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Analytical Methods Applied to Assess the Effects of Gamma Irradiation on Color, Chemical Composition and Antioxidant Activity of *Ginkgo biloba* L

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Abstract The extracts from the leaves of *Ginkgo biloba* are widely used in medicines and food supplements in order to overcome different health problems. To provide decontamination, irradiation is a safe and effective technique, particularly suitable to be integrated in quality control of the postharvest samples. In this study, different analytical methods were applied to assess the effects of gamma irradiation (1 and 10 kGy) in *G. biloba* color, chemical composition and antioxidant properties. Irradiation preserved macronutrients, fatty acids, γ- and δ-tocopherols, fructose, trehalose, quinic and shikimic acids. In particular, 1 kGy protected α-tocopherol, oxalic and malic acids contents, while 10 kGy decreased α-tocopherol, glucose, sucrose, oxalic and malic acids level. Nevertheless, this dose was the most effective for antioxidant activity. Overall, 1 kGy would be the recommended dose to maintain nutritional profile of *G. biloba*, protect specific molecules and also increase antioxidant activity of infusion and methanolic extracts prepared from its leaves.

Keywords Gamma irradiation · *Ginkgo biloba* · Analytical methods · Chemical characterization · Antioxidant activity

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Introduction

Ginkgo (*Ginkgo biloba* L.) is a very old tree widely used in traditional medicine, with its leaf extracts considered one of the most widely sold natural products (Kato-Noguchi et al. 2013). Ginkgo leaf extracts have been extensively studied in humans and animal models. In medicinal applications, ginkgo leaf infusions are used for treatment of asthma, bronchitis, memory, cognitive speed, edema, inflammation, and free-radical toxicity associated with traumatic brain injury (Smith et al. 1996; Diamond et al. 2000). EGb 761 is a standardized extract of *G. biloba* leaves, that contains approximately 24 % flavone glycosides (primarily quercetin, kaempferol and isorhamnetin) and 6 % terpene lactones (2.8–3.4 % ginkgolides A, B and C, and 2.6–3.2 % bilobalide), and these have been used experimentally as natural therapeutic agents in the treatment of Alzheimer's disease (Smith et al. 1996; Diamond et al. 2000; Annaházi et al. 2010). In fact, this plant is widely used the by pharmaceutical industry, which incorporates the leaf extracts in supplements and medicines (Pereira et al. 2013).

Nevertheless, the drying of plants outdoors exposes them to a high level of natural contamination, which may lead to the presence of microorganisms of great relevance to public health, such as *Salmonella* spp., *Escherichia coli*, *Clostridium perfringens*, *Bacillus cereus* and molds (Sádecká 2007).

In the pharmaceutical industry, the use of materials of good microbiological quality is one of the essential requirements, since the microorganisms can contaminate the final product, and could lead to diseases and deteriorate medications (Rosa et al. 1995). This is also important in the food industry, where microbiological decontamination provides a product with higher shelf life, while maintaining its quality (Kamat et al. 2003).

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Irradiation is a promising method for microbial safety (Sádecká 2007; Yordanov et al. 2009); because it is a safe and effective method, it is particularly suitable to be integrated in a comprehensive approach in the quality control of biological materials. It is a physical process in which the high-energy ionizing radiation passes through the target product improving their safety by inactivating microorganisms and without leaving chemical residues (Katusin-Razem et al. 2001; Shim et al. 2009; Khattak and Simpson 2010). One of the advantages of this treatment is its versatility in controlling a variety of microorganisms and insects, as well as the fact that the dietary macronutrients (carbohydrates, proteins and lipids) and micronutrients (e.g., vitamins) are not significantly affected using adequate doses of irradiation (Sádecká 2007; Khattak and Simpson 2010). Nevertheless, the technique should be tested for each particular plant, and only limited studies for gamma irradiation effects in *G. biloba* were performed (Soriani et al. 2005).

Therefore, the aim of this study was to use different analytical methods to evaluate the effects of gamma irradiation in *G. biloba* color, chemical composition and antioxidant properties, since these are related to its use in Alzheimer's disease, as previously noted.

Materials and Methods

Samples and Samples Irradiation

G. biloba L. samples were provided by Américo Duarte Paixão Lda., in Alcanede (Portugal), imported from China, as dry leaves material for infusion preparation (the taxonomical identification of the plant species mentioned in the label was confirmed). The samples were divided into three groups: control (non-irradiated, 0 kGy), group 1 and group 2, where 1 and 10 kGy were, respectively, the predicted doses.

The irradiation was performed in a Co-60 experimental chamber (Precisa 22; Graviner Manufacturing Company Ltd., UK) with a total activity of 177 TBq (4.78 kCi), in September 2013, and the estimated dose rate for the irradiation position was obtained with a Fricke dosimeter. During irradiation process, the dose was estimated using Amber Perspex routine dosimeters (batch V, from Harwell Company, Oxfordshire, UK), following the procedure previously described by Fernandes et al. (2013). The estimated doses, dose rates and dose uniformity ratios (D_{max}/D_{min}) were, respectively: 1.20±0.07 kGy, 2.57±0.15 kGy h^{-1}, 1.20 for sample 1 and 8.93±0.14 kGy, 1.91±0.03 kGy h^{-1}, 1.02 for sample 2. For simplicity, in the text and tables, we considered the values 0, 1 and 10 kGy, for the doses of non-irradiated and irradiated groups 1 and 2, respectively.

After irradiation, the samples were reduced to powder and mixed to obtain homogenized samples for subsequent analysis.

Standards and Reagents

Irradiation

To estimate the dose and dose rate of irradiation it was used a chemical solution sensitive to ionizing radiation, Fricke dosimeter, prepared in the lab following the standards (ASTM American Society for Testing and Materials 1992) and Amber Perspex dosimeters (batch V; Harwell Company). 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) with purity PA (proanalysis), and water treated in a Milli-Q water purification system (model A10; Millipore, Billerica, MA, USA).

Chemical Analyses

Acetonitrile 99.9 %, *n*-hexane 95 % and ethyl acetate 99.8 % were of HPLC grade from Fisher Scientific (Lisbon, Portugal). Fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also were other individual fatty acid isomers, L-ascorbic acid, tocopherol, sugar and organic acid standards. Racemic tocol (50 mg/ml) was purchased from Matreya (Pleasant Gap, PA, USA).

Antioxidant Activity Evaluation

2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Sigma.

Color Measurement

A colorimeter (model CR-400; Konica Minolta Sensing, Inc., Japan), with an adapter for granular materials (model CR-A50) was used to measure the color of the samples. Using the illuminant C and diaphragm aperture of 8 mm, the CIE L^*, a^*, b^* color space values were registered using a data software "Spectra Magic Nx" (version CM-S100W 2.03.0006), from Konica Minolta Company (Japan). Before starting the measurements the instrument was calibrated against a standard white tile (Fernandes et al. 2012).

The color of three samples from each batch was measured in three different points, for each dose and at each time point, being considered the average value. The color difference or total color change for each sample was determined

using the three-dimensional color space coordinates: $\Delta E=(\Delta L^2+\Delta a^2+\Delta b^2)^{0.5}$.

Chemical Composition

Nutritional Value

Protein, fat, carbohydrates and ash were determined following the AOAC procedures (AOAC 1995). The samples crude protein content (N×6.25) was estimated by the macro-Kjeldahl method; the crude fat was determined using a Soxhlet apparatus by extracting a known weight of sample with petroleum ether; the ash content was determined by incineration at 600±15 °C. Total carbohydrates were calculated by difference and total energy was calculated according to the following equation: Energy (kcal)=4×(g protein+g carbohydrates)+9×(g fat).

Lipophilic Compounds

Fatty Acids

Fatty acids were determined after a transesterification procedure as described previously by the authors (Barros et al. 2013a), using a gas chromatographer (DANI 1000; Contone, Switzerland) equipped with a split/splitless injector and a flame ionization detector (GC-FID). Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using CSW 1.7 software (DataApex 1.7, Prague, Czech Republic). The results were expressed in relative percentage of each fatty acid.

Tocopherols

Tocopherols were determined following a procedure previously optimized and described by the authors (Barros et al. 2013a). Analysis was performed by high-performance liquid chromatography (HPLC) system consisted of an integrated system with a pump (Knauer, Smartline system 1000; Berlin, Germany), degasser system (Smartline manager 5000) and autosampler (AS-2057; Jasco, Easton, MD, USA), coupled to a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response of each standard, using the IS (tocol) method and by using calibration curves obtained from commercial standards of each compound. The results were expressed in mg per 100 g of dry weight.

Hydrophilic Compounds

Sugars

Free sugars were determined following a procedure previously optimized and described by the authors (Barros et al. 2013a). Analysis was performed by HPLC (equipment described above) coupled to a refraction index detector (RI detector Knauer Smartline 2300; Berlin, Germany). Sugars identification was made by comparing the relative retention times of sample peaks with standards. Data were analyzed using Clarity 2.4 Software (DataApex). Quantification was based on the RI signal response of each standard, using the internal standard (IS, raffinose) method and by using calibration curves obtained from commercial standards of each compound. The results were expressed in g per 100 g of dry weight.

Organic Acids

Organic acids were determined following a procedure previously optimized and described by the authors (Barros et al. 2013b). Analysis was performed by ultra fast liquid chromatograph (UFLC) coupled to photodiode array detector (PDA), using a Shimadzu 20A series UFLC (Shimadzu Corporation, Kyoto, Japan). Detection was carried out in a PDA, using 215 nm and 245 as preferred wavelengths. The organic acids were quantified by comparison of the area of their peaks recorded at 215 nm with calibration curves obtained from commercial standards of each compound. The results were expressed in g per 100 g of dry weight.

Evaluation of Antioxidant Activity

Preparation of the Extracts

Infusions Each sample (2 g) was added to 0.2 l of boiling distilled water, left to stand at room temperature for 5 min, and filtered through Whatman No. 4 paper.

Methanolic Extracts Each sample (1 g) were stirred with methanol (30 ml) at 25 °C at 150 rpm for 1 h and filtered through Whatman No. 4 paper. The residue was then extracted with an additional portion of methanol. The combined methanolic extracts were evaporated under reduced pressure (rotary evaporator Büchi R-210; Flawil, Switzerland).

The infusion and methanolic extract were redissolved in water and methanol, respectively (final concentration 10 mg/ml). The final solutions were further diluted to different concentrations to be submitted to antioxidant activity evaluation and the results were expressed in

Table 1 CIE color L^* (lightness), a^* (redness) and b^* (yellowness) of non-irradiated and irradiated *G. biloba* samples

	0 kGy	1 kGy	10 kGy
L^*	46.43±1.42[a]	46.15±2.71[a]	42.94±1.58[b]
b^*	22.58±2.17[a]	22.18±1.64[a]	20.52±2.03[a]
ΔE	51.90±1.55[a]	51.37±2.75[a]	49.14±1.92[b]

The results are presented as mean ± SD. In each row, different letters mean significant differences, $p<0.05$

The value of ΔE, total color, was determined using the expression:

$$\Delta E = \sqrt{(L*)^2 + (a*)^2 + (b*)^2}$$

EC_{50} values (sample concentration providing 50 % of antioxidant activity or 0.5 of absorbance in the reducing power assay). Trolox was used as positive control.

Antioxidant Activity In Vitro Assays DPPH radical-scavenging activity was evaluated by using an ELX800 microplate reader (Bio-Tek Instruments, Inc., Winooski, VT, USA), and calculated as a percentage of DPPH discoloration using the formula: $[(A_{DPPH}-A_S)/A_{DPPH}]\times 100$, where A_S is the absorbance of the solution containing the sample at 515 nm and A_{DPPH} is the absorbance of the DPPH solution. Reducing power was evaluated by the capacity to convert Fe^{3+} into Fe^{2+}, measuring the absorbance at 690 nm in the microplate reader mentioned above. Inhibition of β-carotene bleaching was evaluated though the β-carotene/linoleate assay; the neutralization of linoleate free radicals avoids β-carotene bleaching, which is measured by the formula: β-carotene absorbance after 2 h of assay/initial absorbance) × 100. Lipid peroxidation inhibition in porcine (*Sus scrofa*) brain homogenates was evaluated by the decreasing in thiobarbituric acid reactive substances (TBARS); the color intensity of the malondialdehyde–thiobarbituric acid (MDA–TBA) was measured by its absorbance at 532 nm; the inhibition ratio (%) was calculated using the following formula: $[(A-B)/A]\times 100$ %, where A and B are the absorbance of the control and the sample solution, respectively (Fernandes et al. 2013).

Table 2 Macronutrients and energetic value of non-irradiated and irradiated *G. biloba* samples (mean ± SD)

	0 kGy	1 kGy	10 kGy
Ash (g/100 g dw)	12.91±0.20[a]	12.74±0.65[a]	12.34±1.59[a]
Proteins (g/100 g dw)	15.32±0.16[a]	15.19±0.59[a]	12.79±0.15[b]
Fat (g/100 g dw)	4.42±0.13[a]	4.32±0.24[a]	4.56±0.42[a]
Carbohydrates (g/100 g dw)	67.36±0.28[b]	67.75±0.74[b]	70.31±0.88[a]
Energy (kcal/100 g dw)	370.44±0.09[a]	370.64±2.69[a]	373.44±5.98[a]

In each row, different letters mean significant differences ($p<0.05$)

dw dry weight

Statistical Analysis

Three samples from each group were analysed and all the assays were carried out in triplicate. The results are

Table 3 Lipophilic compounds (fatty acids and tocopherols) of non-irradiated and irradiated *G. biloba* samples (mean ± SD) Caproic acid (C6:0); caprylic acid (C8:0); capric acid (C10:0); undecanoic acid (C12:0); tridecanoic acid (C13:0); myristic acid (C14:0); myristoleic acid (C14:1); pentadecanoic acid (C15:0); *cis*-10-pentadecenoic acid (C15:1); palmitic acid (C16:0); palmitoleic acid (C16:1); heptadecanoic acid (C17:0); stearic acid (C18:0); oleic acid (C18:1n9); linoleic acid (C18:2n6); α-linolenic acid (C18:3n3); stearic acid (20:0); eicosenoic acid (C20:1); *cis*-11,14,17-eicosatrienoic acid and heneicosanoic acid (C20:3n3+C21:0); behenic acid (C22:0); tricosanoic acid (C23:0); lignoceric acid (C24:0). In each row, different letters mean significant differences ($p<0.05$)

	0 kGy	1 kGy	10 kGy
C6:0	0.11±0.01	0.17±0.01	0.14±0.03
C8:0	0.14±0.02	0.32±0.01	0.19±0.04
C10:0	0.15±0.03	0.13±0.02	0.18±0.02
C12:0	0.95±0.06	0.88±0.06	1.09±0.14
C13:0	0.21±0.01	0.25±0.01	0.25±0.03
C14:0	9.58±0.08	9.10±0.48	10.19±1.00
C14:1	3.34±0.21	3.17±0.21	3.41±0.19
C15:0	0.52±0.05	0.67±0.03	1.01±0.10
C15:1	0.09±0.00	0.07±0.00	0.10±0.00
C16:0	24.84±0.51	23.50±0.66	25.15±0.57
C16:1	0.90±0.05	1.00±0.03	0.92±0.09
C17:0	0.85±0.03	0.85±0.02	0.90±0.03
C18:0	2.66±0.06	2.41±0.05	2.45±0.04
C18:1n9	7.03±0.06	6.74±0.06	6.66±0.23
C18:2n6	7.94±0.54	8.21±0.47	7.73±0.21
C18:3n3	28.64±2.12	31.63±0.87	28.85±2.31
C20:0	3.63±0.07	2.56±0.04	2.65±0.12
C20:1	0.19±0.02	0.17±0.01	0.27±0.03
C20:3n3+C21:0	1.21±0.01	1.48±0.06	1.29±0.23
C22:0	2.25±0.26	2.34±0.03	2.22±0.11
C23:0	0.87±0.01	0.82±0.06	0.71±0.02
C24:0	3.90±0.20	3.54±0.13	3.65±0.01
Total SFA (relative %)	50.65±2.83[a]	47.54±1.13[a]	50.79±2.24[a]
Total MUFA (relative %)	11.56±0.16[a]	11.16±0.15[a]	11.35±0.51[a]
Total PUFA (relative %)	37.79±2.67[a]	41.31±1.29[a]	37.86±2.74[a]
α-Tocopherol	58.77±0.74[b]	61.18±0.15[a]	52.64±0.92[c]
β-Tocopherol	28.96±0.74[b]	29.59±0.62[ab]	30.30±0.59[a]
γ-Tocopherol	0.92±0.02[a]	0.98±0.13[a]	0.95±0.01[a]
δ-Tocopherol	0.60±0.04[a]	0.54±0.03[a]	0.54±0.04[a]
Total tocopherols (mg/100 g)	89.25±1.53[b]	92.29±0.86[a]	84.43±1.56[c]

expressed as mean values and standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test with α=0.05. This treatment was carried out using SPSS v. 22.0 program (IBM Corp.).

Results and Discussion

Color

The results of CIE color *L** (lightness), *a** (redness) and *b** (yellowness) of non-irradiated and irradiated *G. biloba* samples are presented in Table 1. It seems that for the highest dose, 10 kGy, there was a tendency for the samples to loose lightness, the *L** value diminishes, when compared to non-irradiated (0 kGy) and irradiated samples (1 kGy). For *b** value, which represents the yellowness–blueness tendency, no significant difference was observed with irradiation dose. Data values for *a** parameter are close to zero (data not shown). Color is a parameter of great importance not only for plants but also for other foods. For example, the cosmetics industry has a very stringent selection of the plants color level; the dark color of some natural matrices such as green tea or persimmon leaf makes very difficult their application in food or cosmetic products, and the process to remove

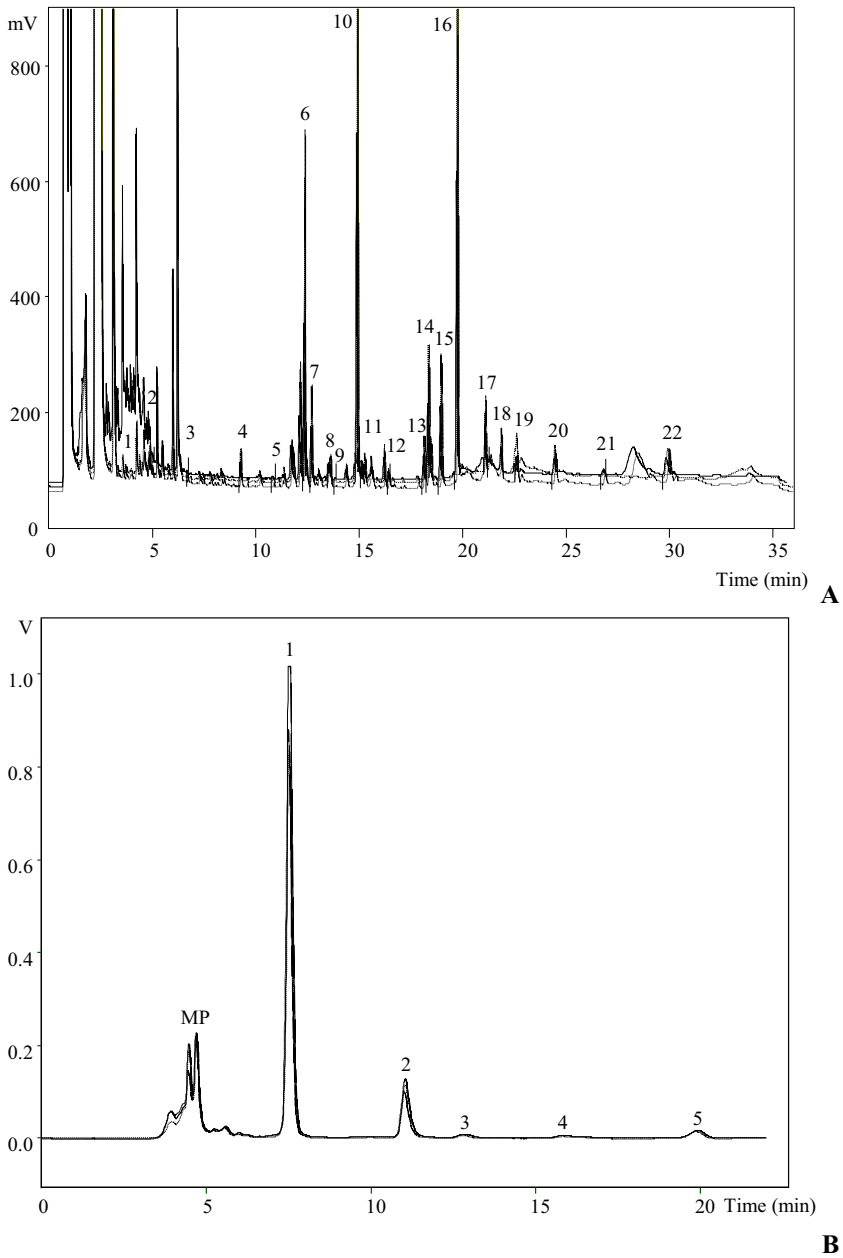


Fig. 1 Individual profile of 0 kGy (*dotted line*), 1 kGy (*solid line*) and 10 kGy (*broken line*) samples in **a** fatty acids: *1* C6:0, *2* C8:0, *3* C10:0, *4* C12:0, *5* C13:0, *6* C14:0, *7* C14:1, *8* C15:0, *9* C15:1, *10* C16:0, *11* C16:1, *12* C17:0, *13* C18:0, *14* C18:1n9, *15* C18:2n6, *16* C18:3n3, *17* 20:0, *18* C20:1, *19* C20:3n3+C21:0, *20* C22:0, *21* C23, *22* C24:0. **b** Tocopherols: *1* α-tocopherol, *2* β-tocopherol, *3* γ-tocopherol, *4* δ-tocopherol, *5* tocol (IS). *MP* mobile phase

color is a difficult, time-consuming, and costly procedure (Jo et al. 2003a,b).

Nutritional Profile

The nutritional profile of non-irradiated and irradiated *G. biloba* samples is shown in Table 2. The control sample showed values very similar to the ones obtained previously by the authors in a sample from different commercial origin (Pereira et al. 2013). Gamma irradiation did not alter significantly the nutritional profile of the samples, regarding ash, fat and energy since similar values were obtained in the control and in samples irradiated with 1 or 10 kGy, being in agreement with previous studies in other foods, such as hazelnuts, walnuts, almonds, and pistachios (Gecgel et al. 2011). Nevertheless, 10 kGy led to a decrease in proteins content (the same was observed in the study of Fernandes et al. 2013 on wild mushrooms) and, consequently, an increase in carbohydrates level (which were determined by difference). Protein values have been previously reported as having no significant changes after irradiation treatments (Fernandes et al. 2012; Kasera et al. 2012; Fernandes et al. 2014). The decrease of protein levels at 10 kGy could be explained by a possible degradation due to the high intensity applied. Proteins are known to be the most reliable irradiation indicators, especially due to degradation reactions such as scission of the C–N bonds in the backbone of the polypeptide chain or splitting of the disulfide bonds, and physical changes like unfolding and aggregation (Molins 2001). Nevertheless, the fact that irradiation induces alterations in the protein content, does not mean a significant problem in the nutritional point of view, since protected amino acids within the structure of the protein complex generally resists to this method (Kausar et al. 2013).

Composition in Lipophilic Compounds

Regarding fatty acids, 22 different molecules were identified (Table 3), which is in accordance with a previous study of the authors (Pereira et al. 2013). Irradiated and control samples revealed the same fatty acids profile, with α-linolenic acid as the major compound, followed by palmitic acid. Some studies showed that the lack of α-linolenic acid in the diet compromises the brain and heart function (Taha et al. 2006; Nguemeni et al. 2013), and therefore, it is important to preserve this and other compounds in irradiated samples. In all the samples, saturated fatty acids appeared in higher concentrations, followed by polyunsaturated and lastly monounsaturated fatty acids. No significant differences were observed between the control and the irradiated samples at two different doses (Table 3; Fig. 1a). Similar results were reported for cashew nuts (Mexis and Kontominas 2009) and lamb meat (Alfaia et al. 2007), where no significant differences were observed in the concentration of SFA, MUFA and PUFA.

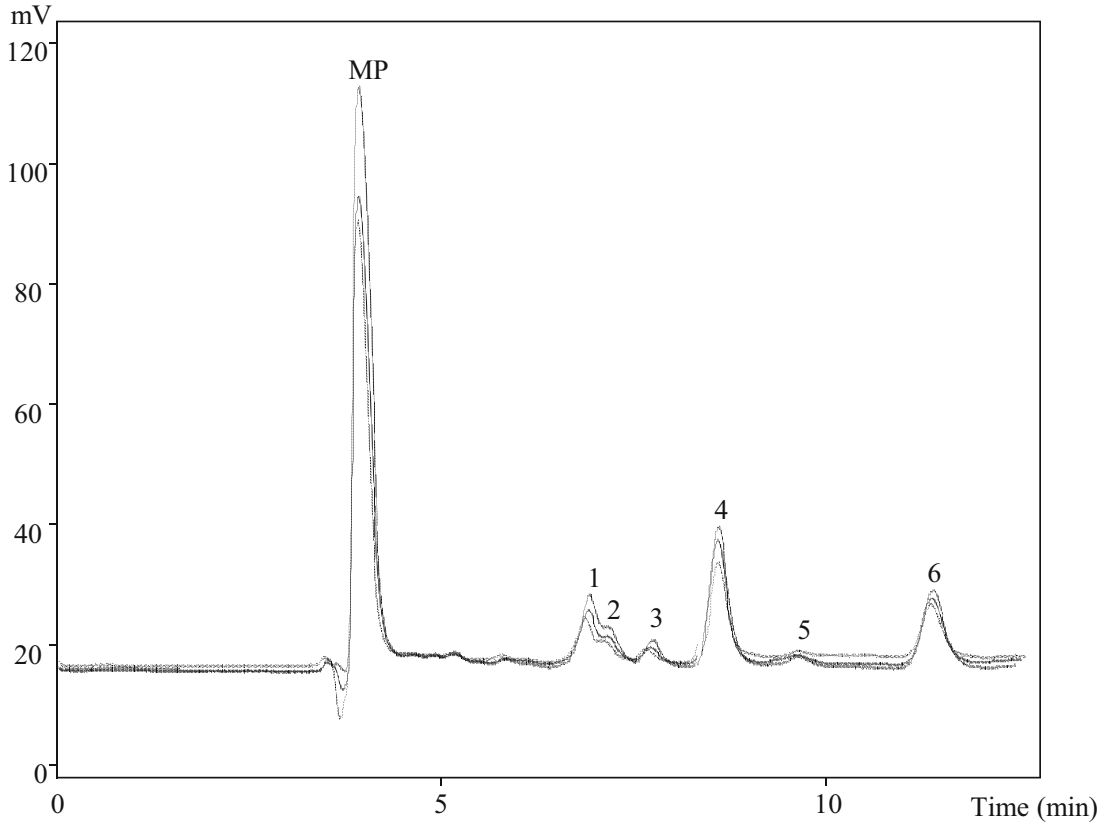
Table 4 Hydrophilic compounds (sugars and organic acids) of non-irradiated and irradiated *G. biloba* samples (mean ± SD) In each row, different letters mean significant differences ($p<0.05$)

Free sugars (g/100 g dw)	0 kGy	1 kGy	10 kGy
Fructose	1.86±0.12^{a}	1.87±0.17^{a}	1.81±0.01^{a}
Glucose	0.98±0.07^{a}	0.73±0.03^{b}	0.79±0.04^{b}
Sucrose	3.78±0.09^{a}	3.83±0.11^{a}	3.60±0.07^{b}
Threalose	0.38±0.04^{a}	0.41±0.00^{a}	0.40±0.02^{a}
Unknown	0.55±0.03^{a}	0.51±0.04^{a}	0.50±0.05^{a}
Total	7.55±0.07^{a}	7.35±0.34^{ab}	7.10±0.04^{b}
Organic acids (g/100 g)	0 kGy	1 kGy	10 kGy
Oxalic	0.82±0.00^{b}	0.89±0.00^{a}	0.80±0.00^{c}
Quinic	1.37±0.09^{a}	1.31±0.01^{a}	1.33±0.01^{a}
Malic	1.09±0.00^{b}	1.21±0.02^{a}	1.05±0.01^{c}
Shikimic	1.49±0.09^{a}	1.43±0.00^{a}	1.42±0.01^{a}
Total	4.78±0.17^{ab}	4.83±0.01^{a}	4.60±0.03^{b}

dw dry weight

Data concerning tocopherols (also lipophilic compounds) concentration are given in Table 3. The four vitamers were found in all the analyzed samples of *G. biloba*, with α-tocopherol as predominant form, as also stated by the authors in a sample from different commercial origin previously studied (Pereira et al. 2013), despite significant differences observed in the concentrations reported. In fact, tocopherols are very sensitive molecules that suffer rapid variation due to oxidation processes (Birringer et al. 2001; Luo et al. 2011). α-Tocopherol was the most susceptible isoform to irradiation process, decreasing with 10 kGy (Fig. 1b). Nevertheless, it should be pointed out that 1 kGy of irradiation dose protected degradation of this vitamer (Fig. 1b) (the same happened with the same irradiation dose, in a study with *Carya illinoensis* (Taipina et al. 2009)), which is very important as α-tocopherol holds several beneficial functions for humans, including antioxidant, anti-inflammatory, anti-carcinogenic and antiatherogenic properties (Manosso et al. 2013). In other studies performed in plants (sage, thyme, and oregano) irradiated with 10 kGy, there were no significant differences in the content of α- and γ-

Fig. 2 Individual profile of 0 kGy (*dotted line*), 1 kGy (*solid line*) and 10 kGy (*broken line*) samples in **a** sugars: *1* fructose, *2* glucose, *3* unknown, *4* sucrose, *5* trehalose, *6* melezitose (IS). **b** Organic acids: *1* oxalic acid, *2* quinic acid, *3* malic acid, *4* shikimic acid. *MP* mobile phase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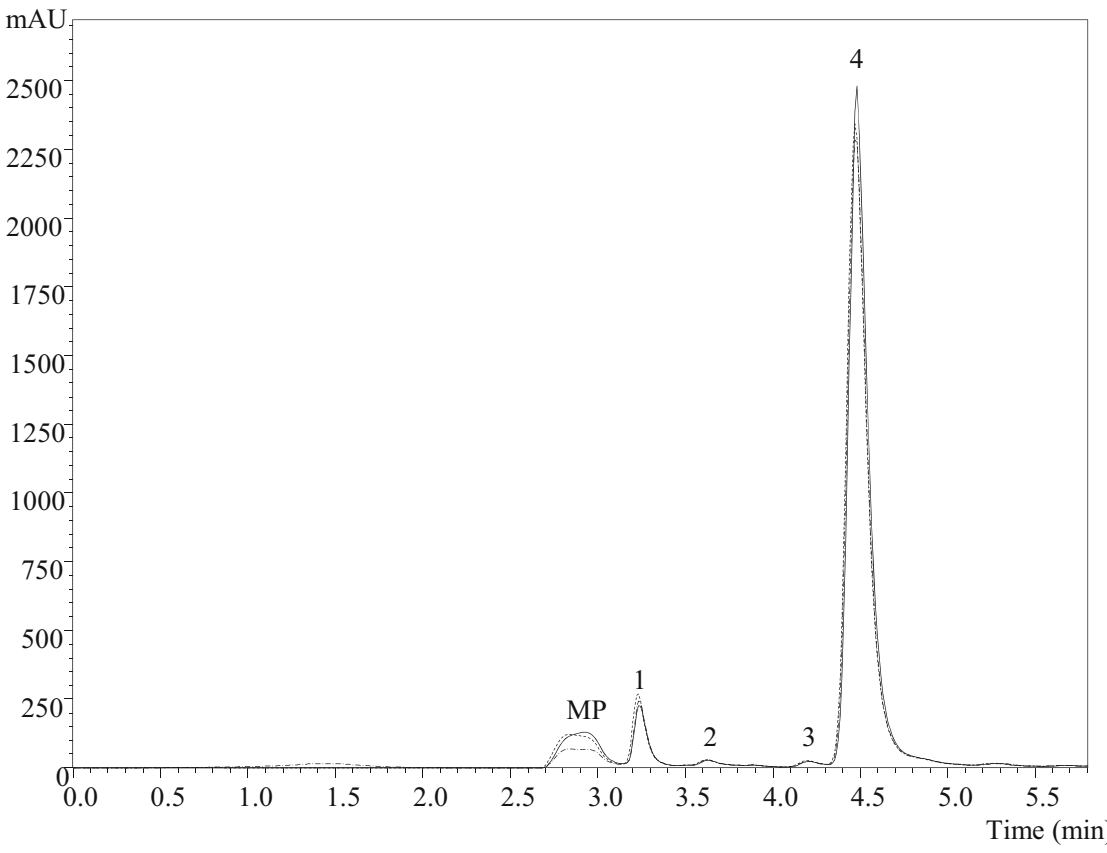
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Time (min)

B

Table 5 Antioxidant activity (EC_{50} values, mg/ml) of infusions and methanolic extracts obtained from non-irradiated and irradiated *G. biloba* samples (mean ± SD)

Antioxidant activity	Infusion			Methanolic extract		
	0 kGy	1 kGy	10 kGy	0 kGy	1 kGy	10 kGy
DPPH scavenging activity	5.80±0.24^{a}	4.09±0.07^{b}	2.88±0.23^{c}	1.64±0.02^{a}	1.54±0.05^{ab}	1.49±0.16^{b}
Reducing power	4.58±0.06^{a}	3.41±0.01^{b}	2.37±0.02^{c}	0.65±0.00^{a}	0.63±0.00^{b}	0.49±0.00^{c}
β-Carotene bleaching inhibition	11.09±0.54^{a}	9.04±0.35^{b}	8.79±0.23^{b}	10.39±0.66^{a}	5.26±0.18^{b}	4.48±0.17^{c}
TBARS inhibition	0.15±0.01^{a}	0.13±0.01^{b}	0.10±0.01^{c}	0.24±0.01^{a}	0.16±0.03^{b}	0.08±0.00^{c}

tocopherol in control and irradiated samples (Brandstetter et al. 2009).

Composition in Hydrophilic Compounds

The composition in hydrophilic compounds was also assessed and the results are shown in Table 4. Fructose, glucose, sucrose and trehalose were identified and quantified in the samples, but with a slight different profile in relation to the one described for other samples of *G. biloba*, in which trehalose was not found (Pereira et al. 2013). In fact, sugar concentration depends on the maturity stage of the sample leaves and other environmental factors that influence the use of these primary metabolites for energy production (Apone et al. 2010). There was an observed decrease in glucose in both irradiated samples, while no significant differences were observed in regard to fructose and trehalose levels, and sucrose (the most abundant sugar) decreased only with 10 kGy of gamma radiation dose (Fig. 2a). Another study attributed the observed increase in sugar levels to a degradation of polysaccharides with the application of gamma irradiation (Kausar et al. 2013); in the present study, this did not occur.

Regarding organic acids (Table 4), oxalic, quinic, malic and shiquimic acids were identified and quantified in all the analyzed samples, which is in agreement with results reported by Pereira et al. (2013). Quinic and shikimic acids concentration was similar in all samples, which shows that gamma irradiation does not affect significantly these compounds. On the other hand, irradiation at 1 kGy protected oxalic and malic acids (higher values), while 10 kGy decreased their concentration (Fig. 2b). The decrease could be explain by a degradation process when 10 kGy is applied, being this doses much higher than 1 kGy, that showed a protective effect, maintaining the content found. According to a study performed by Wen et al. (2006) in irradiated lycium fruit, the concentration of malic and oxalic acids did not change significantly.

Antioxidant Properties

The results of antioxidant properties of infusions and methanolic extracts prepared form non-irradiated and irradiated samples, measured by four in vitro assays, are presented in Table 5. In general, methanolic extracts gave higher antioxidante activity (lower EC_{50} values) than the corresponding infusions (EC_{50} values ranging from 0.24 and 4.48 mg/ml when compared to the infusion 0.13–9.04 mg/ml), which is in agreement with results reported by Pereira et al. (2013). These results are also consistent with a previous study, where the alcoholic extracts showed better results than the corresponding infusions prepared from irradiated Korean medicinal plants (Byun et al. 1999).

For both infusion and methanolic extract, gamma irradiation at both doses increased DPPH scavenging activity, reducing power, β-carotene bleaching and lipid peroxidation inhibition of Ginkgo samples. In general, gamma irradiation at 10 kGy promotes more the antioxidant potential of *G. biloba* infusion and methanolic extract. This is in agreement with the results reported by the research group in a previous study with *Castanea sativa* fruits and skins (Antonio et al. 2011). Khattak et al. (2008) also reported an increase in DPPH scavenging properties of *Nigella sativa* seeds irradiated at 16 kGy).

The analytical methods used proved that irradiation can be a good alternative for *G. biloba* preservation since it maintained macronutrients, fatty acids, γ- and δ-tocpherols, fructose, trehalose, quinic and shikimic acids. Furthermore, 1 kGy protected α-tocopherol, oxalic and malic acids contents, while 10 kGy decreased α-tocopherol, glucose, sucrose, oxalic and malic acids level. Therefore, 1 kGy would be the recommended dose since it maintained the nutritional profile of *G. biloba*, protected specific molecules and increased antioxidant activity of infusion and methanolic extracts prepared from its leaves.

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Conflict of Interest Eliana Pereira declares that she has no conflict of interest. Lillian Barros declares that she has no conflict of interest. Amilcar L. Antonio declares that he has no conflict of interest. Albino Bento declares that he has no conflict of interest. Isabel C.F.R. Ferreira declares that she has no conflict of interest. This article does not contain any studies with human or animal subjects.

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