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The influence of electron beam radiation in the nutritional value, chemical composition and bioactivities of edible flowers of *Bauhinia variegata* L. var. *candida alba* Buch.-Ham from Brazil

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ABSTRACT

As edible flowers are highly perishable, irradiation technology can be applied to increase their shelf life, as also for phytosanitary purposes. Herein, flowers of *Bauhinia variegata* L. var. *candida alba* Buch.-Ham were submitted to electron beam irradiation at the doses of 0.5, 0.8 and 1 kGy, to study the effects in the nutritional and chemical profiles, and also in antioxidant, cytotoxic and anti-inflammatory activities. The petals of white flowers revealed interesting bioactive properties being kaempferol derivatives the most abundant compounds, especially kaempferol-3-*O*-rutinoside. The applied irradiation doses did not highly affect the nutritional profile. No changes were produced in cytotoxicity, but the anti-inflammatory activity slightly decreased. However, the antioxidant activity was increased, especially in the dose of 0.5 kGy, in agreement with the higher content in phenolic compounds found at this dose.

1. Introduction

In the art of cooking, flowers have been increasingly used all around the world for several cultures and for many purposes, becoming a common practice, by providing better sensorial and nutritional quality to foodstuff, in addition to an attractive visual appearance (Koike et al., 2015a; Mlcek & Rop, 2011). Beyond these attractive characteristics, several edible flowers are also rich in different nutrients and bioactive compounds such as proteins, carbohydrates, sugars, organic acids, terpenoids, carotenoids, flavonoids and vitamins (Mlcek & Rop, 2011). Due to the presence of bioactive compounds, especially flavonoids, edible flowers are also described as having different bioactivities such as antioxidant (Kumar, Bhandari, Singh, & Bari, 2009), antihypertensive (Xie & Zhang, 2012), antibacterial (Ammar et al., 2015), or antitumor (Sagdic et al., 2013), among others.

Bauhinia variegata L var. *candida alba* Buch.-Ham white flowers are edible and commonly known as cow's foot due to their unique and characteristic aspect. These flowers belong to the Fabaceae family, and are native from Asia. Other Bauhinia species with white flowers such as *B. forficata* have been described has having different bioactive properties, especially against *Diabetes mellitus* (da Cunha et al., 2010), being the leaves extensively used as an antidiabetic in the folk medicine (Volpato, Damasceno, Rudge, Padovani, & Calderon, 2008). Beyond the mentioned bioactivity, *B. forficata* has also strong antioxidant properties, due to the presence of the glycosides kaempferol and quercetin *O*-glycoside isolated from the leaves (Pinheiro, Johansson, Pizzolatti, & Biavatti, 2006).

Nevertheless, these flowers are highly perishable, suffering oxidation and being easily contaminated by insects that compromise the integrity of the nutrients and the bioactive compounds present, which also decreases their attractiveness.

The irradiation technology has been used to overcome these problems and has been applied to foodstuff for decontamination, preserving the food characteristics and quality and thus increasing the shelf life of the products, being recognized by important authorities such as the World Health Organization – WHO, International Atomic Energy Agency – IAEA, and Food Agriculture Organization – FAO (Farkas & Mohácsi-Farkas, 2011). Brazil has a food irradiation regulation that allows the irradiation of any food product in compliance with the Codex Standard (Roberts, 2016). At the moment, in Brazil, the

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gourmet application to edible flowers is growing and the regulation about irradiation as a phytosanitary treatment to promote the increase in the flowers shelf life is well established. As described by different authors, insect disinfestations by ionizing radiation processing of fresh vegetables, as it concerns of quarantine applications, in some cases is necessary, and the efficacy against the pest and prevention of damage to the fresh commodity provides safe solutions (Ehlermann, 2016; Hallman, 2017).

Lakner, Soos, Vida, and Farkas (2016) reported that in the second half of the 20th century the research in food irradiation application as a preservation method became a new and prospective field of food science and technology following the efforts of Josef Farkas, that made a real bridge between different groups of academic fields, scientists, contributing not just to an enhancement of the knowledge, but also to the proliferation of this path-breaking technology. In a recent report about the Pacific Region and Asia, irradiation is indicated as an environment friendly method for food preservation, as an efficient and safe phytosanitary treatment alternative to the use of chemicals against insects pests (Ihsanullah & Rashid, 2017). Concerning the consumers acceptance, it is time to educate and inform them about the safety and benefits of using irradiation technology in foodstuff (Ehlermann, 2016; Roberts, 2014). With the publication of secure information regarding the irradiation processes, the consumers are more enlightened about the subject and their acceptance towards the irradiated foodstuff is more favourable (Koike et al., 2015a).

In the present study, a mix of petals, stamens and carpel of white flowers of *B. variegata* L. var. *candida alba* Buch.-Ham were submitted to electron beam irradiation applying different doses (0.5, 0.8 and 1 kGy). Furthermore, control samples (non-irradiated) and irradiated samples were studied for their nutritional value, detailed chemical composition, and antioxidant, cytotoxic and anti-inflammatory activities in order to evaluate the irradiation effects in these parameters.

2. Materials and methods

2.1. Samples

Samples of fresh flowers of *B. variegata* L. var. *candida alba* Buch.-Ham were collected in São Paulo (Brazil) in the autumn of 2015. The chosen species were described by Lorenzi and Matos (2002). Brazilian orchid-tree, also known as “pata-de-vaca”, produces white flowers of extreme beauty, robust appearance and edible appeal, being extensively cultivated in Brazil. It has a raceme of flowers with corolla with five oval-elongated white petals, flowering from June to September.

The white fresh flowers samples were collected at a special reserve in São Paulo University (Brazil), and were further labelled and identified with its respective protocol at Nuclear and Energy Research Institute (IPEN-CNEN/SP), Brazil. The samples were divided into four groups: sample 1 control (non-irradiated, 0 kGy), sample 2 (0.5 kGy), sample 3 (0.8 kGy) and sample 4 (1 kGy).

2.2. Electron beam irradiation

The irradiation process was performed at the Nuclear and Energy Research Institute – IPEN-CNEN/SP (São Paulo, Brazil), using an electron beam accelerator (Dynamitron, Radiation Dynamics Inc., Edgewood, NY, USA) and following a procedure reported by Koike et al. (2015a, 2015b). The Flowers samples were irradiated using the doses of 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). In order to measure the radiation dose CTA dosimeters for e-beam machines were used. Afterwards, the samples were lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA) and kept in a desiccator, protected from light for subsequent use.

2.3. Chemical composition

2.3.1. Nutritional value

All samples were analysed in terms of macronutrients (moisture, proteins, fat, carbohydrates and ash), according to the AOAC procedures (AOAC, 2016). Crude protein content (Nx6.25) was estimated using the macro-Kjeldahl method; Soxhlet extraction with petroleum ether was used to determine the crude fat content; incineration at 600 ± 15 °C was used to measure ash content. Total carbohydrates were calculated by difference and the energetic value was calculated as following: Energy (kcal) = 4x (g protein + g carbohydrate) + 9x (g fat).

2.3.2. Free sugars

Free sugars were determined by HPLC coupled to a RI detector (Knauer, Smartline system 1000, Berlin, Germany) using the internal standard (IS, melezitose, Sigma-Aldrich, St. Louis, MO, USA) method, as previously described by the authors (Barros, Pereira, Calhelha, et al., 2013). Mobile phase consisted of acetonitrile:water mixture (70:30 v/v, acetonitrile HPLC-grade, Lab-Scan, Lisbon, Portugal) and separation was achieved using a Eurospher 100-5 NH2 column (4.6 × 250 mm, 5 µm, Knauer). The results were recorded and processed using Clarity 2.4 software (DataApex, Prague, Czech Republic).

2.3.3. Fatty acids

The fatty acids were identified using a gas chromatographer (DANI1000, Contone, Switzerland) provided with a split/splitless injector and a flame ionization detector (GC-FID at 260 °C) operating in the conditions described by Barros, Pereira, Calhelha, et al. (2013). The identification and quantification of the present fatty acids (fatty acid methyl esters (FAME) reference standard mixture 37, Sigma-Aldrich, St. Louis, MO, USA) was achieved by comparing the relative retention times of FAME standard with the ones of the sample’ compounds. The results were recorded and processed using CSW 1.7 software (Data Apex 1.7, Prague, Czech Republic).

2.3.4. Tocopherols

Tocopherols were determined following a procedure previously described by Barros, Pereira, Calhelha, et al. (2013), using a HPLC system (Knauer, Smartline system 1000, Berlin, Germany) coupled to a fluorescence detector (FP-2020; Jasco, Easton, USA) programmed for excitation at 290 nm and emission at 330 nm, using the IS (tocol, Matreya, Pleasant Gap, PA, USA) method for quantification. Mobile phase consisted of a mixture of hexane:ethyl acetate (70:30, v/v, hexane and ethyl acetate HPLC-grade, Lab-Scan, Lisbon, Portugal), and chromatographic separation was performed using a Polyamide II column (250 × 4.6 mm, 5 µm; YMC, Kyoto, Japan). The results were recorded and processed using Clarity 2.4 software (DataApex, Prague, Czech Republic).

2.3.5. Organic acids

Organic acids were determined by ultra-fast liquid chromatography (UFLC) (Shimadzu 20A series UFLC, Shimadzu Corporation, Kyoto, Japan) coupled to a diode-array detector (DAD) operating in the conditions described by Barros, Pereira, and Ferreira (2013). The compounds were identified and quantified by comparing the area of sample’ peaks recorded at 215 nm with calibration curves obtained from commercial standards (Sigma-Aldrich, St. Louis, MO, USA). The results were recorded and processed using LabSolutions Multi LC-PDA software (Shimadzu Corporation, Kyoto, Japan).

2.3.6. Phenolic compounds

Extracts were prepared by macerating the lyophilized flower sample, with a stirring agitation at 150 rpm, using ethanol:water (80:20, v/v, 40 mL) at 25 °C during 1 h, afterwards, the samples were filtered (Whatman No. 4 paper) (Koike et al., 2015a). The residue was