



Electron-beam irradiation as an alternative to preserve nutritional, chemical and antioxidant properties of dried plants during extended storage periods



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ABSTRACT

According to current market demands, there is an increasing need for improved conservation methodologies. In addition to an extension in shelf-life, food products should preserve their compositional integrity and bioactive properties throughout storage time. Irradiation technology has been progressively considered as a feasible conservation technology. Electron-beam irradiation, in particular, might be predominantly suitable to be applied in food products with reduced thickness, such as aromatic and medicinal plants. In this study, the effects of e-beam irradiation on chemical, nutritional and antioxidants parameters of different plant species were evaluated. To assess the potential of this technology over extended periods, plant samples were stored for the first time up to a maximum of 18 months. Despite some heterogeneity among the effects produced in each plant species, electron-beam treatment attenuated the reduction of individual compounds (primarily, free sugars, organic acids, tocopherols and polyunsaturated fatty acids) verified in non-irradiated samples, showing its potential as an alternative conservation technology.

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1. Introduction

Food irradiation is being progressively categorized as a versatile, efficient, safe, secure and highly effective conservation technique. Reasons underlying this classification are related with its ability to provide stability to nutritious foods, besides preserving health-promoting properties during longer storage periods (Cabo Verde et al., 2010; Hunter, 2000; Roberts, 2014).

From a legal standpoint, safety and efficiency of food irradiation have been recognized by authorities such as the World Health Organization (WHO), the International Atomic Energy Agency (IAEA), and the Food Agriculture Organization FAO (Farkas & Mohácsi-Farkas, 2011; Farkas, 2006) regarding three types of ionizing radiation: gamma radiation, X-rays and electron-beam (EU, 1999).

Gamma radiation derives from the spontaneous emission of ⁶⁰Co or ¹³⁷Cs isotopes; X-rays are produced by the impact of

accelerated electrons on a metallic target, which decelerates the electrons, emitting electromagnetic radiation by a physical phenomenon designated as “bremsstrahlung” (literally, braking radiation); electron-beam (e-beam) radiation is produced by accelerating a stream of electrons, focusing them into beams that can be directed to food products on the conveyor belt (mega electron volt) (Farkas, 2008). Despite being able to reach higher energies than X-rays or gamma rays, the maximum energy in food processing is limited to 10 MeV, due to technical and safety reasons (EU, 1999). In addition, e-beam irradiation requires short time, is less expensive and does not produce nuclear waste (Wei et al., 2014).

The specific characteristics of e-beam irradiation make it particularly suitable for food products with low density and small size, such as aromatic and medicinal herbs. These plants might suffer chemical and biological contaminations throughout their production process (harvesting, drying, packaging and storage), causing spoilage, quality deterioration and consequently economic loss (Darfour, Agbenyegah, Oforu, Okyere, & Asare, 2014).

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Considering their wide use in pharmaceutical, food and cosmetic industry (Katusin-Razem, Novak, & Razem, 2001; Haleem, Salem, Fatahallah, & Abdelfattah, 2014), attaining a feasible conservation and decontamination treatment for these matrices might represent good technological advantages.

Besides its decontaminating effectiveness, e-beam irradiation, such as any other preserving treatment, should be able to maintain (or ideally improve) as much characteristics as possible of the treated product (Migdal & Owczarczyk, 1998). Therefore, it is mandatory to evaluate if the chemical profiles (especially considering individual compounds), physical parameters (particularly those related to the product appearance) and bioactive properties (e.g., antioxidant activity) are globally maintained throughout storage time (Nagy, Solar, Sontag, & Koenig, 2011).

Herein, the nutritional, chemical and antioxidant properties of aromatic herbs with highly disseminated use (*A. citrodora* Paláu, *M. officinalis* L., *M. melissophyllum* L. and *M. piperita* L.) were evaluated in non-irradiated and e-beam irradiated samples submitted to a maximum storage of 18 months (Pereira, Antonio, Rafalski, et al., 2015). The main purpose was verifying if e-beam treatment had the ability to preserve the initial characteristics of the plant species, validating the process in natural matrices where this extended storage periods were not tested before, thereby providing additional commercial opportunities.

2. Materials and methods

2.1. Samples

Plant material was obtained from representative species: (*Aloysia citrodora* Paláu, *Melissa officinalis* L., *Melittis melissophyllum* L. and *Mentha piperita* L.) as previously described (Pereira, Antonio, Rafalski, et al., 2015; Pereira, Antonio, Barreira, et al., 2015). Samples were analysed immediately after irradiation (0 months) and after storage in a dry place protected from light for 12 and 18 months. For each period, individual sample groups (unirradiated or irradiated with a 10 kGy dose) were analysed.

2.2. Standards and reagents

Solvents such as acetonitrile, n-hexane and ethyl acetate (HPLC grade) were purchased from Fisher Scientific (Lisbon, Portugal). Fatty acids methyl ester (FAME, standard 47885-U) mixture was purchased from Sigma (St. Louis, MO, USA), as also other individual standards such as trolox, tocopherol (α - and δ -isoforms), sugar ((D-(-)-fructose, D-(+)-sucrose, D-(+)-glucose, D-(+)-trehalose and D-(+)-raffinose pentahydrate) and organic acids (oxalic acid, quinic acid, malic acid, citric acid and fumaric acid). Tocol (50 mg/mL), β -tocopherol and λ -tocopherol were purchased from Matreya (Pleasant Gap, PA, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

2.3. Irradiation treatment

In this process three types of dosimeters were used (a standard dosimeter, a graphite calorimeter, and two routine dosimeters: Gammachrome YR and Amber Perspex) (from Harwell Company; Oxfordshire, UK). Electron-beam irradiation was conducted at the Institute of Nuclear Chemistry and Technology, Warsaw, Poland (Pereira, Antonio, Rafalski, et al., 2015). Irradiation was performed in an e-beam irradiator of 10 MeV of energy with a pulse duration of 5.5 μ s, a pulse frequency of 440 Hz and an average beam current of 1.1 mA; the scan width was 68 cm, the conveyer speed was settled to the range 20–100 cm/min and the scan frequency was 5 Hz. The estimated absorbed dose for irradiated samples was

10.09 kGy, with a maximum uncertainty of 20%. In the Amber Perspex and Gammachrome YR dosimeters, the irradiation dose was estimated by spectrophotometric measurement at 603 nm and 530 nm, respectively, by comparison with a calibration curve. For the graphite calorimeter the electrical resistance was read and converted in dose according to a calibration curve, obtained during the Quality Control procedures of the irradiation equipment and facility.

2.4. Nutritional composition

Protein, fat, carbohydrates and ash content was determined following official procedures (AOAC, 2002). Crude protein content was determined by the macro-Kjeldahl method ($N \times 6.25$); crude fat was determined with a Soxhlet apparatus by extracting (≈ 12 h) a known weight of sample with petroleum ether; ash content was determined by incineration in a muffle furnace (600 ± 15 °C) until a whitish ash appear; carbohydrates were calculated by difference. The energetic value was calculated according to the equation: Energy (kcal) = $4 \times (g_{\text{protein}} + g_{\text{carbohydrates}}) + 9 \times (g_{\text{fat}})$.

2.5. Colour measurement

Colour parameters were evaluated using a colorimeter (model CR-400, from Konica Minolta Sensing, Inc., Japan), with an adapter for granular materials (model CR-A50). The illuminant C and diaphragm aperture of 8 mm were used. CIE $L^*a^*b^*$ colour space values were registered using "Spectra Magic Nx" software (version CM-S100W 2.03.0006, Konica Minolta, Japan).

2.6. Chemical composition

2.6.1. Sugars

Free sugars were determined and analysed by HPLC coupled to a refractive index detector (Barros et al., 2013). Dried extracts (≈ 1 g) were spiked with melezitose (internal standard) (IS, 5 mg/mL), and extracted with 40 mL of 80% aqueous ethanol at 80 °C for 30 min. The resulting suspension was centrifuged (Centurion K240R refrigerated centrifuge, West Sussex, UK) at 15,000 g for 10 min. The supernatant was concentrated at 60 °C under reduced pressure and defatted three times with 10 mL of ethyl ether. After concentration at 40 °C, the solid residues were dissolved in water to a final volume of 5 mL and filtered through 0.2 μ m Whatman nylon filters. Compounds were identified by chromatographic comparisons with authentic standards and quantified using the internal standard method. Data were analysed with a Clarity 2.4 Software (DataApex, Prague, Czech Republic).

2.6.2. Organic acids

Organic acids were determined following a previously optimized procedure (Barros et al., 2013). Samples (≈ 2 g) were extracted by stirring with 25 mL of meta-phosphoric acid (25 °C, 150 rpm, 45 min) and subsequently filtered through Whatman No. 4 paper. Before analysis, samples were filtered again through 0.2 μ m nylon filters. UFLC-DAD (Shimadzu Corporation, Kyoto, Japan) was used for the analyses, using 215 nm as preference wavelength. Organic acids were quantified using calibration curves obtained from commercial standards of each compound. Data were analysed with LabSolutions software (Shimadzu Corporation, Kyoto, Japan).

2.6.3. Tocopherols

Tocopherols were determined by HPLC coupled to a fluorescence detector (Knauer, Berlin, Germany), using a procedure previously described by Pereira, Barros, and Ferreira (2013). Samples

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