



Effect of gamma irradiation on sensory quality and microbial control of table grapes (Sundar khani grapes)

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Abstract

Grapes are one of the world's healthiest foods and it is greatly nutritious. The current study was carried out to inspect the effect of gamma radiation on the surface microbiota of fresh green produce of one of the Pakistan's most eaten variety Sundar khani grapes. The organoleptic characters and sensory attributes along with epiphytic microbial flora of both irradiated and control sample was analyzed after 7 days interval at refrigerated condition. For all trials, the decay assessment and results for sensory evaluation were recorded. Sensory attributes were maintained in low doses and teeth firmness and rachis browning was acceptable in grapes irradiated at 0.8 kGy. Positive effects of irradiation was increase in sweetness and flavor. Irradiation had no effect on berry shatter but weight loss and microbial load was significantly decreased in irradiated grapes. Results showed that low dose of 0.8 kGy was optimum dose at which quality of grapes was most acceptable and shelf life was increased by 2 weeks.

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Introduction Grapes are the world's eighth largest food crops and the variety of colors and flavors of the fruit are liked by the consumers as well as grape berries are appreciated for their convenient bite size. Grapes are utilized for wine production and other fermented beverages, dried into raisins, can also be used for fresh juice and as essence in food (Aujla *et al.*, 2011). Grapes which are consumed fresh are table grapes with large berries and seedless. Grapes cultivars with distinctive aroma and flavor are transformed into unfermented juice (Reisch *et al.*, 2012). The demand of supplemental products in fresh fruits and dietary sources such as grapes, berries and wines is increasing at commercial scale. Grapes have highly bioactive resveratrol derivatives compounds such as trans-viniferin and transresveratrol which have antioxidant and hepatoprotective properties, and can induce apoptosis of leukemia B cells (Rayne *et al.*, 2008). Grapes are the finest source of energy and contain larger quantity of carbohydrates. Resveratrol and its biological activities are considered to be important because it is found in seeds and skin of grapes. (Yadav *et al.*, 2009).

In Pakistan this fruit is consumed both in dried and in fresh form and Balochistan is leading place for grapes production. In Pakistan, *Vitisvinifera* are one of the major crops and mainly cultivated in high elevated valleys of Balochistan. In Balochistan area under cultivation of grapes in 2009 was 15118 hectares and production of fruit was 74758 tons. The leading grape varieties in Balochistan are Haita, Kishmishi, Sundarkhani, Sahibi and Shekhali (Aujla *et al.*, 2011). The important cultivars of table grapes are also grown in some districts of Khyber Pakhtunkhwa and annual production is 122 thousand tons. Average yield calculated was 19 tons per hectare against the potential of 25 tons per hectare (Bashir *et al.*, 2012). Popular cultivars of table grapes can be grown in central area of Punjab (Uddin *et al.*, 2011). Fresh table grapes produced in Pakistan are exported to Bahrain, Bangladesh, Germany, United Arab Emirates and United Kingdom. In 2008-2009, quantity of 184256 kg fresh grapes was exported to these countries which valued 9054000 rupees (Reisch *et al.*, 2012).

Grapes are exposed to several diseases and high moisture leads to the development of certain diseases such as botrytis rot, anthracnose, black rot, powdery mildew and downy mildew. (Ferreira, 1990). There are some other factors which contribute to postharvest losses such as mechanical damage during handling and harvesting, improper postharvest sanitation, environmental conditions and poor cooling. Postharvest losses during transport, storage and distribution are main problems for perishable vegetables and fruits which leads to low profit and less per annum cost of fruit (Aujla *et al.*, 2011).

Gram positive bacteria associated with table grapes berries are mostly *Micrococcus* and *Bacillus* species. Some lactic acid bacteria present on grapes are *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Weissella*, etc (Bae *et al.*, 2006). Other associated bacteria are *Oenococcus oeni*, *Leuconostoc oenos* and *Asaia*. Gram negative bacteria are mostly *Pseudomonas* species, *Actinobacteria*, *Proteobacteria* and *Firmicutes* identified as microflora on grapes (Martins *et al.*, 2012). The most common fungi isolated from grapes were *Botrytis cinerea*, *Rhizopus stolonifer*, *Penicillium notatum*, *Alternaria*, *Cladosporium*, *Fusarium* and *Cladosporium* (Tournas and Katsoudas, 2005). However, the most prevalent yeast species present on grapes are *Saccharomyces cerevisiae* (Renouf *et al.*, 2005).

Ionizing radiation is a revolutionary technique to process foods, pharmaceutical and medical products to disinfect them (Sommers *et al.*, 2004) by eliminating pathogenic bacteria (Crawford and Ruff, 1996). It can be used as quarantine treatment (Dionisio *et al.*, 2009) for fruits and vegetables (Fan and Mattheis, 2001) to extend their shelf life (Kilcast, 1994) by the reduction of parasitic microorganism (Moy and Wong, 2002; Pezzutti *et al.*, 2005) and delay fruit deterioration (Hallman, 1999). Ionizing radiations are of three types consisting of high energy gamma rays, accelerated electron beam and X rays. They are called ionizing because they knock out the electrons and form charged particles called ions. Gamma rays are approved radiation for food and are

produced from radioisotopes cobalt 60 and cesium 137. Most common source of gamma radiation is Co-60 (Arvanitoyannis, 2010).

The other two types have many draw backs such as electron beam caused loss of firmity and change in color, Xrays may lead to change in texture. Gamma irradiation are better than other radiations because less fruit damage , less loss of firmity and extended shelf life is evident. Gamma radiation undergo uniform penetration in fruit and do not increase temperature of fruit. Gamma irradiation are useful for improving hygienic conditions and nutritional quality of fruit leading to increase in shelf life and storage which enhance the trade opportunity and export quality of fruit (Arvanitoyannis, 2010). It is practicable under conditions existing in Pakistan. In Pakistan food can be preserved by using gamma irradiation practically and economically under specific conditions. The study is related to examining the trend in fruit deterioration which is the major constraint in grape marketing system, identification of decay causing microorganisms, delay of fruit decay by using gamma irradiation and enhancement of international competitiveness of grapes. The practice preferably was carried out to find feasibility of gamma irradiation to increase storability of fresh grapes and delay spoilage and post harvest losses

Materials and methods

Sample Collection and Gamma irradiation

Fresh green grapes (*Vitisvinifera* var. Sundarkhani) were collected from local market of Lahore. The selected clusters of grapes were in a good and healthy condition and had uniformity in growth. These grapes were packed in perforated polythene bags properly and divided into control and experimental groups. Then bags were labeled with name of variety of grapes and gamma irradiation doses to be applied. The experimental groups of grapes were subjected to different gamma irradiation doses (0.5kGy, 0.8 kGy and 1.0 kGy) using Co 60 as irradiation source at Pakistan Radiation Service (PARAS) Lahore. However, Control group received no radiation. After irradiation, grapes were stored at refrigerated

temperature (Kim *et al.*, 2014). Grapes for each dose level were examined for sensory evaluation and microbial load at every 7 day interval

Sensory evaluation

9 point hedonic scale was used for sensory attributes assessment(Peryam and Pilgrim, 1957) Sensory evaluation of grapes was performed after making the grapes presentable. Unwashed grapes were washed and dried with paper towel. Then placed in small container and stored in refrigerator after covering with plastic wrap. On evaluation day, grapes were kept out of refrigerator 2h before sensory and analytical testing to set them at room temperature (Kim *et al.*, 2014). Texture, Flavor, appearance acceptability and overall acceptability of irradiated and non-irradiated grapes was evaluated. Analytical attributes including color of grapes and rachis was visually inspected by each panelist from white green to yellow and brown. Control and irradiated grapes both were evaluated for color change and rachis browning (Kim *et al.*, 2014).Bunches were cut into small clusters and 10 to 12 grape berries attached to the rachis were served to the trained panel. Sample was served in paper boats and to prevent biased decision, samples were presented in random order with labeling 1st , 2nd and 3rd(Kim *et al.*, 2014). To assure palate cleaning between taste samples and attributes panelists were provided with tasteless soda crackers and glass of filter water (Guillén *et al.*, 2007). Teeth firmness (firmness of biting a full grape berry in mouth), rachis browning and sweetness of grapes were evaluated using anchored, unstructured 15 point scale having score range of 0 (none), 1, 2.5, 6, 9.5, 14.5 and 15 (Intense). Sensory attributes of cold stored grapes was evaluated after every 7 day interval.

Berry Shatter

Sample bags from Control and Irradiated were selected randomly for each dose level at evaluation day. Each bag was weighed then the grape clusters were removed from the bag in laminar flow and gently shaken for 15 s. The grape berries that became detached were weighed and expressed as a percentage of the total weight of the bag (Kim *et al.*, 2014).

Physiological loss in weight

Healthy bunches of green table grapes were transferred to perforated polythene bags having net weight up to 250 g. Each bag from each dose containing grape clusters was weighed before and after irradiation. Weight loss was evaluated after every 7 day interval and the difference of weight during storage period was noted. Weight loss was estimated according to Sabir *et al.* (2010).

$$\text{Percentage of physiological weight loss} = \frac{W_i - W_f}{W_i} \times 100$$

Decay analysis

To determine decay percentage, total number of normal grape berries was counted. Then, number of affected grape berries was counted in each treatment and percentage of fruit decay was assessed by using this formula (Valero *et al.*, 2006).

$$\% \text{ Decay Analysis} = \frac{\text{No. of affected grape berries in a bunch per treatment}}{\text{Total No. of grape berries in a bunch in that treatment}} \times 100$$

*Estimation of micro flora**Microbial isolation*

Epiphytic micro flora was isolated by using serial dilution method. 10 g of grapes (approximately 3-4 grape berries) was washed in 100 ml of sterile distilled water by shaking it thoroughly. Different dilutions were prepared from this stock solution. 0.1ml of each dilution was spread over the prepared plates of nutrient agar, MacConkey agar, SS agar and potato dextrose agar to determine bacterial and fungal population on grapes (Renouf *et al.*, 2005). After spreading, all the plates except PDA were incubated at 37°C for 24 hours for bacterial growth. The PDA plates were incubated at 28-30°C for 3 days for fungal growth. After specific time period total bacterial and fungal colonies were counted and CFU were calculated by using the following formula.

$$\text{CFU/g} = \frac{\text{No. of Colonies on plate} \times \text{dilution factor}}{\text{amount plated}}$$

The Analytical profile index was used for the identification of bacteria. However, fungi were identified on the basis micro and macroscopic

characteristics.

Statistical analysis

Results and Significant differences between means of every assigned treatment were validated by using Costat 6.4 by completely randomized design. In Costat, means of five replicates of each treatment for each interval were compared and statistical significance was determined using Duncan's New Multiple Range test at $P \leq 0.05$. The mean square error and standard deviation of replicates was also found from mean values

Results and discussion*Decay incidence*

The Table 1 showed that no decay was noticed in irradiated and non-irradiated grapes till 14 days of storage. Green grapes labeled as control started to deteriorate after 2 weeks and by the end of third week they were completely decayed. Grapes irradiated at 0.5 kGy started decaying after 21st day. Grapes remained unaffected when irradiated at 0.8 kGy during cold storage period. After 21st day, significant decrease in firmness and textural alteration was observed in grapes treated at 1 kGy. The dose of 0.8 kGy was found to be most acceptable, showed less bruising and deterioration and its shelf life was increased by 2 weeks up to 28 days.

Sensory evaluation

Figure 1 indicates hedonic scores of 9 point scale significantly showed that degree of likeness for flavor, overall acceptability and appearance acceptability of irradiated grapes was more than control and consumers often scored them "Like Very Much" on evaluation days. Acceptance of irradiated samples was statistically low only in case of texture of Sundarkhani grapes from control evaluated by 9-point hedonic scale (Table 2). Grapes irradiated at low dose of 0.8 kGy for phytosanitary purposes maintained its quality throughout the storage period of 4 weeks and liked more.

*Descriptive Analysis**Teeth Firmness*

Teeth firmness was measured by trained sensory panel. Table 3 depicted that teeth firmness was significantly decreased overall in both irradiated and unirradiated samples. But there was less decrease of teeth firmness in grapes radiated at 0.8 kGy. Textural changes and berry softening is directly related to shelf

life of fruit and quality is related to commercial importance. Textural change and softening was observed in current study in grapes irradiated at high dose of 1 kGy. Degradation of polysaccharides and starch in cell wall and loss of turgor pressure may leads to fruit softening (Prasanna *et al.*, 2007).

Table 1. Effect of gamma irradiation on decay percent of green Sundarkhani grapes kept at refrigerated temperature.

Storage Days	Irradiation Doses (kGy)			
	0	0.5 kGy	0.8 kGy	1.0 kGy
1	0	0	0	0
2	0	0	0	0
4	0	0	0	0
6	0	0	0	0
8	0	0	0	0
10	0	0	0	0
12	0	0	0	0
14	0	0	0	0
16	1	0	0	0
18	2	0	0	0
20	3	0	0	0
22	4	0	0	0
24	Decayed	1	0	0
26	Decayed	2	0	0
28	Decayed	3	0	1

Sweetness of Grapes

Figure 2 showed that sweetness in irradiated and non irradiated grapes during storage was increased. Sweetness of irradiated Sundarkhani grapes was significantly enhanced in all samples for all level of doses. It might be due to increase in carbohydrate

content (Prasanna *et al.*, 2007). There was significant increase of sweetness between control and irradiated but there was no difference between different dose levels. It showed that positive effects of irradiation are increase in sweetness and flavor.

Table 2. Sensory evaluation of Grapes using 9-point Hedonic scale.

Attributes*	Radiated Grapes	Un irradiated Grapes	p value
Overall liking ^s	5.2 ± 2.7 ^a	2.8 ± 1.32 ^a	0.03
Texture liking ^s	2.2 ± 1.32 ^b	4.6 ± 3.38 ^b	0.044
Flavour liking ^s	6.0 ± 1.41 ^c	3.25 ± 0.82 ^c	0.002
Appearance acceptance ^s	5.4 ± 3.44 ^d	2.6 ± 1.85 ^d	0.04

Sample means ± standard deviation and significance for consumer acceptance (n=25) Different superscripts indicate that the mean difference is significant at $p \leq 0.05$ by Duncan's New Multiple Range Test.

Rachis browning

Table 4 depicted increasing trend of rachis browning in irradiated and non irradiated grapes during cold storage. Rachis browning was significantly increased in both non irradiated and irradiated grapes but was noticed less as compared to control in irradiated

samples. Rachis browning in grapes irradiated at 1 kGy was significantly more than others. This showed that high dose induced rachis browning. Less browning was observed at low dose of 0.8 kGy. Rachis due to its more surface to volume ratio is particularly vulnerable to water loss. In grapes, the respiration

rate may be 15 times or higher than that of berries and due to high respiration rate of grape stems may leads to stem browning. Browning of stem does not have any effect on eating quality of grape berries.

Rachis browning is considered as quality defect in sensory evaluation and overall attractiveness of grape bunch is reduced and it differs according to varieties of grapes. (Winkler *et al.*, 1974).

Table 3. Estimated means measured by a trained sensory panel for Sundarkhani grapes using unstructured, anchored 15 cm scale for teeth firmness.

Gamma Radiation doses (kGy)	Days of Storage		
	DAY 7	DAY 14	DAY 21
Control	10.25 ± 0.71 ^a	9.26 ± 0.42 ^a	Decayed
0.5	8.58 ± 0.58 ^b	8.35 ± 0.64 ^b	8.19 ± 0.70 ^a
0.8	7.60 ± 0.56 ^c	7.55 ± 0.65 ^c	7.48 ± 0.70 ^b
1	6.48 ± 0.51 ^d	6.35 ± 0.67 ^d	6.00 ± 0.71 ^c

Teeth Firmness on the scale of 15 cm, 0= none, 15= very intense.

Table 4. Effect of gamma irradiation on rachis browning of grapes.

Gamma Doses (kGy)	Irradiation	Days of Storage		
		DAY 7	DAY 14	DAY 21
Control		8.54 ± 0.15 ^a	11.42 ± 0.13 ^a	Decayed
0.5		3.78 ± 0.24 ^b	4.89 ± 0.56 ^b	6.89 ± 1.09 ^b
0.8		2.43 ± 0.11 ^c	3.67 ± 0.78 ^c	4.56 ± 1.20 ^c
1		4.73 ± 0.27 ^d	5.87 ± 0.57 ^d	7.03 ± 0.91 ^d

Rachis Browning of grapes on the scale of 15 cm, 0=none, 15 = very brown.

Berry shatter

Table 5 describes that there is no significant effect of gamma irradiation on berry shatter (% of grape berries that fell of the rachis with shaking). Berry shatter is mainly related to aging. Berry shatter increases and detachment force of berries in grapes decreases with storage period. Berry shatter is not affected by irradiation except at 0.8 kGy. Grapes

treated at 0.8 kGy have significantly lower level ($p \leq 0.05$) of berry shatter. Berry shatter can take place due to physiological, mechanical, pathological causes (Ben-Tal, 1990). Physiological cause may related to hardening and thickening of the pedicle and abscission layer is produced. It can also be due to mechanical loss during transportation, handling and packaging (Luvisi, 1995).

Table 5. Effect of gamma irradiation on estimated means of berry shatter (% of grape berries that fell of the rachis with shaking) for Sundarkhani grapes.

Gamma Radiation doses (kGy)	Days of Storage		
	7	14	21
Control	20.9 ± 0.102 ^a	20.9 ± 0.102 ^a	Decayed
0.5	18.6 ± 0.178 ^a	18.6 ± 0.19 ^a	18.6 ± 0.21 ^a
0.8	13.56 ± 0.184 ^b	13.56 ± 0.19 ^b	13.56 ± 0.21 ^b
1	17.48 ± 0.15 ^a	17.48 ± 0.19 ^a	17.48 ± 0.22 ^a

Each value is the mean obtained from five parallel replicates ± indicates standard deviation. Different superscripts indicate that the mean difference is significant at $p \leq 0.05$ by Duncan's New Multiple Range Test.

Physiological weight loss

Figure 3 depicts the changes in physiological weight loss in irradiated and non-irradiated grapes stored under refrigerated conditions. Weight loss is noticed

in all irradiated and unirradiated samples. Weight loss was significantly less in irradiated samples. Each value represents the percentage of weight loss during different time intervals at different treatments of

doses. The weight loss was increased with increase in storage period. Weight loss was significantly lower in grapes irradiated at 0.8 kGy among irradiated samples. . It was noticed that gamma irradiation decreased the weight loss in grapes, this reduction may related to respiration rate and malate dehydrogenase (MDH) activity of fruit during

refrigeration period. Irradiation at 1 kGy may have boosted the activity of MDH after treatment and during storage. Higher activity of MDH was observed as compared to irradiated. This may leads to increase in depletion of carbohydrates due increase in MDH and respiration rate and susequently the enhancement of weight loss (Al-Bachir, 1999).

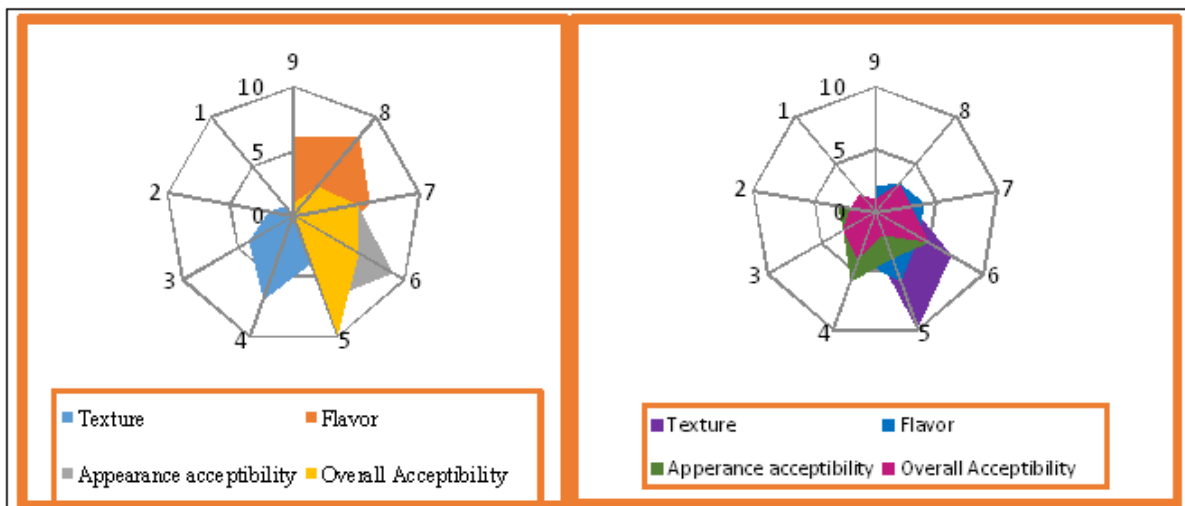


Fig. 1. Sensory evaluation of grapes (a) irradiated grapes sample (b) non irradiated grapes sample.

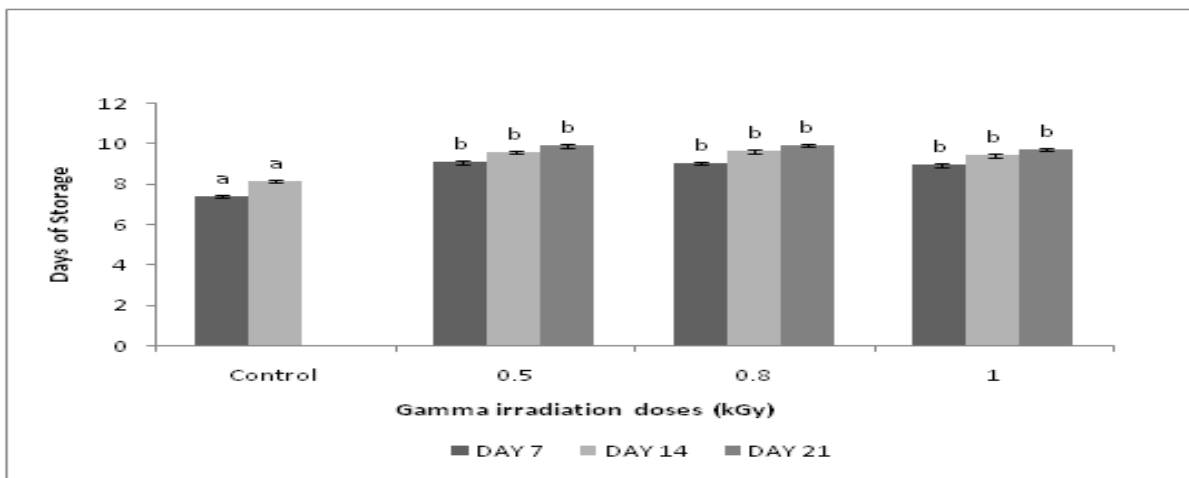


Fig. 2. Effect of gamma irradiation on sweetness of grapes.

Bacterial and Fungal Count

Figure 4 revealed that bacterial colonies gradually decreased with increasing gamma radiation doses. On 7th day, total viable counts of 16.4×10^4 , 8.4×10^4 and 3.8×10^4 cfu/g were evaluated for the doses of 0.5 kGy, 0.8 kGy and 1.0 kGy were significantly lower than control respectively. By the end of third week, bacterial count was enhanced to 69.8×10^4 , 32×10^4 and 24×10^4 cfu/g for grapes irradiated at 0.5 kGy, 0.8

kGy and 1 kGy. According to figure 5 control sample showed bacterial growth on MacConkey agar whereas no growth was observed on irradiated grapes samples indicating their complete absence on the berry surface. . The evaluated viable count of bacteria on control sample was 5.2×10^4 cfu /g and 6.4×10^4 cfu/g at 7th and 14th day. No *Salmonella* and *Shigella* colonies were observed on control and irradiated grapes samples indicating their complete absence on the

berry surface. Figure 6 interpreted the total fungal count of irradiated and control samples. The final total viable fungal count of irradiated samples were less than control i.e 44.8×10^4 , 25.8×10^4 and 18×10^4 cfu/g were evaluated for the doses of 0.5 kGy, 0.8

kGy and 1.0 kGy respectively. Bacterial and fungal count decreased in grapes irradiated at 0.8 and 1 kGy but sensory quality and texture was good and acceptable for grapes irradiated at 0.8 kGy.

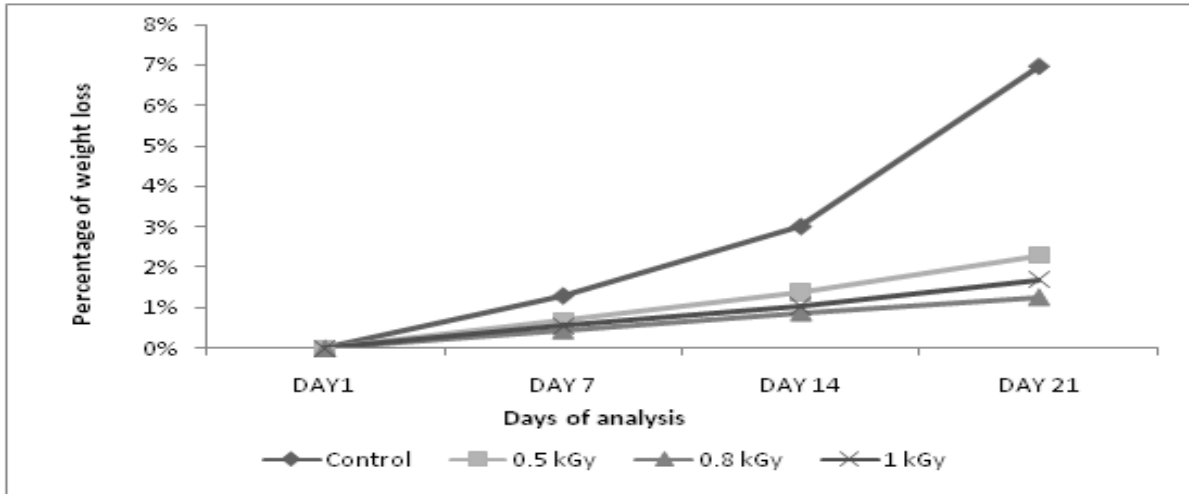


Fig. 3. Effect of different Gamma irradiation doses on the percentage of physiological weight loss in grapes.

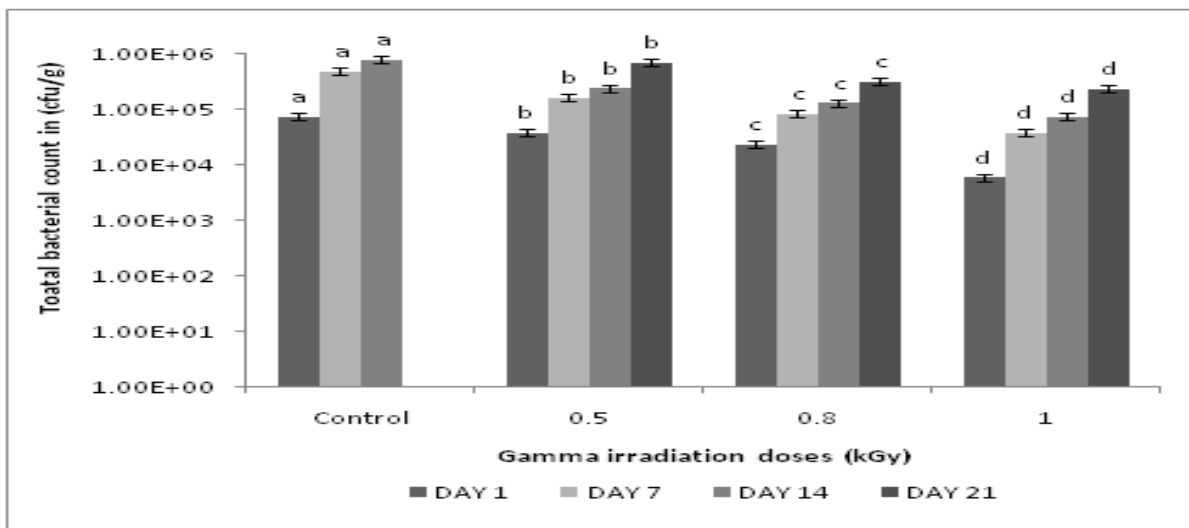


Fig. 4. Impact of different gamma radiation doses on bacteria present on grapes using nutrient agar as testing medium.

It was observed that grapes irradiated at 0.8 and 1 kGy were able to eradicate number of viable cells immediately after irradiation. It might be due to the reason that ionizing radiation may cause radiolysis of water molecules leads to production of free radicals which inactivate or eliminates the microorganisms by destroying DNA of microorganisms in addition to other organelles (Arvanitoyannis, 2010). Irradiation of food was useful to control pathogenic bacteria. *E.coli*

was observed on control but no colonies was observed on irradiated samples. *E.coli* was completely eliminated in grapes irradiated at 0.5, 0.8 and 1 kGy. No growth was observed on Shigella Salomonella agar showing complete absence of pathogenic bacteria on irradiated grapes. Postharvest diseases and infection directly affect the shelf life, storage and market value of grapes. In the present study spoilage due to fungus was significantly delayed in grapes irradiated at 0.8

without affecting the sensory properties of grapes. Fungi isolated from the grapes were *Aspergillusniger*, *Aspergillusflavus*, *Penicilliumsp*, *Cladosporiumsp*, *Alternariasp*, *Rhizopusstolonifer*,

Geotrichumsp and *Curvularia sp*. The identified yeast species were mostly *Saccharomyces sp*, *Candida sp* and *Rhodoturulasp*.

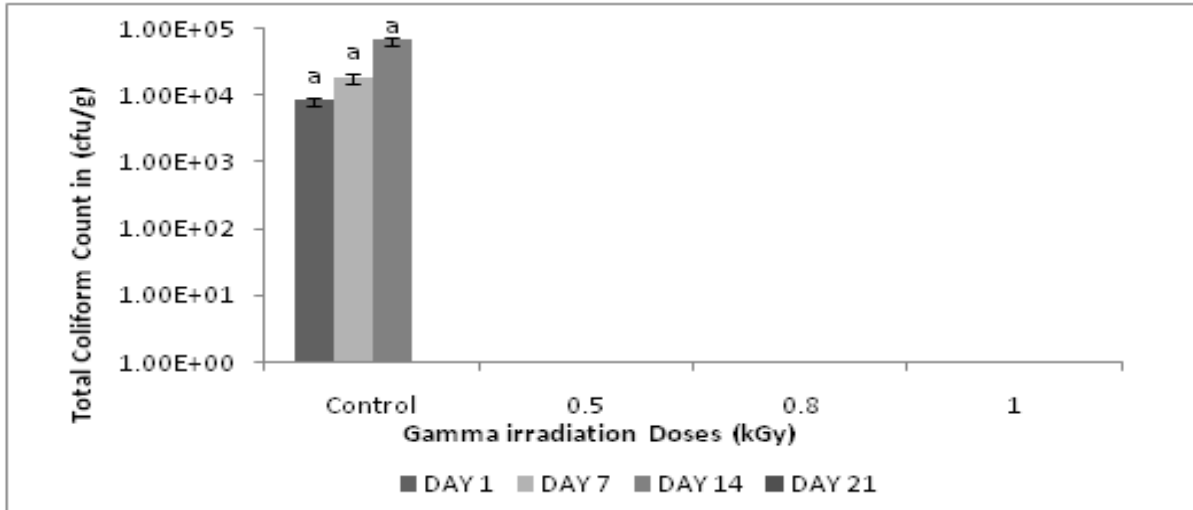


Fig. 5. Impact of different gamma radiation doses on bacteria present on grapes using MacConkey agar.

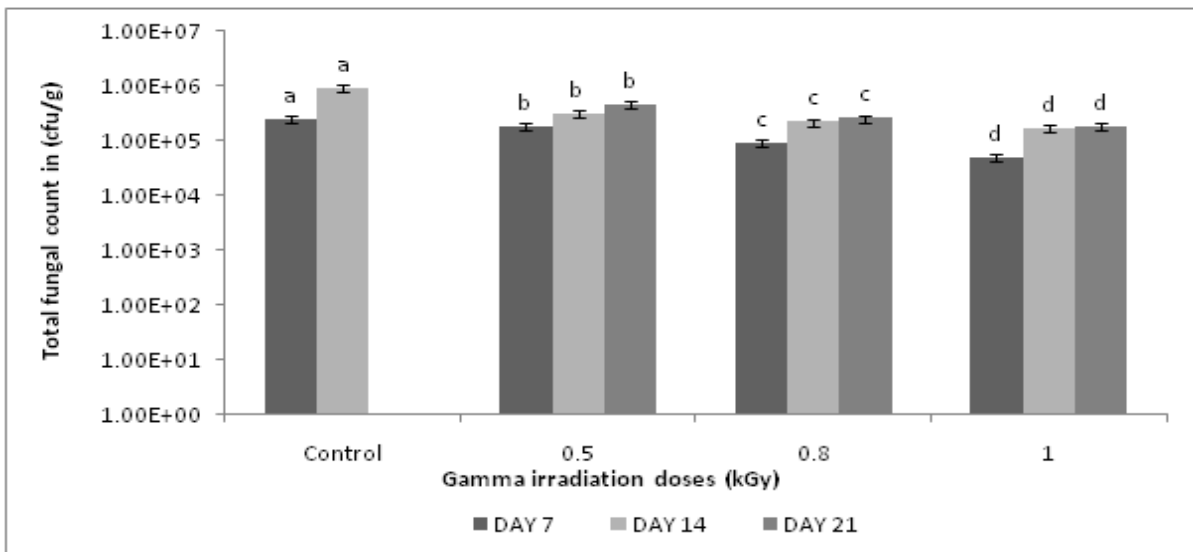


Fig. 6. Effect of different gamma radiation doses on fungal count of grapes using potato dextrose agar.

Conclusion

The results of conducted research reveal that gamma irradiation proved to be beneficial in enhancing shelf life of grapes. Microbial load was significantly reduced at 0.8 kGy. Berry softening and rachis browning was observed after day 21 at high dose of 1 kGy. Low dose irradiation at 0.8 kGy might be suitable for phytosanitary treatment and reduction of epiphytic micro flora associated with grapes because shelf life of grapes irradiated at 0.8 kGy was increased

by two weeks (14 days) without any loss of texture or firmity and remained acceptable for consumer. So optimal dose was found to be 0.8 kGy for green sunder khani grapes.

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