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ANTIOXIDANT ACTIVITY OF *DIANTHUS CHINENSIS* L. FLOWERS PROCESSED BY IONIZING RADIATION

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ABSTRACT

Edible flowers are increasingly used in culinary preparations, which require new approaches to improve their conservation and safety. Irradiation treatment is safe and an effective alternative for food conservation, guaranteeing food quality, increasing shelf-life and disinfestation. This technology offers a versatile way to get good quality food while reducing post-harvest losses. *Dianthus chinensis* L. flowers, popularly known as Chinese pink, are widely used in culinary preparations, being also acknowledged for their bioactive components and antioxidant properties. The purpose of this study was to evaluate the antioxidant activity of *D. chinensis* flowers submitted to electron beam and gamma irradiation at 0, 0.5, 0.8 and 1 kGy. The antioxidant properties were evaluated through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, reducing power and β-carotene bleaching inhibition assays. Total phenolics were determined by the *Folin-Ciocalteu* assay. The antioxidant activity was higher for irradiated samples, especially those treated with 0.5 and 0.8 kGy, independently of the radiation source, which showed the highest capacity to inhibit β-carotene bleaching inhibition. Accordingly, the applied irradiation treatments seemed to represent feasible technology to preserve the quality of edible flower petals, being able to improve the antioxidant activity

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1. INTRODUCTION

The edible flower market is expanding in Brazil and in the world, due to the growing interesting of applying edible flowers in gastronomy, therefore increasing its consumption and consequently generating an economic growth.

Edible flowers have been used for many years in culinary preparations for the purpose of adding beauty, aroma, color and flavor. Currently, this type of application in gastronomy aims to improve the sensory and nutritional quality of foods, since several species have biologically active substances [1-4].

There are several studies showing that edible flowers are rich in bioactive compounds and contain numerous phytochemicals. These bioactive compounds are capable of acting in the

prevention of chronic diseases such as cardiovascular, cancer, age-related and degenerative diseases [4-6].

However, these flowers are highly perishable and must be free from diseases and contaminants, which is a challenge as they are usually grown without the use of pesticides. Its high perishability requires storage in small plastic containers and refrigerated environments, which represents an additional cost in the commercial chain. Various methods are applied by the food industry to increase the shelf life of food products as well as to ensure their quality and safety [7-9]. Alternative treatments capable of increasing shelf life and ensuring the safety of these products could minimize such problems [10].

Studies have shown that the application of ionizing radiation is effective in the disinfestation, conservation, as well as the extension of the shelf life of the food product [9, 11].

International organizations encourage the adoption of international standards for phytosanitary measures, use of technology to prevent the introduction and dissemination of contaminants. Food irradiation is a postharvest quarantine treatment, effective in the disinfestation of food and to maintain its quality, being approved in several countries [11-13]. Flowers are relatively sensitive to ionizing radiation and the sensitivity of cut flowers to the use of radiation varies from species to species [14,15].

Dianthus chinensis L. belongs to the family Caryophyllaceae, which are native to Asia and Europe, popularly known as Chinese pink or just pink. Dianthus flowers are flattened with shades of white, pink, purple and red or bicolor. The petals have a spicy flavor and are used in salads, sandwiches, jellies, pies and in the aromatization of vinegar and wine [16-18].

Therefore, the purpose of this study was to evaluate the antioxidant activity of *D. chinensis* flowers submitted to electron beam and gamma irradiation at 0 (control), 0.5, 0.8 and 1.0 kGy.

2. MATERIAL AND METHODS

2.1 Sample

The edible flowers of *Dianthus majus* L. were purchased from a local market in São Paulo, Brazil, presenting different phenotypes.

2.2 Irradiation

Radiation processing was carried out at the Institute of Energy and Nuclear Research - IPEN/CNEN, São Paulo, SP - Brazil. Non-irradiated samples were used as the control group. After irradiation, the samples were lyophilized (SL404, Solab, São Paulo, Brazil) and stored in a hermetically sealed package.

2.2.1. Gamma irradiation

The samples were irradiated at the Nuclear and Energy Research Institute - IPEN – CNEN/SP (São Paulo, Brazil), using a ^{60}Co source Gammacell 200 (Nordion Inc., Ottawa, ON, Canadá), at room temperature (25 ± 2 °C), with a dose rate of 1.258 kGy/h. Applied doses 0.5, 0.8 and 1 kGy. Harwell Amber 3042 dosimeters were used to measure the radiation dose.

2.2.2. Electron beam irradiation

Samples were irradiated at Nuclear and Energy Research Institute – IPEN - CNEN/SP (São Paulo, Brazil), using an electron beam accelerator (IBA Industrial Inc., Edgewood, NY, USA), at room temperature (25 ± 2 °C). The applied doses were 0.5 kGy (dose rate: 1.11 kGy/s, energy: 1.400 MeV, beam current: 0.3 mA, tray speed: 6.72 m/min), 0.8 kGy (dose rate: 1.78 kGy/s, energy: 1.400 MeV, beam current: 0.48 mA, tray speed: 6.72 m/min) and 1.0 kGy (dose rate: 2.23 kGy/s, energy: 1.400 MeV, beam current: 0.6 mA, tray speed: 6.72 m/min).

2.3 Antioxidant activity

The extracts preparation and antioxidant activity were carried out at the Laboratory of Applied Chemistry and Biochemistry (LQBA) in the School of Agriculture of the Polytechnic Institute of Bragança - Portugal.

2.3.1 Preparation of the extracts

The methanol:water (80:20, v/v) extract was prepared from the powdered flower. Samples (≈0.5 g) were stirred with 20 mL of the solvents mixture, at room temperature, 150 rpm for 1 h. The extract was filtered through Whatman No. 4 paper and the residue was re-extracted with 20 mL of methanol/water (80:20, v/v). The combined hydromethanolic extracts were evaporated at 35 °C (rotary evaporator Büchi R-210, Flawil, Switzerland) to remove the organic solvent, and the aqueous extract was frozen and lyophilized. The dry extract was re-dissolved in methanol:water (80:20, v/v) at a known concentration to perform the antioxidant activity assays.

2.3.2 DPPH radical –scavenging activity

The DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical scavenger activity was evaluated according to previous authors [19]. The samples (30 μL) of different concentrations of the extract solutions were added to the wells of a 96 well microplate with the methanolic solution (270 μL) containing DPPH radicals (6×10^{-5} mol/L). The mixture was left to stand in the dark for 30 minutes, and the absorbance was measured at 515 nm by using an ELX800 microplate reader (Bio-Tek Instruments, Inc; Winooski, USA).

2.3.3 Reducing power

Reduction power was evaluated according to a methodology described previously [20]. This methodology was performed using the Microplate Reader described above and measuring the absorbance at 690 nm. The different concentrations of the extracts (0.5 mL) were mixed with sodium phosphate buffer (200 mmol L^{-1}, pH 6.6, 0.5 mL) and potassium ferricyanide (1% w/v, 0.5 mL) and the mixture was incubated at 50 ºC for 20 min, and trichloroacetic acid (10% w/v, 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48-well plate, as also ferric chloride (0.1% w/v, 0.16 mL) and deionized water (0.8 mL).

2.3.4 β-carotene/linoleate assay

In the inhibition test of β-carotene discoloration, used the previously described method [21]. A solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 mL of chloroform. Two milliliters of this solution were pipetted into a round-bottom flask. The chloroform was removed at 40 ° C under vacuum and linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 mL) were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing 0.2 mL of different concentrations of the extract solutions. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm (Analytik 200-2004 spectrophotometer, Jena, Germany) and tubes were shaken and incubated at 50 °C in a bath for 2 h and the absorption was measured again.

2.3.5 Total phenolics

The total phenolics were determined by the *Folin-Ciocalteu* assay according to a methodology previously described [19]. The extract solution (1 mL) was mixed with *Folin-Ciocalteu* reagent (5 mL, previously diluted with water 1:10 v/v) and sodium carbonate (75 g L^{-1}, 4 mL). The tubes were vortex mixed for 15s and allowed to stand for 30 min at 40 ºC for color development. Absorbance was then measured at 765 nm. Gallic acid was used to

calculate the standard curve and the results were expressed as mg of gallic acid equivalents (GAE) per g of extract.

2.4 Statistical Analysis

The results of the color were submitted to analysis of variance (ANOVA) followed by a Tukey test with a significance level of 95% ($P < 0.05$).

3. RESULTS AND DISCUSSION

The results regarding antioxidant activity of *D. chinensis* processed with electron beam and gamma irradiation are shown in Tables 1 and 2.

Table 1: Antioxidant activity (EC_{50} values, mg/mL) of *D. chinensis* extracts irradiated by ^{60}Co gamma-rays according to the irradiation dose

Assays	EC_{50} values (mg/mL of extract)			
	Irradiation dose			
	control	0.5 kGy	0.8 kGy	1.0 kGy
DPPH radical-scavenging activity	1.53 ± 0.02^{b}	1.66 ± 0.03^{a}	1.43 ± 0.03^{c}	1.65 ± 0.03^{a}
Reducing power	0.80 ± 0.02^{c}	0.82 ± 0.01^{b}	0.75 ± 0.01^{d}	0.95 ± 0.02^{a}
Inhibition of β-carotene bleaching	0.68 ± 0.05^{b}	0.43 ± 0.01^{d}	0.56 ± 0.06^{c}	1.93 ± 0.05^{a}
Folin-Ciocalteu assay*	80.89 ± 0.69^{a}	80.42 ± 0.35^{a}	80.77 ± 0.37^{a}	74.18 ± 0.50^{b}

In each row different letters mean significant differences ($p < 0.05$)
*Results expressed in mg of gallic acid equivalents (GAE) per g of extract

Table 2: Antioxidant activity (EC_{50} values, mg/mL) of *D. chinensis* extracts irradiated by electron beam according to the irradiation dose

Assays	EC_{50} values (mg/mL of extract)			
	Irradiation dose			
	control	0.5 kGy	0.8 kGy	1.0 kGy
DPPH radical-scavenging activity	1.53 ± 0.02^{ab}	1.28 ± 0.03^{c}	1.48 ± 0.08^{b}	1.60 ± 0.06^{a}
Reducing power	0.80 ± 0.02^{d}	0.98 ± 0.01^{c}	1.08 ± 0.01^{b}	1.19 ± 0.01^{a}
Inhibition of β-carotene bleaching	0.68 ± 0.05^{b}	0.58 ± 0.01^{c}	2.17 ± 0.02^{a}	2.16 ± 0.11^{a}
Folin-Ciocalteu assay*	80.89 ± 0.69^{b}	84.46 ± 0.77^{a}	75.07 ± 0.70^{c}	71.14 ± 0.21^{d}

In each row different letters mean significant differences ($p < 0.05$)
*Results expressed in mg of gallic acid equivalents (GAE) per g of extract

In general, irradiated samples presented slightly higher antioxidant activity, especially samples irradiated with 0.5 kGy (irradiated by electron beam) and 0.8 kGy (irradiated by ^{60}Co gamma-rays). Concerning the effect of irradiation technology, only β-carotene bleaching inhibition was similar between gamma and electron-beam irradiated samples, in which 0.5 kGy was the dose that presented higher antioxidant activity (lower EC_{50} values). Similar effects were observed in studies of influence of the irradiation process on antioxidant substances present in foods and edible flowers, which described a significant increase in the phenolic content of samples of petals *Tropaeolum majus*, *Cammellia sinensis* and *Illx paraguariensis* treated with maximum doses of 10.0 kGy [22–24].

4. CONCLUSIONS

According to the results in the present work, it can be concluded that the sample processing by radiation did not compromise the antioxidant activity of the edible flower species. Consequently, the applied irradiation treatments seemed to represent a feasible technology to preserve the quality of edible flower petals.

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