Pre–Ripening Irradiation on Tomato Quality at Market Ripeness

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ABSTRACT

Mechanical and / or hand harvest of full and over – ripe tomato (Solanum lycopersicum L) fruit and management , lead to yield loss . Pre – ripening gamma irradiation on tomato quality at market ripeness was used to minimize loss and prolong shelf – life . P r ior to irradiation , Chemical analysis was conducted on fruit at different stages of ripening .The results revealed that ascorbic acid was increased as titratable acidity decreased , while ph increased slightly as ripening progressed. The carotenoid, phytoene , a precursor for almost all arytenoids lycopene , a potent antioxidant, and principle carotenoid of red–genotype tomato was not detected in fruit at mature – green ,greenish – red and full red . The carotenoid lycopene , the main carotenoid at marketable stage was only detected at low concentration in fruit at greenish – yellow stage , but steadily increased as ripening continued , while β – carotene, a potent antioxidant and vitamin A precursor was present in mature – green and full – red and had its highest level in greenish – red fruit . In mature – green and greenish – yellow fruit irradiated with 3 and 4 Gy at 24 and 48 hours after harvest , ascorbic acid at marketable ripeness accounted for only 17.97 to 34.06 of control fruit . In fruit irradiated with 1 or 2 Gy ,ascorbic acid was less affected and accounted to 38,81 to 84.28 of control fruit . The highest level of severely rotten fruit were in fruit irradiated with 3 or 4 GY , while the lowest levels of severely rotten fruit were in fruit irradiated with 1 or 2 Gy . Fruit irradiated with 1 to 4 Gy at 24 or 48 hrs after harvest , when greenish – yellow , had the highest weight loss at 7 and 16 days after irradiation . Fruit irradiated when mature - greenish had the most weight – loss at 7 and 22 days after harvest. Gamma irradiation from 0.5 to 4 Gy had no effect on pH or titratable acidity at market ripeness when fruit were irradiated at mature – green and greenish – yellow stages 24 and 48 hrs after harvest .

***Keywords**: Solanum lycopersicum*, fruit , pre – mature , market ripeness .

INTRODUCTION

Tomato (Solanum lycopersicum) is an important vegetable mainly grown on large scale in areas with moderate and subtropical areas in the world with China ,USA and India as main producers . The carotenoids , lycopene and β - carotene are important carotenoids of tomato fruit at market ripeness . Lycopene has been reported to have wide ranging medical benefits (Rao and Agarwat, 2000; Alaluf et al., 2002; Khachick et al., 2002; Miller et al., 2002; Mazaffarian et al., 2003; Sesso, 2003; Campbell et al.,2004; Etminan et al.; 2004; Sies and Stahl, 2004; Bhuvaneswari and Nagini; 2005; Kavannaugh et al., 2007; Stahl and Sies, 2007; Moeller et al., 2008; van Breemen and Pajkovic, 2009 Burton–Freeman and Reamer, 2011; Lademan et al., 2011; Mosca et al., 2011; Thurnham et al., 2014) . Carotenoids may also act as scavengers against reactive oxidative species (Rodrigues et al., 2012), and quenching capacity against singlet oxygen (Nishida et al., 2007).Recently (Consumer Health Digest ,2015) it was found consumption of tomato juice significantly improved menopausal symptoms such as anxiety and flashes in mid aged women . Tomato processing waste may be a source for lycopene and several other important materials (Al–Wandawi et al.,1985). Efforts to make this vegetable available throughout the entire season, minimize loss, extend shelf–life, and be available at a reasonable price important goals . Food irradiation is a promising non–thermal food safety technology that can eliminate disease–causing microorganisms, reduce populations of spoilage organisms, and delay ripening and inhibit sprouting (Huhtanen, 1990; Thayer and Boyd, 1993, 2003; Rezaee et al., 2013). Irradiation of meat and poultry has been for use (FDA, 2007) and its use is allowed for fresh vegetables . The nutritional value of irradiated food is essentially unchanged, the food does not become radioactive, and disease–causing microorganisms are reduced, or eliminated. Current mass mechanical harvest and hand harvesting of fully– and over–ripe tomato usually lead to loss of yield and results in large quantity of immature and/or mechanically damaged fruit which are left as agricultural wastes. This study was undertaken to determine if harvesting tomato fruit at immature stages and exposing them to gamma – radiation can reduce loss . ripeness was investigated.

Materials and Methods:

The tomatoes (cv, Pearson) used in this study were obtained from a field used for fruit production for several years. Uniform tomato fruit were hand harvested at mature–green and breaker (greenish–yellow) stages. Four fruit were randomly selected from 100 plants in the field . Fruit were washed thoroughly in running tap–

water and sorted to discard defective and undesirably colored (only fruit still at mature – green and yellowish – green stage were used for irradiation and or as control) .

Irradiation

Each treatment was run in triplicate including 3 groups of un-irradiated fruit as the control. Prior to irradiation, fruit were stored for 24 or 48 hrs at 25± 5°C, 50–55% ± 5% humidity and with 8 hrs illumination at 150–160 lux measured at level of the fruit surface. Fruit with appropriate colors and no defects were used . Each treatment , 40 fruit per maturity stage were irradiated with 100 to 400 krad (1 to 4 Gy) of gamma rays from Co–60. Fruit were left to ripen under the same conditions as above and checked twice daily for 22 days for fruit that were mature–green and 16 days for fruit that were greenish–yellow at time of irradiation and decay levels recorded. The fruit of all treatments including control were kept in an under freezer type Revco, CA, USA at (- 18 to – 20 °C) until analysis.

Analytical Section :

While still frozen each fruit was horizontally cut to several slices (50 g each) , and combined , fruit samples (500 g each) used for analysis. Only defect–free and marketable fruit were analyzed . For pH measurement, 250 g sample sliced tomato fruit (in triplicate) each homogenized using Warding blender Type LB10G, Lab Depot, GA , USA at high speed for 2 minutes .and pH was measured using Metrohm, Model–704 pH meter, Herrisau, Switzerland) using Metrohm pH 4 , pH 7 , and 3M KCl solution for standardization . For determination of titratable acidity, sliced samples (250 g each) were mixed with 250 mL distilled – demonized water and blended for 5 min as above and filtered . The juice was titrated with standard sodium hydroxide solution . For determination of ascorbic acid, N-bromosuccinamide method (Evered, 1960) was used with slight modification to eliminate possible interference due to color. Twenty randomly chosen fruit were homogenized in an ice bath under a fine stream of high purity nitrogen gas in a Warding blender for 5 min. The homogenate was filtered and made up to 25 ml with distilled de- ionized water . The N–bromosuccinamide and potassium iodide were prepared according to Evered (1960). A working solution of ascorbic acid was prepared by dissolving 100 mg of L (+) ascorbic acid in 100 mL of 1% glacial acetic acid to obtain 1000 $\mu g \cdot mL^{-1}$ of peroxide–free diethyl ether obtained by re - distillation from reduced iron. For determination of ascorbic acid in tomato juice 2 sets of tubes were prepared and designated set A and set B. Each tube in set A contained 0.5 mL of ascorbic acid (working solution) and 5 mL of tomato . The contents of each tube were mixed, and to each tube 1 mL of 1% glacial acetic acid was added and the contents of each mixed and titrated with N– bomosuccinamide solution. The ascorbic acid concentration was determined.

Saponification was done by dissolving the total acetone extracted fraction (fat soluble fraction containing carotenoids) in 20 mL of peroxide–free diethyl ether followed by addition of an equal volume of 10% methanolic–potassium hydroxide solution. Saponification was under an atmosphere of high purity nitrogen for 4 hrs in the dark at room temperature with continuous shaking followed by overnight storage at 4^{O}C. The unsaponifiable fraction was mixed in a separating funnel with diethyl ether (20 mL), absolute ethanol (20 mL), and 10 % (w / v) sodium chloride solution. The total unsaponifiable fraction (ether layer) was partitioned between equal volumes of petroleum ether (b. p. 40 ^{O}C) and 95% ethanol and the petroleum ether layer recovered. Only this fraction was used because it contains the hydrocarbon carotenoids including lycopene which is the principle red color carotenoid of ripe tomato, while xanthophylls which may also be present in a negligible amount in the a 95% ethanol–water layer may contribute a little to the color of red tomatoes.

Separation (resolution) of Individual Carotenoids :

The petroleum ether layer was concentrated to a few milliliters, and 0.2 mL carefully added to the top of an alumina grade II column. The various fractions were eluted with petroleum ether (b.p. 40–60 ^{O}C) containing increasing amounts of acetone (0 – 5% v/v). Individual carotenoids were identified by their uv–visible spectra, dsorption characteristics relative to known compounds, and sometimes co- chromatography with authentic compounds or known carotenoids from biological sources. Individual carotenes were quantified based on their extinction coefficient (Davies, 1976; Britton, 1995) .

Results and Discussion :

Prior to irradiation , ascorbic acid increased as titratable acidity decreased, and pH increased slightly as ripening progressed (Table1). The carotenoid phytoene, a precursor for almost all carotenoids, was not detected in fruit at the mature–green, greenish–red and full–red stages (Table 2). This may be due to presence of carotenoids (phytofluene, lycopene and β-carotene) which depend on phytoene desaturation for their biosynthesis. Phytoene seems to be consumed rapidly while other carotenoids have to be increasingly synthesized as a part of enzymatically controlled ripening . Phytoene was not detected in mature–green fruit but had its highest value in full–red (Table 2 , Figs. 1 , 2). The carotenoid lycopene was not detected in mature–green fruit , but had its highest level in full–red fruit, while β- carotene was present in mature–green, greenish–red and full–red fruit and had its highest level in greenish–red fruit (Table 2, Fig . 1) . The presence of β-carotene in mature–green fruit is necessary to prevent chlorophyll pigments from undergoing photo-oxidation. Lycopene, the principle carotenoid giving tomato fruit their red color, was not in mature-green , but increased as ripening progressed. The β–carotene, a potent antioxidant and precursor for vitamin A , was highest in greenish–red fruit and this may indicate that its formation by a pathway not involving lycopene. There was no significant changes in pH and titratable acidity at market ripeness when irradiation of mature. agreen and greenish–yellow fruit 24 or 48 hrs after harvest (Table 3). In mature-green and greenish-yellow fruit irradiated with 3 and 4 Gy at 24 or 48 hrs after harvest, ascorbic acid at marketable ripeness accounted for 20.58, 17.97, 27.23, 26.79, 34.06, 29.26, 28.86, and 24.63% of control fruit (Table 4). In fruit irradiated with 1 or 2 krad, levels of ascorbic acid were 46.09, 36.81, 51.79, 50.81, 84.28 and 58.52 % of control fruit. Fruit irradiated when greenish - yellow at 24 or 48 hrs after harvest had the most weight–loss at 7 and 14 days after irradiation, while fruit irradiated when mature – green had a maximum weight–loss at 7 and 22 days after harvest (Figs. 3-6).

Gamma irradiation unaffected pH and titratable acidity when irradiation was with 1 to Gy when fruit were mature–green and greenish–yellow at 24 or 48 hrs after harvest (Table 5). Irradiation with 100 to 400 krad had little influence pH and titratable acidity . Irradiation of fruit with all doses (1 to 4 Gy) of gamma radiation resulted in reduction in ascorbic acid levels (Table 8). In fruit irradiated with 1 to 4 krad, mature–green fruit at 24 or 48 hrs after harvest, ascorbic acid levels were 46.37 and 21.00% and 38.39 and 28.00%,in compared to control .In fruit harvested when mature–green and greenish–yellow, and irradiated at 24 or 48 hrs after harvest with 3 or 4 Gy, levels of ascorbic acid were reduced and accounted for 20.58, 17.97, 27.23, 26.79 and 34.06, 28.86 and 24.63% in respect to control, (Table 6). Fruit treated with 1 or 4 krad had ascorbic acid levels of 46.09, 36.81, 51.79, 84.28, 58.52, 38.06, and 38.81 % compared to control . Regardless of ripeness stage and pre–irradiation waiting time, doses in excess of 2 Gy induced loss of ascorbic acid, at market ripeness (Tables 3-6). Fruit irradiated when mature–green with 2 Gy at 48 hrs after harvest, and fruit irradiated 4 Gy when greenish–yellow at 48 hrs after harvest had increased level of lycopene over the control (Table - 8) . The lowest lycopene levels were in irradiated with 4 Gy when greenish–yellow at 24 hrs after harvest (Table - 7). The highest level of defect free fruit (visual sorted) were in fruit irradiated when greenish–yellow at 24 hrs after harvest with 1 , 2 or 3 Gy (Table - 9) . The highest levels of severely rotten fruit were in fruit irradiated with 3 and 4 Gy (Table - 10) . Fruit harvested when greenish–yellow and irradiated at 48 hrs after harvest with 1 to 4 Gy had 40, 35, 30 and 39% respectively, of defect–free fruit and the lowest levels of severely rotten fruit were for those irradiated with 1 or 2 krad (Table-10).

Conclusion: Pre-management had affected tomato fruit at market ripeness and irradiation with a dose in excess of 2 Gy is not recommended.

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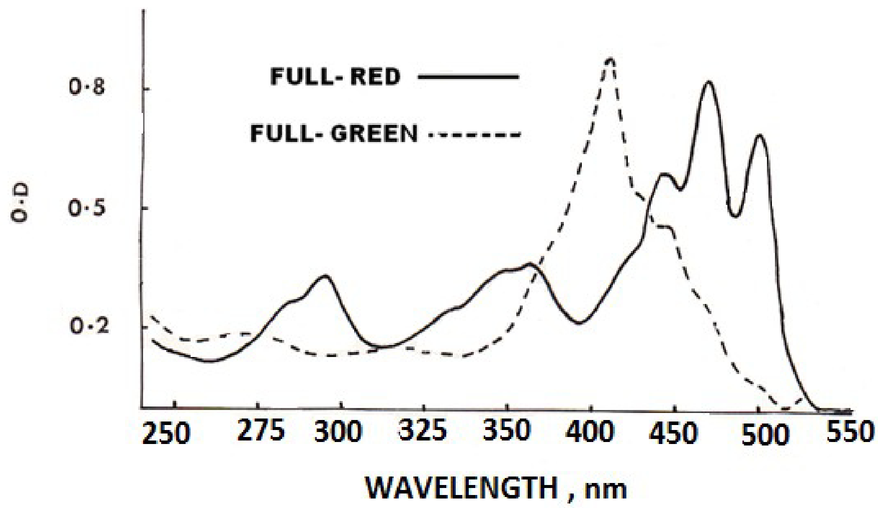


Fig . 1. Absorption spectra of Tomato fruit at mature-green and red-ripe fruit.

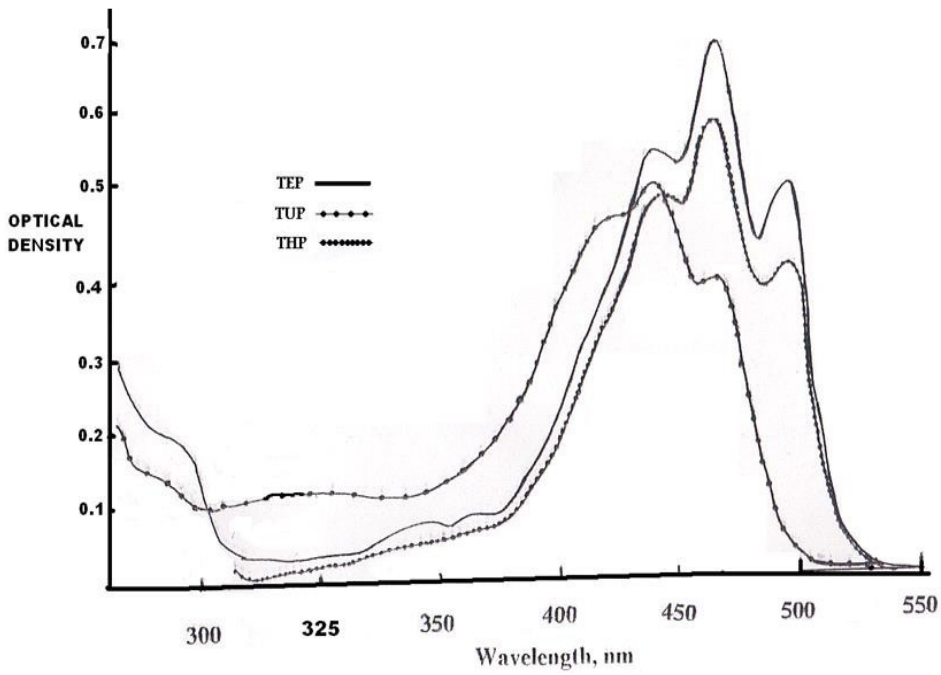


Fig. 2. Absorption spectra in petroleum spirit of total unsaponfiable fraction (TUF), total epiphasic fraction (TEF), and total hypophasic fraction (THF) of tomato fruit at marketable ripeness . (for details see the text) .

Table 1 , Tomato fruit – effect of stage of ripening on pH * , titratable acidity* and ascorbic acid *.

Stage of ripening	pH	Titratable acidity [a]	Ascorbic acid [b]
Mature – green	4.285	20.00	3.39
Greenish – red	4.191	20.25	5.35
Full – red	4.361	18.45	7.34

Each value is average of triplicate measurements .

[a] values in mL of 0.1 N – NaOH tomato fruit .

[b] values in mg ascorbic acid / 100 gm tomato fruit .

Table 2 . Effect of stage of ripening on carotenoids (mg / 100 gm total fat - soluble pigments) of tomato fruit .*

Stage of ripening	Phytoene	Phytofluene	Lycopene	β – Carotene
Mature – green	0.00	0. 00	0.00	2.00
Greenish – red	0.00	2.32	59.13	22.0
Full – red	0.00	6.19	89.51	5.08

* Each value is average of triplicate measurements

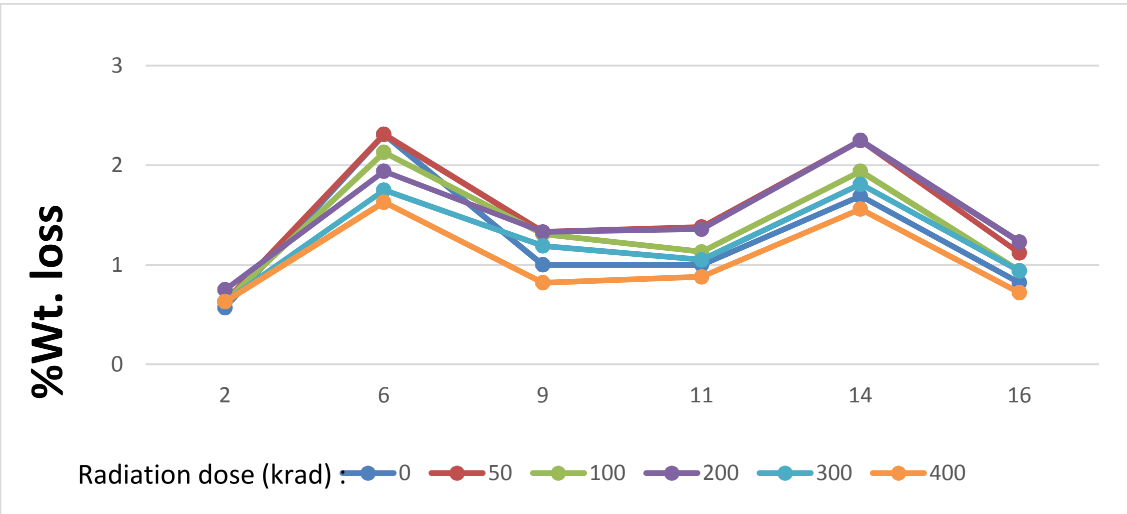


Fig. 3 . Weight loss (%) at days following irradiation with 0, 0.5, 1 , 2, 3, 4 Gy of greenish – yellow fruit 24 hrs after harvest with post–irradiation conditions of

$25^{O}C \pm 5^{O}C$, 50–55 % ± 5 % humidity and with continuous daily illumination of 150 -200 lux for 8 hrs.

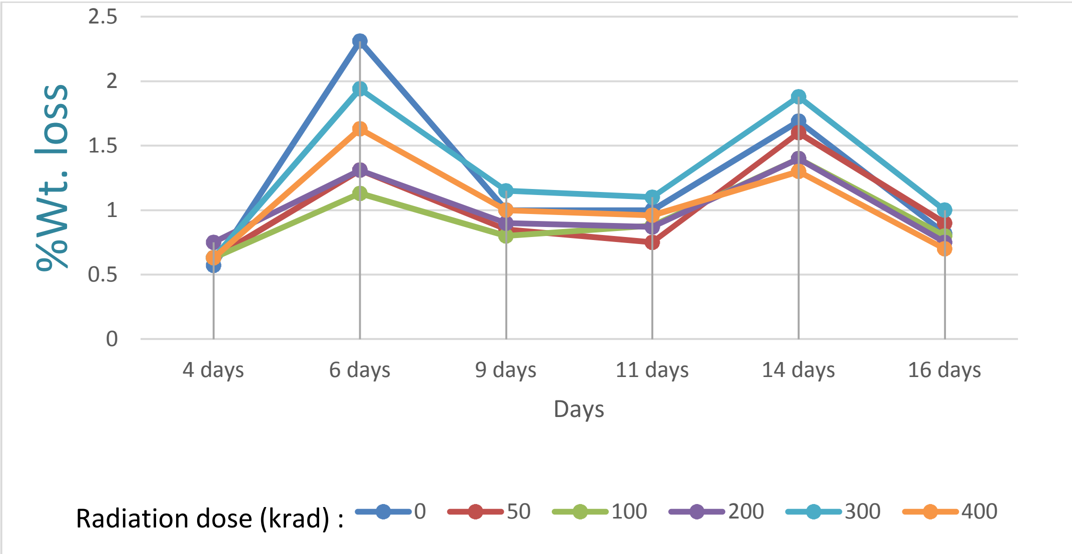
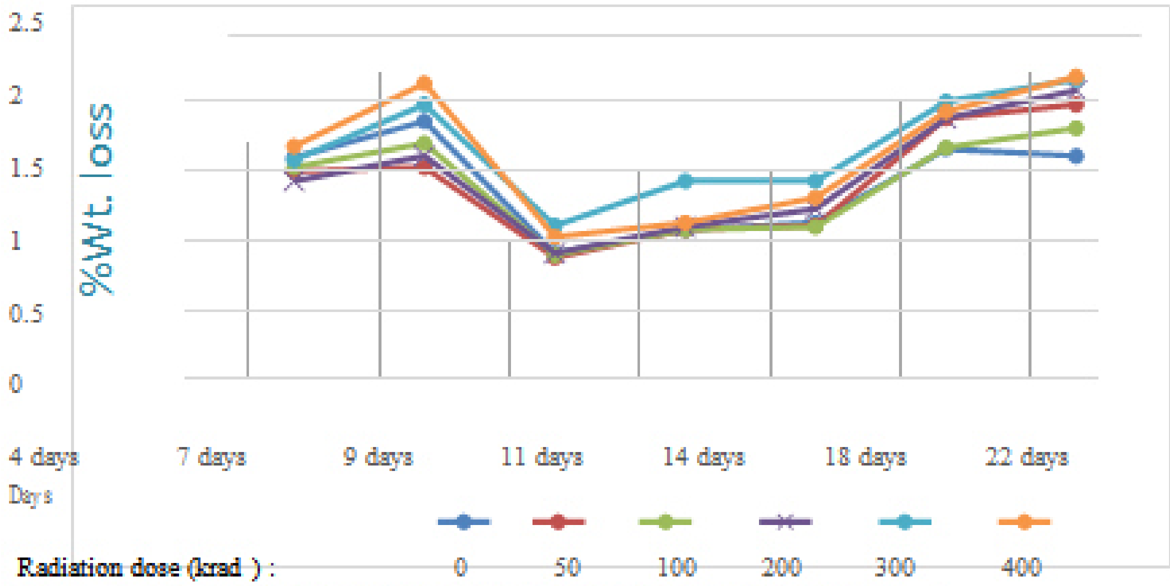


Fig. 4. Weight loss (%) of tomato at days following irradiation with 0, 0.5, 1 , 2 ,
3, 4 Gy of greenish - yellow fruit at 48 hrs after harvest with post–irradiation storage conditions of 25^{O}C ± 5^{O}C ,50–55 % ± 5 % humidity and with continuous daily illumination with 150–
160 lux measured at fruit surface for 8 hrs.



Fig, 5 . Weight loss (%) of tomato at days following irradiation with 0, 0.5, 1, 2,
3, 4 Gy of mature – green fruit at 24 hrs after harvest with post–irradiation storage conditions of 25^{O}C ± 5^{O}C, 50–55 % ±5 % humidity and with continuous daily illumination with 150-
160 lux measured at fruit surface for 8 hrs.

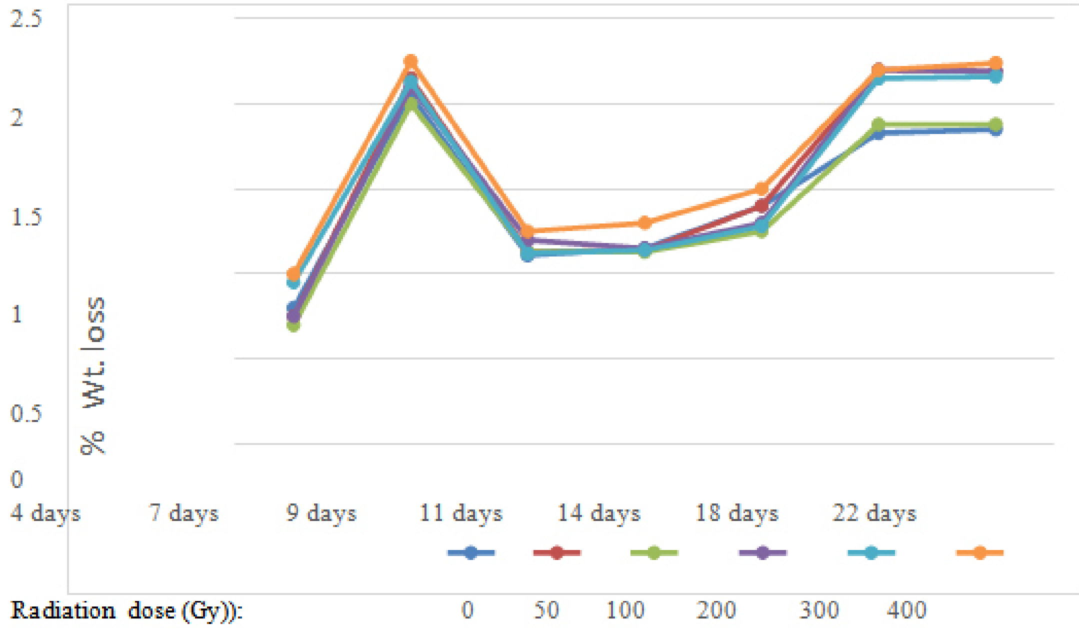


Fig .6 . Weight loss (%)at days following gamma irradiation (0 , 0.5 ,1 , 2 , 3 , 4 Gy of mature – green fruit 48 hrs after harvest with post – irradiation conditions of 25 ° C ± 5 ° C, 50 -55 % ± 5 % humidity and with continuous daily illumination of 150 -200 lux for 8 hrs.

Table 3 . Effect of gamma – irradiation of Tomato at mature – green and greenish – yellow stages at 24 hrs (Group A) and 48 hrs (Group B) after harvest ,on pH and Titratable acidity .

Radiation dose (krad)	Stage of ripening							
	Mature-green				Greenish-yellow			
	pH		Titratable acidity		pH		Titratable acidity	
	A	B	A	B	A	B	A	B
0	4.204	4.331	17.35	21.90	4.450	4.358	26.78	21.67
100	4.256	4.307	16.90	17.10	4.433	4.353	26.40	21.10
200	4.238	4.377	19.30	21.52	4.402	4.310	25.60	22.10
300	4.296	4,247	14.77	14.10	4.343	4.428	23.60	25.60
400	4.277	4.325	15.10	16.20	4.516	4.436	24.75	21.40

Table 5 . Effect of gamma – irradiation on Tomato at mature – green at 24 hrs after harvest on carotenoid composition at market – ripeness .

Total carotene(mg/100g TFF)	β-carotene		lycopene		Phytofluene		Phytoene		Dose(Krad)
	% total carote n-es	mg/ 100g TFF	% total-carot-	mg/1 100g TFF	% total carote-nes	mg/100g TFF	% total carotenes	mg/100g TFF	
680.9S	17.8	120.9	4.5	30.6	18.3	124.5	59.5	404.9	0
814.7	7.9	64.1	2.6	21.1	17.9	145.9	71.7	583.6	100
229.5	36.1	108.7	0	0	13.6	40.8	50.1	150.0	200
444.6	18.8	83.6	4.4	19.6	12.9	57.2	63.9	284.2	300
286.3	4.7	13.4	4.6	13.2	15.7	45.0	75.0	214.7	400

Table 6 . Effect of gamma – irradiation of Tomato at mature - green stage at 48 hrs after harvest on carotenoid composition at market – ripeness .

Dose(krad)	Total carotene (mg /100g TFF)	β–carotene,%total carotenes		Lycopene% TFF		Phytofluene,% total carotenes		Phytoene, %TFF	
0	389.3	4.9	46.5	3.4	13.2	18.5	72.0	66.2	257.6
100	607.2	6.9	41.7	3.7	22.2	16.6	100.7	72.9	442.6
200	524.8	24.8	129.9	4.8	25.0	12.8	67.0	57.7	302.9
300	354.9	8.9	31.5	5.2	18.4	14.1	50.0	71.9	255.0
400	289.5	9.8	28.3	3.4	10.0	17 17	49.1	69.8	02.1

Table 7 . Effect of gamma – irradiation of Tomato at greenish – yellow at 24 hrs after harvest on carotenoid composition at market – ripeness .

Dose Krad	Total Carotenes, mg / 100g TFF	Lycopene		β-carotene		Phytoluene		Phytoene	
		% total carotenes	mg/100 g total TFF	% total carotenes	mg/100 g TFF	% total carotenes	mg/100 g TFF	% total carotenes	Mg/ 100g TFF
0	3058.3	71.0	2171.0	0.2	7.5	7.2	218.8	21.6	661.0
100	2144.8	72.2	1547.4	0.1	1.9	7.6	163.6	20.1	431.9
200	1472.0	75.6	1113.0	3.1	45.0	5.6	82.7	15.7	231.3
300	1215.2	60.8	869.2	3.7	52.3	15.1	215.6	20.5	7. 293
400	706.2	72.8	514.1	0.6	4.5	4.9	34.9	21.6	152.7

Table 8 . Effect of gamma – irradiation of Tomato at greenish – yellow stage at 48 hrs after harvest on carotenoid composition * at market – ripeness .

Dose Krad	Total Carotenes ,mg/100g	Lycopene		β-carotene		Phytofluene		Phytoene	
		mg/100g carotene	mg/100g TFF	mg/100g carotene	mg/100g TFF	mg/100g carotene	mg/ 100g	% total carotene	mg / 100g TFF
0	1194.9	56.0	669.6	0.3	3.5	11.8	140.4	31.9	381.4
100	630.1	59.4	374.2	0.2	1.0	13.0	32.2	27.4	172.77
200	1026.0	74.9	768.1	0	0	6.9	71.2	18.2	186.7
300	525.3	60.8	6o.4	380.4	0	10.7	66.6	28.5	178.3
400	2309.1	71.25	1645.2	0	0	7.0	160.9	21.8	503

Each value is average of triplicate samples .

Table 9 . Effect of gamma – irradiation of Tomato at greenish – yellow stage at 24 hrs after harvest on levels of fruit – rottenness at market ripeness .

Dose (krad)	0	1- 25 (+)	26 -50 (++)	51 -75 (+++)	76 -100 (++++)
0	45	20	25	4	6
25	40	23	27	3	7
50	46	25	18	3	8
100	49	21	16	4	10
200	54	16	15	5	10
300	52	17	10	5	16
400	42	15	11	10	22

Table 10 . Effect of gamma – irradiation of Tomato at greenish – yellow stage at 48 hrs after harvest on levels of fruit rottenness at market ripeness .

Dose (krad)	0	1- 25 (+)	26 -50 (++)	51 -75 (+++)	Ting (++++)
0	46	29	5	8	12
25	49	25	6	9	11
50	44	27	8	8	13
100	40	25	15	13	7
200	35	30	12	18	5
300	30	22	20	12	16
400	39	6	14	16	25