatoes (T) extract came after with total activity of 0.321 unit mg^{-1} protein. The lowest activity was for green beans (G) extract (0.052 unit mg^{-1} protein). *Stolle-Smits et al.* [31] showed that the green beans PG activity was low during the development stages of green beans pod (0.010 – 0.020 nanokatals/ mg protein). Cervone *et al*. [32] claimed that PG from *Phaseolus vulgaris* hypocotyls appears to occur predominately as an endo acting enzyme. They also found a protein from the same plant that binds with PG causing its inhibition, which explain the relatively low amounts and activities of green beans PG.

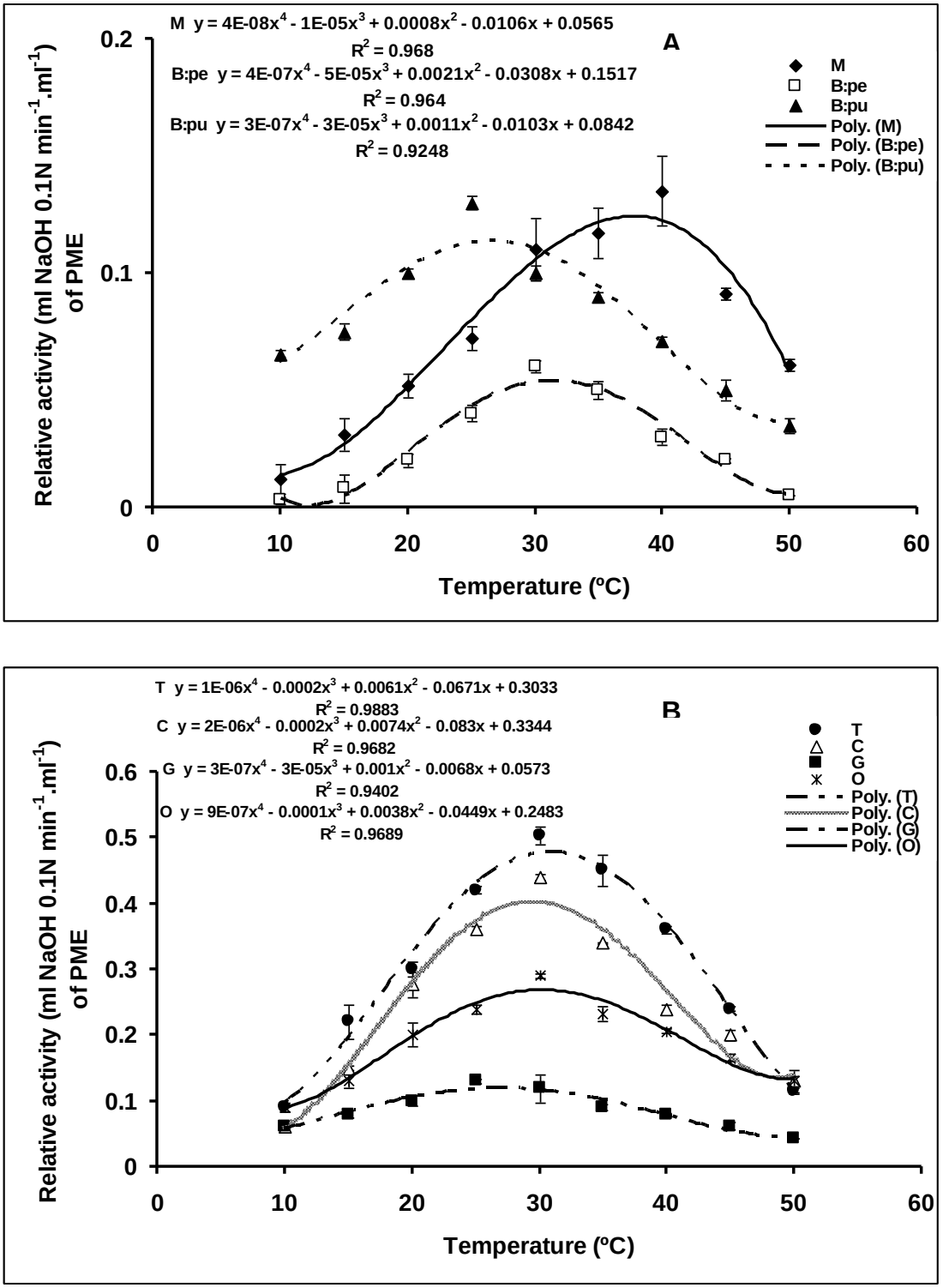


Figure 3. Effect of temperature on PME activity of investigated samples at optimum pH value for each. The reaction medium containing 500 mg/ 20 ml pectin and 0.5 ml of enzyme extract. The activities calculated in the form of relative activity of enzyme at the optimum pH

M, mango; B:pe, banana peel; B:pu, banana pulp; T, tomatoes; C, cucumber; G, green beans; O, olive extracts

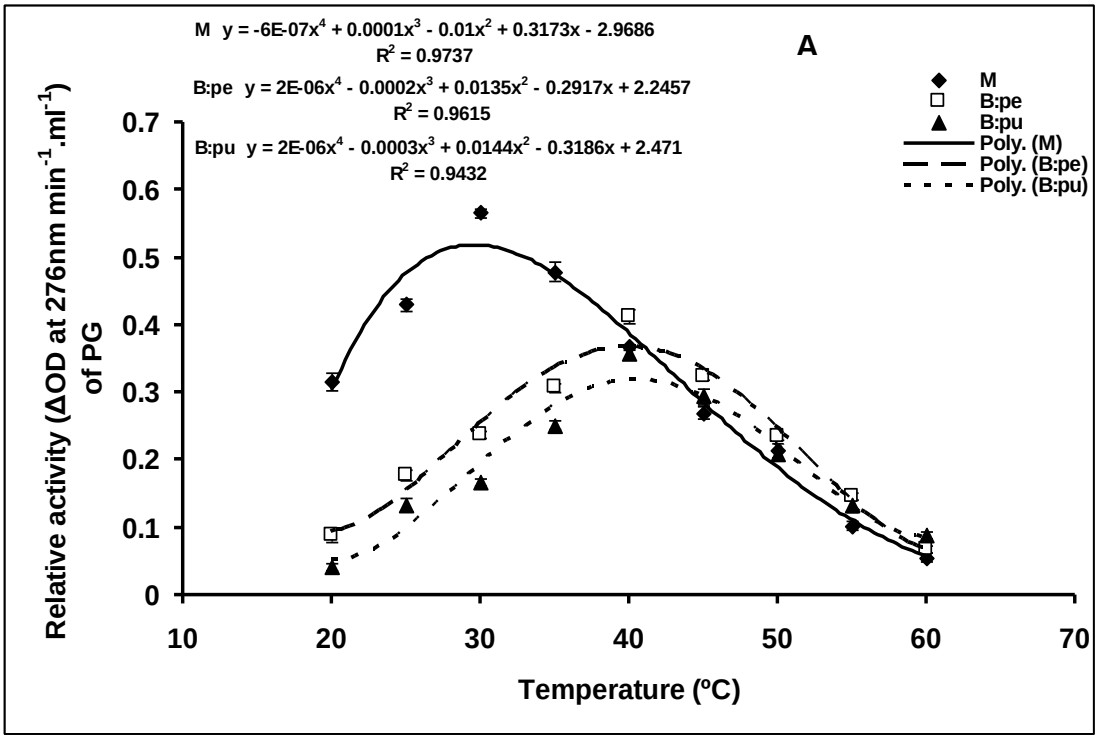


Figure 4. Effect of temperature on PG activity of investigated samples at optimum pH value for each. The reaction medium containing 0.35ml of 3.28mM polygalacturonic acid and 0.05 ml of enzyme extract. The activities calculated in the form of relative activity of enzyme at the optimum pH

M, mango; B:pe, banana peel; B:pu, banana pulp; T, tomatoes; C, cucumber; G, green beans; O, olive extracts

Table 1. Summary of PME activities* in the investigated fruits and vegetables

Fruits and vegetables	PME activity**		
	Relative activity (ml 0.01N NaOH min^{-1}. ml^{-1})	**Total activity (Unit 100 g^{-1})**	**Specific activity (unit mg^{-1} of protein)**
Mango (M)	0.015	0.187	0.008
Banana (B):			
Peel (pe)	0.090	0.106	0.010
Pulp(pu)	0.160	0.215	0.012
Tomatoes (T)	0.503	0.995	0.037
Cucumber (C)	0.445	0.795	0.033
Green beans (G)	0.190	0.190	0.015
Olive (O)	0.260	0.290	0.027

* Means of three replicates

** All reactions have been carried out under the optimum conditions for the enzyme

Table 2. Summary of PG activities* in the investigated fruits and vegetables

Fruits and vegetables	PG activity**		
	Relative activity (ΔOD min^{-1}. ml^{-1})	**Total activity (Unit 100 g^{-1})**	**Specific activity (unit mg^{-1} of protein)**
Mango (M)	0.574	0.033	0.218
Banana (B):			
Peel (pe)	0.414	0.015	0.129
Pulp(pu)	0.370	0.026	0.102
Tomato (T)	0.614	0.026	0.321
Cucumber (C)	0.870	0.028	0.576
Green beans (G)	0.080	0.004	0.052
Olive (O)	0.566	0.032	0.290

* Means of three replicates

** All reactions have been carried out under the optimum conditions for the enzyme

4. Conclusion

The content and properties of PME and PG enzymes in raw sources vary widely with type, and variety of the sources and the environment conditions in which they grew. The optimum pH and temperature values for high PME and PG enzyme activities were varied according to the type of fruits and vegetables grown in Egypt. Tuuhe highest amount of PME was found in tomatoes extract compared with the other homogenates. For PG, the highest content was for cucumber while the green beans extract had the lowest content.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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