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Antioxidant activity of *Dianthus chinensis* 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. Indeed, it can also guarantee food quality, increasing shelf-life and disinfestation of it. This technology gives us a versatile way to get good quality food, reducing post-harvest losses. *Dianthus chinensis* 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 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, reducing power and β-carotene bleaching inhibition assays. Total phenolics were also determined by the *Folin-Ciocalteu* assay. The antioxidant activity was higher for irradiated samples, especially those treated with 0.8 and 1 kGy, independently of the radiation source, which showed the highest capacity to inhibit β-carotene bleaching. 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.

Keywords: edible flowers, *Dianthus chinensis*, ionizing radiation, antioxidant activity

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1. INTRODUCTION

The edible flower market is expanding in Brazil and in the world, due to the growing tendency of the application of flowers in the gastronomy and in recent years there has been an increase in consumption, generating an increase of varied and 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].

Several studies showed that edible flowers are rich in bioactive compounds and contain numerous phytochemicals. Bioactive compounds are capable of acting in the prevention of chronic diseases such as cardiovascular, cancerous and age-related and degenerative diseases [4-6]

However, they are highly perishable product and must be free from diseases and insect pests, which is a challenge as they are usually grown without the use of pesticides. Its high perishability requires storage in small plastic containers in 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 ensure their quality and safety [7-9]. Treatments capable of increasing shelf life and ensuring the safety of these products could be alternatives to minimize such problems [10].

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

International organizations encourage the adoption of international standards for phytosanitary measures, use of technology to prevent the introduction and dissemination of pests. Food irradiation is a postharvest quarantine treatment, effective in the disinfestation of food and to maintain its quality, 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 belongs to the family Caryophyllaceae, is native from Asia and Europe, popularly known as chinese pink or pink. Dianthus flowers are flattened with shades of white, pink,

purple and red or bicolor. The petals have a spicy flavor and are applied 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 0.5, 0.8 and 1.0 kGy and control.

2. MATERIALS AND METHODS

2.1 Sample

Fresh edible flowers of *D chineses* L. were purchased from a local market in São Paulo, Brazil. Chinese pink petals presenting different phenotypes (red, white, pink and multi-colored) were used.

2.2 Sample irradiation

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, Canada), at room temperature (25 ± 2 ° C), with a dose rate of 1.258 kGy/h. Applied doses were 0.5, 0.8 and 1 kGy. Harwell Amber 3042 dosimeters were used to measure radiation dose. 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.

2.2.2. Electron beam irradiation

Samples were irradiated at the 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). 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.

2.3 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 lyophilized flowers. Samples (≈0.5 g) was 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) and lyophilized.

2.3.2 DPPH radical –scavenging activity

The DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical scavenger activity was evaluated according to a methodology described [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 [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, it was used by the method described [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 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 15 s and allowed to stand for 30 min at 40 º C 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) and Tukey test with significance level of 95% ($P < 0.05$).

3. RESULTS AND DISCUSSION

The results of the antioxidant activity of the extract 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
Reducing power	0.81±0.01[a]	0.83±0.01[a]	0.75±0.01[a]	0.95±0.02[a]
Folin-Ciocalteu* assay	78.90±3.07[a]	79.98±0.75[a]	79.85±1.44[a]	74.18±0.50[a]
DPPH	1.53±0.03[a]	1.66±0.03[a]	1.43±0.03[a]	1.67±0.06[a]
Inhibition of β-carotene bleaching	0.65±0.03[a]	0.73±0.26[a]	0.58±0.05[a]	1.97±0.81[b]

Mean ± SD (Standard Deviation)
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
Reducing power	0.81±0.01[a]	0.98±0.01[a]	1.08±0.01[a]	1.19±0.01[a]
Folin-Ciocalteu* assay	78.90±3,07[a]	84.46±0,97[a]	74.61±0.98[a]	71.83±1.07[a]
DPPH	1.53±0.03[a]	1.28±0.03[a]	1.48±0.08[a]	1.60±0.06[a]
Inhibition of β-carotene bleaching	0.65±0.03[a]	0.58±0.01[a]	2.17±0.02[b]	2.16±0.11[b]

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

Samples irradiated by electron beam with 0.8 and 1.0 kGy showed the highest capacity to inhibit β-carotene bleaching inhibition. Concerning the effect of irradiation technology, only β-carotene bleaching inhibition was significantly different for gamma and electron-beam irradiated samples. 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. CONCLUSION

According to the results of the tests presented in the present work, it is concluded that the processing of the samples by radiation did not compromise an antioxidant activity of the edible flower species Chinese pink. Consequently, the applied irradiation treatments seemed to represent feasible technology to preserve the quality of edible flower petals

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