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EVALUATING PINHÃO CONSERVATION BY USING IONIZING RADIATION AND REFRIGERATION

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Abstract

Pinhão is highly perishable due to its high water activity, being easily affected by fungi during storage and also susceptible to infestation by larvae. This seed is usually marketed in the pinhão cones itself, or bulk threshed, and packed in plastic bags, chilled or ground frozen. Pinhão conservation and industrialization techniques should be developed to promote its commercialization and consumption at other times of the year, besides the seasonal period, encouraging its sustainable production, extraction and commercialization, considering its essentially extractive character. The objective of this study was to evaluate the conservation of pinhão by the use of gamma radiation and refrigeration. The pinhões were irradiated with a cobalt–60 source at a dose rate of 1 kGy. A non-irradiated sample was used as a control. The pinhões were packed in high-density polyethylene bags and stored at ambient temperature and refrigerated at 4 ºC, during 90 days. Pinhões were evaluated for weight loss, acidity, reducing sugars, vitamin C, firmness, color, total phenolic compounds, antioxidant activity and microbiological analyzes. The isolated use of gamma radiation was not effective for the maintenance of the evaluated parameters. However, when used in conjunction with refrigerated storage, it reduced the growth of aerobic fungi, as well as mesophilic and psychrotrophic microorganisms. The isolated use of refrigeration showed a reduction in weight loss, reducing sugars and an increase in vitamin C content and antioxidant activity. Thus, to increase the benefits, we suggest evaluating higher doses of radiation as a function of the thick pinhão shell.

Keywords: *Araucaria angustifolia.* Cobalt–60. Seed.

1. Introduction

Pinhão is the seed of *Araucaria angustifolia* (Bertoloni) Otto Kuntze, belonging to the Araucariaceae family. This pine is native to Brazil, mainly found in the states of Paraná, Santa Catarina and Rio Grande do Sul, but can also be found in Argentina and Paraguay (Zandavalli et al. 2004; Reis et al. 2014; Polet et al. 2017). These edible seeds are considered a source of starch, magnesium, phosphorus, copper, iron, ascorbic acid, as well as a large amount of flavonoids and other phenolic compounds present in the shell, which diffuse in the pulp during cooking. These compounds have antioxidant activity and are responsible for inhibiting oxidative mechanisms, preventing degenerative and cardiovascular diseases as well as cancer (CordenunsI et al. 2004; Souza et al. 2014).

Pinhão is highly perishable due to its high water activity (> 0.98), being easily affected by fungi during storage (Balbinot et al. 2008) and also susceptible to infestation by larvae (Olivera 2008). Pinhão is usually marketed in the pinhão cones itself, or bulk threshed, and packed in plastic bags, chilled or ground frozen (Olivera 2008).

Pinhão conservation and industrialization should be developed to promote its commercialization and consumption throughout the year, encouraging its sustainable production, extraction, and commercialization, due to its essentially extractive character (David and Siloch 2010).

An alternative conservation technique is the use of ionizing radiation, which is a non-thermal, chemical-free process and uses less energy due to the minimization of cold chain needs and water consumption (Yun et al. 2012; Maherani et al. 2016). Ionizing radiation also prevents the microbial growth of bacteria and fungi, by altering their molecular structure and induces biochemical modifications in the physiological processes of some vegetables, slowing their maturation, aging as well as sprouting (Stefanova et al. 2010).

This technology has been widely used in the conservation of vegetables such as apricot (Wei et al. 2014), cherry tomatoes (Guerreiro et al. 2016), spinach (Hussain et al. 2016), strawberry (Maraei and Elsawy 2017), chives (Memon et al. 2020), pomegranate (Ashtari et al. 2019), and bamboo shoots (Wang et al. 2019); the results being dependent on the raw material and the radiation dose. To date, there are no studies on the application of ionizing radiation on pinhão seeds. Thus, the objective of this study was to evaluate the conservation of pinhão by the use of gamma radiation and refrigeration.

2. Material and Methods

Pinhões samples [*Araucaria angustifolia* (Bertoloni) Otto Kuntze] were obtained from a producer in Vacaria (Latitude: 28°30'39'' South, Longitude: 50° 55' 47'' West) in the state of Rio Grande do Sul. The seeds were collected, packed in a burlap bag and transported to the city of Pelotas – RS, where they were stored at ambient temperature (18 °C) for two days until processing.

The pinhões were selected, discarding those that showed deterioration or apparent fungal growth and divided into two groups. In the first group, the pinhões were irradiated with a cobalt–60 source (Best Theratronics Ltd., Ottawa, Canada) at a dose rate of 1 kGy with 1.25 MeV particle energy, and 2.0584 $Gy.min^{-1}$ yield, at 20–22 °C. It is worth mentioning that in this first study, we chose to evaluate a low dose of radiation, since there are no related studies on irradiation of pinhão in the literature.

The pinhões (5 kg) were placed in a 22.5 cm hollow-faced cube, coated with a polyvinyl chloride film. Irradiation was applied three-way, from parallel and opposite sides. In the other group, no irradiation was applied. Each kilo of pinhão was subsequently packed in high-density polyethylene bags and stored at ambient temperature (average 18 ºC) and refrigerated at 4 ºC, respectively. The analysis was performed at 0, 30, 60, and 90 days of storage.

The following treatments were evaluated: Treatment A – irradiated pinhão (1 kGy) stored at ambient temperature; Treatment B – non-irradiated pinhão stored at ambient temperature; Treatment C – irradiated pinhão (1 kGy) stored under refrigeration (4 ºC); and Treatment D – non-irradiated pinhão stored under refrigerated temperature (4 ºC).

The experimental design was completely randomized in a 4 × 4 factorial scheme, with 4 treatments (A, B, C, and E), and 4 evaluation periods (0, 30, 60, and 90 days of storage). Each treatment consisted of 330 pinhões seeds.

Weight loss was obtained by calculating the difference between the initial mass of the pinhão and the mass obtained at the end of each storage time (Akhtar et al. 2010). The analysis was performed in triplicate, and the results were expressed as percentage weight loss.

$$\text{Weight loss (\%)} = \left[\frac{\text{(initial mass-final mass)}}{\text{(final mass)}}\right] \text{x100}$$

Total titratable acidity of 10 g pinhões (peeled, ground, and homogenized with 100 mL of distilled water) was determined by potentiometric titration. The sample was titrated with 0.1 mol.L^{-1} NaOH solution to a pH range (8.2–8.4) (IAL 2008). The analysis was performed in triplicate, and the results were expressed in percentage.

An aqueous extract was prepared from peeled and ground pinhões (2.5 g) in 50 mL of water by constant stirring for 2 h and filtered through a qualitative paper. Reducing sugars were determined by the methodology described by Vasconcelos et al. (2013). Aliquots of 1.0 mL of aqueous extract and 1.0 mL of 3,5-dinitrosalicylic acid reagent were pipetted and transferred to a 10 mL volumetric flask, and vortexed for 1 min. Subsequently, the sample was placed in a 100 °C water bath for 5 min and later cooled in a cold water bath. The volume of the flask was filled with distilled water. The absorbance of the resulting solution was measured by a spectrophotometer (AAKER, Brazil) at 540 nm. The results were quantified using a glucose calibration curve at concentrations of 0 to 3 mg.mL^{-1} (517.88x + 45.851, R^2 = 0.9907) and expressed as g per 100 g of pinhões. The analysis was performed in triplicate.

For the determination of vitamin C, the peeled and ground pinhões (20 g) were transferred to Erlenmeyer flask followed by 50 mL of water, 10 mL of 20% (v/v) sulfuric acid solution, 1 mL of 10% (v/v) potassium iodide and 1 mL of 1% (w/v) starch solution. The sample was titrated with 0.02 mol.L^{-1} potassium iodate solution to a pink color. The analysis was performed in triplicate, and the results were expressed as mg.100 g^{-1} sample (IAL 2008).

The pinhões were peeled, and its firmness was determined using a texturometer (Stable Micro Systems model TA.XTplus, UK). The HDP/BS cutting blade and the HDP/90 base plate were used as a tip. A compression test was performed to measure firmness or the strength to cause seed rupture. The operating parameters used were: 1.50 mm.s^{-1} pretest speed, 1.00 mm.s^{-1} test speed, 10.00 mm.s^{-1} post-test speed, distance of 4 mm, and driving force of 0.147 N. The firmness obtained was automatically recorded using the Texture Exponent 32 software. The measurement was performed in the equatorial central region of the pinhão with 8 repetitions, and the results were expressed in Newton (N).

The color was determined using a colorimeter (Minolta, CR 400, Japan). In the CIELAB, the standard was denoted as L*a* b*, where the L* coordinate expresses the degree of lightness of the measured color (L* = 100 = white; L* = 0 = black), the a* coordinate expresses the degree of variation between red (+60) and green (–60) and the b* coordinate expresses the degree of variation between blue (–60) and yellow (+60). The analyses were performed in triplicate

For the preparation of the hydroalcoholic extract, the pinhões with shell were cooked in a pressure cooker for 20 min, peeled, ground, and 5 g were added with 50 mL of methanolic solution (70% methanol/30% water). The extract was stirred for 3 h at ambient temperature, and filtered through a qualitative paper.

The total phenolic compounds were determined by the methodology proposed by Singleton et al. (1999) with some modifications. Aliquots of 1 mL of the hydroalcoholic extract (70% methanol/30% water) were added with 1 mL of Folin-Ciocalteau solution, followed by 8 mL of distilled water. After 3 min of reaction, 1 mL of 1 mol.L^{-1} Na_2CO_3 was added, and the mixture was incubated at 37 °C for 30 min. The absorbance of the resulting solution was measured by spectrophotometer (AAKER, Brazil) at 750 nm. The results were quantified using the calibration curve of gallic acid at concentrations of 0 to 0.5 mg mL^{-1} (y = 1.9772x + 0.072, R^2 = 0.9832) and expressed in mg EAG.100g^{-1} pinhões. The analysis was performed in triplicate.

Determination of antioxidant activity was performed using the DPPH method (2, 2-diphenyl-1-picrylhydraz), according to Brand-Willians et al. (1995). An aliquot of 750 µL of the hydroalcoholic extract (70% methanol/30% water) was homogenized with 3750 µL of DPPH (0.05 mM), and the reading was measured in a spectrophotometer (AAKER, Brazil) at 515 nm after 20 min. The analysis was performed in triplicate and the results were expressed as percentage inhibition.

The present microbiota was evaluated by counting aerobic mesophiles, aerobic psychrotrophs and fungi.

Microbiological analysis was performed according to the procedures proposed by Downes and Ito (2001). Serial dilutions were made in 0.1% buffered peptone water up to 10^{-4} dilution, and the analyses were performed in triplicate.

Quantification of microorganisms was performed by plating dilutions on Standard Counting Agar (PCA). For aerobic mesophilic microorganisms, the plates were incubated at 35 °C for 48 h and aerobic psychrotrophs at 7 °C for ten days. The microbial count was expressed in UFC.g^{-1}.

For fungal enumeration (molds and yeasts), the Potato Dextrose Agar Plating method was used, and the plates were incubated at 25 ºC. Counts were obtained at three and five days of incubation. The result was expressed in UFC.g^{-1}.

The obtained results were subjected to the analysis of variance, and the comparison of means between the treatments was performed by Tukey's test with a significance level of 5% using the Statistix 10 software (USA). For the evaluation of the storage time, the confidence interval was evaluated at 95%.

3. Results

When analyzing the weight loss data of pinhões subjected to different treatments in relation to time, it was observed that there was a significant increase in weight loss ($p \leq 0.05$) during storage, regardless of treatment, as shown in Figure 1. At the end of storage, a significant effect ($p \leq 0.05$) of refrigeration on the reduction in weight loss was observed in non-irradiated (NIRT e NIAT), as well as irradiated pinhões (IRT e IAT). There was no significant influence of irradiation on pinhões weight loss (data not shown). Thus, refrigerated non-irradiated pinhões (NIRT) presented the lowest significant ($p \leq 0.05$) values of mass loss (9.92%), compared to irradiated pinhões stored at ambient temperature (18.26%), which presented the highest values among the evaluated treatments.

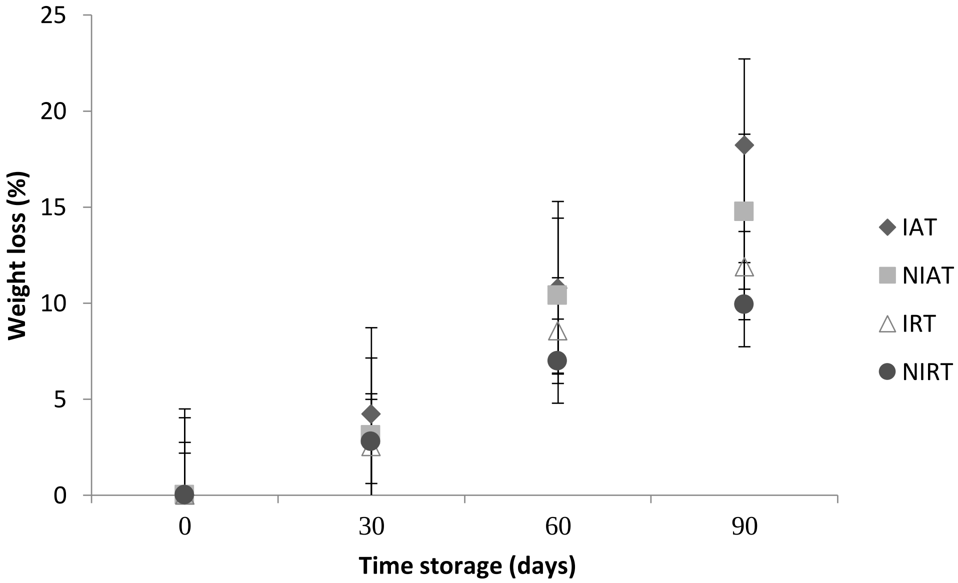

Figure 1. Weight loss (%) in irradiated (1 kGy) and non-irradiated pinhões, stored at ambient temperature, and refrigerated at 4 °C for 90 days. The vertical bars indicate the 95% confidence interval. IAT: irradiated pinhões stored at ambient temperature; NIAT: non-irradiated pinhões stored at ambient temperature; IRT: irradiated pinhões stored at refrigeration temperature; NIRT: non-irradiated pinhões stored at refrigeration temperature.

The acidity data of pinhões in relation to time reveal that there was a significant reduction ($p \leq 0.05$) in acidity during the 90 days of storage in all treatments, as shown in Figure 2. However, at the end of storage there was no significant distinction between treatments (data not shown).

In all treatments, similar behavior in alteration of reducing sugars with relation to time was observed. There was a significant reduction ($p \leq 0.05$) after 30-days for samples stored at ambient temperature (NIAT and IAT), compared to those stored under refrigeration (NIRT and IRT); the reduction occurred at 60 days. However, regardless of treatment, an increase in values was observed within 90 days of storage, as shown in Figure 3.

At the end of the storage, a significant effect (p≤0.05) of refrigeration was observed, showing a lower percentage of reducing sugars on non-irradiated (NIAT and NIRT), and irradiated pinhões (IAT and IRT). Regarding the effect of irradiation, it was observed that it caused significantly (p≤0.05) high levels of reducing sugars, regardless of storage temperature (IAT and IRT). Thus, the highest values were obtained in irradiated pinhões stored at the ambient temperature (IAT) (1.24 g.100g^{-1}) and the lowest in non-irradiated pinhões stored under refrigeration (NIRT) (0.93 g.100g^{-1}) (data not shown).

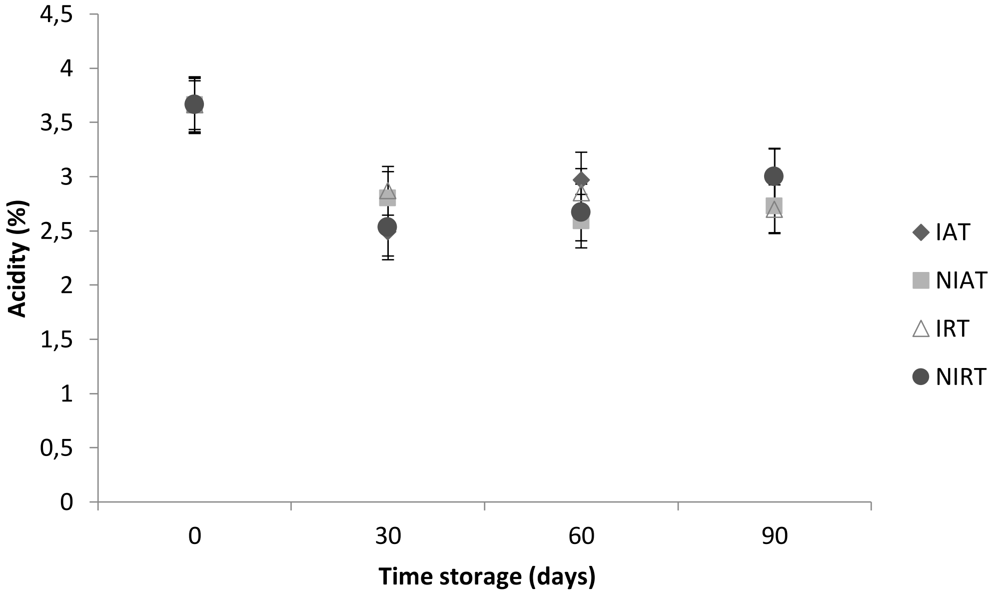

Figure 2. Acidity (%) in irradiated (1 kGy) and non-irradiated pinhões, stored at ambient temperature, and refrigerated at 4 °C for 90 days. The vertical bars indicate the 95% confidence interval. IAT: irradiated pinhões stored at ambient temperature; NIAT: non-irradiated pinhões stored at ambient temperature; IRT: irradiated pinhões stored at refrigeration temperature; NIRT: non-irradiated pinhões stored at refrigeration temperature.

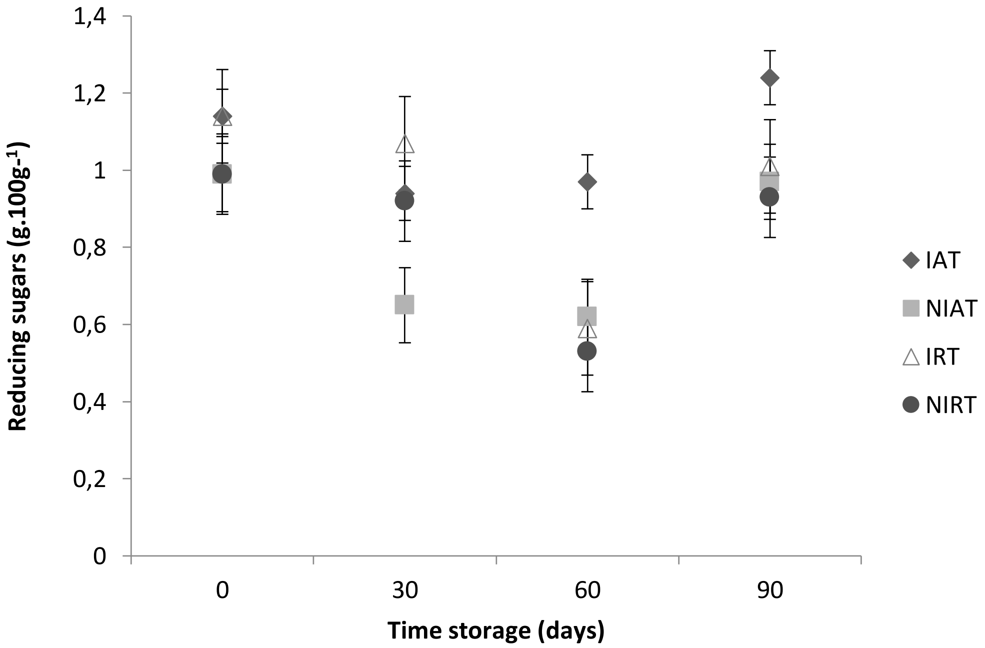

Figure 3. Reducing sugars (g.100g^{-1}) in irradiated 1 kGy and non-irradiated pinhões, stored at ambient temperature, and refrigerated at 4 °C for 90 days. The vertical bars indicate the 95% confidence interval. IAT: irradiated pinhões stored at ambient temperature; NIAT: non-irradiated pinhões stored at ambient temperature; IRT: irradiated pinhões stored at refrigeration temperature; NIRT: non-irradiated pinhões stored at refrigeration temperature.

There was a significant increase ($p \leq 0.05$) in the vitamin C content of non-irradiated pinhão stored at ambient temperature (NIAT) as well as under refrigeration (NIRT). Conversely, the values varied for irradiated pinhão stored at ambient temperature and under refrigeration (IAT and IRT). However, on comparing the first and last storage days of these seeds, there was no significant variation ($p \geq 0.05$) (Figure 4). There was no evident influence of irradiation and cooling observed on the vitamin C content of the pinhão. At the end of storage, vitamin C contents in non-irradiated, refrigerated pinhão (NIRT) (35.22 mg.100g^{-1}) were significantly higher ($p \leq 0.05$) compared to non-irradiated pinhão stored at ambient temperature refrigerated storage (NIAT) (24.95 mg.100g^{-1}) (data not shown).

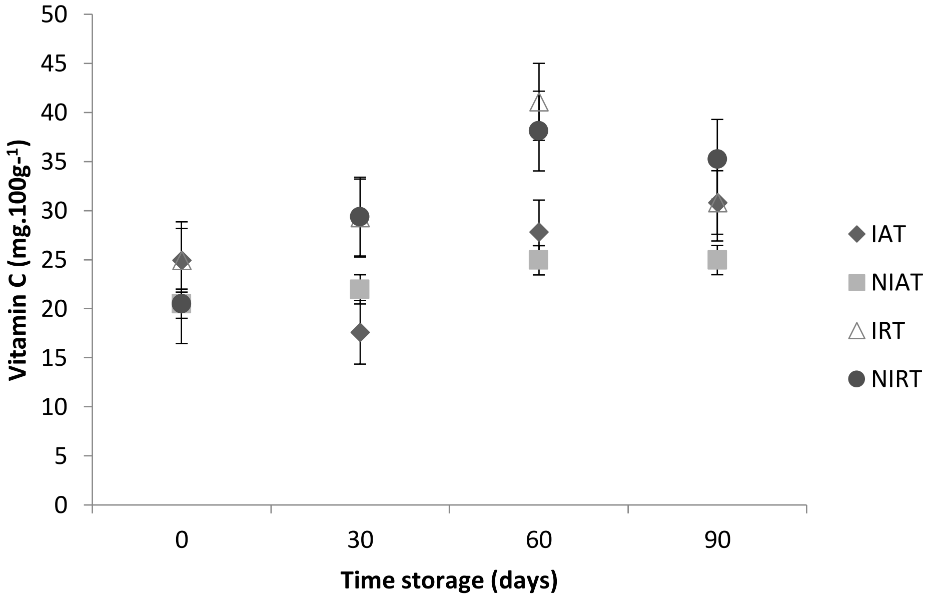

Figure 4. Vitamin C (mg.100g^{-1}) in irradiated 1 kGy and non-irradiated pinhões, stored at ambient temperature, and refrigerated at 4 °C for 90 days. The vertical bars indicate the 95% confidence interval. IAT: irradiated pinhões stored at ambient temperature; NIAT: non-irradiated pinhões stored at ambient temperature; IRT: irradiated pinhões stored at refrigeration temperature; NIRT: non-irradiated pinhões stored at refrigeration temperature.

Significant reduction ($p \leq 0.05$) in firmness of irradiated and non-irradiated pinhão stored under refrigeration (IRT and NIRT) was observed. In the pinhão stored at ambient temperature (IAT and NIAT), the reduction was followed by increased firmness, regardless of the application of irradiation ($p \geq 0.05$). At the end of storage, refrigerated pinhão (NIRT and IRT) showed significantly ($p \leq 0.05$) lower firmness compared to pinhão stored at ambient temperature (NIAT and IAT) (data not shown). There was no influence of the irradiation process on firmness.

A significant reduction ($p \leq 0.05$) in the L* coordinate values of pinhão subjected to different treatments was observed, which refers to the variation from white (100) to black (0), with a tendency to darken during storage (Figure 6). At the end of storage, no significant difference ($p \leq 0.05$) was observed between the treatments (data not shown).

The a* coordinate is associated with the color variation between red (+60) and green (–60), and the b* coordinate reflects the variation between yellow (+60) and blue (–60). When evaluating the a* and b* coordinates, all samples showed a significant increase ($p \leq 0.05$) in the values during storage (Figures 7 and 8), with an intensification of the red and yellow colors, respectively. There was no significant difference for a* (data not shown) and b * (data not shown) values between the treatments at the end of storage.

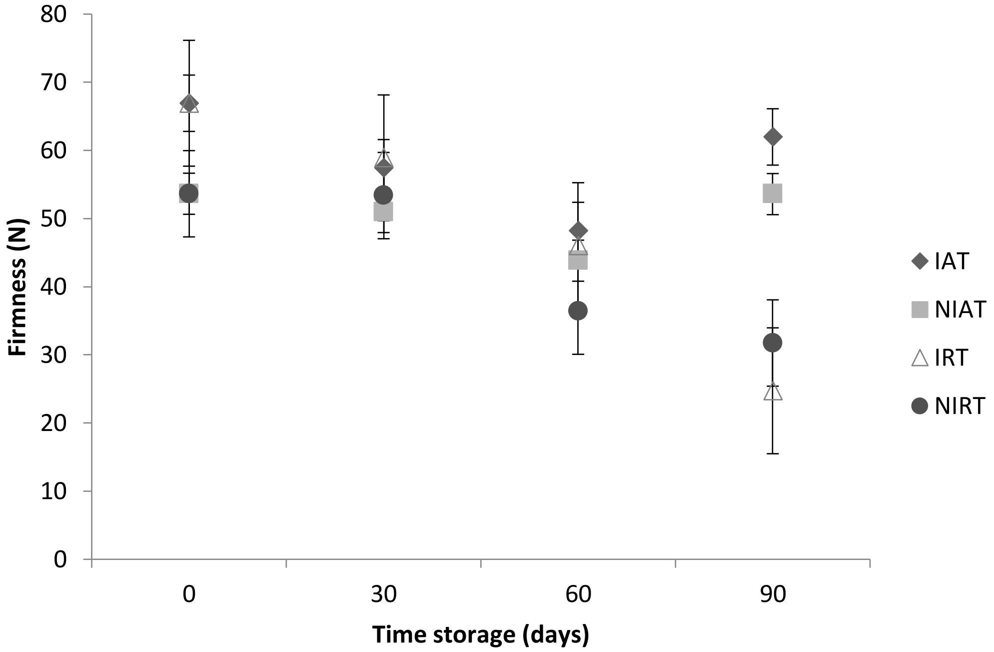

Figure 5. Firmness (N) in irradiated 1 kGy and non-irradiated pinhões, stored at ambient temperature, and refrigerated at 4 °C for 90 days. The vertical bars indicate the 95% confidence interval. IAT: irradiated pinhões stored at ambient temperature; NIAT: non-irradiated pinhões stored at ambient temperature; IRT: irradiated pinhões stored at refrigeration temperature; NIRT: non-irradiated pinhões stored at refrigeration temperature.

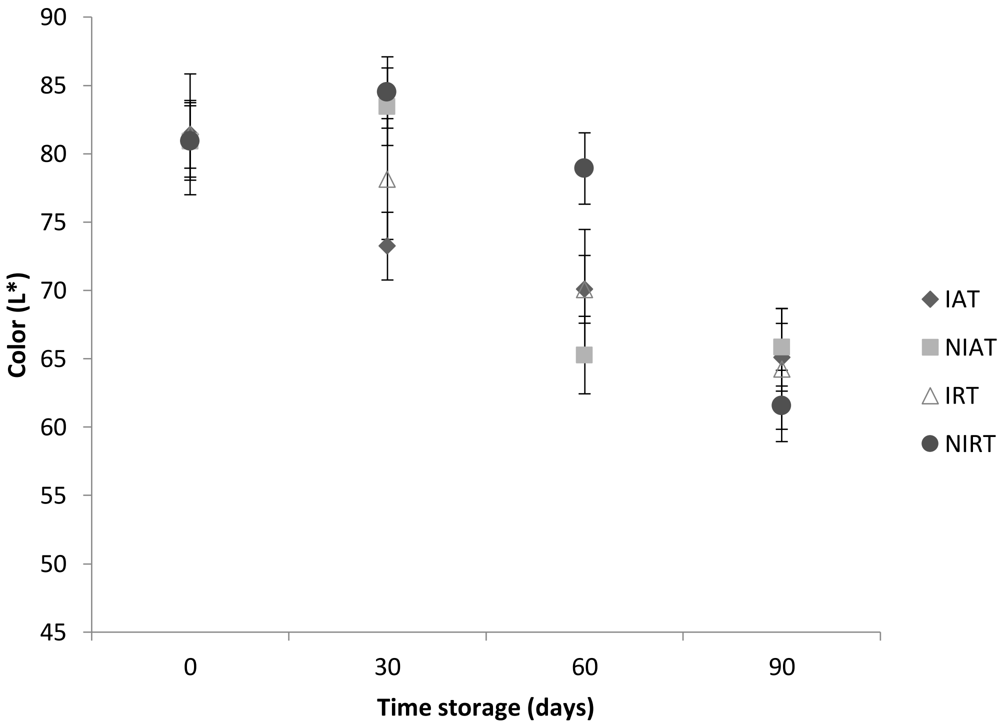

Figure 6. Cor (L*) in irradiated 1 kGy and non-irradiated pinhões, stored at ambient temperature, and refrigerated at 4 °C for 90 days. The vertical bars indicate the 95% confidence interval. IAT: irradiated pinhões stored at ambient temperature; NIAT: non-irradiated pinhões stored at ambient temperature; IRT: irradiated pinhões stored at refrigeration temperature; NIRT: non-irradiated pinhões stored at refrigeration temperature.

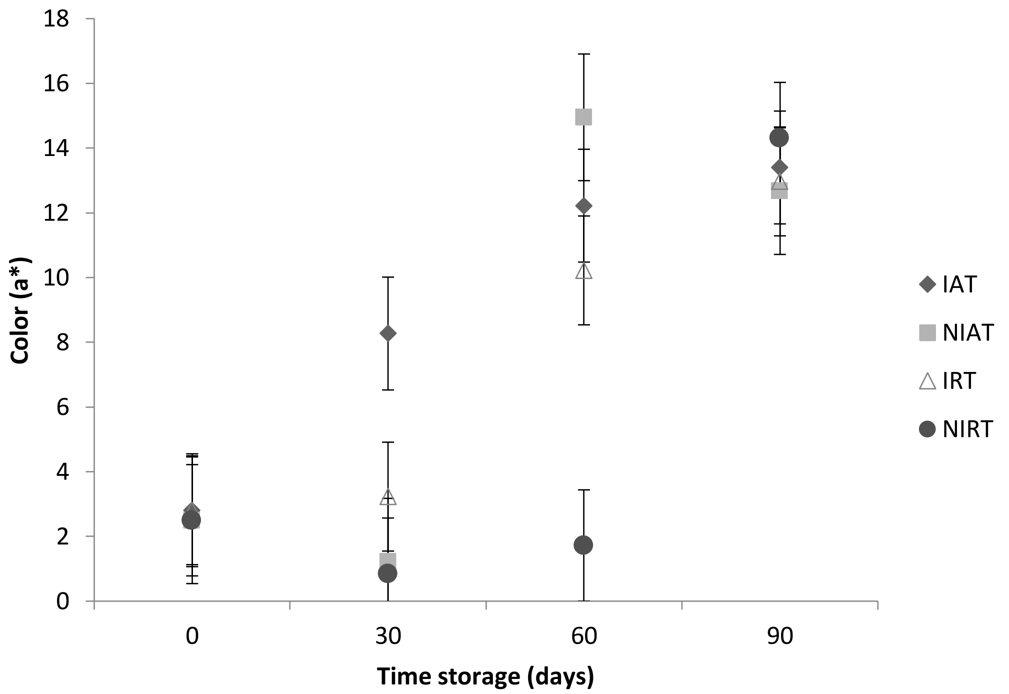

Figure 7. Cor (a*) in irradiated 1 kGy and non-irradiated pinhões, stored at ambient temperature, and refrigerated at 4 °C for 90 days. The vertical bars indicate the 95% confidence interval. IAT: irradiated pinhões stored at ambient temperature; NIAT: non-irradiated pinhões stored at ambient temperature; IRT: irradiated pinhões stored at refrigeration temperature; NIRT: non-irradiated pinhões stored at refrigeration temperature.

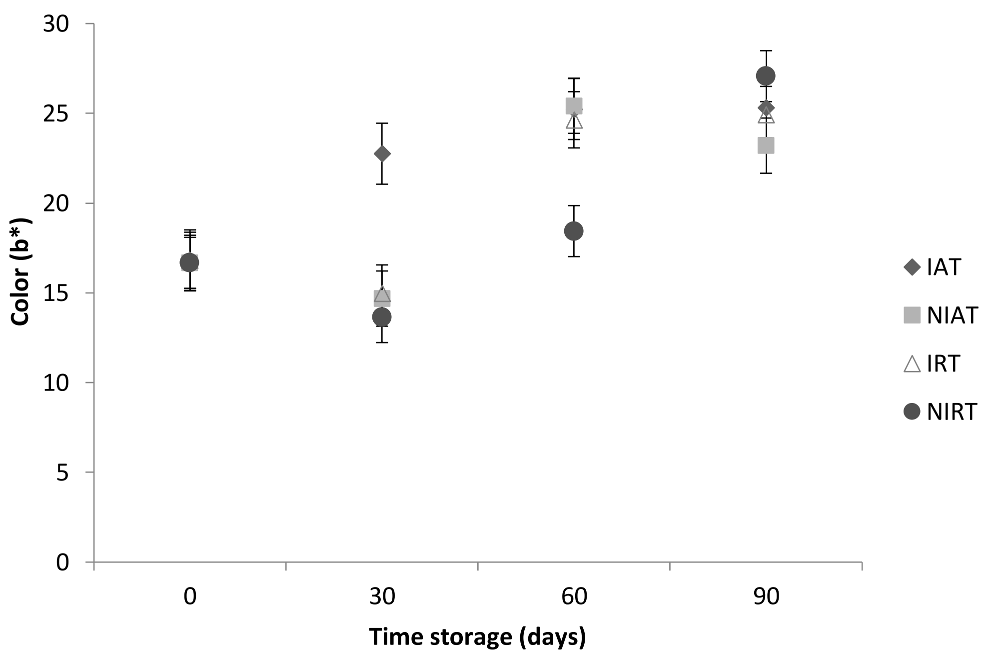

Figure 8. Cor (b*) in irradiated 1 kGy and non-irradiated pinhões, stored at ambient temperature, and refrigerated at 4 °C for 90 days. The vertical bars indicate the 95% confidence interval. IAT: irradiated pinhões stored at ambient temperature; NIAT: non-irradiated pinhões stored at ambient temperature; IRT: irradiated pinhões stored at refrigeration temperature; NIRT: non-irradiated pinhões stored at refrigeration temperature.

By analyzing the content of phenolic compounds in pinhão subjected to different treatments in relation to time, it was observed that there was a significant reduction in values ($p \leq 0.05$) from the thirtieth day of storage, with subsequent maintenance, regardless of the treatment (Figure 9). At the end of storage, a significant effect ($p \leq 0.05$) of irradiation was observed in the reduction of phenolic compounds (IAT and

IRT). The irradiated pinhão stored under refrigeration (IRT) presented significantly (p≤0.05) lowest value of phenolic compounds (292.91 mg.100g^{-1}), while the non-irradiated pinhão stored at ambient temperature (NIAT) had the highest values (306.06 mg.100g^{-1}) (data not shown).

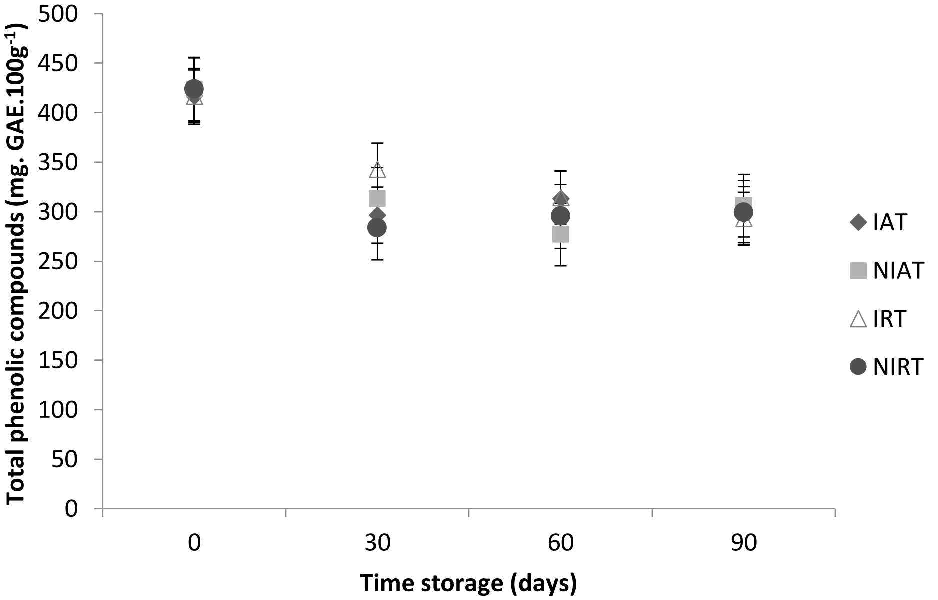

Figure 9. Total phenolic compounds (mg.100g^{-1}) in irradiated 1 kGy and non-irradiated pinhões, stored at ambient temperature, and refrigerated at 4 °C for 90 days. The vertical bars indicate the 95% confidence interval. IAT: irradiated pinhões stored at ambient temperature; NIAT: non-irradiated pinhões stored at ambient temperature; IRT: irradiated pinhões stored at refrigeration temperature; NIRT: non-irradiated pinhões stored at refrigeration temperature.

A significant increase in antioxidant activity (p≤0.05) was observed in all treatments during storage (Figure 10). At 90 days of storage, the non-irradiated pinhão (NIRT and NIAT) presented the highest inhibition percentage, those stored at the refrigerated temperature being significantly (p≤0.05) higher (72.94%). On the other hand, the cooling effect was also significant (p≤0.05), since the lowest values were obtained for irradiated pinhão stored at ambient temperature (IAT) (61.54%) (data not shown).

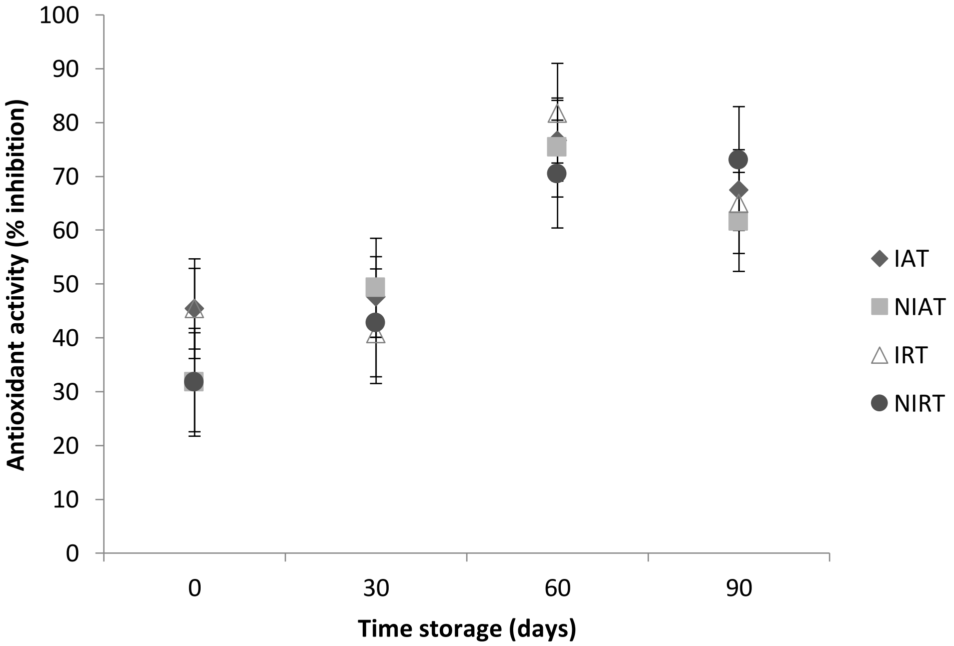

Figure 10. Antioxidant activity (% inhibition) in irradiated 1 kGy and non-irradiated pinhões, stored at ambient temperature, and refrigerated at 4 °C for 90 days. The vertical bars indicate the 95% confidence

interval. IAT: irradiated pinhões stored at ambient temperature; NIAT: non-irradiated pinhões stored at ambient temperature; IRT: irradiated pinhões stored at refrigeration temperature; NIRT: non-irradiated pinhões stored at refrigeration temperature.

A significant increase (p≤0.05) in the fungal count of pinhão stored at ambient temperature was observed and regardless of irradiation (NIAT and IAT). For the pinhão stored under refrigeration, a significant reduction (p≤0.05) in the fungal count was observed for the irradiated (IRT) pinhão. Conversely, a significant increase for the non-irradiated pinhão (NIRT) in 30 days (p≤ 0.05) was detected (Figure 11).

At the end of storage, the pinhão under refrigeration (NIRT and IRT) significantly (p≤0.05) presented the lowest fungal counts. There was no evident influence of irradiation on the fungal count (data not shown) when comparing the effect of the process with different temperatures.

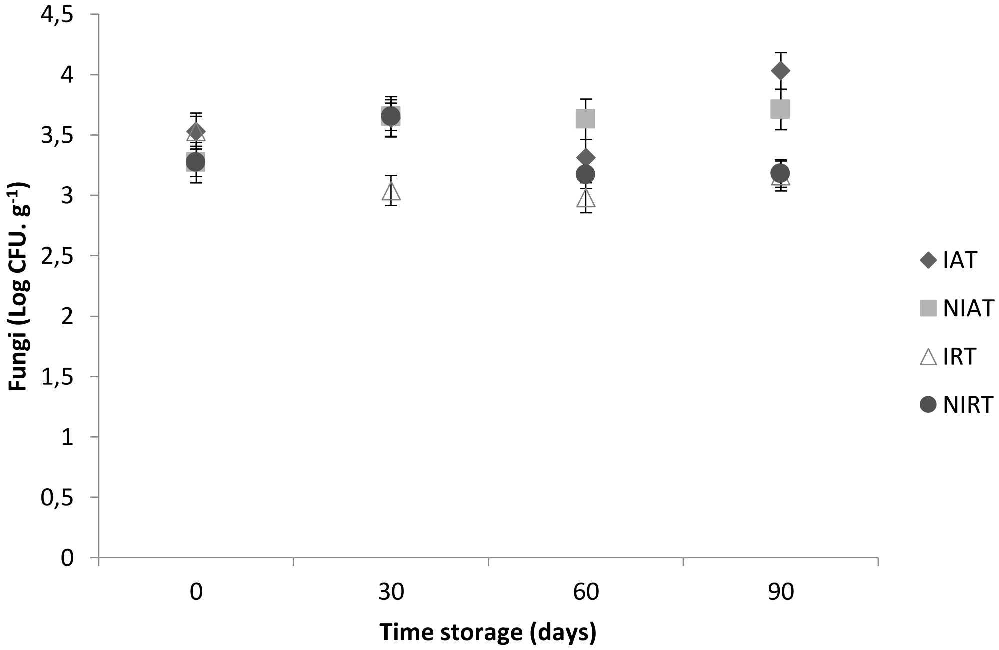

Figure 11. Fungal quantification (Log CFU.g^{-1}) in irradiated 1 kGy and non-irradiated pinhões, stored at ambient temperature, and refrigerated at 4 °C for 90 days. The vertical bars indicate the 95% confidence interval. IAT: irradiated pinhões stored at ambient temperature; NIAT: non-irradiated pinhões stored at ambient temperature; IRT: irradiated pinhões stored at refrigeration temperature; NIRT: non-irradiated pinhões stored at refrigeration temperature.

It was observed that there was a significant increase (p≤0.05) in the count of aerobic psychrotrophic microorganisms on pinhão stored at ambient temperature, independent of irradiation (NIAT and IAT). For pinhão stored under refrigeration, a significant increase (p≤0.05) was observed at 60 days, with subsequent reduction, regardless of the treatment (NIRT and IRT), as shown in Figure 12. At the end of storage, pinhão under refrigeration (NIRT and IRT) showed significantly (p≤0.05) lowest count of aerobic psychrotrophic microorganisms compared to pinhão stored at ambient temperature (NIAT and NIAT).

Among the pinhão stored at ambient temperature, the irradiated ones (IAT) presented higher growth (p≤0.05), but an inverse behavior was observed in the pinhão under refrigeration (data not shown). Despite the significant differences, there is no conclusion on the effect of irradiation, since the behavior varied between pinhão stored at ambient temperature and under refrigeration.

There was a significant increase in the count of aerobic mesophilic microorganisms in all treatments, as shown in Figure 13. At 90 days, the irradiated pinhão, regardless of the storage temperature (IAT and IRT), showed significantly (p≤0.05) lower growth of aerobic mesophilic microorganisms compared to non-irradiated samples (NIAT and NIRT) (data not shown).

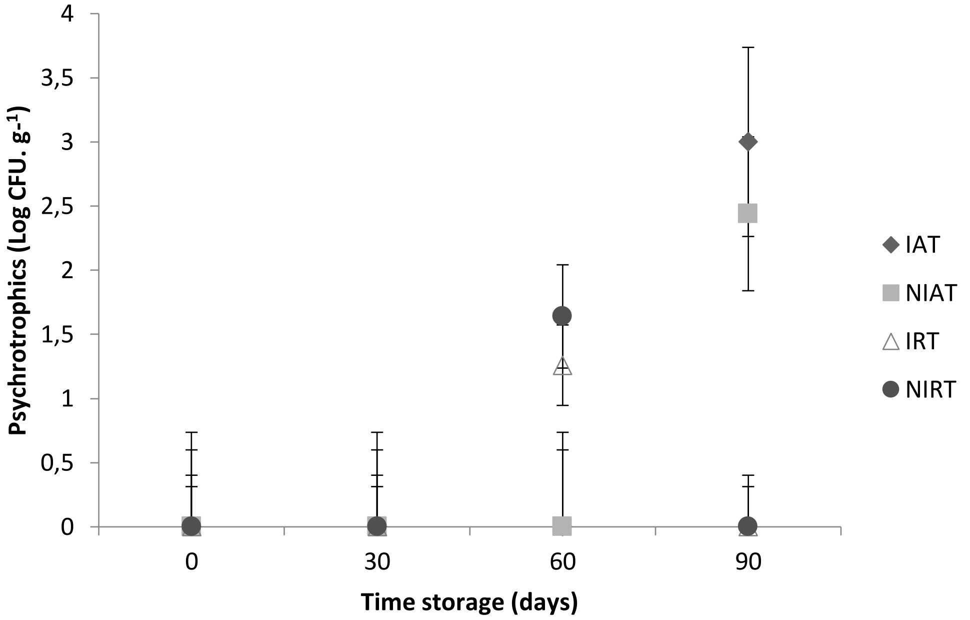

Figure 12. Quantification of aerobic psychrotrophic microorganisms (Log CFU.g^{-1}) in irradiated 1 kGy and non-irradiated pinhões, stored at ambient temperature, and refrigerated at 4 °C for 90 days. The vertical bars indicate the 95% confidence interval. IAT: irradiated pinhões stored at ambient temperature; NIAT: non-irradiated pinhões stored at ambient temperature; IRT: irradiated pinhões stored at refrigeration temperature; NIRT: non-irradiated pinhões stored at refrigeration temperature.

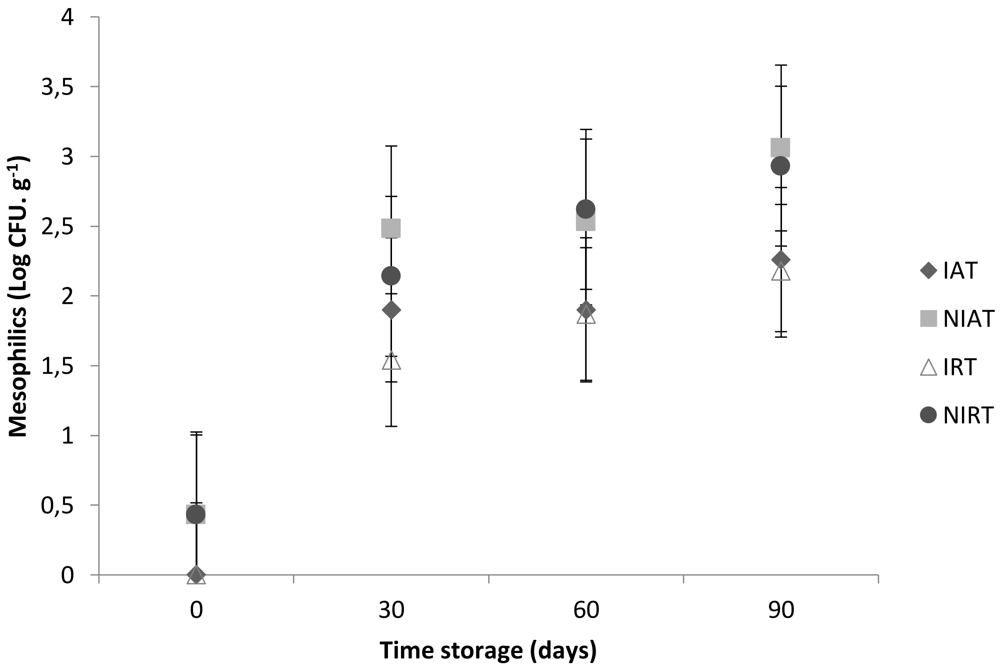

Figure 13. Quantification of aerobic mesophilic microorganisms (Log CFU.g^{-1}) in irradiated 1 kGy and non-irradiated pinhões, stored at ambient temperature, and refrigerated at 4 °C for 90 days. The vertical bars indicate the 95% confidence interval. IAT: irradiated pinhões stored at ambient temperature; NIAT: non-irradiated pinhões stored at ambient temperature; IRT: irradiated pinhões stored at refrigeration temperature; NIRT: non-irradiated pinhões stored at refrigeration temperature.

4. Discussion

Studies have already demonstrated the effect of low temperature in reducing the mass loss of pinhões as a result of decreased respiratory rate (Amarante et al. 2007; Costa 2014).

Irradiation can reduce mass loss, due to reduced respiratory rate, however the effect seems to be dependent on the raw material and the applied radiation dose (Wang and Meng 2016; Maraei and Elsawy 2017). In this study, the dose evaluated seems to have been insufficient to observe the reduction in mass loss, associated with this, it is emphasized that the pinhão has a thick shell that may have contributed for comportment observed.

Memon et al. (2020) observed an increase in mass loss in irradiated (0.5 to 1.5 kGy) spring onions, regardless of the dose applied. Maraei and Elsawy (2017) also observed an increase in mass loss with storage time in irradiated strawberries stored under refrigeration (10 ºC). However, there was a reduction in mass loss with increasing irradiation dose, up to 900 Gy.

The gamma radiation, as well as refrigeration did not slow the maturation of pinhão seeds.

The irradiation process inhibits the maturation of some vegetables by inducing biochemical changes in the physiological processes of the tissue (Da Silva and Da Roza 2010). However, in this study, the dose of 1 kGy was not sufficient to reduce the rate of biochemical processes like respiration and senescence. The thick shell of the pinhão may have contributed, since in other studies with vegetables, higher doses of radiation were necessary to obtain the desired effect (Guerreiro et al. 2016; Najafabadi et al. 2017).

A decrease in acidity levels is probably related to the oxidation of organic acids and their conversion to sugars (Kays 1991; Mattiuz et al. 2003). Ashtari et al. (2019) also observed a decrease in acidity in refrigerated storage (4 ºC) of irradiated pomegranate seeds, being the effect dependent on the applied radiation dose. On the other hand, Maraei and Elsawy (2017) did not observe pH alteration in refrigerated (10 ºC) strawberries, as well as no distinction between irradiated and non-irradiated fruits, were observed.

The reduction in sugars is possibly related to their use during respiration or in the production of vitamin C. On the other hand, the evolution of maturation may cause an increase in sugars due to starch degradation, as well as the conversion of acids into sugars (Chitarra and Chitarra 2005; Hussain et al. 2008). Different behaviors are reported in the literature. Wei et al. (2014) observed an increase in the apricots sugar content stored at ambient temperature, as a function of the irradiation dose applied. Conversely, Wang et al. (2019) observed a reduction in sugars in bamboo shoots subjected to different irradiation doses, stored for 45 days at 4 ºC.

In general, vitamin C results may be related to the sugar content, as they are converted to in this vitamin, mainly for refrigerated samples, regardless of irradiation; being the values obtained higher in these treatments than those reported by Taco (2011) (27.7 $mg.100g^{-1}$).

Maraei and Elsawy (2017) and Ashtari et al. (2019) observed a reduction in vitamin C content in irradiated strawberries and pomegranate seeds, respectively, with increasing applied radiation dose. According to Kilcast (1994), ascorbic acid is one of the most sensitive vitamin to radiation, causing its oxidation.

The firmness of the pinhão may be related to their loss of mass, since a more resistant surface tissue may have formed on the pinhão stored at ambient temperature, due to the greater loss of mass. Costa (2014) observed similar behavior in pinhão stored at ambient temperature where firmness increased, as they showed greater mass loss. In refrigerated samples, there was a reduction in firmness and lower loss of mass which may be related to the hydrolases enzyme activity on the cell wall (Koblitz 2008).

Latorre et al. (2010) observed that irradiation contributed to a greater cell-cell adhesion by increasing calcium cross-linking in the cell wall when beet was subjected to doses of 1 and 2 kGy. Possibly, the application of the 1 kGy dose of irradiation on the pinhão was low and insufficient to cause changes in the cell wall. Wang and Meng (2016) also observed when evaluating the irradiation of blueberries (0.5 to 3 kGy), that increasing the irradiation dose increased fruit firmness, possibly due to the irradiation-stimulated lignin synthesis.

Hussain et al. (2008) and Guerreiro et al. (2016) observed a tendency to reduce the firmness of irradiated strawberries and cherry tomatoes stored under refrigeration. On the other hand, Wang et al. (2019) observed an increase in firmness of bamboo shoots subjected to irradiation stored at 4 ºC, the increase being dose-dependent.

Study has reported that the process of irradiating vegetables can inhibit the activity of enzymes related to browning, such as polyphenoloxidase and peroxidase, being dependent on the applied dose (Wang et al. 2019). However, in the present study, no influence of irradiation on the color of the pinhões was observed, possibly as a function of the applied dose and the thickness of the seed shell. The storage, regardless of the treatment, caused intensification of the red and yellow color of the pinhão. Gama et al. (2010) observed lower values of the color parameters for different samples of pinhão collected at the beginning of the release period, obtaining an average for L*= 70.85, a* = 5.04 and b*= 16.96. Possibly, the color intensification could be related to the diffusion of flavonoids, such as catechin and quercetin, from the shell to the pulp, responsible for the red and yellow colors, respectively (Cordenunsi et al. 2004).

The effect of time on phenolic compound content may vary depending on the plant or cultivar species, geographical conditions, environmental conditions, sample status (dry or wet), phenolic composition, extraction procedure, and temperature (Khattak et al. 2008). The reduction observed in the content of phenolic compounds may be related to tannin complexation and polymerization processes (Menezes 1994; Antunes et al. 2006). Exposure of pinhão to radiation can also lead to oxidation of phenolic compounds, leading to a decrease in these compounds as observed in vegetable juices (Song et al. 2006).

According to Cordenunsi et al. (2004), pinhão presents low values of phenolic compounds; however, during the cooking process, these compounds migrate from the shell to the seed. The main phenolic compounds are catechin, epicatechin, quercetin, apigenin and gallic acid (Koehnlein et al. 2012). Different values of phenolic compounds in pinhão have been reported, ranging from 54 to 5140 mg.100g^{-1} (Cordenunsi et al. 2004; Sant'anna et al. 2016).

Oliveira et al. (2013) did not observe the influence of irradiation on phenolic compounds in camu-camu pulp stored at ambient temperature and under refrigeration, in contrast to this study. Hussain et al. (2016) observed an increase in phenolic compounds as the irradiation dose increased (0.25 – 1.5 kGy) in spinach stored at 4 ºC. This behavior could be attributed to the release of phenolic compounds from glycosidic components, degradation of larger phenolic compounds into smaller ones by gamma irradiation, with a consequent improvement in the extraction yield of the phenolic compounds because of the change in tissue structure by gamma irradiation. Thus, it seems that the dose of 1 kGy, applied to the pinhões in this study, was low as a function of the seed shell, in order to observe the increase in the content of phenolic compounds.

No influence of the phenolic compounds of the pinhão subjected to cooking was observed on the antioxidant activity. Although, there was a relationship between the increase in antioxidant activity and an increase in the a* and b* coordinates, which is possibly related to catechin and quercetin compounds, respectively. However, increased antioxidant activity may also be related to increased vitamin C content.

After cooking, pinhão showed higher antioxidant activity (87.93% inhibition), according to a study by Sant'anna et al. (2016). Maraei and Elsawy (2017) observed an increase in antioxidant activity of strawberries irradiated at different doses, stored for nine days at 10 °C. According to the authors, there was a decline in vitamin C content, but an increase in phenolic compounds and anthocyanins. However, Ashtari et al. (2019) evaluated irradiated pomegranate seeds and found a reduction in antioxidant activity, as well as phenolic compounds, vitamin C, and anthocyanins.

According to Albuzaudi et al. (2017), the reduction of fungi in irradiated foods depends on several factors, such as the characteristics of each plant, humidity, and applied dose.

Pinhão is highly perishable due to its high water activity (0.986), which favors the development of fungi. In addition, during storage at ambient temperature as well as refrigeration, respiration continues causing the moisture around the seed, allowing the growth of these microorganisms (Balbinot et al. 2008; Olivera 2008). The main fungi that affect these seeds are from the genus *Penicillium* sp. and *Cladosporium* sp. (Hennipman et al. 2017).

The Resolution of the College Board of Directors - RDC n° 12 (Brazil 2001) has no parameters for the microorganisms evaluated in this study. However, counts above 5.0 Log UFC.g^{-1} preclude consumption (Verzeletti et al. 2010), as they may compromise sensory and nutritional characteristics, as well as the produce mycotoxins, which may cause liver carcinogenic lesions and teratogenic effect (Vitti et al. 2004; Silva et al. 2007). Thus, the values obtained in this study could enable consumption regardless of the treatments, since the maximum count recorded was 4.03 Log UFC.g^{-1}.

According to the results obtained, cooling was effective in controlling the growth of the fungi. However, the application of 1 kGy was not enough to control the development of fungi, possibly due to the thick pinhão shell, and the fungal growth that occurs below the shell (Fonseca and Freire 2003).

Unlike the results obtained by Al-Bachir (2016) and Filho et al. (2018), who observed that irradiation was effective in inhibiting fungal development in sesame (3 kGy) and strawberries (1 kGy).

Psychrotrophic microorganisms are capable of developing between 0 °C and 7 °C; however, they grow at temperatures up to 43 °C (Franco and Landgraf 2008). This fact justifies the growth observed in pinhão stored at ambient temperature. The main effect observed was the reduction of microbial growth due to refrigeration.

Counts above 5.0 Log UFC.g^{-1} make the commodity inconsumable because of the reduced nutritional value, sensory changes, and infections (Bruno et al. 2005). The risk of contamination by psychrotrophic microorganisms may be associated with the presence of *Listeria monocytogenes*. In addition, many spoilage microorganisms are psychrotrophic, which contributes to reduced shelf life and indicates that sanitizing operations during processing were inefficient (Vitti et al. 2004).

Martins et al. (2007) observed a reduction in the population of psychrotrophic microorganisms in minimally processed watercress subjected to different irradiation doses (1 kGy to 4 kGy).

The RDC nº 12 (Brazil, 2001) has no parameters for aerobic mesophiles, making it difficult to establish limits. According to Prati (2004), counts above 6.0 Log UFC.g^{-1} cause sensory alterations in food, preventing their consumption. However, in the present study, the highest count was 3.06 Log UFC.g^{-1}.

Irradiation damages microbial DNA; the hydrogen bonds responsible for the DNA structure are broken, making it impossible to replicate and causing cell death (Egae et al. 2007; Swailam et al. 2007). Thus, in this study, the irradiation caused cellular damage to the population of aerobic mesophilic microorganisms in the pinhão.

Filho et al. (2018) and Ashtari et al. (2019) also observed a reduction in the mesophilic bacterial count in irradiated strawberries and pomegranate seeds, respectively.

5. Conclusions

The evaluated method made the extension of the life of the pinhão achievable and can be used as an alternative for seed conservation. The isolated use of gamma radiation (1 kGy) was not effective for the maintenance of the evaluated parameters. However, when used in conjunction with refrigerated storage, it reduced the growth of aerobic fungi, as well as mesophilic and psychrotrophic microorganisms. On the other hand, the isolated use of refrigeration showed a reduction in weight loss, reducing sugars and an increase in vitamin C content and antioxidant activity. Thus, to increase the benefits, we suggest evaluating higher doses of radiation (up to 5 kGy) as a function of the thick pinhão shell, as well as evaluating the irradiation in peeled pinhão.

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