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Extending the use of irradiation to preserve chemical and bioactive properties of medicinal and aromatic plants: A case study with four species submitted to electron beam

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ABSTRACT

The effects of gamma irradiation on *Aloysia citrodora*, *Melissa officinalis*, *Melittis melissophyllum* and *Mentha piperita* were previously evaluated. Herein, the same species were treated with electron-beam irradiation (EB) and the same parameters were evaluated. Instead of presenting absolute values for each studied parameter, data were evaluated as percentage of induced variation. Besides the newly obtained results, data from a previous work was recalled and normalized in the same manner. Several examples of percentage variations specific to a plant species or irradiation condition were found. Nevertheless, it was not possible to identify unequivocal trends. Even so, when evaluated in an integrative way, the parameters with highest discriminating ability among irradiation conditions or plant species were fatty acids and bioactive indicators. Comparing the effects of gamma and EB irradiations, it might be concluded that the most suitable solution to irradiate aromatic plants would be EB, independently of the used dose.

1. Introduction

Food irradiation is a non-thermal processing technique, which has been increasingly applied with several purposes. Nowadays, it is highlighted as a preservation and decontamination technique, ensuring the elimination of pathogenic microorganisms, parasites and pests, without changing the nutritional and organoleptic characteristics of the targeted food product (Molins, 2001; Villavicencio et al., 2007; Wen et al., 2010).

Despite the irradiation concept is often misunderstood by most consumers, it is a safe process that exposes food (pre-packaged or unpackaged) to a predetermined dose of radiation according to the food type to be treated, plant-derived products (such as vegetables, fruits and cereals) or even derived from animals, such as meat or fish (Sádecká, 2007; Nagy et al., 2011; Kanatt et al., 2015). It is characterized as a versatile, efficient, safe, secure and highly effective technique, *i.e.*, it is a process that fully satisfies the objective of providing stability to nutritious foods, health conditions and longer storage period (Hunter, 2000; Roberts, 2014). There are several processes of irradiation for food preservation using ionizing radiations, being gamma and electron beam the more well established for industrial purposes (Van Calenberg et al., 1998; Roberts, 2014). Electron beam irradiation is mainly used for food products with low density; the sources can be easily connected/disconnected, whereas the gamma sources are continuously decaying.

Aromatic and medicinal herbs are among the products submitted to decontamination assays based on irradiation treatment. The fact that these matrices are quite popular in the pharmaceutical and food industries requires specific criteria in terms of microbiological safety (Katuŝin-Razĕm et al., 2001; Haleem et al., 2014). *Aloysia citrodora* P., *Melissa officinalis* L., *Melittis melissophyllum* L. and *Mentha piperita* L. are among the studied plants, namely submitted to gamma radiation (Pereira et al., 2015). All of them are characterized by being culinary and medicinal herbs, consumed usually as infusions and used since ancient times as medicinal plants for different diseases, especially in healing and treatment of gastrointestinal and nervous system disorders, displaying antioxidant, antimicrobial and anti-inflammatory properties, due to the presence of bioactive compounds (Ragone et al., 2007; Skrzypczak-Pietraszek and Pietraszek, 2012; Barros et al., 2013; Pereira et al., 2014; Skalicka-Woźniak and Walasek, 2014).

In this study the objective was to compare the effects of gamma irradiation and electron beam irradiation in the chemical parame-

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ters and bioactive indicators of aromatic plants in order to find the most suitable technology in each case.

2. Materials and methods

2.1. Samples and samples irradiation

Samples of *A. citrodora* P. (Verbenaceae; lemon verbena), *M. officinalis* L. (Lamiaceae; lemon balm), *M. melissophyllum* L. (Lamiaceae; bastard balm) and *M. piperita* L. (Lamiaceae; peppermint) were provided as dry leaves by a local producer (Pragmático Aroma Lda, Alfândega da Fé, Bragança, Portugal). After confirmation of the taxonomical identification, the samples were divided into three groups: control (non-irradiated, 0 kGy), groups 1 and 2, where 1 kGy and 10 kGy were, respectively, the predicted doses.

The irradiation was performed at the INCT—Institute of Nuclear Chemistry and Technology, in Warsaw, Poland. To estimate the dose during the irradiation process three types of dosimeters were used: a standard dosimeter, a graphite calorimeter, and two routine Gammachrome YR and Amber Perspex dosimeters, from Harwell Company (UK). The irradiation took place in an e-beam irradiator of 10 MeV of energy with pulse duration of 5.5 ms, pulse frequency of 440 Hz and 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 0.83 kGy for group 1 and 10.09 kGy for group 2, with a maximum uncertainty of 20%. To read the Amber Perspex and Gammachrome YR dosimeters, spectrophotometric methods were used at 603 nm and at 530 nm, respectively, to estimate the dose from the value of absorbance according to a previous calibration curve. For the graphite calorimeter dosimeter the electrical resistance was read and converted in dose according to a calibrated curve, obtained following the standards during the Quality Control procedures of the irradiation equipment and facility.

2.2. Standards and reagents

Acetonitrile 99.9%, *n*-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Fisher Scientific (Lisbon, Portugal). Fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47,885-U) was purchased from Sigma (St. Louis, MO, USA), as well as other individual fatty acid isomers, L-ascorbic acid, tocopherol, sugar and organic acid standards. Racemic tocol, 50 mg/mL, was purchased from Matreya (Pleasant Gap, PA, USA).

2.3. Nutritional value

Protein, fat, carbohydrates and ash were determined following the AOAC procedures (AOAC, 1995). The crude protein content (N × 6.25) was estimated by the macro-Kjeldahl method; the crude fat was determined using a Soxhlet apparatus; the ash content was determined by incineration at 600 ± 15 °C, until a whitish ash was formed. Total carbohydrates were calculated by difference. The results were expressed in g/100 g of dry weight (dw). Total energy was calculated according to the following equation: Energy (kcal) = $4 \times (g_{protein} + g_{carbohydrates}) + 9\ (g_{fat})$, and the results were expressed in kcal/100 g dw.

2.4. Color measurement

A colorimeter (model CR-400, from Konica Minolta Sensing, Inc., Japan), with an adapter for granular materials (model CR-A50) was used to measure the color of the samples. Using the illuminant C and diaphragm aperture of 8 mm, the CIE $L^*a^*b^*$ color space values were registered using a data software "Spectra Magic Nx" (version CM-S100W 2.03.0006), from Konica Minolta company (Japan). Before starting the measurements the instrument was calibrated against a standard white tile (Pereira et al., 2015). The colour of three samples from each batch was measured in three different points, for each dose and at each time point, being considered the average value.

2.5. Chemical composition of hydrophilic compounds

2.5.1. Sugars

Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI), using a previously described procedure (Pereira et al., 2015). Data were analysed using Clarity 2.4 Software (DataApex). The compounds were identified by chromatographic comparisons with authentic standards. Quantification was performed using the internal standard (melezitose) method and the results were expressed in g/100 g dw.

2.5.2. Organic acids

Organic acids were determined following a procedure previously described by the authors (Pereira et al., 2015). Detection was carried out in a DAD, using 215 nm and 245 nm (for ascorbic acid) as preferred wavelengths. The organic acids found were quantified by comparison of the area of their peaks recorded at 215 nm with calibration curves obtained from commercial standards of each compound, and the results were expressed in g/100 g dw.

2.6. Chemical composition in lipophilic compounds

2.6.1. Tocopherols

Tocopherols were determined following a procedure previously described by the authors (Pereira et al., 2015). The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response of each standard, using the IS (tocol) method and by using calibration curves obtained from commercial standards of each compound. The results were expressed in mg/100 g dw.

2.6.2. Fatty acids

Fatty acids were determined by gas–liquid chromatography with flame ionization detection (GC–FID)/capillary column as described previously by the authors (Pereira et al., 2015). Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded, processed using the CSW 1.7 Software (DataApex 1.7, Prague, Czech Republic) and expressed in relative percentages.

2.7. Evaluation of bioactivity

2.7.1. Samples preparation

The methanolic extracts were obtained from the dried plant material. The sample (1 g) was extracted by stirring with 25 mL of methanol (25 °C at 150 rpm) for 1 h and subsequently filtered through Whatman No. 4 paper. The residue was then extracted with 25 mL of methanol (25 °C at 150 rpm) for 1 h. The combined methanolic extracts were evaporated at 40 °C (rotary evaporator Büchi R-210, Flawil, Switzerland) to dryness.

The infusions were also obtained from the dried plant material. The sample (2 g) was added to 200 mL of boiling distilled water (after being taken out from the heating source) and left to stand at room temperature for 5 min, and then filtered under reduced pressure.