EVALUATION OF CAROTENOIDS IN EDIBLE FLOWERS PROCESSED BY RADIATION

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ABSTRACT
The use of flowers in the gastronomy has been growing in recent years, the world market for edible flowers is in full expansion, this kind of flower ensure a special note in the taste and in the decoration, and improve the nutritional aspects when added in food products. Its beneficial properties in health maintenance are also recognized, requiring new approaches to improve its conservation and safety. Food irradiation is an economically viable technology both in quality and safety. The purpose of this study was to evaluate carotenoids in *Rosa chinensis* and *Tagete patula* flowers submitted to gamma irradiation and electron beam doses of 0.5, 0.8 and 1.0 kGy. High performance liquid chromatography (HPLC) was used to carotenoids determination. The most abundant carotenoids were α-carotene for both species of flowers studied. In general, gamma-irradiated samples presented higher amounts in carotenoids (lutein and alfa-carotene) independently of the applied dose. However, the interaction between irradiation and samples did not affect carotenoids present in edible flowers petals.

Keywords: food irradiation; chromatography; *Rosa chinensis; Tagete patula;* biocompounds

1. INTRODUCTION

The edible flower market is in expansion worldwide, due to the growing use of flowers in gastronomy translation in to varieties and increasing its applicabilities and economic growth. Edible flowers are also being extensively explored by the food industry, basically in the production of flower teas, colorings, aromas and beverages (Rop *et al*., 2012; Voon *et al*., 2012).

A large number of studies have reported the bioactive compounds of edible flowers. They are considered a source of chemical compounds that have antioxidant activitythat is directly related to the polyphenolic compounds present in its composition. The variety of colors reflects the different types of carotenoids and anthocyanins present in the flowers chemical composition (Fu and Mao, 2008; Mlcek & Rop, 2011; Rop *et al*., 2012).

Carotenoids are natural pigments controlled by flowers, fruits and vegetables, as well as some birds, insects and marine animals. These pigments confer the yellow, orange and red coloration (Quirós & Costa, 2006; Pinto, 2010). In the last decades, epidemiological studies demonstrated that the carotenoids the promote on health, such as, the elimination to the risk of degenerative diseases, cardiovascular, eye degeneration and the processes of aging (Rodriguez-Amaya *et al*., 2008; Carvalho *et al*., 2013).

Flowers are highly perishable and should be free of insects, which constitutes a challenge, once that they are grown without the use of pesticides aplications. Many methods are used not only to extend the shelf life of these flowers, but also to ensure their quality and safety (Kelly *et al.*, 2003; Newman & O'Conner, 2009; Farkas & Mohácis-Farkas, 2011). Ionizing radiation treatment might be the answer to these problems, ensuring food quality, increasing its shelf-life, food safety and disinfestation of foods.

The species of edible flowers *Rosa chinensis* Jacq. (chinese rose) and *Tagetes patula* L. (French marigold) are applied flowers in the gastronomy, being also acknowledged for their phytochemical and medicinal properties.

Native to central China and known commonly as the Chinese rose, *R. chinensis flowers* can be found in the following colors: redish, pale pink and creamy white. The rose flowers have numerous culinary utilities such as salads, entrees, cakes and pastries (Creasy, 1999; Felippe, 2004). The *T. patula* L. from Central America and Mexico, which are popularly known as French marigold flowers are used in culinary preparations as well, the petals are used in garnishes, salads and in the food colourings extraction (Rop *et al*., 2012; Politi *et al*., 2016).

In this framework, the purpose of this study was to evaluate the effects of gamma and electron beam irradiation doses (0, 0.5, 0.8 and 1.0 kGy) on the carotenoids of *Rosa chinensis* and *Tagete patula* flowers.

2. MATERIAL AND METHODS

2.1. Samples

Samples of fresh flowers of *R. chinensis* and *T. patula* were purchased from a local market in São Paulo, Brazil and petals were used in the present study: the flowers were commercialized inside polyethylene bags.

2.2. Electron-beam irradiation

The samples were irradiated at the Nuclear and Energy Research Institute – IPEN/CNEN (São Paulo, Brazil) using an electron beam accelerator (IBA Industrial Inc., Edgewood, NY, USA), at room temperature. The applied doses were 0.5 kGy (dose rate: 2.22 kGy/s, energy: 1.400 MeV, beam current: 0.3 mA, tray speed: 6.72m/ min), 0.8 kGy (dose rate: 3.56 kGy/s, energy: 1.400 MeV, beam current: 0.48 mA, tray speed: 6.72 m/min) and 1.0 kGy (dose rate: 4.46 kGy/s, energy: 1.400 MeV, beam current: 0.6 mA, tray speed: 6,72 m/min). Non-irradiated samples were used as a control. After irradiation, samples were lyophilized (Solab SL404, São Paulo, Brazil).

2.3. Gamma irradiation

Samples were irradiated at the Nuclear and Energy Research Institute – IPEN/CNEN, (São Paulo, Brazil). Using a ^{60}Co source Gammacell 200 (Nordion Ltd., Ottawa, ON, Canadá), at room temperature, with dose rate of 0.835 kGy/h, at doses 0.5, 0.8 and 1.0 kGy. Non-irradiated samples were used as a control. Harwell Amber 3042 dosimeters were used to measure the radiation dose. After irradiation, the samples were lyophilized (Solab SL404, São Paulo, Brazil).

2.4. Analysis of carotenoid

Carotenoid extraction was done by following a procedure adapted from Sérino *et al.* (2009). Microextraction was performed in 2 mL amber microtubes containing sample (≈0.1 g) of edible flowers lyophilized. Posteriorly was added 100 µL of saturated aqueous NaCl solution to the sample and the microtubes were placed in the vortex (maximum speed) mixed for 30 s to ensure that the solution was mixed. After the addition of 200 µL of dichloromethane, the sample was also mixed in the vortex (maximum speed) for 30 s. Hexane: ethyl mixture (1:1 *v/v*; 500 µL) was added and agitation was vortexed (maximum speed) for 30 s. Subsequent, Centrifugation was applied for 5 min at 4 ºC, 13000 rpm and transferred the organic fraction for 2mL amber microtubes.

The residue was extracted three times by using hexane: ethyl (1:1 *v/v*; 500 µL) under stirring followed per centrifugation. The obtained supernatant was fully evaporated at nitrogen atmosphere, resuspended again in 500 µL of ethyl acetate and filtered through a 0.45-µm disposable LC filter disk for high performance liquid chromatography analysis. Analyses were performed in triplicate.

Carotenoid analyzes were performed using Shimadzu LC-20AT series (Tokyo, Japan) equipped with isocratic pump system (LC-20AT), an automatic injector (SIL 20A), a UV–visible detector with photodiode arrays (SPD-M20A) and column oven (CTO 6A). The conditions for chromatographic separation were: C_{18} column (LiChroCART 250-4 LiChrospher® 100 RP-18 endcapped 100 x 4.6 mm particle size 5 µm - Merck); mobile phase consisting of acetonitrile: water: ethyl acetate (53:7:40, *v/v/v*) and 1 mL/min stream; temperature of 30 °C; injection volume 10 µL; absorbance spectrum of 200-600 nm. The carotenoid present in the edible flowers samples were characterized according to their UV and retention times compared with commercial standards.

2.5. Statistical Analysis

The results were analyzed using the program GraphPad Prism (version 7.0), which was also used for the elaboration of tables. The comparisons between the data were performed using the two-factor ANOVA and Bonferroni post-analysis with a limit for statistical significance of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1.Quantification of carotenoid composition

The results showed statistically that the non-irradiated samples of the flower species *T. patula* as value of lutein and α-carotene (0.0650 mg/100 g and 5.6938 mg/100 g) presented an excellent carotenoid content in relation to flower species *R. chinensis* (0.0207 mg/100 g and 0.1524mg/100 g), showing no significant difference, proving to be an excellent source of carotenoids and a precursor of vitamin A, expressed in table 1.

Table 1- Quantification of carotenoids (mg/100 g) of extracts according to flower species in non-irradiated samples.

Carotenoids	Flower species	
	Tagete patula	***Rosa chinensis***
Lutein	0.0650±0.0001	0.0207±0.0021
α -carotene	5.6938±0.0001	0.1524±0.0064

Values represent the mean ± standard deviation.

Compared with the carotenoid content in the composition of some fruits and vegetables studied in the work of Dias & Oliveira (2015) where was investigating the potential value of carotenoids in fruits and vegetables of Portuguese origin. Leafy vegetables analyzed (leaf of beet and leaf of cabbage) presented higher value in lutein of 4.4 mg/100 g and 7.2 mg/100 g, respectively, in comparison with the flowers of *patagés* species and *R.chinensis.* This is explained by the fact that the lutein content is greater in green and dark green coloring vegetables

For the fruits studied, the value of lutein was lower (apple, cherry, orange, pear and peach with values of 0.0097 mg/100 g; 0.16mg/100 g; 0.072 mg/100 g, 0.0088 mg/100 g and 0.075 mg/100 g, respectively) when compared to *T. patula* and *R. chinensis* flowers. The *T. patula* species showed higher lutein content than the values found in apple, orange and pear, while the values found in *R. chinensis* were higher than apple and pear.

The species of edible flowers analyzed, the high content of α-carotene was found in relation to the fruits studied by Dias & Oliveira (2015), which were 0.037 mg/100 g; 0.027 mg/100 g; 0.0082 mg/100 g for cherry, orange and peach, respectively. On the other hand, the values found in *T .patula* were 5.6938 mg/100 g and for *R. chinensis* were 0.1524 mg/100 g. Vegetable products of the study of Dias & Oliveira (2015) the carotenoid to α-carotene, were not detected.

3.2. The effects of irradiation on flowers.

The results of the carotenoid content of *T. patula* processed with gamma radiation and electron- beam are shown in Tables 2 and 3.

Table 2- Carotenoid content (mg/100 g) of *T. patula* flowers irradiated by an electron accelerator.

	Carotenoids	
Dose (kGy)	**Lutein**	**α -carotene**
Control	0. 0650±0.0001[a]	5. 6938 ±0.0001[a]
0.5	0.0325±0.0007[a]	1.4842±1.2372[a]
0.8	0.0297±0.0023[a]	1. 4743±1.0442[a]
1.0	0.0260±0.0020[a]	1.2137±0.8108[a]

Values represent the mean ± standard deviation.
In column row different letters mean significant difference ($p < 0.05$)

Table 3- Carotenoid content (mg/100 g) of *T. patula* flowers irradiated by ^{60}Co.

	Carotenoids	
Dose (kGy)	**Lutein**	**α -carotene**
Control	0.0650±0.0001 [a]	5.6938 ±0.0001 [a]
0.5	0.0400±0.0291 [a]	3.6678±2.1595 [a]
0.8	0.0505±0.0120 [a]	2.9252±1.3541 [a]
1.0	0.0490±0.0028 [a]	2.2480±1.5659 [a]

Values represent the mean ± standard deviation.
In column row different letters mean significant difference ($p < 0.05$)

According to the results, the lutein and α-carotene content of *T. patula* submitted to the ionizing radiation did not significantly affect carotenoid levels independently of the technology used. In the samples processed by electron-beam a decrease of the carotenoid content was observed according to the increase of the doses. This event was also observed in Lima *et al.* (2004, 2011) with carrots treated with gamma irradiation at low doses (0.25 to 2.0 kGy) and tucumã (*Astrocaryum vulgare* Mart.) wich is a fruits native from Amazonia, and concluded that this reduction of carotenoids with increasing doses is related due to the oxidation and breakage of the chemical bonds present in the sample. Samples irradiated with 0.8 kGy (gamma) presented the highest concentrations of lutein in relation to the dose of 0.5 and 1.0 kGy. However, there was no significant change in carotenoid levels.

The results of the carotenoid content of *R.chinensis* processed with gamma radiation and electron-beam are shown in tables 4 and 5.

Table 4- Carotenoid content (mg/100 g) of *R. chinensis* flowers irradiated by an electron accelerator.

	Carotenoids	
Dose(kGy)	**Lutein**	**α -carotene**
Control	0.0207±0.0021	0.1524±0.0064[a]
0.5	ND	0.0475±0.0320[b]
0.8	ND	0.0399±0.0080[c]
1,0	ND	0.0574±0.0093[d]

Values represent the mean ± standard deviation.
In column row different letters mean significant difference ($p < 0.05$)

The lutein present in the *R.chinensis* species was not detected in the electron-beam irradiated samples, what demonstrated that the irradiation affected the composition of carotenoids. Concerning the content of the α-carotene observed that decreased with increasing of the dose received radiation. The results the samples treated with 1.0 kGy dose indicated high values in relation the dose 0.8 kGy.

Table 5 - Carotenoid content (mg/100 g) of *R. chinensis* flowers irradiated by ^{60}Co.

	Carotenoids	
Dose(kGy)	**Lutein**	**α -carotene**
Control	0.0207±0.0021 [a]	0.1524±0.0064 [a]
0.5	0.0180±0.0014 [a]	0.1017±0.0255 [a]
0.8	0.0350±0.0014 [a]	0.1103±0.0740 [a]
1.0	0.0230±0.0001 [a]	0.0868±0.0132 [a]

Values represent the mean ± standard deviation.
In column row different letters mean significant difference ($p < 0.05$)

The results for samples processed by gamma shows that no significant differences in the values of the carotenoids lutein and α-carotene in relation to the control. However, a favorable effect on lutein content was observed in the samples processed at doses of 0.8 and 1.0 kGy (0.0350 mg/100 g and 0.0230 mg/100 g) compared to the non-irradiated sample (0.0207 mg/100 g).

Similar results were found by Fanaro *et al.* (2015) in samples of green tea irradiated with different doses where was reported the favoring of the its antioxidant capacity. The same was reported by Koike, *et al.* (2015) in the study of edible flowers of *Viola tricolor*, treated by different doses and irradiation technologies (cobalt-60 and electron-beam) where was observed a higher content of bioactive compounds.

4. CONCLUSIONS

According to the results presented in this work, it was concluded that the radiation treatment did not affect the carotenoids present in the species of edible flowers studied. In addition, doses of 0.8 and 1.0 kGy in the samples processed by gamma showed better conservation of the lutein compound.

Thus, the radiation process has proven to be a viable technology to preserve the quality of edible flowers, offering also the possibility of its application in the extension of useful life.

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