PEROXIDASE, PECTIN METHYLESTERASE AND SOME CONSTITUENTS OF PISTACHIO NUTS (*Pistachia vera L.*) TREATED WITH γ-RAYS

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ABSTRACT

Pistachio nuts (*Pistachia vera L.*) variety Halapy, Family *Anacardiaceae* were obtained from "Aleppo City", Arab Republic of Syria. The investigated pistachios were kept in perforated standard carton boxes of 45 × 30 × 12 cm dimensions, under a good ventilation conditions. They were irradiated at the National Centre for Radiation Research and Technology (NCRRT) at "Nasr City", Cairo, Egypt. Irradiation treatments were applied using Cobalt – 60 facilities "Egypt Mega Gamma 1" model AECL 6500 and the dose levels were 250, 500, and 2000 Gy. The irradiated samples were stored at 20°C for 6 months through which the activities of peroxidase (POD/EC. 1.11.1.7) and pectin methylesterase (PME/EC 3.1.1.11) were followed. The kinetic parameters in terms of K_m, V_{max}, angle of activity, catalytic efficiency, slope of activity and inhibition levels were considered within a storage period of 6 months. Amino acids and fatty acids profiles of irradiated pistachio were also given. Experiments proved the preference of a dose 500 Gy for inhibiting both of the two tested enzymes in the investigated pistachios during the whole period of storage.

Key words : Pistachio, irradiation, amino acids, fatty acids, peroxidase and pectin methylesterase.

INTRODUCTION

As a physical process, food irradiation provides opportunities to improve food protection and preservation technologies beside solving known public-health problems, as well as minimize the environmental effects caused by chemical and energy-intense processes. Irradiation is gaining recognition as a physical process for reducing food losses, enhancing hygienic quality of food and facilitating food trade. At present, commercial use of irradiated foods and food ingredients is being carried out in 36 countries. They have approved the use of irradiation for processing collectively over 40 food items either on an unconditional or restricted basis (Bruhn 1995 & Lagunas-Solar, 1995) programs are now also directed toward retailers and processors to explain for consumers that they will buy high quality, safety-enhanced irradiated food (Bruhn, 1998).

Controlling of enzymes activity in the food industry can be used to improve and prevent deterioration in appearance, aroma, flavour, texture and nutritional value of food stuffs. The need to maintain the freshness of food products for extended periods of time underscores the role of some enzymes (Boyle & Hebeda, 1990). Peroxidase, a member of oxidoreductase enzymes is considered to have an empirical relationship to off-flavours and off-colours in fresh fruits and unblanched vegetables. Different doses of gamma rays (i.e. 0.75, 1.0, 1.5 and 2.0 kGy) were tried experimentally, with respect to peroxidase (POD) and polyphenol oxidase (PPO) activities, that are related to browning in foods during cold storage at 5-7°C, 75-85% RH (Romero & Barrett, 1997). On the other hand, pectin methylesterase that catalyzes the hydrolysis of methyl ester groups from pectin is involved in the first step of the fruit-ripening process by producing pectin with a lower degree of methylation, which in turn, becomes the substrate of polygalacturonase. The enzyme also plays a key role in fruit softening during ripening and the control of its activity, is of great practical importance in food industry for the maintenance of the texture characteristics of industrial fruit puree (Versteg *et al.*, 1980).

Pistachio is one of the most important tree nuts that grows naturally in most of the saline and dry areas in Syria. It is one of the most important agricultural products which are exported, as well as being largely consumed locally in the daily diet and in many different ways. Khan (1990) reported that experiments on radiation preservation of dry fruits and nuts (apricots, dates, raisins, figs,

pinenuts, walnuts, almonds and peanuts) have indicated that a dose of 1 kGy can improve quality and easily control the insects in dry fruits and nuts. It was aimed through the scope of the study to look forward for the effect of irradiation on the velocity of oxidoreductase and hydrolase enzymes in pistachio. The investigated enzymes namely peroxidase, representing the former group, while the pectin methylesterase was chosen to represent the latter one. Amino acid and fatty acid composition of the investigated pistachios were also concerned.

MATERIALS AND METHODS

Materials :

Pistachio nuts (*Pistacia vera* L. var. Halapy. Fam. *Anacardiaceae*) used in the present study were obtained from "Aleppo" City. Arab Republic of "Syria". Each lot of pistachio nuts were divided into four groups of 2 kg for each one, while group No. 4 was left without irradiation and served as a control, groups 1,2, and 3 were irradiated with different doses of γ-rays. The tested samples were kept in perforated standard carton boxes of dimensions 45×30×12 cm. under a good ventilation conditions. They were transported to 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 were: 250, 500, 2000 Gy, with a suggested stored period of 6 months. The aforementioned doses were chosen from the outlined literature, in such field, i.e. 0.75 up to 2.5 kGy (Zare *et al.*, 1993) and 0.5 and 1.0 kGy (Othman *et al.*, 1998) in pistachio nuts. All of the aforementioned samples were stored at 20±2°C and 60-70% relative humidity. Samples were withdrawn each month for analysis for a period extended for 6 months.

Analytical Methods:

Dry matter : Determination of dry matter was carried out according to A.O.A.C. (1990)

Procedure assay of peroxidase (POD) activity : The procedure of Halpin & Lee (1987) was applied for measuring the activity of peroxidase. The enzyme was extracted from a response weight of the tested samples using sucrose/phosphate buffer of pH 6.5 (0.05M Na_2HPO_4 containing 0.4M sucrose). The weighed samples were added to high-speed blender with 100 ml of buffer solution and after homogenization for 2.5 min., the sample was centrifuged at 20.000 xg and the supernatant was refrigerated for about 15 min. and recentrifuged. The supernatant was filtered through Whatman paper No.1 and aliquots of the filtrate were taken as a source of soluble enzyme.

Substrate solution was prepared using the procedure of Thomas *et al.*, (1981). The substrate solution of 0.5 (v/v) guaiacol in 0.1M K_2HPO_4 (pH 6.0) was stirred for 30 min. and a solution of 0,008%(v/v) 30% H_2O_2 was added immediately before use. Enzyme solution was added to the substrate in a clean cuvette, the volume was adjusted to 2.5 ml. The POD activity was measured as the changes in absorbency at 470 nm. over a time periods of 165 sec. using spectrophotometer Shimatzu /UV-100-02. One unit of POD activity was defined as a change in absorbance of 0.10 unit per min. per g source of enzyme.

Procedure assay of Pectin Methylesterase (PME) activity : Pectin methylesterase activity was measured using the potentiometric method given continuous titration technique as described by Kertesz (1955) and applied after modification by Fayyaz *et al.*, (1995). The activity was expressed as mg. of methoxy group (CH_3O) liberated at 30°C for 30 minutes per 100g sample, as described by Abd-Allah (1966).

Kinetics aspects of POD, and PME : Kinetic aspects of the tested enzymes in terms of V_o, V_{max}, K_m, $1/K_m$, K_m/V_{max} (slope of reaction), V_{max}/K_m (catalytic efficiency), and R^2 values were calculated. The computer programs namely, "Wilman 4", Fit2, Microsoft Excel 2000 and Statistica version 5.0, were applied through the whole work. Residual activity or percentage of inhibition was calculated by the equation of Baldwin *et al.*, (1996). The corresponding activities within different substrate concentrations were considered as follows:

- In case of POD, the substrate concentrations varied between 1.1 ml (0.6825 mole $\times 10^{-3}$) to 2.5 ml (1.5512 mole $\times 10^{-3}$).

- In case of PME, the substrate concentrations varied between 5 ml of (50 mg pectin/100ml) to 30ml (300 mg pectin/100ml).

Kinetic aspects of investigated enzymes based on different enzyme concentrations were considered in terms of Angle of activity, Pseudo values, R^2, slope of reaction. The applied enzyme concentrations were as follows:

- In case of POD of pistachio, the enzyme concentrations varied between 0.75 to 2.00 unit, Unit = (5×10^{-5} g/µl of enzyme source).
- In case of PME, the enzyme concentrations varied between 0.333 to 1.998 unit, (Unit = 0.0666 g/ml of enzyme source).

Fatty acids identification : Perkin-Elmer gas liquid chromatography, series 8300 was used for identifying the fatty acids of the researched samples. Methylation of the fatty acids was carried out according to the recommendation of the AOAC (1990). The following operating conditions were selected carefully to ensure precise calculations of the peaks.

- Detector: Dual flame ionization.
- Column: 15%OV-275. Chromosorb w. A/W,80/100 mesh/length 2m.
- Carrier gas flow: Nitrogen at 15ml /min.
- Sample size: 1 to 5µl.
- Attenuation: $16x10^{-10}$ AMPS: MV.
- Column temperature: 100°C. for 2min. and increased to 190°C with the rate of 7°C /min. and then isothermally for 20 min. at 190°C.

Amino acid analysis : Amino acids were identified by the method of Moore *et al.*, (1958) after derivatization that carried out according to Landault & Guiochen (1964), using N-butanol and trifluoroacetic anhydride. The separation was accomplished under the following conditions:

- Column: 3% OV-225 on (chromosorb-W-HP, 80-100 mesh) glass.
- Program: 4min. at 80°C and increased to 200°C at rate 4°C/min. then stable for 20min.
- Injected and detected.: Temperature, 250°C.
- Carrier gas (nitrogen, 20ml. /min., hydrogen 20ml /min. and air 200 ml/ min.).
- Attenuation: $16x10^{-10}$ AMPS: MV.

Statistical analysis :

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

RESULTS AND DISCUSSION

Velocity of POD in the unirradiated and irradiated pistachio nuts:

Peroxidases are defined as: H_2O_2 oxidoreductase and belongs to a heme enzymes function either activate dioxygen for incorporation into the substrate (oxygenase activity) or use peroxides for oxidation of the substrate "peroxidsae activity" (Nielsen, 1998). Peroxidase activity was determined in irradiated and unirradiated pistachio before and after storage for 6 months at 20°C. within the applied irradiated doses of 250, 500 and 2000 Gy. Before storage, velocity of POD in the pistachio nuts is characterized by the following kinetic parameters :

- Km of the POD that was 3.856 × (6.205 $\times10^{-4}$ mole) for the unirradiated sample exhibited the highest value when pistachio was treated with 500 Gy, being 5.147 × (6.205 $\times10^{-4}$ mole) as seen in Table (1). V_{max} values showed similar trend as that of the K_m.
- On using a dose of 2000 Gy, both of the Km and V_{max} values were higher than the control sample but still lower than the other irradiated pistachios.

During storage for 4, 5 and 6 months and with the exception of the dose 2000 Gy, it was found that at any given substrate concentration, the activity of peroxidase enzyme tends to be higher than that obtained at zero period of storage as seen in Table (2). Data indicated that affinity of the POD towards the substrate ($1/k_m$) was highly correlated positively with the applied doses after the third month of storage. During the latter period of storage, starting from the fourth up to the six-month, the POD activity recorded the lowest

value for the pistachio sample treated with 2000 Gy. as seen in Fig (1). Analysis of variance in the form of one way was applied to check out the significant levels of the POD activity in pistachio treated with 250, 500 and 2000 Gy. The corresponding $P<0.05$ was found to be 0.0319, 0.0029 and 0.0160 in relation to 0.3852 of the unirradiated sample. So, the dose of 500 Gy could be recommended for inhibiting POD in pistachio nuts

Table 1 : Velocity (Δ OD. $min^{-1}.g^{-1}$) of POD in relation to different substrate concentrations after storage of irradiated pistachio nuts for one month at 20°C and R.H. of 60 – 70 %

Sub.Con. (*)	Un-stored sample				Samples stored for 1 month			
	0	250	500	2000Gy	0	250	500	2000Gy
1.1	5.310	5.995	6.178	5.161	8.358	6.917	6.542	4.272
1.3	6.662	6.575	7.046	6.134	9.418	7.398	7.119	4.952
1.5	7.048	7.059	7.723	7.205	10.279	7.878	8.658	5.437
1.7	7.627	7.736	8.688	7.692	10.952	8.454	9.235	5.632
1.9	8.303	8.219	9.364	8.179	12.393	9.512	9.620	5.826
2.1	8.979	9.380	9.846	8.861	13.257	9.896	10.390	6.118
2.3	9.461	10.057	10.328	9.347	14.218	10.376	10.775	6.992
2.5	9.751	10.637	11.776	9.542	15.371	10.376	11.929	7.379
Kinetic Parameters								
K_m (*)	3.856	4.496	5.147	4.339	5.106	2.068	4.051	2.574
SD	0.679	1.030	1.011	0.876	0.864	0.314	0.826	0.868
V_{max} (Δ OD.$min^{-1}.g^{-1}$)	25.174	29.061	34.683	26.865	45.796	10.352	30.626	14.278
SD	3.611	4.728	5.017	3.8143	5.698	1.581	4.303	2.836
Slope$x10^{-2}$ (**)	15.139	15.47	14.840	16.150	11.150	10.680	13.220	18.030
SD $x10^{-3}$	8.863	10.509	7.863	9.899	5.122	7.668	8.575	25.515
Affinity	0.259	0.222	0.194	0.230	0.195	0.483	0.246	0.388
Catalytic efficiency	6.527	6.463	6.738	6.191	8.968	5.005	7.559	5.545

* X ($6.205 x10^{-4}$ mole)
** X ($6.205 x10^{-4}$ mole) /Δ OD. min^{-1} g^{-1}
Affinity = $1/K_m$,1/ X ($6.205 x10^{-4}$ mole)
Catalytic efficiency = V_{max} / K_m , Δ OD. min^{-1}. g^{-1}/ X($6.205 x10^{-4}$ mole).

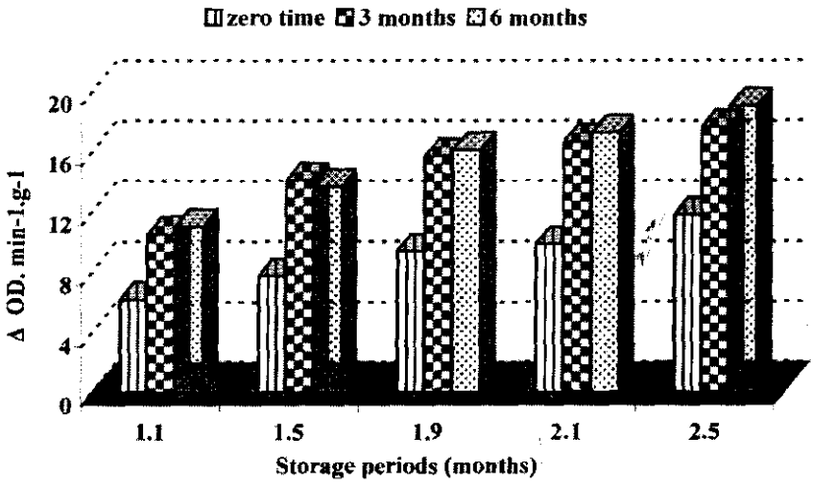

Fig. 1: Velocity of POD in relation to substrate concentration during storage of irradiated pistachio nuts for 6 months.

using of *Eadie-Hofstee* equation (Zubary, 1989) in which a plot of a straight line with a slope of "$-K_m$" could be obtained, the data over the concentration range of the substrate are more evenly weighed.

$$V_0 = [V_{max} - \frac{K_m V_0}{[S]}] \{(V_o) \text{ against } V/[S]\}$$

On such a base, data were recalculated (Table 3) and the intercept on the ordinate and abscissa is related to V_{max} and V_{max}/K_m respectively as seen in Fig. (2). Slope of reaction could be concluded and having the lowest value for the pistachio nuts treated with 500 Gy. Such results confirm the successfully of this dose for inhibiting the velocity of POD.

It was aimed within the scope of the research to study the effect of peroxidase concentration on its activity under the investigated irradiation doses as well as during storage period that extended for 6 months at 20°C. The activity of peroxidase, in the sample treated with a dose of 500 Gy indicates the presence of a down word linear relation between storage and enzyme velocity. This simply means that effect of irradiation on reducing enzyme velocity could be sharply effective when enzyme concentration up to 1.75 or 2 ($5x10^{-5}$ g/µl) was achieved and storage extended more than 3 and less than 6 months at 20°C. (see Fig .3).

Variations of the POD velocity within the investigated pistachio either the unirradiated or the irradiated pistachios could be related to one ore more of the following reasons :

- Saturation of the enzyme with its substrate and the presence of metastable molecules.

Velocity of enzyme reaction should be measured under conditions in which the enzyme is saturated with the substrate, so, the reaction rates are maximum and directly related to enzyme level (Abu Salem *et al.*, 1985 & Foda *et al.*, 1985). With this view in mind, irradiation may affect the surfaces and areas available for the active centers of the POD, a trend that is expected to interfere with the degree of saturation of the enzyme with its substrate, being varied in the tested pistachios and depend on the applied irradiation doses.

Interaction between ionizing radiation and enzymes is of theoretical as well as practical interest. In view of the efforts being made to preserve food, some of the oxidative enzyme molecules are subjected to sufficient

Table 2: Velocity (Δ OD. $min^{-1}.g^{-1}$) of POD in related to different substrate concentration after storage of irradiated pistachio nuts for 4,5 and 6 months at 20 °C R.H. of 60 – 70 %

Sub.con.*	4				5				6 months			
	0	250	500	2000Gy	0	250	500	2000Gy	0	250	500	2000Gy
1.1	17.045	8.586	11.326	6.871	18.622	8.667	11.663	6.865	21.835	14.287	11.006	6.579
1.3	19.317	11.829	12.563	7.825	21.930	12.435	13.733	7.628	24.199	15.886	12.417	7.439
1.5	21.212	13.356	13.514	8.397	24.009	15.074	15.237	8.582	27.129	17.672	13.640	7.914
1.7	22.537	15.741	15.133	9.447	26.184	18.653	16.554	8.677	29.493	18.892	14.862	8.582
1.9	23.484	17.935	16.179	10.306	27.980	22.704	18.059	9.154	32.328	21.619	16.085	9.345
2.1	24.621	20.702	16.941	10.878	29.871	24.117	19.941	9.345	34.408	23.969	17.214	9.917
2.3	26.514	21.560	18.464	11.451	31.099	25.907	20.505	9.536	37.054	25.003	18.061	10.489
2.5	27.462	22.514	19.415	11.832	32.196	26.944	22.198	9.536	39.134	26.695	19.002	10.871
Kinetic Parameters												
Km*	2.126	1.281	3.398	3.545	3.082	1.365	3.664	1.056	4.507	1.020	3.407	2.750
SD	0.165	-	0.357	0.336	0.279	-	0.260	0.174	0.327	-	0.093	0.195
Vmax ($\Delta OD.min^{-1}.g^{-1}$)	50.521	22.51	45.277	29.016	72.97	26.94	52.943	13.982	109.026	26.69	44.890	22.841
SD	2.131	-	3.098	1.816	4.163	-	2.513	0.874	5.634	-	0.8051	0.978
Slope $x10^{-2}$**	4.209	5.693	7.505	12.218	4.224	5.069	6.921	7.558	4.133	3.823	7.590	12.020
SD $x10^{-3}$	1.518	-	2.805	4.011	1.445	-	1.669	7.858	0.889	-	0.741	3.450
Affinity	0.470	0.780	0.294	0.282	0.324	0.730	0.272	0.946	0.208	0.979	0.293	0.363
Catalytic efficiency	23.756	17.562	13.323	8.184	23.674	19.674	14.448	13.230	22.680	26.156	13.174	8.305

Affinity = $1/K_m$; 1/ X (6.205×10^{-4} mole)
Catalytic efficiency = V_{max} / K_m ; Δ OD. min^{-1}. g^{-1}/ X(6.205×10^{-4} mole).
Italic values are calculated using the computer FIT2 Program.

* X (6.205×10^{-4} mole)
** X (6.205×10^{-4} mole) /Δ OD. min^{-1} g^{-1}

Table 3: Correlation between velocity (V) and V/[S] for POD in unirradiated and irradiated pistachio nuts during storage for six months at 20°C R.H. of 60–70%

Storage periods in months	Doses (Gy)	Substrate concentration X(6.205×10^{-4} mole)							
		1.1	1.3	1.5	1.7	1.9	2.1	2.3	2.5
		EADIA-HOFTEE PLOT (V/[S])							
Zero time	0	4.827	5.124	4.698	4.486	4.370	4.275	4.113	3.900
	250	5.450	5.057	4.706	4.550	4.325	4.466	4.372	4.254
	500	5.616	5.420	5.148	5.110	4.928	4.688	4.460	4.710
	2000	4.691	4.718	4.803	4.524	4.304	4.219	4.063	3.816
1	0	7.598	7.244	6.852	6.442	6.522	6.312	6.181	6.148
	250	6.288	5.691	5.252	4.972	5.006	4.712	4.511	4.150
	500	5.947	5.476	5.772	5.432	5.063	4.947	4.684	4.771
	2000	3.883	3.809	3.624	3.312	3.666	2.913	3.040	2.951
2	0	9.548	9.842	9.166	8.368	7.638	7.502	7.099	6.646
	250	6.966	6.116	5.875	5.634	5.545	5.337	5.247	5.057
	500	7.820	7.352	7.136	6.746	6.488	6.189	5.683	5.658
	2000	2.991	2.754	2.711	2.619	2.598	2.535	2.441	2.323
3	0	9.675	9.210	8.742	8.664	8.352	8.101	7.932	7.450
	250	8.499	8.879	8.935	7.968	7.632	7.132	6.968	6.563
	500	9.552	9.480	9.826	8.935	8.245	7.915	7.559	7.031
	2000	6.897	6.205	5.826	5.310	4.902	4.664	4.342	4.225
4	0	15.495	14.859	14.141	13.257	12.360	11.724	11.527	10.984
	250	7.805	9.099	8.904	9.259	9.439	9.858	9.373	9.005
	500	10.296	9.663	9.009	8.901	8.515	7.864	8.027	7.766
	2000	6.249	6.019	5.598	5.557	5.424	5.180	2.252	4.732
5	0	16.929	16.869	16.006	15.402	14.726	14.224	13.521	12.878
	250	7.8791	9.5653	10.049	10.972	12.090	11.484	11.263	10.777
	500	10.662	10.563	10.158	9.737	9.504	9.495	8.915	8.879
	2000	6.240	5.867	5.721	5.104	4.817	4.450	4.146	3.814
6	0	19.850	18.614	18.086	17.348	-17.014	16.384	16.110	15.653
	250	12.988	12.220	11.781	11.112	11.378	11.413	10.871	10.660
	500	10.005	9.551	9.093	8.742	8.465	8.197	7.852	7.601
	2000	5.981	5.722	4.760	5.048	4.918	4.722	4.560	4.348

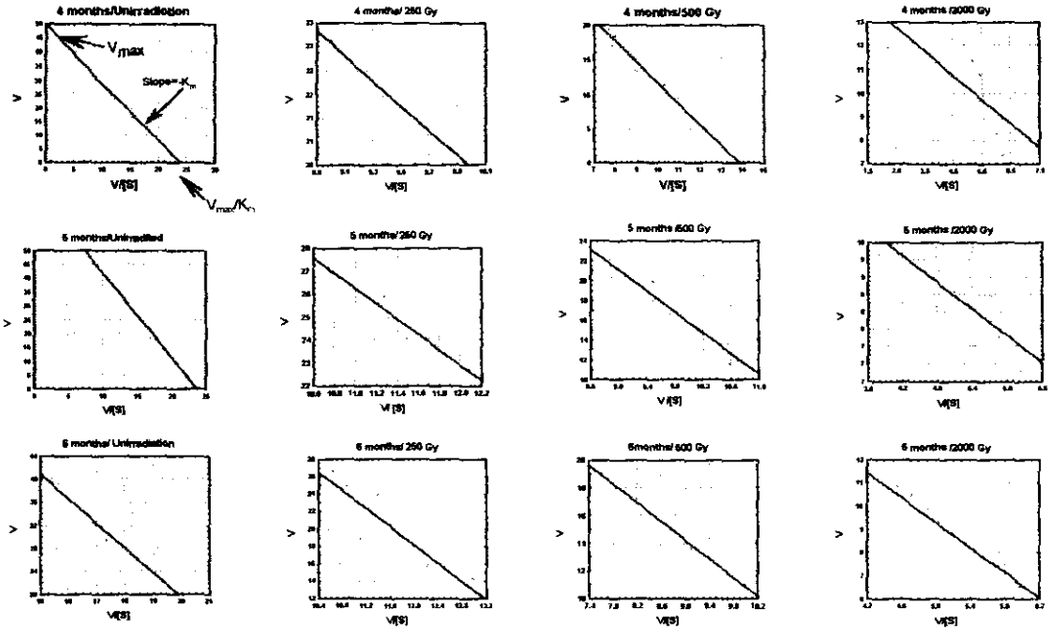

Fig. 2: Correlation between velocity (V) and V/[S] of the irradiated pistachio nuts after storage for 4, 5 and months

Ref. Edia-Hoftee Plot, Zubary, 1989

change to become inactive, other molecules remain intact, and there must be a third category of enzyme molecules which although they have not lost their activity, have become sanitized to further changing leading to inactivation during storage. Such phenomena are known as metastable molecules (Macris & Markakis, 1971). On such a base, metastable molecules may be varied within the investigated pistachios and depend of course on the applied doses and storage periods as well as storage conditions.

■ Mechanism of reaction and catalytic activity:

The peroxidase is found widely distributed and in some cases, the enzyme is unspecific with respect to the hydrogen donor due to the presence of several isoenzymes forms as mentioned by Rodrigo, *et al,* (1996). A total of 42 isoenzymes have been reported, though they are highly specific with respect to the peroxide grouping. In other cases, there was an inverse relationship between V_o and $1/K_m$, so substrate with a high affinity tends to have a low reactivity and vice versa. This trend involves random order binding of the substrates and the existence of alternative pathways of product formation (Dixon *et al.*, 1979). On the other hand, irradiation may interfere with the capability of the POD isoenzymes (that may be present in the investigated pistachios) in acting on or reaction with the substrate, leading to a real variation in the activity of POD within the investigated samples.

It is also has long been known that peroxidsaes display catechol-oxidase (*o*-diphenol-oxidizing) and cresolase activity as given by Chabenet *et al.*, (1993). Thus, paraperoxidsae iosenzymes are capable of exhibiting mixed peroxidsae -oxidsae activity. This is of great importance since the association of oxidsae activities with this basic isoenzyme is capable of oxidizing *o*-diphenols and *p*-diphenols in the absence of H_2O_2, (Lopez-Serrano & Ros-Barcelo, 1996).

The general mechanism of the native ferric enzyme (POD) involves two reaction steps:

- A two-electron oxidation of the enzyme to compound "I" intermediate (POD I), with the peroxide substrate cleaved at the O-O bond.
- A two one-electron reduction of the (POD-I) by electron donor substrate to the native enzyme via compound II intermediate (POD-II).

Since γ-rays could play a role in the internal bonds as well as affecting the severed pathways within the investigated commodities, it is expected that the velocity of the POD will differ from one sample to another and irradiation will inhibit POD to an extent correlated with the applied doses.

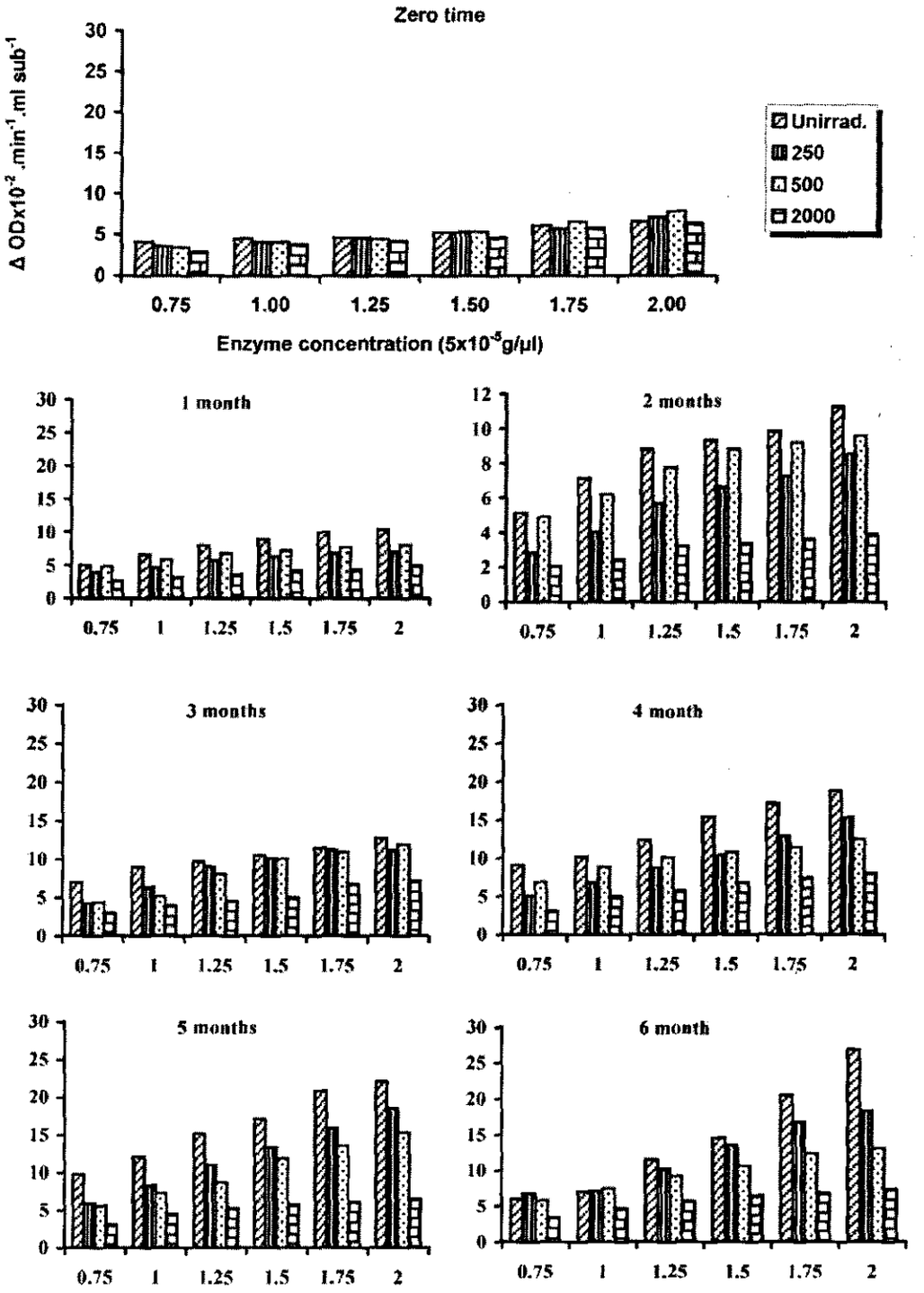

Fig. 3: Relation between peroxidase concentration and irradiation doeses in pistachios during storage for 6 months

Alternatively, the POD-I. H_2O_2 complex formation may lead to the enzyme inactive form. On the other hand, since H_2O_2 behaves as a suicide substrate (suggests in the peroxidsae pathway) that a competition between reductant (donor) substrate and H_2O_2 for POD-I may protect the enzyme form inactivation, (Arnao *et al.*,1990). In the normal cycle, a high ratio of [donor substrate]/ [H_2O_2] favors the POD-I and POD-II cycle, because of the high reaction rate of reduction of POD-I by the donor substrate.

Velocity of PME in un- and irradiated pistachio nuts:

Pectin methylesterase (PME), that removes methoxyl groups in pectin by a nucleophilic attack on the ester, usually leads to the formation of an acyl-enzyme intermediate with the release of a methanol. This in turn is followed by deacylation, which is the hydrolysis of the intermediate to regenerate the enzyme and a carboxylic acid or carboxylate groups along the pectin chain. Multiple forms of citrus PME had been identified most recently by Cameron *et al.*, (1998). The PME activity was measured in pistachio nuts before and after irradiation with the following applied doses, i.e. 250, 500 and 2000Gy. within storage period of 6 months at 20°C. Analysis of the rate of reaction of PME in pistachio nuts before storage showed that, irradiated samples had higher values than the unirradiated one, and such trend correlated positively with substrate concentration. For instance, at a substrate concentration of 300 mg pectin/100ml, the velocity of the PME in the control sample was 1.488 mg CH_3O/100g dried sample /min, which is less than the irradiated samples as seen in Table (4). With respect to the sample stored for 5 and 6 months, the activity of PME measured in the irradiated sample pronounced a sharp down ward trend in relation to the unirradiated one. The previous pattern could be confirmed in terms of V_{max} and k_m values. For instance, the $V_{max.}$ of the sample stored for 6 months that was 7.768 (unirradiated) decreased to 3.913 and 3.049 mg CH_3O/100 g. dried sample/min. for pistachios treated with 500 and 2000 Gy respectively as given in Table (5).

To check out which irradiated dose could lead to a higher inhibition of PME in pistachio, the affinity of the enzyme towards substrate was calculated and compared within irradiation doses in relation to the control sample. Values obtained could be categorized descendingly as follows:

- K_m = 59.1667 mg pectin/100 ml for pistachio sample treated with 500 Gy and stored for 6 months.
- K_m = 121.666 mg pectin/100 ml for pistachio sample having 2000 Gy under similar experimental conditions.
- K_m = 134.166 mg pectin/100 ml , for the sample treated with 250 Gy

The relation between velocity and concentration of PME in pistachio as a result of irradiation doses and storage was studied. Before storage, the unirradiated pistachio having ascending pattern with a slope of 0.369 [(Δmg CH_3O/100 g sample/min) /Δunit of enzyme]. By extending storage to 1 and 2 months, the activity of the PME increased in the unirradiated sample on contrary to the irradiated one at any of the investigated enzyme concentration as seen in Fig. (4). On prolonging storage period up to 6 months, a similar trend was found.

However, when trend line of analysis was considered, irradiation as a whole reduces the activity of the PME up to 50% by the end of 6 months storage at 20°C. Values obtained were 7.768 for the unirradiated one and 3.913 mg CH_3O / 100 g dried sample/min for the pistachio sample received 500 Gy. It is of important to clarify that the dose of 2000 Gy inhibited the activity of PME to the highest reduction rate but it is not recommended because of its effect on the fatty constituents of the pistachio samples, i.e. release the fatty materials inside pistachio nuts. On the aforementioned bases, and statistical analysis of the data, a dose of 500 Gy could be recommended for treating pistachio to improve its quality and to be stored for 6 months at 20°C.

Variations in "PME" velocity could be related to one or more of the following reasons :

■ Presence and /or production of additional substrate:

As to the literature, a breakdown of protopectins could be induced by irradiation of any given samples. Meanwhile, an increase in new H_2O- soluble pectin could be induced as a result of irradiation especially within the last period of storage. Subsequently, the presence of free pectin that produced from protopectins could enhance the PME activity with a level depend on and varied within concentration of the free pectin. Howard *et al.*, (1995) proved that pectin solubility was affected by radiation treatment. Gamma processing caused a reduction in esterification degree of pectin, and conversion of chelator soluble to dilute alkali soluble and non-extractable pectins. These changes were accompanied with an increase in activity of pectin methylesterase.

Table 4 : Radiation-induced changes in activity of PME in pistachio nuts as a result of using different substrate concentrations during storage for 2 months.

Sub.con. (mg pectin / 100 ml)	Activity of the PME (CH_3O/ 100 g sample/ min) within storage periods in months.													
	Zero time					1					2				
	Un.	250	500	2000		Un.	250	500	2000		Un.	250	500	2000	
50	0.249	1.647	0.910	1.574	a	0.521	1.140	1.274	0.935	a	0.836	1.145	1.674	0.811	a
100	0.615	2.283	1.867	2.000	ab	0.962	1.832	2.046	1.302	ab	1.435	1.866	1.864	1.254	ab
150	0.875	2.865	2.795	2.455	ab	1.238	2.657	2.233	1.866	abc	1.952	2.798	2.418	1.488	bc
200	1.089	3.629	3.721	3.167	bc	1.784	2.966	2.976	2.046	bc	2.455	3.162	2.976	1.869	cd
250	1.397	4.277	4.255	3.832	bc	2.093	3.945	3.729	2.523	cd	2.947	3.82	3.348	2.327	de
300	1.488	5.208	5.268	4.348	c	2.445	4.917	4.275	2.981	d	3.556	4.730	3.916	2.678	e
	a	b	b	b		a	b	b	ab		a	a	a	a	
Regression analysis -Linear model: y = a + b x															
Intercept	0.076	0.86:	0.148	0.882		0.151	0.3:	0.675	0.534		0.332	0.505	1.0:	0.444	
Slope	0.005	0.01<	0.017	0.011		0.00:	0.01	0.011	0.008		0.010	0.013	0.0(	0.007	
R^2 %	97.98	99.4(	99.48	99.24		99.4:	98.<	98.4	99.62		99.88	98.95	98.:	99.44	
C.C.	0.989	0.99:	0.997	0.996		0.99:	0.9(	0.992	0.995		0.999	0.994	0.9(	0.997	
S.E.	0.075	0.10:	0.129	0.104		0.061	0.1(	0.156	0.083		0.038	0.148	0.1(	0.058	
V_{max}	1.488	5.20(	5.268	4.348		2.44:	4.91	4.27	2.981		3.556	4.730	3.91	2.678	
K_m	133.33	128.33:	144.166	112.500		138.33:	144.1(	121.66	119.166		135.833	135.00	95.00(	120.833	

N.B. Values of velocity having the same verbal symbol being significance at $P \leq 0.05$

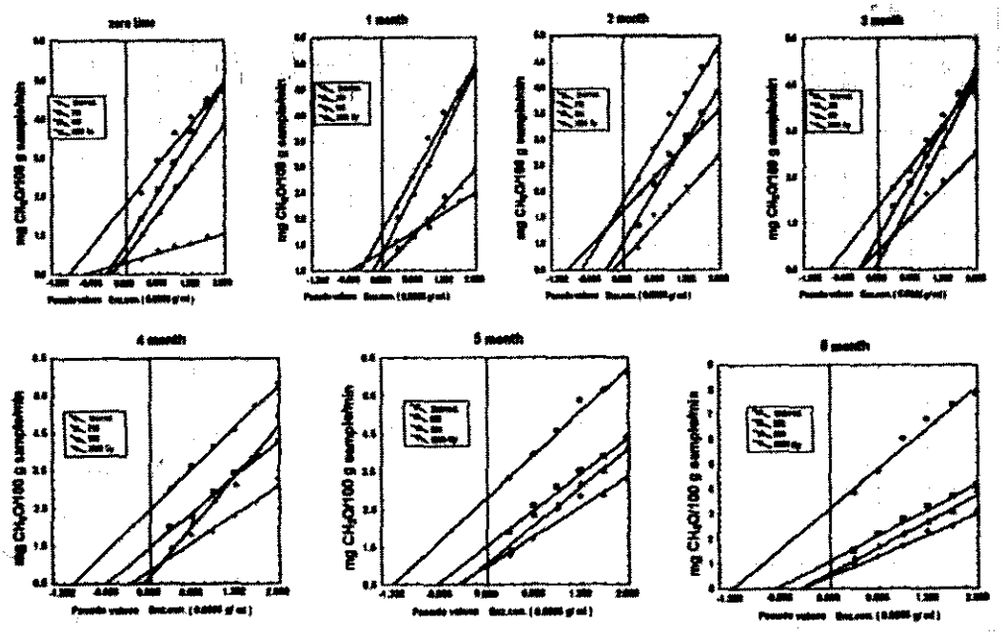

Fig. 4: Irradiation-induced activity of PME under different enzyme concentration in unirradiated and irradiated pistachio nuts stored for 6 months

■ Presence of metal ions and nonesterified galactouronate unit:

The effect of metal ions on the activation of pectinesterase may be related to its interaction with the substrate. On the other hand, polygalacturonic acid has been shown to be a competitive inhibitor in PME-catalyzed hydrolysis reaction. Binding of metal ions to carboxylate groups in this case tends to neutralize the inhibition effect of the pectin substrate on the enzyme. Excess metal ions, however, actually cause inactivation of PME because metal ions are bound to carboxylate groups neighboring to the ester bonds that are required for hydrolysis to occur. The presence of cations prevents also the formation of inactive enzyme-product complexes by competing with the PME for the negative sites on the deesterfied pectin molecule (Macdonald & Evens, 1996). The large number of free carboxyl groups in this latter substrate probably results in the formation of inactive enzyme-substrate complexes.

• Cell wall disassembly and softening:

Recent developments in understanding of the basic biology of fruit and vegetable softening, highlight upon the numerous cell wall modifications, which can potentially occur during ripening or as a result of irradiation, Chen *et al.*, (1998) referred to the causes as to be:

■ The prsence of cell wall hydrolases-pectin enzymes such as α-galactosidase endo-polygalacturonase, exopolygalacturonase, pectin methylesterase, cellulase, β-galactosidase, rhamnogalacturonases, and hemicellulose-degrading activities.

■ Cell wall disassembly

■ Cell wall biosynthesis

■ Nonenzymatic deaggregation of pectin.

In such approach, the pectin content could be increased by irradiation on the expense of protopectin. This may be explained by admitting that irradiation releases some pectins from the protopectins bound with cellulose and hemicellulose of the cell wall. According to this hypothesis, it must be also admitted that radiations are able to release the protopectins bound to the cell wall compounds, either as such, or degraded to pectins. In this case, the amount of free protopectins degraded to pectins is replaced by that part bound to the cell walls and released by radiations. On such a base, a reduction in protopectin and an increase in pectin and pectate fractions due to irradiation of fruits, vegetables and nuts could occur as previously described by Somogy & Romani (1964). These changes were accompanied with a noticeable increase in the activity of pectin methylesterase (PME).

Table 5 : Radiation-induced changes in activity of PME in pistachio nuts as a result of using different substrate concentrations during storage at 3, 4, 5, and 6 months at 20°C

Sub.con. (mg pectin /100 ml)	Activity of the PME (CH_3O/ 100 g sample/ min) within storage periods in months.																			
	3					4					5					6 months				
	Un.	250	500	2000		Un.	250	500	2000		Un.	250	500	2000		Un.	250	500	2000	
50	1.142	1.055	1.837	0.778	a	1.465	0.955	1.85	0.889	a	1.411	0.965	1.865	0.861	a	1.885	0.729	1.567	0.953	a
100	1.715	2.094	2.425	1.331	ab	2.027	1.772	2.325	1.365	ab	2.449	1.539	2.325	1.354	ab	3.256	1.525	2.465	1.355	ab
150	2.568	2.645	3.260	1.722	bc	2.632	2.388	2.796	1.933	bc	3.291	2.265	2.792	1.911	bc	3.970	2.556	2.957	1.667	abc
200	2.936	3.121	3.765	2.128	cd	3.855	2.965	3.457	2.247	cd	4.097	2.722	3.422	2.355	cd	5.055	3.129	3.469	2.122	bc
250	3.372	3.727	4.544	2.625	de	4.855	3.582	4.185	2.822	de	5.122	3.581	3.827	2.821	de	6.473	3.748	3.717	2.733	c
300	4.333	4.805	4.578	3.045	e	5.837	4.284	4.971	3.255	e	6.111	4.362	4.114	3.255	e	7.768	4.134	3.913	3.049	c
	ab	ab	b	a		a	a	a	a		b	ab	ab	a		b	a	a	a	
Regression analyses - Linear model: y = a + b x																				
Intercept	0.548	0.495	1.344	0.283		0.288	0.392	1.079	0.433		0.514	0.215	1.419	0.411		0.719	0.212	1.414	0.472	
Slope	0.012	0.013	0.117	0.009		0.018	0.012	0.012	0.009		0.018	0.013	0.009	0.009		0.022	0.013	0.009	0.008	
R^2 %	98.35	98.65	96.80	99.73		98.38	99.73	98.82	99.65		99.84	99.33	99.05	99.83		99.19	97.82	93.56	99.10	
C.C.	0.991	0.990	0.983	0.998		0.991	0.998	0.994	0.998		0.999	0.996	0.995	0.999		0.995	0.989	0.967	0.995	
S.E.	0.164	0.203	0.223	0.050		0.242	0.070	0.142	0.058		0.077	0.115	0.096	0.041		0.216	0.216	0.250	0.085	
V_{max}	4.333	4.805	4.578	3.045		5.837	4.284	4.971	3.255		6.111	4.362	4.114	3.255		7.768	4.134	3.913	3.049	
K_m	141.00	138.333	80.000	129.166		145.833	135.000	112.500	126.666		142.000	145.833	68.333	126.666		138.333	134.166	59.1667	121.666	

N.B. Values of velocity having the same verbal symbol being significance at $P \leq 0.05$ % level of confidence.

Irradiation effect on amino acid, and fatty acid composition of pistachios:

Amino acids: Diehl (1995) reported that radiation energy deposited in an irradiated protein changes in secondary and in tertiary structure, rather than destruction of constituent amino acids. On the other hand, amino acids, which are sensitive to radical attack when irradiated by themselves are often much less sensitive when they are deeply buried in a protein structure and thus more or less inaccessible to radical reaction

The investigated pistachio nuts were put forward for analysis of their content of amino acids immediately after irradiation. The applied doses were 250, 500, and 2000 Gy in relation to a control sample as seen in Table (6).

With respect to alanine, the decrement level was 31.82 and 34.09 % in response and in between the aforementioned, doses of γ-rays. The other amino acids in the control sample that having a level ranged between 2-3.1% were found to be serine, 2.43%, γ-amino butyric, 2.1%, and arginine, 3%. The valine level in the irradiated pistachio seems to be approximately similar to the unirradiated one with the exception of the sample treated with 250 Gy. On contrary, serine concentration in the irradiated pistachio was higher than the control and its concentration is proportionally correlated with the applied doses. Regarding the γ-amino butyric acid, data of the Table (6) show that the higher applied dose (2000 Gy) led to a very sharp decrement of 71.42% and such trend is in contrast with the arginine since the level of increment under similar dose was 23.33%.

When concentration of the amino acids content around 6% were considered in the investigated pistachio samples, the following identified amino acids were found to be as follows:

• Threonine, 6.2 %, glycine and lysine 6.1% for each one, isoleucine, 6.6% and tyrosine, 6.3 %. These amino acids realized a noticeable increment after irradiation, but with different level with respect to the applied doses of γ-rays.

Table 6: Identified amino acids (g/100g protein) and fatty acids (%) in the tested pistachio nuts.

Amino acids	Irradiated doses (Gy)			
	Unirradiated	250	500	2000
Alanine	2.8	4.4	3.0	2.9
Valine	3.1	4.2	3.2	3.0
Threonine	6.2	8.9	6.7	7.3
Glycine	6.1	8.9	8.1	7.8
Isoleucine	6.6	8.2	7.2	7.7
Leucine	14.1	13.7	14.6	14.5
Serine	2.4	3.1	3.7	4.0
Proline	5.6	8.7	3.6	3.9
γ-Amino butyric	2.1	1.1	1.4	0.6
Methionine	0.6	0.2	0.1	0.8
Cysteine	0.3	0.2	0.8	0.3
Aspartic	5.9	3.6	5.2	4.9
Phenylalanine	13.5	11.7	14.3	12.5
Histidine	0.1	0.1	0.1	0.1
Glutamic	15.4	8.6	12.5	12.2
Tyrosine	6.3	9.0	6.2	7.2
Arginine	3.0	4.1	3.2	3.7
Lysine	6.1	8.8	6.3	7.1

Fatty acids		Irradiated doses (Gy)			
Symbol	Common name	0	250	500	2000
C_8	Caprylic	0.1	0.1	0.1	0.1
C_{10}	Capric	0.6	0.1	0.1	0.1
C_{12}	Lauric	0.2	0.1	0.2	0.1
C_{14}	Myristic	0.3	0.1	0.2	0.2
C_{16}	Palmitic	9.0	8.9	9.4	8.2
C_{18}	Stearic	1.1	1.5	1.5	1.4
$C_{18'}$	Oleic	66.2	67.1	66.2	66.0
$C_{18''}$	Linolenic	0.9	0.5	1.0	1.3
C_{20}	Arachidic	0.1	0.2	0.3	0.3

• It is clear from Table (6) that changes in leucine and phenylalanine contcut after irradiation could be considered within a narrow level, while glutamic decreased sharply after irradiation. The corresponding level of decrement was 2.93, 3.33 and 44.16%. The lowest concentration of the identified amino acids were descendingly ordered in the unirradiated sample as follows: Methionine, 0.6%, cysteine, 0.3% and 0.1% for histidine. These amino acids still being the limiting amino acids in the irradiated pistachio samples at any given of the applied doses. However, the histidine was found to be the first limiting amino acid with a concentration of 0.1%, which is out of change as a result of the applied doses.

Fatty acids: In contrast to the radiation chemistry of food proteins and food carbohydrates, where indirect radiation effects medi-

ated through water radiolysis play a major role, reactions of lipids with the reactive species of water radiolysis play a minor role in most situations-quantitatively at least. Upon irradiation of fats, the primary effect of incident electrons leads to cation radicals and excited molecules. Another primary effect is electron attachment, which may be followed by dissociation and decarbonylation or dimerization. However, the radiolysis of triglycerides of unsaturated fatty acids proceeds similarly. The possible radiolytic products of triglycerides containing fatty acids could cause:

- Formation of aromatic rings
- Condensation of aromatic rings
- Formation of heterocyclic rings

Some of these ring structures are considered to be carcinogens, but the radiotoxin hypothesis has not been confirmed in the dose range of interest in food irradiation. It is important to be aware of the changes that may occur in the constituents of food stuffs in time immediately after irradiation which may give a completely different result from that obtained at an earlier or later time of storage. (Diehl, 1995).

Fatty acids (in the form of even numbers) of the pistachio samples (unirradiated and irradiated one) were identified as given in Table (6). The result obtained could be summarized in the main following points:

- The octanoic acid " C_8 / caprylic acid" of 0.1% was characterized to be out of change as a result of irradiation up to 2000 Gy.
- The capric fatty acid C_{10} which known also as decanoic acid was highly decreased by about 83%, i.e., from 0.6% in the control sample to 0.1% at any of the applied irradiation doses.
- When lauric acid (C_{12}: dodecanoic acid) was considered, the obtained values given in the same table indicate its resistant to a dose of 500 Gy while a reduction of about 50% was found within the sample received 2000 Gy. On contrary, the myristic fatty acid (C14: tetradecanoic acid) possesses its highest destruction (from 0.3 to 0.1 with a 33% level of destruction) on applying a dose of 250 Gy.
- The hexadecanoic acid (palmitic acid: C_{16}) was considered to be approximately staple (around 9%) within the irradiated pistachio. Similar findings were given for oleic acid namely ω_9 ($C_{18:1(9)}$: octadeca-9-enoic acid) and linoleic acid (ω_6: 9,12- octadecadienoic acid), (Belitz & Grosch, 1986). Their concentrations only varied between 66.0 to 67.1% in case of the former fatty acid and from 19.7 to 20.7% in the latter one.
- The palmitoleic acid ($C_{16:\ 1(9)}$: Hexdecaenoic acid) of the same pistachio samples exhibited only about 14.29% increment on receving a dose of 500 Gy, while decreased by about 16.67% on using the other two doses of γ-rays.
- Stearic acid (C18: octadecanoic acid) indicated an upward trend from 1.1 of the control sample to 1.5% for that irradiated with 250 and 500 γ-ray.
- Linolenic acid, which is. Known as "ω_3" ($C_{18:3(9,\ 12,\ 15)}$: octadeca trienoic acid) and
- Rachidic fatty acid (Eicosanoic acid) were of lower values in the irradiated samples received 250 and reached 1.3 and 3% as a result of using 2000 Gy.

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البيروكسيديز والبكتين مثيل أستريز وبعض مكونات الفستق المعامل بأشعة جاما

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أستخدم صنف الفستق *(Pistachia vera L.)* والمعروف باسم صنف الحلبى والذي يتبع عائلة *Anacardiaceae* وتم الحصول عليه من مدينه حلب في الجمهورية العربية السورية. تم حفظ الفستق المتحصل عليه في عبوات كرتونية أبعادها ٣٥×٣٠×١٢ سم تحت ظروف جيدة من التهوية. وقد عوملت عينات الفستق السابقة بأشعة جاما بمركز بحوث وتكنولوجيا الإشعاع بمدينة نصر بالقاهرة باستخدام الكوبلت ٦٠ طراز AECL 6500 واستخدمت الجرعات التالية لمعاملة الفستق وهى ٢٥٠، ٥٠٠، ٢٠٠٠ Gy وقد تم حفظ العينات بعد معاملتها بأشعة جاما على درجة ٢٠°م لمدة ستة أشهر حيث تم تقدير نشاط كل من إنزيم البيروكسيديز والبكتين مثيل أستيريز طوال فترة التخزين.

ومن ناحية أخرى فقد أخذ في الاعتبار تقدير والتعرف على الأحماض الأمينية والأحماض الدهنية لنفس ثمار الفستق كما تشير النتائج إلى أن استخدام الجرعة ٥٠٠ Gy قد أدى إلى تثبيط كل من إنزيم البيروكسيديز والبكتين مثيل أستيريز ولذلك توصى الدراسة بإمكانية استخدام هذه الجرعة لحفظ الفستق.