Physicochemical and microbiological quality of raspberries (*Rubus idaeus*) treated with different doses of gamma irradiation

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Abstract

This study was conducted to evaluate the physicochemical and microbiological characteristics of raspberries exposed to different radiation doses. The fruits were harvested in the city of Campestre, MG, packed in polyethylene bags, and transported to the Federal University of Lavras (UFLA), where they were separated into 4 lots. Irradiation was performed at the Center for Development of Nuclear Technology in Belo Horizonte, MG. The doses used were 0 (control), 0.5, 1.0, and 2.0 kGy. After irradiation, the fruits were transported back to UFLA and stored at 1 °C and 95% relative humidity (RH) for 12 days. The physicochemical analyses for mass loss, total soluble solids, titratable acidity, pH, total soluble sugars, total soluble pectin, firmness, vitamin C content, total antioxidant activity, and total phenolic, and the microbiological assays (coliform at 35 and 45 °C, psychrotrophic and filamentous fungi and yeasts) were performed after 0, 3, 6, 9, and 12 days of storage. Lower loss of mass and filamentous fungi and yeast count were observed in the irradiated fruits, and 2 kGy was determined as the most effective dose for microbial control, but this irradiation dose also resulted in increased loss of fruit firmness.

Keywords: Co^{60} radiation; postharvest; conservation.

1 Introduction

Quality attributes generally considered by consumers in their selection and purchase of most fruits include: appearance, taste, smell, nutritional value, and absence of defects. In the case of raspberries, this condition is not difficult to achieve because this fruit presents a peculiar attraction due to its color (red, black, and yellow), pleasant odor, soft texture, and slightly sour flavor.

The wide acceptability of raspberry is related not only to its sensory characteristics, but also to its chemical composition since it is rich in antioxidant substances such as vitamin C, carotenoids, and phenolic compounds thus rendering it a very healthy fruit. It is also a source of carbohydrates, minerals, and other vitamins and it has low calorie and fat content, while being rich in soluble fiber (BEATTIE; CROZIER; DUTHIE, 2005; PANTELIDIS et al., 2007; PLESSI; BERTELLI; ALBASINI, 2007; TALCOTT, 2007). Raspberries are extremely sensitive to handling and fungal attack, and thus it requires careful handling, harvesting, storage, and transportation; therefore, postharvest cleaning, such as washing or sanitizing, is strongly discouraged.

Irradiation is a common physical process that destroys pathogenic microorganisms and those that cause food spoilage. It is also used to eliminate insects and retard the germination process in plant products. Therefore, irradiation increases food safety and reduces losses caused by deterioration (RESURRECCION et al., 1995). Irradiation has been studied for several years and since 1963, the Food and Drug Administration (FDA) has regulated the use of radiation in wheat and wheat flour processing for human consumption. Application of radiation has also been guided by Good Manufacturing Practices (GMP's) (LAGUNAS-SOLAR, 1995). By the 1980s and 1990s, new regulations emerged with the aim of extending this technology to other foods (ORNELLAS et al., 2006).

Fruit irradiation has been used by countries such as Thailand and India to compete against the Brazilian fruit export, especially after restrictions have been imposed on more common quarantine treatments such as fumigation using methyl bromide or oxide ethylene and submergence in hot water. In order to achieve effective treatments, harvesting should be done before the fruit's optimal maturation period, which otherwise would damage the fruit's flavor and sensory characteristics. Irradiation imparts minimal effect on the chemical and sensory characteristics of food, when the established maximum dose for each product is used. The irradiation process, when properly conducted, does not cause damages to the environment or to human health, and thus it is fully supported by international institutions such as the World Health Organization (WHO), Food and Agricultural Organization (FAO), FDA, and National Health Surveillance Agency (ANVISA). The use of irradiation has also been strongly based on several studies that have confirmed its safety and effectiveness (PEROZZI, 2007).

One major advantage offered by irradiation is that it requires a minimal handling of the food item, thus enabling decontamination without inducing any mechanical damage; it also reduces the time it takes for the product to reach consumers.

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The objective of this study was to evaluate the physicochemical and microbiological effects of irradiating raspberries with different doses of Cobalt-60 gamma and storing at 1 °C and 95% relative humidity (RH), thus creating an alternative approach to postharvest processing that conserves the natural characteristics of the fruit and possibly extends its shelf-life.

2 Materials and methods

2.1 Vegetable material

Raspberries (*Rubus idaeus*), cultivar "autumn bliss", were harvested from a Campestre- plantation, MG-Brazil. The selection criteria for the fruits included: acceptable and attractive appearance, physiological maturity based on size and color, and absence of injuries related to handling and/or diseases. The crop was harvested in December 2011 in the cool morning hours to minimize moisture loss by transpiration. The fruits were harvested manually, stored in polyethylene trays (approximately 100 grams of fruit per tray), and packed in 20-L styrofoam boxes packed with ice to remove field heat and to refrigerate the fruits during transport to the Laboratory of Postharvest Fruit and Vegetable, Department of Food Science, Federal University of Lavras (UFLA).

2.2 Gamma irradiation

Irradiation was performed one day after harvest (approximately 24 hours after collection). Raspberry samples were transported in refrigerated styrofoam boxes to the Gamma Radiation Laboratory (LIG) of the Center for Development of Nuclear Technology, Federal University of Minas Gerais (UFMG), where they were irradiated in a Gammacell-127 GB, IR-214 panoramic irradiator (MDS Nordion, Canada) with Cobalt-60 source. The fruits were placed on a turntable that was positioned around the Co-60 source. It was not necessary to remove the raspberries from its packaging or boxes because radiation can traverse the physical barrier. The doses used for the treatment were 0 (control), 0.5, 1.0, and 2.0 kGy. After the treatment, the fruits were transported back to the UFLA and stored at 1 °C and 95% RH and analyzed at different storage times (0, 3, 6, 9, and 12 days).

2.3 Physicochemical analysis

Fruit weight loss (W. loss) was calculated as the percentage difference between the initial weight of the fruit upon storage and the final weight of the fruits after a specific number of days of storage. The fruits were weighed in a semi-analytical balance.

The Total Soluble solids (SS) were measured using a digital refractometer (ATAGO PR-100) equipped with automatic temperature compensation at 25 °C (ASSOCIATION..., 2000); the total amount of SS was measured as Brix content of SS.

The level of acidity (Titratable acidity -TA) in the fruits was determined by titration with 0.1 N NaOH and a phenolphthalein indicator, according to AOAC (ASSOCIATION..., 2000) and expressed as % citric acid.

The pH level of the fruits was determined following the potentiometric method using a digital potentiometer/pH meter TECNAL (Tec 3MP), according to AOAC (ASSOCIATION..., 2000).

The total amount of soluble sugar (TSS) in the fruits was determined by extraction using ethanol 95% (v/v) using the Anthrone method (DISHE, 1962). The results were expressed in grams of glucose per 100 g of fruit.

The technique of McCready and McComb (1952) was used for the extraction of pectin from the fruits. The total amount of pectin (TP), soluble pectin (SP), and solubility was determined colorimetrically following the study by Bitter and Muir (1962). The measurements were expressed as mg of galacturonic acid per 100 grams of fruit. The percentage of solubility was calculated from the ratio of soluble pectin to total pectin (SP/TP).

Firmness was measured using a texturometer TA-XT2i Texture Analyzer equipped with a needle probe. Ten fruits were randomly selected and texture was measured at 2 different points in each fruit (top and bottom). The measurements were expressed as force (N) used by the probe to penetrate the fruit to a depth of approximately 3 mm.

Total amount of ascorbic acid was colorimetrically determined according to the method by Strohecker and Henning (1967) using 2,4-dinitrophenylhydrazine. The results were expressed as mg of ascorbic acid per 100 g of fruit.

The antioxidant compounds were extracted using the methodology described Rufino et al. (2007). The methodology used to determine total antioxidant activity (TAA) was based on the absorption of 60 mM 2,2-diphenyl-1-picryl hydrazyl (DPPH), as described by Rufino et al. (2007), with some adjustments in the calculation procedure, in which the percentage of the sequestered free radical DPPH was used as the standard. The readings were taken after 30 min using a spectrophotometer at an absorbance wavelength of 515 nm, and the results were expressed as percentage of sequestered free radical (%SFR) using the following Equation 1:

$$\%SFR = \frac{((\text{Absorbance of control} - \text{absorbance of sample}) \times 100)}{\text{Absorbance of control}} \quad (1)$$

For the determination and quantification of phenolic compounds, the colorimetric method developed by Waterhouse (2002) was used with 10% Folin–Ciocalteu reagent. To calculate the total phenolic content, a standard curve using gallic acid solution was constructed. The results were expressed as gallic acid equivalents (grams of GAE per grams of fruit).

2.4 Microbiological analysis

Microbiological analysis was performed in triplicate using 3 dilutions (10^{-1}, 10^{-2}, and 10^{-3}). A sample of approximately 25 g was aseptically collected and transferred to a shaker with 225 ml of sterile 0.1% peptone water. From this stock solution, serial dilutions were prepared in tubes containing 9 ml of 0.1% peptone water using the technique described by Silva et al. (2007).

For determination of Coliform bacteria at 35 and 45 °C (thermotolerant), the protocol of Silva et al. (2007) was followed using lauryl sulfate tryptose (LST) broth and incubating at 35 °C for detection of total coliform and 45 °C for fecal coliform.

To detect the presence of yeast and mold (Filamentous fungi), each extract was inoculated on dichloran chloramphenicol rose bengal (DRBC) agar plates (SILVA et al., 2007).

For psychrotrophic aerobic counts, each extract was inoculated on plate count agar (PCA), as described by Silva et al. (2007).

2.5 Statistical analysis

All data were analyzed using analysis of variance (ANOVA) and when the isolated factors or their interactions were determined to be significant, regression coefficients and the Tukey test were calculated at a 5% significance level using a completely randomized design (CRD) in a 4 × 5 factorial (4 doses of irradiation, 5 days of storage) with 3 replicates; the plot consisted of a tray (approximately 100 g) of fresh raspberries, and by using MINITAB 16. A significant interaction was detected, in which a split in the doses in each storage time was observed.

3 Results and discussion

Adopting a significance level of 5%, it can be said that there was significant interaction between doses of irradiation and storage time for the variables weight loss, firmness, ascorbic acid, total antioxidant activity, total phenolic content and filamentous fungi and yeasts. For the variables titratable acidity, soluble pectin, and solubility, only the storage time was significant, and for soluble sugars there was no significant effect of storage time and irradiation doses separately. There was no significant effect of any of the sources of variation for soluble solids (Table 1).

With regards to the weight loss (Figure 1), there was an increase over time, and the higher the irradiation dose (2 kGy), the lesser the weight loss.

Weight loss is one of the physiological parameters used as quality indicator in fruits. Fruits and vegetables, even when kept under ideal conditions, generally undergo a decrease in mass during storage based on the combined effect of respiration and transpiration (CHITARRA; CHITARRA, 2005). Maxie, Sommer and Mitchell (1971) reported that irradiation increased membrane permeability, transpiration, and metabolic activity, and it disrupted intercellular connections. However, other researchers argued that by using an optimal dose of irradiation, the shelf life of fruits and vegetables could be extended (ARTES-HERNANDEZ et al., 2010; LÓPEZ-RUBIRA et al., 2005). Oliveira et al. (2006) showed that the application of 600 Gy gamma irradiation on postharvest of guava fruits resulted in a lower mass loss as compared to non-irradiated fruits (control) and fruits irradiated with 300 and 900 Gy, which showed the largest loss.

The lower increase in weight loss in irradiated samples during storage could be probably attributed to the modifications caused in cellular integrity and pore size due to the radiation treatment, thereby affecting moisture loss to the atmosphere (RASTOGI et al., 2006; RASTOGI, 2005; HAYASHI; TODORIKI; NAGAO, 1992).

For the variable total soluble sugars (TSS), a tendency to increase over time was observed without interacting with the irradiation doses applied (Figure 2). The lowest TSS value in the non-irradiated fruits was observed in the average TSS value of the treatments, and higher or similar TSS values were observed in the fruits irradiated with doses of 0.5, 1 and 2 kGy (Table 2). The change in sugar content during storage is related to the sensory characteristics of fruits and their conservation potential,

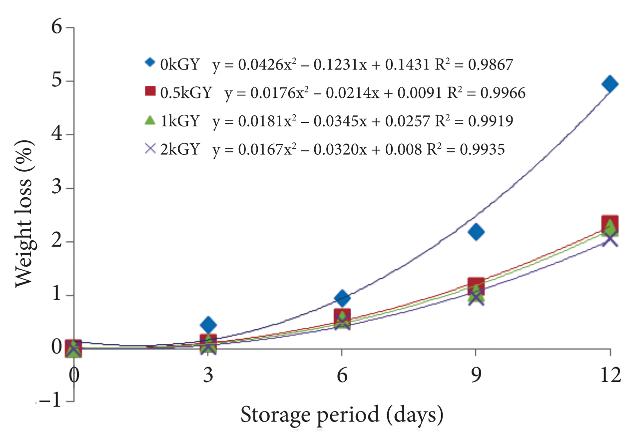

Figure 1. Weight loss during cold storage of raspberries exposed to different irradiation doses (0, 0.5, 1.0, and 2.0 kGy).

Table 1. Statistical significance for the response variables as a function of the major effects and interaction.

SV	DF	p-value					
		TA	pH	SS	TSS	Firmness	SP
Treatment (T)	3	0,539	0,056	0,934	0,036*	0,000*	0,554
Time (t)	4	0,000*	0,003*	0,410	0,000*	0,000*	0,000*
Txt interaction	12	0,269	0,118	0,196	0,114	0,000*	0,874
SV	DF	p-value					
		Solubility	%SFR	Vit C	Phenolic	W. loss	Fungi
Treatment (T)	3	0,273	0,000*	0,001*	0,000*	0,000*	0,000*
Time (t)	4	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*
Txt interaction	12	0,096	0,000*	0,000*	0,000*	0,000*	0,000*

*Probability (p-value <0.05) indicates significant effects.

as well as reserve substances (CHITARRA; CHITARRA, 2005; EPRILIATI et al., 2010).

The pH and titratable acidity were significantly affected by the time of storage, during which an increase in pH values and consequent decrease in acidity were observed (Figure 3). The organic acids, together with the sugar content, comprise an important quality attribute in fruits. Many of these acids are volatile contributing significantly to the characteristic flavor of each fruit (KLUGE et al., 2002). Françoso et al. (2008) reported that the pH and acidity of strawberries treated with different doses of Co-60 gamma irradiation and stored for 29 days were significantly affected only by storage time, during which an increase in pH and a decrease in acidity were observed.

The decrease in acidity can be attributed to the use of organic acids as respiratory substrates and as carbon skeleton for the synthesis of new compounds during ripening (FAN et al., 2009).

Regarding the variable firmness, it was observed a negative effect of irradiation dose since in general all treatments showed a reduction over time, while the treatment with the highest dose (2.0 kGy) resulted in lower firmness at the end of the storage period (Figure 4). Soluble pectin content and solubility increased during storage (Figures 5 and 6).

Table 2. Mean values of total soluble sugar content of raspberries during storage and after exposure to different irradiation doses (0, 0.5, 1.0, and 2.0 kGy).

Irradiation Dose coluna (kGy)	TSS (%)
0	3,14[b]
0,5	3,41[ab]
1	3,72[a]
2	3,54[ab]

Means followed by the same letter do not differ among themselves by Tukey test at 5% probability.

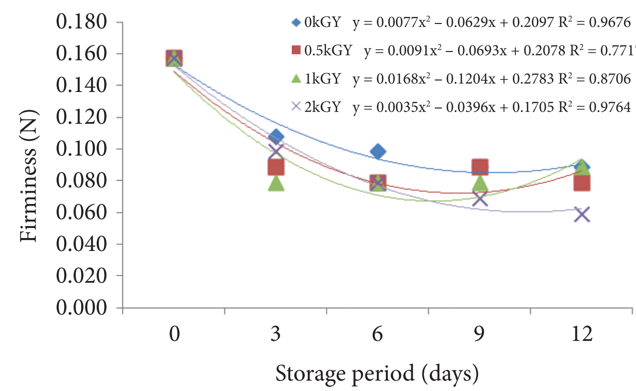

Figure 4. Changes in firmness during cold storage of raspberries exposed to different irradiation doses (0, 0.5, 1.0, and 2.0 kGy).

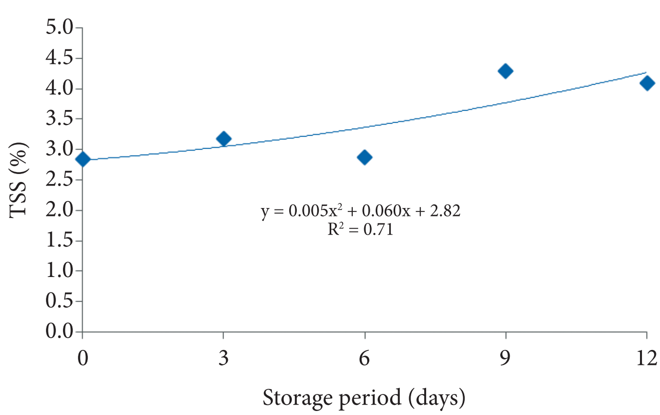

Figure 2. Total soluble sugar content during cold storage of raspberries exposed to different irradiation doses (0, 0.5, 1.0, and 2.0 kGy).

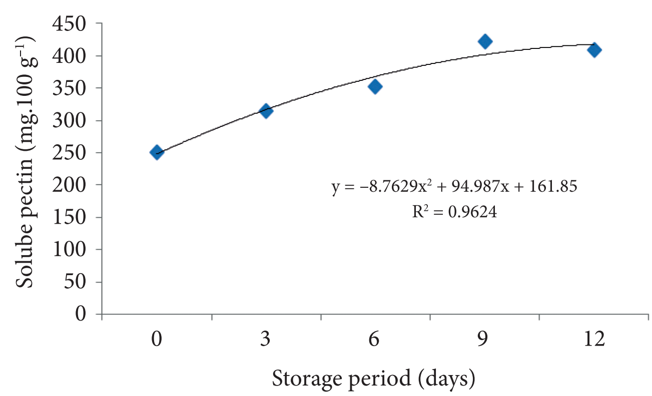

Figure 5. Changes in soluble pectin content during cold storage of raspberries exposed to different irradiation doses (0, 0.5, 1.0, and 2.0 kGy).

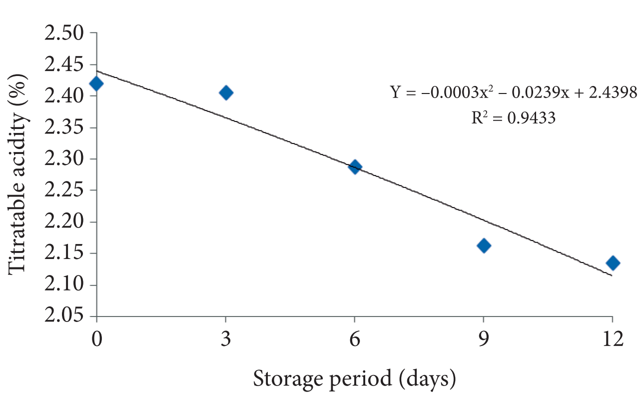

Figure 3. Titratable acidity during cold storage of raspberries exposed to different irradiation doses (0, 0.5, 1.0, and 2.0 kGy).

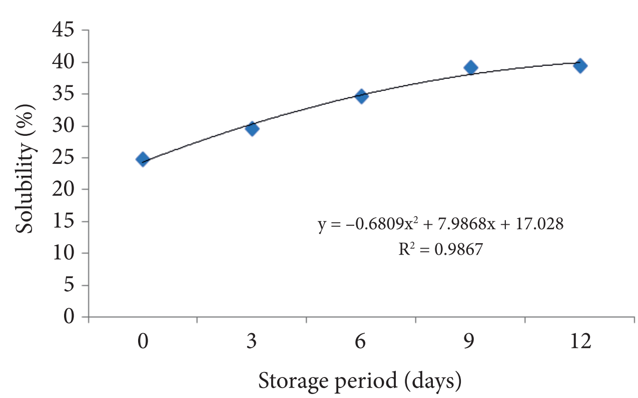

Figure 6. Percentage of solubility during cold storage of raspberries exposed to different irradiation doses (0, 0.5, 1.0, and 2.0 kGy).

Françoso et al. (2008) reported an increase in the amount of soluble pectin and solubility during storage of irradiated strawberries, regardless of the irradiation dose. Pineapple and cashew showed a longer duration of firmness during storage after Co-60 gamma radiation than that of non-irradiated fruits (SILVA; SILVA; SPOTO, 2008; SOUZA et al., 2009). However, Oliveira et al. (2006) showed that guavas irradiated with 600 Gy remained firmer than control guavas irradiated with 300 and 900 Gy. Thus, irradiation can be harmful to the tissue since no adequate doses (higher than that supported by vegetal) can cause cell disruption leading to decrease in firmness; this could be the reason for higher loss of firmness in raspberries radiated with the highest dose (2.0 kGy).

In fruits, the softening of tissue is related to changes in cell wall structure. These changes generally occur due to degradation and solubilization of pectin and the action of enzymes, which result in modification of cell wall structure. The role of protopectinase in the decomposition of macromolecules such as cellulose, hemicellulose, and starch results in the softening of cell walls decreasing the cohesive force that holds cells together thus leading to a decrease in fruit firmness (CHITARRA; CHITARRA, 2005).

For the variable ascorbic acid, a tendency to increase during storage was observed for the doses of 1 and 2 KGy, while doses of 0.5 and 0 KGy showed the highest values of ascorbic acid up to 9 days of storage and decreased close to the end of the storage period showing the lowest values (Figure 7). For the variable total phenolic, similar response occurred between the treatments throughout the storage time, except for the dose of 2 kGy, which showed higher values than those of the 9 and 12 days of storage (Figure 8). It was also observed an increase trend for the variable antioxidant activity at a dose of 2 kGy during storage (Figure 9), corresponding to the observed increase in vitamin C and phenolic compounds, since these two groups of molecules are highly related to *in vitro* antioxidant activity (RUFINO et al., 2010; YE et al., 2011).

The total antioxidant activity represents the action of various compounds such as phenolic compounds and vitamin C (ascorbic acid) to prevent cell oxidation and free radical formation; both of which promote human health. Mechanical damage, decay, and aging promote cell wall disruption releasing degradation enzymes such as polyphenol oxidase and ascorbate oxidase, which are responsible for the oxidation of ascorbic acid (MOKADY; COGAN; LIEBERMAN, 1984). Eichholz et al. (2011) showed that the application of UV-C in blueberries resulted in a higher total phenolic content as compared to control fruits. Sun-dried gamma irradiated apricots showed enhanced capacity to retain high levels of ascorbic acid and antioxidant activity (measured by the method of β-carotene) as compared to control fruits, and the most effective dose was 2.5-3.0 kGy (HUSSAIN et al., 2011). In kiwi, irradiation with 1 and 2 kGy resulted in a 50% decrease in ascorbic acid level compared to than that of control fruits (0 kGy) and to that of 0.5 kGy-treated fruits (HARDER et al., 2009).

In the case of responses related to filamentous fungi and yeasts (Figure 10), an increasing effect related to time was observed; in other words, there was greater fungal growth over

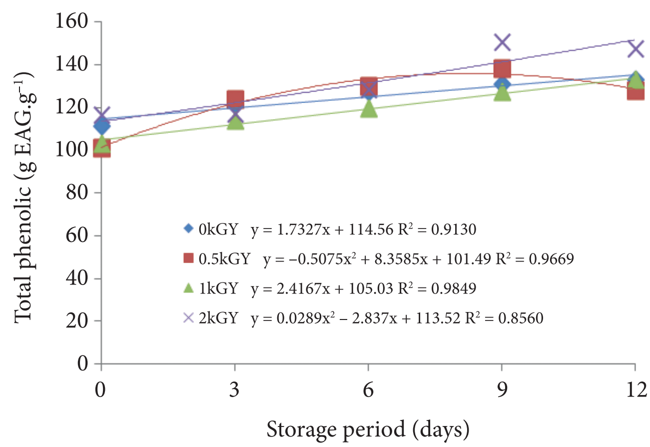

Figure 8. Change in total phenolic content during cold storage of raspberries exposed to different irradiation doses (0, 0.5, 1.0, and 2.0 kGy).

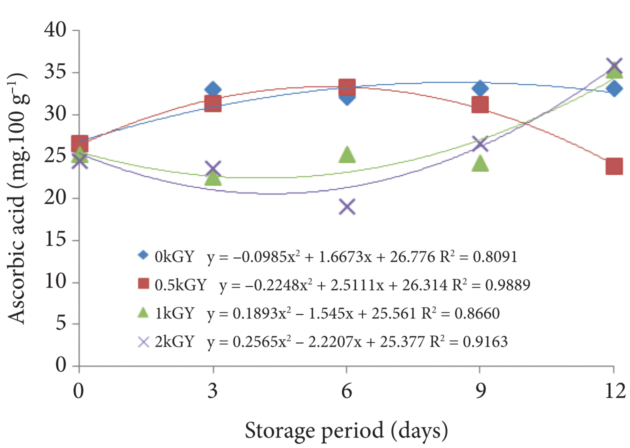

Figure 7. Ascorbic acid levels during cold storage of raspberries exposed to different irradiation doses (0, 0.5, 1.0, and 2.0 kGy).

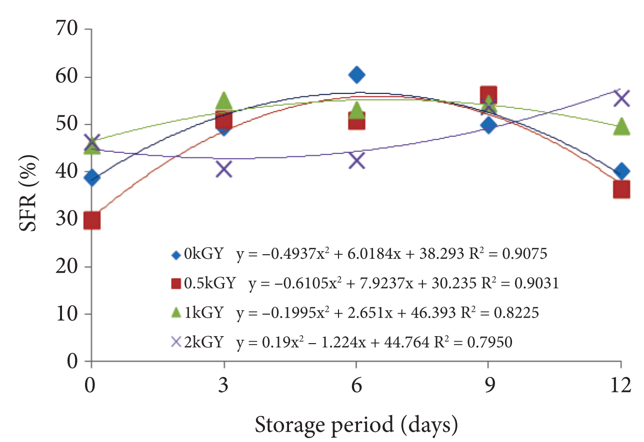

Figure 9. Change in total antioxidant activity (%SFR) during cold storage of raspberries exposed to different irradiation doses (0, 0.5, 1.0, and 2.0 kGy).

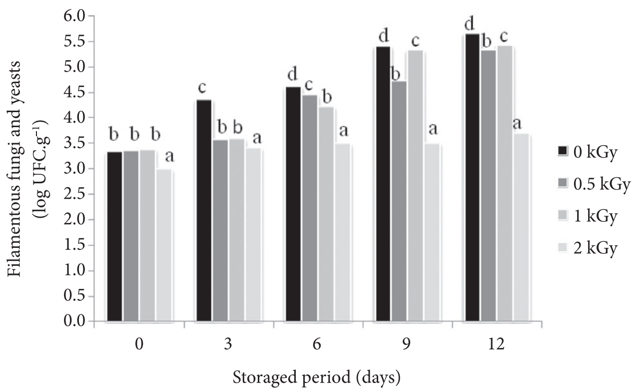

Figure 10. Number of filamentous fungi and yeast in raspberries exposed to different irradiation doses (0, 0.5, 1.0, and 2.0 kGy) and stored under refrigeratation. Treatment means followed by the same letter indicate lack of significance for each time interval based on a 5% significance level by the Tukey test.

time. With respect to the irradiation dose, it can be said that the highest dose resulted in the lowest microbial growth.

Fresh fruits and vegetables are generally considered as carriers of diseases, attributable to the presence of fecal *Escherichia coli* and *Salmonella* sp. that are usually present in the irrigation water, manure in the soil or fertilizer, or due to improper handling of the product (GANGLIARDI; KARNS, 2000).

Bacterial coliforms were not detected at 35 and 45 °C in any of the treated raspberry samples and the controls during the entire storage period. These results indicate adequate sanitary-hygienic conditions during handling, collection, storage, and transport of the fruits. RDC Resolution No. 12, promulgated on January 2, 2001 by ANVISA, Ministry of Health, issued guidelines for fresh, prepared, sterilized, chilled fruits or frozen fruits meant for direct consumption with a maximum allowable microbial level of 5 × 102 $NMP \cdot g^{-1}$ (2.7 log cycles) for coliform bacteria at 45 °C and *Salmonella* in 25 g of the product (BRASIL, 2001). Therefore, the results obtained met the standards set by the legislation for coliform bacteria throughout the storage period.

According to Babic and Watada (1996), yeast and mold populations in the range of 10^3-10^4 $CFU \cdot g^{-1}$ (3-4 log cycles) detected during storage is considered low. Considering this study, a 2 kGy radiation dose should ensure even lower microbial counts in fruits during the 12-day storage period; however, the average microbial content score at the end of the study in fruit radiated at 2 kGy was below 4 log cycles, while the control fruits radiated at 0.5 and 1 kGy showed mean scores of above 4 log cycles.

The total bacterial, fungal, and yeast counts were significantly reduced in Co-60 gamma irradiated lychees stored for 28 days (HAJARE et al., 2010). Irradiation is commonly used in minimally processed fruits and vegetables allowing effective microbial control (ESCALONA et al., 2010; PINTO, 2010; MARTINS et al., 2004, LIMA et al., 2003).

4 Conclusions

On the basis of the experimental conditions used in this study, we conclude the following:

- The use of irradiation on postharvest raspberries is a viable technique;
- Irradiation reduces weight loss and filamentous fungi and yeast count;
- The irradiation dose of 2 kGy is highly effective in controlling microbiological growth, but it is also the dose at which the fruits lose most of their quality and firmness; and
- Further studies using doses between 1 and 2 kGy are required to optimize the raspberry preservation by the irradiation method.

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