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ANALYSIS ON ANTIOXIDANT ACTIVITY BY ORAC METHOD IN *VIOLA TRICOLOR* L. FLOWERS PROCESSED BY IONIZING RADIATION

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ABSTRACT

Edible flowers are increasingly being used in gastronomy, being also recognized for their potential valuable effects beneficial to human health. *Viola tricolor* L. (johnny-jump-up) flowers represents one of the most popular and are widely used in culinary preparations, being also acknowledged for their antioxidant properties. To improve the conservation and safety of flowers the new approaches can be used as ionizing radiation. Radiation treatment contribute to the improve the safety, quality and extends the shelf life of foods by disinfestation insects and reducing or eliminating pathogenic microorganisms. The purpose of this study was to evaluate the dose-dependent effects of electron beam and gamma irradiation in the doses of 0.5, 0.8, 1.0 kGy and control (non-irradiated) on the antioxidant activity of *Viola tricolor* L.by the Oxygen Radical Absorbance Capacity assay (ORAC). Therefore, the ionizing radiation did not affect the antioxidant activity of the flowers.

1. INTRODUCTION

Edible flowers are used to improve the beauty, color, flavor and aroma of culinary preparations for hundreds years. Edible flowers are increasingly being used in culinary preparations, which require new approaches to improve their conservation and safety. In many parts of the world to use them, as food is an ancient tradition. Currently, this type of application aims to improve the sensory quality in gastronomy. On the other hand, several species have active biologically substances, which play an important role in health maintenance [1-4].

The *Viola tricolor* L. (heartseases, johnny-jump-up), from Violaceae family and originating from Europe and Asia, represents one of the most popular sources of edible flowers [1]. The johnny-jump-up are widely used in culinary preparations, recognized for their antioxidant properties and bioactive components. Their biological activities, especially the antioxidant properties, attributed to the presence of flavonoids, being violantina the main compound found [5-7]

These products are highly perishable and must be free of insects, which represents a challenge, as they are usually grown without the use of pesticides. High perishability requires storage in refrigerated environments. Quality and safety of food have to be ensured throughout the food production. Therefore, several methods are applied to increase the shelf life of food products, as well as ensure their quality and safety [8-9].

The process of food irradiation has been shown to be an effective tool in preserving and extending the shelf life of perishable products, improving sanitary quality, insect disinfestation and food safety, and can be used to treat a wide variety of foods [10, 11]. This process consists of the exposure of food to ionizing radiation. Food irradiation has been approved by various authorities such as Food and Agriculture Organization of the United Nations, World Health Organization, Food and Drug Administration, International Atomic Energy Agency, European Union and the Codex Alimentarius [12-14] because its efficiency and safety.

Flowers are relatively sensitive to ionizing radiation and the sensitivity of cut flowers to the use of radiation varies from species to species [15,16]. The advantages of the application of radiation in food is that this process can be applied in packaged products, causing minimal changes in fresh and perishable products, allowing such food to be preserved for longer without losing its quality [17,18].

The purpose of this study was to evaluate the effects of gamma and electron beam irradiation (doses of 0, 0.5, 0.8 and 1.0 kGy) on the antioxidant activity by the Oxygen Radical Absorbance Capacity assay.

2. MATERIAL AND METHODS

2.1 Sample

The fresh flower samples of *Viola tricolor* (Fig. 1) were purchased from a local market in São Paulo, Brazil. Heartsease petals presenting different phenotypes (yellow, orange, purple, white and multi-colored).

Figure 1: Edible flowers *Viola tricolor* L.

2.2 Irradiation

Radiation processing was carried out at Institute of Energy and Nuclear Research - IPEN/CNEN, São Paulo, SP – 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 1.0 kGy. Harwell Amber 3042 dosimeters were used to measure the radiation dose.

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 Oxygen Radical Absorbance Capacity assay – ORAC

The ORAC assay was evaluated according to a method previously described [19] with modifications. The test was conducted at ESALQ-USP, College of Agriculture "Luiz de Queiroz", University of São Paulo, Department of Agribusiness, Food and Nutrition located in Piracicaba, São Paulo, Brazil. The samples were measured fluorescence microplate reader Synergy HT (Bio-Tek Instruments, Inc, Winooski, VT, USA).

Selected 15 mg of the extract samples, which were diluted in 1.5 mL phosphate buffer solution, the test was in 96-well microplate - black color. Therefore, to each well was added 30 µL of sample, 60 µL of the fluorescein solution and 110 µL of AAPH solution. The phosphate buffer was used in the control and white.

It was measured fluorescence microplate reader Synergy HT (Bio-Tek Instruments, Inc, Winooski, VT, USA), where the microplate was incubated at 37 °C with stirring for 2 h, the fluorescence emission 528 nm and excitation 485 nm. Fluorescence was recorded every minute until it reaches zero. Assays were performed in triplicate. In addition, the evaluation of the ORAC value was made by calculating the value of area under the samples of the curve (AUC) and expressed in micromol trolox equivalent/g of dry 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 capacity of the extract of *V. tricolor* processed with electron beams and gamma radiation by Oxygen Radical Absorbance Capacity Assay - ORAC are shown in Fig. 2 and 3.

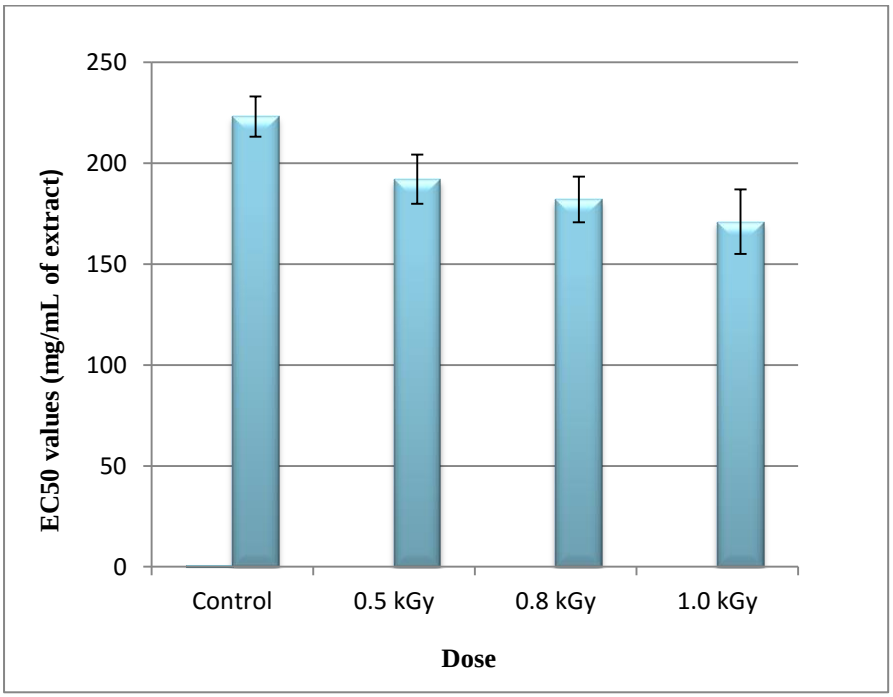

Figure 2: Antioxidant activity (EC_{50} values, mg/mL) of *V. tricolor* extracts irradiated by ^{60}Co

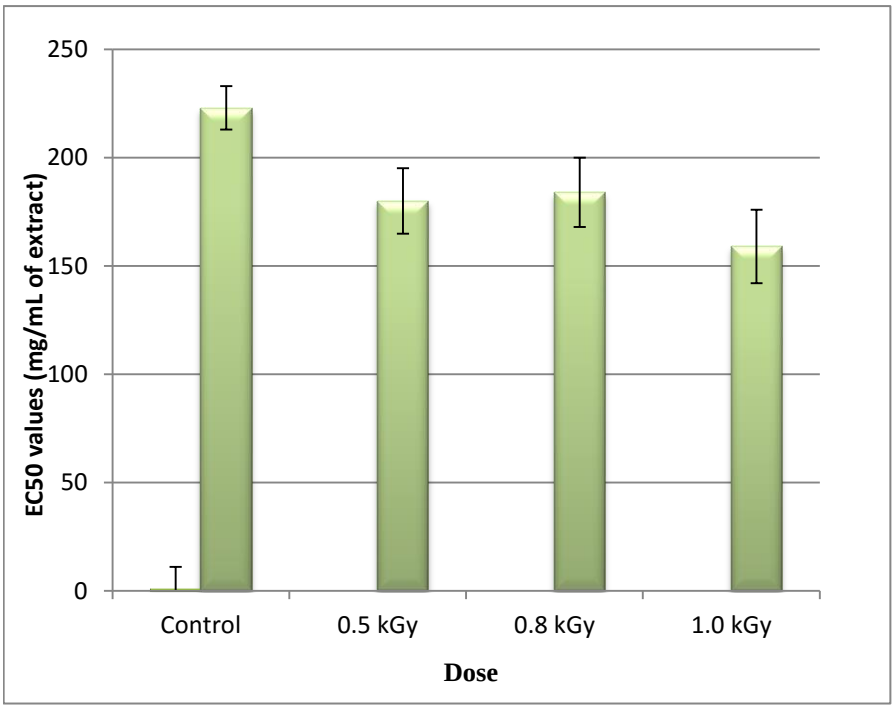

Figure 3: Antioxidant activity (EC_{50} values, mg/mL) of *V.tricolor e*xtracts irradiated by an electron accelerator

According to the obtained results, the antioxidant capacity of *V.tricolor* extract processed with gamma radiation and electron beams the irradiation did not affect negatively the levels of antioxidant activity

Studies have reported that the bioactivity of edible flowers is highly related to their composition in phenolic compounds [16, 20]. Researches on the antioxidant activity and anthocyanins composition in petals of edible flowers were previously reported. Furthermore, aqueous extracts revealed antioxidant properties and high content of phenolics and ascorbic acid [21]. Indeed, antioxidant activity, flavonoids content and total phenolic of methanolic extracts of *Tropaeolum majus* were also reported [6].

4. CONCLUSIONS

Therefore, the radiation treatment did not compromise the antioxidant activity present in the species of edible flowers heartseases studied. The irradiation demonstrated a viable technology to preserve the quality of edible flowers, offering the possibility of its application to expand the shelf-life of edible flower, allowing to achieve new commercial solutions for this food.

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