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How gamma and electron-beam irradiations modulate phenolic profile expression in *Melissa officinalis* L. and *Melittis melissophyllum* L.

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ABSTRACT

Owing to the overall increase in herbal infusions' consumption, there's a progressively higher need of suitable plant material, as well as adequate conservation techniques to maintain its quality. Among, the available technologies, irradiation is gaining interest as a feasible preservation method. In line with this approach, this wok was designed to evaluate the effects of electron-beam and gamma irradiation over the phenolic profiles of two plant species *Melissa officinalis* L. (LB) and *Melittis melissophyllum* L. (BB). Individual phenolics were characterized by high-performance liquid chromatography coupled to a diode array detector and a mass spectrometer (HPLC-DAD-ESI/MS). Irradiated samples showed a general increase in individual phenolic contents, especially in lithospermic acid A in LB and 5-*O*-caffeoylquinic acid in BB. Thus, this study revealed the potential usefulness of both conservation technologies when employed to this type of plants.

1. Introduction

In line with the current food-related concerns, people are generally more receptive to drink herbal infusions, not only due to their pleasant sensory properties, but also for their health-promoting potential (Barroso et al., 2016; Dias et al., 2016), which is mainly provided by their high levels of bioactive agents (Fraga, Galleano, Verstraeten, & Oteiza, 2010; Pereira, Barros et al., 2015).

These bioactive agents correspond to secondary metabolites that might be divided in five main categories: phenolic compounds, carotenoids, alkaloids, nitrogen-containing compounds and organosulfur compounds. Among them, phenolic compounds, owing to their acknowledged antioxidant, anti-inflammatory, antimicrobial and anti-tumor properties, have the highest potential to prevent, reduce and/or treat several pathological conditions (Espín, García-Conesa, & Tomás-Barberán, 2007; Huang, Boxin, & Prior, 2005; Hübsch, Van Zyl, Cock, & Van Vuuren, 2014). Flavonoids and phenolic acids, in particular, are known for their high biological activity, having been reported for their positive effect in the prevention of a several chronic diseases (Bordoloi et al., 2016; Umar Lule & Xia, 2005), or their capacity as food preservatives by stabilizing lipid peroxidation and inhibiting different enzymes associated to oxidation processes (Bernal, Mendiola, Ibáñez, & Cifuentes, 2011; Gonçalves, Gomes, Costa, & Romano, 2013; Shan, Cai, Sun, & Corke, 2005).

In general, phenolic compounds are rarely present in their free form, except when the attached groups have undergone thermal processing or storage at room temperatures, inducing the conversion of low-molecular weight flavan-3-ol derivatives to high-molecular weight tannins, the hydrolysis of flavonol glycosides into the corresponding aglycones and increase in the phenolic acids (among other reactions such as oxidation or isomerisation) (Mäkilä, Laaksonen, Kallio, & Yang, 2017). Accordingly, the employment of a new processing technology should always include the evaluation of potential changes in the phenolic profiles of the food product to which that technology is applied.

Presently, irradiation is one of the most promising processing technologies, being increasingly used to decontaminate dried plants and extend their shelf-life, in order to meet specific food safety requirements (Hallman, 2011). Among the available irradiation types, gamma and electron-beam are the most commonly used in food products (either pre-packaged or unpackaged), being usually applied at room temperature (Alothman, Bhat, & Karim, 2009).

Nevertheless, the validation of irradiation as a food processing technology requires the control of several chemical and biochemical parameters, particularly those with high levels of bioactivity, such as phenolic acids and flavonoids. In fact, the effects of electron-beam or gamma irradiation over phenolic profiles have been characterized in different herbal species, such as *Ginkgo biloba* L., *Mentha x piperita* L. or *Thymus vulgaris* L. (Pereira, Antonio et al., 2015; Pereira, Pimenta et al.,

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2016). However, and despite the reports describing the phenolic profiles of *Melissa officinalis* L. (lemon balm: LB) and *Melittis melissophyllum* (bastard balm: BB) (Barros et al., 2013; Carocho et al., 2015; Duda et al., 2015; Heitz, Carnat, Fraisse, Carnat, & Lamaison, 2000; Maggi et al., 2011; Miron, Herrero, & Ibáñez, 2013; Shakeri, Sahebkar, & Javadi, 2016; Skrzypczak-Pietraszek & Pietraszek, 2012), there are no studies pertaining to the potential effects of irradiation on the phenolic compounds of those species .

Accordingly, the aim of this study was to identify potential changes in the phenolic compounds of irradiated samples of *M. officinalis* and *M. melissophyllum*, in order to achieve a step further in the validation of electron-beam and gamma irradiation as conservation technologies to apply to highly consumed plant species.

2. Materials and methods

2.1. Dried samples and irradiation procedure

2.1.1. Dried samples

M. officinalis (LB) and *M. melissophyllum*, (BB) were provided by a local producer – Pragmático Aroma Lda, Alfândega da Fé, Bragança, Portugal. Dried samples were divided in groups, separating the control sample (non-irradiated, 0 kGy) and samples irradiated with 1 and 10 kGy doses.

2.1.2. Irradiation procedure

For gamma irradiation, a Co-60 experimental chamber (Precisa 22, Graviner Manufacturing Company Ltd., Gosport, UK) was used, with a total activity of 177 TBq (4.78 kCi), as previously described (Pereira, Antonio et al., 2015). The estimated doses, dose rates and dose uniformity ratios (D_{max}/D_{min}) were, respectively: 1.2 ± 0.1 kGy, 2.6 ± 0.2 kGy h^{-1}, 1.2 for sample 1 and 8.9 ± 0.1 kGy, 1.91 ± 0.03 kGy h^{-1}, 1.0 for sample 2. The values 0, 1 and 10 kGy, represent the doses used of non-irradiated and irradiated groups.

For electron-beam irradiation, three types of dosimeters were used to estimate the dose during the irradiation process (Pereira, Barros et al., 2016). The estimated absorbed dose for electron-beam irradiated samples were 0.83 and 10.09 kGy, for group 1 and group 2 respectively, measured with a maximum uncertainty of 20%.

2.2. Standards and reagents

Acetonitrile 99.9% of high-performance liquid chromatography (HPLC) grade was purchased from Fisher Scientific (Lisbon, Portugal). Phenolic compound standards (caffeic acid, ferulic acid, 3-*O*-caffeoylquinic acid, *p*-coumaric acid and rosmarinic acid) were acquired from Extrasynthese (Genay, France). Water was treated in Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA). Ferrous ammonium sulfate(II) hexahydrate, sodium chloride and sulfuric acid, all with PA purity, were purchased from Panreac S.A. (Barcelona, Spain) (proanalysis).

2.3. Infusions preparation

The infusions were prepared as previously described (Pereira, Antonio et al., 2015). After boiling 200 mL of distilled water, the dried sample (1 g) was added and left to stand at room temperature during 5 min, and then filtered under reduced pressure. The obtained infusions were frozen and lyophilized.

2.4. Analysis of phenolic compounds

The lyophilized infusions were dissolved in water at a final concentration of 5 mg/mL. The extracts were analysed using a HPLC chromatograph (Agilent Technologies, Santa Clara, CA, USA) with double online detection using a diode array detector (DAD) with 280 and 370 nm as preference wavelengths, and a mass spectrometer (MS) equipped with an ESI source and a triple quadrupole-ion trap mass analyser (Applied Biosystems, Darmstadt, Germany), following a previously described procedure (Barros et al., 2013). Phenolic compounds quantification was performed through calibration curves of phenolic standards and the results were expressed in mg/L of infusion.

2.5. Statistical analysis

Three independent extractions were performed, and each of these replicates was assayed three times. An analysis of variance (ANOVA) with type III sums of squares was performed using the GLM (General Linear Model) procedure of the SPSS software (IBM Corp., New York, USA). The fulfilment of ANOVA requirements, specifically the normal distribution of the residuals and the homogeneity of variance, was tested by means of the Shapiro-Wilk, and the Levene tests, respectively. The dependent variables were analysed using a 2-way ANOVA, with the factors “irradiation dose” (ID) and “irradiation technology” (IT). Every time both factors acted cooperatively (significant interaction), they were analysed by the estimated marginal mean plots. On the other hand, if no statistical significant interaction was verified ($p > 0.050$), means for different irradiation doses were compared using Tukey’s HSD (for homoscedastic distributions) or Tamhane’s T2 (heteroscedastic distributions). Results for different irradiation types were classified using a simple *t*-test, since there were less than three groups.

In order to obtain some overall conclusions, common to both species, a linear discriminant analysis (LDA) was used to evaluate the effect of irradiation over individual phenolic compounds. A stepwise technique, using the Wilk λ method with the usual probabilities of *F* (3.84 to enter and 2.71 to remove), was applied for variable selection. The stepwise process is based on a combination of forward selection and backward elimination steps, where the inclusion of a new variable is only done after ensuring that all previously selected variables remain significant. In this way, it is possible to identify the significant variables obtained for each factor. A leaving-one-out cross-validation procedure was carried out to assess the model performance.

3. Results and discussion

3.1. Individual phenolic compounds

Compound characteristics and tentative identification of phenolic compounds present in bastard balm (BB) and lemon balm (LB) infusions are given in Tables 1 and 2, respectively. Compounds were identified based on their chromatographic, UV and mass spectra characteristics (Fig. 1). In BB, six compounds were characterized (Table 1), divided into three phenolic acid derivatives (1^{BB}, 2^{BB} and 3^{BB}) and three coumarin derivatives (4^{BB}, 5^{BB} and 6^{BB}). Compounds 2BB (*o*-coumaric acid), 3BB (5-*O*-caffeoylquinic acid) and 4BB (coumarin) have been previously reported in the same plant species (Maggi et al., 2011; Skrzypczak-Pietraszek & Pietraszek, 2012). Nonetheless, to the best of our knowledge, compound 1^{BB} (3-*O*-caffoylquinic acid) and the coumarin derivatives 5^{BB} and 6^{BB} were not detected before. For these compounds (5^{BB} and 6^{BB}), it was not possible to identify the glycosylation pattern, having been indicated as coumarin derivatives, after confirming the presence of the aglycone in their UV–Vis spectra (both similar to the spectrum of compound 4^{BB}).

The phenolic profile of lemon balm (LB) infusions presented higher diversity, including twenty phenolic compounds, mostly corresponding to caffeic acid derivatives (Table 2). Nonetheless, a similar profile was previously observed in the decoctions obtained from the same species (Carocho et al., 2015), presenting differences only in two compounds, specifically yunnaneic acid F (peak 10^{LB}) and salvianolic acid C derivative II (peak 12^{LB}). Even so, peak 10^{LB} was previously identified in infusions obtained with different *M. officinalis* formulations (cultivated, *in vitro* cultured, commercial granulate and commercial “tea”-bag),

Table 1
Retention time (Rt), maximum absorption wavelength (< lambda > max), mass spectral data, and tentative identification of the phenolic compounds found in Bastard Balm infusions prepared from non-irradiated and irradiated samples, and analysed by HPLC and MS techniques.

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification
1^{BB}	5.2	326	353	191(100),179(80),173(5),161(5),135(20)	3-*O*-Caffeoylquinic acid
2^{BB}	7.0	264	325	163(36),191(100)	*o*-Coumaric acid hexoside
3^{BB}	8.1	326	353	191(100),179(40),173(20),161(18),135(21)	5-*O*-Caffeoylquinic acid
4^{BB}	12.7	278,sh314	651	325(100),163(57),119(61)	Coumarin
5^{BB}	22.8	278,sh320	–	–	Coumarin derivative I
6^{BB}	28.3	278,sh312	–	–	Coumarin derivative II

BB: bastard balm; sh: shoulder.

which showed similar profiles to the one observed in the present LB samples (Barros et al., 2013).

3.2. Effects of gamma and electron-beam irradiation on individual phenolic compounds

Values presented for each irradiation dose (ID) were obtained considering samples irradiated with ^{60}Co and those submitted to electron-beam irradiation, aiming to identify the most suitable ID to modulate the expression of each phenolic compound, independently of the used technology. Similarly, the results presented for each irradiation technology (IT) included the contribution of unirradiated and irradiated (1 or 10 kGy) samples, allowing to select the most suitable technology, regardless the applied ID.

The interaction among factors (ID × IT) was significant for all compounds characterized in BB and the great majority of compounds quantified in LB [except for 1^{LB}: 3-(3,4-dihydroxyphenyl) lactic acid; 10^{LB}: yunnaneic acid F; 13^{LB}: rosmarinic acid hexoside; 16^{LB}: salvianolic acid A]. Actually, the cooperative effect of IT and ID was previously observed in *Tropaeolum majus* L. and *Viola tricolor* L. (Koike et al., 2015a, 2015b).

Regarding BB (Table 3), the most abundant compounds were coumarin (4^{BB}), *o*-coumaric acid hexoside (2^{BB}) and 5-*O*-caffeoylquinic acid (3^{BB}). Despite the significant interaction among ID and IT, the estimated marginal mean (EMM) plots (data not shown) allowed to verify a marked tendency towards higher individual phenolics' contents in samples irradiated with 10 kGy (independently of the IT). Complementarily, samples treated with gamma irradiation showed higher phenolic contents (particularly noticeable in *o*-coumaric acid hexoside, 5-*O*-caffeoylquinic acid, coumarin and coumarin derivative II) than the corresponding samples irradiated with electron-beam.

In line with the observed for BB, the effect of ID over the individual phenolics characterized in LB was less pronounced, taking into account that no significant differences were found in half the compounds (1^{LB}, 4^{LB}, 6^{LB}, 7^{LB}, 8^{LB}, 9^{LB}, 11^{LB}, 12^{LB}, 16^{LB} and 20^{LB}) (Table 4). For all the remaining phenolics, irradiation treatment (independently of the source) induced increased contents. This effect was especially observed among compounds 10^{LB} (yunnaneic acid F), 13^{LB} (rosmarinic acid hexoside) and 18^{LB} (lithospermic acid A), for samples irradiated with 1 kGy; compounds 5^{LB} (caffeic acid), 17^{LB} (salvianolic acid C derivative III) and 19^{LB} (salvianolic acid A isomer), for samples irradiated with 10 kGy; and compounds 2^{LB} (caftaric acid), 14^{LB} (sagerinic acid) and 15^{LB} (rosmarinic acid), with no difference among irradiated doses. The tendency to higher phenolic contents in irradiated samples deserves special attention in the case of rosmarinic acid, since this compound was (by far) the most abundant individual phenolic compound in LB (≈90 μg/mL infusion in unirradiated samples), and it increased more than 20% with irradiation treatment (≈110 μg/mL infusion in irradiated samples). This effect is probably due to a lower availability of molecular oxygen inside the polyethylene bag where samples were stored, which is in agreement with results reported in other plant species (Koike et al., 2015a).

Table 2
Retention time (Rt), maximum absorption wavelength (< lambda > max), mass spectral data, and tentative identification of the phenolic compounds found in Lemon Balm infusions prepared from non-irradiated and irradiated samples, and analysed by HPLC and MS techniques.

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification
1^{LB}	4.8	280	197	179(92),135(100)	3-(3,4-dihydroxyphenyl)-lactic acid
2^{LB}	5.3	330	311	179(100),149(98),135(31)	Caftaric acid
3^{LB}	7.0	320	341	179(100),149(7),135(31)	Caffeic acid hexoside
4^{LB}	8.3	324	325	193(100),149(11),145(25),134(43)	Fertaric acid
5^{LB}	11.4	324	179	135(100)	Caffeic acid
6^{LB}	12.8	330	439	359(10),179(8),161(40),135(28)	Sulphated rosmarinic acid
7^{LB}	13.3	270	571	527(14),483(61),439(52),329(23),259(22),241(49),197(100),179(77),135(98)	Yunnaneic acid E
8^{LB}	14.0	276,324sh	537	493(57),359(13),313(27),295(100),269(27),197(19),179(78),135(45)	Lithospermic acid A isomer
9^{LB}	14.9	328	473	311(19),293(19),179(75),149(100),135(28)	Chicoric acid
10^{LB}	16.8	274,334sh	597	359(31),295(27),197(16),179(10),135(12)	Yunnaneic acid F
11^{LB}	17.7	266,336sh	553	491(9),359(3),311(5),197(3),179(21),161(12),135(100)	Salvianolic acid C derivative I
12^{LB}	18.3	266,336sh	553	491(9),359(3),311(5),197(3),179(21),161(12),135(100)	Salvianolic acid C derivative II
13^{LB}	19.0	322	521	359(100),197(16),179(32),161(72),135(16)	Rosmarinic acid hexoside
14^{LB}	21.3	284,328sh	719	539(17),521(15),359(100),197(22),179(26),161(81),135(7)	Sagerinic acid
15^{LB}	24.1	330	359	197(83),179(70),161(100),135(40)	Rosmarinic acid
16^{LB}	27.6	324	493	359(78),313(8),295(52),269(7),197(33),179(44)	Salvianolic acid A
17^{LB}	28.2	328	829	667(86),535(100),491(21),311(39),293(15),179(10)	Salvianolic acid C derivative III
18^{LB}	30.2	288,326sh	537	493(53),359(100),313(5),295(18),269(3),197(44),179(64)	Lithospermic acid A
19^{LB}	30.8	320	493	359(100),313(5),295(6),269(4),197(14),179(34)	Salvianolic acid A isomer
20^{LB}	34.6	288,320sh	715	535(100),491(38),311(69),293(4),179(5),135(20)	Salvianolic acid C derivative IV

LB: lemon balm; sh: shoulder.

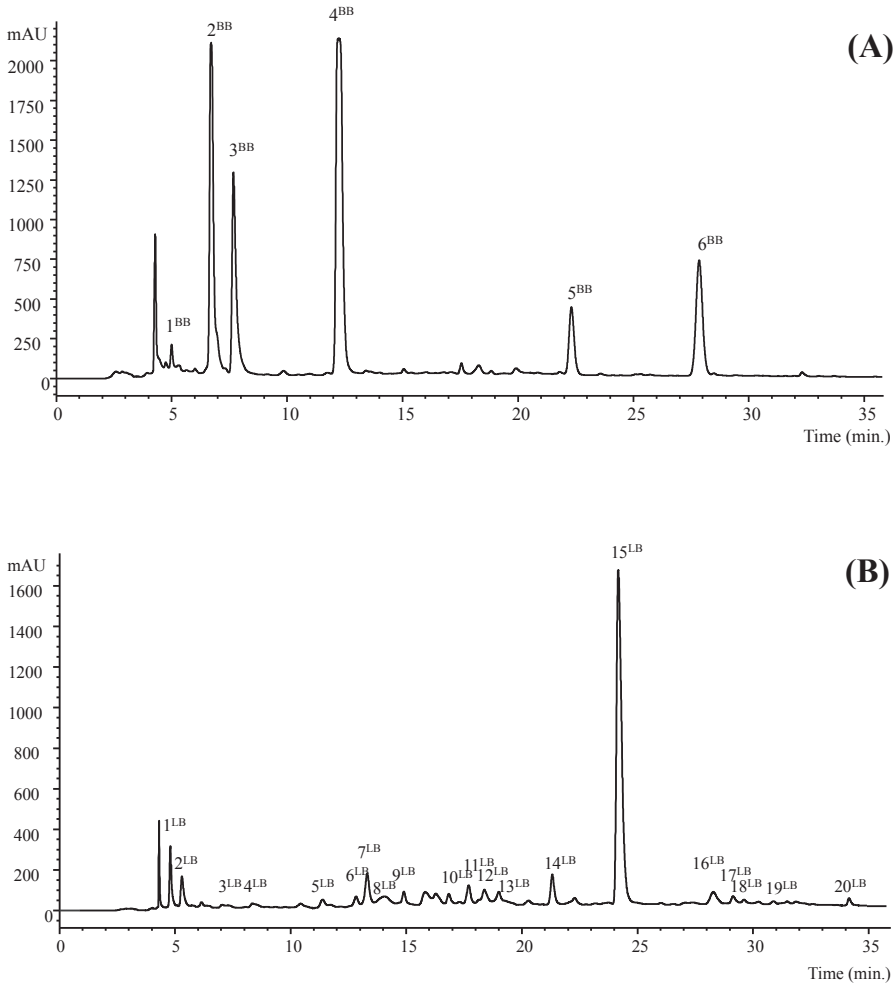

Fig. 1. Phenolic compounds' profile of bastard balm (A) and lemon balm (B) control samples, recorded at 280 nm. Numbers on the chromatograms correspond to peaks identified in Tables 1 and 2, respectively.

Regarding the potential effect induced by IT, no significant differences were observed for compounds 3^{LB}, 10^{LB}, 15^{LB} and 19^{LB}. On the other hand, the electron-beam irradiation was associated with increased contents of compounds 1^{LB}, 4^{LB} to 7^{LB}, 9^{LB}, 12^{LB} and 14^{LB}, while gamma irradiation had the same effect in 2^{LB}, 8^{LB}, 11^{LB}, 13^{LB}, 16^{LB}, 17^{LB}, 18^{LB} and 20^{LB}. In general, the positive effect of gamma irradiation over lithospermic acid and salvianolic acid derivatives should be highlighted, since these compounds represent an important percentage of the individual phenolics found in LB, though not as high as that corresponding to rosmarinic acid, in which the same effect could not be observed.

3.3. Linear discriminant analysis

In the previous section, the differences induced by each assayed factor (ID and IT) were classified considering each plant species individually. In addition, it would be useful to verify which phenolic compounds are affected in higher extension, when compared simultaneously (independently of plant species). Therefore, a linear discriminant analysis (LDA) was performed to evaluate the overall effects of irradiation on the phenolic compounds' profile of both plants. To overcome the potential bias effect induced by different magnitudes of each compound in both species, the results were previously normalized

Table 3
Quantification of phenolic compounds (mg/L infusion) in bastard balm infusions according to the irradiation dose (ID) and irradiation technology (IT).

Compound	Tentative identification (standard used for quantification)	Quantification (mg/L infusion)							
		Irradiation dose (ID)			*p*-value (n = 18)	Irradiation technology (IT)		*p*-value (n = 27)	ID × IT
		0 kGy	1 kGy	10 kGy		Electron-beam	^{60}Cobalt		*p*-value (n = 54)
1^{BB}	3-*O*-Caffeoylquinic acid[1] (3-*O*-Caffeoylquinic acid)	1.9 ± 0.2	2.2 ± 0.2	1.9 ± 0.3	0.009	1.9 ± 0.3	2.1 ± 0.1	0.001	< 0.001
2^{BB}	*o*-Coumaric acid hexoside[2]	15 ± 5	17 ± 2	18 ± 3	0.077	14 ± 2	20 ± 1	< 0.001	< 0.001
3^{BB}	5-*O*-Caffeoylquinic acid[1]	15 ± 2	17 ± 1	19 ± 1	< 0.001	16 ± 2	18 ± 1	< 0.001	0.015
4^{BB}	Coumarin[2]	28 ± 4	29 ± 1	31 ± 3	0.003	27 ± 2	32 ± 2	< 0.001	< 0.001
5^{BB}	Coumarin derivative I*,[2]	6 ± 2	6 ± 2	2 ± 2	< 0.001	5 ± 3	4 ± 1	0.176	< 0.001
6^{BB}	Coumarin derivative II*,[2]	17 ± 3	8 ± 8	11 ± 11	0.011	5 ± 5	20 ± 3	0.001	< 0.001

* These compounds were not detected in samples irradiated with electron-beam at 10 kGy. Phenolic compounds standards used for quantification: [1]3-*O*-caffeoylquinic acid; [2]*p*-coumaric acid.

Table 4
Quantification of phenolic compounds (mg/L infusion) in lemon balm infusions according to the irradiation dose (ID) and irradiation technology (IT).

Compound	Tentative identification	Quantification (mg/L infusion)							
		Irradiation dose (ID)			*p*-value (n = 18)	Irradiation technology (IT)		*p*-value (n = 27)	ID × IT
		0 kGy	1 kGy	10 kGy		Electron-beam	^{60}Cobalt		*p*-value (n = 54)
1^{LB}	3-(3,4-dihydroxyphenyl)-lactic acid[1]	6 ± 1	6 ± 1	6 ± 1	0.121	6.9 ± 0.5	5.1 ± 0.5	< 0.001	0.167
2^{LB}	Caftaric acid[1]	5 ± 1	6 ± 1	6 ± 1	0.013	4.4 ± 0.5	7 ± 1	< 0.001	< 0.001
3^{LB}	Caffeic acid hexoside[1]	0.4 ± 0.2	0.1 ± 0.1	0.01 ± 0.01	< 0.001	0.2 ± 0.2	0.1 ± 0.1	0.562	< 0.001
4^{LB}	Fertaric acid[2]	0.5 ± 0.4	0.5 ± 0.4	0.3 ± 0.1	0.050	0.7 ± 0.3	0.13 ± 0.03	< 0.001	< 0.001
5^{LB}	Caffeic acid[1]	1.3 ± 0.2	1.5 ± 0.4	1.8 ± 0.5	0.018	1.9 ± 0.4	1.2 ± 0.1	< 0.001	< 0.001
6^{LB}	Sulphated rosmarinic acid[3]	2 ± 1	2 ± 1	3 ± 1	0.098	3.3 ± 0.5	1.6 ± 0.3	0.001	< 0.001
7^{LB}	Yunnaneic acid E isomer[3]	6 ± 2	6 ± 2	7 ± 3	0.253	9 ± 2	3.9 ± 0.3	< 0.001	< 0.001
8^{LB}	Lithospermic acid A isomer[3]	26 ± 20	39 ± 21	32 ± 23	0.182	12 ± 5	53 ± 7	< 0.001	< 0.001
9^{LB}	Chicoric acid[2]	2.5 ± 0.5	2.5 ± 0.3	2.8 ± 0.5	0.129	2.8 ± 0.5	2.3 ± 0.4	0.001	< 0.001
10^{LB}	Yunnaneic acid F[3]	3.4 ± 0.3 ^{b}	3.8 ± 0.4^{a}	3.5 ± 0.2 ^{b}	0.001	3.7 ± 0.4	3.5 ± 0.3	0.053	0.364
11^{LB}	Salvianolic acid C derivative I[3]	5.5 ± 0.5	7 ± 3	6 ± 1	0.050	5 ± 1	7 ± 2	< 0.001	< 0.001
12^{LB}	Salvianolic acid C derivative II[3]	4 ± 1	4 ± 1	4 ± 1	0.350	5 ± 1	2.9 ± 0.5	< 0.001	< 0.001
13^{LB}	Rosmarinic acid hexoside[3]	6 ± 1 ^{b}	7 ± 1^{a}	6 ± 1 ^{b}	0.001	5 ± 1	8 ± 1	< 0.001	0.564
14^{LB}	Sagerinic acid[3]	7 ± 1	9 ± 1	9 ± 1	< 0.001	9 ± 1	8 ± 1	0.001	0.007
15^{LB}	Rosmarinic acid[3]	89 ± 7	110 ± 8	112 ± 9	< 0.001	105 ± 16	102 ± 10	0.525	< 0.001
16^{LB}	Salvianolic acid A[3]	10 ± 3^{c}	12 ± 3^{a}	11 ± 3 ^{b}	0.131	8 ± 1	14 ± 1	< 0.001	0.489
17^{LB}	Salvianolic acid C derivative III[3]	7 ± 3	8 ± 1	11 ± 1	< 0.001	7 ± 3	10 ± 1	< 0.001	< 0.001
18^{LB}	Lithospermic acid A[3]	16 ± 14	33 ± 4	29 ± 7	< 0.001	18 ± 12	34 ± 3	< 0.001	< 0.001
19^{LB}	Salvianolic acid A isomer[3]	2.4 ± 0.3	3.3 ± 0.5	3.7 ± 0.4	< 0.001	3 ± 1	3 ± 1	0.073	0.032
20^{LB}	Salvianolic acid C derivative IV[3]	2.4 ± 0.5	2.7 ± 0.4	1.8 ± 0.5	0.087	1.3 ± 0.5	3.3 ± 0.4	< 0.001	< 0.001

In each line, different letters mean significant differences among irradiation doses ($p < 0.05$). Superscript numbers in phenolic compounds names indicate the commercial standards used for quantification: 1 – caffeic acid; 2 – ferulic acid; 3 – rosmarinic acid.

by calculating the percentage of variation in comparison to each corresponding control (unirradiated samples), thereby allowing to analyze all phenolic compounds simultaneously.

Regarding the ID effects, the defined significant functions (Fig. 2A) included 100.0% of the observed variance (function 1: 59.4%; function 2: 40.6%). The individual clustering of markers corresponding to each factor level (0 kGy, 1 kGy and 10 kGy) is very well defined. From the 26 included variables (20 compounds from LB and 6 from BB), only 11 were selected as having discriminant ability: 1) sagerinic acid, 2) rosmarinic acid hexoside, 3) caffeic acid hexoside, 4) lithospermic acid A, 5) salvianolic acid C derivative I, 6) yunnaneic acid F; 7) salvianolic acid A isomer, 8) caffeic acid, 9) coumarin derivative II, 10) chicoric acid and 11) yunnaneic acid E. As it can be observed, differences among the unirradiated samples and those treated with 1 kGy (independently of IT) were associated with function 1, which was more highly correlated to rosmarinic acid hexoside and lithospermic acid A, both showing a higher increase in samples irradiated with 1 kGy. In addition, function 2 highlighted the differences among unirradiated samples and those irradiated with 10 kGy, mainly based in its correlation with salvianolic acid A isomer and sagerinic acid, both reaching a higher increase in the 10 kGy-irradiated samples.

In the case of IT (Fig. 2B), the variables with highest differences among electron-beam and gamma irradiation (*i.e.*, those selected as having discriminant ability) were: 1) coumarin derivative II, 2) caffeic acid hexoside, 3) salvianolic acid C derivative I, 4) salvianolic acid A isomer, 5) coumarin derivative I, 6) sulphated rosmarinic acid, 7) 5-*O*-caffeoylquinic acid, 8) caffeic acid, 9) caftaric acid, 10) lithospermic acid A, 11) coumarin, 12) rosmarinic acid hexoside and 13) sagerinic acid.

The markers corresponding to each factor level were clustered individually according to the distribution defined by the significant functions (Fig. 2B). The changes induced by electron-beam irradiation were more significant, in particular concerning the compounds more correlated with function 1: coumarin derivatives I and II (both with a higher decrease in electron-beam irradiated samples), lithospermic acid A and caffeic acid its derivatives (both with a higher increase in electron-beam irradiated samples). The effects induced by gamma irradiation were mainly evident in salvianolic acid A isomer and sagerinic acid (that did not increase as much as with electron-beam irradiation), caftaric acid and salvianolic acid C derivative I (both with a higher increase in gamma-irradiated samples), which were the variables more correlated to function 2.

Regarding the classification performance, all samples were correctly classified, either for original grouped cases, as well as for cross-validated grouped ones, in both performed LDA.

4. Conclusion

Overall, a general increase in individual phenolics was observed as a result of irradiation, especially (despite not for all compounds) when using gamma irradiation. Together with previously obtained results, which indicate the absence of significant changes in the proximate composition, color parameters, free sugars, organic acids, tocopherols, fatty acids and antioxidant activity of LB and BB samples irradiated with gamma or electron-beam irradiation, the present findings are good indicators of the potential usefulness of both irradiation technologies (when employed to these food matrices). Furthermore, the compounds favored (in terms of overall content) by either using 1 kGy or 10 kGy, as well as electron-beam or gamma irradiation, were fully identified, allowing to select a specific ID or IT to optimize the content of any targeted phenolic compound.

Conflict of interest

The authors declare no conflict of interest.

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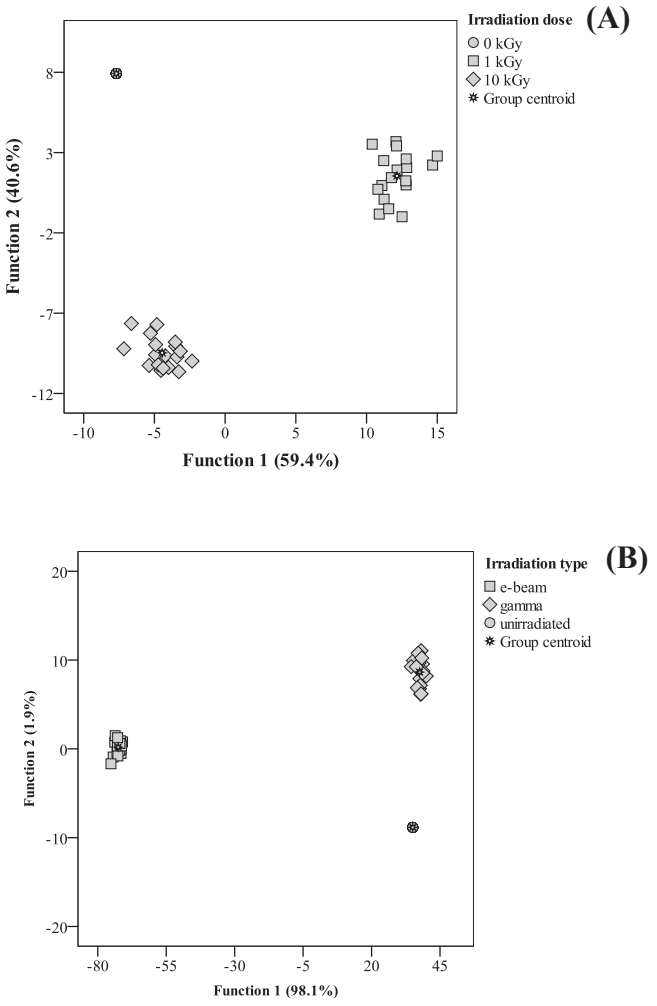

Fig. 2. Mean scores of: (A) different irradiation doses distributed according to the discriminant functions defined from the variations induced by electron-beam and gamma irradiation on the phenolic compounds' profiles of bastard balm and lemon balm. (B) Irradiation types distributed according to the discriminant functions defined from the variations induced by both irradiation doses on the phenolic compounds profiles of bastard balm and lemon balm.

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