Analysis of carotenoids in edible flowers of *Dianthus chinensis* processed by ionizing radiation

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\textbf{ABSTRACT}

*Dianthus chinensis* flowers are widely used in culinary preparations and are appreciated because of its bioactive compounds. It is a perishable food that should be grown without the use of pesticides. In this context, food irradiation is a process that has proven to be an efficient tool in preserving and extending the perishable product shelf life without changing the temperature or leaving residues. 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 for carotenoid determination was used. In the edible flower analyzed was found carotenoid lutein (4.02 to 7.52 mg/100 g). The lutein was higher for irradiated samples, especially those treated with 0.8 and 1.0 kGy in both irradiation technology. In conclusion, the lutein amount in the chinese pink enhances as the dose increases, and the applied irradiation treatments represent a feasible technology to preserve the nutritional quality of edible flower petals as well as attend to food safety requirements.

\textit{Keywords:} food irradiation, chinese pink, bioactive compounds
1. INTRODUCTION

Carotenoids are lipid-soluble pigments widely distributed in nature found in flowers, fruits, and vegetables, as well as some kinds of animals (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 are exclusively from diets (lycopene, lutein, and zeaxanthin) [4,5].

The interest in carotenoids has increased because of its human health benefits and researches reporting that consumption of carotenoid-rich sources auxiliary at reducing the risk of degenerative diseases, disorders, cardiovascular and ophthalmological diseases due to its antioxidant properties [6-8]. Some carotenoids provide additional health benefits since they are pro-vitamin A or can be converted into vitamin A in the human body [9,10].

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

Moreover, to its nutritional value, plant-based foods are considered to be a way of incorporating the concepts of health in the diet. For this reason, the plant-based food manufacturing sector development of innovative products using edible flowers [12,13]. The edible flowers are rich sources of various polyphenolic compounds with antioxidant capacities, carotenoids (color), essential oils (aroma), isothiocyanates (flavor), and cyclotides (nutritious plant peptides) all bioactive compound potential [14-18].

Dianthus chinensis is 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 belongs to the family Caryophyllaceae, originally native to Europe and Asia, popularly known as chinese pink, rainbow pink. Dianthus flower is flattened with shades of white, pink, purple, and red or bicolor, the petals have a pleasant spicy, floral, clove-like taste. It is present in salads, sandwiches, jellies, pies, and the aromatization of vinegar and wine [19-23].
In addition, to the visual importance in gastronomic preparations, the flowers of Chinese 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 ensure food quality and safety. Ionizing radiation treatment answers these problems because it is effective in preserving and extending the shelf life of perishable products, insect disinfestation, improving sanitary quality, and food safety. It also can be used to treat a wide variety of foods [28-30].

Food irradiation is one of the few food technologies that can maintain food quality and address food safety and security problems without significantly affecting a food’s sensory or nutritional attributes [31]. In order to reduce the incidence of food-borne disease, this processing technique exposes food to radiation in order to destroy pathogens organisms [32].

Processing in gamma irradiators exposes products in their final packaging to controlled doses of gamma radiation from Cobalt-60 [33]. In the radiation by accelerated electrons (electron beam or e-beam) the products to be irradiated are exposed to machine-generated electrons calibrated to a conveyor speed necessary to achieve a desired dose [34]. The both technologies provide food products microbiologically safe for consumption.

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

2. MATERIALS AND METHODS

2.1. Samples

Fresh flowers sample 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

2.2.1. Electron beam irradiation

Samples were irradiated at 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: 6.72 m/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 the dosimetry was with ESR/Alanine system. After irradiation, samples were lyophilized (Solab SL404, São Paulo, Brazil) and kept in the best conditions for subsequent use.

2.2.2. Gamma irradiation

The samples were irradiated at 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 a dose rate of 0.835 kGy/h, at doses of 0.5, 0.8 and 1.0 kGy. Harwell Amber 3042 dosimeters were used to measure the radiation dose. After irradiation, samples were lyophilized and kept in the best conditions for subsequent use.
2.3. Analysis of carotenoid by high performance liquid chromatography (HPLC)

The analysis of carotenoid was conducted at the Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Science, University of São Paulo, São Paulo, Brazil. Carotenoid extraction was following a procedure adapted from methodology described Sérino et al. [35].

The powdered samples (~0.1 g) were extracted by vigorous shaking with 100 µL of saturated aqueous NaCl solution, 200 µL of dichloromethane, and Hexane: ethyl mixture (1:1 v/v; 500 µL) at room temperature. Then, it was centrifuged for 5 min at 4 °C, 13000 rpm, and the organic fraction was transferred to amber microtubes.

The residue was re-extracted three times with hexane: ethyl (1:1 v/v; 500 µL). The obtained supernatant was evaporated in the atmosphere of nitrogen and re-dissolved in 500 µL of ethyl acetate and filtered through a 0.45-µm disposable LC filter disk for high performance liquid chromatography (HPLC) analysis. The analysis was performed in triplicate.

Carotenoids were analyzed using a Shimadzu LC-20AT series chromatograph (Tokyo, Japan) with an isocratic pump system (LC-20AT) and UV–visible detector with photodiode arrays (SPD-M20A).

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 of acetonitrile: water: ethyl acetate (53:7:40, v/v/v) and 1 mL/min stream; temperature of 30 ºC; injection volume 10 µL; absorbance spectrum of 200-600 nm.

The carotenoids present in the flower samples were characterized according to their UV and retention times compared with commercial standards.

The results were analyzed 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

In the species of edible flowers analyzed It was found carotenoid lutein of 4.02 to 7.52 mg/100 g (Table 1). The 0.5 and 0.8 kGy doses showed no difference to non-irradiated samples in the electron beam tests, and 1.0 kGy presented the highest lutein content (7.52±0.43). On the other hand, the doses are not different from each other but are from the sample without irradiation in the
gamma irradiation tests. According to the results, the lutein content of Chinese pink submitted to the ionizing radiation did positively and significantly affect carotenoid levels in both irradiation technologies.

**Table 1.** Carotenoid content of Chinese pink (*Dianthus chinensis* L.) irradiated by an electron accelerator and Gamma irradiation.

<table>
<thead>
<tr>
<th>Doses</th>
<th>Electron Accelerator</th>
<th>Gamma irradiation (60Co)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-irradiated</td>
<td>4.02±0.24^a^A</td>
<td>4.02±0.24^a^A</td>
</tr>
<tr>
<td>0.5</td>
<td>4.41±0.61^a^A</td>
<td>5.75±0.23^b^B</td>
</tr>
<tr>
<td>0.8</td>
<td>4.37±0.29^a^A</td>
<td>6.51±0.13^b^B</td>
</tr>
<tr>
<td>1.0</td>
<td>7.52±0.43^b^B</td>
<td>5.75±0.61^b^B</td>
</tr>
</tbody>
</table>

Mean value followed by their standard deviation.
Different small letters in the same column means statistical difference by the Tukey test (*p* > 0.05).
Different capital letters in the same column means statistical difference by the Tukey test (*p* > 0.05).

Similar results have been reported in studies on the potential value of carotenoids in fruits and vegetables of Portuguese origin (Table 2), it was observed that leaf of beet and leaf of cabbage vegetables analyzed presented value in lutein of 4.4 mg/100 g and 7.2 mg/100 g, respectively [36].

**Table 2.** Lutein value in fruits non-irradiated by others authors.

<table>
<thead>
<tr>
<th>Food</th>
<th>Lutein (mg/100 g)</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato (<em>Lycopersicon esculentum</em> M.)</td>
<td>8.1</td>
<td>Dias, Oliveira (2015) [36]</td>
</tr>
<tr>
<td>Cabbage (<em>Brassica oleracea</em> L.)</td>
<td>7.2</td>
<td>Dias, Oliveira (2015) [36]</td>
</tr>
<tr>
<td>Turnip (<em>Brassica rapa</em> L.)</td>
<td>5.6</td>
<td>Dias, Oliveira (2015) [36]</td>
</tr>
<tr>
<td>Purslane (<em>Portulaca oleracea</em> L.)</td>
<td>5.4</td>
<td>Dias, Oliveira (2015) [36]</td>
</tr>
<tr>
<td>Leaf of beet (<em>Beta vulgaris</em> L.)</td>
<td>4.4</td>
<td>Dias, Oliveira (2015) [36]</td>
</tr>
<tr>
<td>Raspberry (<em>Rubus idaeus</em> L.)</td>
<td>0.320</td>
<td>Marinova, Ribarova (2007) [39]</td>
</tr>
<tr>
<td>Blackberry (<em>Rubus fruticosus</em> L.)</td>
<td>0.270</td>
<td>Marinova, Ribarova (2007) [39]</td>
</tr>
<tr>
<td>Blueberry (<em>Vaccinium myrtillus</em> L.)</td>
<td>0.230</td>
<td>Marinova, Ribarova (2007) [39]</td>
</tr>
<tr>
<td>Blackcurrant (<em>Ribes nigrum</em> L.)</td>
<td>0.210</td>
<td>Marinova, Ribarova (2007) [39]</td>
</tr>
<tr>
<td>Cherry (<em>Prunus armeniaca</em> L.)</td>
<td>0.160</td>
<td>Dias, Oliveira (2015) [36]</td>
</tr>
<tr>
<td>Apple (<em>Malus domestica</em> Borkh)</td>
<td>0.097</td>
<td>Dias, Oliveira (2015) [36]</td>
</tr>
<tr>
<td>Pear (<em>Pyrus communis</em> L.)</td>
<td>0.088</td>
<td>Dias, Oliveira (2015) [36]</td>
</tr>
<tr>
<td>Peach (<em>Prunus persica</em> L.)</td>
<td>0.075</td>
<td>Dias, Oliveira (2015) [36]</td>
</tr>
<tr>
<td>Orange (<em>Citrus sinensis</em> L.)</td>
<td>0.072</td>
<td>Dias, Oliveira (2015) [36]</td>
</tr>
<tr>
<td>Redcurrant (<em>Ribes nigrum</em> L.)</td>
<td>0.028</td>
<td>Marinova, Ribarova (2007) [39]</td>
</tr>
<tr>
<td>Strawberry (<em>Fragaria vesca</em> L.)</td>
<td>0.021</td>
<td>Marinova, Ribarova (2007) [39]</td>
</tr>
</tbody>
</table>

However, the carotenoids content at origin Portuguese fruits and vegetables composition studied by [36] were similar to those found in *D. chinensis* and the foods with the highest content of lutein:
purslane (*Portulaca oleracea* L.) 5.4 mg/100 g; turnip (*Brassica rapa* L.) 5.6 mg/100 g; cabbage (*Brassica oleracea* L.) 7.2 mg/100 g and tomato (*Lycopersicon esculentum* M.) 8.1 mg/100 g.

Similar results were found by [37] in green tea samples irradiated with different doses where it was reported the favoring of its antioxidant capacity. The same was reported by [38] in the study of edible flowers of *Viola tricolor*, treated by different doses and irradiation technologies (cobalt-60 and electron-beam) where there was observed a higher content of bioactive compounds.

For the fruits studied, the value of lutein was lower (apple, cherry, orange, pear and peach with values of 0.0097 mg/100 g; 0.16 mg/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 samples 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 [36].

Demonstrating to be an excellent source of carotenoids and a precursor of vitamin A. In Bulgaria, similar studies were conducted on bioactive substances in food in six berry species: raspberry (*Rubus idaeus* L.); strawberry (*Fragaria vesca* L); blackberry (*Rubus fruticosus* L); blueberry (*Vaccinium myrtillus* L); blackcurrant (*Ribes nigrum* L) and redcurrant (*Ribes nigrum* L) for determination of carotenoid content. The foods with the highest lutein levels were raspberry (0.317 mg/100 g) and blackberry (0.271 mg/100 g) [39].

4. CONCLUSION

Carotenoid lutein content was positively affected by the irradiation process. The samples irradiated with an electron beam showed better performance than the gamma at the dose of 1.0 kGy. Both reached the goal of maintaining nutritional quality since the irradiated samples had higher lutein content than the non-irradiated samples. Confirming that irradiation is useful to expand the post-harvest storage also preserving the quality of the edible flowers and promoting their development of new commercial solutions for food.

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REFERENCES


