GC-ECD and GC-MS Analyses of Profenofos Residues and Its Biochemical Effects in Tomatoes and Tomato Products

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GC-ECD and GC-MS analyses of profenofos residues in tomatoes and tomato products were carried out. The effects of the pesticide on some enzyme systems (transaminases and oxidoreductases) and quality attributes of tomato fruits were also studied. The results showed that the GC-ECD analysis allowed an accurate determination of profenofos residues. GC-MS analysis was less susceptible to interfering compounds than the GC-ECD analysis. In addition, the GC-MS technique allowed the identification of the studied pesticide and ensured that profenofos extraction and cleanup steps were acceptable. The fresh tomatoes had considerable amounts of profenofos (26.6-8.7 ppm) depending on the time from pesticide application. Washing tomatoes with tap water resulted in about 15-30% reduction of the pesticide residues. More residues (89%) were removed as the treated tomatoes were processed into tomato juice. Moreover, the pesticide residues did persist in tomato puree (1.57 ppm) and tomato paste (1.16 ppm). Transaminases were differently affected by profenofos residues. While glutamic-pyruvic transaminase was inhibited by the residues, glutamic-oxaloacetic transaminase was stimulated. In addition, peroxidase and polyphenol oxidase activities were induced. The apparent K_m of the profenofos-treated tomatoes was lower (1.410×10^{-4} M) than that (3.891×10^{-4} M) of the untreated tomatoes. Consequently, an increase of the V_{max} was observed (7.353 units) for polyphenol oxidase in the profenofos-treated tomatoes as compared to that (4.629 units) of the untreated tomatoes. The pesticide treatment increased the total soluble solids and acidity contents but decreased the glucose, protein, and ascorbic acid contents of tomatoes.

INTRODUCTION

Heavy use of pesticides on field crops has begun to receive much attention. Many researchers have studied how to remove pesticides from food products (Geisman, 1975; Zidan et al., 1991; Lee et al., 1991). In Egypt, profenofos (Curacron), [*O*-(4-bromo-2-chlorophenyl) *O*-ethyl *S*-propyl phosphorothioate] is a widely used organophosphorus insecticide for the control of various caterpillars, white fly, and mites on cotton and vegetable crops. Some reports have focused on the toxicological aspects of profenofos (Buholzer, 1975; Leader and Casida, 1982; Ghazal et al., 1984; Habiba and Ismail, 1992). Others have looked at the residue analysis and the persistence of this pesticide (Ahmed and Moursy, 1991; Ismail et al., 1991; Habiba et al., 1992). More research is needed to evaluate the effect of preparation and processing methods on the elimination of this pesticide from agricultural commodities.

The aims of this work include (1) studying the persistence of profenofos in tomatoes after its application on a tomato field, to determine if a safety period (time between pesticide application and consumption of tomato fruits) can be established, (2) investigating the effects of the pesticide on some enzymes and quality attributes of tomatoes, and (3) evaluating the effect of some preparative and processing methods on the elimination of this pesticide from tomatoes and tomato products.

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MATERIALS AND METHODS

Field Experiment. Tomato seedlings (var. Super Marmand) were planted at the Experimental Station of the College of Agriculture, Suez Canal University, Ismailia, Egypt. The seedlings were transplanted in the permanent sandy soil in October 1990. Rows were spaced at 0.5 m, with 1 m between the rows in a complete randomized block design. Common cultural and fertilization practices were followed except that a drop irrigation system was used. Profenofos formulated as Selecron 72% emulsifiable concentrate was applied at the manufacturer's (Ciba-Geigy) and The Egyptian Ministry of Agriculture's recommended rate (0.75 L/Feddan = 520 g of active ingredient/acre). The pesticide was applied three times during the season with a 21-day interval between each. The third application was performed 1 h before harvesting. A blower sprayer fitted with one nozzle boom was used. Three replicates were sprayed; the untreated control plots were left unsprayed.

Sampling. Tomato fruits were harvested at firm red maturity stage after 1, 2, 3, and 4 weeks from the final application of profenofos. In addition, a sample of tomatoes was taken 1 h after pesticide spraying to determine the initial deposits of the pesticide residues. Each sample was divided into three subsamples. The first one was used for residue analysis; the others were used for processing and biochemical studies.

Processing. Tomato juice, puree, and paste were made from the treated tomatoes. To prepare tomato juice, the tomato fruits were washed with tap water and crushed in a strainer, and the juice was placed in a cleaned bottle. The juice was subjected to preliminary heating process at 90 °C for 3 min. The heated juice was then transferred into glass containers, heated at 100 °C for 30 min, and then cooled by running water. The juice, obtained as above, was then concentrated at two temperatures (70 and 100 °C) until the total soluble solids (TSS) reached 17% (which was considered tomato puree) and 28% (regarded as tomato paste with the addition of 2.5% NaCl). The puree and paste were hot-filled in glass containers, heated in a boiling water bath at 100 °C for 20 min, sealed, inverted, allowed to stand for several minutes, and cooled.

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1. Residue Analysis. Profenofos residues in fresh tomatoes (tap water washed and unwashed), tomato juice, puree, and paste were determined.

1.1. Extraction and Cleanup. Tomato samples, 25 g (fresh or processed), were homogenized (1:3 w/v) with a mixture of hexane/acetone (1:1) using a Virtus 23 mechanical homogenizer. To clean up the resultant homogenate, charcoal and anhydrous sodium sulfate were added, and the homogenate was filtered through Whatman No. 1 filter paper. The filter cake was extracted twice more with the same solvent mixture. The filtrates were combined and evaporated to dryness using a rotary evaporator and then transferred quantitatively in 1 mL of *n*-hexane into small glass vials ready for analysis.

1.2. GC-ECD and GC-MS Analyses. 1.2.1. GC-ECD Analysis. A Shimadzu GC-12A gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD) was used for all samples. The glass column employed was packed with 3% silicone XE-60 supported on 60/80 mesh Chromosorb W-AW. Deoxygenated nitrogen was used as a carrier gas at 15 mL min^{-1} flow rate. Column temperature was isothermal at 170 °C. Injector and detector temperatures were maintained at 260 and 280 °C, respectively. Each sample was diluted to the suitable concentration with *n*-hexane, and then 1 μL of this solution was injected into the instrument. The chromatographic response was calibrated by injecting a range of profenofos standards in *n*-hexane. The resulting linear regression of profenofos concentrations vs corresponding peak areas was used to calculate the amount of profenofos residues in (parts per million) in each sample. Results were corrected according to the recovery percentages obtained from fortified untreated samples (about 85%).

1.2.2. GC-MS Analysis. A Shimadzu GCMS-QP1000 EX instrument operating in the electron impact mode (EI) and equipped with a glass column packed with 1.5% OV-17 coated on 80/100 mesh Chromosorb W-AW was used. Column temperature was isothermal at 200 °C, while the injector and detector temperatures were 240 and 260 °C, respectively. Full-scan EI mass spectra (*m/e* 20–1030, 1 s/scan) were recorded for peak identification.

2. Biochemical Studies. Fresh treated and untreated tomato samples were homogenized at 4 °C with (1:4 w/v) 0.2 M sodium phosphate buffer, pH 7.0, for polyphenol oxidase (PPO) and transaminases extraction and with 0.01 M sodium acetate buffer, pH 5.0, for peroxidase extraction. The homogenates were filtered, and the filtrates were centrifuged at 4000 rpm for 20 min at 4 °C. The supernatants were used for enzyme assay.

2.1. Enzyme Assay. An assay was established where the initial rate of the enzyme-catalyzed reaction was proportional to the concentration of the enzyme extract.

2.1.1. PPO Assay. Enzyme activity was measured according to the method of Benjamin and Montgomery (1973). The reaction mixture was prepared using 0.2 M phosphate buffer, pH 7.0, and 10 mM catechol (2:1). Then, 0.1 mL of the enzyme extract was added to 2.9 mL of the reaction mixture at 30 °C. The increase in absorbance at 400 nm was recorded for 3 min. One unit of enzyme activity was defined as the amount of enzyme that caused a change in absorbance of 0.001/min.

2.1.2. Peroxidase Assay. Peroxidase activity was measured according to the procedure of Lu and Whitaker (1974). The enzyme extract (0.1 mL) was added to 2.9 mL of freshly prepared reaction mixture consisting of 0.01 M acetate buffer, pH 5.0; 0.1 M guaiacol; and 0.3% H_2O_2 (4:2:1). The reaction was carried out at 30 °C, and the increase in absorbance at 470 nm was recorded for 3 min. One unit of enzyme activity was defined as the amount of enzyme that caused a change in absorbance of 0.001/min. Specific activity was calculated as units of enzyme activity per milligram of protein under the assay conditions.

2.1.3. Glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) activities were measured according to the method of Reitman and Frankel (1957).

2.2. PPO K_m and V_{max}. The Michaelis–Menten constant (K_m) and maximum velocity (V_{max}) of PPO were calculated from Lineweaver–Burk plots for the profenofos treated and the untreated tomato enzyme extracts. The enzyme activity was determined under the assay conditions except that the substrate concentration was varied over the range 0.02–20 mM.

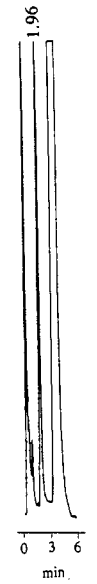

Figure 1. GC-ECD chromatogram for profenofos residues in a fresh washed tomato sample 1 week after pesticide application. The retention time for profenofos is 1.96 min.

3. Determination of Some Quality Attributes. 3.1. Protein. The protein content of tomatoes was determined by the dye binding method according to the method of Bradford (1976).

3.2. Glucose. The glucose content of treated and untreated tomato samples was measured according to the methods of Peterson and Young (1968) as mentioned in Sigma Technical Bulletin No. 15 U.V. Glucose is converted to glucose 6-phosphate by hexokinase in the presence of adenosine triphosphate (ATP), coupled with a subsequent reduction of nicotinamide adenine dinucleotide phosphate (NADP) to the reduced form (NADPH). The increase in absorbance at 340 nm due to the formation of NADPH is directly proportional to the amount of glucose present.

3.3. Ascorbic Acid, TSS, Acidity, and pH. Ascorbic acid (vitamin C), TSS, acidity, and pH of the treated and untreated tomatoes were determined as set forth by the AOAC (1984).

RESULTS AND DISCUSSION

Residue Analysis. It has been reported that gas chromatography with electron capture detector (GC-ECD) is greatly sensitive to halogenated compounds (McNair and Bonelli, 1969; Eberbach and Douglas, 1991); in addition, it is a more common analytical technique than GC–MS analysis. Figure 1 shows a typical GC-ECD chromatogram of profenofos residues in a fresh washed tomato sample 1 week after pesticide application. As shown in the chromatogram, the retention time for profenofos was 1.96 min, and it was well resolved from other eluting compounds in the chromatogram. This allowed accurate determination of profenofos residues in all samples.

The profenofos residues in fresh unwashed tomatoes ranged from 26.6 to 8.7 ppm 1 h and 4 weeks after application, respectively (Table I). These results are higher than those of Rady (1991). This could be due to the differences in the number of profenofos applications in each study. In this investigation, the studied pesticide was applied three times to be as close as possible to the normal practice of most farmers in Egypt, who use profenofos mainly for white fly control. Washing the treated tomatoes with tap water decreased the profenofos residues between 22.9 and 12.6% depending on time from pesticide application to fruit harvest. These findings are in agreement with those of Ahmed and Moursy (1991). The results obtained indicate that even after washing with water, the fresh tomatoes still contained unacceptably high

Table I. Profenofos Residues in Fresh Tomatoes after Different Time Intervals from Its Application on Tomato Field As Affected by Water Wash

time from application	profenofos residues,[a] ppm		
	unwashed	washed	% reduction
1 h (initial)	26.6 ± 1.6	20.5 ± 1.2	22.9
1 week	23.6 ± 1.1	19.3 ± 0.9	18.2
2 weeks	19.4 ± 0.8	16.5 ± 0.9	14.5
3 weeks	14.3 ± 0.6	12.4 ± 0.5	13.3
4 weeks	8.7 ± 0.4	7.6 ± 0.3	12.6

[a] Results are expressed as means ± standard deviation for three determinations of profenofos residue levels for each tomato sample. Results were corrected according to the recovery percentages obtained from fortified untreated samples (84.65–85.44%).

Table II. Effect of Some Processing Methods on the Level of Profenofos Residues in Tomato Products

attribute	profenofos residues,[a] ppm	% reduction[b]
tomato juice, fresh	1.57 ± 0.08	89.0
tomato juice, processed	1.16 ± 0.05	91.9
tomato puree (17%, 70 °C)	3.18 ± 0.19	77.8
tomato puree (17%, 100 °C)	2.40 ± 0.14	83.2
tomato paste (28%, 70 °C)	1.36 ± 0.07	90.5
tomato paste (28%, 100 °C)	1.07 ± 0.04	92.5

[a] Results are expressed as means ± SD for three determinations of profenofos residue levels for each attribute. Results were corrected according to the recovery percentages obtained from fortified untreated sample (84.09–85.87%). All tomato samples were taken 3 weeks after the last application of profenofos. [b] Based on 14.3 ppm of profenofos residues in the treated fresh tomatoes.

levels of profenofos residues, which are likely to cause health hazards to consumers. This is supported by the toxicological studies by Ghazal et al. (1984), who concluded that profenofos exerts an inhibitory effect on both adrenergic and cholinergic transmission as well as its direct inhibitory effect on the smooth muscles. Also, Habiba and Ismail (1992) stated that direct and indirect contamination of edible crops by profenofos may alter normal metabolism and cause elevated concentration of undesirable compounds in animal tissues.

The level of profenofos residues was greatly reduced (89%) in the juice made from treated tomatoes taken 3 weeks after the final application of profenofos (Table II). The fresh and processed tomato juices contained 1.57 and 1.16 ppm of profenofos, respectively, as compared to 14.3 ppm in the treated fresh tomatoes. This means that the pesticide was mainly eliminated by removing tomato peels, seeds, and other remnants during juice making. The heat treatment also decreased the profenofos residues, but this was not the major cause of pesticide reduction in tomato juice as compared to fresh tomatoes. Similar results were reported (Kamil, 1987) for dimethoate residues, which were reduced by 92–97% during tomato juicing, i.e., washing and straining.

Tomato puree and paste made from tomatoes harvested from a profenofos-treated tomato field contained profenofos residues between 1.07 and 3.18 ppm depending on temperature (70 vs 100 °C) and degree of concentration (17 vs 28%). As the temperature used in the concentration process increased, the level of profenofos residues decreased. Moreover, as the time of concentration increased (to reach higher TSS), the pesticide residues decreased, with the temperature being constant (Table II). The technological processes involved in the preparation of tomato puree decreased the profenofos residues by about 80% relative to those of unwashed fresh tomatoes. However, the puree still contained considerable amounts of the studied pesticide (2.4–3.18 ppm). In addition, the

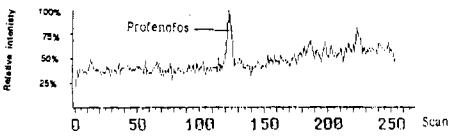

Figure 2. GC–MS chromatogram for profenofos residues in a fresh washed tomato sample 1 week after pesticide application.

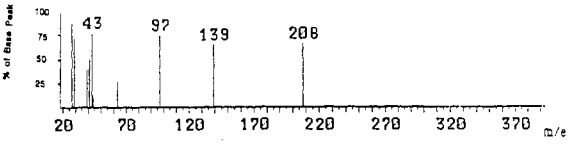

Figure 3. Mass spectrum (EI) of profenofos separated from a fresh washed tomato sample 1 week after pesticide application.

processes for tomato paste preparation effectively reduced profenofos residues (1.36–1.07 ppm) by 90.5–92.5%. The above results were in agreement with those of Rady (1991), who reported that steps for tomato paste preparation reduced profenofos residues by 79.19–89.37%. Moreover, Kamil (1987) found that processes for making tomato paste reduced dimethoate residues 89–95% and actellic residues 97–98%.

EI GC–MS analysis has been performed in comparison to GC-ECD determination of profenofos residues. It was reported (Rutschmann and Buser, 1991) that the GC–MS method in some cases was less susceptible to interfering compounds than electron capture detection. Figure 2 presents the mass chromatogram of profenofos residues in a fresh washed tomato sample 1 week after pesticide application, which can be compared to the GC-ECD chromatogram for the same sample (Figure 1). As can be seen, the number of eluting compounds in the mass chromatogram is smaller than in the ECD chromatogram; nevertheless, the latter still permits accurate measurement with a well-separated peak for profenofos. The EI mass spectrum for the same sample is shown in Figure 3. The base peak at m/e 43 represents the propyl ion $[CH_3CH_2CH_2]^+$. Fragment 139 resulted from McLaffery rearrangement, which could undergo further hydrogen transfer and elimination of propene to give fragment 97 as demonstrated in Figure 4. It is also known (Silverstein et al., 1981) that when the alkyl portion of an aromatic alkyl ether is C_2 or longer, cleavage beta to the ring is accompanied by hydrogen migration as illustrated in Figure 4. The resulting ion has chlorine and bromine atoms with M^{2+} peak at m/e 208.

Biochemical Studies. The effect of profenofos residues on the activities of transaminases (GOT and GPT), peroxidase, and polyphenol oxidase in tomatoes is shown in Tables III and IV.

Transaminases. As shown in Table III, 1 h after profenofos application, GPT and GOT of tomatoes were inhibited by 37.5 and 20%, respectively. However, as the time for the pesticide application increased, GPT and GOT behaved differently. While GPT continued to be inhibited, GOT activity constantly increased until the end of the investigation period (4 weeks). In potatoes, Habiba et al. (1992) reported that profenofos residues induced the level of transaminases. Other studies on profenofos indicated that GOT in the brain, muscle, and kidney tissues of the New Zealand white rabbit fed clover contaminated with drifted profenofos was inhibited over the test period (8 days), while GOT from heart tissue of the same animal was stimulated only over the last 4 days (Habiba and Ismail, 1992). Moreover, El-Gendy et al. (1990) found that pyrazophos and glyphosate also caused spontaneous activation of liver GOT and muscle GPT, while significant

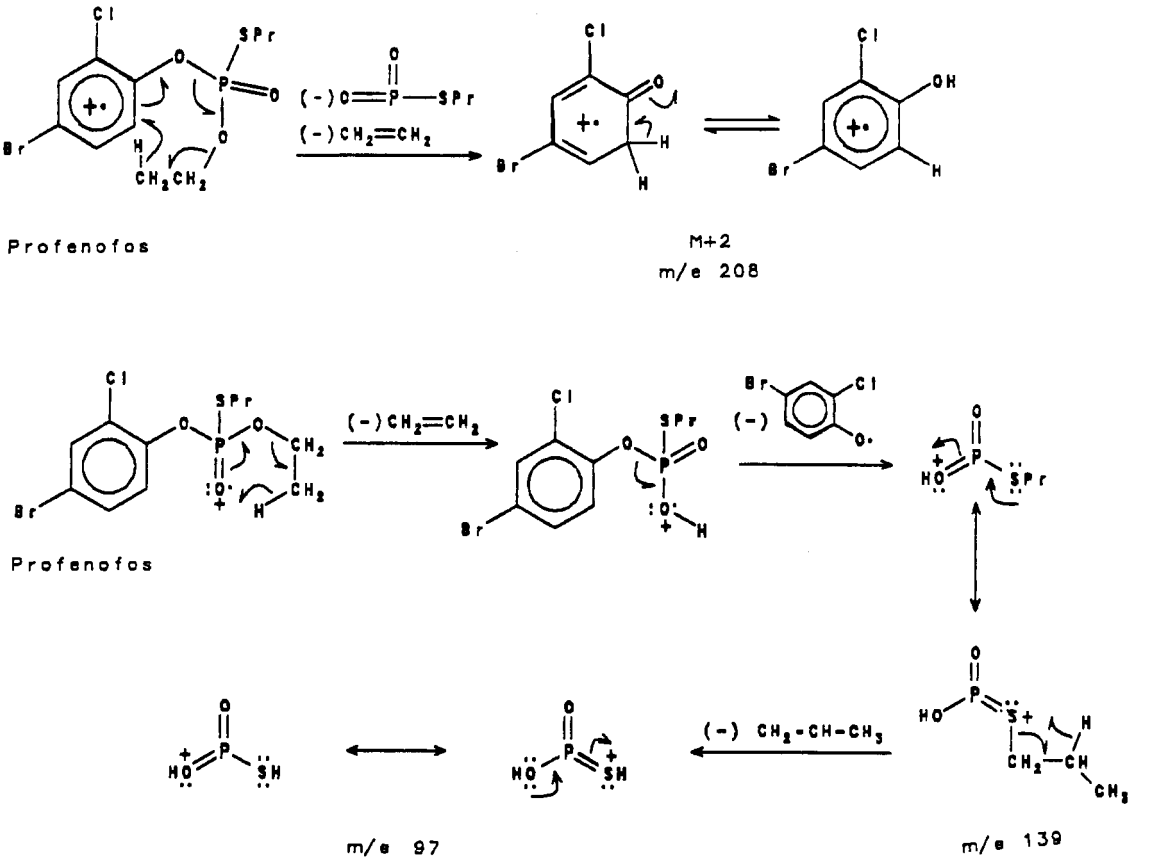

Figure 4. Fragmentation of profenofos by EI-mass spectrometer.

Table III. Effect of Profenofos Residues on the Activity of Transaminases GPT and GOT in Tomatoes

time from treatment	activity,[a] units/L			
	GPT	% change[b]	GOT	% change[b]
1 h	2.40 ± 0.12	−37.5	2.04 ± 0.08	−20
1 week	1.60 ± 0.06	−58.3	6.12 ± 0.30	+140
2 weeks	1.28 ± 0.07	−66.6	6.80 ± 0.34	+167
3 weeks	1.20 ± 0.04	−68.7	8.67 ± 0.43	+240
4 weeks	1.44 ± 0.06	−62.5	12.24 ± 0.73	+380

[a] Results are means ± SD of two experiments, each performed in triplicate. [b] From control (untreated) tomatoes which had 3.84 ± 0.23 and 2.55 ± 0.12 units/L for GPT (glutamic-pyruvic transaminase) and GOT (glutamic-oxaloacetic transaminase), respectively.

Table IV. Effect of Profenofos Residues on the Activity of Peroxidase and Polyphenol Oxidase (PPO) in Tomatoes

time from treatment	Activity,[a] units			
	peroxidase	% change[b]	PPO	% change[b]
1 h	130.8 ± 6.54	+30.0	310.0 ± 15.5	+40.9
1 week	150.5 ± 6.02	+49.6	290.2 ± 11.6	+31.9
2 weeks	136.3 ± 5.45	+35.5	280.5 ± 11.2	+27.5
3 weeks	132.6 ± 5.30	+31.8	250.8 ± 12.5	+14.0
4 weeks	128.4 ± 3.85	+27.6	239.0 ± 9.60	+8.6

[a] Results are means ± SD of two experiments, each performed in triplicate. [b] From control (untreated) tomatoes which had 100.6 ± 6.2 and 220.0 ± 9.5 units for peroxidase and PPO, respectively. Unit of enzyme activity = change in absorbance of 0.001/min.

inhibition of brain GOT was found in common carp (*Cyprinus caprio* L.). The results from this study showed that profenofos residues from tomatoes resulted in disruption of transaminase activities from normal values, indicating biochemical events of tissues and cellular functions.

Peroxidase and Polyphenol Oxidase. Changes in the activity of peroxidase and PPO, important oxidoreductases, by profenofos residues are shown in Table IV. Both

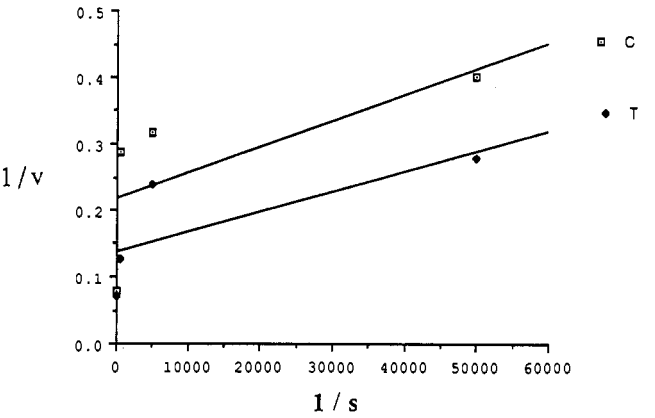

Figure 5. Lineweaver–Burk plot of tomato polyphenol oxidase as affected by profenofos treatment (T) vs untreated (C).

enzymes were induced by the pesticide residues. Peroxidase level increased by 30% 1 h after pesticide application. The increase in enzyme activity reached 49.6% after 1 week, and then the level of activation decreased to about 30% at the end of the studied period (4 weeks). PPO activity was maximal (310.0 ± 15.5 units, 40.9%) 1 h after profenofos application; then the enzyme activation rate decreased (239.0 ± 9.6 units, 8.6% 4 weeks after pesticide application). The untreated tomatoes had 100.6 ± 6.2 and 220.0 ± 9.5 units for peroxidase and PPO, respectively. It has been reported (Habiba et al., 1992) that the activity of peroxidase in profenofos-treated potatoes decreased by 79% of that of control, while PPO activity was stimulated by 39.4%. The above enzymes are involved in oxidative deterioration and off-flavor production in fruits and vegetables. Therefore, the increase in the activity of these enzymes is not desirable.

Kinetic Parameters of PPO. The effect of profenofos on Michaelis–Menten constant K_m and maximal velocity

Table V. Effect of Profenofos Residues on Some Quality Attributes of Tomatoes

time from treatment	% TSS[a]			% acidity			ascorbic acid, mg %			glucose, mg %			protein, mg %		
	C	T	%[b]	C	T	%	C	T	%	C	T	%	C	T	%
1 week	4.3 ± 0.22	5.25 ± 0.31	+22	0.51 ± 0.02	0.63 ± 0.04	+23.5	14.0 ± 0.70	11.2 ± 0.34	−20.0	220.0 ± 11.0	228.8 ± 9.2	−4.0	348.6 ± 13.9	303.4 ± 12.1	−12.9
2 weeks	4.2 ± 0.13	4.83 ± 0.19	+15	0.48 ± 0.02	0.61 ± 0.03	+27.1	13.3 ± 0.53	11.4 ± 0.57	−14.3	218.2 ± 10.9	225.4 ± 9.0	−3.3	325.0 ± 16.3	290.0 ± 14.5	−10.8
3 weeks	4.7 ± 0.24	5.25 ± 0.21	+12	0.50 ± 0.03	0.63 ± 0.04	+26.0	15.1 ± 0.60	14.7 ± 0.59	−2.6	222.5 ± 13.4	229.1 ± 11.5	−2.9	350.2 ± 20.0	336.3 ± 13.5	−4.0
4 weeks	4.5 ± 0.14	5.00 ± 0.20	+11	0.45 ± 0.02	0.56 ± 0.03	+24.4	15.0 ± 0.9	15.0 ± 0.6	0.0	221.4 ± 6.6	224.7 ± 8.9	−1.5	340.0 ± 17.3	340.0 ± 17.0	0.0

[a] C and T refer to control and profenofos-treated tomatoes, respectively. Results are means ± standard deviation of two experiments, each performed in triplicate. [b] % = percentage change.

V_{max} of tomato PPO was also studied. The apparent K_m, calculated from Lineweaver–Burk plots (Figure 5), of PPO from profenofos-treated tomatoes was lower (1.410×10^{-4} M) than that (3.891×10^{-4} M) of the untreated tomatoes. Consequently, an increase of the V_{max} was observed (7.353 units) for PPO in the profenofos-treated tomatoes as compared to that (4.629 units) of the untreated tomatoes. Similar effects were reported by Habiba et al. (1992) for profenofos-treated potatoes.

Effect of Profenofos on Some Quality Attributes. The effect of profenofos residues on TSS, acidity, ascorbic acid, glucose, and protein contents is shown in Table V. TSS and acidity contents of profenofos-treated tomatoes were increased during the test period. The percentage of TSS increase was gradually decreased as the time from pesticide application increased; however, the level of acidity increase was almost constant (about 25%) regardless of the time from treatment. On the contrary, ascorbic acid, glucose, and protein contents decreased in the treated tomatoes. This effect lessened as the time from pesticide application increased. Habiba et al. (1992) reported that profenofos residues slightly decreased the glucose content of potatoes. Other pesticides (Nexion, Birlane, and Dyeonate), however, generally increased the free sugar concentration of summer carrots as reported by Rouchaud et al. (1983).

The results indicate that much more attention should be directed toward the profenofos application on fruits and vegetables, especially those that can be eaten fresh such as tomatoes. It is therefore recommended that a maximum residue limit (MRL) be established for this pesticide and its use regulated to avoid adverse effects.

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