



Infusions from *Thymus vulgaris* L. treated at different gamma radiation doses: Effects on antioxidant activity and phenolic composition



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ABSTRACT

The use of ionizing radiation dates back to many years ago, and is accredited for application in different foods with several purposes. It has been increasingly used in many countries for the treatment of aromatic plants. *Thymus vulgaris* L. (thyme) is a plant commonly used by food, pharmaceutical and cosmetic industries representing a natural source of several bioactives such as phenolic compounds. The aim of this work was to evaluate the effects of gamma radiation on the antioxidant activity (measured through the free radical scavenging activity, reducing power and lipid peroxidation inhibition) and phenolic compounds profile (obtained by HPLC-DAD-ESI/MS) of infusions prepared from irradiated thyme. The results showed that gamma irradiation at the dose of 10 kGy improved the free radical scavenging activity, reducing power and lipid peroxidation inhibition capacity of the studied infusions, while increasing significantly the concentrations of methylerythritol-O-pentosylhexoside, luteolin-7-O-glucoside, eriodictyol and total flavonoids content. Thus, gamma radiation could be considered as a suitable treatment to be used in *Thymus vulgaris* L., herein validated for its bioactive parameters.

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1. Introduction

Irradiation is a physical decontamination and preservation method, which processes the food at room temperature (Allothman, Bhat & Karim, 2009). It is used by the industry for different purposes, such as disinfestation, shelf life extension, decontamination and improvement of product quality (Singh & Datta, 2010). Despite being an increasingly popular technology, the number of irradiated foods is very low, even in countries where this preservation process is permitted. The main reasons are not only related with the high cost of the equipment but, especially, with the lack of acceptance by consumers (Roberts, 2014; Sádecká, 2007). The irradiation can be applied by using gamma rays, electron beams or X-rays, each one with different properties and comprising technological advantages and disadvantages (Allothman et al., 2009).

Gamma radiation has been applied to several matrices,

including mushrooms and aromatic plants, being verified that guarantees the quality of the products for the authorized radiation doses (Fernandes et al., 2012; Kausar, Akram, & Kwon, 2013; Pereira et al., 2015b). It is also characterized by its high penetration ability, being effective in irradiation of large volume foods (IAEA, 2002). Irradiation has been applied to aromatic and medicinal plants with a maximum permitted dose of 10 kGy in Europe; this treatment allows the decontamination of such matrices, while maintaining their chemical, nutritional and organoleptic properties (EU, 1999).

For a long period of time, the plants were almost the only source of therapeutic agents for humans. Indeed plants are natural sources of phytochemicals, especially polyphenols, which have been related to the prevention of chronic diseases and improved health quality (Hayta, Polat, & Selvi, 2014; Zhang et al., 2011). Presently, plants continue being the main sources of substances for drug development by the pharmaceutical industry (Caleja et al., 2015; Carvalho, Costa, & Carnelossi, 2010). Several studies have been performed in order to test the effects of irradiation on phytochemicals present in dried plants, and in favorable conditions (irradiation source, dose,

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humidity, etc.) the antioxidant potential of the species can be increased (Alothman et al., 2009). The intention of these studies is to follow the process from the irradiation until the consumer and to study the impact of this industrial process on the main components of herbal infusions (DeRuiter & Dwyer, 2002).

Thyme (*Thymus vulgaris* L.) is an herbaceous and perennial aromatic plant belonging to the Lamiaceae family, commonly consumed as herbal infusion and as a condiment and spice (flavoring agent) (Balladin & Headley, 1999; Helmy, Farrag, & Hasaballah, 2015). It is one of the most cultivated herbs of *Thymus* genus, being used in food, pharmaceutical and cosmetic industries. It is listed in current editions of the European Pharmacopoeia, US Pharmacopoeia and other official papers (Damianova, Tasheva, Stoyanova, & Damianov, 2008; Gavarić et al., 2015), exhibiting carminative, antispasmodic, antitussive, expectorant, bactericidal, antihelmintic and astringent effects. Traditionally, the herb has been used for the treatment of dyspepsia, chronic gastritis and diseases of the upper respiratory tract (Fecka & Turek, 2008; Helmy et al., 2015). It has also been reported that thyme infusions are low caloric beverages (Pereira, Barros, & Ferreira, 2015a), while containing high levels of phenolic compounds with antioxidant properties (Martins et al., 2015).

The aim of the present work was to go further on the study of antioxidant activity and phenolic composition of thyme, previously reported (Brandstetter, Berthold, Isnardy, Solar, & Elmadfa, 2009; Martins et al., 2015; Pereira et al., 2016), evaluating the effects of gamma irradiation on antioxidant potential and phenolic composition of infusions prepared from non-irradiated (0 kGy) and irradiated samples of thyme with 1 and 10 kGy. These doses were chosen since 1 kGy guarantees insects' disinfestation and 10 kGy is the highest dose permitted by the EU legislation that allows also microbiological decontamination (EU, 1999). The obtained results will assess if the expected benefits of these beverages are maintained after the application of this decontamination/preservation technique.

2. Materials and methods

2.1. Standards and reagents

Acetonitrile 99.9% was of HPLC grade from Fisher Scientific (Lisbon, Portugal). Phenolic compound standards (apigenin-6-C-glucoside, caffeic acid, luteolin-7-O-glucoside, naringenin, quercetin-3-O-rutinoside and rosmarinic acid) were from Extrasynthese (Genay, France). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Sigma (St. Louis, MO, USA) and 2,2-diphenyl-1-picrylhydrazyl (DPPH•) was obtained from Alfa Aesar (Ward Hill, MA, USA). β -Carotene and linoleic acid were acquired from Sigma-Aldrich (St. Louis, Missouri, USA) and Tween 80 from Panreac (Barcelona, Spain). All other solvents and reagents were acquired from scientific retailers. Ferrous ammonium sulfate (II) hexahydrate, sodium chloride and sulfuric acid, all with PA purity, were purchased from Panreac S.A. (Barcelona, Spain), in order to prepare the acid aqueous Fricke dosimeter solution. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

2.2. Samples and samples irradiation

The samples (dry leaves of *Thymus vulgaris* L.) were provided by a local producer (Pragmático Aroma Lda, Alfândega da Fé, Bragança, Portugal), and divided in three groups: control (non-irradiated), 1 (irradiated at 1 kGy) and 2 (10 kGy). Each group consisted of three samples, each one with 40 g of dry material, with a total amount of 120 g for each group.

A gamma radiation equipment (Precisa 22, Grainer Manufacturing Company Ltd., UK) with four ^{60}Co sources, with a total activity 140 TBq (3.77 kCi), was used for sample irradiations, in May 2015. During the irradiation procedure, the dose and dose rate were estimated using Amber Perspex (Batch X, from Harwell Company, Didcot, Oxfordshire, UK) and Fricke reference dosimeters, respectively (ASTM, 1992; Pereira et al., 2015b).

The estimated radiation doses for groups 1 and 2 were 1.2 ± 0.1 kGy and 10.4 ± 0.9 kGy, respectively. For simplicity, the values 1 kGy, and 10 kGy are considered for presentation and discussion of the results. The dose rate and dose uniformity ratio (D_{\max}/D_{\min}) were 1.7 and 1.2 kGy/h, respectively.

2.3. Infusions preparation

The infusions were prepared according to Pereira et al. (2015b) and were obtained from dried plants irradiated. Briefly, 1 g of the sample 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.

2.4. Total phenolic content, total flavonoid content and in vitro antioxidant activity

The Folin-Ciocalteu method was used to estimate total phenolic content following a method explained by Wolfe, Wu, and Liu (2003) and total flavonoid content were determined by a colorimetric assay using aluminum trichloride, as previously described by Jia, Tang, and Wu (1999). The results were expressed as mg of gallic acid equivalents (GAE) per mL of infusion for total phenolic content and as mg of (+)-catechin equivalents (CE) per mL of infusion for total flavonoid content.

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: $[(A_{\text{DPPH}} - A_s)/A_{\text{DPPH}}] \times 100$, 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 (Pereira, Barros, & Ferreira, 2013).

Reducing power was evaluated by the capacity to convert Fe^{3+} into Fe^{2+} , measuring the absorbance at 690 nm in the microplate reader (Pereira et al., 2013). Inhibition of β -carotene bleaching was evaluated through the β -carotene/linoleate assay; the neutralization of linoleate free radicals avoids β -carotene bleaching, the reaction was measured at 470 nm in a spectrophotometer (AnalytikJena, Jena, Germany) and β -carotene bleaching inhibition (%) was calculated using the equation: $(\beta\text{-carotene absorbance after 2 h of assay}/\text{initial absorbance}) \times 100$ (Amarowicz, Pegg, Rahimi-Moghaddam, Barl, & Weil, 2004).

Lipid peroxidation inhibition in porcine brain homogenates was evaluated by the reduction of thiobarbituric acid reactive substances (TBARS); the colour intensity of the malondialdehyde-thiobarbituric acid (MDA-TBA) was measured by its absorbance at 532 nm; the inhibition ratio (%) was calculated using the formula: $[(A - B)/A] \times 100\%$, where A and B were the absorbance of the control and the sample solution, respectively (Fernandes et al., 2013).

The results were then converted to EC_{50} values (mg/mL) by using the graphs of the antioxidant activity percentage or absorbance at 690 nm (in the case of reducing power assay) against the extract concentration. Trolox was used as positive control.

2.5. Analysis of individual phenolic compounds

The aqueous preparations (infusions) obtained above were

filtered through a 0.45 μm Whatman syringe filter and transferred to an amber HPLC vial for chromatographic injection. The analysis was performed in an HPLC equipment (Agilent Technologies, Santa Clara, CA, USA) with a double online detection in a diode array detector (DAD), using with 280, 330 and 370 nm as preference wavelengths, and a mass spectrometer (MS) equipped with an ESI source and a triple quadrupole-ion trap mass analyser, which was connected to the HPLC system via the DAD cell outlet. The DAD was coupled to an HP Chem Station (rev. A.05.04) and the MS was controlled by the Analyst 5.1 software. The separation was achieved using a Spherisorb S3 ODS-2 C_{18} column (3 μm , 4.6 \times 150 mm) thermostatted at 35 $^{\circ}\text{C}$, using a gradient elution with the following solvents: 0.1% formic acid in water (A) and acetonitrile (B). The elution gradient was 15% B for 5 min, 15% B to 20% B over 5 min, 20–25% B over 10 min, 25–35% B over 10 min, 35–50% B for 10 min, and re-equilibration of the column (10 min), using a flow rate of 0.5 mL/min, as previously described by Barros et al. (2013). The MS detector was programmed for recording in two consecutive modes: Enhanced MS (EMS), employed to record full scan spectra, and enhanced product ion (EPI) analysis. Air (zero graded) was used as the nebulizer gas (30 psi) and turbo gas for solvent drying (400 $^{\circ}\text{C}$, 40 psi). Nitrogen functioned as the curtain (20 psi) and collision gas (medium). The ion spray voltage was set at -4500 V and spectra were recorded in negative ion mode between m/z 100 and 1700. The settings used were: declustering potential (DP) -450 V, entrance potential (EP) -6 V, collision energy (CE) -10 V. EPI mode was performed in order to obtain the fragmentation pattern of the parent ion(s) in the previous scan using the following parameters: DP -50 V, EP -6 V, CE -25 V, and collision energy spread (CES) 0 V. Compounds were tentatively identified comparing the obtained information (retention times, UV-vis and mass spectra) with available data reported in the literature and by comparison with standard compounds, when available. For the quantification a calibration curve for each available phenolic standard (apigenin-6-C-glucoside, caffeic acid, luteolin-7-O-glucoside, naringenin, quercetin-3-O-rutinoside, rosmarinic acid) was constructed based on the UV signal (maximum wavelength of absorption of each standard compound). For the identified phenolic compounds for which a commercial standard was not available, the quantification was performed through the calibration curve of another compound from the same phenolic group. The results were expressed in μg per mL of infusion.

2.6. Statistical analysis

Three independent samples were analysed for each dose of irradiation with three aqueous extractions performed for each sample, and all the assays were carried out in triplicate ($n = 27$). The results for control (non-irradiated) and irradiated samples were expressed as mean values \pm standard deviation (SD). The results were analysed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $p = 0.05$. In the case of phenolic composition, a Student's t -test was used to determine the significant difference among two different samples, with $p = 0.05$. When the p value was lower than 0.05, significant differences between samples were considered. Furthermore, a Pearson's correlation analysis between the antioxidant activity and all the analysed compounds was carried out, with a 95% confidence level. Analyses were carried out using IBM SPSS Statistics for Windows, version 23.0. (IBM Corp., Armonk, New York, USA).

3. Results and discussion

The results for the total phenolic content and total flavonoid content of infusions prepared from non-irradiated and irradiated

samples of *T. vulgaris* and the evaluation of the antioxidant properties tested by four *in vitro* assays (DPPH scavenging activity, reducing power, β -carotene bleaching and lipid peroxidation inhibition) are presented in Table 1. Significantly higher values of total phenolic and flavonoid contents were found in the samples irradiated at 10 kGy (0.168 mg GAE/mL of infusion and 0.06 mg CE/mL of infusion, respectively). The increase in total phenolic and flavonoid contents with the irradiation dose could be related with the release of these compounds from the matrix structures, increasing extractability of certain compounds and the degradation of larger compounds into smaller ones (Polovka & Suhaj, 2010; Taheri, Abdullah, Karimi, Oskoueian, & Ebrahimi, 2014). Statistically significant differences were observed in the EC_{50} values of all the antioxidant assays among samples subjected to different radiation doses, with 10 kGy leading to the highest antioxidant potential (lowest EC_{50} values). The increase in the antioxidant capacity of the *T. vulgaris* samples submitted to 10 kGy is in agreement with previous findings in infusions of irradiated *Ginkgo biloba* L. (Pereira et al., 2015c), Borututu (a folk medicine plant obtained from the African tree *Cochlospermum angolense* Welw.) (Pereira et al., 2014) and ethanolic extracts of *Hizikia fusiformis* Harvey (edible brown seaweed consumed in Korea and Japan) (Kim et al., 2009).

Table 2 presents data related to the phenolic compounds identification (retention time, λ_{max} in the visible region, pseudomolecular ion, main fragment ions in MS^2 , and tentative identities) obtained by HPLC-DAD-ESI/MS analysis. The phenolic profile of *T. vulgaris* infusions revealed the presence of thirteen phenolic compounds, from which seven were phenolic acids derivatives and six were flavonoid glycosides (Table 2). The phenolic profile of thyme irradiated with 10 kGy was shown in Fig. 1. In this study, the phenolic profile was only evaluated for samples irradiated with the dose of 10 kGy, and compared with the control sample; this is because 10 kGy showed higher antioxidant potential comparatively with 1 kGy, i.e., lower EC_{50} values. A similar phenolic profile was previously observed by the authors in methanolic extracts obtained from irradiated samples of *T. vulgaris* (Pereira et al., 2016). Similarly, Martins et al. (2015) reported apigenin 6,8-di-C-glucoside (peak 2), caffeic acid (3), rosmarinic acid hexoside (6), luteolin-7-O-glucuronide (7), luteolin-7-O-glucoside (8), rosmarinic acid (10), lithospermic acid A (12) and eriodictyol (13) in non-irradiated samples of *T. vulgaris*. The presence of caffeic acid, luteolin-7-O-glucuronide, rosmarinic acid and eriodictyol was also found in thyme hydrophilic extracts by Fecka and Turek (2008), whereas Boros et al. (2010) detected apigenin 6,8-di-C-glucoside, caffeic acid, rosmarinic acid and eriodictyol in hydroalcoholic extracts of different *Thymus* species (*Thymus pannonicus*, *Thymus glabrescens*, *Thymus pulegioides*, *Thymus praecox*, *Thymus serpyllum*).

The majority phenolic compounds found in *T. vulgaris* infusions were rosmarinic acid hexoside (peak 6), luteolin-7-O-glucuronide (7), caffeic acid trimer (9) and rosmarinic acid (10), as previously found in methanolic extracts of irradiated thyme samples (Pereira et al., 2016). Also, the presence of luteolin-7-O-glucuronide and rosmarinic acid as major compounds in thyme infusions was reported by Martins et al. (2015).

In general, gamma radiation at a dose of 10 kGy causes statistically relevant changes in the concentration of the phenolic compounds. Small but significant increases were observed in the levels of methyleriodictyol-O-pentosylhexoside, luteolin-7-O-glucoside and eriodictyol, and consequently in total flavonoid content. This is in agreement with Pereira et al. (2015c) and Allothman et al. (2009) who also observed that irradiation at 10 kGy dose caused an increase in some individual phenolic compounds in the infusions of *Ginkgo biloba*. These results could be explained by an increase in compounds extractability due to depolymerization and release from cell wall polysaccharides produced by the irradiation process,

Table 1Total phenolic content, total flavonoid content and *in vitro* antioxidant properties of infusions prepared from *Thymus vulgaris* subjected to gamma radiation.

Dose (kGy)	0	1	10
Total phenolic content (mg GAE/mL of infusion)	0.138 ± 0.001c	0.150 ± 0.001b	0.168 ± 0.001a
Total flavonoid content (mg CE/mL of infusion)	0.048 ± 0.001c	0.053 ± 0.001b	0.060 ± 0.001a
DPPH scavenging activity (EC ₅₀ value, mg/mL)	0.87 ± 0.05a	0.76 ± 0.02b	0.66 ± 0.02c
Reducing power (EC ₅₀ value, mg/mL)	0.48 ± 0.01a	0.43 ± 0.01b	0.41 ± 0.01c
β-carotene bleaching inhibition (EC ₅₀ value, mg/mL)	1.63 ± 0.01a	1.66 ± 0.04a	1.25 ± 0.01b
TBARS inhibition (EC ₅₀ value, mg/mL)	0.22 ± 0.01a	0.22 ± 0.01a	0.13 ± 0.01b

GAE-Gallic acid equivalents; CE-Catechin equivalents. In each row different letters mean significant differences ($p < 0.05$).**Table 2**Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{\max}), mass spectral data, identification and quantification of phenolic compounds in infusion prepared from *Thymus vulgaris* subjected to gamma irradiation ($\mu\text{g/mL}$ of infusion).

Peak	Rt (min)	λ_{\max} (nm)	Pseudomolecular ion [M-H] ⁻ (m/z)	MS ² (m/z)	Tentative identification	Infusion		t-Students test p-value
						0 kGy	10 kGy	
1	7.3	320	341	179(100), 135(88)	Caffeic acid hexoside	3.1 ± 0.1	2.5 ± 0.2	0.003
2	10.8	338	593	473(20), 383(33), 353(27), 297(5)	Apigenin 6,8-di-C-glucoside	6.6 ± 0.4	6.2 ± 0.1	0.122
3	11.3	326	179	135(100)	Caffeic acid	4.54 ± 0.1	4.19 ± 0.4	0.091
4	17.4	284,336sh	595	301(47), 286(100)	Methylerythrodityol-O-pentosylhexoside	5.03 ± 0.01	6.34 ± 0.04	<0.001
5	18.1	350	461	301(100)	Quercetin-O-glucuronide	0.21 ± 0.01	0.13 ± 0.02	0.002
6	18.8	322	521	359(100), 197(13), 179(36), 161(62), 135(21)	Rosmarinic acid hexoside	33 ± 1	32 ± 1	0.035
7	20.0	348	461	285(100)	Luteolin-7-O-glucuronide	24.0 ± 0.1	23.8 ± 0.1	0.062
8	20.8	350	447	285(100)	Luteolin-7-O-glucoside	4.7 ± 0.1	5.57 ± 0.01	<0.001
9	23.4	286,320sh	537	493(20), 359(70), 295(5), 197(13), 179(28), 161(100), 135(63)	Caffeic acid trimer	19.36 ± 0.02	17.8 ± 0.1	<0.001
10	24.0	330	359	197(17), 179(35), 161(100), 135(29)	Rosmarinic acid	22.3 ± 0.4	21 ± 1	0.012
11	25.6	282	567	535(23), 493(49), 387(32), 285(25), 197(13)	Caffeic acid derivative	3.0 ± 0.1	2.6 ± 0.3	0.034
12	27.5	290,326sh	537	493(50), 359(17), 295(33), 179(75), 135(100)	Lithospermic acid A	3.53 ± 0.04	2.1 ± 0.4	0.004
13	30.6	288,334sh	287	151(35), 135(100)	Eriodictyol	1.51 ± 0.02	1.6 ± 0.1	0.019
					Total phenolic acids	89 ± 1	82 ± 2	0.001
					Total flavonoids	42.0 ± 0.4	43.7 ± 0.4	0.002
					Total phenolic compounds	131 ± 1	125 ± 1	0.001

as stated above. On the contrary, small but statistically significant decreases were produced in the levels of caffeic acid hexoside, quercetin-O-glucuronide, rosmarinic acid hexoside, caffeic acid trimer, rosmarinic acid, caffeic acid derivative and lithospermic acid A, after irradiation at 10 kGy, being also, in total phenolic acids and total phenolic compounds. Otherwise, non-significant changes were observed in other phenolic compounds, such as apigenin 6,8-di-C-glucoside (peak 2), caffeic acid (peak 3) and luteolin-7-O-glucuronide (peak 7). It could be supposed that phenolic acids have lower stability against gamma irradiation and, therefore, they are degraded to some extent at higher irradiation doses. Thus, radiation may contribute to increase compounds extractability, on the other hand, it may also lead to degradation of some less stable compounds.

In order to try to relate antioxidant capacity and phenolic composition of the samples, correlation factors were obtained between the sums of phenolic acid derivatives, flavonoids and total phenolic compounds. A Pearson's correlation between all antioxidant activities tested was performed, because the normality was verified through a Shapiro-Wilk test. The correlations of all antioxidant assays (DPPH scavenging activity, reducing power, β-carotene bleaching and lipid peroxidation inhibition) and total phenolic compounds are presented in Table 3. In general, the majority of the compounds showed correlations statistically

significant with the antioxidant activity assays. Methylerythrodityol-O-pentosylhexoside, luteolin-7-O-glucoside and eriodictyol (peaks 4, 8 and 13) presented highly significant correlations with all antioxidant activities tested but these correlations were negative, meaning that the antioxidant activity increased (lower EC₅₀ values), for irradiated samples with 10 kGy, with increasing concentrations of these compounds at the same dose; therefore, inducing a similar behavior in the total flavonoid content. However, the compounds corresponding to peaks 1, 5, 7, 6, 10 and 12 also revealed statistically significant correlations, presenting p -values < 0.05. Otherwise, apigenin 6,8-di-C-glucoside (peak 2) and caffeic acid (peak 3) were the only compounds that did not statistically significantly relate to any of the antioxidant assays, presenting p -value > 0.05. The major individual phenolic compound (peak 6 – rosmarinic acid hexoside) also had a high correlation with all the antioxidant activity assays, showing a variation between 0.815 and 0.865. Overall, total phenolic acids, total flavonoids and total phenolic compounds showed statistically significant correlations with all assays, displaying p -values ≤ 0.05.

4. Conclusion

Infusions are a form of consumption of thyme leaves and evaluation of their bioactive potential depending on the type of plant

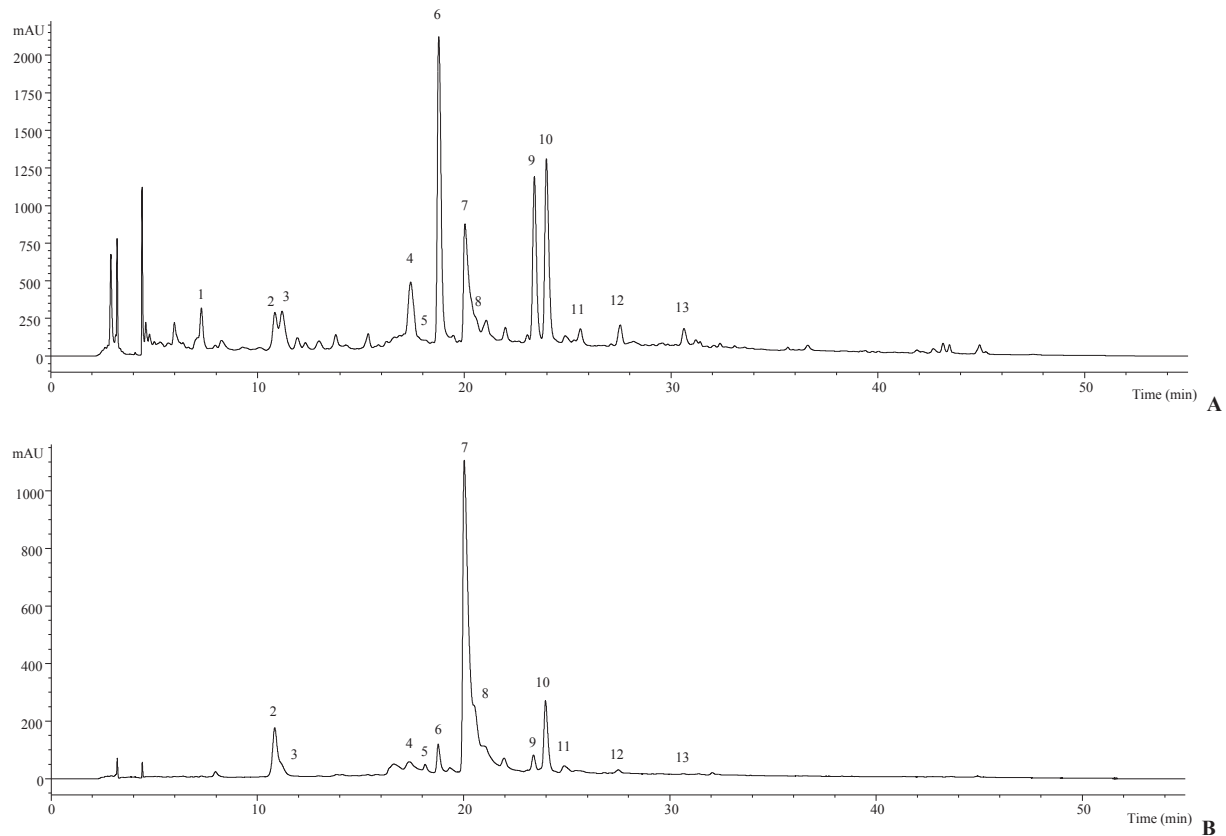


Fig. 1. Phenolic compounds profile in infusions prepared from thyme samples irradiated with 10 kGy, recorded at 280 nm (A) and 370 nm (B).

Table 3

Correlation coefficients of phenolic compounds with *in vitro* antioxidant activity of infusions prepared from *Thymus vulgaris* subjected to gamma radiation.

Peaks	Compounds	DPPH scavenging activity		Reducing power		β-Carotene bleaching inhibition		TBARS inhibition	
		Correlation factor	<i>p</i> -value	Correlation factor	<i>p</i> -value	Correlation factor	<i>p</i> -value	Correlation factor	<i>p</i> -value
1	Caffeic acid hexoside	0.878	0.021	0.969	0.001	0.952	0.003	0.956	0.003
2	Apigenin 6,8-di-C-glucoside	0.530	0.280	0.742	0.091	0.689	0.137	0.698	0.123
3	Caffeic acid	0.719	0.107	0.724	0.104	0.750	0.086	0.742	0.091
4	Methyleriodictyol- <i>O</i> -pentosylhexoside	-0.954	0.003	-0.997	<0.001	-1.000	<0.001	-1.000	<0.001
5	Quercetin- <i>O</i> -glucuronide	0.945	0.004	0.962	0.002	0.965	0.002	0.967	0.002
6	Rosmarinic acid hexoside	0.865	0.026	0.815	0.048	0.851	0.032	0.843	0.035
7	Luteolin-7- <i>O</i> -glucuronide	0.679	0.138	0.817	0.047	0.778	0.068	0.788	0.063
8	Luteolin-7- <i>O</i> -glucoside	-0.926	0.008	-0.998	<0.001	-0.992	<0.001	-0.993	<0.001
9	Caffeic acid trimer	0.956	0.003	0.997	<0.001	0.999	<0.001	1.000	<0.001
10	Rosmarinic acid	0.906	0.013	0.890	0.017	0.916	0.010	0.911	0.012
11	Caffeic acid derivative	0.791	0.061	0.856	0.029	0.837	0.038	0.844	0.035
12	Lithospermic acid A	0.906	0.013	0.941	0.005	0.953	0.003	0.950	0.004
13	Eriodictyol	-0.808	0.052	-0.885	0.019	-0.887	0.018	-0.884	0.011
	Total phenolic acids	0.945	0.004	0.961	0.002	0.975	0.001	0.972	0.001
	Total flavonoids	-0.955	0.003	-0.943	0.005	-0.965	0.002	-0.961	0.002
	Total phenolic compounds	0.940	0.005	0.964	0.002	0.977	0.001	0.974	0.001

processing is extremely important. According to the results, it was evident that gamma radiation had an influence on the antioxidant activity of the samples, with treatment at 10 kGy leading to higher antioxidant potential (lower EC₅₀ values in all the assays). This irradiation dose (10 kGy) also evidenced higher total phenolic and flavonoid contents. The composition in phenolic compounds was determined and thirteen compounds were detected in the infusions prepared either from irradiated or non-irradiated samples and, in general, the gamma irradiation treatment at a dose of 10 kGy caused relevant changes in the concentration of several

compounds. Methyleriodictyol-*O*-pentosylhexoside, luteolin-7-*O*-glucoside, eriodictyol and total flavonoid content were the only ones that increase with the irradiation dose applied (10 kGy), and apigenin 6,8-di-C-glucoside, caffeic acid and luteolin-7-*O*-glucuronide were the only compounds with no statistically significant changes observed. The results show that, for the legally permitted maximum radiation dose in EU (10 kGy), gamma radiation may even improve the antioxidant potential and total flavonoid content of *T. vulgaris* infusion without changing its chemical profile.

Conflict of interest

The authors declare they have no conflict of interest.

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References

- Allothman, M., Bhat, R., & Karim, A. A. (2009). Effects of radiation processing on phytochemicals and antioxidants in plant produce. *Trends Food Science and Technology*, 20, 201–212.
- Amarowicz, R., Pegg, R. B., Rahimi-Moghaddam, P., Barl, B., & Weil, J. A. (2004). Free radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry*, 84, 551–562.
- ASTM. (1992). *Practice for using the fricke reference standard dosimetry system, ASTM E1026*. Philadelphia, PA: American Society for Testing and Materials. Annual Book of ASTM Standards, 12.02.
- Balladin, D. A., & Headley, O. (1999). Evaluation of solar dried thyme (*Thymus vulgaris* Linné) herbs. *Renewable Energy*, 17, 523–531.
- Barros, L., Pereira, E., Calhelha, R. C., Dueñas, M., Carvalho, A. M., Santos-Buelga, C., et al. (2013). Bioactivity and chemical characterization in hydrophilic and lipophilic compounds of *Chenopodium ambrosioides* L. *Journal of Functional Foods*, 5, 1732–1740.
- Boros, B., Jakabová, S., Dörnyei, Á., Horváth, G., Pluhár, Z., Kilár, F., et al. (2010). Determination of polyphenolic compounds by liquid chromatography–mass spectrometry in thymus species. *Journal of Chromatography A*, 1217, 7972–7980.
- Brandstetter, S., Berthold, C., Isnardy, B., Solar, S., & Elmadfa, I. (2009). Impact of gamma-irradiation on the antioxidative properties of sage, thyme, and oregano. *Food and Chemical Toxicology*, 47, 2230–2235.
- Caleja, C., Barros, L., Antonio, A. L., Ciric, A., Soković, M., Oliveira, M. B. P. P., et al. (2015). *Foeniculum vulgare* Mill. as natural conservation enhancer and health promoter by incorporation in cottage cheese. *Journal of Functional Foods*, 12, 428–438.
- Carvalho, L. M., Costa, J. A. M., & Carnelossi, M. A. G. (2010). *Qualidade em plantas medicinais* (p. 56). Aracaju, SE: Embrapa Tabuleiros Costeiros.
- Damianova, S., Tasheva, S., Stoyanova, A., & Damianov, D. (2008). Investigation of extracts from thyme (*Thymus vulgaris* L.) for application in cosmetics. *Journal of Essential Oil-Bearing Plants*, 11, 443–450.
- DeRuiter, F. E., & Dwyer, J. (2002). Consumer acceptance of irradiated foods: Dawn of a new era? *Food Service Technology*, 2, 47–58.
- EU. (1999). Directive 1999/3/EC of the European Parliament and of the Council of 22 February 1999 on the establishment of a community list of foods and food ingredients treated with ionising radiation. *Official Journal of the European Communities* L66, 24–25.
- Fecka, I., & Turek, S. (2008). Determination of polyphenolic compounds in commercial herbal drugs and spices from Lamiaceae: Thyme, wild thyme and sweet marjoram by chromatographic techniques. *Food Chemistry*, 108, 1039–1053.
- Fernandes, Á., Antonio, A. L., Barreira, J. C. M., Oliveira, M. B. P. P., Martins, A., & Ferreira, I. C. F. R. (2012). Effects of gamma irradiation on physical parameters of *Lactarius deliciosus* wild edible mushrooms. *Postharvest Biology and Technology*, 74, 79–84.
- Fernandes, Á., Barreira, J. C. M., Antonio, A. L., Santos, P. M. P., Martins, A., Oliveira, M. B. P. P., et al. (2013). Study of chemical changes and antioxidant activity variation induced by gamma-irradiation on wild mushrooms: Comparative study through principal component analysis. *Food Research International*, 54, 18–25.
- Gavarić, N., Kladar, N., Mišan, A., Nikolić, A., Samojlik, I., Mimica-Dukić, N., et al. (2015). Postdistillation waste material of thyme (*Thymus vulgaris* L., Lamiaceae) as a potential source of biologically active compounds. *Industrial Crops and Products*, 74, 457–464.
- Hayta, S., Polat, R., & Selvi, S. (2014). Traditional uses of medicinal plants in Elazığ (Turkey). *Journal of Ethnopharmacology*, 154, 613–623.
- Helmy, W. A., Farrag, A. A., & Hasaballah, A. A. (2015). Chemical composition and biological activity of aqueous and methanolic extracts of thyme (*Thymus vulgaris*). *International Journal of Advanced Research*, 3, 1285–1305.
- IAEA. (2002). *Dosimetry for food irradiation*. Technical report series 490. Vienna: International Atomic Energy Agency.
- Jia, Z., Tang, M., & Wu, J. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64, 555–559.
- Kausar, T., Akram, K., & Kwon, J.-H. (2013). Comparative effects of irradiation, fumigation, and storage on the free amino acids and sugar contents of green, black and oolong teas. *Radiation Physics and Chemistry*, 86, 96–101.
- Kim, H.-J., Choi, J.-I., Kim, D.-J., Kim, J.-H., Chun, B. S., Ahn, D. H., et al. (2009). Effect of ionizing radiation on the physiological activities of ethanol extract from *hizikia fusiformis* cooking drips. *Applied Radiation and Isotopes*, 67, 1509–1512.
- Martins, N., Barros, L., Santos-Buelga, C., Silva, S., Henriques, M., & Ferreira, I. C. F. R. (2015). Decoction, infusion and hydroalcoholic extract of cultivated thyme: Antioxidant and antibacterial activities, and phenolic characterization. *Food Chemistry*, 167, 131–137.
- Pereira, E., Antonio, A. L., Barreira, J. C. M., Barros, L., Bento, A., & Ferreira, I. C. F. R. (2015b). Gamma irradiation as a practical alternative to preserve the chemical and bioactive wholeness of widely used aromatic plants. *Food Research International*, 67, 338–348.
- Pereira, E., Barros, L., Dueñas, M., Antonio, A. L., Santos-Buelga, C., & Ferreira, I. C. F. R. (2015c). Gamma irradiation improves the extractability of phenolic compounds in *Ginkgo biloba* L. *Industrial Crops and Products*, 74, 144–149.
- Pereira, E., Barros, L., & Ferreira, I. C. F. R. (2013). Chemical characterization of *Ginkgo biloba* L. and antioxidant properties of its extracts and dietary supplements. *Industrial Crops and Products*, 51, 244–248.
- Pereira, C., Barros, L., & Ferreira, I. C. F. R. (2015a). A Comparison of the nutritional contribution of thirty-nine aromatic plants used as condiments and/or herbal infusions. *Plant Foods for Human Nutrition*, 70, 176–183.
- Pereira, C., Calhelha, R. C., Antonio, A. L., Queiroz, M. J. R. P., Barros, L., & Ferreira, I. C. F. R. (2014). Effects of gamma radiation on chemical and antioxidant properties, anti-hepatocellular carcinoma activity and hepatotoxicity of borututu. *Innovative Food Science and Emerging Technologies*, 26, 271–277.
- Pereira, E., Pimenta, A. I., Calhelha, R. C., Antonio, A. L., Cabo Verde, S., Barros, L., et al. (2016). Effects of gamma irradiation on cytotoxicity and phenolic compounds of *Thymus vulgaris* L. and *Mentha x piperita* L. *LWT – Food Science and Technology*. <http://dx.doi.org/10.1016/j.lwt.2016.04.004>.
- Polovka, M., & Suhaj, M. (2010). The effect of irradiation and heat treatment on composition and antioxidant properties of culinary herbs and spices — A Review. *Food Reviews International*, 26, 138–161.
- Roberts, P. B. (2014). Food irradiation is safe: Half a century of studies. *Radiation Physics and Chemistry*, 105, 78–82.
- Sádecká, J. (2007). Irradiation of spices — A review. *Czech Journal of Food Sciences*, 25, 231–242.
- Singh, B., & Datta, P. S. (2010). Effect of low dose gamma irradiation on plant and grain nutrition of wheat. *Radiation Physics and Chemistry*, 79, 819–825.
- Taheri, S., Abdullah, T. L., Karimi, E., Oskoueian, E., & Ebrahimi, M. (2014). Antioxidant capacities and total phenolic contents enhancement with acute gamma irradiation in *Curcuma alismatifolia* (Zingiberaceae) leaves. *International Journal of Molecular Sciences*, 15, 13077–13090.
- Wolfe, K., Wu, X., & Liu, R. H. (2003). Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry*, 51, 609–614.
- Zhang, J.-Y., Wang, Y.-Z., Zhao, Y.-L., Yang, S.-B., Zuo, Z.-T., Yang, M.-Q., et al. (2011). Phytochemicals and bioactivities of Paris species. *Journal of Asian Natural Products Research*, 13, 670–681.