Gamma radiation: An efficient technology to conserve the quality of fresh raspberries

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A B S T R A C T
Raspberries have short postharvest life and decay rapidly. A technology that is increasingly being used to inhibit the growth of these microorganisms and simultaneously delay fruit senescence is gamma ray irradiation. The aim of this study was to evaluate the effectiveness of this technology in cold-stored fresh raspberries postharvest and to find the best irradiation dose. Raspberries were purchased from a commercial orchard. The doses applied were of 0.5, 1.0 or 2.0 kGy, and the fruit was stored at 0 °C and 90% RH after the irradiation. Non-irradiated fruit was used as the control. The results indicated that the application of gamma rays did not alter the respiratory rate, ethylene production, flesh firmness, anthocyanins content or color index of the raspberries. The use of gamma radiation in doses of 1.0 and 2.0 kGy, associated with cold storage, extended the postharvest life of fresh raspberries by 8 days. The 1.0 kGy dose is considered the most useful one that reduces the decay incidence and weight loss and presents the lowest reduction in ascorbic acid. However, to recommend its use, it is necessary further studies about the effect of this dose in the bioactive and nutritional compounds of the raspberries.

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1. Introduction

The raspberry fruit (Rubus idaeus L.) has a wide antioxidant, anti-inflammatory, anticancer and cardio protective capacity, providing numerous beneficial effects for human health when consumed fresh (Bomser et al., 1996; Heinonen et al., 1998). The raspberry also has a pleasant appearance and flavor if consumed fresh, which justifies its increased production and consumption. However, the short postharvest life and the fast proliferation of postharvest pathogens hinder its distribution in the market. In ambient conditions (±25 °C, 70% RH), the raspberry can be sold only up to 48 h after harvest (Raseira et al., 2004).

According to numerous published studies, the use of postharvest technologies like cold storage, high CO₂, controlled atmosphere or postharvest application of volatile compounds result in maintaining the postharvest quality of the raspberry for a period of 10–12 days (Agar et al., 1996; Raseira et al., 2004; Wang, 2003). However, these technologies are still having problems with high postharvest decay, and it is necessary to apply pre-harvest synthetic fungicides.

As an alternative to the use of chemicals, a technology that has been increasingly used for inhibiting the growth of pathogenic microorganisms and simultaneously delaying fruit senescence is gamma ray irradiation. Gamma radiation is a kind of ionizing radiation that is transmitted by high frequency electromagnetic waves (Abdalla and Villela Neto, 2005) and that leaves no residue on the food post-application.

The main obstacles of gamma radiation use are the high cost and the rate of the dose required to control the decay and not result in fruit damage. Studies have shown that doses above tolerance result in browning and the loss of firmness, texture and flavor for oranges, grapefruits, pineapples and strawberries (Damayanti et al., 1992).

As the raspberry is in high demand for consumption in North America and Europe and has high value in South America, the irradiation cost would become feasible if the benefit brought with its use reduced the postharvest losses. However, there are no studies examining the best dose for this fruit, nor are their studies showing the influence of gamma radiation use in raspberry conservation.

Thus, the aim of this study was to evaluate the effectiveness of this technology in fresh raspberries stored postharvest at 0 °C and to find the best irradiation dose. These results will help the producer and distributor of this fruit to choose effective treatments that do not leave residues on the product.

2. Materials and methods

2.1. Fruit material

Mature ‘Autumn Bliss’ raspberries of uniform shape, size and pinkish color were purchased from a commercial orchard in Ibiúna, SP, Brazil, as part of the 2011/2012 crop. The fruit had no
visible mechanical or pathogen damage. After harvest, the fruit were directly placed in the sales package (perforated polyethylene terephthalate trays), and these were placed into polystyrene boxes containing ice sheets. The polystyrene boxes were sealed and immediately transported to the laboratory in Piracicaba, SP.

2.2. Gamma irradiation treatment and storage

The day after the fruits were harvested, they were randomly divided into eight polystyrene boxes with ice sheets. The boxes were sealed and transported to the Instituto de Pesquisas Energéticas e Nucleares (IPEN), USP, São Paulo, SP, where the fruit were subjected to gamma irradiation using a Gammabeam YR-530 nm irradiator having 60Co as a gamma ray source. The doses applied were 0.5, 1.0 and 2.0 kGy, maintained for 8.40 min, 19 min and 42 min, respectively. Two of the eight boxes transported to the IPEM received no gamma ray, serving as controls. Thus, the experiment consisted of four treatments (doses), with 4 replications, each composed of 120 g fruit (or about 35 raspberries). After irradiation, the fruit was transported back to the laboratory and then put into cold storage at 0 ± 1°C and 90 ± 5% RH.

2.3. The determination of respiration rate and ethylene production

The determination of the respiratory rate and ethylene production were carried out at harvest, after the irradiation treatments and every two days thereafter for seventeen days. For the analysis, eight raspberries were placed into 80 mL glass flasks and hermetically sealed for 30 min at the same temperature and relative humidity conditions of the cold storage. A silicon septum was fitted in the flask lids to allow for the collection of a 0.5 mL of internal atmosphere. Gas samples were injected in a gas chromatograph (Thermo Electron Corporation, model Trace GC Ultra), which was equipped with two flame ionization detectors (FID), two injectors, two Porapack H columns and one methanator set to 350°C. The results were determined considering the chromatographic values, fruit mass, flask volume and the time it remained closed. The results concerning the respiratory rate were expressed in mL CO2 kg−1 h−1 and ethylene production in μL C2H4 kg−1 h−1.

2.4. Fruit quality evaluations

Qualitative analyses were carried out at harvest (day 0) and thereafter every four days for 20 days. Decay and weight loss were measured in the same four packages, containing 35 raspberries each one, from the beginning to end of the experiment. Decay incidence was visually inspected, counting the number of fruit that showed signs of fungal growth and mealiness (a condition of extreme softness and oozing) and the results were expressed in %. To determine weight loss, the raspberries were weighted at the harvest (day 0) and thereafter each day, being the results expressed as the percentage loss of the initial total weight. For the following analyses, the fruit were removed from the cold 24 h before the analysis to simulate the marketing period. The flesh firmness was determined by an application technique, as described by Calbo and Nery (1995), with the results expressed in Newtons. The pectin solubilization percentage was executed according toMcCready and McCoomb (1952), and the determination was performed colorimetrically using methods described by Bitter and Muir (1962). We had to reduce the fruit mass from 5 to 2 g in each sample, and the aliquot used for the quantification from 1 to 0.2 mL. The ascorbic acid content was determined by titration (Carvalho et al., 1990), and the results were expressed in mg 100 g−1 of fruit. The titratable acidity was determined from 10 g possed juice sample diluted with 90 mL distilled water, titrated with 1 N NaOH to pH 8.1 and expressed in % citric acid. The anthocyanin and quercetin contents were determined according to Lees and Francis (1972), and they were expressed in mg g−1 of fruit. The pulp masses were reduced from 100 to 10 g and homogenized in a 10 mL ethanol and HCl solution. The color index (CI) was determined in a Minolta colorimeter, model CR-400, and calculated as CI = 100 × a/(L × b). The a and b values are the chroma values a* and b*, and L is the luminosity. The CI ranged from 39 to 75, with a greater IC value indicating a more intense red color of the fruit.

2.5. Statistical analysis

An analysis of variance was performed using the SISVAR version 4.2 software according to a completely randomized experiment in factorial design (treatment × day of analysis). Mean comparisons were performed by Tukey’s test at P ≤ 0.05.

3. Results

3.1. The influence of irradiation on the respiration and ethylene production rates

We postulate that irradiated fresh raspberry could maintain its life for a longer period after harvest. To test this hypothesis, we applied different gamma radiation doses to the fruit and evaluated the resulting respiratory and ethylene production rates. The irradiated fruit showed the same performance than the not treated fruit (Table 1). However, both respiration and ethylene production rates decreased after three days of cold storage. Regarding the respiratory rate, there was a reduction of 90%, with this value remaining constant until the end of the period. The ethylene production decreased 88% by the 3rd day, but from the 7th day it began to increase, reaching values that were similar to those of the irradiation day. These results indicated that the gamma irradiation did not alter the gas metabolism of the fresh raspberries.

3.2. Evolution of the physical-chemical quality of the irradiated fruits

As one of the biggest benefits of gamma radiation use is the control of pathogens, we have evaluated their incidence in irradiated

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Respiration rate (mL kg⁻¹ h⁻¹ of CO₂)</th>
<th>Ethylene production (μL kg⁻¹ h⁻¹ of C₂H₄)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiation (kGy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>28.98 a</td>
<td>7.05 a</td>
</tr>
<tr>
<td>0.5</td>
<td>27.72 a</td>
<td>7.10 a</td>
</tr>
<tr>
<td>1.0</td>
<td>27.66 a</td>
<td>8.00 a</td>
</tr>
<tr>
<td>2.0</td>
<td>25.80 a</td>
<td>7.05 a</td>
</tr>
<tr>
<td>Pr &gt; Fc</td>
<td>0.8155</td>
<td>0.2163</td>
</tr>
</tbody>
</table>

Days after harvest

| | | |
| 0 | 111.65 a | 18.03 a |
| 1 | 42.72 b | 11.15 b |
| 3 | 11.18 c | 2.14 e |
| 5 | 11.24 c | 3.63 e |
| 7 | 13.25 c | 5.72 d |
| 9 | 14.05 c | 5.85 d |
| 11 | 14.23 c | 4.27 d |
| 13 | 12.02 c | 4.58 d |
| 15 | 15.20 c | 8.85 c |
| 17 | 17.90 c | 12.26 b |
| Pr > Fc | 0.0000 | 0.0000 |

CV (%)

| 21.23 | 29.79 |

Means followed by the same letter within the column are not significantly different by Tukey’s test, P < 0.05 (n = 4).
raspberries. The results confirmed the effectiveness of this technology: non-irradiated fruit differed from the other treatments, reaching approximately 20% decay on the 12th day after harvest, at which time they were discarded (Fig. 1a). According to the analysis made in the Clinica Fitopatológica of ESALQ/USP, the microorganisms present in the fruit were Pucciniastrum americanum (Farl.) Arthur and Botrytis cinerea. Fruit irradiated at 0.5 kGy also was discarded at day 12, when 18% of the fruit showed symptoms of infection. Fruits irradiated to 1.0 and 2.0 kGy did not differ until the 12th day after harvest, with an average decay of 6%. Thereafter, the 2 kGy dose was more efficient as determined by the lower percentage of decayed fruit at the end of the study period.

The weight loss of the raspberries was also influenced by the radiation dose (Fig. 1b). Non-irradiated fruit and fruit irradiated at 0.5 kGy showed similar behavior, losing on average 10.5% of its mass on the 12th day after harvest. Fruit irradiated at 1.0 kGy lost 12.2% of its mass, and at 2.0 kGy, 15% on the 20th day. Thus, the 1.0 kGy dose resulted in the lowest raspberry weight loss.

The fruit firmness was reduced more than 30% from the day of harvest to day 4, irrespective of use or the radiation dose (Fig. 1c). From day 4 to 20, the firmness remained constant, and there was no difference between the treatments; however, the fruit were soft, and their handling was difficult. With respect to the pectin solubilization, there was an increase in the solubilization percentage from day 0 to 8. On this day, the irradiated fruit at 1.0 and 2.0 kGy presented the lowest percentage, and they differed from the non-irradiated fruits (Fig. 1d). From the 8th day, there was a reduction in the pectin solubilization, and the irradiated fruit, regardless of dose, showed the lowest values. Thus, the use of radiation at any dose resulted in a decreased pectin solubility of the raspberries.

The fruit showed a titratable acidity reduction during the evaluated period regardless of treatment; however, the fruit irradiated at 2.0 kGy had the greatest reduction (Fig. 1e). Non-irradiated fruit and fruit irradiated at 0.5 and 1.0 kGy did not differ from each other. Thus, we observed that the 2.0 kGy dose accelerated the acidity reduction of the raspberries.

There was a decrease in the ascorbic acid content of all of the treatments, especially those that received the highest irradiation dose (Fig. 1f). Fruit irradiated at 0.5 and 1.0 kGy did not differ for this variable. Thus, we observed that the higher the irradiation dose presented, the greater the ascorbic acid reduction in the raspberries.
Table 2
Flavonoids and color measured in ‘Autumn Bliss’ raspberries irradiated and stored at 0°C and 90% RH for 20 days.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Anthocyanins (mg 100 g⁻¹ of fruit)</th>
<th>Quercetin (mg 100 g⁻¹ of fruit)</th>
<th>Color index (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiation (kGy)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>32.75 a</td>
<td>12.18 a</td>
<td>57.63 a</td>
</tr>
<tr>
<td>0.5</td>
<td>43.36 b</td>
<td>13.94 a</td>
<td>57.55 a</td>
</tr>
<tr>
<td>1.0</td>
<td>32.93 a</td>
<td>8.62 b</td>
<td>59.43 a</td>
</tr>
<tr>
<td>2.0</td>
<td>32.62 a</td>
<td>8.14 b</td>
<td>58.98 a</td>
</tr>
<tr>
<td>Pr &gt; Fc</td>
<td>0.0048</td>
<td>0.0128</td>
<td>0.6466</td>
</tr>
<tr>
<td>Days after harvest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>21.27 a</td>
<td>8.60 a</td>
<td>44.10 a</td>
</tr>
<tr>
<td>4</td>
<td>34.79 b</td>
<td>8.37 a</td>
<td>60.66 b</td>
</tr>
<tr>
<td>8</td>
<td>40.07 bc</td>
<td>9.58 ab</td>
<td>61.18 b</td>
</tr>
<tr>
<td>12</td>
<td>44.50 c</td>
<td>17.51 b</td>
<td>61.73 b</td>
</tr>
<tr>
<td>16</td>
<td>35.47 bc</td>
<td>5.71 a</td>
<td>63.23 b</td>
</tr>
<tr>
<td>20</td>
<td>37.54 bc</td>
<td>8.73 a</td>
<td>67.02 b</td>
</tr>
<tr>
<td>Pr &gt; Fc</td>
<td>0.0000</td>
<td>0.0009</td>
<td>0.0000</td>
</tr>
<tr>
<td>CV (%)</td>
<td>27.67</td>
<td>24.87</td>
<td>7.56</td>
</tr>
</tbody>
</table>

Means followed by the same letter within the column are not significantly different by Tukey's test, P<0.05 (n=4).

3.3. Flavonoids and coloring of irradiated raspberries

In this study, we evaluate two of the four flavonoids present in raspberry: anthocyanins and quercetin. According to the results, the application of 0.5 kGy increased the anthocyanin content in the fruit, making this treatment different from the others (Table 2). Regarding the quercetin, we observed that non-irradiated fruits and fruit irradiated at 0.5 kGy had higher levels, so that both differed from the fruit irradiated to 1.0 and 2.0 kGy. Over the analyzed period, we also observed that both flavonoids exhibited a content increase until the 12th day. Subsequently, the anthocyanins content remained constant and the quercetin decreased. With these results, we conclude that the doses of gamma radiation that had showed better results in the conservation of raspberries (1.0 and 2.0 kGy), had no effect on the anthocyanins content and decreased the quercetin content. The raspberries CI were not influenced by the treatments (Table 2). It was observed that the index increased 37.5% from day 0 to 4 and remained constant thereafter.

4. Discussion

We found that the doses of gamma radiation tested in this project did not increase the respiration rate or the ethylene production of the raspberries, as was observed in irradiated lemon (Maxie et al., 1965), mangosteen (Sritananan et al., 2005) and pluot (Prunus salicina × Prunus armeniaca) (Duvenhage et al., 2012). It is likely that the increase in the respiratory rate was not observed in the raspberries because they were stored at 0°C. The cold storage resulted in a sharp initial drop of the fruit metabolism and reduced the action of Krebs cycle enzymes, thus reducing the release of CO₂ and ATP synthesis. As ATP is required for the synthesis of ethylene, there was also a decrease in its output, regardless of the gamma radiation use. We therefore conclude that gamma radiation up to a 2.0 kGy dose, in association with cold storage, may be used on raspberries without changing the respiratory rate and ethylene production.

The synergistic effect of cold storage with gamma radiation delayed the decay, extending the postharvest life of fruit irradiated at 1.0 and 2.0 kGy by eight days. For irradiated raspberry, pineapple (Damayanti et al., 1992), strawberry (Hussain et al., 2012) and plum (Hussain et al., 2013), the highest doses were more effective as they were of sufficiently high energy to disrupt the atomic and molecular components of the microorganisms (Hagenaier and Baker, 1998). Thus, the highest dose resulted in the lowest pathogen survival and in a greater raspberry shelf-life. However, the use of higher doses can increase the membrane permeability and transpiration through the cuticles, increasing weight loss (Maxie et al., 1973). This may have been the reason that the raspberries irradiated at 2.0 kGy lost more fresh weight than did those irradiated at 1.0 kGy.

The fast reduction in the flesh firmness is an important postharvest change for the raspberry that is necessary to control with conservation technologies. However, the use of radiation was ineffective in slowing the initial loss of firmness and the increase in the solubilization of pectin. It is possible that the energy from the gamma radiation did not act on the activities of the enzymes that degrade or solubilize the cell wall.

All of the treatments presented a reduction in the titratable acidity. This behavior is due to the low sugar content of the raspberry composition, so that in the postharvest, the fruit makes use of organic acids, such as respiratory substrate and a carbon skeleton for the synthesis of new compounds, mainly those that are volatile (Hussain et al., 2012). Regarding the radiation dose, it was verified that 2.0 kGy intensifies the organic acid reduction of the raspberries. As this behavior is not related to the respiratory rate, it is possibly connected to the synthesis of volatile compounds. However, more studies are needed to confirm this assumption.

With respect to the ascorbic acid, we observed a decrease in its levels, including in the non-irradiated raspberries. It is known that this reduction is due to the antioxidant activity of the acid, further accentuating the reduction in cold storage conditions (Davey et al., 2000). We also observed that the reduction of ascorbic acid was higher with increases in the gamma radiation dose. This increase occurs because when the dose is raised, the water radiolysis from inside the fruit generates more OH⁻ radicals, which oxidize ascorbic acid (Wong and Kitts, 2001). However, Hussain et al. (2012) observed that the decrease in ascorbic acid of irradiated strawberies was accompanied by an increase in dehydroascorbic acid (oxidized form of vitamin C). To an even lesser extent, this compound is biologically active, and the use of radiation did not result in the drastic loss of the activity of this vitamin in the fruit. Moreover, it should be considered that the non-irradiated fruit also lost ascorbic acid upon postharvest and that, although the use of gamma radiation at 1.0 and 2.0 kGy resulted in losses of ascorbic acid, irradiation extended the life of the raspberries. Thus, we conclude that, for this fruit, a gamma radiation dose of 1.0 kGy may be recommended.

The data analysis showed that the use of different doses of gamma radiation did not increase the anthocyanin content of the raspberries, as occurred with irradiated peach (Hussain et al., 2008), strawberry (Hussain et al., 2012) and plum (Hussain et al., 2013). The increased anthocyanin content due to the increase in the gamma ray dose was mediated by the increased ethylene production and, consequently, the activity of two key enzymes in the biosynthesis of anthocyanin, phenylalanine ammonia lyase (PAL) and glucosyltransferase (GT) (Hussain et al., 2008). As the raspberries had not increased their ethylene production with increasing doses, there was also no increase in the anthocyanin biosynthesis. However, the quercetin content of the raspberries was influenced by the use of gamma radiation. The decrease in quercetin in fruit irradiated to 1.0 and 2.0 kGy may have occurred because of exposure to more drastic oxidative conditions; just as for ascorbic acid, the main function of quercetin is in the removal of free radicals (Lakhanpal and Rai, 2007). Regarding the behavior over the evaluated period, we observed that the content of the two flavonoids increased until day 12, which may be related to the reduction in the acidity. We also observed that the CI of the raspberries was not influenced by the use of radiation. This result is consistent with other previously obtained data because the main raspberry pigments are the anthocyanins.
5. Conclusion

The use of gamma radiation in doses of 1.0 and 2.0 kGy, associated with cold storage, extends the postharvest life of fresh raspberries by 8 days; therefore, this postharvest technology is effective for raspberry storage. The 1.0 kGy dose is considered to be the most useful given that it reduces the decay incidence and weight loss and presents the lowest reduction in ascorbic acid. However, to recommend its use it is necessary further studies about the effect of this dose in the bioactive and nutritional compounds of the raspberries.

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