

Color, physicochemical parameters and antioxidant potential of whole grape juices subject to different UV-C radiation doses

Coloração, parâmetros físico-químicos e potencial antioxidante de sucos de uva integrais submetidos a diferentes doses de radiação UV-C

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ABSTRACT

Knowing that moderate stress such as UV radiation can activate defense mechanisms in plants, the use of UV-C radiation appears as hypothesis of a promising technique that would help to stimulate and enhance beneficial compounds for health, through a clean and healthy technology. In this study, the possible induction of secondary metabolism, the increase in the content of phytochemical compounds and physicochemical changes through the use of UV-C radiation were evaluated on whole grape juices produced with *Vitis labrusca* grapes, cultivar Isabel Precoce. Grapes were harvested, sanitized, exposed to UV-C radiation at doses of 0, 2, 4 and 6 KJ m⁻², and then the juices were prepared and packed into amber glass bottles at room temperature. Analyses were performed at 0, 30, 60, 90 and 120 days of storage. Based on results obtained and conditions in which the experiment was performed, UV-C treatment in grapes caused abiotic stress in the fruits, affecting color, titratable acidity, soluble solids/titratable acidity ratio, vitamin C and percentage of protection against oxidation. Application of UV-C did not change levels of phenolic compounds in fruit juices or the percentage of scavenging free radicals, pH and soluble solids.

Index terms: Phenolics; antioxidant activity; vitamin C.

RESUMO

Sabendo que estresses moderados como a incidência de radiação UV podem ativar mecanismos de defesa nos vegetais, o uso da radiação UV-C surge como hipótese de uma técnica promissora que ajudaria a estimular e a potencializar compostos benéficos para a saúde, por meio de uma tecnologia limpa e saudável. Neste estudo, avaliou-se a possível indução ao metabolismo secundário, o incremento do teor de compostos fitoquímicos e as alterações físico-químicas, por meio do uso da radiação UV-C em sucos de uva integrais produzido com uvas *Vitis labrusca*, cv. Isabel Precoce. As uvas foram colhidas, sanitizadas, submetidas à exposição à radiação UV-C, nas doses 0; 2; 4; e 6 kJ m⁻² e, em seguida, destinadas à elaboração de sucos, que foram acondicionados em frascos de vidro âmbar e armazenados à temperatura ambiente, sendo as análises realizadas nos dias 0, 30, 60, 90 e 120 dias de armazenamento. Com base nos resultados obtidos e nas condições em que foi realizado o experimento, verificou-se que o tratamento UV-C nas uvas provoca um estresse abiótico nas frutas, afetando a coloração, acidez titulável e relação sólidos/acidez titulável, vitamina C e porcentagem de proteção contra a oxidação. A aplicação da radiação UV-C não resultou em alterações nos teores de compostos fenólicos dos sucos, nem na porcentagem de sequestro de radicais livres, não alterando também o pH e sólidos solúveis.

Termos para indexação: Fenólicos; atividade antioxidante; vitamina C.

INTRODUCTION

In recent decades, population is increasingly concerned with a diet rich in plant products with high content of vitamins, anthocyanins and phenolic compounds with antioxidant capacity, able to maintain a good health.

Among fruits, grapes stand out for presenting large amount of phenolic compounds capable of capturing free radicals, countering oxidative stress and preventing cancer, neurological disorders, among others. Compounds present in grape and its products, which provide positive health effects,

are mainly flavanols, procyanidins, anthocyanins and phenolic acids (Andrade et al., 2001; Chedea; Braicu; Socaciu, 2010).

Phenolic compounds, resulted from the secondary metabolism of plants, are formed under stress conditions, such as infections, injuries, ultraviolet (UV) radiation, among others and are essential to plant growth and reproduction (Angelo; George, 2007; Melo et al., 2008). These compounds play important roles in nature, acting as defense mechanism and as antipathogenic agents. In foods, they are responsible for color, astringency,

aroma and oxidant stability (Angelo; George, 2007). From the medical point of view, several studies have shown that these compounds have anticancer, anti-inflammatory, anti-hepatotoxic, antiviral, anti-allergic, anti-thrombotic and antioxidant effects (Pimentel; Francki; Gollücke, 2005).

In vegetables, many factors influence the biosynthesis of compounds derived from secondary metabolism, such as macro and micronutrient content of the soil, climate, photoperiod, cultural practices and the incidence of UV radiation. Knowing that moderate stress, such as UV radiation can activate defense mechanisms in plants, the use of UV-C radiation appears as hypothesis of promising technique that would help stimulate and enhance the beneficial compounds for healthcare, applying through a clean and healthy technology.

Thus, the objective of the study was to evaluate color, vitamin C content, physicochemical aspects and antioxidant potential of whole grape juices under different UV-C radiation doses.

MATERIAL AND METHODS

Grape juices were obtained in the Laboratory of Postharvest Physiology of Fruits and Vegetables of the Federal University of Lavras, using grapes from 2013/2014 crop, grown in southwestern Minas Gerais, at the institutional orchard.

After harvest, grapes (with means of 17+1 of diameter and 18+1 cm of length) were kept in cold chamber at 10 ± 2 °C for 12 hours. Then, manual threshing and sanitization with sodium hypochlorite 100 mg L^{-1} for 10 minutes were performed. 500 g of grapes were used for each treatment. After these steps, fruits were exposed or not to UV-C rays, following methodology proposed by Sauter et al. (2008) and kept under refrigeration for 5 days to allow physiological response to UV-C irradiation.

Irradiation was at a distance of 20 cm from the source to the surface in anti-reflective chamber with ultraviolet source type C (UV-C) (trademark Ecolume, Power 15 W with circulating air at 10 °C). The irradiance of 0.787 W m^{-2} at wavelength of 254 nm was measured by a spectroradiometer (International Light® brand, model

RPS900). The doses were calculated by integrating the exposure time and the irradiance of the source, using Origin™ package version 5.1. It is important to highlight that the irradiation were applied on the both sides of the berries at the same time, ensuring that the both sides were achieved and promoting an uniform irradiation on the berries placed on a glass support in the chamber.

Irradiation time of grapes was determined according to Diffey (2002) by the ratio of dose and irradiance emitted by the ultraviolet lamp, as shown in the equation: $t = D / I$ where: t is the exposure time (s), D is the exposure dose (J m^{-2}) and I is the irradiance of the ultraviolet lamp (W m^{-2}).

The irradiation doses and exposure time of grapes for each treatment are shown in Table 1.

For the production of juices, technological protocol suggested by Rizzon, Manfroi and Meneguzzo (1998) was employed using a handmade equipment by vapor dragging performed at 75 ± 5 °C for 1 hour. For each liter of grape juice, 0.05 g of potassium metabisulfite (Synth®, Diadema, Brazil) was added. After this stage, the juices were immediately bottled in amber glass flasks of 100 mL and maintained at room temperature until the time of analysis, which were performed at 0, 30, 60, 90 and 120 days of storage.

To evaluate the color of the juices, a colorimeter Minolta, Model CR 400 was employed according the *Commission Internationale de l'Eclairage* (Commission Internationale de l'Eclairage-Cie 1978), researching the coordinate L^* , which measure the lightness or brightness of the sample, ranging between black (0) and white (100).

Measurement of pH was done using pHmeter Tecal (Tec 3M) with glass electrode, as recommended by Association of Official Analytical Chemists-Aoac (2007). The soluble solids of the juices were determined using a digital refractometer ATAGO PR-100 and the results expressed in%, according to Aoac (2007). Titratable acidity (TA) was also determined by method suggested by AOAC (2007), and the titration were carried out with sodium hydroxide (NaOH) solution 0.1 mol L^{-1} . Results were expressed in (%) of tartaric acid. SS/TA ratio was calculated dividing the total soluble solids content by titratable acidity.

Table 1: Treatments and irradiation doses applied in the experiment.

Treatment	Dose	Exposure time
Control	0 KJ m^{-2}	0 minutes
Irradiated with UV-C	2 KJ m^{-2}	2.54 minutes
Irradiated with UV-C	4 KJ m^{-2}	5.08 minutes
Irradiated with UV-C	6 KJ m^{-2}	7.62 minutes

Phenolic compounds were obtained according to the colorimetric method developed by Singleton and Rossi (1965), using Folin-Ciocalteu method, in solution with concentration of 10% (v/v). The extraction procedure involved sequential steps of centrifugation, filtration and rest, as described by Larrauri, Rupérez and Saura-Calixto (1997).

Determination of antioxidant activity of the juices was performed by the method of sequestration of DPPH (2,2-diphenyl-1-picryl-hydrazyl) by antioxidants according to Rufino et al. (2007a). Extracts used to determine total phenolics were used in the antioxidant activity analysis. For purposes of comparison with literature results, the percentage of scavenging free radicals (% SFR) was calculated according to equation suggested by Duarte-Almeida et al. (2006): % SFR = $(CA - SA) \times 100 / CA$, where CA (Abs Control) and SA (Abs Sample). In this parameter, higher values indicate greater antioxidant capacity of the sample studied.

Antioxidant activity of the juices were evaluated by β -carotene/linoleic acid system followed protocol recommended by Rufino et al. (2007b). The extract sample was obtained according to the methodology of Larrauri, Rupérez and Saura-Calixto (1997). Results were expressed in % inhibition of the system against oxidation.

Total content of anthocyanins was carried out following pH differential method proposed by Giusti and Wrolstad (2001). Results were expressed in mg L^{-1} . Quantification of vitamin C was done by colorimetric method, using 2,4-dinitrophenylhydrazine, according to Strohecker and Henning (1967). Reading were performed at 520 nm in spectrophotometer. Results were expressed in mg ascorbic acid per 100 ml juice.

A completely randomized (CRD) in a factorial design (5x4) composed of five storage periods (0, 30, 60, 90 and 120 days) and four irradiation doses (0, 2, 4 and 6 kJ m^{-2}) with 4 replicates. The polynomial regression models, used for storagetime were selected based on the significance of F test for each model tested and on the coefficient of determination.

RESULTS AND DISCUSSION

Appearance of food is the first factor to be considered by the consumer in the purchase process, influencing on acceptance or rejection of the observed product. Color is the primary attribute of appearance

(Bayarri et al., 2001). Thus, Figure 1 shows L^* values of grape juice subjected to different UV-C radiation doses.

L^* values ranging from black to white, increased linearly throughout the storage period when the juices were irradiated with a dose of 2 kJ m^{-2} and these juices were less dark, particularly in the final period of storage. At the end of 120 days, the juice irradiated with the dose of 6 kJ m^{-2} had the lowest values for this parameter, indicating darker juices with characteristic color of grape juice.

Figure 2 shows the pH values and soluble solids of the juices during storage time.

There was no significant interaction between radiation doses and storage time for the parameters pH and soluble solids. The third-order polynomial model shows the trend for pH during storage. Until thirty days of storage, a decrease in pH values was observed, followed by an increase up to 90 days of storage and before that, a decrease was also observed. Rizzonand Link (2006) in a comparative study with different grape cultivars, found that cultivar Isabel presents more acidic characteristics. This information confirms the pH value found for Isabel Precoce (pH = 2.93), as Ritschel and Camargo (2007) stated cultivar Isabel Precoce presents agronomic features and physicochemical characteristics very similar to the cultivar Isabel.

Juices from cultivar Isabel Precoce, regardless of irradiation dose showed lower soluble solids, on average 9.06%, which experienced a slight increase during the storage period. This value is below the minimum recommended by the Brazilian legislation for grape juice, which is 14% (Brasil, 2000). Rizzon and Link (2006), studying the composition of homemade juice from different cultivars (Isabel, Claret, Concord and Cabernet Sauvignon) also found soluble solids under the law, with values between 12.2 and 13.1 °Brix. According to Freitas (2006) and Rizzonand Link (2006), these variations may occur probably due to the dilution effect of the water steam used in the heating and extraction of the pigments of grape due to the type of equipment and the conditions used.

Figure 3 shows titratable acidity and soluble solids/titratable acidity ratio of juices subjected to UV-C radiation doses.

Except for the juices produced with grapes treated with 0 kJ m^{-2} , the titratable acidity of the juices presented a linear increase during storage. Irradiated

juices with UV-C radiation dose of 4 KJ m⁻² had higher acidity throughout the storage period, with mean values of 1.2 % tartaric acid, for that dose. This increase in acidity levels may be due to the degradation of pectin, which generates galacturonic acid. Freitas (2006) obtained 0.85% of acidity in grape juice of the same variety. Rizzon and Link (2006) found acidity varies due to the varieties characteristics and, studying grape

juice from different cultivars, higher values of acidity for Isabel and Cabernet cultivars and lower values for Claret and Concord cultivars were obtained. Nagatto et al. (2003) found values between 0.5 and 0.9% acidity in grape juice from Isabel cultivar. It is noteworthy the values obtained in this study are within the limits established by Brazilian legislation (minimum of 0.41g tartaric acid/100 g).

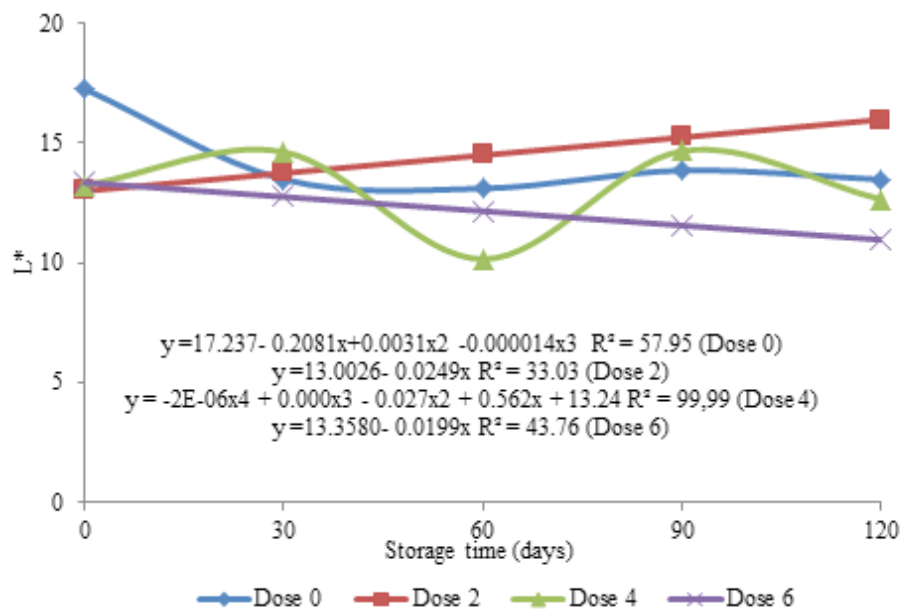


Figure 1: L* values of grape juice subjected to UV-C radiation doses (KJ m⁻²).

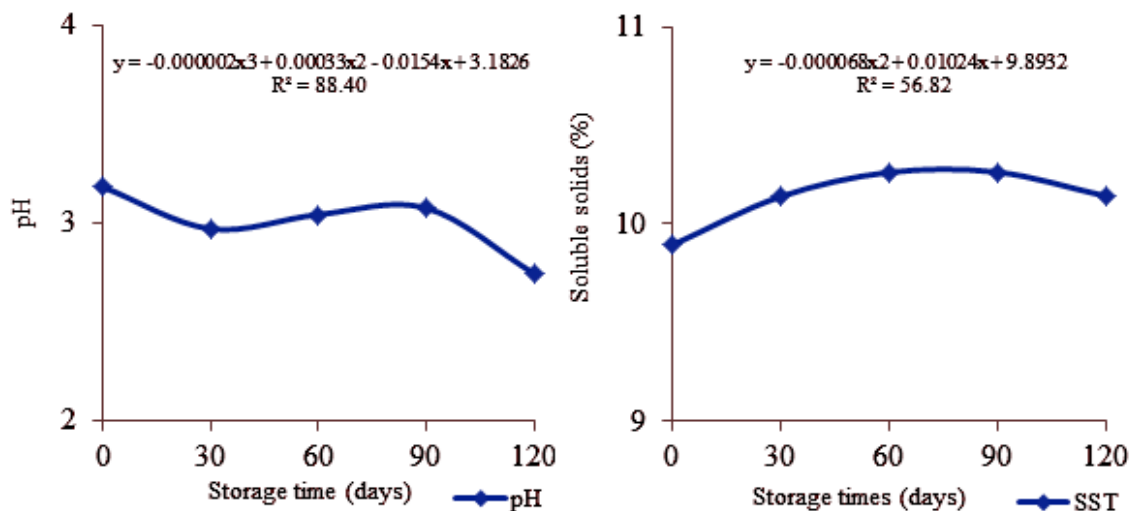


Figure 2: pH and soluble solids of grape juice subjected to UV-C radiation doses (KJ m⁻²).

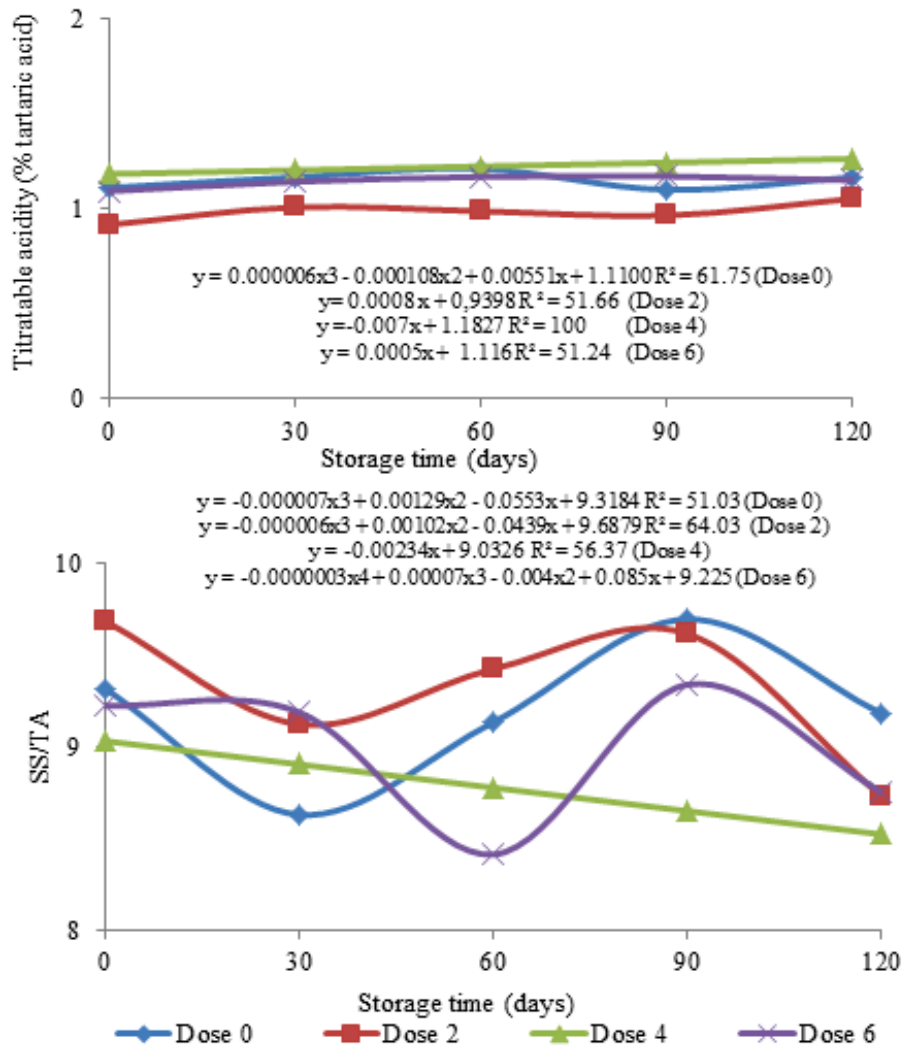


Figure 3: Titratable acidity and soluble solids/titratable acidity ratio of grape juice subjected to UV-C radiation doses (KJ m^{-2}).

Soluble solids/titratable acidity ratio is a parameter used to determine maturity of fruits, and the lower is the acidity of the juice, the higher is the ratio (Kimball, 1991; Volpe; Schöffef; Barbosa, 2002). Fruits destined for processing need to be in adequate maturation in order to obtain juice with sensory and physicochemical characteristics that meet consumer expectations and legislation (Brazil, 2000). The SS/TA ratio, which is indicative of the quality of the grape juice, showed values outside the limits set by the legislation, i.e., between 15 and 45. The values obtained in this study ranged from 8.5 to 9.5. Freitas (2006) found higher values for SS/TA ratio for the same variety under study. This difference may be

due to a lower content of soluble solids in the present work, which generates a smaller relationship between sweet and acid in the juice.

The total anthocyanins content is directly related to the pigmentation of juices. Thus, the content of anthocyanins of whole grape juice is shown in Figure 4.

Monomeric anthocyanin content decreased linearly during the storage period independent of the dose applied. During processing, and especially during storage of the juice, the content of monomeric anthocyanins decreased progressively and irreversibly forming more stable polymeric pigments. These pigments are responsible for changes in flavor, color and

flavor of the juice (Francia-Aricha, 1997). Furthermore, cultivar, maturity, year of cultivation and other environmental factors affect the content of anthocyanins of grapes and consequently the grape juice (Mazza, 1995). On the first day of analysis, juices irradiated with a dose of 4 KJ m⁻² had the highest anthocyanin content (292.8652 mg L⁻¹), unlike the non-irradiated juices, which demonstrated the lowest level (192.1754 mg L⁻¹). At the end of the storage period, the juices evaluated did not differ statistically, with anthocyanin values of 24.35 ± 10.04 mg L⁻¹.

Phenolic compounds or polyphenols are a heterogeneous group of substances found in vegetables in high concentrations, which are interesting, especially due to their antioxidant potential (Scalbert; Williamson, 2000). The content of phenolic compounds showed no significant differences between the doses evaluated or storage times, with mean values of 318.54 mg 100 mL⁻¹. Pala and Toklucu (2013) studying the effects of UV-C radiation on some quality characteristics of grape juice, also reported that UV-C treatment did not affect the level of phenolic compounds.

Various techniques have been used to determine the *in vitro* antioxidant activity of vegetables. Among the methods used are auto-oxidation of β-carotene/linoleic acid system (percentage of protection) and DPPH (percentage of scavenging free radicals). Thus, the antioxidant activity of juices by both methods are shown in Figure 5.

The irradiated juices presented a constant antioxidant activity during storage, except for juices irradiated with 6 KJ m⁻², that presented an increase until 90 days of storage. This increase can be related to the higher fruit stress, with consequent production of metabolites with antioxidant action. Juices undergoing radiation in dose of 2 KJ m⁻² had higher percentage of protection in the last periods evaluated when compared to other treatments. In this case, the UV-C radiation may have caused stability in antioxidant activity for the percentage of protection by β-carotene/linoleic acids system. The ability that grape products have to prevent lipid oxidation is a major benefit, because these antioxidant compounds active in blocking the oxidation of plasma lipoproteins.

By DPPH method (percentage of scavenging free radicals), sample activity is measured by the capacity thereof to inhibit the action of free radicals that are generated during lipid peroxidation. In this case, there were no significant differences between the applied radiation treatments.

Vitamin C content of the juices over 120 days of storage is shown in Figure 6.

Grape juices receiving UV-C radiation dose of 2 KJ m⁻² showed linear increase in vitamin C content during the storage period, resulting in 15 mg mL⁻¹ on the last day of storage. After 120 days of storage, all doses applied showed higher vitamin C values than the control group, indicating the effectiveness of UV-C radiation in this parameter.

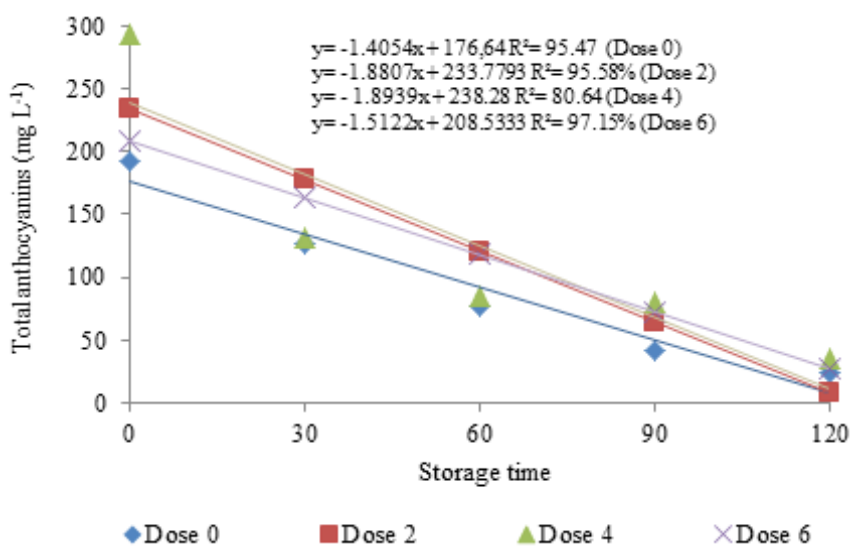


Figure 4: Anthocyanin of grape juice subjected to UV-C radiation doses (KJ m⁻²).

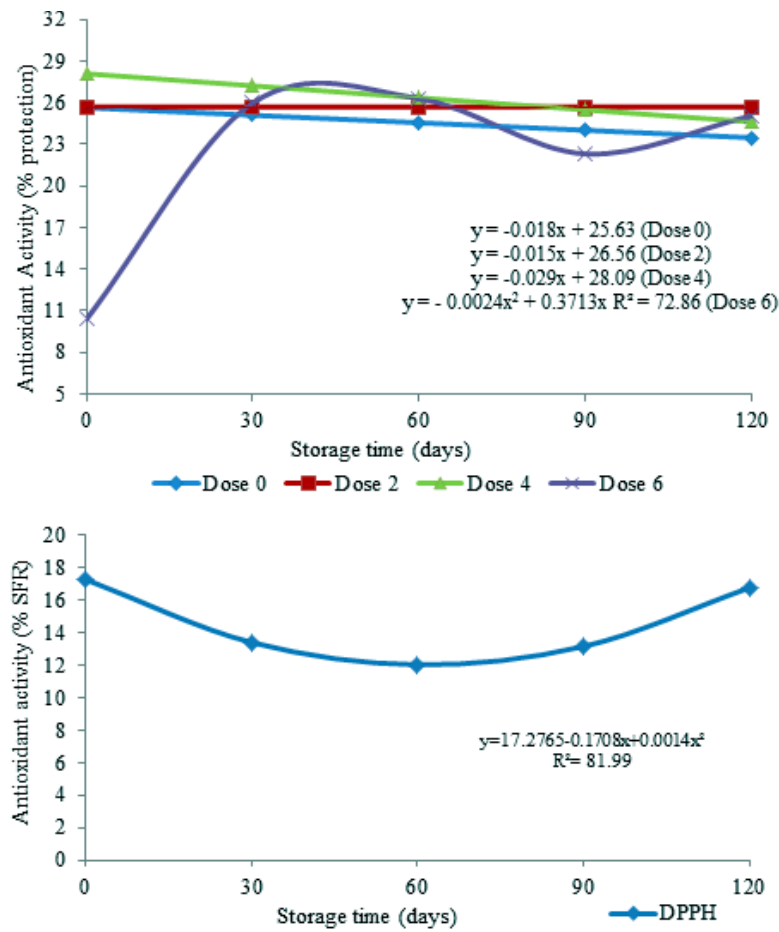


Figure 5: Antioxidant activity (% protection and % of scavenging free radicals) of grape juice subjected to UV-C radiation doses (KJ m^{-2}).

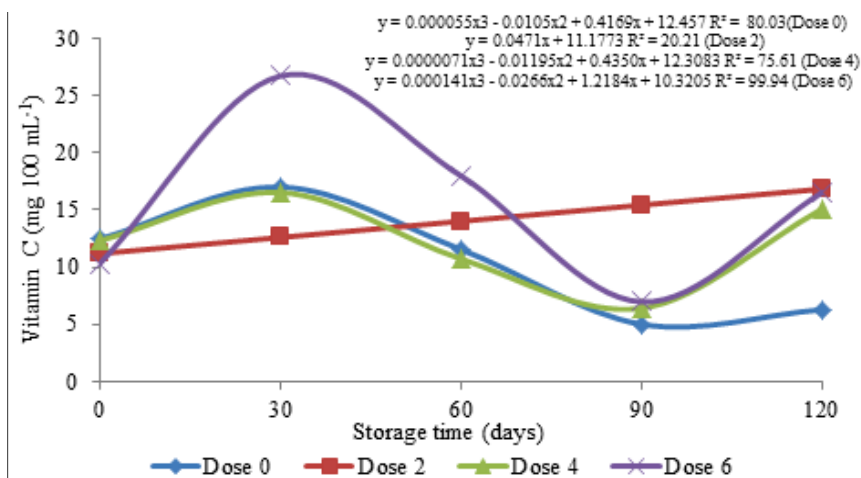


Figure 6: Content of vitamin C of grape juice subjected to UV-C radiation doses (KJ m^{-2}).

CONCLUSION

Treating grapes, cultivar Isabel Precoce, with UV-C radiation had positive effect especially in color, vitamin C content and percentage of protection against oxidation in grape juice, not affecting parameters such as phenolic compounds, pH, soluble solids and antioxidant activity (% SFR).

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