

ANALYSIS OF CAROTENOIDS IN EDIBLE FLOWERS OF *DIANTHUS CHINENSIS* PROCESSED BY IONIZING RADIATION

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ABSTRACT

Over the last few years, the trend of using flowers in gastronomy, edible flowers are used in food preparations in order to add beauty, color and flavor. On the other hand, several species have active biologically substances, which play an important role in health maintenance. These highly perishable food should be grown without the use of pesticides. Thus, several methods are applied to increase the shelf life of food products, as well as ensure their quality and safety. Among the treatments, the food irradiation process has proven to be an effective tool in preserving and extending the shelf life of perishable product. *Dianthus chinensis* flowers, popularly known as Chinese pink (cravina), belongs to the family Caryophyllaceae, are native to Asia and Europe, are widely used in culinary preparations, being also acknowledged for their bioactive components and antioxidant properties. The purpose of this study was to evaluate carotenoids in *D. chinensis* flowers submitted to gamma irradiation and electron beam doses of 0.5, 0.8 and 1.0 kGy. High performance liquid chromatography was used to carotenoids determination. In the specie of edible flowers analyzed it was found carotenoid lutein (2.77 to x 7.52 mg/ 100 g). In general, the lutein was higher for irradiated samples, especially those treated with 0.8 and 1.0 kGy independently of irradiation technology. Accordingly, the applied irradiation treatments seemed to represent a feasible technology to preserve the quality of edible flower petals.

1. INTRODUCTION

Carotenoids are lipid-soluble pigments widely distributed in nature, which can be found in flowers, fruits and vegetables, as well as some kinds of animals (e.g. birds, insects and marine animals). These pigments naturally exhibit yellow, orange and red colors [1-3]. Plants, algae, fungi and bacteria can produce carotenoids through biosynthesis, whereas carotenoids found in humans (lycopene, lutein and zeaxanthin) are only from diets [4,5].

The growing interest in carotenoids has increased because of their human health benefits and researchers have reported consumption of carotenoids-rich sources can help reduce the risk of degenerative diseases; several disorders; cardiovascular diseases and ophthalmological diseases due to their antioxidant properties [6-8]. In addition, some carotenoids provide additional health benefits since they are pro-vitamin A or can be converted into vitamin A in human body [9,10].

Thus, several carotenoids are used in food-related industries as natural food colorants and increased the searching for new natural sources, including wild vegetables and fruits [10, 11]. In this sense, edible flowers can be a source of phytochemicals, including carotenoids and natural dyes. The edible flowers market is in full expansion in the worldwide and flowers can be regarded as Unconventional Edible Plants.

Moreover, to its nutritional value, plant-based foods are considered to be currently a way of incorporating the concepts of health in the diet. Furthermore, the plant-based food manufacturing sector is currently under expansion through development of innovative products using edible flowers [12,13].

Once the edible flowers are rich sources of various polyphenolic compounds with antioxidant capacities and contain carotenoids (color); essential oils (aroma); isothiocyanates (flavor) and cyclotides (nutritious plant peptides) all compounds bioactive potential [14-18].

Hundreds of edible flowers have been identified. Among them, *Dianthus chinensis* one of the popular edible flowers which has been used from ancient times, their petals are one of the ingredients of the famous French liqueur *Chartreuse*.

The *D. chinensis* (China pink) is belongs to the family Caryophyllaceae, originally native to Europe and Asia, popularly known as Chinese pink, Rainbow pink and pink. Dianthus flowers are flattened with shades of white, pink, purple and red or bicolor. The petals have a pleasant spicy, floral, clove-like taste and are applied in salads, sandwiches, jellies, pies and in the aromatization of vinegar and wine [19-23].

In addition to the visual importance in gastronomic preparations, the flowers of China Pink contain non-nutrient compounds, antioxidant compounds, which present physiological and/or metabolic effects in the human organism, such as phenolic compounds and carotenoids [24,25].

However, the edible flowers are highly perishable foods and must be insect free, which represents a challenge. Its high perishability requires storage in small plastic containers in refrigerated environments, which is an additional cost in the commercial chain. Treatments that extend the life and ensure the safety of these products could be alternatives to minimize such problems [26-28].

Several methods are applied by the food industry to increase the shelf life of perishable food products, as well as ensure quality and safety. Ionizing radiation treatment might be the answer to these problems, ensuring food quality, increasing shelf-life, food safety and disinfestation of foods. The process of food irradiation has been shown to be an effective tool in preserving and extending the shelf life of perishable products, insect disinfestation, improving sanitary quality and food safety, can be used to treat a wide variety of foods [28-30].

Thus, the purpose of this study was to evaluate the effects of different gamma and electron beam irradiation doses (0, 0.5, 0.8 and 1.0 kGy) on the carotenoids of *Dianthus chinensis*.

2. MATERIAL AND METHODS

2.1. Samples

Samples of fresh flowers of *D. chinensis* were purchased from a local market in São Paulo, Brazil. The edible flowers were commercialized inside polyethylene bags. Chinese pink petals demonstrate different phenotypes were used in the study: pink, red, white and multicolored.

2.2. Samples irradiation

Edible flowers irradiation processing was carried out at the Nuclear and Energy Research Institute – IPEN/CNEN (São Paulo, Brazil).

Samples were irradiated 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) and a ⁶⁰Co source Gammacell 200 (Nordion Ltd., Ottawa, ON, Canadá), at room temperature, with a dose rate of 0.835 kGy/h, at doses 0.5, 0.8 and 1.0 kGy. Harwell Amber 3042 dosimeters were used to measure the radiation dose.

Non-irradiated samples were used as a control and after irradiation, the samples were lyophilized (Solab SL404, São Paulo, Brazil).

2.3. Analysis of carotenoid by high performance liquid chromatography (HPLC)

The analysis of carotenoid was conducted at Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Science, University of São Paulo, São Paulo, Brazil.

2.3.1 Sample preparation

Carotenoid extraction was following a procedure adapted of methodology described Sérino et al. [31]. Microextraction was performed in 2 mL amber microtubes containing sample (≈0.1 g) of edible flowers lyophilized. The sample was addition of 100 µL of saturated aqueous NaCl

solution and the microtubes were vortex (maximum speed) mixed for 30 s. After the addition of 200 µL of dichloromethane, agitation was vortex (maximum speed) for 30 s. Hexane: ethyl mixture (1:1 v/v; 500 µL) was added and agitation was vortex (maximum speed) for 30 s. Centrifugation for 5 min at 4 °C, 13000 rpm and transferred the organic fraction for 2 mL amber microtubes.

2.3.2 Analysis of carotenoid

The residue was extracted three times with hexane: ethyl (1:1 v/v; 500 µl) and the stirring and centrifugation. The obtained supernatant was fully evaporated in an atmosphere of nitrogen, resuspended 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 by 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: C₁₈ 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.

2.4. Statistical Analysis

The results were analysed using the program GraphPad Prism (version 8.0). 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

Comparing the carotenoid content in the composition of some fruits and vegetables studied in the work of [] where was investigated the potential value of carotenoids in fruits and vegetables of Portuguese origin, it was observed that leafy vegetables analyzed (leaf of beet and leaf of cabbage) presented higher value in lutein of 4.4 mg/100g and 7.2 mg/100g, respectively, in comparison with the flowers of *patagés* species and *R. chinensis*. This is explained by the fact that the lutein content is more presented in greener and darker 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 *D. chinensis* flowers. The *D. patula* species showed higher lutein content than the values found in apple, orange and pear, while the values found in *D. 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 [] 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 [] the carotenoid to α -carotene, were not detected.

The results of the carotenoid content of *D.chinensis* processed with gamma radiation and electron- beam are shown in Tables 1 and 2.

Table 1- Carotenoid content (mg/100g) of *D. chinensis* flowers irradiated by an electron accelerator.

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

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

Table 2- Carotenoid content (mg/100g) of *D. chinensis* flowers irradiated by an ⁶⁰Co.

Carotenoids			
Dose(kGy)	Lutein	α -carotene	β -carotene
Control	0.1524±0.0064 ^a	ND	ND
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0.8	0.0399±0.0080 ^c	ND	ND
1,0	0.0574±0.0093 ^d	ND	ND

Values represent the mean \pm 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 [] 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 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.

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 [] in samples of green tea irradiated with different doses where was reported the favoring of the its antioxidant capacity. The same was reported by [] 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.

3. 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 flower 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.

ACKNOWLEDGMENTS

The authors would like to thank CNPq, IPEN-CNEN/SP, CNEN and FCF/USP for the structure and financial support.

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