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Influência do tratamento com radiação gama no perfil de compostos fenólicos e nos parâmetros de qualidade de morangos cv. Albion durante o armazenamento.

Influence of gamma radiation treatment on the profile of phenolic compounds and on the quality parameters of strawberries cv. Albion during storage

Influencia del tratamiento con radiación gamma en el perfil de compuestos fenólicos y en los parámetros de calidad de las fresas cv. Albion durante el almacenamiento

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Resumo

Este estudo avaliou os efeitos do tratamento com radiação gama no perfil de compostos fenólicos e nos parâmetros de qualidade de morangos irradiados durante o armazenamento. Os frutos foram submetidos a doses de irradiação 0, 0,8; 1,6 e 2,4 kGy e armazenados por até 9 dias a 10°C. Foram avaliados os níveis de antocianinas, ácido ascórbico e compostos fenólicos totais, assim como o perfil desses compostos e parâmetros colorimétricos e microbiológicos. Nossos resultados mostram que as doses utilizadas preservaram os compostos fenólicos estudados. A variação nos valores de ácido ascórbico pode ser devido ao armazenamento pós-colheita dos frutos. As doses aplicadas não interferiram na característica cor vermelha do morango. A dose de irradiação de 2,4 kGy foi a mais eficiência e a dose ideal para aumentar a vida útil dos morangos.

Palavras-chave: Compostos bioativos; Conservação de alimentos; Irradiação de alimentos; Segurança microbiana de alimentos.

Abstract

This study evaluated the effects of gamma radiation treatment on the profile of phenolic compounds and the quality parameters of strawberries irradiated during storage. The fruits were submitted to irradiation doses 0, 0.8; 1.6, and 2.4 kGy and stored for up to 9 days at 10°C. The levels of anthocyanins, ascorbic acid, and total phenolic compounds were evaluated, as along with the profile of these compounds and colorimetric and microbiological

parameters. Our results show that the doses used preserved the studied phenolic compounds. The variation in ascorbic acid values may be due to post-harvest storage of fruits. The applied doses did not interfere in the characteristic red color of the strawberry. The irradiation dose of 2.4 kGy more efficiently controlled microorganisms and is the ideal dose to increase the shelf life of strawberries.

Keywords: Bioactive composition; Food preservation; Food irradiation; Microbial food safety.

Resumen

Este estudio evaluó los efectos del tratamiento con radiación gamma sobre el perfil de compuestos fenólicos y los parámetros de calidad de las fresas irradiadas durante el almacenamiento. Los frutos fueron sometidos a dosis de irradiación 0, 0.8; 1.6 y 2.4 kGy y se almacenan hasta 9 días a 10°C. Se evaluaron los niveles de antocianinas, ácido ascórbico y compuestos fenólicos totales, junto con el perfil de estos compuestos y los parámetros colorimétricos y microbiológicos. Nuestros resultados muestran que las dosis utilizadas conservaron los compuestos fenólicos estudiados. La variación en los valores de ácido ascórbico puede deberse al almacenamiento de frutas después de la cosecha. Las dosis aplicadas no interfirieron en el color rojo característico de la fresa. La dosis de irradiación de 2.4 kGy controla los microorganismos de manera más eficiente y es la dosis ideal para aumentar la vida útil de las fresas.

Palabras clave: Composición bioactiva; Conservación de alimentos; Irradiación de alimentos; Seguridad alimentaria microbiana.

1. Introduction

The strawberry belonging to the Rosaceae family, of the genus Fragaria and is one of the most cultivated fruits in the world (Nizioł, Misiorek & Ruman, 2019). This fruit is essential in the diet due to its large content of essential nutrients and phytochemicals beneficial to human health (Giampieri et al., 2012).

The fruits are a source of ascorbic acid, minerals and contain a variety of bioactive polyphenolic compounds, such as anthocyanins and flavonoids, which have antioxidant and anti-inflammatory activities (Giampieri et al., 2013; Skrovankova et al., 2015). The physical,

sensory, and nutritional qualities of strawberries are associated with size, firmness, color, flavor, aroma, and contents of ascorbic acid and phenolic compounds (Mazur et al., 2014).

Gamma radiation is a method of food preservation, which aims to maintain the quality of fruits and vegetables (Hussain et al., 2012; Fante et al., 2015; Nassur et al., 2016). The combination of this technology of food preservation with refrigeration reduces microbiological contamination and increases the shelf life of food (Hussain et al., 2010; Fante et al., 2015).

Radiation technology can inactivate microorganisms with no change in temperature. This avoids deterioration of the taste, coloring, and nutritive value of foods caused by heat. The irradiation of food can be performed after packaging, without any additional intervention, to reduce cross contamination, until the product makes it to consumers (Guerreiro et al., 2016).

Lima Filho et al. (2014) conducted a sensory analysis with irradiated strawberries and found that the changes caused by low doses of radiation are perceived by consumers, but do not cause their rejection, requiring a dose of 3.6 kGy to provoke sensory rejection by the consumer. The effects of radiation treatment on antioxidant and phytochemical levels depend on the dose applied, the solvents used for extraction, the characteristics of each product, and the sensitivity of each phytochemical (Ito et al., 2016).

Studies investigating the chemical modifications induced by gamma radiation during the storage period in individual components, such as gallic acid, catechin, quercetin, chlorogenic acid and ellagic acid, in sparse strawberries. Thus, it is necessary to study the effects that gamma radiation can have on each phenolic compound and also on other physical-chemical characteristics and microbiologies of strawberries cv. Albion on storage.

The objective of the present study is to evaluate the effects of gamma radiation treatment on the profile of phenolic compounds and the quality parameters of strawberries (Albian cv.) irradiated during storage at the refrigeration temperature used in commercial establishments, considering both the dose of irradiation and storage time.

2. Methodology

2.1. Fruit material, irradiation treatment, and statistical analysis

The fruits were purchased from the State Supply Center (CEASA) in Contagem, Minas Gerais, Brazil, where they were selected for absence of defects and injuries, and then

packaged in plastic containers. Subsequently, the strawberries were transported to the Nuclear Technology Development Center/National Nuclear Energy Commission (CDTN/CNEN), in Belo Horizonte, Minas Gerais, Brazil, for the application of irradiation through the commercial colbato-60 source. The total amount (15 kilograms) was randomly divided into four parts of 3.75 kilograms each. Each part received one of the four irradiation treatments: control (samples that did not receive irradiation) and irradiation doses of 0.8, 1.6, and 2.4 kGy, maintained for 32, 65, and 98 min, respectively. The packages were positioned within 32 cm of the fountain protector. The choice of treatment was also random.

After irradiation, the fruits were sent to the Operations, Processes, and Technology Sector of the Faculdade de Farmacia of the Universidade Federal de Minas Gerais (Belo Horizonte, Minas Gerais, Brazil) where they were stored at 10±1°C and RH 90 ± 5% for up to 9 days, simulating the commercialization temperature of grocery stores and supermarkets. In each storage period (0, 3, 6, and 9 days), three portions of 250 grams were randomly selected from each treatment. These portions were evaluated for anthocyanins, ascorbic acid, and total phenolic compounds, as well as the profile of these compounds (gallic acid, catechin, chlorogenic acid, ellagic acid, and quercetin), colorimetric and microbiological parameters in four storage periods.

According to the literature (Françoso et al., 2008), soluble solids contents, titratable total acidity, and pH are not altered with irradiation. Thus, these analyzes were performed only on the day that the strawberries were irradiated (day zero) to characterize them.

The soluble solids, expressed as °Brix, were determined using a digital refractometer (Instrutherm, model RTD-45, São Paulo, Brazil) according to the methodology recommended by the Instituto Adolfo Lutz (IAL, 2008). Titratable total acidity was determined by titrating using NaOH 0.1 N, with phenolphthalein as indicator, and the results were expressed as mg of citric acid.100 g^{-1} of fruit, according to the standards of the Association of Official Analytical Chemists (AOAC, 1998). The pH was determined using a benchtop pH meter (Bante Instruments, model 922, USA).

This is a qualitative and quantitative laboratory research. For statistical analysis, a completely randomized design was used in the factorial 4 × 4, with four levels of irradiation factor (0, 0.8, 1.6 and 2.4 kGy) and four levels of storage time factor (0, 3, 6 and 9 days) at a temperature of 10 ± 1°C and RH 90 ± 5%, with three repetitions for each irradiation combination and for each time. Each of the three 250 grams portions provided a replica.

The results were submitted to analysis of variance and comparison of means by the Tukey test at a significance level of 5%. The Box-Cox transformation was used for all

measures that were not normal. The nonparametric versions for analysis of variance and comparison of means (Kruskal Wallis test at the significance level of 7%) were used for the measurement of gallic acid, since the Box-Cox transformation was not sufficient to correct the lack of normality in this case. Statistical analysis was performed using the RStudio Team statistical program (2015).

2.2. Anthocyanin Content

The anthocyanins were determined following the differential pH method described by Giusti and Wrolstad (2001). The strawberries were weighed (50 g) and ground, then 100 ml of acidified ethanol was added with hydrochloric acid 0.01% (v/v). Subsequently, the solution was stirred manually and filtered.

Two samples with extracts were prepared for each treatment, in which one was mixed with buffer hydrochloric acid/potassium chloride pH 1.0 and another with buffer sodium acetate/acetic acid pH 4.5.

After 15 min, these samples were measured at a maximum wavelength of 496 (A_{max}) and at 700 nm (A_{700}) using a spectrophotometer (Micronal, model AJX-1900, São Paulo, Brazil). The result was expressed in mg pelargonidin-3-glycoside.100 g^{-1} of fruit.

2.3. Total Phenolic Content

The phenolic compounds were determined according to a colorimetric method developed by Singleton and Rossi (1965), using Folin Ciocalteu reagent, in solution 10% (v/v). To obtain the extracts, 5 grams of strawberries were weighed, 10 ml of methanol 80% was added, homogenized using a magnetic stirrer (model 752, Fisatom, São Paulo, SP, Brazil) and filtered. The supernatant was then transferred to a 100 ml volumetric flask. To the first extraction residue, 10 ml of methanol 80% (v/v) were added, homogenized using a magnetic stirrer (Model 752, Fisatom, Sao Paulo, SP, Brazil), and filtered.

Thereafter, the supernatant was filtered and transferred to the volumetric flask, which was the first supernatant to make up the final volume with methanol 80% (v/v). An aliquot 0.5 ml of extract was transferred to a 10 ml volumetric flask and 2.5 ml of Folin Ciocalteau reagent 10% and 5 ml of distilled water added. After stirring for 3 min, 1 ml of sodium carbonate 7.5% was added. The volumetric flasks were allowed to stand for 1 h under light, then read at 765 nm in a spectrophotometer (Micronal model AJX-1900, São Paulo, SP,

Brazil). To calculate total phenolic contents, a standard curve was constructed with gallic acid solution. The result was expressed as mg of gallic acid.100 g-¹ of fresh fruit.

2.4. Analysis of phenolic compounds by UPLC

The phenolic compounds in strawberries were determined using the chromatographic method described by Chisté et al., (2012) and Eça et al., (2015) with some modifications. The extract was obtained using 5 g of crushed strawberries and extracted with methanol: water (8:2). After centrifugation for 15 min at 19754 x g (Sigma, model 2K15, Germany), the supernatant was filtered with a 0.22 µm nylon syringe filter and injected into an ultra efficient liquid chromatograph (Waters, Acquity UPLC® Class, Milford, MA, USA) equipped with a diode array UV detector, quaternary pump, on-line decanter and autosampler. Data were processed using Empower® software. Chromatographic conditions consist of Acquity UPLC® BEH C18 (2.1 × 50 mm id; 1.7 µm) column, under constant flow of 0.1 ml/min^{-1}, column temperature at 29 °C, with two mobile phases (A = water: formic acid, 99.5: 0.5 and B = acetonitrile: formic acid, 99.5: 0.5), using linear gradient. The spectra were obtained at 271, 320, and 367 nm. The phenolic compounds were calculated by calibration with external standards. The results were expressed in mg.100 g^{-1} of fresh fruit.

2.5. Analysis of ascorbic acid by UPLC

Ascorbic acid was analyzed according to De Velde et al., (2013) with changes by ultra-high-performance liquid chromatography. The extract was obtained using 0.3 g of crushed strawberries and extracted with 1 ml of 8% acetic acid and 1 mM EDTA (Ethylenediaminetetraacetic Acid) solution. After centrifugation for 15 min at 19754 x g (Sigma, model 2K15, Germany), the supernatant was filtered with a nylon syringe filter 0.22 µm and was injected into a liquid chromatograph (Waters, Acquity UPLC® Class, Milford, MA, USA) equipped with a diode array UV detector, quaternary pump, on-line degasser, and autosampler. Data were processed using Empower® software. Chromatographic conditions were a column of Acquity UPLC® BEH C18 (2.1 × 50 mm i.d.; 1.7 µm) under a constant flow of 0.1 ml/min^{-1}. The mobile phase consisted of a solution of methanol and 50 mM potassium phosphate (30:70 v/v), in isocratic mode. Chromatograms were run at 254 nm. Ascorbic acid was quantified by comparison with the standard. The result was expressed as mg ascorbic acid.100 g-¹ of fresh fruit.

2.6. Color

The colorimetric properties were determined using colorimeter (Konica Minolta model CM-2600D, Osaka, Japan) with determination in the CIE L*a*b* system. The coordinate a* represents the axis of green (negative values) to red (positive values) and coordinate b* represents the axis of yellow (positive values) to blue (negative values). The chroma (c*) evaluates the intensity or saturation of the color, and the angle Hue shows the location of the color in a diagram, where the angle 0° represents pure red, 90° pure yellow, 180° pure green, and 270° blue (Wang and Meng, 2016).

2.7. Microbiological Analysis

Microbiological analyzes were performed according to the official methodology (BRASIL, 2003). For each strawberry sample with different treatments, 25g were weighed and diluted in 225 ml of 0.1% sterile peptone water. Each dilution was plated in triplicate. The coliforms were quantified using the most probable number technique (NMP) in the Lauryl Sulfate Triptose broth (LST) in the presumptive test, with incubation at 35°C for 24-48 hours, Brilliant Green Broth (BV) to confirm the presence of total coliforms, and E. coli broth to confirm the presence of thermotolerance at 45°C. The results were expressed in the most probable number per gram (MPN g^{-1}).

The fungi and yeasts were quantified by the surface plating method, in which 25 g of the strawberries from each treatment were weighed and homogenized with 225 ml of 0.1% sterile peptone water. Decimal dilutions were performed (10^{-2} and 10^{-4}). Then, 1 ml of each dilution were added on the plates together with the Agar Dextrose Potato medium and incubated at 37°C for 5 days. After the incubation period, the counts were performed, and the results were expressed in colony forming units per gram (UFC g^{-1}).

For the *Salmonella* spp detection, 25 g of the strawberries of each treatment were weighed. These strawberries were homogenized in 225 ml of 1% buffered peptone water and incubated at 37°C for 24 hours. Aliquots of 1 ml of this pre-enriched culture were transferred to two tubes, each containing 10 ml of selective enrichment broth composed of the Tetrathionate Broth and the Selenite Cystine Broth and incubated at 37°C for 24 hours. Each was seeded in Enteric Agar of Hecktoen (HE), *Salmonella-Shigella* Agar (SS), and Xylose Lysine Deoxycholate Agar (XLD), incubated at 37°C for 24 hours. The typical Salmonella

colonies observed were seeded in Triple Iron Agar (TSI) and Iron Lysine Agar (LIA), incubated at 37°C for 24 hours to verify whether there was a typical reaction of *Salmonella*.

3. Results and Discussion

3.1. Soluble solids, total titratable acidity, and pH

The fruits used in this study presented on the first day, after irradiation, average values of 7.0 ° Brix for soluble solids, 1.40 mg of citric acid.100 g-¹ of fruit of titratable acidity and pH of 3.2. These results were similar to those determined by Françoso, Couto, Canniatti-Brazaca, & Arthur (2008), who found an average value of 8.5°Brix, 1.48 mg of citric acid. 100 g-¹ of fruit and pH of 3.4 in irradiated strawberries and Nassur et al. (2016) found pH of 3.5. Andrade Júnior et al. (2016) found 8.2 ° Brix for non-irradiated strawberries cv. Toyonoka and Alves et al. (2019) found a pH of 3.5 for non-irradiated strawberries.

3.1. Anthocyanin Content

No significant difference in anthocyanin values was observed in strawberries irradiated with different doses of gamma radiation during the storage period studied. Values ranged from 4.08 and 6.28 mg of pelargonidin-3-glycoside.100 g-¹ of fruit (Table 1).

Table 1. Anthocyanin contents in strawberries treated with different doses of gamma irradiation (0.8, 1.6, 2.4 kGy) and control sample (0 kGy) stored for up to 9 days under refrigeration (10±1ºC, RH 90± 5 %).

Doses (kGy)	Anthocyanin (mg.100 g-¹ de fruto)			
	Storage time (days)			
	0	3	6	9
0	6.28 (1.17) A	4.06(0.98) A	4.75(0.24) A	*
0,8	4.08 (0.59) A	5.89(0.38) A	5.75 (1.45) A	4.92 (0.94) A
1,6	5.45 (1.29) A	4.80 (0.43) A	4.14(0.97) A	5.17(1.20) A
2,4	5.42(1.29) A	5.25(1.54) A	4.92(0.90) A	5.15(0.70) A

Means followed by the same letter do not differ from each other, by the Tukey test at 5% significance. The data represent the mean value (SD). * Lost portion of control treatment (0kGy) on day 9. Source: Authors.

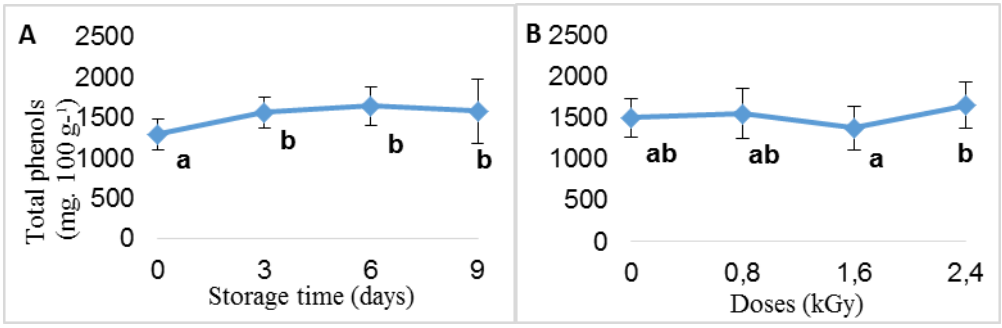
These data show that the gamma irradiation applied did not interfere with the anthocyanin content of the strawberries, since the treatment without irradiation was similar to the others.

That result corroborates with Hussain, Dar, and Wani (2012), who analyzed strawberries coated with carboxymethyl cellulose and irradiated at the dose of 2.0 kGy, kept under refrigeration for 21 days, and did not observe difference between the values of anthocyanins in irradiated and non-irradiated fruits. Tezotto-Uliana, Berno, Saji, & Kluge (2013) examined different doses of gamma radiation (0.5, 1.0, 2.0 kGy) and control sample (0 kGy), for raspberries stored for 20 days at 0±1°C and 90% UR and found that gamma irradiation doses 1.0 and 2.0 kGy had no effect on anthocyanin content. These authors observed an increase until day 12 in the content of anthocyanins and after that day they remained constant.

3.2. Total Phenolic Content

For total phenolic compounds, there was no significant interaction between irradiation doses and storage time. On days evaluated during storage, there was a significant difference, and on day zero, strawberries had the lowest content of phenolic compounds (Figure 1A). Irradiated strawberries at the dose 1.6 kGy differed statistically from strawberries irradiated with the dose 2.4 kGy (Figure 1B).

Figure 1. Total phenols of cv *Albion* strawberries stored for up to 9 days under refrigeration (10±1°C, RH 90±5%) (A) and submitted to different doses of gamma irradiation (0.8, 1.6, 2.4 kGy) and control sample (0 kGy). (B).



Averages followed by the same letter do not differ from each other, by the Tukey test at 5% significance. The vertical bars represent a standard deviation above and below the mean value. Source: Authors.

Mridha et al. (2017) evaluated the effects of different doses of gamma irradiation (0.5, 1.0, 1.5 kGy) and control sample (0 kGy), on strawberries during 6 days of storage and found

that the content of total phenolic compounds increased in all irradiated samples. Guimarães et al. (2013) studied raspberries subjected to gamma irradiation (0.5, 1.0, 2.0 kGy) and control sample (0 kGy), stored at 1°C for 12 days and noticed that the fruits treated with the dose 2 kGy had the highest levels of total phenolic compounds at the end of storage time, which is different from the present study.

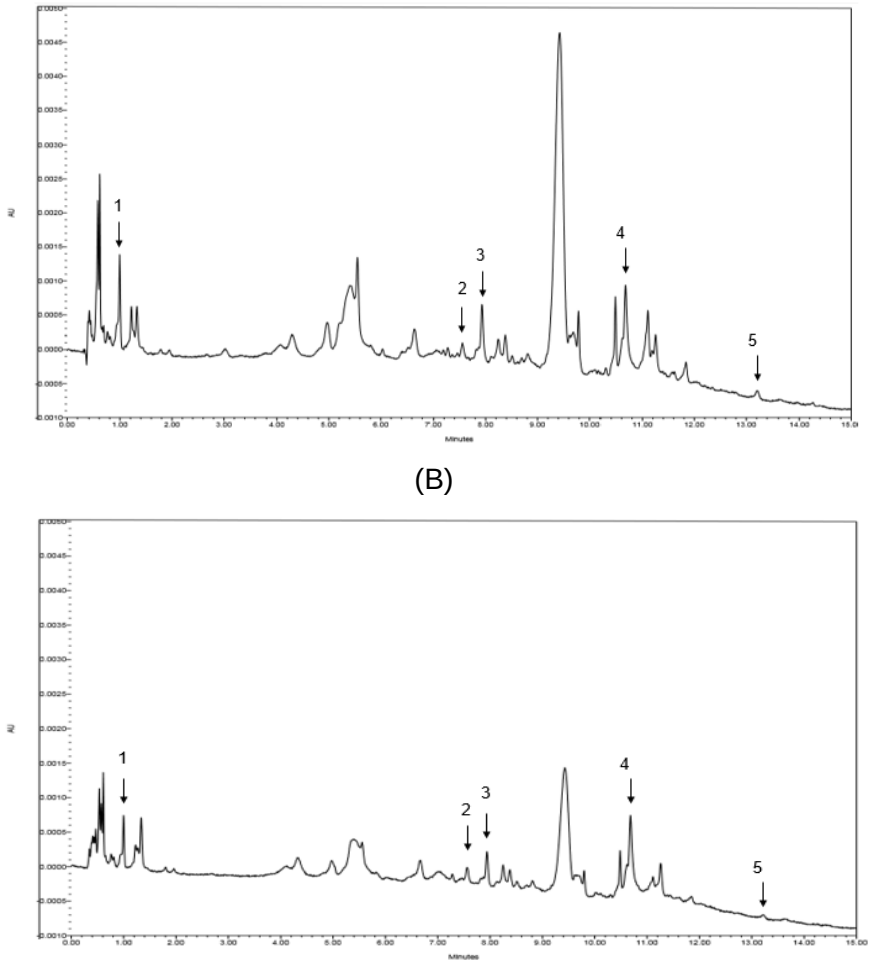
The increase of total phenolic compounds during gamma irradiation may be due to the release of phenolic compounds from glycosidic components and the degradation of larger phenolic compounds into smaller ones (Harrison & Were, 2007). In addition, fruit phytochemical values can be affected by the degree of maturation and environmental conditions during harvesting, by genetic differences among cultivars, by fruit handling and treatment, and by post-harvest storage conditions (Severo et al., 2011).

Our study observed that the phenolic compounds increased after the first day of storage and that the doses used did not influence the loss of the phenolic compounds, maintaining the nutritional quality of the fruits.

3.3 **. Analysis of phenolic compounds by UPLC**

The chromatographic profile of phenolic compounds using ultra-high performance liquid chromatography is shown in Figure 2. In this chromatogram, the peaks of phenolic compounds identified according to the order of elution in the chromatographic column were gallic acid, catechin, chlorogenic acid, ellagic acid and quercetin, respectively.

Figure2. Chromatographic profile of phenolic compounds in non-irradiated (A) and irradiated (B) strawberries analyzed by ultra-high performance liquid chromatography at wavelength 271 nm.



1. Acidoglycan, 2. Catechin, 3. Chlorogenic acid, 4. Ellagic acid, and 5. Quercetin (A).

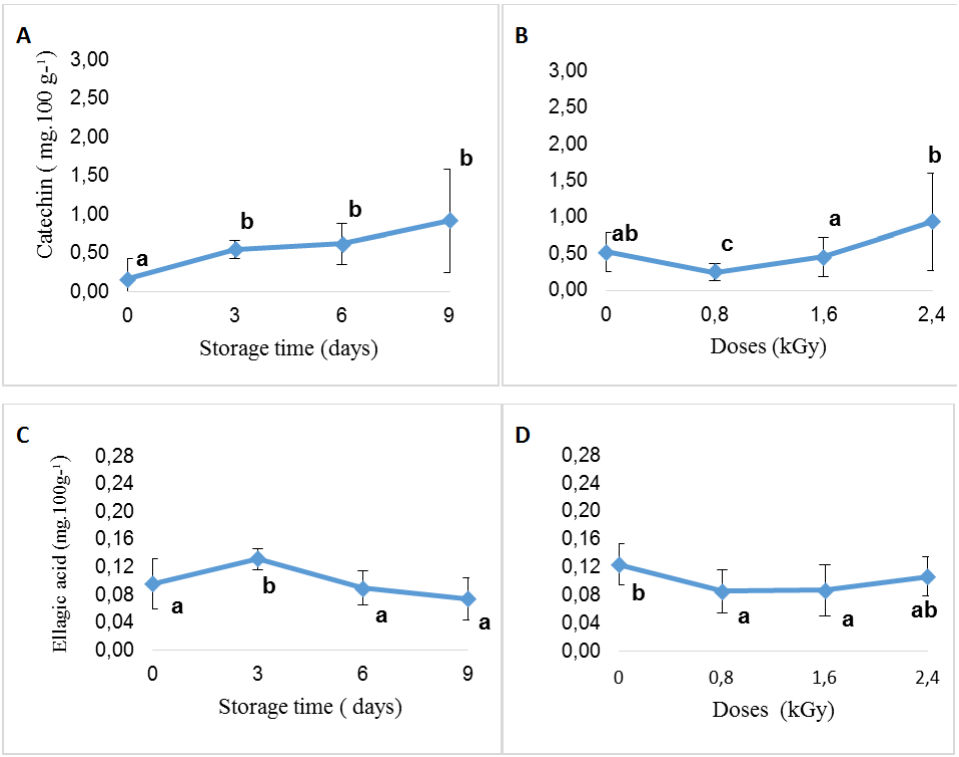
Source: Authors.

For the catechin, the interaction of the factors irradiation doses and storage time were not significant. Figure 3A illustrates that the first time evaluated (day 0) was statistically different from the others, with lower catechin concentration. In Figure 3B, strawberries irradiated at doses 0.8 and 1.6 kGy showed different behavior than the fruits irradiated at the dose 2.4 kGy, which did not differ statistically from the control (0 kGy). The strawberries irradiated at the dose 0.8 kGy had the lowest catechin content.

In this study, for the variable ellagic acid, the interaction of the factors irradiation doses and days of storage was not significant. Figure 3C shows that the days of storage made a significant difference, and the third day presented the highest values (0.1320 mg.100 g^{-1}). For the irradiation doses, the control (0 kGy) statistically differed from the doses of 0.8 kGy

and 1.6 kGy (Figure 3D). Furthermore, the control and dose of 2.4 kGy had the highest concentrations of ellagic acid.

Figure 3. Mean values of catechin (A and B) and ellagic acid (C and D) of strawberries cv Albion stored for up to 9 days under refrigeration (10±1°C, RH 90±5%) (A and C) and submitted to different doses of gamma irradiation (0.8, 1.6, and 2.4 kGy) and control sample (0kGy) (B and D).



Averages followed by the same letter do not differ from each other by the Tukey test at 5% significance. The vertical bars represent a standard deviation above and below the mean value. Source: Authors.

For gallic acid, the interaction of the factors irradiation doses and storage time were significant (Table 2). Although each dose did not present significant difference over time, the non-irradiated fruits presented gallic acid contents higher on day three than the fruits treated with the dose 0.8 kGy did on day nine.

For chlorogenic acid, the interaction of the factors irradiation doses and storage time were significant. Table 1 shows that control strawberries on day zero and irradiated fruits at the dose of 2.4 kGy on day three presented statistical similarity for this variable, while the fruits irradiated at the dose of 0.8 kGy on the last three days of analysis, strawberries irradiated at the 1.6 kGy dose in the last two days, and the fruits irradiated at the dose 2.4 kGy on the last day were different from the previously mentioned treatments. The last day of

analysis strawberries irradiated at the dose of 2.4 kGy presented a statistically significant difference when compared to irradiated fruits at the dose of 0.8 kGy.

There was a significant interaction between the irradiation doses and the storage time for quercetin as observed in Table 2.

Table 2. Gallic acid, chlorogenic acid, and quercetin contents in strawberries treated with different doses of gamma irradiation (0.8, 1.6, 2.4 kGy) and control sample (0 kGy) stored for up to 9 days under refrigeration (10±1ºC, RH 90±5 %).

Phenolic compound (mg.100g-¹)	Doses (kGy)	Storage time (days)			
		0	3	6	9
Gallic acid	0	0.103(0.02) AB	0.119(0.01) B	0.054(0.03) AB	*
	0.8	0.089(0.03) AB	0.102(0.01) AB	0.042(0.01) AB	0.024(0.01)A
	1.6	0.095(0.02) AB	0.086(0.01) AB	0.040(0.02) AB	0.036(0.02)AB
	2.4	0.086(0.04) AB	0.097(0.01) AB	0.029(0.02) AB	0.041(0.01)AB
Chlorogenic acid	0	1.019(0.37) D	0.884(0.18) CD	0.384(0.05)ABCD	*
	0.8	0.442(0.13)ABCD	0.263(0.07) AB	0.154(0.09) A	0.168(0.08)A
	1.6	0.433(0.25)ABCD	0.391(0.09)ABCD	0.343(0.16)ABC	0.241(0.03)AB
	2.4	0.391(0.15)ABCD	0.936(0.07) D	0.569(0.31)ABCD	0.751(0.11)BC
Quercetin	0	0.061(0.022) AB	0.049(0.005)AB	0.059(0.016)AB	*
	0.8	0.048(0.003) AB	0.069(0.017)AB	0.072(0.010) AB	0.058(0.010)AB
	1.6	0.043(0.012) A	0.064(0.010)AB	0.037(0.023) A	0.069(0.004)AB
	2.4	0.045(0.007) A	0.066(0.013)AB	0.054(0.007) AB	0.084(0.010) B

Means followed by the same letter do not differ from each other, by the Tukey test at 5% significance for chlorogenic acid and quercetin and by the Kruskal-Wallis test at 7% significance for gallic acid. The data represent the mean value (SD). * Lost portion of control treatment (0kGy) on day 9. Source: Authors.

Strawberries irradiated at 2.4 kGy increased quercetin content during the days studied, presenting the highest content of the compound evaluated on the last day of analysis, which is statistically different from the irradiated fruits at the dose of 1.6 kGy on days zero and six and from the irradiated fruits at the dose of 2.4 kGy on day zero. Different results for quercetin were found in a study with irradiated raspberries at doses of 0.5 to 2.0 kGy and stored at 0°C for 20 days, treatments with the dosages of 1.0 and 2.0 kGy showed the lowest values of

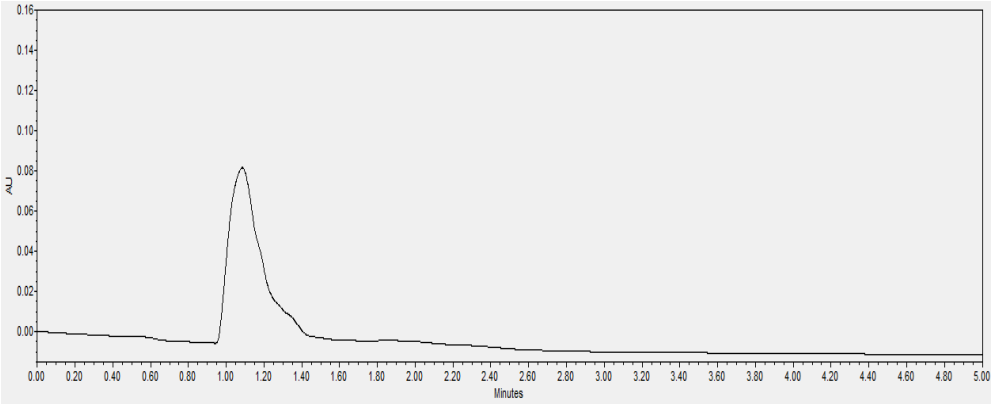
quercetin. As for the analyzed period, an increase in quercetin content was observed up to day 12, and after that, a reduction was observed (Tezotto-Uliana et al., 2013).

The radiation dose may influence the content of phenolic compounds, as well as other factors such as solvents used in the extraction, treatments and technological processes, levels of water activity during storage, and especially the characteristics of each phenolic compound and of each food (Ito et al. al., 2016). Furthermore, the composition of phenolic compounds in fruits can be modified by post-harvest factors such as storage and processing, which promote enzymatic and chemical oxidation of phenolic compounds, contributing to their reduction (Kaur and Kapoor, 2001; Tremocoldi et al., 2014). The gamma radiation may have preserved the phenolic compounds during storage.

3.4. Analysis of ascorbic acid by UPLC

The strawberry (*Fragaria x ananassa* Duch.) is one of the most consumed fruits in the world due to a pleasant sensorial characteristic and nutritive value, especially vitamin C (Proteggente et al., 2002). An example of a typical ascorbic acid chromatogram in strawberries is shown in Figure 4.

Figure4. Example of a typical ascorbic acid chromatogram in strawberries by ultra-high-performance liquid chromatography at the wavelength of 254 nm.



Source: Authors.

For ascorbic acid, this study found significant interaction between irradiation doses and storage time (Table 3).

Table 3. Ascorbic acid in strawberries treated with different doses of gamma irradiation (0.8, 1.6, and 2.4 kGy) and control sample (0kGy), stored for up to 9 days under refrigeration (10±1°C, RH 90±5%).

Doses (kGy)	Ascorbic acid (mg.100 g-¹)			
	Storage time (days)			
	0	3	6	9
0	98.07(9.22) AB	103.63(8.86) AB	104.33(10.76) AB	*
0.8	80.46(5.89) A	112.31(9.12) AB	106.00(16.05) AB	106.25(11.67) AB
1.6	91.33(6.59) A	104.94(7.11) AB	121.08(9.50) B	85.26(9.16) A
2.4	86.56(13.18) A	107.59(14.74) AB	109.41(12.37) AB	107.85(8.09) AB

Means followed by the same letter do not differ from each other, by the Tukey test at 5% significance. The data represent the mean value (SD). * Lost portion of control treatment (0kGy) on day 9. Source: Authors.

Strawberries irradiated with the dose of 1.6 kGy on the sixth day of storage had a higher value of ascorbic acid, when fruits irradiated with the doses 0.8, 1.6, and 2.4 kGy on the first day of study and with strawberries irradiated with the dose 1.6 kGy on the last day of analysis.

Results similar to the present study were found in camu-camu submitted to gamma irradiation at 1.0, 2.0 kGy and control sample (0 kGy). and stored under refrigeration for 21 days, in that ascorbic acid values were different between treatments and storage times and the use of irradiation generally maintained ascorbic acid levels during storage (Sanches et al., 2017). Results different from that of the present study were found for raspberries subjected to gamma irradiation (0, 0.5, 1, and 2 kGy) and stored at 1°C for 12 days. That study recorded an increase in ascorbic acid content during storage for the doses of 1 and 2 kGy, whereas fruits irradiated with dose 0.5 kGy and control sample (0 kGy) had the highest ascorbic acid values up to 9 days of storage (Guimarães et al., 2013).

Hussain et al. (2010) found in a study with irradiated peaches that the decrease of vitamin C is largely due to storage and non-irradiated treatment. The present study verified that the variation in ascorbic acid values may be due to the post-harvest storage of the fruits and not to the irradiation treatment.

3.5. Color

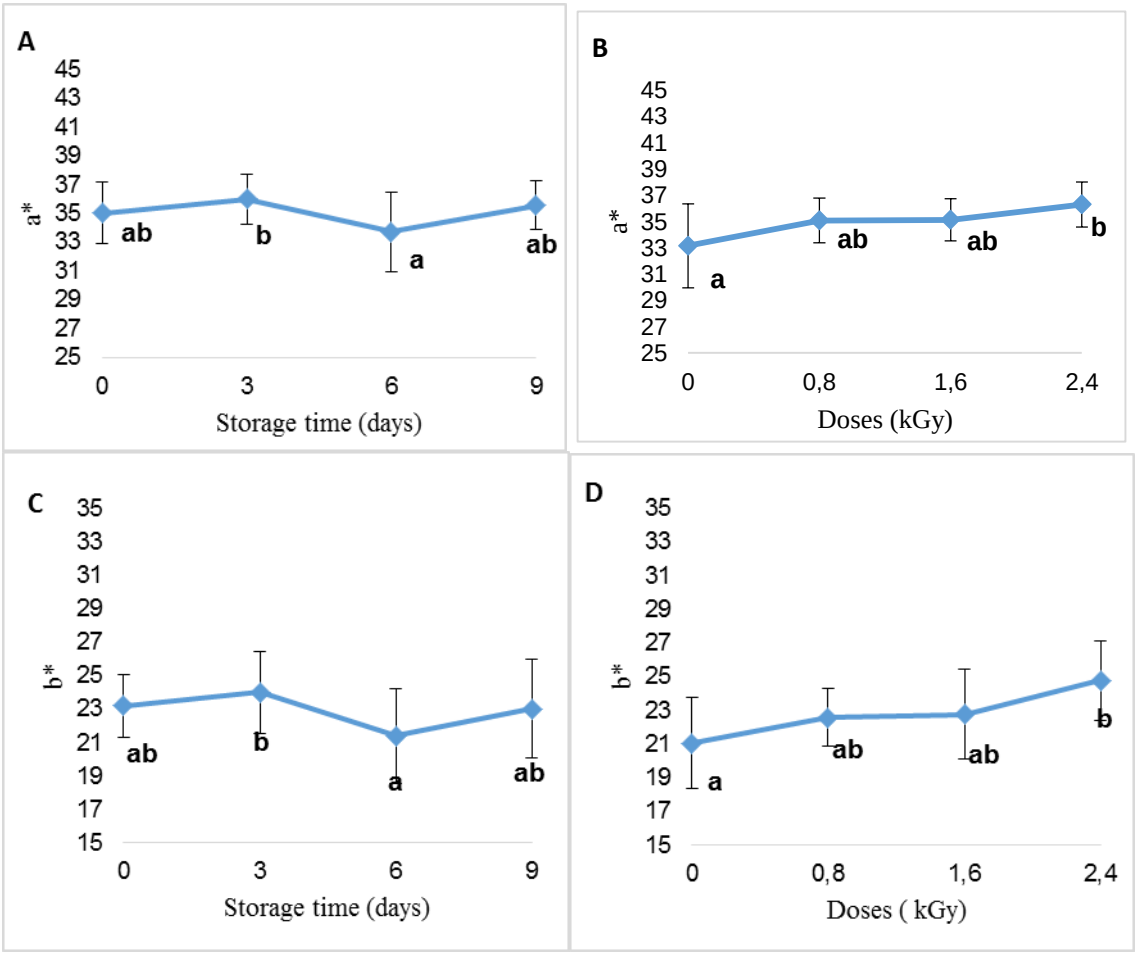
According to Guerreiro et al. (2016), color is one of the most attractive factors influencing the consumer when buying fresh food. Polyphenoloxidase and peroxidase enzymes can cause color changes in vegetables (Gokmen, 2010).

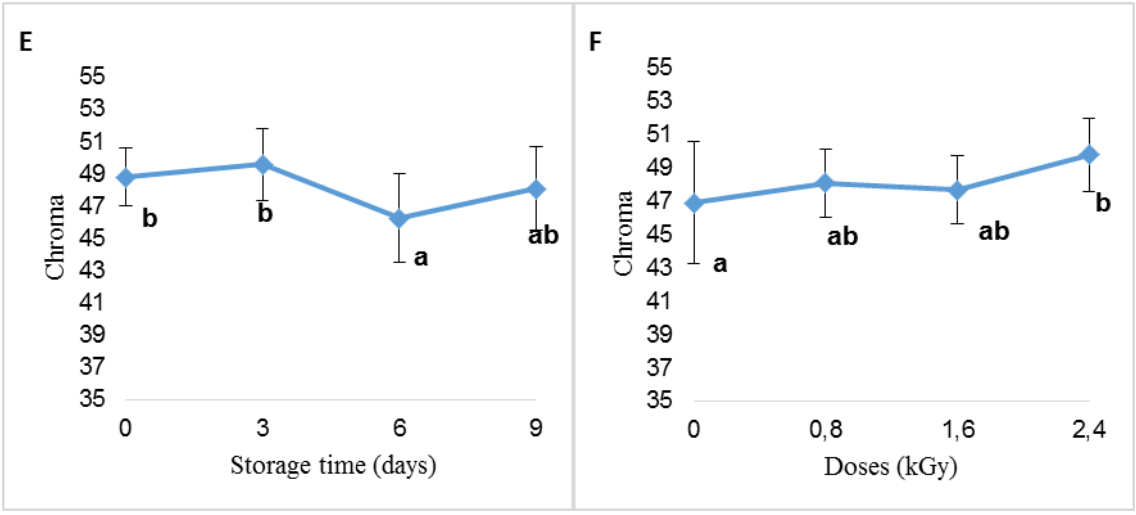
Irradiation, as well as other food conservation technologies, can cause the destruction of pigments in fruits; thus, it is important to check if the color is different in irradiated

strawberries and non-irradiated (Shahbaz et al., 2014). The irradiation may also alter the structure of the polyphenoloxidase enzyme affecting fruit color (Mishra et al., 2012).

The analysis of color properties was performed for control and irradiated strawberries, the results are presented in Figure 5 and Table 3. The parameters a*, b*, and chroma demonstrated similar behaviors. For these variables, the interaction of the factors irradiation doses and storage time were not significant. The storage days showed a significant difference, with a decrease of these variables from the third day to the sixth day of storage (Figure 5A; 5C; 5E). As for irradiation doses, there was a significant difference between the control strawberry (0 kGy) and the dose 2.4 kGy, which had the highest values for the parameters a*, b*, and chroma (Figure 5B; 5D; 5F).

Figure5. Parameters a* (A), b* (C), and Chroma (E) of strawberries cv Albion stored for up to 9 days under refrigeration (10±1ºC, RH 90±5%). Parameters a* (B), b*, (D), and Chroma (F) of strawberries cv Albion submitted to different doses of gamma irradiation (0.8, 1.6,2.4 kGy) and control sample (0 kGy).





Averages followed by the same letter do not differ from each other, by the Tukey test at 5% significance. The vertical bars represent a standard deviation above and below the mean value. Source: Authors.

The value of a* always remained positive, indicating that the strawberries presented characteristic red color. According to Conti, Minami, and Tavares (2002), chroma values greater than 36 are considered fruits with homogeneity of color, therefore, the strawberries of the present study had homogeneous colors.

No significant differences were observed in the for the strawberries submitted to the different treatments during the storage period studied, the values found varied from 30.60° to 34.,80°, which represents the red tonality. Han et al., (2004) report that Hue angle values may decrease during storage due to the synthesis of anthocyanins, which are pigments that contribute to the red color of strawberries. As present work, the Hue angle and the anthocyanins showed no significant difference due to the different doses of gamma radiation during the evaluated period of time; therefore, there were no synthesis of anthocyanins during storage.

For the parameter L*, the interaction of irradiation dose and storage time was significant. Table 4 shows that the fruits irradiated with the dose 2.4 kGy on the third day presented the highest value of the L* variable when compared to the control fruits (0 kGy) and the irradiated strawberries at the dose 0.8 kGy in the sixth day and the irradiated fruits at dose 1.6 kGy on the ninth day.

Table 4. Parameter L* in strawberries treated with different doses of gamma irradiation (0.8, 1.6, 2.4 kGy) and control sample (0 kGy) stored for up to 9 days under refrigeration (10±1ºC, RH 90±5%).

L *				
Doses	Storage time (days)			
(kGy)	0	3	6	9
0	34.49(1.69) ABCD	34.52(0.21) BCD	30.34(1.80) A	*
0.8	35.01(0.62) CD	32.53(1.47) ABCD	31.12(0.99) ABC	32.66(0.90) ABCD
1.6	32.56(0.82) ABCD	33.38(1.39) ABCD	32.32(1.11) ABCD	30.47(2.39) AB
2.4	33.62(1.28) ABCD	35.77(1.81) D	32.74(0.63) ABCD	34.03(1.64) ABCD

Means followed by the same letter do not differ from each other, by the Tukey test at 5% significance. The data represent the mean value (SD). * Lost portion of control treatment (0kGy) on day 9. Source: Authors.

Serapian & Prakash (2016) studied irradiated strawberries at the dose of 400 Gy and verified that the parameters a *, b *, and L * were not affected, stating that the use of low doses of irradiation does not affect the colors of these fruits. Nassur et al., (2016) also evaluated the application of different doses of gamma radiation (0.5, 1.0, 1.5 kGy) and control sample (0 kGy) in refrigerated stored strawberries (0±1°C and 90±5% RH) for 15 days and did not observe significant difference for the variables L * and chroma.

According to Youssef et al., (2002), gamma radiation may increase polyphenoloxidase activity, possibly in response to stress during the process. Silva and Koblitz (2010) reported that darkening of plants occurs when they present some type of injury or simply have contact with oxygen, causing darkening due to the joint action of enzymatic activity mainly of polyphenoloxidase together with peroxidase. The results of the colorimetric analysis of the present study demonstrated that the irradiation doses analyzed preserved the color of the strawberry, which is essential for its quality.

3.6. Microbiological Analysis

According to Mridha et al. (2017), the presence of high concentration of bacteria and fungi in strawberries may be due to irrigation water, soil dung, or food handlers' poor hygiene. One of the greatest benefits of the use of gamma radiation is the control of pathogenic microorganisms; therefore, their incidence in irradiated and non-irradiated strawberries was evaluated.

The Resolution RDC Nº. 12, promulgated on January 2, 2001 by the National Health Surveillance Agency of the Brazilian Ministry of Health establishes for fresh, whole, prepared, sanitized, chilled, or frozen fruits, for direct consumption, a maximum limit of 5 ×

10^2 NMP.g-¹ for coliforms at 45°C and absence of *Salmonella* in 25 g of the product (Brazil, 2001). The sanitary legislation does not establish limit for the counting of fungi and yeasts.

Table 5 records that the most probable number of coliforms at 35°C and 45°C was within the limits established by the legislation in control (0 kGy) and irradiated strawberries throughout the analysis period. The presence of *Salmonella* was not detected. Increasing doses of irradiation reduced the most probable number of total coliforms, fungi, and yeasts, because the fruits irradiated with the dose of 2.4 kGy showed the most efficient control of these microorganisms during the evaluated period.

Table 5. Results of microbiological analyzes for strawberries treated with different doses of gamma irradiation (0.8, 1.6, 2.4 kGy) and control sample (0 kGy), stored for up to 9 days under refrigeration (10±1°C) and 90±5% RH .

Storage time (days)	Doses (kGy)	Total coliforms (NMP g -¹)	Fungi and yeasts (UFC g-¹)
0	0	< 3,0	6,55 x 10^5
	0,8	< 3,0	5,90 x 10^5
	1,6	< 3,0	7,35 x 10^3
	2,4	< 3,0	7,75 x 10^3
3	0	1100	1,46 x 10^6
	0,8	150	> 5,00 x 10^5
	1,6	15	7,50 x 10^5
	2,4	3,6	> 5,00 x 10^5
6	0	1100	5,00 x 10^5
	0,8	460	9,65 x 10^5
	1,6	43	1,94 x 10^6
	2,4	9,2	4,30 x 10^5
9	0	*	*
	0,8	1100	5,05 x 10^5
	1,6	12	5,50 x 10^4
	2,4	23	2,64 x 10^4

* Lost portion of control treatment (0kGy) on day 9. Source: Authors.

The results confirm the benefit of gamma radiation, since the non-irradiated fruits had a large growth of fungi after the sixth day of storage, when they were discarded leading to the lost of the control treatment (0 kGy) on day 9.

The effectiveness of radiation in the inactivation of microorganisms is mainly attributed to the DNA damage (DeRuiter and Dwyer, 2002; Hussain, Dar & Wani, 2012). The effects of irradiation on the DNA molecule lead to the inability of the cell to replicate itself leading to its death. Radiation generates products of water radiolysis, which in turn combines with cellular components especially bases of the DNA molecule, leading to mutations in the cell. Induced mutations can both be repairable and irreparable, the latter leading to cell death (*Ibidem*, 2012).

Strawberries irradiated with the dose of 2.4 kGy showed more efficient control of the microorganisms studied during the nine days of storage, with this ideal dose being perceived to increase the useful life of strawberries cv. Albion. The fruits treated by gamma radiation maintained the physical-chemical and microbiological qualities during the studied period, so the irradiation was efficient.

4. Final Considerations

This study showed that non-irradiated samples became unfit for consumption after the sixth day of storage, while samples treated by gamma radiation maintained the physico-chemical and microbiological qualities over the nine days of storage. Irradiation in low doses and under refrigeration increased by three days the commercialization period of the strawberries.

The irradiation treatment did not interfere with the anthocyanin content over the assessed storage time. The doses used did not influence the loss of the phenolic compounds, as these compounds were maintained throughout the storage with the application of the gamma radiation. The variation in ascorbic acid values may be due to the post-harvest storage of the fruits and not to the irradiation treatment.

The applied doses did not interfere in the characteristic red color of the strawberry. The fruits irradiated at dose 2.4 kGy showed more efficient control of the studied microorganisms during the evaluated period, this being the most ideal dose to increase the useful life of strawberries cv. Albion.

As a suggestion for future work, we can include the combination of food preservation methods such as modified atmosphere as a means of preserving quality and extending the life

of irradiated strawberries. In addition to sensory analysis to see if the sensory characteristics of the fruits have changed.

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