Food Chemistry 179 (2015) 6–14

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Edible flowers of *Viola tricolor* L. as a new functional food: Antioxidant activity, individual phenolics and effects of gamma and electron-beam irradiation

Amanda Koike [a,b], João C.M. Barreira [a,c], Lillian Barros [a], Celestino Santos-Buelga [c], Anna L.C.H. Villavicencio [b], Isabel C.F.R. Ferreira [a,*]

[a] Mountain Research Centre (CIMO), ESA, Polytechnic Institute of Bragança, Campus de Santa Apolónia, Ap. 1172, 5301-855 Bragança, Portugal
[b] Nuclear and Energy Research Institute, National Commission of Nuclear Energy – IPEN/CNEN-SP, Av. Professor Lineu Prestes 2242, Butantã 05508-000, São Paulo, Brazil
[c] Grupo de Investigación en Polifenoles (GIP-USAL), Faculty of Pharmacy, University of Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain

ARTICLE INFO

Article history:
Received 29 October 2014
Received in revised form 22 December 2014
Accepted 27 January 2015
Available online 31 January 2015

Keywords:
Viola tricolor
HPLC–DAD–ESI/MS
Phenolic compounds
Antioxidant activity
Linear discriminant analysis

ABSTRACT

Edible flowers are used in food preparations, being also recognized for their beneficial effects on human health. Nevertheless, these species are highly perishable, and irradiation treatment might be applied to ensure food quality and increase their shelf life. *Viola tricolor* L. is a typical edible flower, with multiple applications and biological properties, mainly provided by the flavonoid content. In the present work, the phenolic compounds were analyzed by HPLC–DAD–ESI/MS, and the antioxidant activity was evaluated using biochemical assays. Linear discriminant analyses (LDA) were performed in order to compare the results obtained with flowers submitted to different irradiation doses and technologies (cobalt-60 and electron-beam). In general, irradiated samples (mostly with 1 kGy) showed the highest phenolic content and antioxidant activity. Furthermore, the significant differences observed in the LDA allow determination of which dose and/or technology is suitable to obtain flowers with higher antioxidant potential.

1. Introduction

Edible flowers have been used in culinary preparations to improve the sensorial and nutritional qualities of food, by adding color, flavor, taste and visual appeal to culinary preparations. They are used in sauces, jellies, syrups, liquors, vinegars, honey, oils, candied flowers, ice cubes, salads, teas and other beverages, and different desserts. Furthermore, edible flowers are important for human health due to their richness in bioactive and nutraceutical compounds, which offers additional marketing opportunities (Creasy, 1999; Mlcek & Rop, 2011; Anderson, Schnelle, & Bastin, 2012).

Nevertheless, edible flowers are highly perishable and must be free of insects, which is difficult because these flowers are usually cultivated without using any pesticide. Their high perishability requires storage in small plastic packages under refrigerated environments, which poses an additional cost in the commercial chain (Kelley, Cameron, Biernbaum, & Poff, 2003; Newman & O'Conner, 2009).

Several methods are used by the food industry to increase the shelf life of food products, but also to ensure their quality and safety, which is important to guarantee the public health (Farkas & Mohácsi-Farkas, 2011). Food irradiation is an economically viable technology to extend shelf life of perishable commodities, improving their hygiene and quality, but also allowing the disinfestation of insects (Fan, Niemira, & Sokorai, 2003). Radiation processing (the use of ionizing radiations from isotopes of cobalt or from electron-accelerators) has been used in several countries, and its efficiency and safety has been approved by authorities, namely the FDA, USDA, WHO, FAO, and also by scientific societies based on extensive research (Morehouse, 2002; Farkas, 2006; Komolprasert, 2007).

Viola tricolor L. (heartseases), from *Violaceae* family, represents one of the most popular sources of edible flowers. Disseminated through Europe and Asia, the flowers are small and delicate, with blue, yellow, purple, pink or white color, and sometimes, combined colors in the same flower (Mlcek & Rop, 2011; Jauron, Beiwel, & Naeve, 2013). *V. tricolor* has a refreshing taste and velvety texture which allow its use in sweets, salads, soups, vinegars, drinks, and also in the extraction of blue and yellow food colourings (Creasy, 1999; Newman & O'Conner, 2009). Heartsease flowers, apart from being used as food, have also been used as medicinal agents for thousands of years. Their biological activities (Hellinger et al., 2014), mainly antioxidant properties, are attributed to the

* Corresponding author. Tel.: +351 273 303219; fax: +351 273 325405.
E-mail address: iferreira@ipb.pt (I.C.F.R. Ferreira).

http://dx.doi.org/10.1016/j.foodchem.2015.01.123
0308-8146/

presence of flavonoid compounds (Vukics, Kery, Bonn, & Guttman, 2008; Vukics, Ringer, Kery, Bonn, & Guttman, 2008; Piana et al., 2013), with violanthin reported as the major compound (Vukics, Kery, et al., 2008).

The purpose of this study was to characterize the phenolic profile and the antioxidant activity of *V. tricolor* flowers of Brazilian origin, and to evaluate the effects of different electron-beam and gamma irradiation doses on these two bioactive indicators (*i.e.*, phenolic compounds and antioxidant activity).

2. Materials and methods

2.1. Samples

Fresh flowers of *V. tricolor* were purchased from a local market in São Paulo, Brazil, in November 2013. Heartsease petals with different phenotypes (yellow, orange, purple, white and multicolored) were pooled together prior to laboratory analyses.

2.2. Samples irradiation

2.2.1. Gamma irradiation

The samples were irradiated at the Nuclear and Energy Research Institute – IPEN/CNEN (São Paulo, Brazil), using a ^{60}Co source Gammacell 200 (Nordion Ltd., Ottawa, ON, Canada), at room temperature, with a dose rate of 1.230 kGy/h, at doses of 0.5, 0.8 and 1 kGy. Harwell Amber 3042 dosimeters were used to measure the radiation dose. Non-irradiated samples were used as control. After irradiation, the samples were lyophilized (Solab SL404, São Paulo, Brazil), powdered and stored inside polyethylene bags kept in a desiccator at room temperature for subsequent use.

2.2.2. Electron-beam irradiation

Samples were irradiated at the Nuclear and Energy Research Institute – IPEN/CNEN (São Paulo, Brazil), using an electron-beam accelerator (Dynamitron, Radiation Dynamics Inc., Edgewood, NY, USA), at room temperature. 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 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 a control. After irradiation, the samples were lyophilized (Solab SL404, São Paulo, Brazil), powdered and stored inside polyethylene bags, and kept in a desiccator at room temperature for subsequent use.

2.3. Analysis of phenolic compounds

2.3.1. Analysis of non-anthocyanin phenolic compounds

The samples (≈0.5 g) were extracted by stirring with 20 ml of methanol/water 80:20 (*v*/*v*), at room temperature, 150 rpm, for 1 h. The extract was filtered through Whatman No. 4 paper. The residue was then re-extracted with additional portions (20 ml) of methanol/water 80:20 (*v*/*v*). The combined extracts were then evaporated at 35 °C (rotary evaporator Büchi R-210, Flawil, Switzerland) to remove methanol. The aqueous phase was lyophilized, and 10 mg were re-dissolved in 2 ml of 20% aqueous methanol and filtered through a 0.22-μm disposable LC filter disk for high performance liquid chromatography (HPLC–DAD–MS) analysis. The extracts were analyzed using a Hewlett–Packard 1100 chromatograph (Agilent Technologies, Santa Clara, CA, USA) with a quaternary pump and a diode array detector (DAD) coupled to an HP Chem Station (rev. A.05.04) data-processing station. A Waters Spherisorb S3 ODS-2 C_{18}, 3 μm (4.6 mm × 150 mm) column thermostatted at 35 °C was used. The solvents used were: (A) 0.1% formic acid in water, (B) acetonitrile. The elution gradient established was 10% B to 15% B over 5 min, 15–25% B over 5 min, 25–35% B over 10 min, isocratic 50% B for 10 min, and re-equilibration of the column, using a flow rate of 0.5 ml/min. Double online detection was carried out in the DAD using 280 nm and 370 nm as preferred wavelengths, and in a mass spectrometer (MS) connected to the HPLC system via the DAD cell outlet.

MS detection was performed in an API 3200 Qtrap (Applied Biosystems, Darmstadt, Germany) equipped with an ESI source and a triple quadrupole-ion trap mass analyzer that was controlled by Analyst 5.1 software. Zero grade air served as the nebulizer gas (30 psi) and turbo gas for solvent drying (400 °C, 40 psi). Nitrogen served as the curtain (20 psi) and collision gas (medium). The quadrupoles were set at unit resolution. The ion spray voltage was set at −4500 V in the negative mode. The MS detector was programmed to perform a series of two consecutive modes: enhanced MS (EMS) and enhanced product ion (EPI) analysis. EMS was employed to record full scan spectra in order to obtain an overview of all of the ions in the sample. The settings used were: declustering potential (DP) −450 V, entrance potential (EP) −6 V, and collision energy (CE) −10 V. Spectra were recorded in negative ion mode between *m*/*z* 100 and 1500. Analysis in EPI mode was further performed in order to obtain the fragmentation pattern of the parent ion(s) detected in the previous experiment using the following parameters: DP −50 V, EP −6 V, CE −25 V, and collision energy spread (CES) 0 V.

The phenolic compounds present in the samples were characterized according to their UV and mass spectra, and retention times compared with commercial standards when available. Otherwise, peaks were tentatively identified by comparing the obtained information with available data reported in the literature. For the quantitative analysis of phenolic compounds, a calibration curve was obtained by injection of known concentrations (1–100 μg/ml) of different standard compounds: isorhamnetin-3-*O*-rutinoside ($y = 258.42x + 10.647$; $R^2 = 0.999$), quercetin-3-*O*-rutinoside ($y = 222.79x + 243.11$; $R^2 = 1.000$), kaempferol-3-*O*-rutinoside ($y = 175.02x - 43.887$; $R^2 = 1.000$) and apigenin-6-*C*-glucoside ($y = 246.05x - 309.66$). Quantification was performed based on DAD results from the areas of the peaks recorded at 280 nm or 370 nm, and results were expressed in mg/g of extract.

2.3.2. Analysis of anthocyanins

Each sample (≈0.5 g) was extracted with 20 ml of methanol containing 0.5% trifluoroacetic acid (TFA), and filtered through a Whatman n° 4 paper. The residue was then re-extracted with additional 20 ml portions of 0.5% TFA in methanol. The combined extracts were evaporated at 35 °C to remove the methanol, and re-dissolved in water. For purification, the extract solution was deposited onto a C-18 SepPak® Vac 3 cc cartridge (Phenomenex, Torrance, CA, USA), previously activated with methanol followed by water. Sugars and more polar substances were removed by passing through 10 ml of water, and anthocyanin pigments were further eluted with 5 ml of methanol/water (80:20, *v*/*v*) containing 0.1% TFA. The extract was concentrated under vacuum, lyophilized, re-dissolved in 1 ml of 20% aqueous methanol and filtered through a 0.22-μm disposable LC filter disk for HPLC analysis.

The extracts were analyzed in the HPLC system indicated above using previously described conditions (García-Marino, Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón, 2010). Separation was achieved on an AQUA®(Phenomenex, Torrance, CA, USA) reverse phase C_{18} column (5 μm, 150 mm × 4.6 mm i.d) thermostatted at 35 °C. The solvents used were: (A) 0.1% TFA in water, and (B) 100% acetonitrile. The gradient employed was: isocratic 10% B for 3 min, from 10% to 15% B for 12 min, isocratic 15% B for 5 min, from