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Physicochemical characterization, antioxidant activity and total phenolic content in 'Gala' apples subjected to different UV-C radiation doses

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ABSTRACT. UV-C radiation is a food preservation method aimed to extend the life of the product, inactivate microorganisms, and stimulate the synthesis of phenolic compounds. This study aimed to physicochemically characterize and evaluate the antioxidant activity and phenolic content of 'Gala' apples subjected to different UV-C radiation doses. The fruits were harvested, sanitized, selected and inserted into a UV-C radiation chamber, and different radiation doses were applied as follows: 0 KJ m^{-2} (0 min.), 0.68 KJ m^{-2} (2 minutes), 2.73 KJ m^{-2} (4 minutes), and 4.10 KJ m^{-2} (6 minutes). The apples were stored for 120 days at 5 ± 1°C and analyzed after 0, 30, 60, 90, and 120 days of storage. Radiation doses had no influence on parameters, such as weight loss, firmness and Hue angle, and physicochemical aspects, such as pH, soluble solids, titratable acidity and the soluble solids/titratable acidity ratio. The 4.10 KJ m^{-2} dose was effective and increased the phenolic content and antioxidant activity for up to 90 days while maintaining the content of vitamin C during storage.

Keywords: *Malus* sp., preservation, quality, bioactive compounds.

Caracterização físico-química, atividade antioxidante e teor de fenólicos totais em maçãs 'Gala' submetidas a diferentes doses de radiação UV-C

RESUMO. A radiação UV-C é um método de conservação de alimentos que visa o prolongamento da vida útil do produto, a inativação de microrganismos, além de estimular a síntese de compostos fenólicos. O presente trabalho teve como objetivo a caracterização físico-química, bem como a avaliação da atividade antioxidante e teor de fenólicos totais de maçãs cultivar Gala submetidas a diferentes doses de radiação UV-C. Os frutos foram colhidos, sanitizados, selecionados e inseridos em uma câmara de radiação UV-C, onde diferentes doses foram aplicadas: 0 KJ m^{-2} (0 minutos); 0,68 KJ m^{-2} (2 minutos); 2,73 KJ m^{-2} (4 minutos); 4,10 KJ m^{-2} (6 minutos). As maçãs foram armazenadas por 120 dias a 5±1ºC e analisadas após 0, 30, 60, 90 e 120 dias de armazenamento. As doses de radiação não apresentaram influência em alguns parâmetros como perda de massa, firmeza e ângulo Hue, e em alguns aspectos físico-químicos, como pH, sólidos solúveis, acidez titulável e relação sólidos solúveis/acidez titulável. A dose de 4,10 KJ m^{-2} foi eficaz no aumento do teor de fenólicos e atividade antioxidante por até 90 dias, além de manter os teores de vitamina C ao longo do armazenamento.

Palavras-chave: *Malus* sp., conservação, qualidade, compostos bioativos.

Introduction

Apples are known to be fruits rich in vitamins, minerals and fibers. According to Brackmann, Pinto, Neuwald, and Sestari (2005), the 'Gala' apple is extensively studied due to its properties of being crunchy, juicy and tasty, which have made it widely accepted in the market. However, its early cultivation and high respiratory activity reduce its longevity in storage, hindering the market supply in the off season. Among the various techniques that can be adopted to decrease the respiratory activity, the use of low temperatures during storage is interesting because it delays ripening.

Several post-harvest methods have been used to maintain the quality of apples during storage, such as modification of the atmosphere (Fante, Vilas Boas, Paiva, Pires, & Lima, 2014), controlling the atmosphere (Steffens, Giehl, & Brackmann, 2005; Brackmann, Weber, Pinto, Neuwald, & Steffens, 2008), the use of 1-methylcyclopropene (Fante et al., 2013) and the use of different temperatures and concentrations of O_2 and CO_2 (Brackmann, Argenta, & Mazaro, 1996). In addition to these methods, ultraviolet (UV-C) radiation has been recently used to preserve fruits. This method has been demonstrated to maintain quality, control rot and increase or maintain the content of bioactive compounds that are beneficial for human health (Nascimento, Santos, Valdebenito-Sanhueza, & Bartnicki, 2014; Alothman, Bhat, & Karim, 2009). Fruits and vegetables play an important role in preserving health, and their beneficial effects are especially associated with the antioxidant activity of phytochemicals, which are widely distributed in fresh fruits (Genova et al., 2012).

The use of UV-C radiation can activate defense mechanisms in certain fruits, promoting the synthesis of compounds of interest to the human body, such as phenolic compounds. Phenolic compounds can be divided into the following two groups: non-flavonoids and flavonoids, both of which are considered low-molecular-weight compounds and secondary metabolites of fruits and vegetables (Volp, Renhe, Barra, & Stringueta, 2008). One of the most outstanding features of phenolic compounds is their intense antioxidant activity.

Thus, the objective of this study was to evaluate the color, vitamin C content, physicochemical aspects and antioxidant potential of 'Gala' apples subjected to different UV-C radiation doses.

Material and methods

'Gala' apples were collected in the city of Barbacena, Minas Gerais, Brazil and transported to the Postharvest Laboratory of the Federal University of Lavras (Lavras, Minas Gerais, Brazil), where they were sanitized with sodium hypochlorite (200 mg L^{-1}) and selected after checking for the absence of injuries or rot. The fruits were placed in a UV-C radiation chamber, and different radiation doses were applied as follows: 0 KJ m^{-2} (0 min.); 0.68 KJ m^{-2} (2 minutes); 2.73 KJ m^{-2} (4 minutes); and 4.10 KJ m^{-2}(6 minutes). The doses were measured using a portable spectroradiometer (USB-850 RED TIDE) coupled to a R400-7-VIS-NIR probe (US Bio Solutions Ocean Optics). The distance between the lamp and the irradiated material within the chamber was 20 cm above and below. Four germicidal, fluorescent lamps (model Ecolume 15 W 09/11) were used in the process. The dose was calculated by integrating the time of exposure and the irradiance of the source.

After radiation, the fruits were packed in rigid polypropylene trays with lids and kept in cold storage at a temperature of approximately 5 ± 1°C for 120 days. Five fruits were placed in each package. Three packages were used for each treatment. Analyses were performed every 30 days starting at day 0.

Weight loss was determined by weighing the product in a semi-analytical balance. The results were expressed as percentage (%) of weight loss, considering the difference between the initial weight of the package containing the fruit and that obtained after each sampling period.

The color of the apples was measured using a Minolta Colorimeter, Model CR 400, using the system of the Commission Internationale de Eclairage (Comission Internationale de I'eclairage [CIE], 1978), according to which the coordinates L*, a* and b* were obtained. The coordinate L* measures the lightness or brightness of the sample, ranging from black (0) to white (100). The values of a* and b* were used for calculating the chromaticity and tonality, respectively, according to McGuire (1992).

The soluble solids (SS) of the fruits were determined using an ATAGO PR-100 digital refractometer, and the results were expressed as percentages, according to Association of Official Analytical Chemists (AOAC, 2007). The measurement of the pH was carried out by employing a Tecnal (Tec 3M) pH meter with a glass electrode, as recommended by AOAC (2007). The titratable acidity (TA) was also determined by the method suggested by AOAC (2007); the titrations were performed with a 0.1 mol L^{-1}sodium hydroxide (NaOH) solution, and the results were expressed as % of malic acid. The SS/TA ratio was calculated by dividing the total soluble solid content by the titratable acidity.

Vitamin C (ascorbic acid) was quantified by a colorimetric method using 2,4-dinitrophenylhydrazine according to Strohecker and Henning (1967). The data were acquired at 520 nm on a spectrophotometer using a computerized system, and the results were expressed as mg of ascorbic acid per 100 g^{-1} of fruit.

Phenolic compounds were obtained according to the colorimetric method developed by Singleton and Rossi (1965) using the Folin-Ciocalteu reagent in solution at a concentration of 10% (v/v).

Antioxidant activities were determined by the method of DPPH (2,2-diphenyl-1-picryl-hydrazyl) sequestration by antioxidants, according to Rufino et al. (2007a). For the purpose of comparing the results with the literature, the percentage of free radical sequestration (% FRS) was calculated according to the equation suggested by Duarte-Almeida, Santos, Genovese, and Lajolo (2006):

$$\%FRS = (Ac - Am)x100 / Ac$$

where Ac is the absorbance of the control, and Am is the absorbance of the sample. Higher values of this parameter indicate greater antioxidant capacity of the evaluated sample.

The antioxidant activity of the fruits was evaluated using the β-carotene/linoleic acid system, following the protocol recommended by Rufino, Alves, Brito, Filho, and Moreira (2007b), and the results were expressed as % of the system protected against oxidation.

The experiment followed a completely randomized 4x5 factorial design composed of four doses of UV-C radiation and five storage periods (0, 30, 60, 90, and 120 days) with three repetitions. Tukey´s test at a 5% probability level was used to compare the treatments within each time period. The polynomial regression model, which was used to assess storage time, was selected based on the significance of F test and the coefficient of determination for each tested model.

Results and discussion

Color is an important attribute observed during the purchase of fruits and is a determining factor for consumers' purchasing decisions. Furthermore, color can undergo important changes during storage. Figure 1 shows the L*, chroma and Hue angle values of apples exposed to different UV-C radiation doses.

The L* values showed a downward trend over the storage period for all treatments, showing no differences between the doses except at the last storage period. In this period, lower values were found in fruits subjected to a dose of 0.68 KJ m-2, resulting in darker or less yellow fruits. Chroma values, on the other hand, increased slightly over time, indicating a stronger and redder color. The highest values were found after treatments with a dose of 0.68 KJ m-2. Thus, the dose of 0.68 KJ m^{-2} was less effective in maintaining the yellow color of the apples. UV-C radiation can delay fruit ripening, which is manifested as lower color development, pigment accumulation and softening (Cote et al., 2013).

Hue angle values did not differ between the treatments but were significantly influenced by time. The Hue angle decreased during storage (Figure 1), as the fruits lost their yellowness and gained redness due to the normal process of fruit ripening. Lower values of this parameter were noted with increasing storage time, indicating redder fruits. Cabia, Daiuto, Vieites, Fumes, and Carvalho (2011), who studied 'Hass' avocados subjected to different UV-C radiation doses, did not observe the influence of the dose on parameters, such as polyphenol oxidase activity, color and total phenolic content.

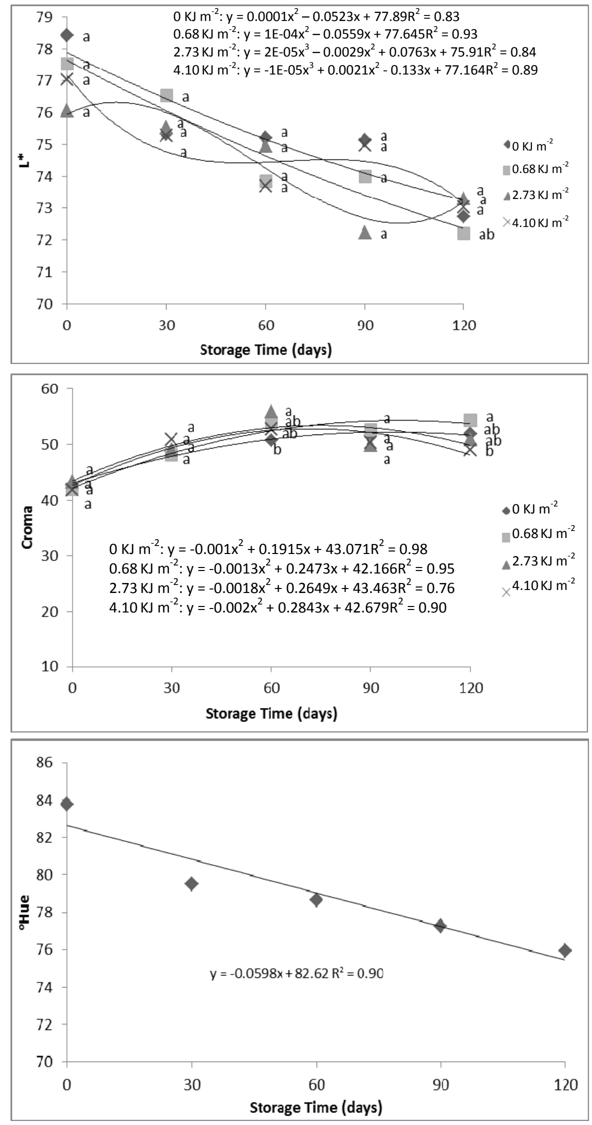

Figure 1. Values of L*, chroma and Hue angle of 'Gala' apples under different UV-C radiation doses (0 KJ m^{-2}; 0,68 KJ m^{-2}; 2,73 KJ m^{-2}; and 4,10 KJ m^{-2}). Different letters indicate significant differences among the treatments for various time periods, based on Tukey's test ($p \leq 0.05$).

Figure 2 shows the weight loss and firmness of apples subjected to UV-C radiation during cold storage.

Weight loss increased during the storage period for all treatments, reaching 9.26% during the last time period. These values are in agreement with the results found by Fante et al (2014), in which the weight losses of 'Eva' apples were between 3.63 and 8.79% after 135 days of storage, depending on the packaging used to wrap the fruit. The firmness of the apples decreased over time because of normal ripening of the fruit. No significant differences were observed between the treatments, and at 120 days of storage, the fruits showed mean values of 19.87 N.

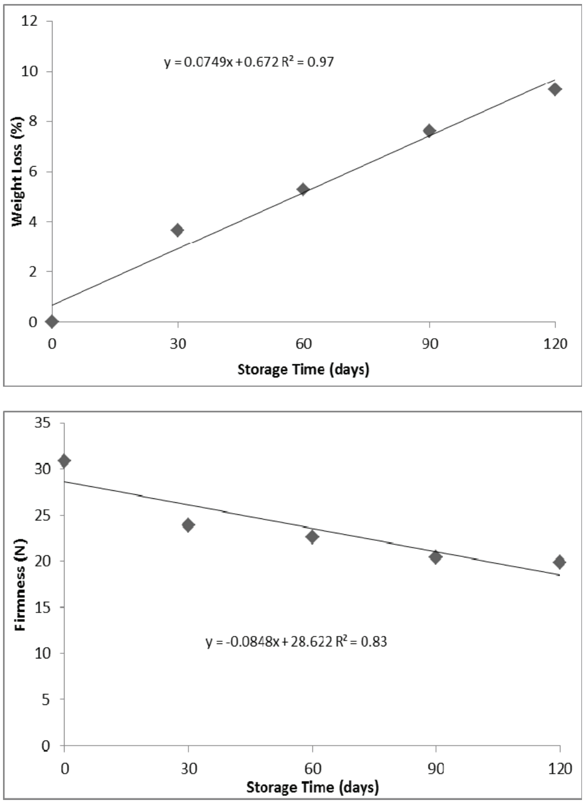

Figure 2. Weight loss and firmness of 'Gala' apples under different UV-C radiation doses (0 KJ m^{-2}; 0,68 KJ m^{-2}; 2,73 KJ m^{-2}; and 4,10 KJ m^{-2}).

The progressive loss of firmness or softening of a fruit usually occurs as a consequence of normal ripening, a complex process which involves different mechanisms, such as the loss of cell turgor, a decrease in the size and distribution of cell wall polymers and the action of hydrolytic enzymes (Chitarra & Chitarra, 2005). Firmness analysis and weight loss measurements were conducted to verify the influence of the radiation on the general preservation of the fruit as indicated by water loss and softening. Fante et al. (2013), working with apples subjected to different levels of 1-MCP, also noted a decrease in the firmness of fruit during storage due to the action of ethylene regardless of the dose of 1 -MCP.

Apple production mainly targets the fresh fruit market, where high quality is a requirement of consumers, who are looking for a regular supply of healthy foods throughout the year (Vieites et al., 2014). Thus, physicochemical characterization of the fruits becomes extremely important as these attributes directly influence their flavor and aroma. Figure 3 shows the pH values, titratable acidity and SS/TA ratios of apples during storage.

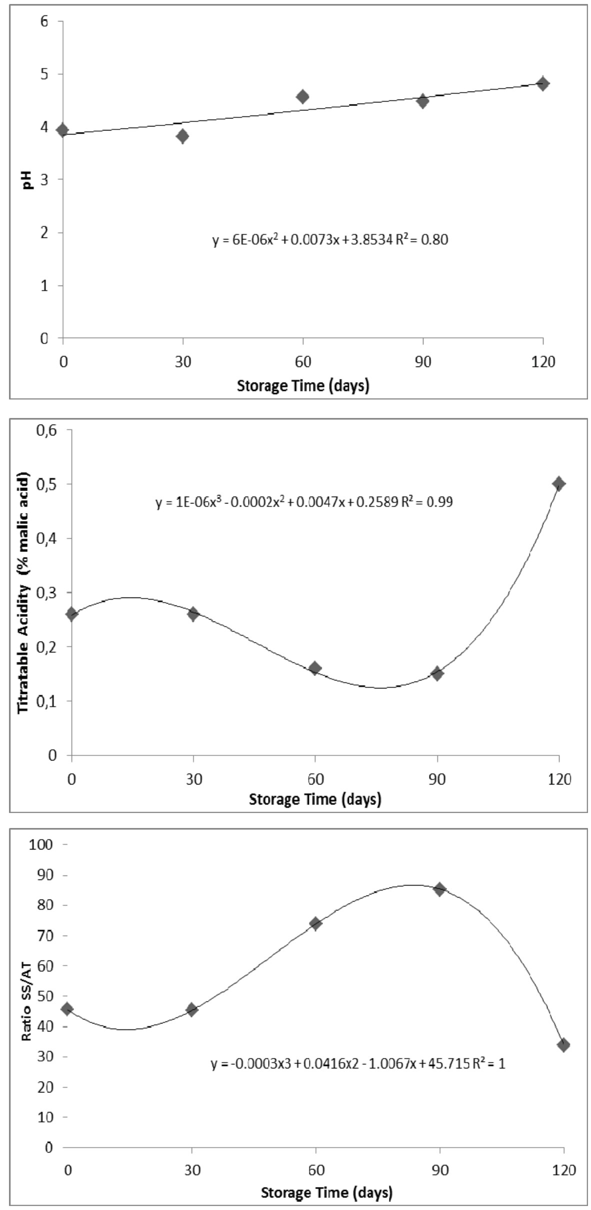

Figure 3. pH, titratable acidity and SS/TA ratio of 'Gala' apples under different UV-C radiation doses (0 KJ m^{-2}; 0,68 KJ m^{-2}; 2,73 KJ m^{-2}; 4,10 KJ m^{-2}).

The pH, titratable acidity and SS/TA ratios did not show significant differences between the doses, but storage time influenced these parameters. The pH increased over the storage period, reaching mean values of 4.82 at 120 days, which indicates that the fruit become less acidic at all applied doses. As a consequence of the increase in pH, the titratable acidity decreased over time, except for the last day of evaluation. On the last day, an increase in % malic acid was observed due to the conversion of the reduced sugars in the acid as a result of the respiration of fruits. The SS/TA ratio increased, especially at 60 and 90 days of storage, and decreased again at 120 days. The lower the SS/TA ratio, the more interesting the fruit becomes for industrial processes. Thus, samples with values below 20 are of the greatest interest from an industrial point of view because they have a high acidity (Czelusniak, Oliveira, Nogueira, Silva, & Wosiacki, 2003). In this context, it can be observed that all samples had values above 20, indicating that they were suitable for fresh consumption.

The SS content did not differ between treatments, and interactions between the factors were not significant. The mean values obtained for this parameter were 11.46, 11.70, 11.86 and 11.9 for the control, 0.68 KJ m^{-2}, 2.73 KJ $m^{-2}$and 4.10 KJ m^{-2} treatments, respectively. Fante et al. (2014), while studying 'Eva' apples in different modified atmospheres, did not observe differences in SS up to 225 days of storage in all of the tested films.

Figure 4 shows the vitamin C content of apples treated over 120 days of cold storage.

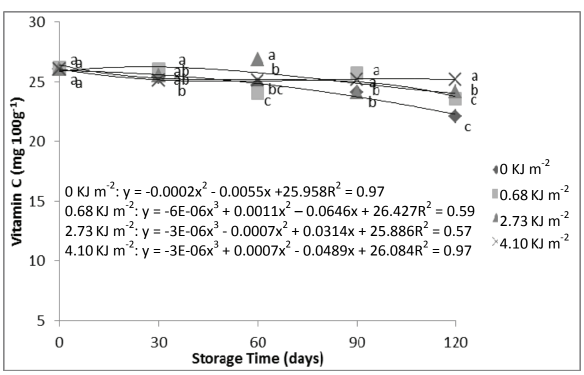

Figure 4. Vitamin C content of 'Gala' apples under different UV-C radiation doses (0 KJ m^{-2}; 0,68 KJ m^{-2}; 2,73 KJ m^{-2}; 4,10 KJ m^{-2}). Different letters indicate significant differences among the treatments for each time period based on Tukey's test ($p \leq 0.05$).

The vitamin C content of the apples showed a downward trend during storage and at the end of the last time period, was most effectively maintained with the dose 4.10 KJ m^{-2}, reaching values of 25.19 mg 100 g^{-1} at 120 days of storage. The lowest values were noted for the control group, showing a decrease of 15,07% at the end of the last time period. It is noteworthy that vitamin C is involved in several important functions in humans, and its intake through fruit plays an important role in the diet.

Fruits are the main dietary sources of polyphenols, and their components vary in quantitative and qualitative terms. This variation is due to intrinsic factors, such as cultivation, variety and stage of maturity, and extrinsic factors, such as climate and soil conditions, emphasizing that the effectiveness of antioxidant activity depends on the chemical structure and concentration of these phytochemicals in the food (Melo, Maciel, Lima, & Nascimento, 2008). The total phenolic content and antioxidant activity of the treated apples stored for 120 days is shown in Figure 5.

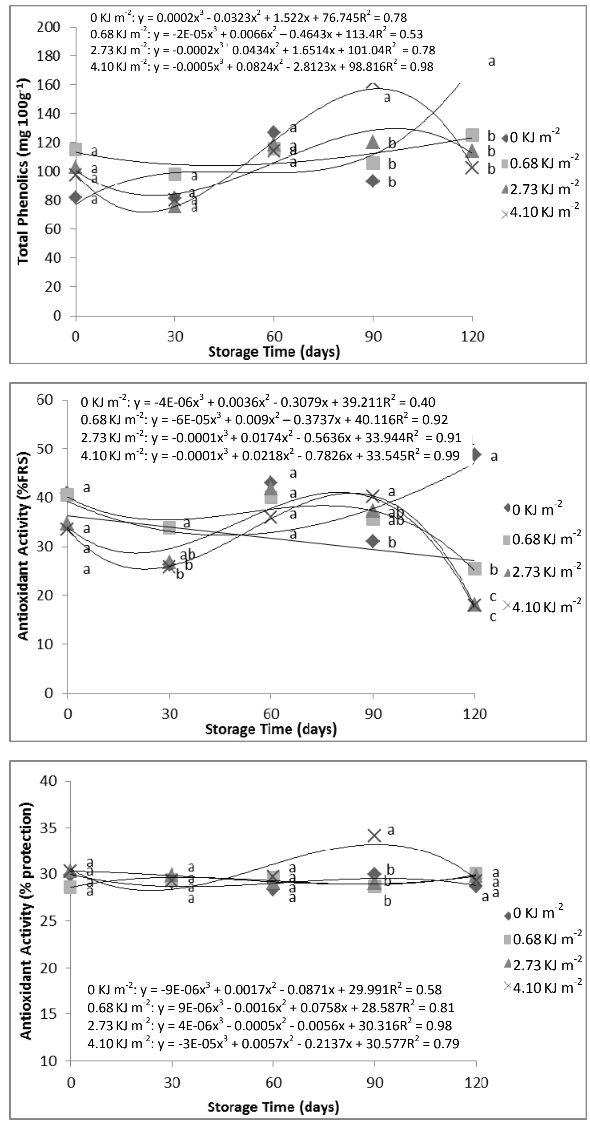

Figure 5. Total phenolic content and antioxidant activity (DPPH and β-carotene/linoleic acid system) of 'Gala' apples subjected to different UV-C radiation doses (0 KJ m^{-2}; 0,68 KJ m^{-2}; 2,73 KJ m^{-2}; and 4,10 KJ m^{-2}). Different letters indicate significant differences among the treatments for each time period based on Tukey's test ($p \leq 0.05$).

The total phenolic content showed no significant differences over storage time for the various radiation doses, but the control group had higher levels at 120 days of storage, showing mean values of 177.33 mg 100 g^{-1}. However, observations of the mean values of total phenolic content throughout the storage time showed that fruits treated with doses of 0.68 KJ m^{-2} showed the highest means (111.98 mg 100 g^{-1}). In addition, it is noteworthy that the apples treated with the dose of 4.10 KJ m^{-2}

showed a higher content of phenolic compounds at 90 days (increased by 65,37% from day 0) compared to the other treatments.

The antioxidant activity of the fruits measured by the DPPH method was not positively affected by the radiation doses at 120 days of storage, and the control group showed higher values. However, after 90 days of storage, the apples treated with the dose of 4.10 KJ m^{-2} had higher values of % FRS (increased by 19,55% from day 0), differing statistically from the other samples. The same result was obtained by the second method of analysis of antioxidants. After 90 days of storage, fruits treated with a dose of 4.10 KJ m^{-2} had higher % protection values compared to the other fruits, reaching 34.14% (increased 12,48% from day 0).

Figure 6 shows the principal component analysis of apples treated over 120 days of cold storage.

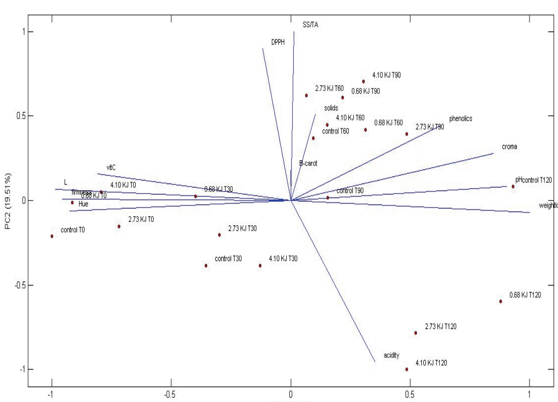

Figure 6. Principal component analysis of apples treated over 120 days of cold storage

A principal component analysis showed that the dose of 4.10 KJ m^{-2} after 90 days of storage showed intermediate values of antioxidant activity and total phenolic content compared to those obtained by both methods (DPPH and B-carotene/linoleic acid). These results demonstrate the influence of UV-C radiation on these parameters. Thus, this method of data analysis confirms the influence of UV-C radiation on bioactive compounds in 'Gala' apples. The samples in the initial period of storage showed larger values of L*. The Hue angle and firmness were observed to decrease over time as shown in figure 6, in which samples stored for 120 days are located on the opposite side of the figure. Samples with similar chroma values at 120 days of storage also showed higher values of weight loss and acidity at 120 days.

Conclusion

UV-C radiation had no influence on parameters, such as weight loss, firmness and Hue angle, and physicochemical aspects, such as the pH, soluble solids, titratable acidity and SS/TA ratio, of apples. However, the dose of 4.10 KJ m^{-2} increased the total phenolic levels and antioxidant activity for up to 90 days of cold storage, while maintaining the levels of vitamin C over the experimental period.

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