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Changes in total ascorbic acid and carotenoids in minimally processed irradiated Arugula (*Eruca sativa* Mill) stored under refrigeration



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HIGHLIGHTS

- There is a lack of information about the effect of gamma radiation on the content of vitamin C and carotenoids compounds in some vegetables such as arugula.
- This research shows that doses up to 2 kGy on fresh arugula do not impair the content of vitamin C and carotenoids besides improving the safety of the product.
- The content of vitamin C reduced during the period of storage in control as well as irradiated samples.

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ABSTRACT

This work investigated the effects of irradiation (0, 1 and 2 kGy) on the content of bioactive compounds such as vitamin C and carotenoids with provitamin A activity in arugula during the storage at 5 ± 1 °C for up to 13 and 16 days, respectively. The vitamin C content decreased in non-irradiated as well as irradiated (1 and 2 kGy) samples during the storage period. On the other hand, no significant change in the content of carotenoids with provitamin A activity was observed after irradiation or storage period. Thus, the irradiation had minimal detrimental effects on the contents of carotenoids in arugula.

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1. Introduction

The market of minimally processed vegetables has increased in the last 10 years because of changes in consumer's habits in relation to healthier foods. In addition, the industry, researchers and consumers have demonstrated great interest in bioactive compounds present in vegetables which protect against degenerative diseases, improving the quality of life (Ness and Powles, 1997; Ragaert et al., 2007; Rao and Rao, 2007).

Arugula is the third most popular vegetable consumed in Brazil. It contains a wide range of phytonutrients, such as provitamin A, vitamin C, flavonoids and glucosinolates, as well as potassium, sulfur, and fiber (Bennett et al., 2004; Kawashima and Soares, 2003; Trani and Passos, 1998).

Carotenoids are a class of natural fat-soluble pigments found in a great variety of fruits and vegetables. These pigments also play important roles in human health, preventing certain types of cancer, cardiovascular diseases and eye disorders. Carotenoids are divided into two major groups, the precursors of vitamin A (e.g. β -cryptoxanthin, α -carotene and β -carotene) and the non-precursors (e.g. lycopene, lutein and zeaxanthin). Most of the vitamin A in the human diet comes from provitamin A carotenoids (Rao and Rao, 2007).

Ascorbic acid is a water-soluble vitamin, which is required for a range of essential metabolic reactions; in addition it plays an important role for prevention of scurvy, maintenance of healthy skin, gums and blood vessels. This vitamin is also an effective antioxidant and protects proteins, lipids and carbohydrates from damage by free radicals and reactive oxygen species (Davey et al., 2000).

Minimally processed vegetables have been associated with foodborne disease outbreaks (Center For Disease Control and

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Prevention, 2006a, 2006b, Food Safety Network – FSNET, 2007), showing that the sanitizing process usually used does not minimize the problem. Ionizing radiation is a process that can be used to reduce or even overcome this problem. On the other hand, this technology can cause chemical changes in foods, which are directly related to the radiation dose (Labuza and Breene, 1989).

Relative little information is available regarding changes on nutritional aspects in irradiated vegetables. The contents of vitamin C and carotenoids in arugula are around 49 mg/100 g and 50 µg/g (Hassimotto et al., 2005; Moreira et al., 2005), respectively, and it is important to consider the action of irradiation on these substances since they are important micronutrients and antioxidants. Therefore, the purpose of this study was to evaluate the effect of gamma radiation on the content of total ascorbic acid and provitamin A carotenoids in minimally processed arugula stored at 5 ± 1 °C.

2. Materials and methods

Substrate: Arugula (*Eruca sativa* Mill.) samples were kindly provided by a local minimally processed produce industry located in São Roque-SP, Brazil, harvested between September and December. The time elapsed between the harvest, their processing and the beginning of the tests did not exceed 24 h.

2.1. Vegetable sanitation (according to Niemira et al. (2002) with modifications)

The damaged leaves were discarded. Arugula leaves and stalks were rinsed thoroughly in cold water and sanitized by immersion in ozone treated water (0.08 ppm) for 5 minutes. Later, the leaves and stalks were centrifuged to remove excess of water, and packed into polyethylene bags for posterior irradiation.

2.2. Irradiation process

Samples were irradiated using a gamma radiation ^{60}Co source (dose rate: 2.0 kGy/h) located at the Nuclear and Energy Research Institute (IPEN), São Paulo, Brazil. The dosimetric system used was the Harwell Amber Perspex (United Kingdom) dosimeter. The minimally processed samples of arugula were exposed to 1.0 and 2.0 kGy and, after processing, they were kept at 5 °C during the analysis period. A non-irradiated sample was used as a control.

Each trial included three samples per dose and was repeated in three different days.

2.3. Reduced ascorbic and total ascorbic acid extraction (Pasternak et al., 2005)

Irradiated and non-irradiated samples were maintained under refrigeration (5 ± 1 °C) during 16 days. In this period, the samples were analyzed on days 0, 5, 9, 12 and 16. Day 0 was considered 24 h after the radiation treatment. **Total ascorbic acid** (Pasternak et al., 2005). Total ascorbic acid was extracted with aqueous solution of metaphosphoric acid (0.3% w/v) and analyzed by reversed-phase HPLC in a Hewlett Packard 1100 system with an autosampler and a quaternary pump coupled to a diode array detector (DAD). A µ-Bondapak (300 mm × 3.9 mm i.d., Waters, Milford, MA) column was used; the elution was performed under isocratic conditions at flow rate of 1.5 mL/min with 0.2 M sodium acetate/acetic acid buffer (pH 4.2) and monitored at 262 nm. The total AA was estimated after the reduction of dehydroascorbic acid (DHA) with 10 mM dithiothreitol.

Results were expressed as milligrams of ascorbic acid per 100 g of fresh weight (FW), as mean [standard deviation (SD)]. The

dehydroascorbic acid was calculated as the difference between total ascorbic and reduced ascorbic acid.

2.4. Provitamin A carotenoids determination (de Rosso and Mercadante, 2007a)

After irradiation, samples were maintained under refrigeration (5 ± 1 °C) during 13 days. The contents of provitamin A carotenoids were analyzed at days 0, 3, 7, 10 and 13. Day zero was considered 24 h after the radiation treatment. Standards of all-*trans*-β-cryptoxanthin, all-*trans*-α-carotene, all-*trans*-β-carotene, 15-*cis*-β-carotene, 13-*cis*-β-carotene and 9-*cis*-β-carotene were provided by DSM Nutritional Products (Basel, Switzerland) showing purity between 93 and 99%, determined by HPLC.

The carotenoids were exhaustively extracted from the arugula samples (10 g) with acetone, transferred to petroleum ether (30–70 °C)/diethyl ether (2:1), and saponified overnight at room temperature with 10% methanolic KOH (De Rosso and Mercadante, 2007a). The extracts obtained were concentrated until solvent elimination on a rotatory evaporator (Rotavapor RE 120, Buchi, Flawil, Switzerland) at 40 °C and stored at –35 °C for posterior HPLC analysis.

2.4.1. HPLC-DAD and HPLC-DAD-MS/MS (De Rosso and Mercadante, 2007a)

The quantitative analyzes were carried out with a Waters HPLC (Milford, USA) equipped with quaternary pumps (model 600), on-line degasser, a Rheodyne injection valve (Rheodyne LCC, Rohnert Park, EUA) with a 20 µL loop and a DAD detector (Waters, model 996). The UV–visible spectra were obtained between 250 and 600 nm and the chromatograms were processed at 450, 350 and 280 nm in the Millennium Waters software.

Mass spectrometry analysis (MS) was carried out with a Shimadzu HPLC (Kyoto, Japan) equipped with quaternary pumps (model LC-20AD), on-line degasser, and a Rheodyne injection valve (Rheodyne LCC, Rohnert Park, CA) with a 20 µL loop. The equipment included, connected in series, a DAD detector (Shimadzu, model SPD-M20A) and a mass spectrometer with an ion-trap analyzer (model Esquire 4000) and atmospheric pressure chemical ionization source from Bruker Daltonics (Bremen, Germany). The MS parameters were set as follows: positive mode; current corona, 4.0 µA; source temperature, 450 °C; dry gas, N₂, temperature, 350 °C; flow, 60 L/h; nebulizer, 5 psi; MS/MS fragmentation energy, 1.4 V. The mass spectra were acquired with scan range of *m/z* from 100 to 700.

With both instruments, Waters and Shimadzu, carotenoid separation was carried out on a C₃₀ YMC column (5 µm, 250 × 4.6 mm i.d.) (Waters, Wilmington, USA) using as mobile phase with a linear gradient of methanol (MeOH) with 0.1% triethylamine (TEA)/methyl *tert*-butyl ether (MTBE) from 95:5 to 70:30 in 30 min, following to 50:50 in 20 min. The flow rate was 1.0 mL/min, and the column temperature was set at 29 °C, when the analysis was carried out with the Waters HPLC. For HPLC-MS/MS, TEA was excluded from the mobile phase, and the flow rate and column temperature values were reduced to 0.9 mL/min and 26 °C, respectively.

The carotenoids were identified according to the following combined information: elution order on the C₃₀ column, UV–visible spectrum features (λ_{max} , spectral fine structure, peak *cis* intensity), and mass spectrum characteristics (protonated molecule and MS/MS fragments) as compared to standards analyzed in the same conditions and data available in the literature (Back and Enzell, 1995; Britton, 1995; Britton et al., 2004; De Rosso and Mercadante, 2007a, 2007b). The carotenoids were quantified by HPLC, using external calibration curves for all-*trans*-β-cryptoxanthin, all-*trans*-α-carotene and all-*trans*-β-carotene. The *cis*

isomers of β -carotene were estimated using the curve of the corresponding all-*trans* isomer.

2.4.2. Calculation of the vitamin A value

The Nas-Iom (2001) conversion factor was used to calculate the vitamin A value, with 12 μg of dietary all-*trans*- β -carotene and 24 μg of other dietary provitamin A carotenoids corresponding to 1 μg of retinol activity equivalent (RAE), and the activities used were 100% for all-*trans*- β -carotene, 50% for all-*trans*- β -cryptoxanthin, all-*trans*- α -carotene (Bauerfeind, 1972) and for all *cis*-isomers of β -carotene.

2.4.3. Statistical analysis

Data were analyzed using StatSoft[®], version 5.5 (Tulsa, USA). For all the results, mean and standard deviations were calculated. ANOVA was used to detect significant differences between control and irradiated (1 and 2 kGy) samples for all parameters, and significant variation within the same sample during the storage period was also analyzed by ANOVA.

3. Results and discussion

3.1. Vitamin C of irradiated arugula in storage

During the storage period (16 days at 5 °C), a significant decrease ($P \leq 0.05$) on the free ascorbic acid (AA) and dehydroascorbic acid (DHA) contents in irradiated and non-irradiated arugula were observed.

As shown in Fig. 1, at day zero the free ascorbic acid content from non-irradiated sample (0 kGy) was significantly high than those from irradiated samples (1 and 2 kGy), probably due to the high oxidative stress caused by the irradiation process.

From the 9th day on, however, this significant effect ($P > 0.05$) of the radiation doses (1 and 2 kGy) on the free ascorbic acid content was no longer observed. It was also observed that the storage time caused a very significant decrease ($P \leq 0.05$) in both control and irradiated samples, reaching levels not detectable after 16 days of storage for all treatments.

Lester and Hallman (2010) also observed that free ascorbic acid in baby leaf spinach decreased with increasing dose of irradiation, however, Reyes and Cisneros-Zevallos (2007) noticed that the free ascorbic acid content in irradiated mangoes (3.1 kGy) was not significantly reduced ($P \leq 0.05$) until the 18th day of storage.

As a product of oxidation reaction of ascorbic acid, it was expected that the content of dehydroascorbic acid increased in irradiated samples, as was observed by Graham and Stevenson (1997) in irradiated strawberries and by Lester and Hallman (2010) in baby leaf spinach. However, in our experiments, DHA content

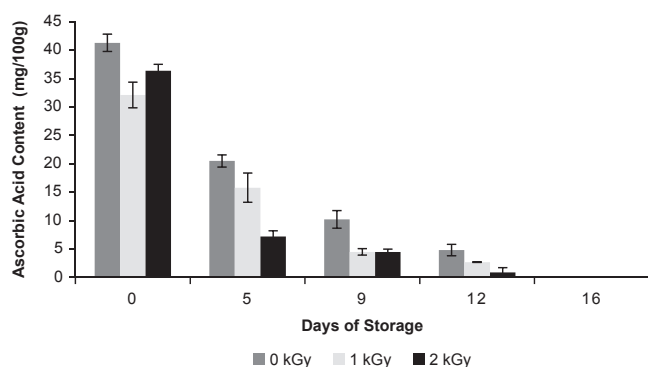


Fig. 1. Free ascorbic acid content in arugula samples exposed to 1 kGy and 2 kGy during 16 days of refrigerated storage.

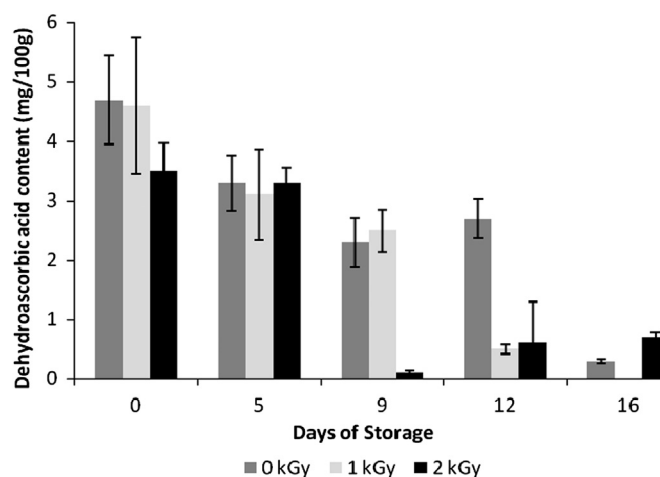


Fig. 2. Dehydroascorbic acid content in arugula samples exposed to 1 kGy and 2 kGy during 16 days of refrigerated storage.

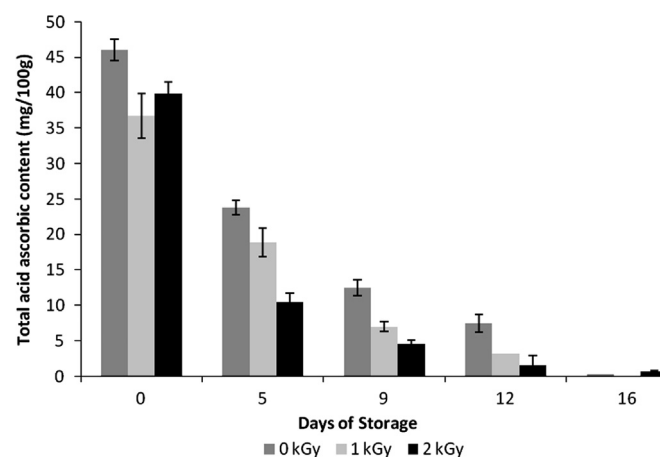


Fig. 3. Total acid ascorbic content in arugula samples exposed to 1 kGy and 2 kGy during 16 days of refrigerated storage.

was significantly reduced ($P \leq 0.05$) by the 9th day of storage. This would indicate that there was a rapid oxidation of dehydroascorbic acid content to 2,3-diketogulonic acid, which has no vitamin C activity and therefore was not detected by the method employed in this study (Tannenbaum et al., 1985).

DHA contents significantly reduced ($P \leq 0.05$) during the storage period, independently of the applied dose (Fig. 2). As was also observed for AA, DHA content did not significantly differ ($P > 0.05$) on all the analyzed samples on the 16th day of storage, independent of the applied dose, with values near zero.

Fig. 3 shows a significant decrease of total ascorbic acid content during storage for 16 days at 5 °C for both control and irradiated samples. The irradiation dose also affected significantly ($P \leq 0.05$) the reduction of total ascorbic acid content until the 5th day of storage. From the 9th day on, these levels were not different from each other ($P > 0.05$), regardless of the dose of 1 or 2 kGy. As expected, at the 16th day, total ascorbic acid content was not detected in any sample analyzed. This was probably because the biosynthesis of this vitamin does not occur during storage, as well as transformation of DHA to 2,3-diketogulonic acid. Moreover, many reactions may also have occurred during vegetable senescence such as color transformation, sugar and cell wall degradation. All these phenomena may cause tissue stresses which would require antioxidant action, especially by ascorbate, preventing cell damage, as well as neutralization of radiolytic products through

the antioxidant activity of vitamin C (Tannenbaum et al., 1985; Klein, 1987; Barata-Soares et al., 2004).

Graham and Stevenson (1997) observed, as in the present study, that the levels of vitamin C and free AA content in strawberries were significantly reduced by irradiation. However, they found that the storage period increased the levels of these acids, which was not observed in minimally processed arugula. Fan and Sokorai (2007) also observed a reduction of the vitamin C in frozen irradiated corn and peas (1.8 and 4.5 kGy) stored for 12 months.

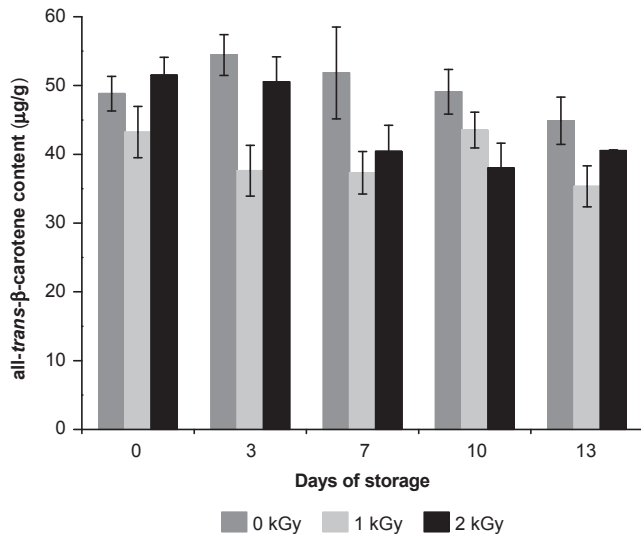


Fig. 4. Contents of all-trans-β-carotene in arugula samples exposed to 1 kGy and 2 kGy during 13 days of refrigerated storage.

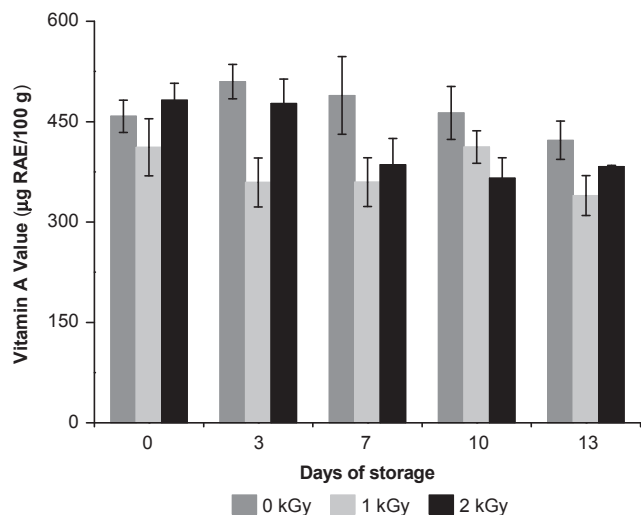


Fig. 5. Vitamin A values in arugula samples exposed to 1 kGy and 2 kGy during 13 days of refrigerated storage.

Table 1

All-trans-β-carotene (µg g⁻¹) content in minimally processed arugula samples exposed to 0 (control), 1 and 2 kGy and stored at 5 °C during 13 days.

Dose (kGy)	Storage period (days)				
	0	3	7	10	13
0	48.82 ^{aA} ± 2.52	54.44 ^{aA} ± 2.96	51.83 ^{aA} ± 6.68	49.10 ^{aA} ± 3.25	44.88 ^{aA} ± 3.44
1	43.23 ^{aA} ± 3.72	37.61 ^{bA} ± 3.71	37.31 ^{aA} ± 3.09	43.53 ^{aA} ± 2.60	35.35 ^{aA} ± 2.98
2	51.50 ^{aA} ± 2.60	50.57 ^{abA} ± 3.6	40.46 ^{aAB} ± 3.7	38.03 ^{ab} ± 3.6	40.55 ^{aAB} ± 0.1

Values in a row followed by the different upper case letters are significantly ($P < 0.05$) different (Tukey's test) in relation to storage period. Values in a column followed by the different lower case letters are significantly ($P < 0.05$) different (Tukey's test) in relation to irradiation dose. Mean value ± standard error.

On the other hand, while Lu et al. (2005) have also observed reduction in the levels of ascorbic acid in irradiated (1 kGy) and non-irradiated celery during the storage period, they found that the irradiated samples had higher levels than non-irradiated. However, the difference between the contents of the samples disappeared at the end of the storage period (9 days). Similar behavior was observed by Zhang et al. (2006) in irradiated lettuce with 1 kGy, but the vitamin C content of the irradiated samples was significantly higher until the last day of storage (9 days).

Fan and Sokorai (2008), in a study about the nutritional value of 13 fresh-cut vegetables observed that 1 kGy irradiation dose did not affect vitamin C content of most vegetables; however, irradiated green and red leaf lettuce had lower vitamin C contents than controls both at day one and day 14, with losses of 24% to 53%, respectively, at the end of the storage period. Fan and Sokorai (2011) reported losses in the content of ascorbic acid in irradiated fresh-cut spinach during storage at 4 °C for 14 days with 33.0% for control sample, 73.7% in samples at 1 kGy, and increased to 92.3% when samples were irradiated to 4 kGy.

3.2. Irradiation effect on the contents of provitamin A carotenoids in irradiated arugula

Among all the carotenoids with provitamin A activity present in arugula, all-trans-β-carotene was the major one (80%), followed by its *cis*-isomers (17%), with all-trans-β-cryptoxanthin and all-trans-α-carotene corresponding to the remaining 3%. The effects of irradiation on the provitamin A carotenoids from arugula are shown in Fig. 5 and Table 5.

The contents of all-trans-β-carotene (Fig. 4) decreased during storage in arugula treated with both irradiation doses, however, a significant decrease ($P > 0.05$) was only observed in the samples treated with 2 kGy. The significant decrease ($P > 0.05$) observed during stored of the samples treated with 2 kGy was not expected and may be partially attributed to the inherent variability between the leafy samples Fig. 5.

On the other hand, in general, the contents of the minor provitamin A carotenoids, were not significantly affected either by the exposure to 1 or 2 kGy and storage period at 5 ± 1 °C ($P > 0.05$) (see Tables 1–5). Exceptions were also observed, such as the all-trans-α-carotene which significantly increased ($P \leq 0.05$) after treatment with 2 kGy as compared to non-treated arugula at day 0 (Table 3).

Our results, together with those of other researchers, reveal a tendency of carotenoids to remain stable to radiation, as occurred for coriander leaves (Kamat et al., 2003) and sprouts (Nagar et al., 2012), probably due to the presence of other compounds offering protection to isoprenoids against radiation damage ((Bhushan and Kumta, 1997). On the other side, during storage carotenoids tend either to remain almost unchanged, as observe in our study and in sprouts (Nagar et al., 2012) or to decrease (Kamat et al., 2003).

Table 2Contents of all-*trans*- β -cryptoxanthin ($\mu\text{g g}^{-1}$) in minimally processed arugula samples exposed to 0 (control), 1 and 2 kGy and stored at 5 °C during 13 days.

Dose (kGy)	Storage period (days)				
	0	3	7	10	13
0	1.28 ^{aA} ± 0.03	1.26 ^{aA} ± 0.01	1.22 ^{aA} ± 0.01	1.29 ^{aA} ± 0.06	1.18 ^{aA} ± 0.02
1	1.30 ^{aA} ± 0.04	1.26 ^{aA} ± 0.07	1.20 ^{aA} ± 0.05	1.24 ^{aA} ± 0.09	1.26 ^{aA} ± 0.13
2	1.28 ^{aAB} ± 0.01	1.31 ^{aAB} ± 0.01	1.33 ^{aB} ± 0.08	1.33 ^{aB} ± 0.01	1.16 ^{aA} ± 0.01

Values in a row followed by the different upper case letters are significantly ($P < 0.05$) different (Tukey's test) in relation to storage period. Values in a column followed by the different lower case letters are significantly ($P < 0.05$) different (Tukey's test) in relation to irradiation dose. Mean value \pm standard error.

Table 3All-*trans*- α -carotene ($\mu\text{g g}^{-1}$) content in minimally processed arugula samples exposed to 0 (control), 1 and 2 kGy and stored at 5 °C during 13 days.

Dose (kGy)	Storage period (days)				
	0	3	7	10	13
0	0.52 ^{aA} ± 0.03	n.d. ^{aB} ± 0.0	0.0 ^{aB} ± 0.0	0.0 ^{aB} ± 0.0	0.0 ^{aB} ± 0.0
1	0.35 ^{aA} ± 0.10	0.0 ^{aB} ± 0.0	0.0 ^{aB} ± 0.0	0.0 ^{aB} ± 0.0	0.0 ^{aB} ± 0.0
2	0.65 ^{aAB} ± 0.11	0.53 ^{bA} ± 0.14	0.33 ^{bA} ± 0.01	0.0 ^{aB} ± 0.0	0.0 ^{aB} ± 0.0

Values in a row followed by the different upper case letters are significantly ($P < 0.05$) different (Tukey's test) in relation to storage period. Values in a column followed by the different lower case letters are significantly ($P < 0.05$) different (Tukey's test) in relation to irradiation dose. Mean value \pm standard error.

Table 4*cis*- β -carotene ($\mu\text{g g}^{-1}$) content in minimally processed arugula samples exposed to 0 (control), 1 and 2 kGy and stored at 5 °C during 13 days.

Dose (kGy)	Storage period (days)				
	0	3	7	10	13
0	10.49 ^{aA} ± 0.78	12.23 ^{aA} ± 0.20	12.83 ^{aA} ± 0.54	11.60 ^{aA} ± 2.94	10.39 ^{aA} ± 0.08
1	10.66 ^{aA} ± 2.86	9.70 ^{aA} ± 1.29	10.48 ^{aA} ± 2.58	10.57 ^{aA} ± 0.53	9.52 ^{aA} ± 1.07
2	10.81 ^{aA} ± 0.73	11.56 ^{aA} ± 1.45	9.99 ^{aA} ± 1.82	10.38 ^{aA} ± 0.12	9.66 ^{aA} ± 0.13

Values in a row followed by the different upper case letters are significantly ($P < 0.05$) different (Tukey's test) in relation to storage period. Values in a column followed by the different lower case letters are significantly ($P < 0.05$) different (Tukey's test) in relation to irradiation dose. Mean value \pm standard error.

Table 5Total of provitamin A carotenoids ($\mu\text{g g}^{-1}$) content in minimally processed arugula samples exposed to 0 (control), 1 and 2 kGy stored at 5 °C during 13 days.

Dose (kGy)	Storage period (days)				
	0	3	7	10	13
0	61.10 ^{aA} ± 3.24	67.93 ^{aA} ± 3.17	65.57 ^{aA} ± 7.23	61.99 ^{aA} ± 6.25	56.45 ^{aA} ± 3.46
1	55.54 ^{aA} ± 6.52	48.58 ^{bA} ± 5.06	49.00 ^{aA} ± 5.92	54.34 ^{aA} ± 3.22	46.13 ^{aA} ± 4.18
2	64.24 ^{aA} ± 3.42	63.96 ^{abA} ± 5.15	52.11 ^{aA} ± 5.66	9.74 ^{aA} ± 3.71	51.37 ^{aA} ± 0.24

Values in a row followed by the different upper case letters are significantly ($P < 0.05$) different (Tukey's test) in relation to storage period. Values in a column followed by the different lower case letters are significantly ($P < 0.05$) different (Tukey's test) in relation to irradiation dose. Mean value \pm standard error.

4. Conclusion

The present study demonstrated that vitamin C content showed identical behavior for non-irradiated and irradiated (1 and 2 kGy) arugula samples at the end of storage period. On the other hand, the contents of carotenoids with provitamin A activity did not show significant changes after irradiation. The results from this research demonstrate that gamma irradiation can be used on minimally processed arugula without affecting the levels of these micronutrients when compared to non-irradiated controls.

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