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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.

Table 1
Proximate composition (g/100 g dw), energy (kcal/100 g dw) and color parameters (L*: lightness, a*: redness, b*: yellowness) of the aromatic species (controls; non-irradiated samples). Values for irradiated samples are presented as percentage of variation in comparison to the control.[a]

		Fat	Protein	Ash	Carbohydrates	Energy	L^*	a^*	b^*
Dose	Irradiation type								
Aloysia citrodora (Lemon verbena)									
0 kGy	Control	1.6 ± 0.1	3.0 ± 0.1	8.2 ± 0.1	87.1 ± 0.1	375 ± 1	49 ± 1	-8.4 ± 0.2	27.2 ± 0.3
1 kGy	Electron beam	20 ± 4^{b}	1 ± 2^{b}	-1 ± 2^{b}	-1 ± 1^{b}	1 ± 1	1 ± 2	-8 ± 8	7 ± 7
	Gamma rays	32 ± 5^{a}	-42 ± 5^{c}	3 ± 2^{a}	1 ± 1^{a}	1 ± 1	3 ± 2	5 ± 5	3 ± 1
10 kGy	Electron beam	19 ± 8^{b}	45 ± 10^{a}	-1 ± 1^{b}	-2 ± 1^{c}	1 ± 1	2 ± 3	-10 ± 10	3 ± 2
	Gamma rays	6 ± 3^{c}	-2 ± 3^{b}	4 ± 2^{a}	-1 ± 1^{b}	-1 ± 1	-2 ± 2	-1 ± 3	-3 ± 1
p-values	Homoscedasticity*	0.012	0.172	0.073	0.016	0.008	0.310	0.003	0.030
	1-way ANOVA**	<0.001	<0.001	<0.001	<0.001	0.081	0.104	0.087	0.117
Melissa officinalis (Lemon balm)									
0 kGy	Control	1.2 ± 0.1	2.5 ± 0.3	8.4 ± 0.4	88 ± 1	372 ± 2	48 ± 1	-5.1 ± 0.5	20.9 ± 0.4
1 kGy	Electron beam	-7 ± 4^{c}	4 ± 2^{b}	-3 ± 2	1 ± 1^{a}	1 ± 1	1 ± 1^{b}	-10 ± 4^{b}	-2 ± 1^{c}
	Gamma rays	65 ± 5^{a}	167 ± 11^{a}	-3 ± 2	-5 ± 1^{b}	1 ± 1	-1 ± 1^{bc}	-1 ± 2^{a}	-1 ± 1^{b}
10 kGy	Electron beam	11 ± 3^{b}	5 ± 2^{b}	-1 ± 2	-1 ± 1^{a}	1 ± 1	4 ± 1^{a}	-13 ± 2^{b}	6 ± 1^{a}
	Gamma rays	60 ± 2^{a}	156 ± 20^{a}	1 ± 1	$-5 \pm 1^{*}$	1 ± 1	-2 ± 1^{c}	-2 ± 4^{a}	-3 ± 1^{d}
p-values	Homoscedasticity*	0.731	0.002	0.045	0.009	0.003	0.850	0.180	0.261
	1-way ANOVA**	<0.001	<0.001	0.082	<0.001	0.071	<0.001	<0.001	<0.001
Melittis melissophyllum (Bastard balm)									
0 kGy	Control	1.8 ± 0.1	4.6 ± 0.2	7.6 ± 0.1	86.0 ± 0.4	378 ± 1	42 ± 2	-8.4 ± 0.5	18 ± 3
1 kGy	Electron beam	-7 ± 7	-7 ± 5^{b}	-2 ± 4^{bc}	1 ± 1^{a}	1 ± 1	-1 ± 3	36 ± 11^{a}	1 ± 2
	Gamma rays	-8 ± 5	-45 ± 4^{c}	7 ± 2^{ab}	2 ± 1^{a}	1 ± 1	3 ± 3	-3 ± 4^{b}	-1 ± 2
10 kGy	Electron beam	-13 ± 8	2 ± 4^{b}	-4 ± 5^{c}	1 ± 1^{a}	-1 ± 1	-2 ± 4	28 ± 13^{a}	-4 ± 4
	Gamma rays	-13 ± 5	22 ± 5^{a}	13 ± 3^{a}	-2 ± 1^{b}	-1 ± 1	-3 ± 4	-4 ± 4^{b}	-5 ± 5
p-values	Homoscedasticity*	0.064	<0.001	0.059	0.053	0.012	0.111	0.188	0.962
	1-way ANOVA**	0.400	<0.001	<0.001	<0.001	0.082	0.743	<0.001	0.698
Mentha piperita (Peppermint)									
0 kGy	Control	2.4 ± 0.1	5.1 ± 0.3	9.2 ± 0.2	83.3 ± 0.5	375 ± 1	40 ± 1	-5.9 ± 0.1	23.9 ± 0.3
1 kGy	Electron beam	-5 ± 2^{b}	19 ± 8^{b}	-4 ± 3^{a}	1 ± 1^{b}	1 ± 1	-1 ± 2^{a}	17 ± 8	3 ± 2^{a}
	Gamma rays	13 ± 5^{a}	-45 ± 10^{d}	-10 ± 2^{b}	3 ± 1^{a}	1 ± 1	-3 ± 3^{a}	-5 ± 2	-3 ± 2^{b}
10 kGy	Electron beam	-4 ± 5^{b}	3 ± 4^{c}	-3 ± 2^{a}	1 ± 1^{b}	1 ± 1	1 ± 2^{a}	-26 ± 15	2 ± 2^{ab}
	Gamma rays	-21 ± 6^{c}	91 ± 3^{a}	-6 ± 3^{ab}	-6 ± 1^{c}	1 ± 1	-7 ± 3^{b}	-25 ± 14	-16 ± 5^{c}
p-values	Homoscedasticity*	0.056	0.045	0.306	0.544	0.053	0.376	0.064	0.580
	1-way ANOVA**	<0.001	<0.001	0.002	<0.001	0.082	<0.001	0.077	<0.001

[a] The results are presented as the mean ± SD.
* Homoscedasticity among obtained ratios was tested by the Levene test: homoscedasticity, $p > 0.05$; heteroscedasticity, $p < 0.05$.
** $p < 0.05$ indicates that the mean value of at least one ratio differs from the others (in this case multiple comparison tests were performed). For each species, means within a column with different letters differ significantly ($p < 0.05$).

2.7.2. Antioxidant activity

DPPH radical-scavenging activity was evaluated by using an ELX800 microplate reader (Bio-Tek Instruments, Inc; Winooski, VT, USA), and calculated as a percentage of DPPH discoloration using the formula: $[(A_{DPPH}\text{-}A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution containing the sample at 515 nm, and A_{DPPH} is the absorbance of the DPPH solution. Reducing power was evaluated by the capacity to convert Fe^{3+} into Fe^{2+}, measuring the absorbance at 690 nm in the microplate reader mentioned above. Inhibition of β-carotene bleaching was evaluated though the β-carotene/linoleate assay; the neutralization of linoleate free radicals avoids β-carotene bleaching, which is measured by the formula: (β-carotene absorbance after 2 h of assay/initial absorbance) × 100% (Pereira et al., 2013). The results were expressed as EC_{50} values (μg/mL).

2.7.3. Phenolics and flavonoids content

Total phenolics were estimated by Folin–Ciocalteu colorimetric assay, while total flavonoids were determined by a colorimetric assay using aluminum trichloride, according to procedures previously described (Pereira et al., 2013). The results were expressed in mg GAE (gallic acid equivalents)/g of extract and mg CE (catechin equivalents)/g of extract for phenolics and flavonoids, respectively.

2.8. Statistical analysis

For each irradiation dose and plant species, three independent samples were analysed. Each of the samples was taken after pooling the plants treated in the same conditions together. Data for control (non-irradiated) samples were expressed as mean ± standard deviation. Data for irradiated samples were presented as the normalized difference ((irradiated sample value-control value)/control value × 00) among the values obtained for each irradiated sample and the respective control.

The obtained values were evaluated using 1-way ANOVA. The homogeneity of variance, was tested by means of the Levene's tests. All dependent variables were compared using Tukey's honestly significant difference (HSD) or Tamhane's T2 multiple comparison tests, when homoscedasticity was verified or not, respectively.

Owing the high number of evaluated parameters, a LDA was used to evaluate the association of variations in the measured parameters with, sequentially, irradiation condition and plant species. A stepwise technique, using the Wilks' λ method with the usual probabilities of F (3.84 to enter and 2.71 to remove), was applied for variable selection. This procedure uses a combination of forward selection and backward elimination procedures, where before selecting a new variable, it is verified whether all variables previously selected remain significant (Palacios-Morillo et al., 2013). With this approach, it is also possible to identify the significant variables that contribute most to the possible discrimination of a determined irradiation treatment or plant species. To verify which canonical discriminant functions were significant, the Wilks' λ test was applied. A leaving-one-out cross-validation procedure was carried out to assess the model performance.

All statistical tests were performed at a 5% significance level using IBM SPSS Statistics for Windows, version 22.0. (IBM Corp., Armonk, NY, USA).

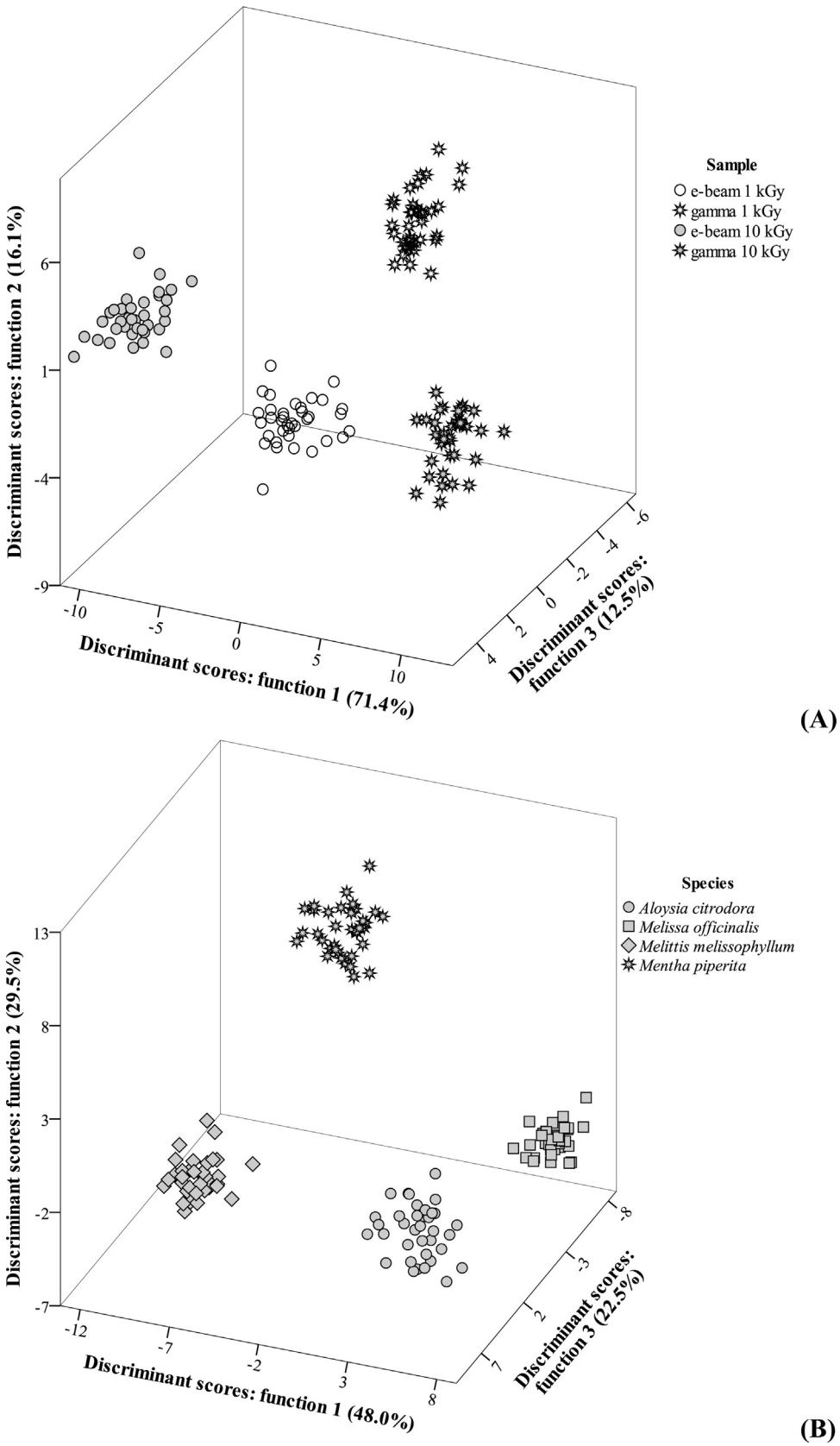

Fig. 1. Mean scores of different irradiation conditions (A) and different plant species (B) projected for the three discriminant functions defined variations measured in all evaluated parameters.

Table 2
Hydrophilic compounds (free sugars and organic acids) composition (g/100 g dw) of the aromatic species (controls; non-irradiated samples). Values for irradiated samples are presented as percentage of variation in comparison to the control.[a]

		Fructose	Glucose	Sucrose	Trehalose	Total sugars	Oxalic acid	Quinic acid	Malic acid	Shikimic acid	Citric acid	Organic acids
Dose	Irradiation type											
Aloysia citrodora (Lemon verbena)												
0 kGy	Control	1.0 ± 0.1	1.3 ± 0.1	7.1 ± 0.3	1.2 ± 0.1	10.7 ± 0.4	1.1 ± 0.1	nd	0.14 ± 0.03	1.4 ± 0.1	1.4 ± 0.1	4.1 ± 0.1
1 kGy	Electron beam	1 ± 5	−7 ± 6	16 ± 6^{b}	12 ± 8^{ab}	3 ± 5^{b}	−3 ± 3^{ab}	–	−5 ± 7^{ab}	-6 ± 5^{b}	−4 ± 3^{b}	−4 ± 2^{c}
	Gamma rays	−2 ± 4	−9 ± 7	−10 ± 7^{c}	−1 ± 2^{b}	−8 ± 2^{c}	−2 ± 2^{ab}	–	29 ± 16^{a}	29 ± 5^{a}	40 ± 9^{a}	24 ± 9^{a}
10 kGy	Electron beam	18 ± 13	−2 ± 6	28 ± 9^{a}	18 ± 7^{a}	13 ± 6^{a}	−9 ± 6^{b}	–	−10 ± 6^{b}	−11 ± 4^{c}	−3 ± 4^{c}	−8 ± 4^{c}
	Gamma rays	−1 ± 3	−5 ± 5	−8 ± 4^{c}	−2 ± 5^{b}	−6 ± 3^{c}	5 ± 7^{a}	–	3 ± 7^{ab}	12 ± 3^{b}	20 ± 7^{b}	12 ± 5^{b}
p-values	Homoscedasticity*	0.023	0.029	0.003	0.012	0.002	0.354	–	0.056	0.390	0.059	0.459
	1-way ANOVA**	0.131	0.726	<0.001	0.007	<0.001	0.035	–	0.044	<0.001	<0.001	<0.001
Mellissa officinalis (Lemon balm)												
0 kGy	Control	1.2 ± 0.1	1.0 ± 0.1	4.8 ± 0.2	0.49 ± 0.05	7.5 ± 0.2	0.5 ± 0.1	0.26 ± 0.04	0.4 ± 0.1	4.1 ± 0.2	nd	5.3 ± 0.3
1 kGy	Electron beam	9 ± 5^{a}	21 ± 9^{a}	−11 ± 7^{b}	5 ± 3^{c}	11 ± 4^{ab}	−48 ± 3^{c}	−24 ± 5^{b}	−27 ± 3^{b}	−30 ± 3^{c}	–	−36 ± 2^{c}
	Gamma rays	9 ± 3^{a}	1 ± 1^{b}	12 ± 5^{a}	37 ± 17^{b}	11 ± 4^{ab}	−2 ± 3^{a}	−12 ± 5^{c}	−8 ± 8^{b}	1 ± 2^{b}	–	−1 ± 2^{b}
10 kGy	Electron beam	1 ± 2^{b}	8 ± 6^{b}	−59 ± 16^{c}	16 ± 4^{c}	4 ± 4^{b}	−10 ± 3^{b}	25 ± 5^{a}	8 ± 4^{a}	16 ± 4^{a}	–	6 ± 3^{a}
	Gamma rays	5 ± 3^{ab}	1 ± 2^{b}	17 ± 1^{a}	72 ± 8^{a}	17 ± 1^{a}	−3 ± 5^{a}	−4 ± 4^{b}	−1 ± 4^{b}	−1 ± 2^{b}	–	−1 ± 2^{b}
p-values	Homoscedasticity*	0.030	0.026	<0.001	0.004	0.095	0.188	0.934	0.009	0.306	–	0.160
	1-way ANOVA**	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	–	<0.001
Melittis melissophyllum (Bastard balm)												
0 kGy	Control	1.0 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.28 ± 0.03	5.5 ± 0.3	1.4 ± 0.1	0.17 ± 0.01	6.0 ± 0.3	0.97 ± 0.05	0.022 ± 0.001	8.6 ± 0.4
1 kGy	Electron beam	−11 ± 5^{b}	5 ± 5^{ab}	4 ± 3^{a}	−11 ± 8^{b}	−1 ± 2^{b}	−11 ± 4^{b}	−31 ± 8^{c}	8 ± 5^{a}	−24 ± 9^{b}	41 ± 17^{a}	−3 ± 2^{a}
	Gamma rays	−8 ± 6^{ab}	−3 ± 5^{b}	4 ± 4^{a}	84 ± 20^{a}	6 ± 4^{b}	−16 ± 5^{b}	−10 ± 5^{b}	−26 ± 4^{b}	−12 ± 6^{ab}	−12 ± 6^{c}	−22 ± 2^{b}
10 kGy	Electron beam	−24 ± 4^{c}	−26 ± 3^{c}	−17 ± 4^{b}	−21 ± 10^{b}	−21 ± 3^{c}	−12 ± 2^{b}	−45 ± 9^{d}	12 ± 4^{a}	−13 ± 7^{ab}	18 ± 7^{b}	1 ± 2^{a}
	Gamma rays	1 ± 2^{a}	9 ± 6^{a}	8 ± 6^{a}	119 ± 32^{a}	17 ± 4^{a}	1 ± 2^{a}	10 ± 4^{a}	−1 ± 1^{b}	−3 ± 4^{a}	16 ± 6^{b}	−1 ± 1^{a}
p-values	Homoscedasticity*	0.040	0.030	0.017	0.511	0.338	0.575	0.055	0.064	0.364	0.369	0.032
	1-way ANOVA**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.045	<0.001	<0.001
Mentha piperita (Peppermint)												
0 kGy	Control	0.47 ± 0.05	0.30 ± 0.05	0.7 ± 0.1	1.0 ± 0.1	2.4 ± 0.2	1.1 ± 0.1	0.040 ± 0.003	0.9 ± 0.1	nd	8.5 ± 0.2	10.6 ± 0.3
1 kGy	Electron beam	−3 ± 5	5 ± 5^{a}	10 ± 6^{a}	−6 ± 6^{ab}	3 ± 3^{a}	−11 ± 8	−4 ± 5	9 ± 5^{a}	–	7 ± 7^{a}	6 ± 6^{a}
	Gamma rays	−12 ± 8	−1 ± 2^{ab}	12 ± 8^{a}	3 ± 4^{a}	3 ± 4^{a}	6 ± 5	−10 ± 8	−2 ± 4^{a}	–	−30 ± 4^{c}	−20 ± 4^{b}
10 kGy	Electron beam	−1 ± 4	−11 ± 5^{b}	−26 ± 10^{b}	−29 ± 10^{c}	−11 ± 5^{b}	4 ± 5	−21 ± 10	8 ± 8^{a}	–	11 ± 7^{a}	9 ± 8^{a}
	Gamma rays	−1 ± 2	5 ± 5^{a}	5 ± 5^{a}	−24 ± 8^{bc}	−6 ± 4^{b}	−11 ± 5	−17 ± 8	−32 ± 12^{b}	–	−10 ± 3^{b}	−10 ± 2^{b}
p-values	Homoscedasticity*	0.742	0.199	0.065	0.011	0.660	0.311	0.720	0.255	–	0.033	0.164
	1-way ANOVA**	0.157	0.004	<0.001	<0.001	<0.001	0.052	0.118	<0.001	–	<0.001	0.062

[a] The results are presented as the mean ± SD.
* Homoscedasticity among obtained ratios was tested by the Levene test: homoscedasticity, $p > 0.05$; heteroscedasticity, $p < 0.05$.
** $p < 0.05$ indicates that the mean value of at least one ratio differs from the others (in this case multiple comparison tests were performed). For each species, means within a column with different letters differ significantly ($p < 0.05$).

3. Results and discussion

In a previous study, the effect of gamma irradiation was evaluated by measuring changes in the same parameters as those assayed herein. The values obtained in non-irradiated samples are recalled for each plant species and assayed parameter. To allow a more immediate comparison of the effects of gamma and electron-beam irradiations the percentages of variances (calculated as explained in Section 2) are indicated for both types of irradiation. These percentages were obtained from previously published results for gamma irradiation (GI) (Pereira et al., 2015) and from the newly assessed values resulting from applying electron-beam irradiation (EB) in the same doses as those used for gamma-irradiation. In every cases where the variation laid below 5% (either representing an increase or a decrease), it was assumed that the irradiation had no identifiable effect.

3.1. Effects on chemical parameters

Regarding the proximate composition and color parameters (Table 1), it became obvious that fat and protein are the ones suffering higher changes with irradiation treatment. Nevertheless, the observed effect was highly dependent on the plant species. Fat content, for instance, tended to increase in *A. citrodora* (lemon verbena) and *M. officinalis* (lemon balm), but an opposite effect was produced in *M. melissophyllum* (bastard balm) and *M. piperita* (peppermint). Likewise, no general trend could be identified for the effect on protein content, despite the similar variation in lemon verbena, bastard balm and peppermint obtained with 1 kGy of GI. Furthermore, the effects on the remaining parameters, despite lower in magnitude, were significantly different ($p < 0.05$) for each of the applied conditions in most occasions (21 out of 32 cases). Nevertheless, the 10 kGy dose tended to have a more pronounced effect than the 1 kGy dose, independently of the irradiation technology (except for a^* in all plants and fat content in bastard balm).

In this first approach, it is important to highlight the slight effects caused on L^* and b^*, since colour parameters are usually used in the quality control of post-harvest preservation processes (Hsu et al., 2010). In the case of a^*, the results are even better, since a general decrease was observed in response to irradiation treatment, which should be interpreted as an increase of samples greenness, resulting more appealing to the consumers. The variation in colour parameters is in general agreement with those available from similar reports (Jo et al., 2003; Hsu et al., 2010).

Concerning free sugars composition (Table 2), the induced variations were more pronounced, despite the specificity of effect towards the plant species. Sucrose and trehalose seemed to be the most susceptible sugars to irradiation, as they suffered significant ($p < 0.05$) changes in all cases. Fructose, on the other hand, showed significant changes only in in lemon balm and bastard balm, while glucose remained nearly unchanged in lemon verbena. This result might be an indicator of the vulnerability of the glycosidic bond, since the monosaccharides presented higher resistance. Whereas total sugars, only minor variations were detected, which could be anticipated from the changes in individual sugars, since the decrease in sucrose and trehalose contribute to an increase in fruc-

Table 3
Tocopherols composition (mg/100 g dw) of the aromatic species (controls; non-irradiated samples). Values for irradiated samples are presented as percentage of variation in comparison to the control.[a]

	α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total tocopherols	
Dose	Irradiation type					
Aloysia citrodora (Lemon verbena)						
0 kGy	Control	15.3 ± 0.4	0.41 ± 0.04	1.8 ± 0.1	nd	17.5 ± 0.4
1 kGy	Electron beam	22 ± 5^{a}	2 ± 4^{a}	5 ± 5	–	17 ± 3^{a}
	Gamma rays	14 ± 4^{a}	7 ± 9^{a}	4 ± 5	–	13 ± 4^{a}
10 kGy	Electron beam	5 ± 5^{b}	−12 ± 10^{ab}	−5 ± 6	–	2 ± 2^{b}
	Gamma rays	−12 ± 4^{c}	−29 ± 10^{b}	−5 ± 5	–	−12 ± 3^{c}
p-values	Homoscedasticity*	0.053	0.279	0.168	–	0.426
	1-way ANOVA**	<0.001	0.004	0.050	–	<0.001
Melissa officinalis (Lemon balm)						
0 kGy	Control	29 ± 1	1.3 ± 0.1	1.5 ± 0.1	0.37 ± 0.05	32 ± 1
1 kGy	Electron beam	−10 ± 2^{d}	−22 ± 5^{b}	−15 ± 3^{d}	1 ± 1^{b}	−10 ± 2^{d}
	Gamma rays	16 ± 1^{a}	−15 ± 4^{a}	18 ± 5^{a}	2 ± 4^{b}	14 ± 1^{a}
10 kGy	Electron beam	−2 ± 2^{c}	−30 ± 3^{c}	−7 ± 3^{c}	2 ± 2^{b}	−3 ± 1^{c}
	Gamma rays	2 ± 1^{b}	−25 ± 3^{bc}	12 ± 6^{b}	31 ± 9^{a}	1 ± 1^{b}
p-values	Homoscedasticity*	<0.001	0.148	0.802	<0.001	0.304
	1-way ANOVA**	<0.001	<0.001	<0.001	<0.001	<0.001
Melittis melissophyllum (Bastard balm)						
0 kGy	Control	0.88 ± 0.05	13.4 ± 0.3	0.18 ± 0.02	0.14 ± 0.02	14.6 ± 0.4
1 kGy	Electron beam	1 ± 3^{b}	−22 ± 5^{d}	−25 ± 10^{bc}	−34 ± 10^{b}	−21 ± 4^{d}
	Gamma rays	−8 ± 5^{b}	−1 ± 1^{c}	−8 ± 5^{b}	3 ± 3^{a}	−2 ± 1^{c}
10 kGy	Electron beam	60 ± 24^{a}	21 ± 5^{b}	14 ± 8^{a}	−39 ± 7^{b}	21 ± 5^{b}
	Gamma rays	−48 ± 6^{c}	115 ± 6^{a}	−40 ± 9^{c}	−44 ± 6^{b}	102 ± 6^{a}
p-values	Homoscedasticity*	0.002	0.559	0.749	0.098	0.363
	1-way ANOVA**	<0.001	<0.001	<0.001	<0.001	<0.001
Mentha piperita (Peppermint)						
0 kGy	Control	16.5 ± 0.4	1.1 ± 0.1	1.8 ± 0.1	0.23 ± 0.03	19.7 ± 0.5
1 kGy	Electron beam	18 ± 6^{a}	27 ± 10^{a}	8 ± 10	−4 ± 2^{b}	18 ± 6^{a}
	Gamma rays	−5 ± 3^{c}	−42 ± 12^{b}	−3 ± 5	15 ± 6^{a}	−6 ± 3^{c}
10 kGy	Electron beam	7 ± 4^{b}	15 ± 10^{a}	−2 ± 5	5 ± 7^{b}	6 ± 4^{b}
	Gamma rays	−25 ± 4^{d}	−29 ± 10^{b}	−1 ± 4	22 ± 7^{a}	−21 ± 4^{d}
p-values	Homoscedasticity*	0.648	0.229	0.097	<0.001	0.906
	1-way ANOVA**	<0.001	<0.001	0.278	<0.001	<0.001

[a] The results are presented as the mean ± SD.

tose and especially glucose. Other less coherent variations might be explained by changes in the optical rotation, which is a common occurrence under irradiation treatment (Molins, 2001).

Significant variations were also detected in the organic acids (Table 2), with quinic and citric acids as the compounds more prone to suffer quantitative changes. It could also be observed that the species with the highest contents in organic acids (bastard balm and peppermint) were the ones with higher number of significant variations. Another interesting observation was the higher propensity of lemon verbena and peppermint to have increased levels of organic acids when GI was applied, while lemon balm and bastard balm showed a general trend to lower amounts of organic acids when irradiated with EB.

Among tocopherols (Table 3), α and β isoforms were the ones presenting higher number of significant variations, but the produced effect was once again highly dependent on the assayed plant species. α-and β-Tocopherols are known for being less stable to irradiation than γ-tocopherol (Warner et al., 2008). Regarding total tocopherols, this dissimilarity among effects was also observed. For instance, lemon verbena present higher amounts in samples irradiated with 1 kGy, while the 10 kGy had a very positive effect on bastard balm (independently of irradiation technology in both case) and peppermint' tocopherols were increased when EB was applied. The significant changes in tocopherols profile in response to irradiation treatment had already been published in different species (Taipina et al., 2009).

Due to the high number of individual fatty acids (FA), these compounds were divided as those quantified below 1% in all species (Table 4A) and those above 1% at least in one species (Table 4B). Like it was verified for the previous parameters, the variations in FA were highly dependent on the analyzed plant species. Nevertheless, it is easily observable that irradiated samples (except for bastard balm) presented higher percentages of monounsaturated fatty acids (MUFA), which represents an interesting result. A similar result was also obtained for some particular polyunsaturated fatty acids (PUFA), such as C18:2n6, C18:3n6 (bastard balm), C18:3n3 (lemon balm) and C20:5n3 (peppermint). Besides C18:2n6, the variations for the remaining predominant FA (C16:0 and C18:3n3) were not particularly noticeable (exempting the decrease of C18:3n3 in bastard balm samples irradiated with 10 kGy). Among the studied plants, lemon balm was the one showing less variation in the FA profiles, especially those samples irradiated with EB. The higher effect in the remaining species might be related with their higher fat contents (Table 1), that might have boosted mechanisms of lipid radiolysis, involving primary ionization, followed by migration of the positive charge toward the carbonyl group or double bonds (Molins, 2001).

3.2. Effects on antioxidant parameters

The effects on the antioxidant activity, namely the scavenging effects on DPPH radicals, reducing power and inhibition of β-carotene bleaching, as well as the amounts of total phenols and flavonoids were also compared (gamma irradiation). In general, EB produced an increase in the ability to scavenge DPPH radicals and in the reducing power (especially the 10 kGy dose), while GI caused the opposite effect. On the other hand, the effect of irradiation on β-carotene bleaching inhibition did not seem to be ruled by any overall trend, being highly dependent on the extract type (aqueous or methanolic) and on the plant species. Regarding bioactive com-

Table 4A
Minor (<1% in all species) fatty acids of the aromatic species. The results are presented in relative percentage (controls; non-irradiated samples). Values for irradiated samples are presented as percentage of variation in comparison to the control.[a]

	C6:0		C8:0	C11:0	C12:0	C13:0	C15:0	C15:1	C17:0	C20:1n9	C20:2n6	C20:3n3+C21:0	C22:1n9
Dose	Irradiation type												
Aloysia citrodora (Lemon verbena)													
0 kGy	Control	0.30 ± 0.01	0.11 ± 0.01	0.26 ± 0.02	0.26 ± 0.02	0.32 ± 0.01	0.58 ± 0.02	0.10 ± 0.01	0.22 ± 0.01	0.25 ± 0.03	0.21 ± 0.01	0.30 ± 0.01	0.27 ± 0.02
1 kGy	Electron beam	−37 ± 6^{c}	−49 ± 12^{c}	-23 ± 8^{c}	10 ± 6^{b}	−50 ± 3^{d}	−19 ± 5^{c}	−26 ± 6^{c}	2 ± 4^{bc}	−16 ± 7^{b}	−14 ± 6^{b}	28 ± 9^{a}	83 ± 18^{b}
	Gamma rays	−7 ± 7^{a}	−4 ± 5^{b}	-19 ± 5^{bc}	9 ± 5^{b}	41 ± 9^{b}	5 ± 5^{b}	−14 ± 3^{b}	10 ± 5^{b}	62 ± 20^{a}	−19 ± 3^{b}	−12 ± 1^{bc}	36 ± 11^{c}
10 kGy	Electron beam	−22 ± 8^{b}	−42 ± 9^{c}	15 ± 6^{a}	5 ± 4^{b}	83 ± 6^{a}	−13 ± 8^{c}	−13 ± 3^{b}	−6 ± 6^{c}	−18 ± 9^{b}	−46 ± 6^{c}	−17 ± 4^{c}	181 ± 43^{a}
	Gamma rays	−24 ± 8^{b}	17 ± 6^{a}	-6 ± 8^{b}	40 ± 11^{a}	9 ± 5^{c}	23 ± 7^{a}	2 ± 4^{a}	27 ± 4^{a}	−11 ± 7^{b}	27 ± 5^{a}	−8 ± 4^{bc}	−32 ± 5^{d}
p-values	Homoscedasticity*	0.104	0.836	0.374	0.055	0.021	0.272	0.007	0.097	0.147	0.078	<0.001	<0.001
	1-way ANOVA[4]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Melissa officinalis (Lemon balm)													
0 kGy	Control	0.22 ± 0.01	0.40 ± 0.02	0.13 ± 0.01	0.46 ± 0.01	0.14 ± 0.01	0.44 ± 0.03	0.55 ± 0.01	0.81 ± 0.01	0.18 ± 0.02	nd	0.28 ± 0.01	nd
1 kGy	Electron beam	1 ± 2^{a}	−3 ± 2^{a}	1 ± 2^{b}	1 ± 2^{a}	−1 ± 5^{a}	−3 ± 4^{a}	−2 ± 2^{a}	2 ± 4	−1 ± 3^{a}	–	−1 ± 2^{b}	–
	Gamma rays	−30 ± 4^{b}	−25 ± 12^{b}	−2 ± 1^{bc}	−27 ± 1^{b}	15 ± 2^{a}	−4 ± 4^{a}	−12 ± 1^{b}	7 ± 1	−18 ± 2^{b}	–	25 ± 1^{a}	–
10 kGy	Electron beam	−3 ± 4^{a}	1 ± 2^{a}	−10 ± 5^{c}	−45 ± 6^{d}	−40 ± 10^{b}	−8 ± 6^{ab}	−30 ± 4^{c}	8 ± 10	−20 ± 12^{bc}	–	−8 ± 5^{b}	–
	Gamma rays	−36 ± 2^{b}	−27 ± 1^{b}	27 ± 2^{a}	−36 ± 2^{c}	1 ± 2^{a}	−19 ± 6^{b}	−7 ± 2^{a}	−1 ± 1	−33 ± 12^{c}	–	28 ± 1^{a}	–
p-values	Homoscedasticity*	<0.001	0.008	0.001	0.008	0.002	0.006	0.025	0.006	0.026	–	0.001	–
	1-way ANOVA[d]	<0.001	<0.001	<0.001	<0.001	<0.001	0.015	<0.001	0.578	<0.001	–	<0.001	–
Melittis melissophyllum (Bastard balm)													
0 kGy	Control	0.18 ± 0.01	0.07 ± 0.01	0.04 ± 0.01	0.18 ± 0.01	0.05 ± 0.01	0.90 ± 0.02	0.09 ± 0.01	0.24 ± 0.02	0.16 ± 0.01	0.09 ± 0.02	0.24 ± 0.01	nd
1 kGy	Electron beam	78 ± 24^{a}	79 ± 34^{b}	1 ± 2^{b}	5 ± 5^{b}	26 ± 5^{b}	−10 ± 4^{b}	14 ± 4^{a}	−20 ± 6^{b}	−18 ± 7^{c}	−20 ± 3^{b}	−10 ± 7^{b}	–
	Gamma rays	−64 ± 2^{c}	4 ± 5^{c}	10 ± 7^{b}	32 ± 6^{a}	35 ± 10^{b}	−7 ± 2^{b}	−9 ± 5^{b}	−2 ± 2^{a}	26 ± 7^{a}	68 ± 16^{a}	10 ± 4^{a}	–
10 kGy	Electron beam	29 ± 13^{b}	118 ± 13^{a}	3 ± 5^{b}	−23 ± 5^{c}	−11 ± 2^{c}	−29 ± 7^{c}	−3 ± 3^{b}	−18 ± 7^{b}	−12 ± 6^{c}	−24 ± 3^{b}	−36 ± 9^{b}	–
	Gamma rays	−58 ± 4^{c}	33 ± 8^{c}	127 ± 12^{a}	37 ± 2^{a}	48 ± 7^{a}	7 ± 3^{a}	17 ± 5^{a}	1 ± 2^{a}	10 ± 2^{b}	93 ± 21^{a}	1 ± 1^{a}	–
p-values	Homoscedasticity*	0.001	0.002	0.130	0.005	0.078	0.038	<0.001	0.143	0.023	<0.001	0.022	–
	1-way ANOVA**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	–
Mentha piperita (Peppermint)													
0 kGy	Control	0.15 ± 0.02	1.0 ± 0.1	0.12 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.59 ± 0.05	0.04 ± 0.01	0.44 ± 0.01	0.25 ± 0.01	0.19 ± 0.01	0.45 ± 0.04	0.11 ± 0.01
1 kGy	Electron beam	−23 ± 4^{b}	−22 ± 6^{b}	−14 ± 2^{b}	−8 ± 2^{d}	−72 ± 5^{c}	−15 ± 4^{b}	−43 ± 2^{c}	−6 ± 5^{b}	7 ± 7^{b}	32 ± 9^{a}	−18 ± 6^{c}	35 ± 5^{d}
	Gamma rays	2 ± 4^{ab}	−8 ± 5^{b}	27 ± 8^{a}	7 ± 5^{c}	−19 ± 6^{b}	−23 ± 6^{b}	6 ± 6^{a}	7 ± 2^{ab}	7 ± 7^{b}	−6 ± 6^{b}	6 ± 5^{b}	48 ± 10^{c}
10 kGy	Electron beam	21 ± 2^{a}	37 ± 9^{a}	−13 ± 2^{b}	53 ± 9^{a}	28 ± 7^{a}	16 ± 4^{a}	15 ± 1^{a}	8 ± 8^{a}	26 ± 13^{b}	−8 ± 8^{b}	16 ± 5^{a}	79 ± 7^{a}
	Gamma rays	−60 ± 16^{c}	−19 ± 6^{b}	−9 ± 4^{b}	29 ± 5^{b}	−71 ± 12^{c}	−12 ± 5^{b}	−9 ± 7^{b}	2 ± 2^{ab}	52 ± 2^{a}	−20 ± 7^{b}	18 ± 5^{a}	61 ± 3^{b}
p-values	Homoscedasticity*	<0.001	0.229	0.136	0.011	<0.001	0.017	<0.001	<0.001	<0.001	<0.001	0.316	0.018
	1-way ANOVA**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.030	<0.001	<0.001	<0.001	<0.001

[a] The results are presented as the mean ± SD.

* Homoscedasticity among obtained ratios was tested by the Levene test: homoscedasticity, $p > 0.05$; heteroscedasticity, $p < 0.05$.

** $p < 0.05$ indicates that the mean value of at least one ratio differs from the others (in this case multiple comparison tests were performed). For each species, means within a column with different letters differ significantly ($p < 0.05$).

Table 4B
Major (>1%, at least in one species) fatty acids of the aromatic species. The results are presented in relative percentage (controls; non-irradiated samples). Values for irradiated samples as presented as percentage of variation in comparison to the control.[a]

		C10:0	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1n9	C18:2n6	C18:3n6	C18:3n3	C20:0	C20:5n3	C22:0	C23:0	C22:6n3	C24:0	SFA	MUFA	PUFA
Dose	Irradiation type																			
Aloysia citrodora (Lemon verbena)																				
0 kGy	Control	nd	1.1 ± 0.1	nd	15.7 ± 0.2	0.50 ± 0.02	1.17 ± 0.01	0.95 ± 0.02	12.6 ± 0.1	nd	56.2 ± 0.3	0.87 ± 0.02	nd	1.00 ± 0.02	5.4 ± 0.1	nd	1.4 ± 0.1	28.6 ± 0.2	2.07 ± 0.03	69.3 ± 0.3
1 kGy	Electron beam	–	-20 ± 5^{c}	–	5 ± 4^{a}	-18 ± 5^{b}	12 ± 4^{a}	8 ± 6^{b}	5 ± 2^{a}	–	-3 ± 1^{c}	10 ± 5^{a}	–	29 ± 9^{a}	15 ± 5^{a}	–	9 ± 7^{bc}	4 ± 2^{b}	9 ± 5^{b}	-2 ± 1^{b}
	Gamma rays	–	26 ± 7^{a}	–	1 ± 2^{ab}	25 ± 5^{a}	-5 ± 1^{b}	1 ± 2^{bc}	-1 ± 1^{b}	–	1 ± 1^{b}	13 ± 2^{a}	–	-18 ± 2^{c}	-22 ± 1^{c}	–	22 ± 2^{ab}	-2 ± 2^{c}	17 ± 3^{a}	1 ± 1^{a}
10 kGy	Electron beam	–	10 ± 8^{b}	–	-3 ± 2^{b}	35 ± 12^{a}	-8 ± 4^{b}	-3 ± 4^{c}	-8 ± 4^{c}	–	3 ± 2^{a}	-11 ± 5^{b}	–	11 ± 8^{b}	-4 ± 2^{b}	–	-7 ± 7^{c}	-4 ± 2^{c}	18 ± 6^{a}	1 ± 1^{a}
	Gamma rays	–	-15 ± 6^{c}	–	5 ± 4^{a}	27 ± 5^{a}	13 ± 1^{a}	19 ± 3^{a}	-1 ± 1^{b}	–	-3 ± 1^{c}	-33 ± 6^{c}	–	-7 ± 5^{c}	10 ± 4^{a}	–	32 ± 6^{a}	6 ± 2^{a}	10 ± 2^{b}	-3 ± 1^{b}
p-values	Homoscedasticity*	–	0.051	–	0.620	0.012	0.001	0.002	0.001	–	0.129	0.038	–	0.001	<0.001	–	<0.001	0.600	0.002	0.470
	1-way ANOVA**	–	<0.001	–	<0.001	<0.001	<0.001	<0.001	<0.001	–	<0.001	<0.001	–	<0.001	<0.001	–	<0.001	<0.001	0.002	<0.001
Melissa officinalis (Lemon balm)																				
0 kGy	Control	0.29 ± 0.02	2.9 ± 0.1	0.53 ± 0.01	22.7 ± 0.3	nd	3.6 ± 0.1	4.9 ± 0.2	15.3 ± 0.4	nd	33.2 ± 0.5	3.4 ± 0.1	3.9 ± 0.1	1.3 ± 0.1	3.3 ± 0.2	nd	1.2 ± 0.2	41.2 ± 0.5	6.2 ± 0.2	52.6 ± 0.5
1 kGy	Electron beam	1 ± 2^{a}	-3 ± 4^{a}	-2 ± 4^{c}	1 ± 1^{a}	–	1 ± 2^{a}	-1 ± 2^{a}	-1 ± 1^{bc}	–	-1 ± 1^{c}	1 ± 2^{bc}	-1 ± 2^{b}	1 ± 2^{b}	1 ± 2^{b}	–	-10 ± 5^{b}	1 ± 1^{a}	-1 ± 2^{a}	-1 ± 1^{c}
	Gamma rays	-13 ± 3^{b}	-9 ± 2^{ab}	-1 ± 2^{c}	-8 ± 1^{c}	–	1 ± 1^{a}	-2 ± 2^{a}	-1 ± 1^{c}	–	4 ± 1^{b}	17 ± 2^{a}	16 ± 1^{a}	12 ± 5^{a}	-1 ± 2^{b}	–	12 ± 5^{a}	-4 ± 1^{b}	-3 ± 1^{a}	3 ± 1^{b}
10 kGy	Electron beam	-18 ± 5^{b}	-48 ± 6^{c}	39 ± 10^{a}	-15 ± 2^{d}	–	-2 ± 2^{a}	-4 ± 4^{a}	5 ± 2^{a}	–	8 ± 2^{a}	-1 ± 2^{c}	-16 ± 7^{c}	-5 ± 5^{b}	29 ± 8^{a}	–	-8 ± 4^{b}	-9 ± 2^{d}	1 ± 2^{a}	7 ± 1^{a}
	Gamma rays	-26 ± 3^{c}	-15 ± 1^{b}	18 ± 2^{b}	-5 ± 1^{b}	–	-11 ± 2^{b}	-12 ± 3^{b}	2 ± 2^{b}	–	9 ± 1^{a}	4 ± 2^{b}	-9 ± 1^{c}	11 ± 5^{a}	-5 ± 5^{b}	–	-8 ± 6^{b}	-6 ± 1^{c}	-10 ± 2^{b}	6 ± 1^{a}
p-values	Homoscedasticity*	0.106	<0.001	<0.001	0.196	–	0.045	0.005	0.621	–	0.080	0.177	<0.001	0.093	0.274	–	0.072	0.581	0.010	0.659
	1-way ANOVA**	<0.001	<0.001	<0.001	<0.001	–	<0.001	<0.001	<0.001	–	<0.001	<0.001	<0.001	<0.001	<0.001	–	<0.001	<0.001	<0.001	<0.001
Melittis melissophyllum (Bastard balm)																				
0 kGy	Control	nd	0.58 ± 0.03	nd	14.3 ± 0.2	1.29 ± 0.05	2.41 ± 0.05	11.5 ± 0.3	14.8 ± 0.4	5.8 ± 0.1	36 ± 1	0.88 ± 0.02	nd	1.3 ± 0.1	6.2 ± 0.2	nd	3.0 ± 0.1	30.4 ± 0.2	13.1 ± 0.2	56.5 ± 0.2
1 kGy	Electron beam	–	36 ± 7^{b}	–	-7 ± 3^{c}	12 ± 6^{a}	5 ± 4^{ab}	16 ± 4^{c}	10 ± 5^{c}	33 ± 10^{a}	-7 ± 3^{a}	5 ± 5^{a}	–	-13 ± 4^{b}	-10 ± 4^{a}	–	1 ± 2	-5 ± 2^{b}	15 ± 3^{c}	-2 ± 1^{a}
	Gamma rays	–	39 ± 5^{b}	–	-1 ± 1^{b}	-11 ± 4^{bc}	1 ± 1^{b}	13 ± 2^{c}	9 ± 3^{c}	1 ± 1^{b}	-7 ± 1^{a}	8 ± 2^{a}	–	3 ± 3^{a}	-4 ± 2^{a}	–	-5 ± 4	-1 ± 1^{a}	10 ± 2^{c}	-2 ± 1^{a}
10 kGy	Electron beam	–	-2 ± 4^{c}	–	-7 ± 4^{c}	-25 ± 8^{c}	1 ± 2^{b}	51 ± 5^{a}	31 ± 6^{a}	18 ± 6^{ab}	-21 ± 3^{b}	-26 ± 8^{b}	–	-35 ± 7^{c}	-11 ± 8^{a}	–	-5 ± 4	-10 ± 3^{c}	46 ± 4^{a}	-7 ± 1^{b}
	Gamma rays	–	59 ± 12^{a}	–	6 ± 1^{a}	-3 ± 3^{ab}	14 ± 2^{a}	31 ± 6^{b}	23 ± 1^{b}	9 ± 4^{b}	-21 ± 2^{b}	10 ± 4^{a}	–	11 ± 4^{a}	-33 ± 2^{b}	–	1 ± 2	-1 ± 1^{a}	27 ± 5^{b}	-6 ± 1^{b}
p-values	Homoscedasticity*	–	0.463	–	0.014	0.012	0.008	0.024	0.003	<0.001	0.003	0.001	–	0.802	<0.001	–	0.993	0.045	0.007	0.053
	1-way ANOVA**	–	<0.001	–	<0.001	<0.001	0.001	<0.001	<0.001	0.005	<0.001	<0.001	–	<0.001	<0.001	–	0.216	<0.001	<0.001	<0.001
Mentha piperita (Peppermint)																				
0 kGy	Control	0.07 ± 0.01	1.4 ± 0.1	1.2 ± 0.1	10.4 ± 0.3	0.88 ± 0.05	2.47 ± 0.03	1.62 ± 0.05	7.3 ± 0.1	nd	46 ± 1	15.8 ± 0.5	2.8 ± 0.2	2.6 ± 0.1	0.24 ± 0.0	1.4 ± 0.1	2.1 ± 0.1	38 ± 1	4.1 ± 0.1	58 ± 1
1 kGy	Electron beam	-34 ± 2^{a}	-2 ± 4^{c}	6 ± 6^{a}	-5 ± 3^{b}	-12 ± 5^{b}	8 ± 4^{b}	33 ± 6^{b}	3 ± 3^{b}	–	1 ± 1^{a}	2 ± 4^{b}	-17 ± 4^{b}	1 ± 2^{b}	2 ± 4^{ab}	-6 ± 4^{b}	-5 ± 2^{ab}	-2 ± 2^{c}	8 ± 4^{b}	1 ± 1^{a}
	Gamma rays	-76 ± 9^{a}	11 ± 5^{bc}	-1 ± 2^{a}	-1 ± 1^{b}	9 ± 5^{a}	3 ± 1^{b}	-1 ± 2^{d}	2 ± 1^{b}	–	-4 ± 2^{b}	5 ± 3^{b}	8 ± 4^{a}	7 ± 2^{ab}	-20 ± 8^{b}	9 ± 3^{a}	-12 ± 5^{b}	2 ± 2^{b}	4 ± 2^{b}	-2 ± 1^{b}
10 kGy	Electron beam	-20 ± 3^{a}	30 ± 9^{a}	4 ± 4^{a}	13 ± 4^{a}	8 ± 8^{a}	21 ± 6^{a}	42 ± 4^{a}	8 ± 3^{a}	–	-6 ± 2^{bc}	4 ± 2^{b}	8 ± 6^{a}	10 ± 5^{a}	-5 ± 8^{ab}	-17 ± 3^{c}	4 ± 4^{a}	10 ± 2^{a}	20 ± 3^{a}	-5 ± 1^{c}
	Gamma rays	-376 ± 53^{b}	16 ± 4^{ab}	-20 ± 6^{b}	-3 ± 3^{b}	-9 ± 4^{b}	5 ± 3^{b}	15 ± 3^{c}	-2 ± 1^{c}	–	-7 ± 2^{c}	12 ± 2^{a}	15 ± 4^{a}	9 ± 4^{a}	9 ± 2^{a}	10 ± 3^{a}	-12 ± 3^{b}	5 ± 2^{b}	10 ± 2^{b}	-4 ± 1^{c}
p-values	Homoscedasticity*	<0.001	0.001	0.265	0.104	0.179	0.014	0.014	0.001	–	0.143	0.007	0.013	0.093	<0.001	0.090	0.124	0.787	0.007	0.092
	1-way ANOVA**	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	–	<0.001	<0.001	<0.001	0.002	0.042	<0.001	<0.001	<0.001	<0.001	<0.001

[a] The results are presented as the mean ± SD.

* Homoscedasticity among obtained ratios was tested by the Levene test: homoscedasticity, $p > 0.05$; heteroscedasticity, $p < 0.05$.

** $p < 0.05$ indicates that the mean value of at least one ratio differs from the others (in this case multiple comparison tests were performed). For each species, means within a column with different letters differ significantly ($p < 0.05$).

Table 5
Antioxidant properties of extracts from the aromatic species.[1] EC_{50} values (μg/mL) for the controls (non-irradiated samples) are presented for all assays except phenols and flavonoids, which are expressed as mg GAE/g extract and mg CE/g extract, respectively. Values for irradiated samples are presented as percentage of variation in comparison to the control.[a]

		DPPH scavenging activity		Reducing power		β-carotene bleaching inhibition		Phenols		Flavonoids	
Dose	Irradiation type	Infusion	MeOH	Infusion	MeOH	Infusion	MeOH	Infusion	MeOH	Infusion	MeOH
Aloysia citrodora (Lemon verbena)											
0 kGy	Control	232 ± 8	39 ± 4	169 ± 1	22.8 ± 0.3	580 ± 31	208 ± 9	134 ± 8	665 ± 13	92 ± 1	369 ± 5
1 kGy	Electron beam	−1 ± 2^{a}	13 ± 8^{c}	−13 ± 2^{d}	−9 ± 1^{c}	−10 ± 5^{c}	254 ± 63^{a}	4 ± 4^{d}	5 ± 2^{a}	3 ± 6^{a}	7 ± 2^{a}
	Gamma rays	2 ± 1^{a}	130 ± 16^{b}	9 ± 1^{a}	115 ± 1^{b}	73 ± 7^{a}	14 ± 7^{c}	41 ± 11^{b}	−20 ± 6^{b}	−35 ± 2^{c}	-3 ± 3^{b}
10 kGy	Electron beam	−8 ± 5^{b}	−13 ± 5^{d}	−11 ± 1^{c}	−10 ± 1^{c}	67 ± 25^{a}	60 ± 28^{b}	30 ± 5^{c}	6 ± 1^{a}	12 ± 6^{a}	7 ± 2^{a}
	Gamma rays	−12 ± 6^{b}	177 ± 15^{a}	1 ± 1^{b}	172 ± 2^{a}	43 ± 6^{b}	−5 ± 3^{c}	54 ± 8^{a}	−31 ± 3^{c}	−18 ± 4^{b}	−25 ± 1^{c}
p-values	Homoscedasticity*	<0.001	<0.001	<0.001	0.014	<0.001	<0.001	0.038	<0.001	<0.001	0.001
	1-way ANOVA**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Melissa officinalis (Lemon balm)											
0 kGy	Control	101 ± 3	67 ± 1	80 ± 1	44 ± 1	165 ± 4	125 ± 3	100 ± 1	829 ± 6	63 ± 1	448 ± 4
1 kGy	Electron beam	−7 ± 5^{c}	17 ± 8^{a}	1 ± 1^{c}	20 ± 1^{b}	86 ± 8^{b}	−14 ± 3^{b}	−5 ± 2^{c}	−12 ± 1^{c}	8 ± 8	−12 ± 1^{d}
	Gamma rays	1 ± 1^{b}	9 ± 3^{b}	−6 ± 1^{d}	8 ± 1^{c}	−21 ± 2^{c}	−10 ± 1^{a}	8 ± 1^{a}	−5 ± 2^{b}	9 ± 1	11 ± 1^{a}
10 kGy	Electron beam	−14 ± 5^{d}	−9 ± 3^{c}	9 ± 1^{b}	1 ± 1^{d}	118 ± 15^{a}	−14 ± 4^{b}	−6 ± 2^{c}	1 ± 1^{a}	5 ± 5	4 ± 1^{b}
	Gamma rays	7 ± 2^{a}	8 ± 2^{b}	28 ± 1^{a}	25 ± 1^{a}	-18 ± 1^{c}	−13 ± 1^{ab}	4 ± 1^{b}	−10 ± 1^{c}	4 ± 1	−7 ± 1^{c}
p-values	Homoscedasticity*	<0.001	<0.001	0.075	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	1-way ANOVA**	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	<0.001	0.499	<0.001
Melittis melissophyllum (Bastard balm)											
0 kGy	Control	583 ± 24	354 ± 39	512 ± 16	249 ± 2	1648 ± 154	447 ± 66	70 ± 4	160 ± 3	29 ± 2	108 ± 4
1 kGy	Electron beam	−12 ± 4^{c}	36 ± 7^{a}	−7 ± 2^{c}	35 ± 1^{a}	−13 ± 2^{c}	−22 ± 4^{b}	10 ± 1^{a}	6 ± 2^{a}	12 ± 1^{a}	1 ± 1^{b}
	Gamma rays	19 ± 7^{b}	2 ± 4^{b}	18 ± 4^{a}	−20 ± 2^{c}	28 ± 5^{b}	21 ± 5^{a}	3 ± 4^{ab}	−37 ± 2^{d}	−45 ± 5^{c}	−32 ± 3^{d}
10 kGy	Electron beam	−1 ± 2^{c}	−24 ± 2^{c}	6 ± 1^{b}	−38 ± 2^{d}	−14 ± 4^{c}	−15 ± 4^{b}	−1 ± 1^{c}	−5 ± 1^{b}	-3 ± 2^{b}	15 ± 4^{a}
	Gamma rays	45 ± 8^{a}	1 ± 2^{b}	−11 ± 2^{c}	16 ± 2^{b}	40 ± 10^{a}	35 ± 7^{a}	−1 ± 2^{c}	−16 ± 2^{c}	−49 ± 3^{c}	−23 ± 2^{c}
p-values	Homoscedasticity*	<0.001	<0.001	<0.001	0.487	<0.001	0.081	<0.001	<0.001	0.002	0.001
	1-way ANOVA**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.011	<0.001	<0.001	<0.001
Mentha piperita (Peppermint)											
0 kGy	Control	184 ± 5	83 ± 7	119 ± 2	52 ± 2	597 ± 44	184 ± 5	218 ± 2	591 ± 19	117 ± 2	319 ± 6
1 kGy	Electron beam	−12 ± 4^{c}	18 ± 3^{a}	16 ± 1^{c}	1 ± 1^{a}	−27 ± 4^{c}	92 ± 19^{b}	−1 ± 1^{a}	−6 ± 1^{a}	−1 ± 1^{a}	−8 ± 1^{b}
	Gamma rays	4 ± 2^{b}	15 ± 3^{b}	13 ± 1^{a}	−22 ± 3^{c}	−28 ± 8^{b}	−35 ± 4^{a}	21 ± 1^{ab}	−4 ± 1^{d}	−23 ± 3^{c}	10 ± 1^{d}
10 kGy	Electron beam	−14 ± 3^{c}	−7 ± 2^{c}	35 ± 1^{b}	15 ± 1^{d}	63 ± 15^{c}	32 ± 6^{b}	1 ± 1^{c}	−6 ± 1^{b}	−1 ± 2^{b}	−11 ± 1^{a}
	Gamma rays	18 ± 3^{a}	4 ± 2^{b}	18 ± 3^{c}	1 ± 2^{b}	15 ± 5^{a}	−64 ± 10^{a}	10 ± 1^{c}	−12 ± 2^{c}	−51 ± 4^{c}	−20 ± 2^{c}
p-values	Homoscedasticity*	0.140	0.086	0.002	0.066	0.003	<0.001	0.006	<0.001	0.499	0.001
	1-way ANOVA**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

MeOH- Methanol; GAE- Gallic acid equivalents; CE- Catechin equivalents.

[a] The results are presented as the mean ± SD.

* Homoscedasticity among obtained ratios was tested by the Levene test: homoscedasticity, $p > 0.05$; heteroscedasticity, $p < 0.05$.

** $p < 0.05$ indicates that the mean value of at least one ratio differs from the others (in this case multiple comparison tests were performed). For each species, means within a column with different letters differ significantly ($p < 0.05$).

pounds, irradiation tended to increase the levels of total phenols in infusions, while methanolic extracts suffered the opposite effect. Flavonoids tended to diminish with irradiation, independently of plant species, extract type or irradiation technology.

3.3. Linear discriminant analysis (LDA)

In the former sections, the differences resulting from irradiation treatment at different doses were compared for each individual parameter within each species. Despite the significant variations verified in several cases, it was not possible to identify unequivocal tendencies. Accordingly, the results were evaluated considering data for all irradiation conditions and evaluated parameters simultaneously. In the performed LDA, irradiation conditions and plant species were sequentially used as grouping factors. All those parameters not detected in the four species were not used in the analysis.

The significant independent variables (evaluated parameters) were selected using the stepwise procedure of the LDA, according to the Wilks' λ test. Only those with a statistical significant classification performance ($p < 0.050$) were kept in the analysis.

In the discriminant model obtained to verify if the different irradiation treatments (EB, 1 kGy; EB 10 kGy; GI, 1 kGy; GI 10 kGy) exerted variations in the evaluated parameters in a specific way, the three defined functions (plotted in Fig. 1A) integrated 100% of the observed variance (first: 71.4%; second: 16.1%; third: 12.5%). Among the tested variables 26 were selected as having discriminant ability: fat, carbohydrates, energy, sucrose, organic acids, C6:0, C11:0, C13:0, C14:0, C15:0, C18:0, C18:2n6, C20:0, C20:1, C20:3n3 + C21:0, MUFA and all those in Table 5, which indicates that the fatty acids profile and the antioxidant activity were the most affected variables considering the overall results of the different irradiation treatments. The groups corresponding to each condition were completely individualized, thereby indicating that its effects are highly specific. Function 1 (more correlated with DPPH scavenging activity in infusions, total phenols and flavonoids in methanolic extracts) separated mainly the groups corresponding to the 10 kGy dose of both types of irradiation; function 2 (more correlated with C13:0, β-carotene bleaching inhibition in methanolic extracts and flavonoids in infusions) separated mainly EB at 1 kGy dose, while function 3 (more correlated with C20:0, carbohydrates, β-carotene bleaching inhibition in infusions and MUFA) was more effective in separating the doses of 1 kGy and 10 kGy for both irradiation sources.

In the assessment of the interaction with the plant species the three defined functions included also 100% of the observed variance (first: 48.0%; second: 29.5%; third: 22.5%), selecting 30 variables (fat, protein, ash, fructose, sucrose, trehalose, oxalic acid, organic acids, α-tocopherol, tocopherols, C6:0, C8:0, C13:0, C14:0, C16:0, C18:1n9, C18:3n3, C20:0, C20:1, C23:0, C24:0, SFA, MUFA and all the variables in Table 5, except DPPH scavenging activity and flavonoids content in infusions). Likewise, the defined functions separate the markers corresponding to each of the assayed species (Fig. 1B). Function 1 (highly correlated to C18:3n3, C8:0, C18:1n9, C14:0 and fat) separated mainly bastard balm (*M. melissophyllum*); function 2 (more correlated to reducing power in infusions, trehalose and C13:0) contributed mainly to discriminate peppermint (*M. piperita*); finally, function 3 (closely correlated to phenols in infusions, MUFA, protein and β-carotene bleaching inhibition in methanolic extracts) allowed to separate lemon verbena (*M. officinalis*).

Overall, when analyzed individually, the chemical parameters and bioactive indicators of the tested aromatic plants showed that the effects of EB and GI irradiation were highly dependent on the plant species. After, when evaluated together it became evident that changes in fatty acids profiles and antioxidant activity were those showing the highest differences, either when discriminating among irradiation conditions, as well as plant species. Combining this information with that obtained in Tables 1–5, that highlight irradiated samples as having higher MUFA (and some PUFA) percentages and a beneficial effect of EB irradiation on the antioxidant activity, it might be concluded that the most suitable solution to irradiate aromatic plants would be EB. Nevertheless, the dependence on the plant species and irradiation dose was strongly demonstrated, advising for accurate studies of any plant species to be considered for irradiation.

Conflict of interest

The authors declare no conflict of interest.

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