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Evaluation of gamma-irradiated aromatic herbs: Chemometric study of samples submitted to extended storage periods

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ABSTRACT

The preserving capacity of gamma radiation (10 kGy) on the chemical, nutritional and antioxidant components of *Aloysia citrodora* Paláu, *Melissa officinalis* L., *Melittis melissophyllum* L. and *Mentha piperita* L., stored for 12 and 18 months, was evaluated. Despite the maintenance of the main characteristics during the first 12 months of storage, the additional 6 months induced several significant changes in individual compounds. In general, the analyzed species reacted dissimilarly throughout time, but it was possible to verify that the fatty acids, tocopherols and antioxidant capacity presented the most significant changes after 18 months of storage, inclusively in samples submitted to gamma radiation. In fact, the applied treatment (10 kGy) did not seem to be effective to prevent the decrease of free sugars, organic acids and tocopherols, especially considering the 18 months period. On the other hand, the evolution in color parameters indicated a greener color (yet slightly more yellow) among irradiated samples. Likewise, gamma radiation had a positive effect on oleic acid, β-carotene bleaching inhibition (in infusions), DPPH scavenging activity and reducing power (in methanolic extracts). Nevertheless, it might be generally concluded that gamma radiation is less suitable than electron-beam to maintain the characteristics of dried herbs during extended storage periods.

1. Introduction

The interest in providing better quality and durability to food products led to the development and improvement of alternative decontamination and conservation methods (Alothman, Bhat, & Karim, 2009; Pereira & Vicente, 2010). Food irradiation is a previously validated technique, which is been growing remarkably in recent years in Asia, USA, Australia and other regions (Diehl, 2002; Roberts, 2014). This technology is characterized as a physical process that, despite involving an energy-input, does not induce radioactivity in foods. Actually, that energy is similar to the one associated with thermal infrared or microwave treatments (Siddhuraju, Osoniyi, Makkar, & Becker, 2002). There are three types of ionizing radiation permitted in food processing: gamma radiation, electron-beam and x-rays. Each technique has different technological characteristics, besides presenting diverse levels of penetration in the irradiated material (EU, 1999; Jung et al., 2015; Kim et al., 2009). An important feature of ionizing radiation is that it acts not only as a decontamination method, eliminating bacteria, insects and other pathogens, but it also preserves nutrients and other molecules, increasing shelf-life without modifying the organoleptic characteristics (Chmielewski & Migdał, 2005; Lado & Yousef, 2002).

Several organizations such as WHO (World Health Organization), FAO (Food and Agriculture Organization), IAEA (International Atomic Energy Agency) and CAC (Codex Alimentarius Commission) have been defending and enabling the use of food irradiation throughout the world (Ihsanullah & Rashid, 2016). In Europe, food irradiation is regulated by different legal dispositions, such as the Directives 1999/2/CE (European Parliament and Council, L 66/16, 1999) and 1999/3/CE (European Parliament and Council, L 66/24, 1999), which define several technical requirements and marketing guidelines, including the labeling of irradiated products. According to these directives, the symbol “radura” must be present not only in irradiated products themselves, but also on products including ingredients that have been irradiated (EU, 1999).

Several studies have been carried out to allow a better understanding of the effects caused by irradiation (Brandstetter, Berthold,

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Isnardy, Solar, & Elmadfa, 2009; Farkas, 2008; Roberts, 2014), often reporting the absence of significant changes in chemical, nutritional and organoleptic profiles. However, when planned to evaluate the potential effects of storage conditions, those studies are routinely limited to one year (Byun, Yook, Kwon, & Kang, 1997; Kausar, Akram, & Kwon, 2013; Pereira, Antonio, Barreira, Barros, Bento, and Ferreira, 2015; Pereira, Antonio, Rafalski, Barreira, Barros, and Ferreira, 2015; Tissot, Grdanovska, Barkatt, Silverman, & Al-Sheikhly, 2013), which justifies studying extended periods.

The effects of gamma radiation were previously studied in the species selected herein (*A. citrodora* Paláu, *M. officinalis* L., *M. melissophyllum* L. and *M. pipperita* L.), but without considering the potential changes induced by storage time (Pereira, Antonio, Rafalski, et al., 2015). In another study, the effects of electron-beam irradiation were assessed together with different storage periods, but that technology proved to have some limitations, since the observed effects were highly modulated by the plant species (Pereira, Antonio, Rafalski, Barreira, Barros, Oliveira, and Ferreira, 2017). Accordingly, gamma radiation was evaluated in the present study, considering extended storage periods (12 and 18 months), to verify its suitability to maintain/improve chemical and antioxidant profiles of aromatic dried plants throughout storage.

2. Materials and methods

2.1. Samples and samples irradiation

The plant material (*Aloysia citrodora* Paláu, *Melissa officinalis* L., *Melittis melissophyllum* L., *Mentha pipperita* L.) used was previously described by Pereira, Antonio, Barreira, et al., 2015; Pereira, Antonio, Rafalski, et al., 2015). Three samples of each plant species were used in each assayed condition and all analysis were conducted in triplicate. Samples were analyzed immediately after irradiation (0 months) and after storage (12 or 18 months) in a dry place protected from light.

The irradiation was performed in a ^{60}Co experimental chamber (Precisa 22, Graviner Manufacturing Company Ltd., UK) with total activity 177 TBq (4.78 kCi), in September 2013 (Pereira, Antonio, Barreira, et al., 2015). The estimated dose, dose rate and dose uniformity ratio (D_{max}/D_{min}) were: 8.93 ± 0.14 kGy, 1.91 ± 0.03 kGy·h^{-1} and 1.02, respectively. For simplicity, the values 0 and 10 kGy were considered as the doses of non-irradiated and irradiated groups.

After irradiation, samples were grinded to powder (20 mesh) and mixed to obtain homogenized samples for subsequent analysis.

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 (standard 47,885-U) was obtained from Sigma (St. Louis, MO, USA), as other individual fatty acid isomers, tocopherols (α-, β-, λ-, and δ-isoforms), sugars ((D-(−)-fructose, D-(+)-sucrose, D-(+)-glucose, D-(+)-trehalose and D-(+)-raffinose pentahydrate) and organic acid standards. Racemic tocol, 50 mg/mL, was purchased from Matreya (Pleasant Gap, PA, USA).

Amber Perspex dosimeters (batch V, from Harwell Company, Oxfordshire, UK) were used to assess the absorbed dose by the samples and Fricke dosimeter was applied to estimate the dose rate of irradiation geometry. To prepare the acid aqueous Fricke dosimeter solution, the following reagents were used: ferrous ammonium sulfate(II) hexahydrate, sodium chloride and sulfuric acid, all purchased from Panreac S.A. (Barcelona, Spain). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

2.3. Nutritional composition

The protein, fat, carbohydrates and ash contents were determined following official procedures (AOAC, 2002). Macro-Kjeldahl method was applied to determine protein content (N × 6.25); the crude fat was analyzed using a Soxhlet apparatus by extracting a known weight of sample with petroleum ether, ash content was evaluated by incineration at 600 ± 15 °C, total carbohydrates content were calculated by difference and total energy was calculated according to the equation: Energy (kcal) = 4 × ($g_{protein}$ + $g_{carbohydrates}$) + 9 × (g_{fat}).

2.4. Color measurement

The color parameters were evaluated as described previously (Pereira, Antonio, Barreira, et al., 2015), using a colorimeter (model CR-400, Konica Minolta Sensing, Inc., Japan), with an adapter (model CR-A50) for granular materials. The illuminant C and diaphragm aperture of 8 mm were used. The CIE L^*, a^* and b^* color space values were registered using "Spectra Magic Nx" software (version CM-S100W 2.03.0006), from Konica Minolta company (Japan).

2.5. Chemical composition

2.5.1. Sugars

Free sugars were characterized by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI, Knauer, Berlin, Germany; degasser system: Smartline manager 5000; autosampler: AS-2057, Jasco, Easton, MD, USA) and analyzed using Clarity 2.4 Software (DataApex, Prague, Czech Republic) (Barros et al., 2013). The compounds were identified by chromatographic comparisons with authentic standards. Melezitose (Sigma Chemical Co.; Saint Louis, Missouri, USA) was used as internal standard for quantification.

2.5.2. Organic acids

Organic acids were characterized by ultra-fast liquid chromatography coupled to a photodiode array detector (UFLC-DAD, ultra-fast liquid chromatography coupled to a photodiode array detector Shimadzu Corporation, Kyoto, Japan). The wavelengths of 215 nm and 245 nm (for ascorbic acid) were used based on Barros et al. (2013). Quantification was done by comparing the area of peaks recorded at 215 nm with calibration curves obtained from commercial standards of each compound.

2.5.3. Phenolics and flavonoids content

Total phenolics were estimated by Folin-Ciocalteu colorimetric assay, while total flavonoids were determined by a colorimetric assay using aluminium trichloride, according to previously described procedures (Pereira, Barros, & Ferreira, 2013). Results were respectively expressed in mg GAE (gallic acid equivalents)/g of extract and mg CE (catechin equivalents)/g of extract.

2.5.4. Tocopherols

Tocopherols were determined following a previously described procedure (Pereira et al., 2013). The compounds were identified by chromatographic comparisons with commercial standards. Quantification was based on the fluorescence signal response of each standard, using tocol (Matreya (Pleasant Gap, PA, USA) as internal standard method. Calibration curves were obtained from the standards of each compound.

2.5.5. Fatty acids

Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID)/capillary column (DANI 1000, Contone, Switzerland) (Pereira et al., 2013) and identified by comparing the relative retention times of the FAME peaks from samples with standards. The CSW 1.7 Software (DataApex, Prague, Czech Republic) was used for the results interpretation.

Table 1
Proximate composition, energy and color parameters variation (differential percentage in comparison to the control values: Pereira, Antonio, Barreira, et al., 2015) in response to gamma radiation at 10 kGy and storage time. The results are presented as the mean ± SD.

		Fat	Protein	Ash	Carbohydrates	Energy	L^*	a^*	b^*
12 months									
Gamma-radiation (GR)	0 kGy	-3 ± 24	−6 ± 8	3 ± 5	1 ± 1	−1 ± 1	1 ± 4	−12 ± 10	−8 ± 8
	10 kGy[1]	−10 ± 13	−23 ± 9	1 ± 3	2 ± 1	−1 ± 1	4 ± 5	−9 ± 11	−3 ± 7
	p-value (n = 36)[2]	0.134	< 0.001	0.013	< 0.001	0.259	0.034	0.252	0.005
Plant species (PS)	*A. citrodora*	−2 ± 6	−13 ± 8	1 ± 3	1 ± 1	−1 ± 1	4 ± 2	-9 ± 8^{ab}	-8 ± 2^{b}
	M. officinalis	9 ± 26	−16 ± 19	2 ± 4	1 ± 2	−1 ± 1	−1 ± 3	-14 ± 12^{b}	-2 ± 4^{a}
	M. melissophyllum	−26 ± 7	−15 ± 11	3 ± 6	1 ± 1	−1 ± 1	4 ± 7	-4 ± 7^{a}	-1 ± 10^{a}
	M. piperita	−6 ± 12	−12 ± 9	1 ± 4	2 ± 1	−1 ± 1	3 ± 4	-16 ± 9^{b}	-11 ± 4^{b}
	p-value (n = 18)[2]	< 0.001	0.772	0.256	0.077	< 0.001	0.012	0.001	< 0.001
(GR × PS)	*p*-value (n = 72)[3]	< 0.001	0.034	< 0.001	< 0.001	< 0.001	0.034	0.628	0.357
18 months									
Gamma-radiation (GR)	0 kGy	−15 ± 34	−19 ± 13	−2 ± 4	2 ± 1	−1 ± 1	3 ± 4	−17 ± 10	−9 ± 8
	10 kGy	−27 ± 9	−25 ± 37	−2 ± 2	3 ± 4	−1 ± 1	4 ± 4	−11 ± 11	−5 ± 8
	p-value (n = 36)[2]	0.061	0.393	0.406	0.059	0.567	0.145	0.017	0.018
Plant species (PS)	*A. citrodora*	−24 ± 6	−30 ± 8	−2 ± 3	2 ± 1	−1 ± 1	4 ± 2^{ab}	−16 ± 7	-14 ± 3^{c}
	M. officinalis	10 ± 29	−20 ± 22	−5 ± 4	2 ± 2	1 ± 1	2 ± 3^{b}	−16 ± 15	-3 ± 3^{b}
	M. melissophyllum	−43 ± 11	1 ± 34	−1 ± 3	1 ± 2	−1 ± 1	6 ± 5^{a}	−8 ± 11	3 ± 6^{a}
	M. piperita	−27 ± 9	−40 ± 22	−2 ± 2	6 ± 3	−1 ± 1	4 ± 3^{ab}	−16 ± 8	-14 ± 4^{c}
	p-value (n = 18)[2]	< 0.001	< 0.001	0.001	< 0.001	< 0.001	0.003	0.081	< 0.001
(GR × PS)	*p*-value (n = 72)[3]	< 0.001	< 0.001	0.005	< 0.001	< 0.001	0.412	0.830	0.881

[1] For each species, means within a column with different letters differ significantly ($p < 0.05$)
[2] $p < 0.05$ indicates that the mean value of at least one ratio differs from the others.
[3] $p < 0.05$ indicates a significant interaction (in this case multiple comparison tests results could not be indicated).

2.6. Evaluation of antioxidant properties

2.6.1. Extracts preparation

Methanolic and aqueous (infusions) extracts were obtained following a procedure described by Pereira, Antonio, Barreira, et al., 2015, Pereira, Antonio, Rafalski, et al., 2015).

2.6.2. Antioxidant activity

DPPH radical-scavenging activity, reducing power, inhibition of β-carotene bleaching, total phenols and total flavonoids were evaluated by colorimetric assays (Pereira et al., 2013). The EC_{50} values (mg/mL) were obtained in all assays by using the graphs of the antioxidant activity percentage (for DPPH radical-scavenging activity and inhibition of β-carotene bleaching) or absorbance at 690 nm (in the case of reducing power assay) against the extract concentration. Trolox (Sigma (St. Louis, MO, USA), a water-soluble analogue of vitamin E, was used for a positive control.

2.7. Statistical analysis

For each irradiation dose, storage time and plant species, three independent samples (each corresponding to approximately 40 g of dried leaves obtained from several plants) were analyzed. Each sample was taken after pooling the plants treated in the same conditions together. Data were presented as the difference among values obtained for each irradiated sample and the respective control ((irradiated sample value - control value)/control value × 100) (Pereira, Antonio, Barreira, et al., 2015). This normalization was done to prevent the occurrence of biased outcomes due to the natural differences in the magnitude of the analyzed parameters among the four assayed species.

An analysis of variance (ANOVA) with type III sums of squares was performed using the GLM (General Linear Model) procedure of the SPSS software version 22.0 (IBM Corp., Armonk, NY: USA). The dependent variables were analyzed using 2-way ANOVA, with the factors “gamma radiation” (GR) and “plant species” (PS). When a statistically significant interaction (GR × PS) was detected, the two factors were evaluated simultaneously by the estimated marginal means plots for all levels of each single factor. Alternatively, if no statistical significant interaction was verified, means within each factor were compared using the Tukey HSD test, regarding the ST effect, or the *t*-student test, in the case of GI effect (since only 2 different levels were available in this last case).

To understand which parameters were more affected by GR throughout the storage, independently of the plant species, principal components analysis (PCA) was used to evaluate their variations all together, checking for the highest correlations with the defined principal components. The number of dimensions kept for data analysis was assessed by the respective eigenvalues (which should be greater than one), by the Cronbach's alpha parameter (that must be positive) and also by the total percentage of variance (that should be as high as possible) explained by the number of selected components. The number of plotted dimensions (two) was chosen in order to obtain meaningful interpretations.

3. Results and discussion

In a previous work, the potential effects of gamma radiation on the nutritional profiles, individual compounds and antioxidant parameters of the plant species studied herein were evaluated in non-stored samples (Pereira, Antonio, Rafalski, et al., 2015). Those results were now used as reference values to assess the chemometric variations arising from storage time (ST) effect over irradiated and non-irradiated samples of the same plant species. In general, it was intended to verify if gamma irradiation could prevent the adverse changes that typically occur throughout ST in non-irradiated plants. As an overall rule, differences below 5% were considered irrelevant. To validate the suitability of this technique independently of plant species (PS), the results from all assayed plants were combined, both for non-irradiated and irradiated samples. Therefore, values presented for 0 kGy and 10 kGy assays were calculated including all species simultaneously. Likewise, values presented for each PS comprise the results of 0 and 10 kGy assays together. Accordingly, the standard deviation values should not be regarded as a certainty measure, since they represent the variability of results among parameters assayed for different GR or PS.

Only those parameters detected in all PS and GR were considered for comparison purposes.

3.1. Effects on chemical parameters

Changes observed in proximate composition (Table 1) showed significant interaction among both factors (GR and PS), either in samples stored 12 months, as well as those stored 18 months, thereby indicating that changes induced by GR depended on PS and *vice-versa*. Regarding the effects of GR and PS individually, it was verified that changes in nutritional parameters (particularly the decrease in fat and protein contents) among different PS tended to increase after 18 months. Conversely, some of the significant differences (specifically protein, ash and carbohydrates) observed among irradiated and non-irradiated samples after 12 months of storage tended to diminish after 18 months. Actually, after this longer ST, none of the nutritional parameters showed significant ($p < 0.05$) differences among irradiated and non-irradiated samples.

On the other hand, the effects of ST and PS on color parameters were independent, except for L^* after 12 months ($p = 0.034$). The values obtained for a^* (greenness-redness) and b^* (blueness-yellowness) showed higher variation than L^*, especially after 18 months of storage. Independently, of PS and GR, a^* and b^* tended to decrease along time, indicating slightly greener samples on one hand, but also a minor development of yellow color, which is in agreement with the results observed in gamma-irradiated green tea extracts (Jo, Son, Shin, & Byun, 2003).

Considering now the evaluation of differences in free sugars and organic acids, the interaction of PS and GR was significant among samples stored 12 months (except for oxalic acid), but not, in general terms, after 18 months of storage (Table 2). This might be explained by the lack of significant changes in sugar contents after 12 months, which on the other hand decreased after the additional six months (especially noticeable in the case of trehalose), without differences among non-irradiated and irradiated samples. In this particular, the results achieved with e-beam irradiation were more satisfactory, as it showed ability to attenuate the decrease of both groups of compounds (Pereira, Antonio, et al., 2017). Organic acids, on the other hand, showed similar variation in both ST. Concerning the PS effect, several significant differences were found. For instance, it was verified that *M. melissophyllum* was less prone to suffer a decrease in free sugars content (except trehalose) and that *A. citrodora* had the highest capacity in maintaining organic acids contents. In previous reports, the contents in free sugars tended to increase after gamma radiation (Byun et al., 1997; Pereira, Antonio, Rafalski, et al., 2015), probably due to the shortening or depolymerization of polysaccharide molecules, but those studies were conducted in non-stored samples or samples stored for shorter periods (up to 6 months).

The effects induced by ST on tocopherols contents varied within each PS (significant interaction), similarly with the verified for the individual effect of PS (Table 3). However, no significant differences were detected among irradiated and non-irradiated samples in any case. In general, around 75% of tocopherols contents were lost, without relevant differences among the 12 months and 18 months periods, thereby indicating that gamma irradiation could not reduce these losses, contrarily to the observed in the previous study using e-beam irradiation (Pereira, Antonio, et al., 2017).

The changes in fatty acids percentages (including only those quantified over 0.5% in all PS) are presented in Table 4. In addition to the tabled fatty acids, C6:0, C8:0, C10:0 (except in *A. citrodora* and *M. melissophyllum*), C11:0 (except in *M. melissophyllum*), C13:0, C14:1, (except in *A. citrodora* and *M. melissophyllum*), C15:1, C16:1 (except in *M. officinalis*), C18:3n6 (only in *M. melissophyllum*), C20:1, C20:2 (except in *M. officinalis*), C20:5n3 (except in *A. citrodora* and *M. melissophyllum*), C22:1 (except in *M. officinalis* and *M. melissophyllum*), C22:6n3 (only in *M. piperita*) and C23:0 were also detected. Nevertheless, all the quantified fatty acids were included in the PCA presented in Section 3.3.

The interaction among PS and GR was significant in both ST (except C15:0 and C17:0 in samples stored for 12 months), as it were, in general, the effects of PS individually. On the other hand, and exempting a few cases, there were no significant changes on fatty acids contents between non-irradiated and irradiated samples. Overall, polyunsaturated fatty acids (PUFA) tended to decrease along time, particularly among samples stored during 18 months.

Conversely, saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) showed a general tendency to increase, more noticeable after 18 months storage. After the same period, the application of GR prevented the loss of oleic acid, independently of PS. A similar effect

Table 2
Hydrophilic compounds content variation (differential percentage in comparison to the control values: Pereira, Antonio, Barreira, et al., 2015) in response to gamma radiation at 10 kGy and storage time. The results are presented as the mean ± SD.

		Fructose	Glucose	Sucrose	Trehalose	Total sugars	Oxalic acid	Malic acid	Organic acids
12 months									
Gamma-radiation (GR)	0 kGy[1]	1 ± 15	7 ± 32	1 ± 19	−5 ± 26	−4 ± 10	−11 ± 17	−11 ± 18	−8 ± 8
	10 kGy	−1 ± 14	−2 ± 20	−9 ± 15	−22 ± 21	−9 ± 12	−10 ± 19	−2 ± 25	−6 ± 9
	p-value (n = 36)[2]	0.710	0.147	0.029	0.002	0.126	0.864	0.087	0.264
Plant species (PS)	*A. citrodora*	−2 ± 17	−16 ± 15	−11 ± 8	−14 ± 12	−11 ± 7	10 ± 13^{a}	−24 ± 19^{c}	−2 ± 7
	M. officinalis	2 ± 15	−10 ± 14	−19 ± 9	−9 ± 39	−14 ± 12	−14 ± 18^{b}	−6 ± 21^{b}	−8 ± 12
	M. melissophyllum	4 ± 9	9 ± 15	9 ± 11	−17 ± 25	3 ± 7	−25 ± 9^{c}	−11 ± 9^{bc}	−10 ± 8
	M. piperita	−6 ± 15	28 ± 32	5 ± 22	−15 ± 19	−3 ± 11	−12 ± 10^{b}	14 ± 19^{a}	−7 ± 5
	p-value (n = 18)[2]	0.218	< 0.001	< 0.001	0.805	< 0.001	< 0.001	< 0.001	0.020
(GR × PS)	*p*-value (n = 72)[3]	0.011	0.006	0.003	< 0.001	< 0.001	0.091	0.050	0.022
18 months									
Gamma-radiation (GR)	0 kGy	−12 ± 17	5 ± 48	−13 ± 17	−63 ± 38	−16 ± 3	−11 ± 15	2 ± 25	−11 ± 10
	10 kGy	−13 ± 14	−6 ± 32	−14 ± 20	−64 ± 37	−21 ± 12	−13 ± 14	1 ± 18	−8 ± 10
	p-value (n = 36)[2]	0.817	0.253	0.796	0.882	0.065	0.527	0.892	0.209
Plant species (PS)	*A. citrodora*	−22 ± 9^{b}	−37 ± 10	−19 ± 7^{bc}	−30 ± 10^{b}	−23 ± 6	−11 ± 10	1 ± 29^{ab}	−6 ± 7^{a}
	M. officinalis	−19 ± 12^{b}	−22 ± 11	−29 ± 9^{c}	−100*^{c}	−32 ± 7	−5 ± 18	5 ± 20^{a}	−5 ± 13^{a}
	M. melissophyllum	4 ± 13^{a}	2 ± 12	8 ± 14^{a}	−100*^{c}	−10 ± 10	−13 ± 11	−14 ± 10^{b}	−11 ± 9^{ab}
	M. piperita	−14 ± 14^{b}	53 ± 41	−13 ± 16^{b}	−23 ± 9^{a}	−9 ± 8	−18 ± 14	16 ± 13^{a}	−16 ± 7^{b}
	p-value (n = 18)[2]	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.049	< 0.001	0.002
(GR × PS)	*p*-value (n = 72)[3]	0.065	0.015	0.279	0.136	0.014	0.004	0.291	0.181

* This parameter was not detected in the samples treated under these conditions.
[1] For each species, means within a column with different letters differ significantly ($p < 0.05$).
[2] $p < 0.05$ indicates that the mean value of at least one ratio differs from the others.
[3] $p < 0.05$ indicates a significant interaction (in this case multiple comparison tests results could not be indicated).

Table 3
Tocopherols content variation (differential percentage in comparison to the control values: Pereira, Antonio, Barreira, et al., 2015) in response to gamma radiation at 10 kGy and storage time. The results are presented as the mean ± SD.

		α-Tocopherol	β-Tocopherol	γ-Tocopherol	Total tocopherols
12 months					
Gammaradiation (GR)	0 kGy[1]	−76 ± 13	−87 ± 23	−53 ± 41	−65 ± 12
	10 kGy	−74 ± 7	−88 ± 21	−61 ± 28	−65 ± 8
	p-value (n = 36)[2]	0.378	0.868	0.316	0.810
Plant species (PS)	*A. citrodora*	−74 ± 3	−100*	−69 ± 6	−74 ± 3
	M. officinalis	−74 ± 2	−100*	−50 ± 7	−74 ± 2
	M. melissophyllum	−89 ± 5	−50 ± 3	−100*	−52 ± 2
	M. piperita	−61 ± 5	−100*	−8 ± 20	−59 ± 6
	p-value (n = 18)[2]	< 0.001	< 0.001	< 0.001	< 0.001
(GR × PS)	*p*-value (n = 72)[3]	< 0.001	< 0.001	< 0.001	< 0.001
18 months					
Gamma radiation (GR)	0 kGy	−76 ± 11	−88 ± 21	−59 ± 35	−66 ± 10
	10 kGy	−73 ± 7	−90 ± 18	−64 ± 25	−67 ± 6
	p-value (n = 36)[2]	0.210	0.746	0.471	0.919
Plant species (PS)	*A. citrodora*	−74 ± 3	−100*	−71 ± 5	−74 ± 3
	M. officinalis	−73 ± 2	−100*	−54 ± 5	−73 ± 3
	M. melissophyllum	−87 ± 5	−56 ± 4	−100*	−58 ± 3
	M. piperita	−62 ± 4	−100*	−20 ± 15	−61 ± 4
	p-value (n = 18)[2]	< 0.001	< 0.001	< 0.001	< 0.001
(GR × PS)	*p*-value (n = 72)[3]	< 0.001	< 0.001	< 0.001	< 0.001

* This parameter was not detected in the samples treated under these conditions.
[1] For each species, means within a column with different letters differ significantly ($p < 0.05$).
[2] $p < 0.05$ indicates that the mean value of at least one ratio differs from the others.
[3] $p < 0.05$ indicates a significant interaction (in this case multiple comparison tests results could not be indicated).

was observed in linolenic acid, the major fatty acid in all analyzed species (Pereira, Antonio, Barreira, et al., 2015; Pereira, Antonio, Rafalski, et al., 2015), despite not statistically significant ($p = 0.170$). Nevertheless, this was not reflected in grouped PUFA, once again pointing e-beam irradiation as a better conservation technology for this type of product (Pereira, Antonio, et al., 2017).

3.2. Effects on antioxidant parameters

In line with the information described in Tables 1–4, the effects of GR and PS in the antioxidant parameters also had a significant interaction (Table 5).

Samples stored for 12 months and further submitted to aqueous extraction (infusions) did not show relevant changes in DPPH scavenging activity and reducing power, both in non-irradiated and irradiated plants. On the other hand, β-carotene bleaching inhibition was preserved in irradiated plants, contrarily to the decrease observed in non-irradiated plants. The evaluated bioactive compounds tended to increase independently of GR, but the same effect could only be observed in *M. officinalis* (in what regards PS), which might contribute to explain the higher reducing power detected in this species. On the other hand, no significant effects of PS were observed in DPPH scavenging activity, while β-carotene bleaching inhibition was lower in all species except *M. melissophyllum*.

In the case of methanolic extracts, a higher dissimilarity was observed in result of GR, as exemplified by the decrease of reducing power and DPPH scavenging activity measured in non-irradiated samples. Similarly, irradiation treatment exerted a protective effect on phenolics content, but not in flavonoid contents, which decreased independently of GR. Considering the PS effects, *M. melissophyllum* and *M. piperita* showed lowered DPPH scavenging activity and reducing power, which were maintained in *A. citrodora* and *M. officinalis*.

Concerning the infusions prepared with plants stored during 18 months, the absence of noticeable effects of GR over DPPH scavenging activity was maintained; however, contrarily to the observed in samples stored during 12 months, reducing power decreased in 18 months-stored samples, irradiated or not. On the other hand, the ability to inhibit β-carotene bleaching increased after this longer storage period. The most significant difference among non-irradiated and irradiated samples was observed in flavonoids content, which was higher in irradiated plants. Nevertheless, the same effect was not verified in total phenolics. As observed after 12 months, *M. officinalis* showed the most significant differences among all the assayed PS. Actually, this was the only species in which total phenolics and flavonoids contents increased, contrarily to the reduction observed in all other species. However, these differences were only in agreement with reducing power assay results, since no effect was observed for DPPH scavenging activity, and *M. officinalis* showed a decline in β-carotene bleaching inhibition, contrarily to the other species.

In the case of methanolic extracts, DPPH scavenging activity and β-carotene bleaching inhibition were generally decreased (in less percentage in irradiated plants), while reducing power was maintained in non-irradiated plants and increased in irradiated ones. Total phenolics and flavonoids contents decreased independently of GR.

Regarding PS, DPPH scavenging activity and β-carotene bleaching inhibition also decreased (except in *M. melissophyllum*), in line with the overall decrease in total phenolics and flavonoids contents. Nevertheless, there was an increase in reducing power capacity (except in the case of *A. citrodora*).

In general, and independently of ST and GR treatment, the analyzed species showed different responses in antioxidant activity assays, either when evaluating their infusions or methanolic extracts. A similar effect was observed with total phenolics and flavonoids, which might be explained by their dissimilar profiles in each PS. In fact, the major individual phenolics in the infusions of *A. citrodora*, *M. melissophyllum*, *M. officinalis* and *M. piperita*, were, respectively, luteolin-7-*O*-diglucuronide (68.7 ± 0.3 mg/mL in non-irradiated plants, 70.0 ± 0.5 mg/mL in

Table 4
Major fatty acids variation (differential percentage in comparison to the control values: Pereira, Antonio, Barreira, et al., 2015) in response to gamma radiation at 10 kGy and storage time. The results are presented as the mean ± SD.

		C12:0	C14:0	C15:0	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3n3	C20:0	C22:0	C24:0	SFA	MUFA	PUFA
12 months																
Gamma radiation (GR)	0 kGy[1]	13 ± 22	−23 ± 15	27 ± 43	29 ± 5	43 ± 28	39 ± 15	47 ± 22	2 ± 5	−14 ± 10	10 ± 54	82 ± 23	−15 ± 45	11 ± 10	23 ± 21	−9 ± 7
	10 kGy	16 ± 41	−30 ± 16	31 ± 46	24 ± 12	37 ± 24	34 ± 23	39 ± 23	−4 ± 8	−15 ± 11	10 ± 53	91 ± 36	−29 ± 21	14 ± 14	17 ± 19	−11 ± 10
	p-value (n = 36)[2]	0.697	0.054	0.720	0.018	0.318	0.288	0.132	0.002	0.910	0.988	0.222	0.085	0.282	0.202	0.469
Plant species (PS)	*A. citrodora*	−16 ± 21	−17 ± 10	−18 ± 13[c]	23 ± 9	63 ± 21[a]	43 ± 11	50 ± 19	5 ± 3	−7 ± 2	−74 ± 4	113 ± 31	−58 ± 6	7 ± 5	45 ± 14	−4 ± 2
	M. officinalis	37 ± 36	−29 ± 8	17 ± 21[b]	32 ± 8	37 ± 13[b]	59 ± 15	51 ± 15	−1 ± 3	−31 ± 3	67 ± 11	78 ± 15	8 ± 50	28 ± 5	25 ± 10	−23 ± 3
	M. melissophyllum	18 ± 18	−29 ± 25	22 ± 13[b]	23 ± 9	46 ± 23[b]	24 ± 12	14 ± 9	−8 ± 8	−13 ± 6	18 ± 19	106 ± 12	−25 ± 13	−1 ± 3	11 ± 8	−2 ± 2
	M. piperita	21 ± 29	−32 ± 8	94 ± 20[a]	28 ± 8	12 ± 17[c]	21 ± 9	56 ± 16	1 ± 6	−7 ± 5	28 ± 5	51 ± 11	−12 ± 12	16 ± 8	−2 ± 6	−11 ± 5
	p-value (n = 18)[2]	< 0.001	0.016	< 0.001	0.008	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
(GR × PS)	*p*-value (n = 72)[3]	< 0.001	< 0.001	0.475	< 0.001	0.055	< 0.001	< 0.001	< 0.001	< 0.001	0.027	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
18 months																
Gamma radiation (GR)	0 kGy	48 ± 26	34 ± 55	76 ± 50	39 ± 16	59 ± 42	85 ± 80	44 ± 50	−14 ± 22	−43 ± 23	−17 ± 35	39 ± 27	−31 ± 26	32 ± 19	51 ± 53	−32 ± 20
	10 kGy	36 ± 30	32 ± 43	59 ± 52	34 ± 9	41 ± 17	48 ± 34	20 ± 13	−17 ± 21	−35 ± 21	−22 ± 37	41 ± 35	19 ± 40	31 ± 21	24 ± 20	−28 ± 23
	p-value (n = 36)[2]	0.070	0.856	0.163	0.105	0.080	0.013	0.008	0.533	0.170	0.560	0.789	0.003	0.853	0.008	0.367
Plant species (PS)	*A. citrodora*	51 ± 36	−34 ± 7	24 ± 25	49 ± 14	95 ± 36	144 ± 76	82 ± 49	−8 ± 12	−27 ± 12	−75 ± 5	39 ± 30	48 ± 47	32 ± 16	86 ± 53	−24 ± 12
	M. officinalis	71 ± 17	80 ± 12	138 ± 28	29 ± 8	36 ± 8	79 ± 11	26 ± 2	−48 ± 4	−69 ± 1	−14 ± 7	25 ± 15	−63 ± 8	58 ± 8	48 ± 4	−63 ± 2
	M. melissophyllum	24 ± 10	63 ± 39	31 ± 13	28 ± 8	44 ± 12	19 ± 15	4 ± 3	−2 ± 5	−45 ± 11	−12 ± 13	74 ± 30	47 ± 12	15 ± 5	16 ± 13	−16 ± 7
	M. piperita	22 ± 7	24 ± 16	78 ± 27	39 ± 6	25 ± 11	24 ± 7	16 ± 2	−4 ± 6	−15 ± 9	22 ± 4	23 ± 12	−56 ± 12	20 ± 13	15 ± 6	−17 ± 9
	p-value (n = 18)[2]	< 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
(GR × PS)	*p*-value (n = 72)[3]	< 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

[1] For each species, means within a column with different letters differ significantly ($p < 0.05$).
[2] $p < 0.05$ indicates that the mean value of at least one ratio differs from the others.
[3] $p < 0.05$ indicates a significant interaction (in this case multiple comparison tests results could not be indicated).

Table 5
Variation in the EC_{50} values of different antioxidant assays, phenols and flavonoids contents (presented as differential percentages in comparison to the control values: Pereira, Antonio, Barreira, et al., 2015) in response to gamma radiation at 10 kGy and storage time. The results are presented as the mean ± SD.[1]

		DPPH scavenging activity		Reducing power		β-carotene bleaching inhibition		Phenols		Flavonoids	
		Infusion	MeOH	Infusion	MeOH	Infusion	MeOH	Infusion	MeOH	Infusion	MeOH
12 months											
Gamma-radiation (GR)	0 kGy	−2 ± 18	110 ± 62	−2 ± 11	32 ± 39	97 ± 69	87 ± 36	84 ± 30	−24 ± 26	52 ± 36	−38 ± 30
	10 kGy	−7 ± 8	13 ± 16	−9 ± 17	−18 ± 16	−1 ± 45	93 ± 89	66 ± 26	−1 ± 34	81 ± 27	−17 ± 38
	p-value (n = 36)[2]	0.100	0.001	0.028	< 0.001	< 0.001	0.089	0.548	0.001	0.322	0.009
Plant species (PS)	*A. citrodora*	−2 ± 8	110 ± 81	6 ± 5	64 ± 35	75 ± 84	98 ± 60	5 ± 19	4 ± 25	2 ± 15	−1 ± 21
	M. officinalis	−9 ± 5	22 ± 12	−27 ± 8	−30 ± 9	89 ± 79	83 ± 50	93 ± 14	−63 ± 5	73 ± 11	−75 ± 4
	M. melissophyllum	5 ± 21	10 ± 10	6 ± 4	2 ± 13	−31 ± 36	−45 ± 26	−2 ± 6	−1 ± 16	−10 ± 21	−41 ± 13
	M. piperita	−11 ± 14	3 ± 7	−7 ± 6	−8 ± 12	58 ± 13	43 ± 23	3 ± 11	8 ± 5	2 ± 6	6 ± 8
	p-value (n = 18)[2]	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
(GR × PS)	*p*-value (n = 72)[3]	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
18 months											
Gamma radiation (GR)	0 kGy	3 ± 5	33 ± 28	32 ± 11	−5 ± 14	−31 ± 33	19 ± 38	−19 ± 28	−54 ± 13	−26 ± 29	−55 ± 9
	10 kGy	−5 ± 6	13 ± 18	26 ± 32	−18 ± 14	−47 ± 38	15 ± 53	−28 ± 33	−40 ± 18	−6 ± 19	−44 ± 7
	p-value (n = 36)[2]	0.001	0.001	0.310	< 0.001	0.068	0.697	0.242	< 0.001	0.001	< 0.001
Plant species (PS)	*A. citrodora*	−3 ± 5	12 ± 38	28 ± 6	9 ± 8	−64 ± 12	26 ± 2	−37 ± 11	−39 ± 13	−34 ± 9	−48 ± 8
	M. officinalis	1 ± 4	9 ± 12	2 ± 15	−23 ± 9	18 ± 3	18 ± 2	28 ± 1	−73 ± 2	23 ± 1	−54 ± 2
	M. melissophyllum	−3 ± 9	28 ± 14	62 ± 15	−17 ± 12	−48 ± 18	−50 ± 18	−44 ± 3	−40 ± 10	−26 ± 22	−57 ± 9
	M. piperita	2 ± 2	43 ± 13	24 ± 7	−15 ± 3	−61 ± 15	73 ± 18	−41 ± 7	−37 ± 4	−27 ± 11	−39 ± 4
	p-value (n = 18)[2]	0.472	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
(GR × PS)	*p*-value (n = 72)[3]	< 0.001	0.046	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

[1] For each species, means within a column with different letters differ significantly ($p < 0.05$).
[2] $p < 0.05$ indicates that the mean value of at least one ratio differs from the others.
[3] $p < 0.05$ indicates a significant interaction (in this case multiple comparison tests results could not be indicated).

10 kGy gamma-irradiated plants), coumarin (28 ± 4 mg/mL in non-irradiated plants, 31 ± 3 mg/mL in 10 kGy gamma-irradiated plants), rosmarinic acid (89 ± 7 mg/mL in non-irradiated plants, 112 ± 9 mg/mL in 10 kGy gamma-irradiated plants) and luteolin-7-*O*-rutinoside (34 ± 0.3 mg/mL in non-irradiated plants, 41 ± 1 mg/mL in 10 kGy gamma-irradiated plants) (Pereira, Barros, Antonio, Verde, Santos-Buelga, Ferreira, and Rodrigues, 2017). Furthermore, there were differences among infusions and methanolic extracts; for instance, verbascoside was the main compound in methanolic extracts of *A. citrodora*, while eriodictyol-*O*-rutinoside predominated in *M. piperita* extracts (Pereira et al., 2016; Pereira, Pimenta, Calhelha, Antonio, Barros, Santos-Buelga, et al., 2017).

In addition, the interaction among PS and GR was significant for most assayed parameters, implying that their variation in response to GR would not be the same, and making it difficult to find statistically significant changes among irradiated and non-irradiated samples. It is true that if the analysis was performed by considering each PS individually, some particular differences among irradiated and non-irradiated samples could have been found. However, the main objective of this work was validating GR treatment as an alternative to improve the storage quality of different aromatic plants, not of a single PS.

3.3. Principal component analysis (PCA)

In the former sections, changes induced by GR throughout ST were analyzed. Despite the detected differences, the dissimilarity among species did not allow obtaining overall conclusions regarding the effect of GR in each evaluated parameter.

Accordingly, PCA was applied to the percentage differences obtained in all parameters simultaneously. The total matrix of obtained results was used, corresponding to 52 variables (columns) × 144 assay results (lines), 72 from each assayed storage time. The results were not pre-processed, as they were already normalized to be included in Tables 1–5.

In the performed analysis, five significant dimensions were obtained, from which the first three (1st: Cronbach's α = 0.942; eigenvalue = 13.176; 2nd: Cronbach's α = 0.880; eigenvalue = 7.320; 3rd: Cronbach's α = 0.852; eigenvalue = 6.071) were plotted, sequentially using GR and ST as labelling variables.

In the first case (GR effect), the plot of object scores (Fig. 1A) shows that the markers corresponding to non-irradiated and irradiated samples were not clearly separated, which validates the lack of significant differences, inclusively when considering the contribution of all parameters together.

On other hand, groups corresponding to each storage time correlated differently with the defined principal components (Fig. 1B). The markers corresponding to 12 months are mainly located in the positive end of dimension 1 axis and in the negative end of dimension 2 axis, while those corresponding to 18 months are negative end of dimension 1 axis and in the positive end of dimension 2 axis. Dimension 3 had no relevant contribution to separate the markers corresponding to each assayed ST. Besides highlighting their separation, PCA also allow to identify the parameters more highly correlated to the markers corresponding to each ST (Table 6). Values in bold correspond to the highest overall correlations among each variable and dimension. As it might be concluded, variables with highest correlation with dimension 1 are mostly fatty acids, while tocopherols and antioxidant activity results stood out as the variables more highly correlated with dimension 2. Therefore, the former parameters are the ones with highest changes according to ST. The former inferences are applicable the all studied PS.

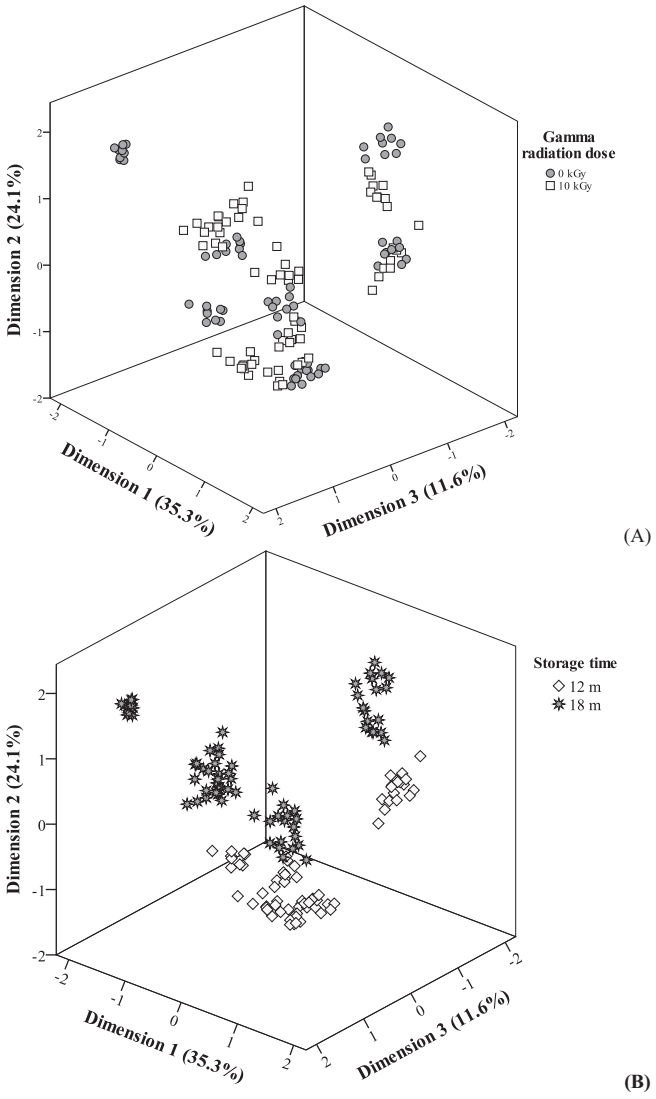

Fig. 1. Plots of objects scores and component loadings using gamma irradiation doses (**A**) or storage time (**B**) as scoring objects.

4. Conclusion

Overall, the nutritional parameters presented similar profiles after the assayed ST, independently of treating samples with GR. Changes in color parameters *a** and *b** indicated a greener color (yet slightly more yellow) among irradiated samples. Furthermore, gamma radiation could not prevent the decrease in free sugars, organic acids and tocopherols, particularly after 18 months. Conversely, gamma radiation had a positive effect in oleic acid, β-carotene bleaching inhibition (in the infusions), DPPH scavenging activity and reducing power (in the methanolic extracts). Even so, and taking into account previous results obtained after treating the same species herein with electron-beam radiation, it seems obvious that gamma radiation is a less suitable conservation alternative, mainly for extended storage periods.

In either case, and despite the dissimilarity observed among PS, it was possible to identify the most significant variations along ST, in addition to their correlations with the markers corresponding to 12 months or 18 months (independently of PS).

Table 6
Component loadings for all included variables (only the three most).

Variable	Dimension 1	Dimension 2	Dimension 3
Fat	−0.26	**−0.66**	0.23
Proteins	0.29	−0.10	−0.07
Ash	0.44	0.00	0.01
Carbohydrates	−0.26	−0.01	−0.09
Energy	−0.43	−0.49	0.22
L*	0.14	0.37	0.01
a*	0.29	0.18	−0.18
b*	0.16	0.09	**−0.63**
Fructose	0.45	−0.04	−0.25
Glucose	0.43	−0.25	−0.27
Sucrose	**0.68**	0.16	−0.26
Trehalose	0.45	−0.35	**0.54**
Sugars	**0.76**	−0.09	−0.10
Oxalic acid	−0.13	−0.13	0.37
Malic acid	−0.21	−0.22	−0.06
Organic acids	−0.04	−0.09	0.30
α-TF	−0.25	**−0.58**	0.35
β-TF	**0.52**	**0.53**	**−0.59**
γ-TF	−0.19	**−0.67**	0.19
Tocopherols	**0.62**	0.16	**−0.50**
C6:0	**−0.81**	0.23	−0.31
C8:0	**−0.68**	**0.62**	−0.02
C11:0	**−0.57**	−0.29	−0.25
C12:0	**−0.85**	0.41	−0.08
C13:0	**0.51**	0.33	0.39
C14:0	−0.43	0.13	**−0.64**
C15:0	**−0.55**	−0.22	−0.31
C15:1	0.06	0.40	0.13
C16:0	−0.39	0.30	0.22
C17:0	−0.39	**0.73**	0.28
C18:0	**−0.80**	0.38	0.22
C18:1	**−0.69**	0.45	0.37
C18:2	**0.71**	−0.11	0.31
C18:3	**0.77**	−0.21	0.48
C20:0	0.16	**−0.56**	**−0.56**
C20:1	**0.59**	0.37	−0.49
C20:3n3 + C21:0	−0.18	**−0.59**	0.02
C22:0	**0.55**	0.02	0.09
C24:0	0.25	0.24	−0.18
SFA	**−0.93**	−0.07	−0.09
MUFA	**−0.74**	0.48	0.30
PUFA	**0.93**	0.06	0.21
DPPH (infusion)	−0.03	0.14	−0.09
Reducing power (infusion)	0.08	**0.78**	−0.04
β-Carotene (infusion)	−0.39	**−0.55**	−0.03
Phenols (infusion)	**0.73**	−0.02	0.33
Flavonoids (infusion)	**−0.51**	**−0.57**	−0.32
DPPH (methanolic extract)	−0.06	0.30	**0.60**
Reducing power (methanolic extract)	−0.03	**0.50**	**0.60**
β-Carotene (methanolic extract)	−0.26	−0.18	**0.72**
Phenols (methanolic extract)	**−0.62**	**−0.51**	−0.29
Flavonoids (methanolic extract)	0.42	−0.26	**0.51**

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Conflict of interest

The authors declare no conflict of interest.

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