VELOCITY OF SPECIFIC SELECTIVE ENZYMES IN MANGO (*Mangifera indica* L.) TREATED WITH γ- RAYS

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ABSTRACT

The problem at hand is dealing with; to what extend velocities of polyphenol oxidase (PPO), peroxidase (POD) and pectinmethylesterase (PME) are affected by irradiation?. To reach such target, the investigated mango fruits were irradiated with different doses of γ- rays namely .500, 1000, 1500, 2500 and 5000 Gy produced from Co^{60}. Velocity reaction of the aforementioned enzymes in relation to different substrate and enzyme concentrations were studied. Kinetic properties in terms of affinity, maximum velocity, activity coefficient, and slope of reaction were calculated through a specific computer program namely "Enzyme kinetics". Changes in the velocity patterns of the investigated enzymes as affected by storage for three weeks at 20±2°C were also studied. On the basis of the obtained results, it was found that a dose of 2500 Gy could be recommended to reach higher inhibition level of the three tested enzymes, a pattern which improves the quality and extends shelf-life of mango fruit.

Key Words : Mango, irradiation , PPO , POD and PME enzymes..

INTRODUCTION

Irradiation is now one of the major attractions for food producers, traders and consumers due to its capability of extending shelf-life, increased safety of food stuffs, reduction of spoilage and inhibition of the growth of microorganisms, parasites and insect infestation as well as preventing sprouting and ripening without the use of chemical additives, reduces the risk of food poisoning and can help alleviate starvation by retarding food spoilage. The FDA Currently allows irradiation of wheat, and variety of fruits and vegetables. Irradiated foods are eaten by astronauts, and now, 36 countries have approved the process for more than 49 different foods (IAEA, 1989; Anonymous, 1990 & Loaharanu, 1990). In all cases, irradiation is a cold technological treatment and the treated foods maintain raw, fresh-like character and the process is also considered safe by the World Health Organization, the American Medical Association, U.S. Food and Drug Administration, and various health and safety authorities in over 36 countries. On the other hand, in USA, the Congress specified in the 1950s that irradiation should be treated as a "food additive" and FDA has the responsibility for processing conditions and labeling (Taub & Singh, 1997).

The FDA requires the "radura" symbol and the words "treated with radiation" or "treated by irradiation" to appear on packages of irradiated foods. These comments need only to appear if the entire product has been irradiated and not if the product contains ingredients that have been irradiated (The Referee, 1990).

Newer techniques more-or-less derived from the traditional methods of preservation include the successful application of ionizing radiation, which is increasingly being employed in food preservation. At the same time, there is a reawakening of interest towards the presence of free radicals. The reasons for this ; derive principally from consumers requirements for foods of higher quality; more natural, so less heavily preserved; i.e. nutritionally healthier, or preferably improved with deep assurance of safety (Gould, 1995). On the other hand, there are many groups of enzymes primarily responsible for the quality deterioration of fruits and vegetables. Polyphenol oxidase and peroxidase (to a lesser extent) may cause color changes while ascorbic acid oxidase and thiaminase as well as pectinmethylesterase can cause nutritional loss and firminess changes (Williams *et al*., 1986).

Interaction between ionizing radiation and enzymes activities is of theoretical as well as practical interest in view of efforts being made to preserve foods and food products as well as sterilize commercial enzyme preparations (Macris & Markakis, 1971). The

purpose of irradiating foodstuffs is often to achieve a strong interference with their normal metabolic functions. For instance, mango fruits my be irradiated with the intention of delaying maturation and extension of shelf-life. Subsequently, it may be expected that a radiation dose, which has such a pronounced effect on such physiol-ogical properties, will also have other unintended effects (Shapton & Shapton, 1998).

The problem at hand is dealing with; to what extend the velocity of polyphenol oxidase (PPO), peroxidase (POD) and pectin-methylesterase (PME) are affected by irradiation?. To reach such target, the inves-tigated mango fruits were irradiated with different doses of γ- rays produced from Co^{60}.

Velocity reaction of the aforementioned enzymes as well as their kinetic properties in terms of affinity, maximum velocity, activity coefficient, and slope of reaction were calculated through a specific computer program. Changes in velocity patterns of the same enzymes as affected by storage were also studied for different periods of storage at 20±2°C.

MATERIALS AND METHODS

Materials :

Mango fruits (*Mangifera indica* L.) var. Hindy Sinnara. Family *Anacardiaceae* were obtained from El-Ebor retail market, Cairo Governorate, Egypt. The length of the investigated mango variety was up to 12 cm. of 250 g. in weight with an oval oblong shape, orange/green color, flesh firm, very sweet and rich in flavor with smooth fibers

Methods :

Samples preparation: Mango fruits of about 60 kg were divided into seven groups of 7-8 kg for each one. Group No. 7 was left without irradiation and served as a control, while the other six groups were irradiated with γ-rays of different doses. Mango fruits were stored at room temperature (about 20°C±2°C) under good ventilation in perforated standard carton boxes of a dimension 45×30×12 cm.

Irradiation process: The investigated samples were transported to the National Center for Radiation Research and Technology (NCRRT) at Nasr City, Cairo, Egypt where irradiation treatments were applied using Cobalt-60 facility "Egypt Mega Gamma 1" Model AECL 6500. The exposure dose levels that applied for mango samples were: 500, 1000, 1500, 2000, 2500 and 5000 Gy. Storage of the treated samples was extended for three weeks at 20±2°C and RH (relative humidity) of 60-70%. The activity of poly phenol oxidase (PPO), peroxidase (POD) and pectin methylesterase (PME) was measured weekly in the tested mango samples.

Activity measurements of PPO, POD and PME: The procedure of Halpin & Lee (1987) was applied for measuring the activities of polyphenol oxidase and peroxidase; while that of Fayyaz, *et al*., (1995) was applied for pectin methylesterase. Substrates were selected to be catechol for (PPO), guaicol 0.5% for (POD) and pectin 1% for (PME).

Changes in absorbency at 420 and at 470 nm were measured over a period of 165 sec. for (PPO) and (POD), respectively. One unit of activity was expressed as a change of 0.1 absorbance for each enzyme. On the other hand, the activity of (PME) was expressed as the amount of methoxy group (CH_3O) liberated at 30°C for 30 min. per 100g sample.

Kinetic aspects of PPO, POD, and PME: Kinetic aspects were calculated by using the Trinity software program ; analytical tools based on a Macintosh computer as described by Stanislawski , (1991).

Kinetic aspects of the investigated enzymes in terms of Vo, V_{max}, K_m, $1/K_m$, and K_m/V_{max} (slope of reaction) as well as V_{max}/K_m (catalytic efficiency), and correlation coefficient "R^2" were calculated after measuring the corresponding activities within different substrate concentrations as follows:

- In case of PPO; substrate concentrations varied between 0.7 to 2.8 ml ($mole.ml^{-1}$); while they varied between 1.1 ml ($0.68255 \text{ mole} \times 10^{-3}$) to 2.5 ml ($1.5512 \text{ mole} \times 10^{-3}$) for the POD.
- In case of PME; substrate concentrations varied between 5 ml (50 mg pectin/100ml) to 30 ml (300 mg pectin/ 100ml).

Kinetic aspects of the investigated enzymes based on different enzyme concentrations were considered in terms of Angles of activities, Pseudo values, "R^2", slope of reaction. The applied variations in enzyme concentration were as following:

- From 0.25 to 1.00 unit for the PPO; Unit = (5×10-4 g/µl of enzyme source).
- From 2.50 to 10.0 unit for the POD; Unit = (5×10-5 g/µl of enzyme source).
- From 0.20 to 1.20 unit for the PME; Unit = (0.04 g/ ml of enzyme source).

Statistical analysis:

Analysis of Variance and Duncans new multiple range testing method was used to compare mean values of the tested factors. Linear and multiple regressions were also applied (where appropriate) according to Montgomery, (1984). The level of significance is accepted as being $P \geq 0.05$ (unless otherwise stated).

RESULTS AND DISCUSSION

Velocity of polyphnol oxidase (PPO) in the investigated mango fruits :

The activity of PPO in the investigated mango fruits was expressed as unit of activity in terms of $\Delta OD.\ min^{-1}.\ g^{-1}$ within substrate concentrations ranged from 0.7 to 2.8 mole ml-1. .The activity of the tested enzyme was followed during storage of mango fruits either the unirradiated or the irradiated samples for three weeks at 20±2°C. The kinetic aspects of the investigated PPO in the control samples in terms of K_m, V_{max} and slope of activities with their corresponding standard deviations were also calculated. The obtained data proved the presence of higher activity of the investigated enzyme in the control sample up to the second week of storage at 20 ± 2°C as seen in Fig (1). Such a trend was held true and also confirmed by the V_{max} values i.e. 31.220 $\Delta OD.min^{-1}.g^{-1}$. of the tested enzyme within the given storage period, as seen in Table (1).

The activity of PPO in the irradiated mango fruits as a function of any given substrate concentration tended to decrease by prolonged storage periods as seen in Fig. (1). On the basis of calculating the values of Km, V_{max}, and slope of activity for the irradiated mango fruits "500 Gy" stored for three weeks; a proportional relation of the Km values and storage period was found. The corresponding values of K_m were 0.1758 for the control; while it progressively increased to 0.3316, 0.7534 and 1.0596 mole ml^{-1} for the stored samples.

With respect to the unstored mango fruits treated with "1000 Gy", the proportional relation between the activity of PPO and substrate was performed up to a substrate concentration of 2.2 mole min^{-1} as seen in Fig. (1), after which any further addition of substrate declines the activity. This could be explained by the structure deformation of the PPO within substrate specificity since it is recently proved that the occurrence of a distinct functional group in the substrate is the only prerequisite for few enzymes such as hydrolases while more restricted specificity is found in other enzymes such as oxidoreductases. This means that PPO activity requires a substrate molecule which contains a distinct structural feature in addition to the reactive functional group to form a close steric with the enzyme in its deformed structure, (Belitz & Grosch, 1999). On the contrary; during storage, the activity of the same enzyme was correlated positively with substrate concentration at any given period of storage that extended for three weeks.

On the basis of the previous results, it seems that irradiation dose of 1000 Gy could act as induce in regaining maximum velocity of the PPO enzyme; a pattern which was really found in the investigated mango fruits that stored for 3 weeks especially at a substrate concentration of 2.8 moles ml-1. However, a reversible relation was pronounced between reaction velocity of PPO in mango fruits received "1500 Gy" and storage period. These results proved that using a radiation dose of 1500 Gy beside storage of more than 7 days at 20±2°C enhanced specific constituents in mango fruits that may be acting as inhibitors for the PPO enzyme. The aforementioned trend was proved since the overall activity of the enzyme in mango fruits treated with a dose of 2000 Gy showed a downward trend during storage for three weeks, a pattern which was held true after the calculation of residual activity as seen in Fig. (1). Similar findings were found on using a dose of "2500 Gy"; which caused more inhibition of PPO during storage of mango fruits; and so it could be considered the more pronounced dose for inhibiting PPO in the investigated mango fruits. Subsequently, the main following points could be assured :

- Velocity of PPO as a function of the applied doses of irradiation tends to decrease by elongation storage period at any given substrate concentration.

Table 1 : Relation between kinetic parameters of PPO in the investigated mango fruits as a function of irradiated doses

Irradiation doses "Gy"	Kinetic parameters											
	K_m (mole. ml^{-1})				V_{max} (Δ OD. min^{-1}. g^{-1})				Slope ($x10^{-2}$) (Δ OD. min^{-1}. g^{-1}) / (mole. ml^{-1})			
	0	I	II	III	0	I	II	III	0	I	II	III
0	0.8661	1.1997	0.4979	1.2730	25.6565	31.5056	31.2205	27.3204	3.3757	3.8078	1.5949	4.6603
500	0.1758	0.3316	0.7534	1.0596	24.5045	26.9760	27.3530	23.9782	0.7177	1.2300	2.7545	4.4189
1000	2.5637	0.6454	0.8435	1.9166	31.3905	17.6788	19.9825	27.8454	8.1672	3.6505	4.2213	6.8830
1500	0.5944	0.5452	1.0982	0.2959	19.7872	22.2582	20.6099	12.4307	3.0038	2.4494	5.3286	2.3808
2000	3.0827	1.1131	0.4969	0.1564	41.7872	26.4394	14.7436	10.9314	7.3771	5.4581	3.3702	1.4310
2500	0.3172	0.2365	1.1361	0.4464	16.3851	13.3107	14.5530	9.3911	2.2657	1.7764	7.8075	4.7532
5000	1.0737	0.5554	1.1752	0.9639	40.1836	40.1139	36.0737	27.8809	2.6714	1.3845	3.2577	3.4573

I, II, III = Storage periods (weeks at 20 ± 2°C)

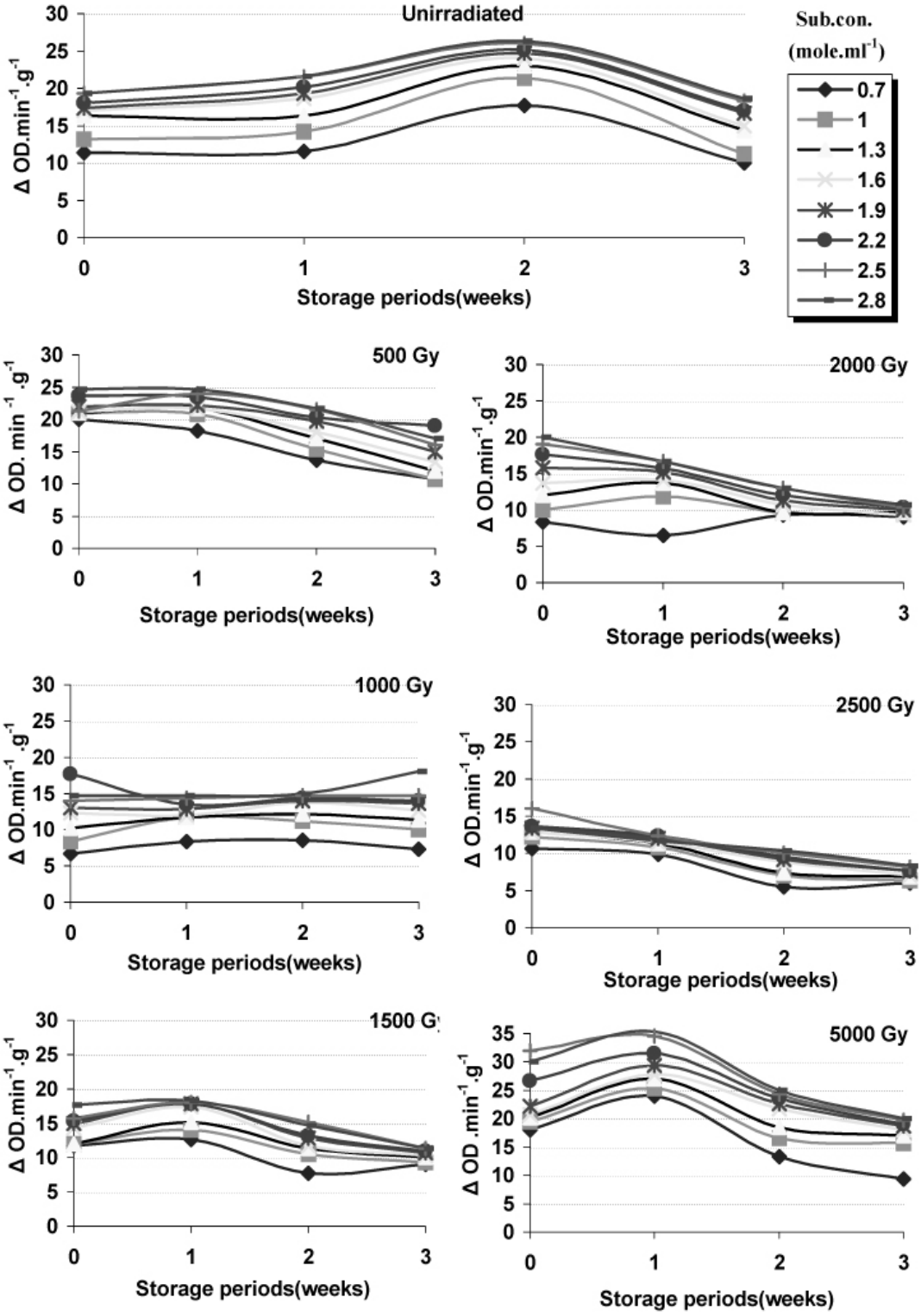

Fig. 1: Activity pattern of PPO during storage for three weeks of the tested mango fruits as a result of applying different irradiation doses.

- All of the activity of PPO realized proportional relation within the substrate concentration under any of the tested storage periods.

Robinson, *et al*., (1993) observed considerable activity of PPO (catechol oxidase) in the sap and skin of ripe 'Kensington' mangoes. The sap enzyme was found to be active on both *p*-and *o*-diphenol substrates, while the skin was active only on o-diphenol substrates. They concluded that sap PPO is a laccase-type enzyme (EC 1.10.3.2), whereas the skin contained the more common catechol oxidase-type enzyme (EC 1.10.3.1). On the other hand, irradiated mango fruits also remain edible for longer periods before they pass into the senescent phase. Doses between 250 and 1000 Gy have been reported as optimal for different varieties, although some authors found skin spotting and other indications of phyto-toxicity at doses of 750 Gy or higher, while others insist that doses above 250 Gy should be avoided in order to prevent radiation injury in mango fruits (Diehl, 1995).

Relation between enzyme concentration and the activity of the PPO in irradiated and unirradiated mango fruits was studied and the data obtained are given in Table (2) and Fig. (2). Before storage, the activity of PPO enzyme was 2.500 $\times 10^{-2}$ (ΔOD.min^{-1}. ml sub^{-1}) at a PPO concentration of 0.25 (5×10^{-4}g.µl^{-1}) in the unirradiated mango fruits. On the other hand, as a result of increasing the enz-yme concentration up to 1.0 (5×10^{-4}g.µl^{-1}), the activity of the enzyme reached 6.893×10^{-2} (ΔOD.min^{-1}.ml sub^{-1}). This simply means that velocity of PPO increased by 2.757 folds as a result of the elevation enzyme levels by 4 times of its original value; i.e. from 0.25 to 1 (5×10^{-4} g.µl^{-1}). However, a comparison was performed between the different applied doses (500, 1000, 1500, 2000, 2500 and 5000 Gy) of irradiation and the kinetic parameters of PPO in mango fruits. By calculating the degree of folds of reaction rate of the PPO in the investigated mango fruits, the following points could be concluded:

- Activity folds were 2.826, 2.926 and 1.435 of its original value in case of the unirradiated mango fruits stored for 1, 2 and 3 weeks, respectively.
- All irradiated mango fruits revealed a similar pattern with respect to the degree of activity folds of the PPO. For instance the most pron-ounced variation within the activity of the PPO in terms of "degree of folds" was noticed in the mango samples having a dose of 2500 Gy values were 4.00 for the unstored and 3.50, 2.61 and 2.50 for the same samples stored for 1, 2 and 3 weeks. This result supported the view of considering the 2500 Gy to be the best-fit dose for inhibiting PPO in mango fruits.

The data given in Table (2) were statistically analyzed to obtain slope of activity; correlation coefficient and standard error of estimation. In such a case the following multiplicative model was used:

"y = a × b"

Where: a = Log of intercept. b = Slope of reaction and y = Enzyme velocity as ΔOD at 420 nm .min^{-1}.ml sub^{-1}.

The obtained values indicate a strong "R^2" between concentration and velocity of PPO in the unirradiated and irradiated mango fruits within any given storage period.

Velocity of POD in the unirradiated and irradiated mango fruits:

Peroxidase activity in mango fruits was measured in the unirradiated mango samples and followed up during 3 weeks of storage at 20±2°C. A proportional correlation between both substrate concentration and storage period was performed as seen in Fig. (3). Calculation of kinetic parameters proved that Vmax possess a real upward line trend in a positive relation with storage; being 2.031 before storage and 5.311, 10.285 and 16.654 (Δ OD. sec^{-1}. g^{-1}) on storage for 1, 2 and 3 weeks, respectively at 20±2°C as seen in the Table (3) . The obtained results could be also represented in the following terminology :

- 1\K_m (affinity of the enzyme towards substrate); recently published by Espin *et al*.,(1998) proved the presence of lower affinity of the POD towards guaiacol as a function of storage of the control samples for 3 weeks at 20±2°C. The aforementioned trend copes with the application of a dose of 1500 and 2500 Gy.

Table 2: Relation between concentration and velocity of PPO in unirradiated and irradiated mango fruits during storage for 3 weeks

Doses "Gy"	Storage periods in weeks	Enzyme concentration Multiplied by ($5x10^{-4}$g/µl)						Statistical parameters (Regression analysis-Multiplicative model : y = a x^b)				
		0.25	0.4	0.55	0.699	0.85	1.0	Intercept	Slope	R^2 %	C.C.	S.E.
0	0	2.500	3.678	4.643	5.893	6.786	6.893	2.000	0.768	98.83	0.994	0.047
	1	2.738	4.405	5.476	6.905	7.500	7.738	2.134	0.767	97.45	0.987	0.071
	2	3.214	3.809	6.964	8.512	9.166	9.405	2.337	0.882	93.46	0.966	0.134
	3	4.643	5.119	5.476	6.190	6.428	6.666	1.897	0.273	97.80	0.988	0.023
500	0	0.893	2.083	5.119	6.190	8.333	8.809	2.380	1.727	96.54	0.982	0.187
	1	3.095	4.583	6.131	8.036	8.452	8.809	2.264	0.796	97.61	0.987	0.071
	2	3.988	5.357	6.309	6.845	7.381	7.738	2.082	0.475	98.03	0.990	0.038
	3	3.095	3.571	4.047	4.643	5.238	6.071	1.736	0.474	96.15	0.980	0.054
1000	0	1.071	1.190	3.333	4.405	5.357	5.238	1.816	1.355	89.93	0.948	0.260
	1	2.619	3.333	4.345	4.881	5.00	5.238	1.713	0.524	96.44	0.982	0.057
	2	1.607	2.559	4.523	4.940	5.238	5.357	1.833	0.919	92.24	0.960	0.153
	3	1.428	2.143	3.690	4.762	6.190	6.428	1.943	1.167	98.37	0.991	0.086
1500	0	1.309	3.750	4.286	5.119	5.357	6.309	1.954	1.030	87.36	0.934	0.225
	1	2.262	4.166	5.833	6.309	6.547	6.547	2.040	0.770	88.70	0.941	0.158
	2	2.262	3.095	3.512	4.047	4.881	5.238	1.649	0.601	99.05	0.995	0.033
	3	1.428	2.262	2.857	3.690	4.166	4.047	1.512	0.796	97.08	0.985	0.079
2000	0	1.190	1.547	1.726	4.881	6.666	7.143	1.929	1.453	96.60	0.931	0.328
	1	1.964	2.559	3.452	5.059	5.833	5.952	1.841	0.883	96.79	0.983	0.092
	2	1.666	2.262	3.690	4.285	4.524	4.643	1.647	0.804	94.43	0.971	0.112
	3	1.785	2.143	2.857	3.571	3.809	3.809	1.406	0.613	96.05	0.980	0.071
2500	0	1.190	1.905	2.50	3.33	4.166	4.762	1.563	1.010	99.79	0.998	0.026
	1	1.190	2.381	2.857	3.333	3.809	4.166	1.502	0.863	94.98	0.974	0.114
	2	1.369	1.547	2.262	2.976	3.214	3.571	1.281	0.758	95.43	0.976	0.095
	3	1.190	1.428	2.262	2.50	2.738	2.976	1.131	0.709	95.49	0.977	0.088
5000	0	3.690	5.238	6.428	7.619	9.405	10.74	2.345	0.761	99.46	0.997	0.032
	1	5.595	7.857	8.809	10.00	11.54	12.61	2.531	0.568	99.07	0.995	0.031
	2	3.452	4.881	6.905	7.619	8.095	8.690	2.229	0.682	96.56	0.982	0.074
	3	2.50	2.976	4.166	6.309	6.785	7.143	2.012	0.852	94.60	0.972	0.117

Activity unit of PPO = ΔOD .min^{-1} .ml sub^{-1} $x10^{-2}$

Unit of enzyme concentration = $5x10^{-4}$ g/µl

The inercept is equal to log a .

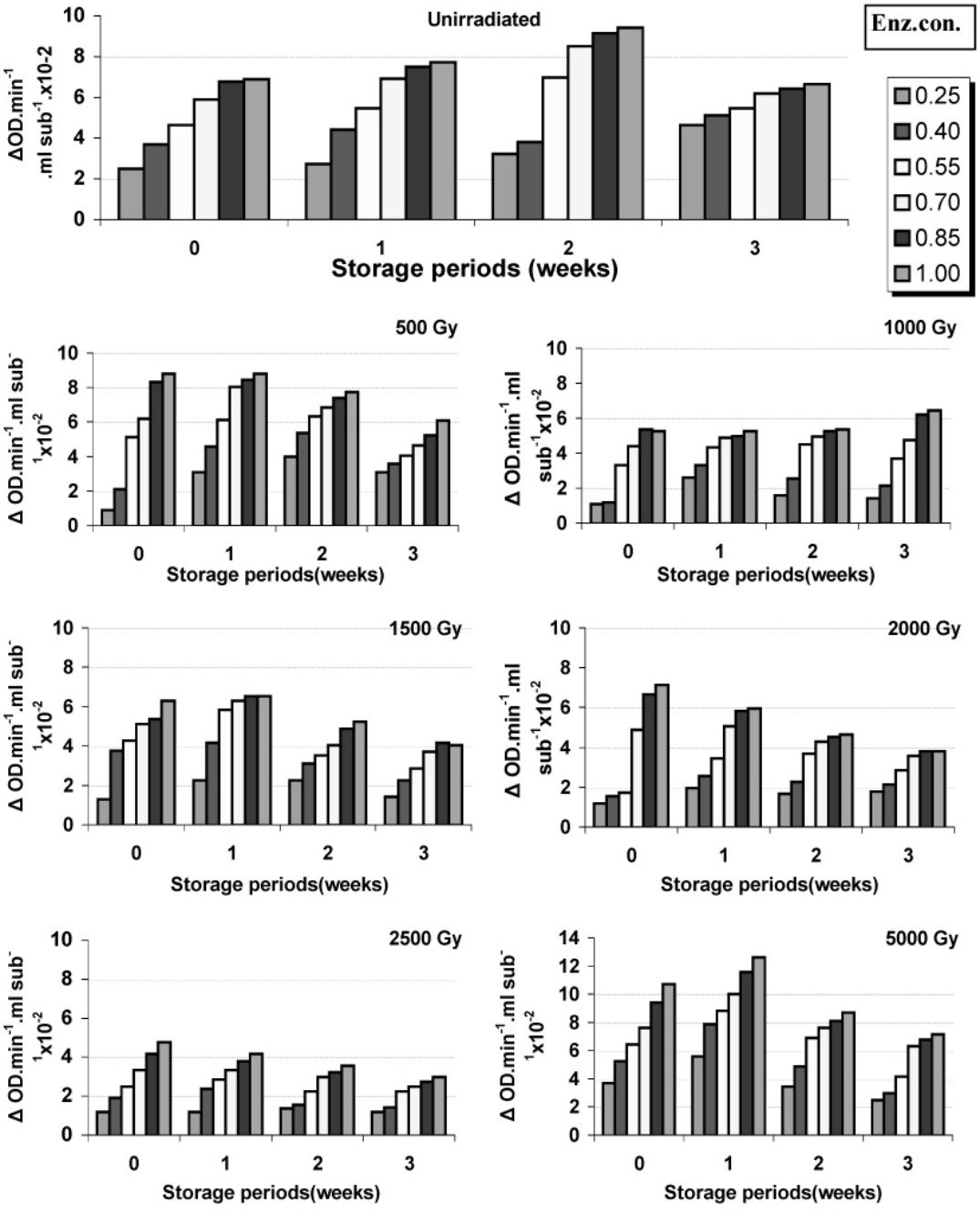

Fig. 2 : Velocity of PPO in irradiated and unirradiated mango fruits as a result of changing enzyme concentration during storage for three weeks

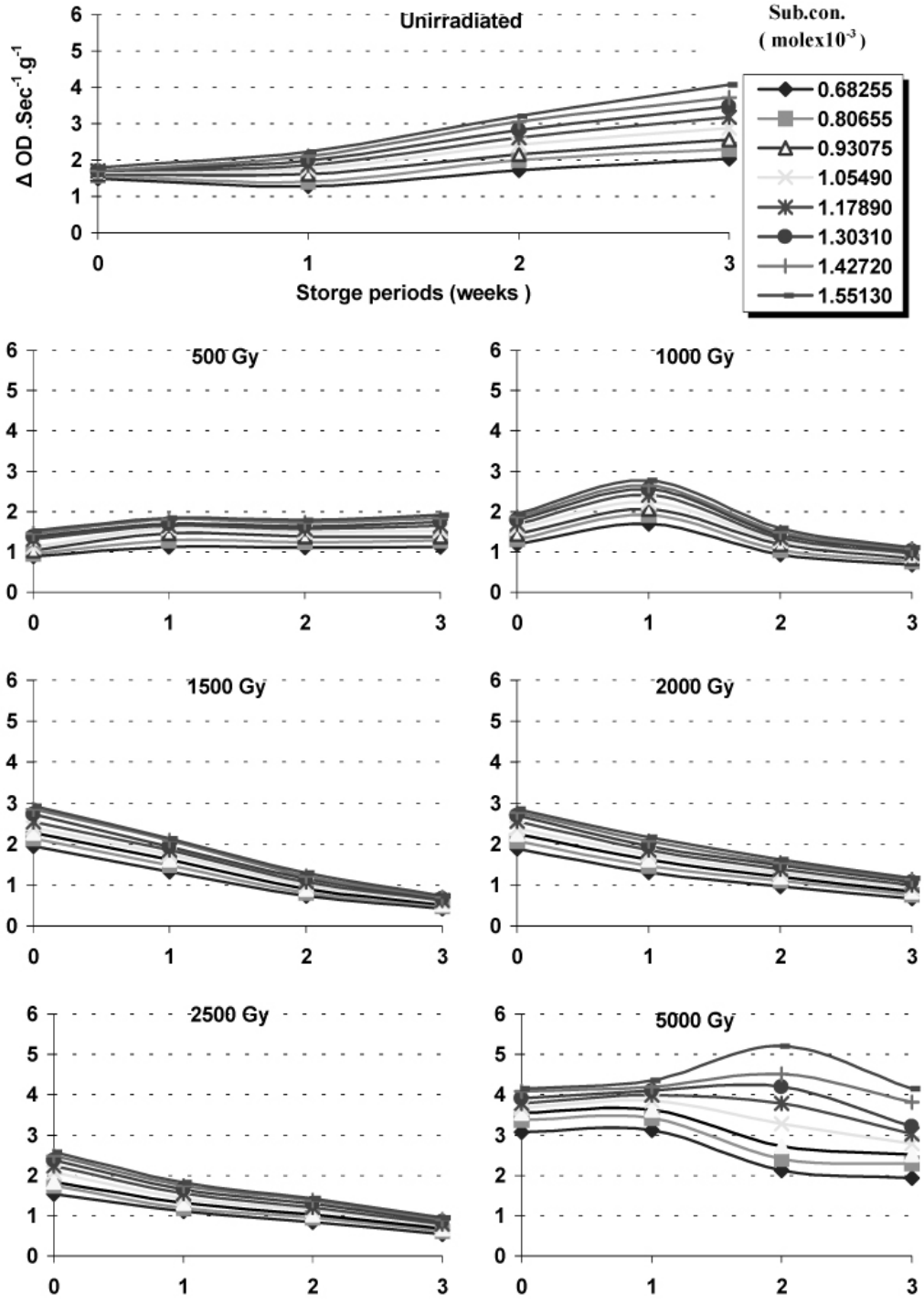

Fig. 3 :Relation between substrate concentration and activity of POD in mango fruits irradiated with different doses of gamma rays within storage for 3 weeks

Table 3: Kinetic values of POD in the investigated mango fruits

Irradiation Doses	Parameters	Kinetic values during storage for three weeks			
		0	I	II	III
Control Sample	K_m (mole X 10^{-3}) S.D.	$2.394x10^{-4}$	$2.170x10^{-3}$	$3.424x10^{-3}$	$5.008x10^{-3}$
		$2.742x10^{-5}$	$1.646x10^{-4}$	$2.532x10^{-4}$	$6.280x10^{-4}$
	V_{max} (Δ OD. Sec^{-1}. g^{-1}) S.D.	2.031	5.311	10.285	16.654
		$4.358x10^{-2}$	1.2649	0.569	1.696
	Slope mole X 10^{-3} / Δ OD. Sec^{-1}. g^{-1} S.D.	$1.1785x10^{-4}$	$4.086x10^{-4}$	$3.329x10^{-4}$	$3.007x10^{-4}$
		$1.104x10^{-5}$	$1.082x10^{-5}$	$6.313x10^{-6}$	$7.2485x10^{-6}$
	Affinity	4176.411	460.723	291.979	199.656
	C.E.	8485.215	2447.270	3003.240	3325.220
500 Gy	K_m S.D.	$2.394x10^{-3}$	$1.418x10^{-3}$	$1.364x10^{-3}$	$1.945x10^{-3}$
		$1.742x10^{-4}$	$2.106x10^{-4}$	$5.882x10^{-5}$	$7.525x10^{-5}$
	V_{max} S.D.	3.891	3.581	3.360	4.331
		0.192	0.298	$7.989x10^{-2}$	$10.614x10^{-2}$
	Slope S.D.	$6.153x10^{-4}$	$3.960x10^{-4}$	$4.059x10^{-4}$	$4.491x10^{-4}$
		$1.469x10^{-5}$	$2.629x10^{-5}$	$7.991x10^{-6}$	$6.493x10^{-6}$
	Affinity	417.606	705.019	733.030	514.006
	C.E.	1625.240	2524.816	2463.568	2226.419
1000 Gy	K_m S.D.	$1.695x10^{-3}$	$1.526x10^{-3}$	$1.746x10^{-3}$	$1.591x10^{-3}$
		$6.559x10^{-5}$	$6.948x10^{-5}$	$1.0124x10^{-4}$	$5.822x10^{-5}$
	V_{max} S.D.	4.109	5.495	3.349	2.279
		$9.574x10^{-2}$	0.144	0.118	$4.898x10^{-2}$
	Slope S.D.	$4.125x10^{-4}$	$2.778x10^{-4}$	$5.215x10^{-4}$	$6.984x10^{-4}$
		$6.469x10^{-6}$	$5.435x10^{-6}$	$1.204x10^{-5}$	$1.072x10^{-5}$
	Affinity	589.796	655.007	572.442	628.180
	C.E.	2423.768	3599.528	1917.224	1431.873
1500 Gy	K_m S.D.	$1.071 x10^{-3}$	$1.478 x10^{-3}$	$2.481 x10^{-3}$	$2.118 x10^{-3}$
		$7.220 x10^{-5}$	$5.378 x10^{-5}$	$1.010 x10^{-4}$	$1.168 x10^{-4}$
	V_{max} S.D.	4.931	4.206	3.345	1.736
		0.1640	$8.727 x10^{-2}$	$9.345 x10^{-2}$	$6.247 x10^{-2}$
	Slope S.D.	$2.173 x10^{-4}$	$3.514 x10^{-4}$	$7.416 x10^{-4}$	$1.220 x10^{-3}$
		$7.533 x10^{-6}$	$5.594 x10^{-6}$	$9.679 x10^{-6}$	$2.388 x10^{-5}$
	Affinity	932.922	676.452	403.014	472.076
	C.E.	4600.615	2845.227	1348.285	819.525

Affinity = $1/K_m$; 1/(mole $x10^{-3}$) S.D. = Standard Deviation

C.E. = Catalytic Efficiency = V_{max} / $K_{m;}$ (Δ OD. sec^{-1}. g^{-1})/(mole $x10^{-3}$).

- V_{max}/K_m (catalytic efficiency); recently published by Mar Sojo *et al*. (1998) indicated the presence of reversible correlation between catalytic efficiency of the POD towards its substrate within the storage of mango fruits for 3 weeks at 20±2°C. All of the irradiated samples having higher catalytic efficiency of POD at the beginning of storage but still less than the unirradiated samples, a trend which confirmed the aforementioned conclusion.

As a function of using the dose of 1000 Gy, the velocity declined sharply by prolonging the storage period; a pattern which was found at any of the given substrate as seen in the same figure from which activity was around 57 to 58 % of the original value. Calculation of the catalytic efficiency (Vmax/Km) further confirmed the aforementioned conclusion as seen in Table (3).

The velocity and residual activity of peroxidase in mango samples irradiated with 1500 Gy are given in Fig (4). The effect of substrate concentration along with storage periods extended to 3 weeks at 20 ±2°C was recorded in Table (3) from which the over-all trend of enzyme activity was negatively correlated by extending storage period at any of the given substrate concentration. Kinetic parameters of the peroxidase activity for the mango samples of 1500 were as follows:

- V_{max} values were 4.931 , 4.206 , 3.345 and 1.736 (ΔOD. $sec^{-1}.g^{-1}$) for the unstored and samples stored for 1, 2 and 3 weeks at 20±2°C. These results show the decline tendency of the V_{max} upon extending storage period indicating the efficiency of such previous dose in decreasing the velocity of the POD in mango fruits. Affinity of the POD extracted from irradiated samples (1500 Gy) against guaiacol and its residual activity given in Fig. (4) confirmed the previous conclusion.
- V_{max}/K_m is also matching with the previous conclusion. The obtained values were 4600.615 (ΔOD.$sec^{-1}.g^{-1}$ /mole$\times 10^3$) at the beginning of storage and subsequently decline to 819.525 (ΔOD. $sec^{-1}.g^{-1}$/mole $\times$ 10^{-3}) by the end of storage periods as seen in Table (3).

Residual activity of peroxidase was calculated for the mango fruits irradiated by 2000 Gy and during storage for 3 weeks at 20±2°C within the used substrate concentration. It seems evident from the data given in Table (4) and Fig. (4) that higher substrate concentration reduced enzyme activity within the applied storage periods.

It is worth to refer to the opinion of Diehl (1995) who proved that, when a dilute solution is irradiated the extent of degradation of the solute depends on the number of reactive radicals available for reaction with the solute molecules. The higher the concentration of the solute, the more solute molecules will not find a reaction partner and will remain unchanged. A dose of 10 kGy caused 20% loss of POD activity in a 1% solution and 60% loss in a 0.5% solution, while a dose of less than 1 kGy inactivated the 0.01% solution. This elevation of radiation sensitivity with increasing dilution is known as the dilution effect.

The kinetic parameters of the same POD in mango fruits stored under similar conditions were obtained and results showed the main following points :

- K_m values realized a lower affinity of the enzyme towards the substrate during storage of irradiate mango fruits (2000 Gy) for 3 weeks at 20±2°C as seen in Table (4). With the previous view in mind, the active center of the enzyme may have a steric change due the irradiation treatment (2000 Gy) leading to unfit adherence between the substrate and the enzyme a trend which was confirmed by residual activity given in Fig. (4).
- Maximum velocity of the same enzyme under experimental conditions confirmed also the previous conclusion. Values of V_{max} were 4.896 ΔOD.sec^{-1}.g-1 before storage and subsequently were minimized to 2.969 ΔOD. $sec^{-1}.g^{-1}$ after 3 weeks of storage at 20±2°C.
- V_{max}/K_m are going with aforementioned trend; the obtained value that was 4477.867 (ΔOD. $sec^{-1}.g^{-1}$/molx10^3) before storage represents 1.6426, 2.2353, and 3.5307 folds of the corresponding values after 1, 2 and 3 weeks of storage, respectively.

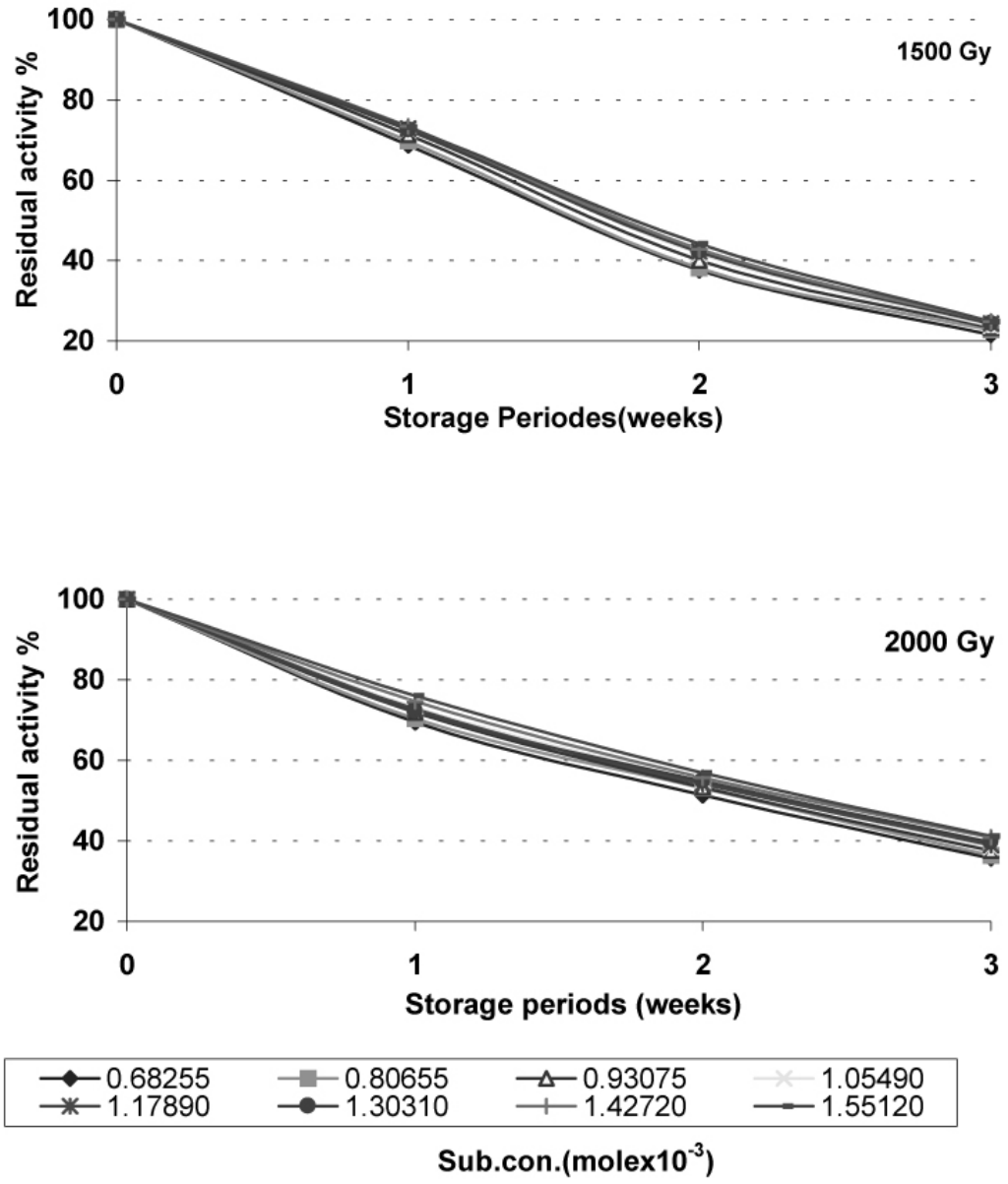

Fig. 4 : Residual activity of POD in mango fruits irradiated with 1500 and 2500 Gy 1500 and 2500 Gy within 3 weeks of storage.

Table 4: Velocity of POD in mango fruits treated with 2500 Gy in relation to different substrate concentration during storage for 3 weeks.

Sub.con. (mole X10^{-3})	Storage periods (weeks)							
	0		I		II		III	
	Δ OD/ Sec.g.	Residual activity %	ΔOD/ Sec.g.	Residual activity %	Δ OD/ Sec.g.	Residual activity %	Δ OD/ Sec.g.	Residual activity %
0.6826	1.536	100	1.102	71.744	0.837	54.492	0.543	35.351
0.8066	1.738	100	1.183	68.066	0.942	54.200	0.611	35.155
0.9308	1.883	100	1.317	69.942	1.023	54.328	0.677	35.953
1.0549	2.081	100	1.425	68.476	1.139	54.733	0.743	35.703
1.1789	2.232	100	1.559	69.847	1.209	54.166	0.799	35.797
1.3031	2.376	100	1.641	69.065	1.291	54.335	0.854	35.942
1.4272	2.492	100	1.748	70.144	1.372	55.056	0.910	36.516
1.5512	2.579	100	1.828	70.880	1.418	54.983	0.954	36.991
Kinetic parameters	**Kinetic values**							
K_m (mole X 10^{-3})	1.854 x10^{-3}		1.911 x10^{-3}		1.973 x10^{-3}		2.363 x10^{-3}	
SD	9.166 x10^{-5}		1.737 x10^{-4}		1.035 x10^{-4}		5.785 x10^{-5}	
V_{max} (Δ OD. $Sec^{-1}.g^{-1}$)	5.714		4.063		3.242		2.4072	
SD	0.1758		0.232		0.108		3.982 x10^{-2}	
Slope (ΔOD/Sec.g.)/ (mole.10^{-3})	3.245x10^{-4}		4.704 x10^{-4}		6.087 x10^{-4}		9.820 x10^{-4}	
SD	6.169 x10^{-6}		1.616 x10^{-5}		1.183 x10^{-5}		7.951 x10^{-6}	
Affinity	539.199		523.176		506.662		423.029	
Catalytic efficiency	3081.311		2125.771		1642.752		1018.355	

Affinity = $1/K_m$ (mole^{-1}x10^{-3})

Catalytic efficiency = V_{max} / K_m (Δ OD at 470nm. sec^{-1} . g^{-1}) / (mole x10^{-3}).

Velocity of peroxidase in mango fruits irradiated with 2500 and 5000 Gy tended to decline through storage for 3 weeks since inhibition percentage within the previous conditions of storage reached 64.65, 64.20 and 63.48% when the mango fruits treated with 2500Gy and the substrate concentrations were 0.6826, 1.1789 and 1.4272 mole × 10^{-3}, respectively. The V_{max} values proved the presence of downward trend with respect to storage periods. These results support the idea that irradiation of mango fruits (2500 Gy) followed by storage for 3 weeks at 20±2°C. minimized the velocity of POD, a trend which was confirmed within the calculation of the affinity as well as the catalytic efficiency of the POD against substrate concentration as seen in Table (4). Such a conclusion is out of order in the mango samples irradiated with 5000 Gy.

It is of interest to refer to the idea of Spalding and Reeder (1986) who found that a delay in ripening of mango fruits was observed with irradiation doses of 150-250 and 750 Gy. Other study given by Boag *et al.* (1990) also showed a delay and reduction of respiratory climacteric and degreening of mango peel with an irradiation dose of 200 Gy for 'Kensington Pride' mango fruits. The damage appeared to be a function of irradia-

tion dose, fruit ripeness, and fruit maturity at time of treatment.

The relation between enzyme concentration and irradiation doses giving for mango fruits is given in Table (5) from which irradiated samples of 500 and 1000 Gy showed a real increment relation with storage periods whether the enzyme concentration was lower as 2.5 (5×10^{-5}g/µl) unit of the investigated enzyme source or higher up to10 ($5x10^{-5}$g/µl) unit of the same enzyme. On the contrary, on using a dose level of 1500, 2000 and 2500 Gy, the velocity of peroxidase tended to decline during storage at higher enzyme concentration. On such a base, radiation doses up to 1000 Gy failed to reduce enzyme velocity during storage while higher irradiation doses; i.e. 1500, 2000, and 2500 Gy succeeded in reducing enzyme activity during storage. Subsequently, to check out the activity of the POD in mango fruits, higher enzyme concentration should be considered within the studied irradiation doses due to the presence of several active centers, which accelerate enzyme velocity in the form of substrate hydrolyses.

It was found that during storage of mango fruits, increment of ethylene production, along with a breakdown in carot-enoids; peel (yellowing), enhanced respir-ation and softening. Together with the ethylene evolution and respiratory climacteric in mango fruits, the catalase and peroxidsae activities were found to increase considerably, due to the disappearance of the heat-labile and non-dialysable inhibitor of these enzymes .The patterns of respiration and ripening behaviour vary among the varieties, the climatic conditions and the places where the fruits are grown (Mitra & Baldwin, 1997).

Velocity of PME in the investigated mango fruits :

Recent developments in the understanding of the basic biology of fruit softening were reviewed by Seymour, and Gross, (1996). They highlighted the numerous wall modifications, which can potentially occur during ripening. Aspects of fruit softening are usually considered within the following patterns: cell wall disassembly; cell wall hydrolases-pectin degrading enzymes (endo-polygalactureonase, exopolygalactur-onase, pectinmethylesterase, β-galactosid-ase, rhamnogalactureonases, γ-galactosidase, hemicellulose-degrading activity and cellulase); cell wall biosynthesis; and nonenzymic deaggregation of pectin.

The reaction pattern of pectin methylesterase was considered through the tested mango samples; a control and those irradiated with 500, 1000, 1500, 2000, 2500 and 5000Gy.

Regarding the activity of PME in the unstored irradiated mango fruits, data of Table (6) show slight increase due to irradiated treatments up to the level of 2500 Gy then sharply enhanced when the dose of 5000 Gy was used; but still much lower than the control. Such a trend could be noticed at any of the substrate concentration since the correlation coefficients (r and R^2) are highly found between velocity and substrate concentration at any investigated doses as confirmed by regression analysis. Trend analysis of the velocity of the other mango samples under similar conditions and received irradiation doses of 500 to 2000 Gy was in between of the previous pattern of activity. The increase in β-galactosidase and PME activities in response to irradiation is in sharp contrast to the behaviour for polygalacturenase "PG" as early reported by Somogy & Romani (1964). Such a trend was also noticed on measuring the activity of PME activity in sweet cherries irradiated at 2.0 and 5.0 kGy, as well as in oranges irradiated at 1.0 to 3.0 kGy (Dennison *et al*., 1967). Other enzymes reported to increase in response to irradiation include, peroxidsae as well as phenylalanine ammonialyase in mango fruits as mentioned by Frylinck *et al*. (1987) and (1-aminocyclopropane-1-carboxylic) synthase in cherry tomato (Larrigaudiere *et al*., 1990).

By extending storage for 2 and 3 weeks at 20±2°C, the PME of the unirradiated samples reached the highest velocity in relation to the irradiated samples leading to a soften tissues with the weakening of the responsed structure and the appearance of brown and black spots on mango surfaces in addition to noticeable mold infection as clearly indicated in Fig. (5) These physiological changes were also noticed in samples treated with a dose level of 5000Gy. and so, in such latter case, mango fruits became also unsuitable for consumption. To trace which dose is the most pronounced one for reducing the "PME" activity, a comparison was carried out between the applied doses on the base of using regression analyses. The obtained data put the dose 2500 Gy in the first order followed by the dose 2000 Gy. Such a pattern was towards the corresponding lowest slope of reaction which reached [0.081 and 0.113 [(mg CH3O/ 100g sample)/(mg pectin /100 ml)] as seen in Fig. (6).

Table 5 : Activity of POD as Δ OD x10^{-3}. Sec^{-1} . ml sub^{-1} with respect to different enzyme concentrations during storage of the unirradiated or irradiated mango fruits for 3 weeks

Enz.Con.	Unirradiated				500 Gy				1000 Gy			
5 x 10^{-5} g / µl	0	I	II	III weeks	0	I	II	III weeks	0	I	II	III weeks
2.5	0.089	0.333	0.400	0.622	0.156	0.177	0.333	0.422	0.244	0.288	0.311	0.355
4.0	0.422	0.711	0.800	0.955	0.222	0.244	0.689	0.822	0.400	0.478	0.578	0.667
5.5	0.889	1.155	1.577	1.788	0.444	0.488	1.000	1.178	0.911	0.800	1.000	0.933
7.0	1.222	1.644	2.355	2.977	0.844	0.844	1.311	1.566	1.222	0.989	1.377	1.600
8.5	1.489	1.911	2.755	3.644	1.222	1.288	1.667	1.767	1.400	1.111	1.533	1.933
10.0	1.622	2.111	3.288	4.555	1.333	1.678	1.778	2.066	1.578	1.200	1.667	2.533
Regression analysis -Linear model : y = a + b x												
Intercept	-0.378	-0.234	-0.647	-1.022	-0.401	-0.522	-0.116	-0.058	-0.229	0.019	-0.115	-0.491
Slope	0.213	0.247	0.401	0.551	0.176	0.209	0.199	0.217	0.190	0.126	0.190	0.292
R^2 %	97.40	98.16	98.90	98.35	95.93	95.67	98.64	98.99	96.63	96.62	96.45	98.16
C.C.	0.986	0.990	0.994	0.991	0.979	0.978	0.993	0.994	0.982	0.979	0.982	0.990
S.E.	0.109	0.106	0.132	0.221	0.114	0.139	0.073	0.069	0.111	0.080	0.114	0.125

Continued of Table (5)

Enz.Con.	1500 Gy				2000 Gy				2500 Gy				5000 Gy			
5 x 10^{-5} g / µl	0	I	II	III	0	I	II	III	0	I	II	III	0	I	II	III
2.5	0.400	0.355	0.288	0.155	0.238	0.733	0.355	0.278	0.222	0.378	0.288	0.200	0.600	0.465	0.488	0.400
4.0	0.711	0.667	0.556	0.288	0.476	1.089	0.689	0.344	0.556	0.778	0.422	0.288	1.399	1.466	0.822	0.667
5.0	1.200	1.111	0.689	0.378	0.793	1.378	1.022	0.656	1.00	1.044	0.711	0.556	2.664	2.800	1.577	1.444
7.0	1.778	1.533	0.911	0.556	1.031	1.556	1.311	0.856	1.355	1.289	1.022	0.644	3.333	3.867	2.222	2.200
8.5	2.088	1.711	1.089	0.711	1.458	1.889	1.467	1.100	1.578	1.488	1.244	0.867	4.065	4.333	3.288	3.088
10.0	2.378	1.822	1.200	0.733	2.083	1.911	1.567	1.222	1.978	1.511	1.355	0.955	4.665	5.133	4.800	4.111
Regression analysis -Linear model : y = a + b x																
Intercept	-0.312	-0.096	0.029	-0.046	-0.466	0.417	0.034	-0.113	-0.337	0.124	-0.125	-0.081	-0.663	-0.918	-1.324	-1.178
Slope	0.278	0.207	0.121	0.082	0.236	0.161	0.165	0.136	0.232	0.151	0.154	0.106	0.552	0.628	0.563	0.506
R^2 %	98.74	95.97	98.74	97.55	96.54	96.40	96.05	98.07	99.38	94.11	98.17	97.99	98.56	97.91	95.11	97.71
C.C.	0.993	0.979	0.993	0.987	0.982	0.981	0.980	0.990	0.996	0.970	0.990	0.989	0.992	0.989	0.975	0.988
S.E.	0.096	0.133	0.043	0.041	0.142	0.097	0.105	0.060	0.057	0.108	0.066	0.047	0.020	0.288	0.400	0.243

Table 6 : Activity of PME in mango fruits treated with different doses of γ rays at zero time of storage

Sub. con.**	Control Sample		Irradiation doses (Gy)											
			500		1000		1500		2000		2500		5000	
	*	NF	*	NF	*	NF	*	NF	*	NF	*	NF	*	NF
50	10.333	-	26.655	-	35.133	-	40.150	-	45.667	-	46.511	-	55.667	-
100	23.524	2.276	41.322	1.550	44.433	1.265	50.422	1.255	51.324	1.124	52.142	1.121	72.833	1.308
150	31.817	1.352	50.420	1.220	56.833	1.279	67.166	1.332	62.000	1.208	65.265	1.252	96.667	1.327
200	43.167	1.356	64.333	1.276	71.300	1.254	78.547	1.169	77.50	1.250	76.667	1.175	124.32(	1.286
250	51.667	1.196	77.500	1.204	80.600	1.130	85.833	1.092	84.131	1.085	83.544	1.089	156.56:	1.259
300	60.255	1.266	82.667	1.066	87.833	1.089	93.000	1.083	97.133	1.154	95.666	1.145	185.65:	1.185

Regression analysis -Linear model : y = a + b x

Intercept	2.254	16.898	24.041	30.999	32.500	34.827	22.406
Slope	0.197	0.230	0.220	0.218	0.212	0.200	0.530
R^2 %	99.47	98.80	99.05	97.75	98.64	99.11	99.06
C.C.	0.997	0.993	0.995	0.988	0.993	0.995	0.995
S.E.	1.507	2.652	2.258	3.463	2.608	1.989	5.411
V_{max}	**60.255**	**82.667**	**87.833**	**93.000**	**97.133**	**95.666**	**185.655**
K_m	**141.666**	**105.833**	**90.000**	**70.833**	**75.833**	**65.000**	**132.500**

* mg CH_3O /100 g sample/ 30 min ** mg pectin / 100 ml NF : Number of folds

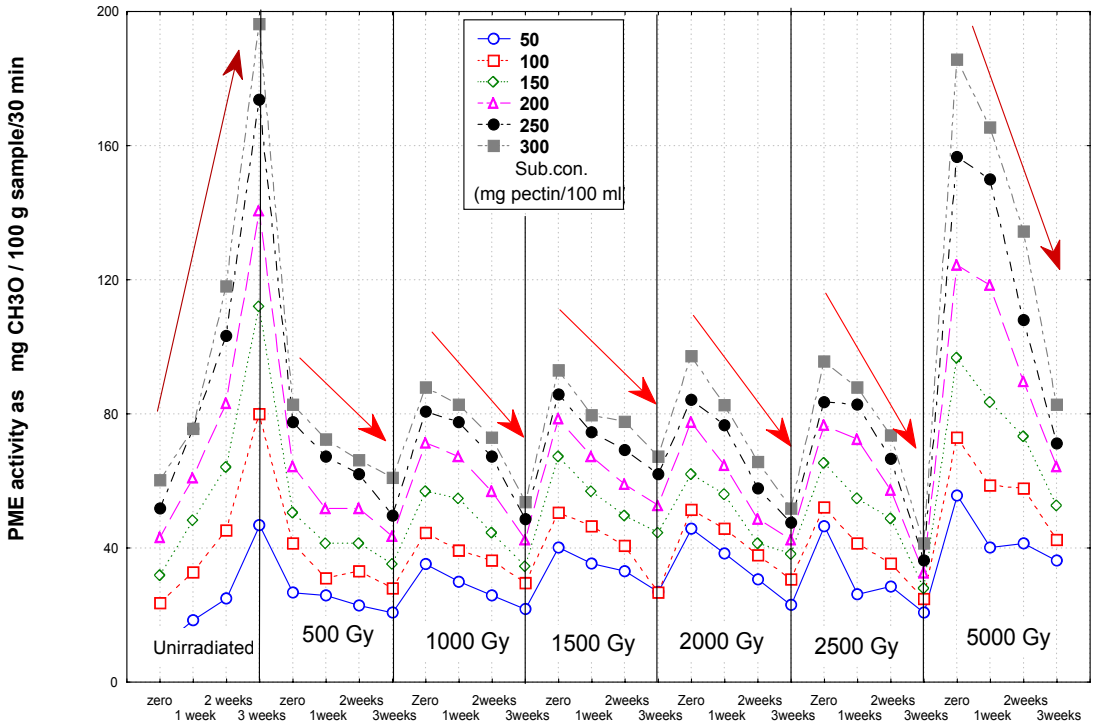

Fig. 5 : Relation comparison between the activity of PME in the unirradiated and the irradiated mango fruits during storage for 3 weeks.

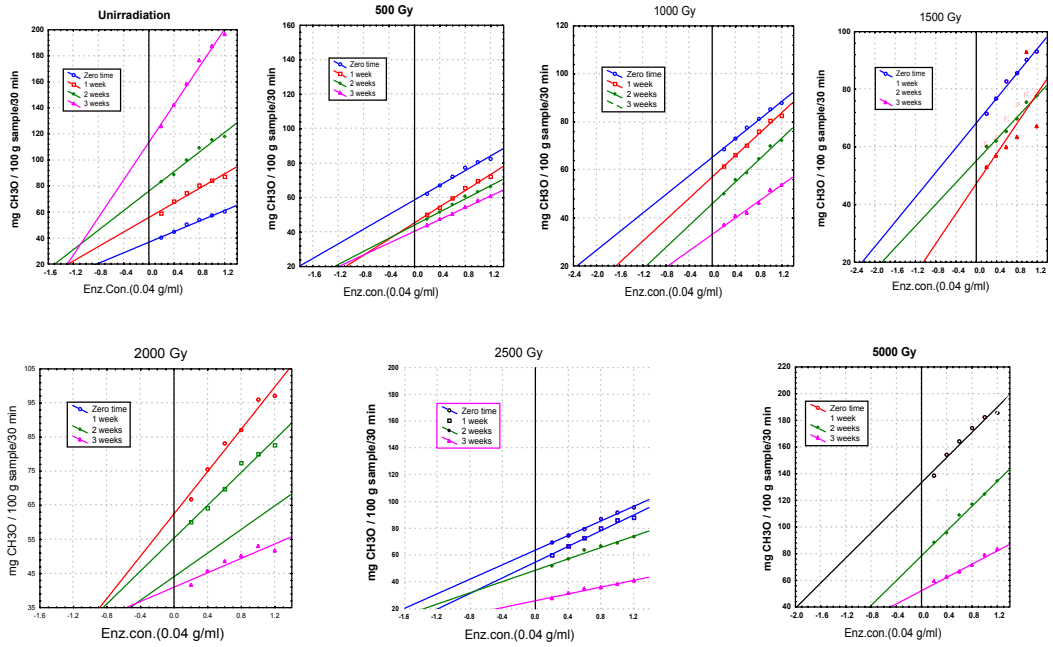

Fig 6: Pseudo values of PME velocity measured in unirradited and irradiated fruits stored for three weeks

The aforementioned results indicated the impossible 100% inhibition of the PME under any of the investigated doses. This could be explained by the opinion of Vas, (1969) who studied the radiation resistance of a pectinmethylesterase and a cellulase preparation in the dry state and in aqueous solutions of different concentrations. He proved that radiation resistance was established with both enzyme preparations.

To study the effect of enzyme concentration on the velocity pattern of PME, it was aimed to figure the obtained data as illustrated in Fig. (6) and predict the pseudo theoretical value of the enzyme activity in both of the unirradiated and the irradiated mango samples.

- The pseudo value of the unirradiated mango fruits that was –0.130 with an angle of 27°, changed to –1.21/29.0° (500Gy), –0.79/20.05° (1000 Gy), –0.04/30.0° (1500 Gy), –0.56/7.50° (2000 Gy), –0.48/11.5° (2500 Gy) and –0.50/12.0° (5000 Gy). Variations in pseudo values and their corresponding angles due to storage for three weeks at 20±2°C. may be as a result of the direct reduction effect of irradiation on the velocity of the PME.
- The correlation coefficient varied slightly between the applied doses during storage that extended for three weeks.

To shed light on the variation in PME activity within storage of irradiated mango fruits, analysis of variance and the multiple range Duncan test were performed. All of the investigated mango samples i.e. unirradiated and the irradiated samples exhibited significant differences within the velocity of PME under the tested storage conditions and the highest level of significance was found to be for the dose of 2500 Gy followed by the dose 2000 Gy and so, the former one could be recommended for minimizing the activity of PME. It is favorite over the other doses because of its sharing aspects when the relation between velocity of PME and substrate concentration was concerned.

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نشـاط بعض الإنزيمـات المختـارة فى المانجـو المعامـل بأشـعة جامـا

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تتعلق المشكلة موضع الدراسة إلى طرح السؤال الأتى إلى أى مدى يتأثر نشاط كل من إنزيم البولي فينول أوكسيديز (PPO) والبيروكسديز (POD) والبكتين مثيل أستيريز (PME) عند معاملة ثمار المانجو بأشعة جاما þ وللإجابة عن هذا التساؤل فقد أخذ في الاعتبار تعريض ثمار المانجو لجرعات مختلفة من أشعة جاما وهى ٥٠٠، ١٠٠٠، ١٥٠٠، ٢٠٠٠ Gy Ď والمتحصل عليها من مصدر الكوبلت ٦٠.

من ناحية أخرى فقد تم دراسة سرعة التفاعل لأنواع الإنزيمات السابق الإشارة إليها عند مستويات مختلفة من تركيز كل من مادة التفاعل والإنزيمات تحت الدراسة. بالإضافة إلى ما سبق فقد تم دراسة الخصائص الحركية للإنزيمات السابق الإشارة إليها من حيث ميل الإنزيمات لمادة التفاعل، والسرعة القصوى لكل منها ومعامل النشاط الخاص بها وميل التفاعل مع تطبيق برنامΝ الحاسب الآلي المعروف باسم " حركيات النشاط الإنزيمي" لتقدير المعايير السابق الإشارة إليها.

وقد أخذ في الاعتبار دراسة التغيرات التي تحدث في نشاط الإنزيمات التي موضع أثناء تخزين ثمار المانجو لمدة ثلاثة أسابيع على ٥°Ď±Ď٠م وقد ثبت أنه يمكن التوصية باستخدام الجرعة ٥٠٠ Gy Ď في حفظ ثمار المانجو حيث أدت إلى حدوث تثبيط واضح لنشاط الإنزيمات (PPO, POD, PME) وبالتالي تحسين جودة وطول فترة تخزين ثمار المانجو