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Original Research Article

Effect of radiation and chemical treatments on guava (*Psidium guajava* L.) to delay ripening in relation to organoleptic biochemical and microbiological properties

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Keywords

Guava (*Psidium guajava* L.), Ripening, Irradiation, Chemical, Microbiological, Post-Harvest

A B S T R A C T

The present study was carried out to investigate the effectiveness of chemical (0.2% and 0.3% citric acid and potassium sorbate) and radiation (0.5 kGy and 1.0 kGy) at room and low temperature (4°C) in extending the post-harvest life in relation to delay ripening of guava (*Psidium guajava* L.) during storage. During ripening, skin color of guava changes from green to completely yellow with the texture firm to moderately soft. During ripening of guava, the most delayed ripening was reported as 5 days without any decay at room temperature. At 4°C temperature, 0.3% potassium sorbate and 1.0 kGy treated samples took 19 days to fully ripe whereas control sample took 14 days for complete ripening. A significant weight loss was found in 0.3% potassium sorbate and irradiated sample (1.0 kGy) at both temperature for guava compare to other treatments during storage. At the same time, a significant increase of moisture was recorded in guava during ripening. A reversible result was attributed between titratable acidity (TA) and pH where TA decreases and pH increases in all treatments at both temperatures during storage period. Total phenol (TP) and total flavonoid (TF) content in guava was decreased in all treatments during preservation at both temperatures and highest TP and TF content was found in 0.3% potassium sorbate treated guava compare to other treatments. An increased rate of total bacterial count (TBC), total coliform count (TCC) and total mold count (TMC) was recorded during the increase of storage period in both temperatures. The lowest microbial count (TBC, TMC, and TCC) was recorded for 0.3% potassium sorbate and 1.0 kGy radiation treated sample for all temperatures and for both fruits. The present study revealed that postharvest treatment of 0.3% potassium sorbate and 1.0 kGy radiation was most effective to delay ripening resulted in extending shelf life of guava.

Introduction

Bangladesh is a tropical country with six seasons. It has lot of fruits which are available only in certain season (Fuad et al., 2014). Guava (*Psidium guajava* L.) is one of the most common fruit grown widely in tropical and sub-tropical regions of the world and available in rainy season in Bangladesh. It originated in tropical

America, stretching from Mexico to Peru, and gradually became a crop of commercial significance in several countries because of its hardy nature, prolific bearing, high vitamin-C content and high remuneration even without much care (Negi and Rajan, 2007). Guava has well-established markets in more than 60 countries. Guava (*Psidium guajava* L.) is an important resource in the domestic economy of many countries in the tropics (Yavada, 1996). The largest producers of guava in world are India, Mexico, Brazil, Cuba, Venezuela, Australia, South Africa, Thailand, Malaysia, Indonesia, China, Sri Lanka, the Philippines, Bangladesh, Myanmar, Dominican Republic, USA (Hawaii, Florida and California) and Haiti. In Bangladesh this fruits are mostly available from August to September but the fruit has very short shelf life and uneven ripening pattern. This short shelf life aggravates postharvest losses and does not allow for efficient distribution and marketing (Kolade et al., 1992). This generates the necessity to search for new technologies to increase shelf-life, reach distant markets and thus improve the marketing process. In this regard, development of postharvest technology related to quality maintenance and extending the postharvest life are an important to consumer acceptability and marketing consideration along with export option (Zhong et al., 2006; Chien et al., 2007). The purpose of the present study was to find out the most suitable postharvest treatments for preservation of guava by using available resources. Potassium sorbate (0.2% and 0.3%), citric acid (0.2% and 0.3 %), radiation (0.5 and 1.0 kGy) and temperature (room and 4°C) treatments were used in the proposed study. It is expected that the results of this research will assist in acquiring information about the effectiveness of temperature, chemical and radiation in extending the postharvest life to delay the ripening of guava under tropical room and controlled temperature which will reflect to the minimization of post-harvest losses, nutritional improvement, food and financial security to the peoples of Bangladesh and employment generation for local population.

Materials and Methods

Guava variety, sampling and treatments

Selected, freshly hand-harvested, uniformly sized, mature-green fruits (locally available wild type) were obtained from the orchard. All fruits were free from physical injury and other blemishes. Guavas were initially washed with chlorinated water (125ppm of active chlorine) for 5 minutes to prevent contamination. For chemical treatments, guavas were dipped into 0.2% (citric acid and potassium sorbate) and 0.3% (citric acid and potassium sorbate) solution (w/v) for three minutes. Then water from the surface of guava was removed by using paper towel. For irradiation the guava were irradiated with two selected doses of gamma radiation which were 0.5 and 1.0 kGy with a 50kCi Co^{60} gamma source at dose rate of 6.4kGy/hr located at Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Savar, Dhaka. Guavas without any treatments were treated as control. For each treatment one piece of sample was placed into low-density polyethylene pouches (150µ gauge) and sealed tightly. The sealed polythene bags were labeled by indicating the name of the product and both treated and untreated samples were stored at room temperature (25°C) and 4°C temperature for biochemical and microbiological analysis. For analysis fruit samples were cut and sampled according to the different chemical analyses. Guava slices were cut longitudinally. Each sample analyzed represents tissue from a single fruit. Each assay was sampled with

three replications using one independent extraction per fruit.

Determination of color change and % of decay

Storage life was measured at the completely ripened stage or at the limit of acceptability was determined by the method of Fuad et al. (2014).

Determination of moisture content

The moisture content of guava sample was determined by drying at an oven at 105°C for 5–6 hrs according to the standard method of AOAC, 1975.

Determination of weight loss

Weight loss was measured by the method of Fuad et al. (2014).

Determination of pH

The pH of the guava varieties were measured by digital pH meter (type H1 98106; HANNA) at ambient temperature using juice extracted directly from pulp.

Determination of titratable acidity (TA)

Titratable acidity was determined by dissolving a known amount of guava pulp in distilled water and then titrated against 0.1N sodium hydroxide (NaOH) using phenolphthalein as an indicator (Srivastava et al., 2003). The results were calculated as percent of citric acid.

Determination of total soluble solid (TSS)

Total Soluble Solids (TSS) content was determined using an Abbe refractometer (TAGO 9099, Japan); pulp samples were homogenized in a blender. By placing a drop of thoroughly mixed sample on its prism, a direct refractometer reading was taken by the method described by Rababah (Rababah et al., 2011).

Determination of ascorbic acid (Vitamin-C)

Ascorbic acid was determined by 2,6-dichloroindophenol titrimetric method (Rangana et. al., 1986). Briefly, sample (2g) was homogenized with 3% metaphosphoric acid (25ml) and was filtered through filter paper (Whatman 1, 7.0cm). Then an aliquot (5ml) of filtrate was titrated with the 2,6-dichloroindophenol dye (standardized by the metaphosphoric acid) to a pink end-point. Results were expressed on a fresh weight basis as mg ascorbic acid equivalent/100gm. The estimation of ascorbic acid (vitamin-C) content of guava fruits was carried out by the titration result of the sample extract with 2,6-dichloroindophenol dye (Rangana et al., 1986).

Determination of total phenolic compounds

Phenolic compounds in guava were estimated by a modified spectrophotometric Folin–Ciocalteu method (Singleton and Rossi, 1965). Briefly, 200 µL of guava extract were mixed with 1 mL Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL of 10% Na_2CO_3 solution was added to the mixture and adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm by a T 80 UV/VIS spectrophotometer (ChromoTek GmbH, Germany). Gallic acid was used to calculate the standard curve (20, 40, 60, 80, and 100 μg/mL). Estimation of the phenolic compounds was carried out in triplicate. The results were expressed as micrograms (µg)

of gallic acid equivalents (GAEs) per gram (g) guava.

Determination of total flavonoids

Total flavonoid content (TF) of each guava sample was determined according to the colorimetric assay developed by Zhishen and others (1999). Guava extract (200 μL) was mixed with 4 mL of distilled water. At baseline, 0.3 mL of $NaNO_2$ (5%, w/v) was added. After 5 min, 0.3 mL $AlCl_3$ (10% w/v) was added, followed by the addition of 2 mL of NaOH (1 M) 6 min later. Immediately after that, the volume was increased to 10 mL by the addition of 2.4 mL distilled water. The mixture was vigorously shaken to ensure adequate mixing, and the absorbance was read at 510 nm. A calibration curve was prepared using a standard solution of Querchetin (20, 40, 60, 80 and 100 μg/mL). The results were also expressed as micrograms (µg) of Querchetin equivalents (QEQ) per gram (g) guava.

Statistical analysis

All determinations were obtained from triplicate measurements and results were expressed as mean ± standard deviation. Data were analyzed by the SPSS.16.0 (Statistical Package for Social Sciences) software. Statistical significance was declared at $p < 0.05$.

Results and Discussion

Color change and decay

The scores for color changes in guava attained in different treatments at room and 4°C refrigerated temperature have been represented at Figure 1 and Figure 2, respectively. Control sample was fully ripened on 3rd day at room temperature. Whereas, citric acid (0.2%) treated sample was ripened on 4th day. The most delayed ripening result was observed for all other treatments but 0.3% Citric acid and 0.3% Potassium sorbate and irradiated (1.0kGy) sample which took 5 days to ripe fully (Fig. 1). Decay was observed only in control samples on 5th day of preservation. At 4°C temperature, The most delayed ripening was found at 0.3% Potassium sorbate and 1.0 kGy irradiation treatment which was 19th day (Fig. 2) whereas the control sample was ripened on 14 days. On day 19, no decay was observed in all samples except 15% decay was found only in control sample. In the present study, 0.3% potassium sorbate and 1.0 kGy irradiation were most effective for delaying ripening of guava at both room and 4°C temperature compare to other treatments. It is reported that color changes are primarily associated with several biochemical changes; both degradation and synthesis of various classes of molecules including carotenoids in fruit (Aina and Oladunjoye, 1993). Guava fruits become overripe and mealy within a week under ambient conditions, whereas, in cold storage the shelf-life can be manipulated up to two weeks at 6–8°C and 90–95% RH (Tandon et al., 1989 and Mahajan et al., 2009). During ripening of fruits chlorophyll content decreased while carotenoid content increased. Simultaneously, starch content decreased with concomitant increase in alcohol-soluble sugars. The most notable metabolic changes occurred between mature green and color turning stage, implying that for improved postharvest handling, guava fruits may be harvested at color turning stage (Jain et al., 2003).

Events that occur during ripening and senescence reflect the deterioration of cellular structures, and in particular the cell membrane, which results in a loss of compartmentalization of ions and ultimately homeostasis (Paliyath et al., 2009). The process of membrane degradation during ripening and senescence is enhanced by

Reactive Oxygen Species (ROS) produced during stress conditions that occur after harvest such as cold conditions of storage, post-harvest handling etc. (Paliyath and Droillard, 1992).

Moisture and weight loss

Changes of Moisture in guava in different treatments at both room and 4°C temperature are shown in the Figure 3 and Figure 4 respectively. At room temperature, between day 0 and day 5 the significantly lowest increase in moisture was found for radiation treated samples (1.0 kGy and 0.5 kGy, respectively) followed by potassium sorbate (0.3%) treated samples, respectively. Significantly highest increase in moisture was found in control sample. At 4°C temperature, between day 0 and day 19 the lowest increase in moisture was also found for radiation and 0.3% potassium sorbate treated samples. The increase in moisture content of the fruits pulp during ripening could be attributed to loss of moisture from peel to the pulp. During ripening, carbohydrates are hydrolysed into sugars increasing osmotic transfer of moisture from peel to pulp (Joseph et al., 1990).

For both temperatures the Table 1 showed that the % of weight loss fall down initially with preservation days then it gradually increases with days. Similar findings were also reported by (Orathai and Lih-Shang, 2012). The rate at which water is lost depends on the water pressure gradient between the fruit tissue and the surrounding atmosphere and the storage temperature (Hemandez-Munoz et al., 2008). At the same time the lowest weight loss was found for potassium sorbate (0.2% and 0.3%) and radiation (0.5 kGy and 1.0 kGy) treated samples (Table 1). In case of % of weight loss potassium sorbate (0.2% and 0.3%) and irradiation (1.0 kGy and 0.5 kGy) were most effective for delaying ripening of mango at both room and 4°C temperature compare to other treatments. The percentage (%) of weight loss of guava during storage at room and 4°C temperature are shown in Table 1. For both temperatures the Table 1 showed that the mean of the % of weight loss fall down initially with preservation days then it gradually increases with days. Similar findings were also reported by another study (Orathai and Lih-Shang, 2012, Gill et al., 2014). At the same time the lowest weight loss was found for potassium sorbate (0.3%) and radiation (1.0 kGy) treated samples (Table 1). The rate at which water is lost depends on the water pressure gradient between the fruit tissue and the surrounding atmosphere and the storage temperature (Hemandez-Munoz et al., 2008).

Weight loss of 5% during storage is the maximum permissible limit in the case of fruits, above which the fruits show shriveling and become unmarketable (Mahajan et al., 2009). In the current study, using this as a standard it can be assumed that potassium sorbate (0.3%) and irradiation (1.0 kGy) treatments helped in maintaining the marketability of mango up to 7 days at room temperature and 28 days at 4°C temperature and guava up to 5 days at room temperature and 19 days at 4°C temperature.

Ascorbic acid (Vitamin-C)

A significant decline in ascorbic acid (AA) was found in all treatments under both temperatures during storage (Table 1). Storage period has also significant effect on AA. Ascorbic acid is one of the effective nutrient stability indexes during fruit storage operations and has been generally seen that if it is well retained, the other nutrients are also well retained (Fennema, 1996; Rueda, 2005). Our results depict that AA was much

higher in citric acid (0.2 and 0.3%), potassium sorbate (0.2 and 0.3%) and radiation (0.5 and 1.0 kGy) treated guava during storage as compared to control under both temperature. These results agree with those reported by Ayranci and Tunc (2004) who stated that ascorbic acid loss rate was much lower in stored apricots treated with citric acid as compared to control fruits. González-Aguilar et al., (2007) have reported the negative effect of UV-C irradiation on AA in mango "Tommy Atkins" fruits when compared with control fruits which disagree with our findings. Decline in AA is attributable to susceptibility of ascorbic acid to oxidative destruction during ripening (Aina, 1990). Similarly, much lower values of AA were found in green preclimacteric wild mangoes relative to ripe fruits (Aina and Oladunjoye, 1993). The decrease in ascorbic acid during storage is due to conversion of ascorbic acid to dehydroascorbic acid due to the action of ascorbic acid oxidase (Mapson, 1970; Singh et al., 2005). The gradual decline in citric acid and potassium sorbate treated fruits might be due to its increased biosynthesis, or decreased oxidation during storage.

Total soluble solids (TSS)

Total soluble solid (TSS) content is an indicator of good quality of fruits (Palaniswamy et al., 1975). In the present study TSS increased significantly during storage period in all treatments with both temperatures and storage period had also a significant effect on TSS during ripening of guava. Highest TSS was found in control sample at room and 4°C temperature respectively which is showed at Figure 5. Present study showed that TSS was increasing with the decrease of TA during ripening of guava. The lowest TSS was recorded on 0.3% potassium sorbate and 1.0 kGy radiation treated samples of guava during the preservation period due to its effect on delayed ripening. Increasing in TSS during fruit ripening was attributed to the increased activity of enzymes responsible for the hydrolysis of starch to soluble sugars (Hemandez-Munoz et al., 2008). Generally, taste and particularly sweetness of the fruits depend on the percentage of TSS content (Shafique et al., 2006). It was also reported that, on completion of hydrolysis, no further increase occurred and subsequently the TSS content declined as the sugars were metabolized to organic acids during respiration (Wills et. al., 1980).

Titratable acidity (TA) and pH

Titratable acidity (TA) gives a measure of the amount of acid present in a fruit (Dadzie et al., 1997, Ihekoronye and Ngoddy, 1985). In the present study, TA was decreasing with increasing the storage period in all treatments at both temperatures. A significantly reduced rate of TA was found in 0.3 % potassium sorbate at room temperature. In case of 4°C temperature, significantly reduced TA was found in 0.2 % citric acid treated sample. At room temperature, there were no significant differences between 0.2% citric acid and 0.5 kGy radiation along with 0.3% citric acid and 0.2% potassium sorbate treated sample but control was highly significant with all other treatments (Table 2). On the contrary, at 4°C temperature, the changes of TA were not significantly different in control, radiation (0.5 kGy and 1.0 kGy) and 0.3% potassium sorbate treated sample. Thus temperature had a significant effect on TA. The decline in acidity could be due to susceptibility of citric acid to oxidative destruction as impacted by the ripening environment (Aina, 1990). The decline in acidity during ripening is a consequence of starch hydrolysis leading to an increase in

total sugars and a reduction in acidity (Fuchs et al., 1980). Variation in acidity among different treatment may be attributed to the extent of degradation of citric acid as a function of the activity of citric acid glyoxylase during ripening (Doreyappy and Hudder, 2001; Rathore et al., 2007). Similarly, a decrease in titratable acidity of mango fruits during ripening has been reported (Vazquez-Salinas and Lakshminarayana, 1985).

The acid content of guava followed by declining trend throughout the storage period are represented in Table 2 indicated that there was noticeable increase in the titratable acidity during the first few days when the ripening is towards the peak followed by a sizable decrease till the end of the storage period (19 days). This sizable decrease could be attributed to its use as a substrate for respiration. Control fruits showed the higher acid content value in the both room and 4°C temperature. The decrease in acid content of fruits with the increase in storage period could be attributed to the use of organic acids in respiratory process by the fruit cells and conversion of acids into total sugars (Echeverria and Valich, 1989). Guava treated with 0.3% potassium sorbate and 1.0 kGy radiation maintained a higher titratable acidity value during the storage at both room temperature and 4°C temperature possibly due to delayed ripening. Similar results have been reported in guava (Jayachandran et al., 2005) and ber fruits (Siddiqui and Gupta, 1995).

A remarkably highest pH value was found in control sample compare to the all other treatments during storage. Reduced rate of pH value was found in 1.0 kGy radiation at both temperatures. At room temperature, pH changes in control sample were significant compare to all other treatments. Changes between 1.0 kGy radiation, 0.3% potassium sorbate and 0.2% citric acid were not significant. At 4°C temperature, a pH change in 1.0 kGy radiation was significantly different from all other treatments and it was lowest. Increase in pH during ripening of mango fruits has been reported by some authors (Tovar et al. 2000, Saeed et al., 2010) and was similar to what was observed in the present study. The increase in pH (decline in acidity) could be due to utilization of acids as respiration substrates (Dadzie et al., 1997). Another study (Kudachikar et al., 2001) also confirmed the changes in pH and acidity in mangoes during ripening process.

Polyphenol and flavonoid

The action of phenolic compounds in foods has been drawn a lot of attention because of their biological activity in cancer and heart diseases prevention (Rocha et al., 2007). At day 5 the highest amount of phenol was recorded for radiation (1.0 kGy) and 0.3% potassium sorbate treated samples in guava at room temperature (Fig. 6). At 4°C temperature 0.3% potassium sorbate and 1.0 kGy showed highest phenol content among other treatments in guava (Fig. 7).

Irradiation induces the accumulation of phenolic compounds and flavonoids in plants as a defense mechanism against irradiation, also the increase in total phenol and total flavonoid can be attributed to the phenylalanine ammonialyase activity, which is one of the key enzymes in the synthesis of phenolic compounds in plant tissues (Frohnmeyer and Staiger, 2003; Gitz et al., 2004). Increase in phenolic compounds of irradiated plant produce has also been attributed to depolymerization and dissolution of cell wall polysaccharides, which facilitated higher extractability (Bhat et. al., 2007). The variation of phenolics in fruits depends on many factors, it is known

that the different stages in the process of fruits development. e.g., in red pepper, it increases during the ripening stage due to maximum deposition of anthocyanin and flavonoids (Srivastava et al., 2013).

The maximum total phenols of guava fruits were recorded at harvest which, decreased with the progression of cold storage irrespective of treatment (Fig. 6). Treated guava maintained significantly higher amount of phenol content as compared to control. A sudden decline in the phenol content was observed in control sample at room temperature at day 3 (Fig. 6) and just after 7 days of cold storage (Fig. 7) synchronizing with the ripening and fruit softening. This result was supported by another study carried out by Gill et al. (2014). Quick ripening in untreated fruits during storage led to a decline in total phenol contents earlier than the treated ones, which is consistent with the observations of Bashir and Abu-Goukh (2003) in guava. Singh and Pal (2008) also reported similar declining trend of phenolic content during storage in guava. Similar results were reported by Ling et al., (2007) in 'Yali' pear (*Pyrusbretschneideri Rehd.)* treated with 10.0mM Ascorbic acid (10.0 mM).

A Significant highest amount of flavonoid in guava was found in 1.0 kGy radiation treated samples at room temperature compare to other treatments. At 4°C temperature 0.3% potassium sorbate and 1.0 kGy showed highest flavonoid content among other treatments in guava (Fig. 8).

Total bacterial count (TBC), total mould count (TMC) and total coliform count (TCC)

In the initial period of guava storage, the control sample of room temperature was shown maximum ($0.01x10^3$ cfu/gm) bacterial colony and the samples of other treatments showed no bacterial growth (Table 3). Data for total mould count during storage of guava in room and 4°C temperature is illustrated in Table 4. The table showed that, TMC also was affected by chemical, radiation and low temperature treatments. At day 5, all the samples at room temperature was observed for mould colony but the sample treated with 0.3% potassium sorbate and 1.0 kGy radiation treated guava had TMC as nill.

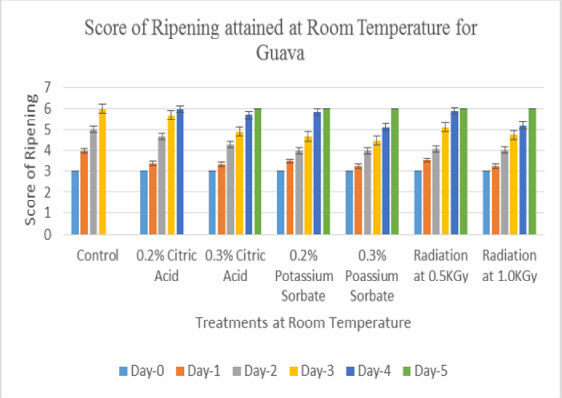

Figure.1 Scores of ripening at room temperature in Guava

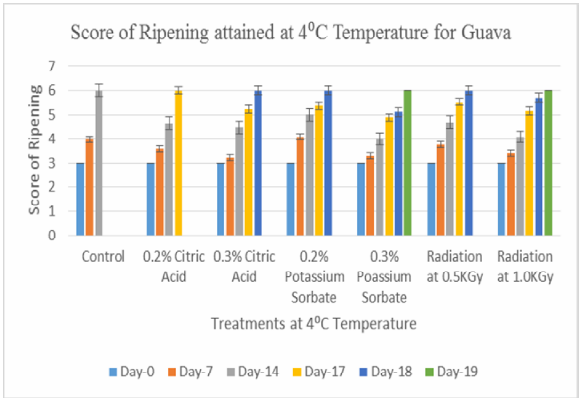

Figure.2 Scores of ripening at 4°C in Guava

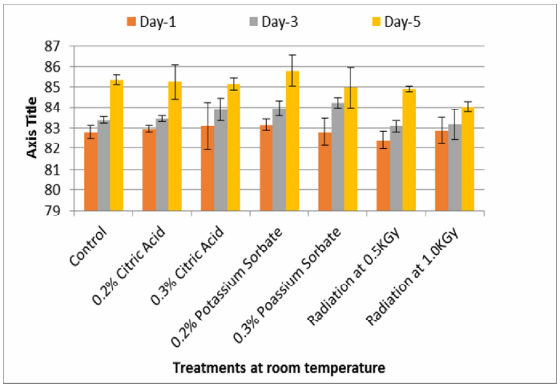

Figure.3 Moisture changes at room temperature in Guava

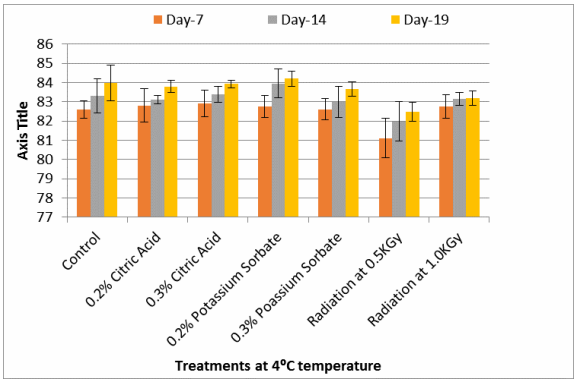

Figure.4 Moisture changes at 4°C in Guava

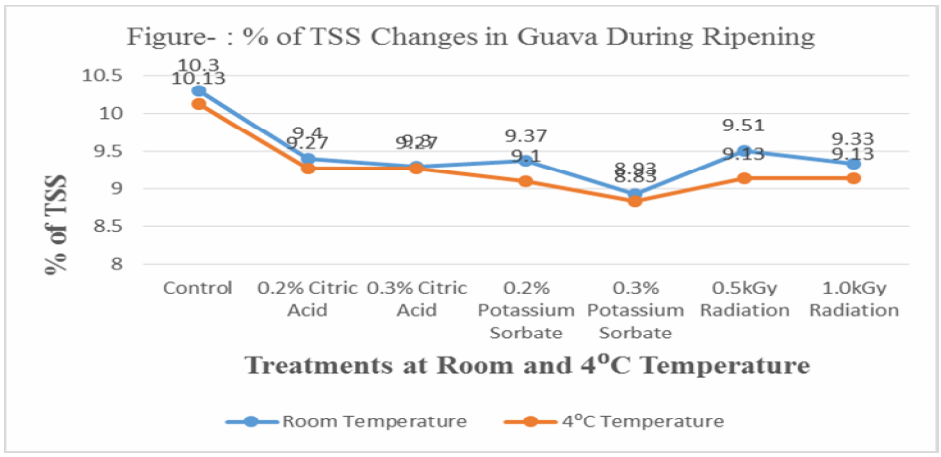

Figure.5 Changes of TSS in guava during ripening under different treatments

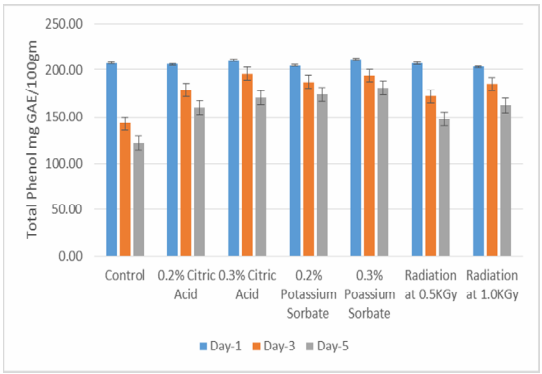

Figure.6 Total phenol changes in guava at room temperature under

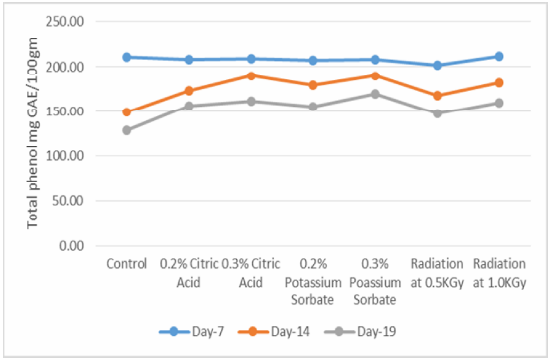

Figure.7 Total phenol changes in guava at 4°C temperature under different treatments

different treatments

Figure.8 Mean of Flavonoid content in guava during ripening

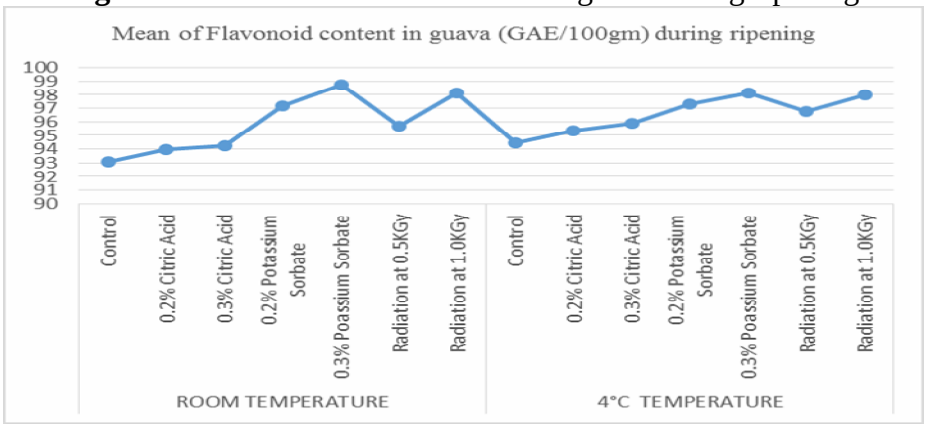

Table.1 Weight loss percentage and Ascobic acid change in guava at different treatments at room temperature and at 4°C temperature during ripening

	ROOM TEMPERATURE										4°C TEMPERATURE								
	Day	Control	0.2% Citric Acid	0.3% Citric Acid	0.2% Potassium Sorbate	0.3% Poassium Sorbate	Radiation at 0.5 KGy	Radiation at 1.0 KGy	**Mean**	Day	Control	0.2% Citric Acid	0.3% Citric Acid	0.2% Potassium Sorbate	0.3% Poassium Sorbate	Radiation at 0.5 KGy	Radiation at 1.0 KGy	**Mean**
% of Weight Loss	Day-1	2.91	1.98	1.47	2.03	0.87	2.76	1.1	1.87	Day-7	4.7	2.69	1.88	2.97	2.38	4.11	2.93	3.09
	Day-3	4.28	4.11	3.8	3.13	1.5	3.88	1.56	3.18	Day-14	4.3	3.81	4.76	3.66	3.13	4.45	2.21	3.76
	Day-5	9.6	5.78	4.22	4.07	2.01	4.4	1.91	4.57	Day-19	7.8	5.07	4.97	4.35	3.09	6.78	3.11	5.02
	Mean	5.60	3.96	3.16	3.08	1.46	3.68	1.52		**Mean**	5.60	3.86	3.87	3.66	2.87	5.11	2.75	
Ascorbic Acid mg/100g	Day-1	251.12	255.21	252.91	241.77	249.29	241.89	245.66	248.26	Day-7	214.80	218.12	226.18	219.26	229.61	201.30	221.71	218.71
	Day-3	170.05	201.73	204.80	200.08	211.33	191.36	210.59	198.56	Day-14	170.05	191.29	217.23	177.97	189.44	179.76	181.79	186.79
	Day-5	121.60	161.31	186.54	177.51	197.81	151.54	181.26	168.22	Day-19	136.35	169.90	171.12	156.56	162.98	147.81	152.41	156.73
	Mean	180.92	206.08	214.75	206.45	219.48	194.93	212.50			173.73	193.10	204.84	184.60	194.01	176.29	185.30	

Table.2 Changes of titratable acidity (TA) under different treatments in guava during storage at room and 4°C temperature

Treatments	Room Temperature						4°C Temperature					
	Storage periods/day											
	0	1	2	3	4	5	0	7	14	17	18	19
Control	0.17	0.184	0.2	0.224	0.149	0.193	0.17	0.195	0.217	0.249	0.203	0.148
Citric Acid 0.2%	0.17	0.175	0.186	0.202	0.167	0.184	0.17	0.19	0.203	0.221	0.189	0.185
Citric Acid 0.3%	0.17	0.179	0.19	0.197	0.162	0.18	0.17	0.186	0.2	0.215	0.201	0.18
Potassium Sorbate 0.2%	0.17	0.182	0.211	0.265	0.207	0.17	0.17	0.187	0.194	0.213	0.2	0.175
Potassium Sorbate 0.3%	0.17	0.172	0.184	0.195	0.217	0.168	0.17	0.19	0.201	0.21	0.2	0.195
Radiation 0.5 kGy	0.17	0.177	0.19	0.2	0.187	0.166	0.17	0.185	0.201	0.213	0.198	0.168
Radiation 1.0 kGy	0.17	0.175	0.18	0.195	0.200	0.16	0.17	0.182	0.2	0.214	0.191	0.188
LSD at 5%	**0**	**0.2**	**0.11**	**0.1**	**0.16**	**0.12**	**0**	**0.3**	**0.13**	**0.15**	**0.19**	**0.1**

Table.3 Total Bacterial Count (TBC) in control, chemical and radiation treated sample stored at room temperature and refrigerated temperature (4°C) in guava

Storage Time (Day)	Storage Temperature	Control[b]	0.2% Citric Acid[b]	0.3% Citric Acid[b]	0.2% Potassium Sorbate[b]	0.3% Potassium Sorbate[b]	Radiation at 0.5 kGy[b]	Radiation at 1.0 kGy[b]
0	RT 4°C	$0.01x10^3$ $0.01x10^3$	Nil Nil	Nil Nil	Nil Nil	Nil Nil	Nil Nil	Nil Nil
3	RT 4°C	$0.03x10^3$ $0.01x10^3$	$0.01x10^3$ Nil	$0.01x10^3$ Nil	$0.01x10^3$ Nil	Nil Nil	Nil Nil	Nil Nil
5	RT 4°C	$0.05x10^3$ $0.01x10^3$	$0.03x10^3$ $0.01x10^3$	$0.02x10^3$ Nil	$0.03x10^3$ $0.01x10^3$	$0.01x10^3$ Nil	$0.03x10^3$ $0.01x10^3$	$0.01x10^3$ Nil
7	RT 4°C	NA $0.1x10^3$	NA $0.05x10^3$	NA $0.04x10^3$	NA $0.08x10^3$	NA $0.03x10^3$	NA $0.1x10^3$	NA Nil
14	RT 4°C	NA $0.16x10^3$	NA $0.07x10^3$	NA $0.06x10^3$	NA $0.09x10^3$	NA $0.06x10^3$	NA $0.11x10^3$	NA $0.02x10^3$
19	RT 4°C	NA $0.29x10^3$	NA $0.14x10^3$	NA $0.12x10^3$	NA $0.19x10^3$	NA $0.12x10^3$	NA $0.3x10^3$	NA $0.1x10^3$
19	RT 4°C	NA $0.29x10^3$	NA $0.14x10^3$	NA $0.12x10^3$	NA $0.19x10^3$	NA $0.12x10^3$	NA $0.3x10^3$	NA $0.1x10^3$

Table.4 Total Mold Count (TMC) in control, chemical and radiation treated sample stored at room temperature and refrigerated temperature (4°C) in guava

Storage Time (Day)	Storage Temperatur	Control[b]	0.2% Citric Acid[b]	0.3% Citric Acid[b]	0.2% Potassium Sorbate[b]	0.3% Potassium Sorbate[b]	Radiation 0.5 kGy[b]	Radiation 1.0 kGy[b]
0	RT 4°C	$0.01x10^3$ $0.01x10^3$	Nil Nil	Nil Nil	Nil Nil	Nil Nil	Nil Nil	Nil Nil
3	RT 4°C	$0.03x10^3$ $0.01x10^3$	Nil Nil	Nil Nil	Nil Nil	Nil Nil	Nil Nil	Nil Nil
5	RT 4°C	$0.07x10^3$ $0.02x10^3$	$0.03x10^3$ $0.01x10^3$	$0.01x10^3$ $0.01x10^3$	$0.02x10^3$ $0.01x10^3$	Nill Nill	$0.01x10^3$ Nill	Nill Nill
7	RT 4°C	NA $0.06x10^3$	NA $0.03x10^3$	NA $0.03x10^3$	NA $0.04x10^3$	NA $0.02x10^3$	NA $0.06x10^3$	NA $0.01x10^3$
14	RT 4°C	NA $0.09x10^3$	NA $0.07x10^3$	NA $0.07x10^3$	NA $0.06x10^3$	NA $0.03x10^3$	NA $0.23x10^3$	NA $0.03x10^3$
19	RT 4°C	NA $0.25x10^3$	NA $0.16x10^3$	NA $0.14x10^3$	NA $0.12x10^3$	NA $0.09x10^3$	NA $0.3x10^3$	NA $0.1x10^3$

Table.5 Total Coliform Count (TCC) in control, chemical and radiation treated sample stored at room temperature and refrigerated temperature (4°C) in guava

Storage Time (Day)	Storage Temperature	Control[b]	0.2% Citric Acid[b]	0.3% Citric Acid[b]	0.2% Potassium Sorbate[b]	0.3% Potassium Sorbate[b]	Radiation 0.5 kGy[b]	Radiation 1.0 kGy
0	RT 4°C	$0.01x10^3$ $0.01x10^3$	Nil Nil	Nil Nil	Nil Nil	Nil Nil	Nil Nil	Nil Nil
3	RT 4°C	$0.05x10^3$ $0.03x10^3$	$0.03x10^3$ Nil	$0.02x10^3$ Nil	$0.03x10^3$ Nil	$0.03x10^3$ Nil	$0.02x10^3$ Nil	$0.01x10^3$ Nil
5	RT 4°C	$0.1x10^3$ $0.07x10^3$	$0.06x10^3$ $0.02x10^3$	$0.06x10^3$ $0.02x10^3$	$0.05x10^3$ $0.01x10^3$	$0.04x10^3$ Nill	$0.07x10^3$ $0.01x10^3$	$0.03x10^3$ Nill
7	RT 4°C	NA $0.1x10^3$	NA $0.05x10^3$	NA $0.04x10^3$	NA $0.07x10^3$	NA $0.05x10^3$	NA $0.14x10^3$	NA $0.02x10^3$
14	RT 4°C	NA $0.17x10^3$	NA $0.11x10^3$	NA $0.1x10^3$	NA $0.13x10^3$	NA $0.08x10^3$	NA $0.19x10^3$	NA $0.06x10^3$
19	RT 4°C	NA UC	NA $0.21x10^3$	NA $0.19x10^3$	NA $0.23x10^3$	NA $0.18x10^3$	NA $0.28x10^3$	NA $0.09x10^3$

NA= Not Assessed due to sample spoilage, Data represents mean values of three replications

b Represents colony forming unit per gm (cfu/gm) sample., UC= Represents sample contained uncountable mold colony or >300 colonies.

At day 19, the total mould colony at 4°C temperature, the lowest TMC was recoded for 1.0 kGy radiation treatments which was $0.1x10^3$ cfu/gm followed by 0.3% potassium sorbate treatments ($0.09x10^3$ cfu/gm). Data for total coliform count during storage of guava in room and 4°C temperature is illustrated in Table 5. The table showed that, TCC also was affected by chemical, radiation and low temperature treatments. At the day 5, all the samples at room temperature it was observed coliform colony but the sample treated with 0.3% potassium sorbate and 1.0 kGy radiation treated sample showed the lowest count. At day 19, the TCC in control sample was uncountable (>300 colonies) at 4°C temperature. In this period at 4°C temperature the lowest TCC was recorded for 1.0 kGy radiation treatments which was $0.09x10^3$ cfu/gm followed by 0.3% potassium sorbate treatments ($0.18x10^3$ cfu/gm).

The number of total bacterial count (TBC), total mould count (TMC) and total coliform count (TCC) was gradually increased for all samples; but rapidly increased in the samples which were stored at room temperature than the samples which were stored at 4°C. TBC, TMC and TCC was rapidly increased in control samples than any other samples of both temperatures. After the day 5, all the guava samples at room temperature colony count can't be done and only the controlled temperature samples were remained for experiments.

Bacteria are one of the major organisms contributing to the fruit quality deterioration and spoilage. Loaharanu (1996) discussed about the effectiveness of irradiation as a cold pasteurization method to control food borne diseases caused by pathogenic microorganisms and parasites especially in food to be consumed raw or particularly processed which is now been established. Total bacterial count (TBC) varied in different treatments. Aytae et al., (2000) reported that radiation has beneficial effects in controlling bacterial growth which is observed in current study. It was also reported that maximum inhibition was achieved using radiation dose of 2.0 kGy (Aytae et al., 2000). Shashidhar et al., (2007) reported the effectiveness of radiation processing for elimination of *Salmonella typhimurium* from minimally processed pineapple. The pathogen was not detected from radiation-processed samples (1.0 kGy and 2.0 kGy) up to 12 days during storage at 4°C and 10°C temperature. Ghaly et al., (2000) concluded that irradiation dose of 3 kGy was effective at reducing total bacteria. Pathogenic *Staphylococcus aureus* and *E. coli* were completely eliminated at this dose. It was important to note that TBC load reach $1.0X10^7$ cfu/gm or more in any food and food products these food are considered as spoiled (Lee et al., 2001). It was also reported that at 5 kGy radiation dose, shelf life of fruits was enhanced effectively by suppression of microbial growth and proliferation. Chakrabarti and Verma (2000) reported that microorganism like- bacteria, fungi etc. are more sensitive to potassium sorbate. Another study carried out by Garcia et al., (1998) reported that treatments with potassium sorbate reduced microbial counts, extending strawberry storage life from 14 days (for control fruits) to 28 days in coated strawberries (Garcia et al., 1998).

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