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# Phytochemical characterization and bioactive properties of strawberries treated by gamma radiation

Salma Barkaoui[1,2,3] · Joana Madureira[3,5] · Maria Inês Dias[4] · Lillian Barros[4] · Isabel C. F. R. Ferreira[4] · Fernanda M. A. Margaça[3,5] · Nourhène Boudhrioua[2] · Sandra Cabo Verde[3,5]

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**Abstract**
Strawberry is one of the most consumed berries worldwide and the compounds on its extracts present numerous health benefits, including antioxidant potential. In this work, the effects of processing strawberries by gamma radiation at 2 kGy and 7 days storage at 4 °C were evaluated regarding their phenolic profile and bioactive properties. From the HPLC–DAD-ESI/MS analysis, pelargonidin-3-*O*-glucoside was the main compound identified in extracts from non-stored and non-irradiated strawberries (1040 ± 53 mg/100 g dry weight). Gamma radiation and refrigerated storage preserved or improved the antioxidant activity of the strawberry extracts, while keeping the phenolic and ascorbic acid contents. Furthermore, all strawberry extracts exhibited high α-glucosidase inhibition, whereas α-amylase inhibition seemed to be enhanced by gamma radiation treatment at 2 kGy. These findings can provide useful information to develop new ingredients from irradiated fruits to be used by industries, with high added value especially as natural and promising antidiabetic agents.

**Keywords** Food irradiation · Strawberry · Phenolic compounds · Antioxidant capacity · Antidiabetic activity · Antimicrobial activity

## Introduction

Strawberry (*Fragaria x ananassa* Dutch.) is one of the most consumed berries across the world due to its unique flavor [1]. The high nutritional value of strawberry has been linked to its high levels of micronutrients, such as vitamin C and folate, as well as phenolic compounds including flavonoids, hydrolysable and condensed tannins and phenolic acids [2–5], which have promising benefits for human health [6]. In fact, these compounds demonstrated their potential in preventing hyperglycemia and hypertension linked to type 2 diabetes and cancer, cardiovascular, neurological and neurodegenerative diseases [5, 7–10]. Agarwal et al. [10] and Miller, Thangthaeng, Rutledge, Scott, & Shukitt-Hale [11] reported that the consumption of strawberries could improve the cognitive functions and reduce the risk of Parkinson and Alzheimer diseases due to the neuroprotective action of flavonoids contained in these fruits [10, 12]. In addition, the ellagitannins purified from strawberries presented high α-amylase inhibitory activity [5] and the anthocyanins reduced the coronary heart disease [7]. Mandave et al. [13] also demonstrated the protective and therapeutic effects of strawberry extracts against liver and kidney injuries.

Sandra Cabo Verde
sandracv@ctn.tecnico.ulisboa.pt

[1] Higher School of Food Industries of Tunis (ESIAT), University of Carthage, 58 Avenue Alain Savary, 1003 Tunis El Khadra, Tunisia

[2] Laboratory of Physiopathology, Food and Biomolecules, LR17ES03, Institut Supérieur de Biotechnologie de Sidi Thabet, Université de La Manouba, BP-66, 2020 Ariana-Tunis, Tunisia

[3] Centro de Ciências e Tecnologias Nucleares (C2TN), Instituto Superior Técnico, Universidade de Lisboa, E.N. 10 Ao Km 139.7, 2695-066 Bobadela LRS, Portugal

[4] Centro de Investigação de Montanha (CIMO), LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

[5] Departamento de Engenharia e Ciências Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10, Ao Km 139.7, 2695-066 Loures, Portugal

However, in spite of its richness in micronutrients, strawberry is a highly perishable fruit with a shelf life of two or three days at room temperature, being prone to post-harvest rot due to their high respiration rate and microbial growth [14]. Pre-harvest treatments can be applied to extend the shelf life of strawberries and enhance their adaptability to post-harvest processes, as highlighted in a recent review [15]. In order to reduce the post-harvest losses and increase the fresh produce shelf life, various preservation treatments including dehydration, refrigeration and freezing, modified atmosphere packaging, vacuum packaging, heat treatment, ultraviolet radiation, ionizing radiation and high hydrostatic pressure have been employed and were recently discussed by the authors [16]. Ionizing radiation is a suitable technology with a potential use for extending the shelf life of fresh produce and eliminating potential pathogenic microorganisms without dramatically changing its sensory quality [17–19]. In fact, food irradiation was developed during the second part of the twentieth century as an scientifically approved technology and a safe food process [20]. In this minimally processing technology, food is exposed to ionizing radiation, such as gamma rays from radioisotopes $^{60}$Co or less frequently $^{137}$Cs, machine-produced X-rays ("Bremsstrahlung") with a maximum energy of 5 MeV, or accelerated electrons with a maximum energy of 10 MeV [21]. Compared to accelerated electrons, electromagnetic radiation from the radioisotopes has deeper penetration. It is established that none of these energy sources induce radioactivity in the food and that the food can be treated in its final packaging, avoiding recontamination handling the products and making it immediately available for distribution to food suppliers [22]. As mentioned above, this treatment demonstrated to have several scientifically and technically feasible uses, such as the enhancement of microbiological safety and/or storage stability of foods [23]. Several studies reported the preservation of strawberries using gamma radiation. Barkaoui et al. [24] indicated that gamma radiation could extend the shelf life of strawberries and ensure their quality for 14 days at 4 °C, with a significant decrease in the proliferation of total microbiota. Also an improvement on phenolic and flavonoid content as well as on antioxidant activity of the extracts from 2 kGy irradiated strawberries was found after at least 7 days of refrigerated storage [24]. Furthermore, a decrease in vitamin C was observed at 2 kGy, with no anti-proliferative effect on tumor and non-tumor cell lines [17]. Regarding the sensory analysis, the strawberries irradiated at 2 kGy were the blind samples preferred by the evaluation panel [24]. When strawberries are stored at 10 °C, gamma radiation at low doses (600 Gy) could increase phenolic content and antioxidant activity while reducing the ascorbic acid content with no change in fruit acidity [25]. According to Majeed et al. [26], radiation doses of 1 and 1.5 kGy can be used to extend strawberry shelf life and cause only minor weight loss and decay.

The aim of this study was to assess the effects of gamma radiation at 2 kGy on the bioactive content (total phenolic and total flavonoid contents, and ascorbic acid content), and antioxidant, antidiabetic and antimicrobial activities of strawberries during refrigerated storage for 7 days at 4 °C. The radiation dose and storage period were selected based on the results previously obtained by the authors [24], which indicated that an absorbed dose of 2 kGy could be suitable to ensure the safety of strawberries and extend their shelf life by at least 7 days, enhancing their sensory quality and bioactive content. The identification and quantification of anthocyanins and other phenolic compounds was performed for all the extracts from irradiated and non-irradiated samples, as well as an evaluation of their antidiabetic activity. To our knowledge, this is the first study reporting the antidiabetic activity of the extracts from irradiated strawberries and the effect of gamma radiation on the concentration of individual phenolic compounds. The results obtained from this work will increase the awareness of consumers and food industry about the safety and quality of fruits treated by irradiation, increasing the acceptance of these products. The outputs of this study will also contribute to leverage the use of extracts from irradiated fruits as a natural resource of bioactive compounds for health promotion.

## Materials and methods

### Sampling and irradiation experiments

Strawberries (*Fragaria* x *ananassa* Duch) cv. "Monterey" were purchased in August 2021 from a local supermarket in Lisbon, Portugal, and immediately stored at 4 ± 1 °C until analysis.

Irradiation experiments were carried out in a cobalt-60 experimental chamber (model Precisa 22, Graviner Lda, UK, 1971, with four radioactive sources with a total activity of 1.67 kCi (62 TBq, August 2021) located at Instalação de Radiações IonizanteS (IRIS) from Centro de Ciências e Tecnologias Nucleares (C$^{2}$TN) of Instituto Superior Técnico, Universidade de Lisboa. Fresh strawberries (500 g) in a transparent PET box were irradiated at room temperature at an absorbed dose of 2.3 kGy (for simplicity, the absorbed dose will be mentioned as 2 kGy). The absorbed dose was estimated using calibrated Amber Perspex dosimeters (Harwell Dosimeters, United Kingdom) [27] with a dose uniformity (DUR) of 1.02. Non-irradiated samples (0 kGy) were used as control and followed all the experiments.

## Storage

All the experiments were carried out immediately after irradiation (0 day of storage; T0) and after 7 days (T7) of refrigerated storage and irradiation at 2 kGy. As mentioned above, the previous results obtained by the authors [24] suggested that this absorbed dose could be appropriate to ensure the safety of strawberries and extend their shelf life by at least 7 days, and therefore these conditions were selected to continue the evaluation and understand the effect of gamma radiation on bioactive properties of strawberry extracts.

## Phytochemical characterization

After gamma irradiation, the strawberries were manually mashed and lyophilized (Heto CD8, Allerod, Denmark) in the dark. The extractions of phenolic compounds from irradiated and non-irradiated strawberries were performed according to the following methodologies.

### Non-anthocyanin compounds

The strawberry extracts of non-anthocyanin compounds were prepared in triplicate by a solid–liquid extraction as previously described by Barkaoui et al. [17], using ethanol/water solution (80:20, v/v) as solvent. In the end of extractions, the ethanol was evaporated in a rotary evaporator Büchi R-210 (Flawil, Switzerland) and then the aqueous phase was lyophilized to obtain the dry extracts.

For the compounds identification, the extracts were dissolved in ethanol:water (20:80, v/v) (10 mg/mL) and filtered through a 0.22 µm syringe filter. The phenolic composition was analysed by high performance liquid chromatography (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA) coupled to a diode array detector (280, 330 and 370 nm wavelengths) and electrospray ionization mass spectrometry (HPLC-DAD-ESI/MS), using the analytical conditions described by Bessada, Barreira, Barros, Ferreira, & Oliveira [28].

### Anthocyanin compounds

The lyophilized samples of strawberries (1 g) were extracted with 30 mL of an ethanol:water solution (80:20 v/v) acidified with 0.1% citric acid, according to the methodology described by Albuquerque et al. [29]. The final lyophilized extracts were re-dissolved in 2 mL of ethanol:water (20:80 v/v) (10 mg/mL) and filtered through a 0.22 µm syringe filter into an amber vial for HPLC analysis. The analysis was carried out by HPLC–DAD-ESI/MS using a detection wavelength of 520 nm and operating under the conditions previously described [30].

### Identification and quantification

Data were collected and analysed using the Xcalibur® program (ThermoFinnigan, San Jose, CA, USA). The identification of the phenolic compounds (non-anthocyanins and anthocyanins) was performed using standard compounds, when available, by comparing their retention times, UV–vis and mass spectra. Also, it can be achieved comparing the obtained information with available data in the literature giving a tentative identification. For quantitative analysis, a calibration curve for each available phenolic standard was constructed based on the UV signal. For the identified phenolic compounds for which a commercial standard was not available, the quantification was performed from the calibration curve of the most similar standard. The results were expressed in mg per 100 g of strawberry dry weight (dw).

### Total phenolic content

The Total Phenolic Content (TPC) was determined by Folin–Ciocalteau method [31], using extract solutions concentrated at 5 mg/mL. TPC results was determined by measuring the absorption at 765 nm (Barkaoui et al., 2020). The assay was carried out in triplicate and the results were expressed as mg of gallic acid equivalents (GAE) per 100 g of strawberry dry weight (dw).

### Total flavonoid content

The Total Flavonoid Content (TFC) was determined using the Aluminum Chloride Colorimetric method as described by [24], with extract solutions concentrated at 5 mg/mL. The absorbance was measured at 510 nm and the results were expressed as mg of catechin equivalents (CAE) per 100 g strawberry dry weight (dw). The assay was made in triplicate.

### Ascorbic acid content

L-ascorbic acid content was determined by High Performance Liquid Chromatography (HPLC) (Prominence CBM 20-A, Shimadzu, Japan) with UV-DAD detector, following the procedure described by Barkaoui et al. (2020). The assay was performed in triplicate. For quantification purposes, a calibration plot was performed under the experimental conditions used. Results were expressed as mg per 100 g of strawberries dry weight (dw).

## Evaluation of the bioactive properties

### Antioxidant activity

Antioxidant activity was evaluated using two assays: DPPH radical scavenging activity [32] with some modifications, and Ferric Reducing Antioxidant Power (FRAP) [33], as previously described [17]. For FRAP assay, the extracts were dissolved in distilled water at a concentration of 1.25 mg/mL while for DPPH the extracts were dissolved in distilled water at a concentration of 10 mg/mL and then successive dilutions were prepared (5–0.078 mg/mL). The results of FRAP were expressed as mmol of ferrous sulfate equivalent (FSE) per 100 g strawberry dry weight (dw) and DPPH in terms of $IC_{50}$ values (µg/mL), indicating the extract concentrations that provided 50% of antioxidant activity [17]. Both assays were carried out in triplicate.

### Antimicrobial activity

The antimicrobial activity was assessed against three Gram-negative bacteria: *Escherichia coli* (ATCC 8739TM), *Pseudomonas fluorescens* (ATCC 13525TM) and *Salmonella enterica* serotype Typhimurium (ATCC 14028TM), three Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538TM), *Bacillus cereus* (SSI C1/1) and *Listeria monocytogenes* (ATCC 19111TM) and a yeast: *Candida albicans* (ATCC 10231™). The minimum inhibitory (MIC) and minimum microbicidal (MBC) concentrations were determined by the microdilution method [34], according to the procedure previously described by Madureira et al. [35]. Samples were tested in triplicate and each experiment was repeated three times.

### Antidiabetic activity

The antidiabetic activity of strawberry extracts from non-irradiated and irradiated samples was assessed using two different enzymes: α-amylase and α-glycosidase, following the procedures described by Madureira et al. [35]. For both assays, different concentrations of extract solutions (10–1 mg/mL) were prepared in 0.1 M phosphate-buffered saline (PBS, pH 6.9)). The results were expressed as $IC_{50}$ values (µg/mL) and acarbose was used as the positive control. The assays were performed in triplicate.

## Statistical analysis

SPSS 22.0 software (SPSS Inc., Chicago, USA) was used for data analysis. Confidence intervals for means values were estimated considering a significance level of $p<0.05$ and the number of replicates for each assay. The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test with $\alpha=0.05$.

# Results and discussion

## Chemical characterization

### Anthocyanin and non-anthocyanin compounds

Phenolic compounds are well recognized as important contributors for the nutritional and sensory quality of the fruits, as well as their health benefits for human and animal. The phenolic compounds present in strawberry extracts were characterized and tentatively identified by their UV–vis and MS spectra as well as by the comparison with literature (Table 1). An illustrative phenolic profile of the hydroethanolic extracts of strawberry fruits non-irradiated and irradiated at 2 kGy and stored for 7 days at refrigerated temperatures is presented in Fig. 1. Thirteen phenolic compounds were identified in all the samples (Table 1), namely ten flavonoids including four anthocyanins, two hydroxycinnamic acid derivatives and one ellagitannin.

According to their retention time, mass and UV–Vis characteristics and by comparison with the commercial standards, the anthocyanins identified in all the extracts were cyanidin-3-*O*-glucoside (peak 1), pelargonidin-3-*O*-glucoside (peak 2), pelargonidin-3-*O*-rutinoside (peak 3) and pelargonidin-3-*O*-acetylglucoside (peak 4). All of these anthocyanins were previously described in strawberry extracts [3, 36–38]. Also (+)-catechin (peak 6) was identified in all the samples, which displayed a pseudomolecular ion $[M-H]^{-}$ at m/z 289 and characteristic fragmentation at m/z 245 (loss of $CO_2$), 205, 151 and 137. This compound was previously reported in strawberry extracts [3, 37]. Peak 5 had $[M-H]^{-}$ at m/z 577 and $MS^2$ fragmentation patterns coherent with B-type (epi)catechin dimers with m/z at 451 (−126 mu), 425 (−152 mu) and 407 (-18 mu). These fragment ions could be attributed to the heterocyclic ring fission (HRF) reaction, retro-Diels–Alder (RDA) and further loss of water from (epi)catechin unit, respectively, as described by Gu et al. [39]. The other fragment ions at m/z 289 and 287 could be ascribed as the lower and upper (epi)catechin unit, respectively [40]. The comparison of its retention time (6.51 min) with the literature allowed to identify this compound as the procyanidin dimer B1 (epicatechin-4,8-catechin) [40]. Peak 12 and peak 14 exhibited $[M-H]^{-}$ at m/z 609 and 477, respectively, with fragment ions at m/z 301, which indicated that these compounds were originated from quercetin. Based on the information reported in the literature, these compounds were characterized as quercetin-3-*O*-rutinoside and quercetin-*O*-hexurunoside [38]. Two

**Table 1** Chromatographic and mass spectral characteristics, tentative identification and quantification of phenolic compounds in extracts from irradiated (2 kGy) and non-irradiated (0 kGy) strawberry fruits before and after 7 days of storage at 4 °C

Anthocyanin compounds

| Peak | $R_t$ (min) | $\lambda_{max}$ (nm) | $[M\text{-}H]^+$ (m/z) | $MS^2$ (m/z) | Tentative identification | T0 | | T7 | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 0 kGy | 2 kGy | 0 kGy | 2 kGy |
| | | | | | | (mg/100 g dry weight) | | | |
| 1 | 29.27 | 515 | 449 | 287(100) | Cyanidin-3-*O*-glucoside[1] | $10\pm1^{a}$ | $10\pm1^{a}$ | $14.00\pm0.02^{a}$ | $7\pm1^{b}$ |
| 2 | 31.09 | 500 | 433 | 271(100) | Pelargonidin-3-*O*-glucoside[2] | $1040\pm53^{b}$ | $455\pm41^{c}$ | $1378\pm59^{a}$ | $834\pm19^{b}$ |
| 3 | 32.25 | 502 | 579 | 579(100) | Pelargonidin-3-*O*-rutinoside[3] | $23\pm2^{a}$ | $20\pm3^{a}$ | $25\pm2^{a}$ | $23\pm2^{a}$ |
| 4 | 41.97 | 502 | 475 | 271(100) | Pelargonidin-3-*O*-acetyl-glucoside[3] | n.d | $0.73\pm0.04^{a}$ | $8\pm4^{a}$ | $1.7\pm0.5^{a}$ |

Non-anthocyanin compounds

| Peak | $R_t$ (min) | $\lambda_{max}$ (nm) | $[M\text{-}H]^-$ (m/z) | $MS^2$ (m/z) | Tentative identification | T0 | | T7 | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 0 kGy | 2 kGy | 0 kGy | 2 kGy |
| | | | | | | (mg/100 g dry weight) | | | |
| 5 | 6.51 | 278 | 577 | 451(33), 425(65), 407(100), 289(75), 287(17) | B-type (epi) catechin dimer[4] | $60\pm2^{c}$ | $52\pm2^{c}$ | $77\pm4^{b}$ | $106\pm4^{a}$ |
| 6 | 7.22 | 280 | 289 | 245(100),205(33),151(28), 137(14) | (+)-Catechin[5] | $76\pm1^{a}$ | $68\pm7^{a}$ | $91\pm4^{a}$ | $66\pm10^{a}$ |
| 7 | 7.74 | 315 | 325 | 163(100),145(189),119(17) | *p*-Coumaric acid hexoside[6] | $11.4\pm0.4^{b}$ | $13\pm1^{b}$ | $13\pm1^{b}$ | $20.2\pm0.3^{a}$ |
| 8 | 8.19 | 290, 310 | 519 | 477(<5),331(89) | Acylated methylmyricetin deoxyhexoside[7] | $40.6\pm0.4^{a}$ | $43\pm3^{a}$ | $35\pm3^{a}$ | $39.8\pm0.5^{a}$ |
| 9 | 11.87 | 298, 325 | 613 | 477(<5),331(89) | Acylated methylmyricetin deoxyhexoside[7] | $50\pm1^{a}$ | $41\pm4^{a}$ | $43\pm5^{a}$ | $40\pm1^{a}$ |
| 10 | 15.74 | 280, 339 | 415 | 283(100) | Unknown | n.q | n.q | n.q | n.q |
| 11 | 16.90 | 234 | 935 | 633(54),301(21) | Galloyl-bis-HHDP-glucose isomer[8] | $16.2\pm0.2^{a}$ | $17\pm2^{a}$ | $14\pm1^{a}$ | $15.89\pm0.04^{a}$ |
| 12 | 17.83 | 342 | 609 | 301(100) | Quercetin-3-*O*-rutinoside[9] | n.q | n.q | n.q | n.q |
| 13 | 17.93 | 284 | 355 | 309 (100),207(12),179(8),161(5),147(5) | Cinnamic acid hexoside[10] | $38.4\pm0.5^{a}$ | $41\pm4^{a}$ | $38\pm3^{a}$ | $42\pm1^{a}$ |
| 14 | 18.3 | 256, 355 | 477 | 301(100) | Quercetin-*O*-hexuronoside[11] | $13\pm1^{a}$ | $11.6\pm0.6^{a,b}$ | $8.8\pm0.4^{b}$ | $8.1\pm0.5^{b}$ |

The results are presented as the mean ± standard error

*HHDP* hexahydroxydiphenoyl. Values within a row with similar letters do not differ significantly ($p > 0.05$). n.q.: not quantified. Calibration curves used for quantification: [1]cyanidin-3-*O*-glucoside y = 163508x + 4378.4, $R^2 = 0.9994$; [2]pelargonidin-3-*O*-glucoside y = 33872x + 2,000,000, $R^2 = 0.9988$; [3]pelargonidin-3-*O*-glucoside y = 155053x + 19,343, $R^2 = 0.9979$; [4]epicatechin: y = 10314x + 147,331, $R^2 = 0.9994$; [5]catechin: y = 84950x– 23,200, $R^2 = 1$; [6]coumaric acid: y = 466578x + 527,324; [7]myricetin: y = 23287x– 581,708, $R^2 = 0.9988$; [8]ellagic acid: y = 26719x–317,255; [9]quercetin-3-*O*-rutinoside: y = 13343x + 76,751, $R^2 = 0.9998$; [10]cinnamic acid: y = 1E + 06x– 222,204, $R^2 = 0.9993$; [11]quercetin-3-*O*-glucoside: y = 34843x– 160,173, $R^2 = 0.9998$

hydroxycinnamic acid derivatives (peak 7 and peak 13) were also detected in the studied strawberry extracts, being tentatively identified as *p*-coumaric acid hexoside with $[M-H]^-$ at m/z 325, and $MS^2$ fragments at m/z 163 (loss of hexose moiety), 145 and 119, and cinnamic acid hexoside with $[M-H]^-$ at m/z 355 and $MS^2$ fragments at m/z 309, 207 and 179. Both compounds were previously reported in strawberries [3, 38]. Peak 8 ($[M-H]^-$ at m/z 519) and peak 9 ($[M-H]^-$ at m/z 613) were presumably identified as methylmyricetin derivatives based on their common fragments at m/z 331. Although MS analysis does not allow to conclude about the nature and the position of the substituting sugar, the sequential loss of 42 mu (m/z 477) and 146 mu (m/z 331) contributed for the identification of peak 8

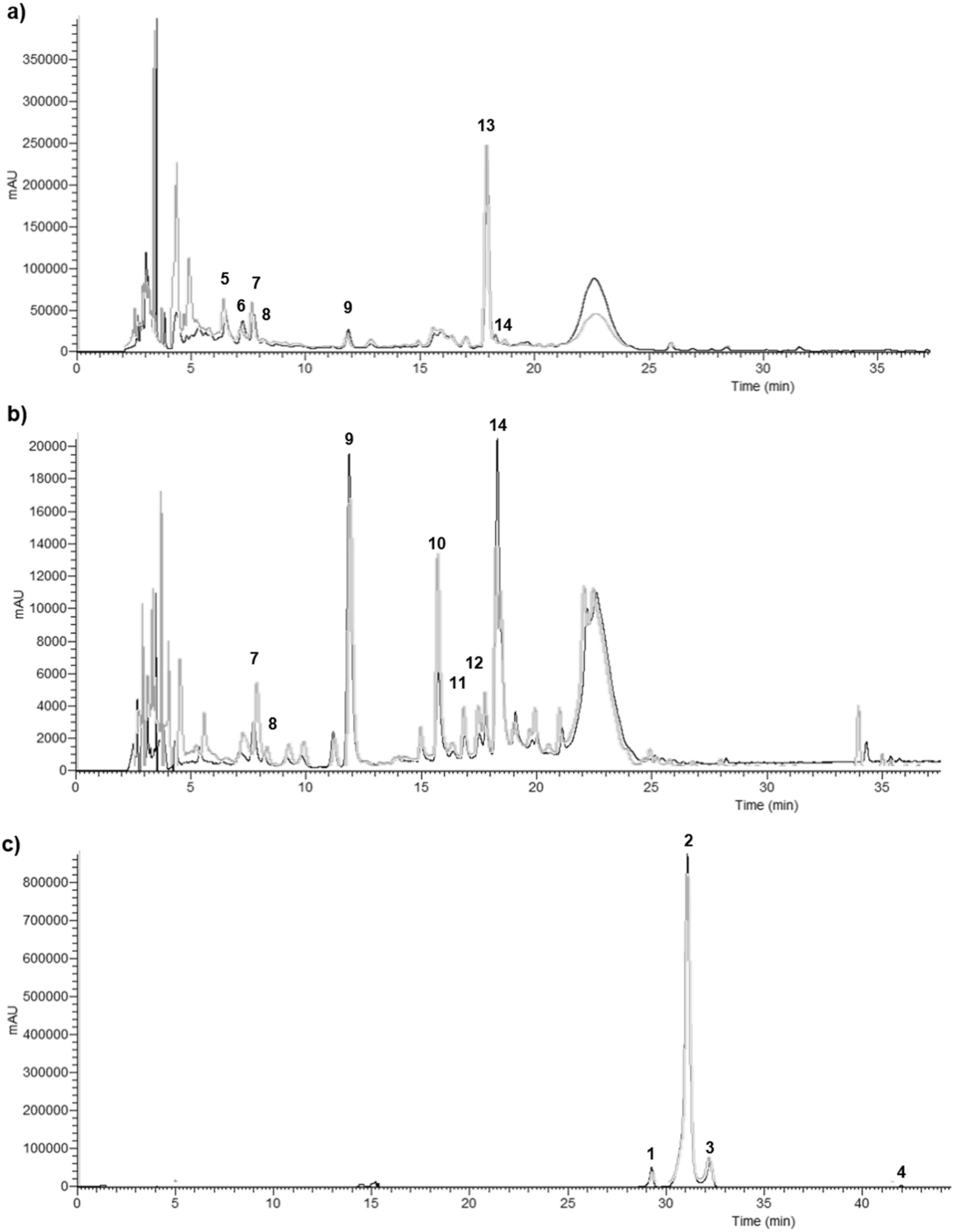

**Fig. 1** Chromatographic profile of the hydroethanolic extracts prepared from strawberry fruits non-irradiated (black line) and irradiated at 2 kGy and stored for 7 days (grey line), recorded at 280 nm (**a**), 370 nm (**b**) and 520 nm (**c**)

as an acylated methylmyricetin deoxyhexoside [41]. Peak 9 was also identified as acylated methylmyricetin deoxyhexoside based on its fragmentation pattern. To the best of authors' knowledge, this is the first report of these derivatives in strawberry fruits. Peak 10 presented $[M-H]^-$ at m/z 415 with $MS^2$ fragment at m/z 283 (loss of 132 mu, that could correspond to a pentose residue) and $MS^3$ fragments at m/z 249, 179 and 161 (data not shown). The fragmentation pattern did not allow a complete identification of this compound, being considered as unknown. Comparing its fragmentation pattern with previously studies about phenolic profile of strawberry extracts [40], peak 11 was the only ellagitannin detected in all the samples and it was identified as galloyl-bis-HHDP-glucose isomer. This peak exhibited a pseudomolecular ion at m/z 935 and the main fragmentation ions at m/z 633 and 301, corresponding to the loss of one HHDP unit and a galloyl-hexose unit, respectively.

The most abundant anthocyanin observed in the samples was pelargonidin-3-*O*-glucoside (1040 ± 53 mg per 100 g of strawberry dry weight in non-irradiated and non-stored sample) (Table 1), as previously shown by Karaaslan & Yaman [42], Dias et al. [37] and Lopes da Silva et al. [36]. Pelargonidin-3-*O*-glucoside was also pointed out as the most abundant anthocyanin present in wild strawberries from the North-eastern of Portugal [37], in local produced strawberries from Madeira Island of Portugal [38], in all the 27 strawberry cultivars grown in Norway [3] and in fully ripe Mexican strawberries [43]. Pelargonidin-3-*O*-glucoside was demonstrated to be responsible for the anti-inflammatory potential of strawberries [44] improving cardiovascular, liver and metabolic functions in metabolic syndrome [45]. In fact, this anthocyanin was the most abundant compound in the studied samples of the present work, followed by (+)-catechin (76 ± 1 mg per 100 g of strawberry dry weight), B-type (epi)catechin dimer (60 ± 2 mg per 100 g of strawberry dry weight), both acylated methylmyricetin deoxyhexoside (40.6 ± 0.4 mg per 100 g of strawberry dry weight and 50 ± 1 mg per 100 g of strawberry dry weight, respectively) and cinnamic acid hexoside (38.4 ± 0.5 mg per 100 g of strawberry dry weight) (Table 1).

Although several studies in the literature have described the main phenolic compounds present in strawberry extracts [3, 37, 38, 40], it is important to mention that this is the first one exploring the effect of ionizing radiation, in particular gamma radiation, on their individual concentrations together with the effect of the storage at refrigerated temperature of 4 °C. It seemed that refrigerated storage of 7 days promoted a significant increase ($p < 0.05$) of pelargonidin-3-*O*-glucoside amount (1378 ± 59 mg per 100 g of strawberry dry weight) (Table 1). Nevertheless, a significant ($p < 0.05$) decrease was noticed on the concentration of pelargonidin-3-*O*-glucoside present in the strawberry extracts analysed immediately after irradiation (455 ± 41 mg per 100 g of strawberry dry weight), while for the stored samples, gamma radiation could preserve its quantity (834 ± 19 mg per 100 g of strawberry dry weight) when compared to the control samples (0 kGy, T0). Regarding the concentration of the minor anthocyanins identified, non-significant ($p > 0.05$) differences were observed with the storage and gamma radiation of the fruits, except for the strawberries irradiated at 2 kGy and stored for 7 days since cyanidin-3-*O*-glucoside concentration significantly decreased (7 ± 1 mg per 100 g of strawberry dry weight). Pelargonidin-3-*O*-acetylglucoside was not detected in the control samples, but its low concentrations in the other samples did not differ significantly along the storage at refrigerated temperatures or after gamma radiation treatment (Table 1). Given that strawberries are a significant dietary source of anthocyanins, it is important to consider the various technological processes used to isolate these compounds or enhance their stability in commercially fortified products [46].

Concerning the non-anthocyanin compounds, the concentration of the majority of them seemed to be preserved by gamma radiation and refrigerated storage, with exception of B-type (epi)catechin dimer, *p*-coumaric acid hexoside and quercetin-*O*-hexurunoside (Table 1). For quercetin-*O*-hexurunoside, the refrigerated storage indicated to have negative effect on its extractability, since its concentration significantly ($p < 0.05$) decreased in both non-treated and treated strawberries. On the other hand, the refrigerated storage seemed to significantly ($p < 0.05$) increase the concentration of B-type (epi)catechin dimer with the highest value in the extracts obtained from the treated strawberries (106 ± 4 mg per 100 g of strawberry dry weight). Similar results were observed for *p*-coumaric acid hexoside, indicating a significant increase of its concentration in stored and treated samples (20.2 ± 0.3 mg per 100 g of strawberry dry weight). The increase of the phenolic concentrations in irradiated strawberries could be attributed to the release of fractions associated to polysaccharides and other matrix components and/or to the degradation of larger polyphenols into smaller ones, both induced by the radiation which could increase their extractability [47, 48].

### Total phenolic content

The TPC of the extracts from the studied samples ranged from 2376 ± 175 and 2739 ± 219 mg GAE per 100 g of strawberry dry weight (Table 2). These values of TPC were higher than those reported by Ganhão, Pinheiro, Tino, Faria, & Gil [49] for different cultivars ('Primoris', 'Endurance' and 'Portola') also produced in Portugal. In fact, the phenolic content of strawberry depends on many factors, including the fruit cultivar, production location, degree of maturity

**Table 2** Bioactive properties of the extracts obtained from irradiated (2 kGy) and non-irradiated (0 kGy) strawberry fruits before and after 7 days of storage at 4 ºC. The results are presented as the mean ± standard error

| Storage Time (days) | Dose (kGy) | TPC (mg GAE/100 g dw) | TFC (mg Catechin/100 g dw) | Ascorbic acid (mg/100 g dw) | Antioxidant activity | | Antidiabetic activity | |
|---|---|---|---|---|---|---|---|---|
| | | | | | FRAP (mmol of FSE/100 g dw) | DPPH $IC_{50}$ (μg/mL) | α-glucosidase $IC_{50}$ (μg/mL) | α-amylase $IC_{50}$ (μg/mL) |
| 0 | 0 | $2622 \pm 92^{a}$ | $560 \pm 12^{c}$ | $270 \pm 14^{a}$ | $29 \pm 1^{a}$ | $1152 \pm 41^{a}$ | $2519 \pm 10^{b}$ | $6090 \pm 31^{a}$ |
| | 2 | $2739 \pm 219^{a}$ | $775 \pm 33^{a}$ | $295 \pm 23^{a}$ | $39 \pm 2^{a}$ | $1093 \pm 28^{a,b}$ | $2921 \pm 46^{a}$ | $2444 \pm 9^{c}$ |
| 7 | 0 | $2376 \pm 175^{a}$ | $728 \pm 48^{a,b}$ | $291 \pm 10^{a}$ | $35 \pm 2^{a}$ | $802 \pm 34^{c}$ | $2915 \pm 60^{a}$ | $5728 \pm 59^{b}$ |
| | 2 | $2504 \pm 215^{a}$ | $623 \pm 35^{b,c}$ | $228 \pm 19^{a}$ | $38 \pm 2^{a}$ | $985 \pm 31^{b}$ | $2504 \pm 12^{b}$ | $2288 \pm 45^{d}$ |

*TPC* Total Phenolic Content, *TFC* Total Flavonoid Content, *FRAP* Ferric Reducing Antioxidant Power, *DPPH* 2,2 Diphenyl 1 picrylhydrazyl. $IC_{50}$ values correspond to the sample concentration achieving 50% of antioxidant activity or antidiabetic activity. Values within a column with similar letters do not differ significantly ($p > 0.05$)

at harvest and the time of year [50, 51], turning difficult the comparison of the results from the literature. In this work, non-significant ($p > 0.05$) differences between all the analysed samples were observed, more specifically, among the control (0 kGy) and the irradiated samples (2 kGy) immediately after irradiation (T0) and after 7 days of refrigerated storage (T7) (Table 2). These results indicated that refrigerated storage and gamma radiation at 2 kGy could preserve the TPC of strawberries. Similar results were reported by Barkaoui et al. [56] for non-irradiated samples, although an increase of TPC was observed at 2 kGy and T7, differently from the obtained results in the present work. Also Elias et al. [19] reported a preservation of TPC in raspberry extracts with refrigerated storage during 7 days and electron-beam radiation at 3 kGy. On the other hand, an increase of TPC was reported for the three cultivars ('San Andreas', 'Benicia' and 'Albion') of strawberries stored at refrigerated temperatures during 7 days [52].

### Total flavonoid content

The flavonoid content of strawberry extracts varied from 560 ± 12 to 775 ± 33 mg catechin per 100 g of strawberry dry weight (Table 2). Different trends were observed during refrigerated storage. It was denoted a significant ($p > 0.05$) increase on non-treated samples (560 ± 12 mg catechin per 100 g of strawberry dry weight for T0 and 728 ± 48 mg catechin per 100 g of strawberry dry weight for T7) and a significant ($p < 0.05$) decrease on treated samples at 2 kGy after the 7 days of storage (775 ± 33 mg catechin per 100 g of strawberry dry weight for T0 and 623 ± 35 mg catechin per 100 g of strawberry dry weight for T7). Other authors have also reported the increase of flavonoid content in strawberries stored for 7 days [52]. When combined gamma radiation and storage, the results showed a preservation of TFC, which it is not in agreement with the previous results [24]. Nevertheless, Barkaoui et al. [56] had studied strawberries from Tunisia, and, as mentioned before, the geographical and environmental conditions can affect the bioactive content of the fruits.

### Ascorbic acid content

Ascorbic acid, also known as vitamin C, is a powerful antioxidant and its deficiency can cause scurvy. The human body does not produce this vitamin, turning the fruits and vegetables (such as strawberries) as its only source. The ascorbic acid content for non-irradiated samples (T0, 0 kGy) was 270 ± 14 mg per 100 g of strawberry dry weight (Table 2) which is lower than the content obtained in a previous study of the authors (340 ± 6 mg per 100 g of strawberry dry weight) [17]. As mentioned before, this difference can be related with the influence of strawberries genotype, harvesting time, ripening stage and culture conditions. Furthermore, the results showed that both gamma radiation and 7 days storage at 4 ºC did not cause any variation on this amount, contrarily to other studies reporting the decrease of vitamin C in fruits with gamma radiation and during storage [17, 53, 54] due to the conversion of ascorbic acid to dehydroascorbic acid [55].

## Bioactive properties of strawberry extracts

### Antioxidant activity

The antioxidant activity of the extracts from irradiated and non-irradiated strawberries were measured by two assays: FRAP and DPPH scavenging activity. For FRAP assay, a preservation of antioxidant activity was noticed for all the samples (Table 2), which could probably be related with the same tendency observed for TPC and ascorbic acid content in stored and treated strawberries (Table 2). No variation on FRAP values for non-stored samples had also been verified in a previous study even at higher doses (3 kGy) [17]. Furthermore, the preservation of antioxidant activity of extracts from irradiated stored strawberries was reported using gamma and electro-beam radiation [24, 56]. In the DPPH assay, the results were expressed as $IC_{50}$ values, with higher values corresponding to lower antioxidant potentials ($IC_{50}$: extract concentration corresponding to 50% of antioxidant

**Table 3** Antimicrobial activity of the extracts obtained from irradiated (2 kGy) and non-irradiated (0 kGy) strawberry fruits before and after 7 days of storage at 4 ºC

| Storage Time (days) | Dose (kGy) | *Escherichia coli* | *Salmonella* Typhimurium | *Pseudomonas fluorescens* | *Listeria monocytogenes* | *Staphylococcus aureus* | *Bacillus cereus* | *Candida albicans* |
|---|---|---|---|---|---|---|---|---|
| | | Minimum Inhibitory Concentration (mg/mL) | | | | | | |
| 0 | 0 | 50 | 100 | 50 | 100 | 50 | 50 | 100 |
| | 2 | 50 | 100 | 50 | 100 | 50 | 50 | 100 |
| 7 | 0 | 50 | 100 | 50 | 100 | 50 | 50 | 100 |
| | 2 | 50 | 100 | 50 | 100 | 50 | 50 | 100 |
| | | Minimum Microbicidal Concentration (mg/mL) | | | | | | |
| 0 | 0 | 50 | 100 | 50 | 100 | 50 | 50 | 100 |
| | 2 | 50 | 100 | 50 | 100 | 50 | 50 | 100 |
| 7 | 0 | 50 | 100 | 50 | 100 | 50 | 50 | 100 |
| | 2 | 50 | 100 | 50 | 100 | 50 | 50 | 100 |

activity). The highest scavenging activity was obtained in the samples stored during 7 days ($IC_{50}$ values of 802 ± 34 and 985 ± 31 µg/mL for non-irradiated and irradiated samples, respectively) (Table 2). The improvement of antioxidant activity in these samples could be attributed to the accumulation of anthocyanins, namely pelargonidin-3-glucoside, during cold storage (Table 1) and consequent enhancement of phenylalanine ammonia-lyase (PAL) activity [57] as it was reported by Hussain, Wani, Meena, & Dar [58] in peach samples. For non-irradiated samples, the DPPH scavenging activity significantly ($p > 0.05$) increased after 7 days of storage ($IC_{50}$ values of 1152 ± 41 and 802 ± 34 µg/mL for T0 and T7, respectively), which is in agreement with the results observed by Tudor et al. [52]. For non-stored strawberries (T0), gamma radiation at 2 kGy could preserve the scavenging activity of the extracts ($IC_{50}$ values of 1152 ± 41 and 1093 ± 28 µg/mL at 0 and 2 kGy, respectively), while after storage (T7) gamma radiation significantly decreased ($p < 0.05$) the radical scavenging potential of strawberry extracts ($IC_{50}$ values of 802 ± 34 µg/mL 985 ± 31 µg/mL at 0 and 2 kGy, respectively), although indicating significantly higher scavenging activity than non-stored strawberries (T0). The overall results indicated that the storage and the irradiation treatment could preserve the antioxidant activity of the strawberry extracts. These results are in agreement with those obtained by Barkaoui et al. [56] which detected that the irradiated stored samples presented higher antioxidant activity.

## Antimicrobial activity

The results of antimicrobial activity of the extracts from non-irradiated and irradiated strawberries showed that all the samples had antimicrobial potential (MIC 50–100 mg/mL) (Table 3). Among all the studied microorganisms, *E. coli*, *P. fluorescens*, *S. aureus* and *B. cereus* presented higher sensitivity towards strawberry extracts (MBC 50 mg/mL). The antimicrobial activity of ethanolic extracts of strawberries was previously verified against *E. coli*, *S. aureus* and *C. albicans* [59, 60]. Puupponen-Pimiä, Nohynek, & Meier [61] presented a significant antimicrobial effect of strawberry extracts against Gram-negative bacteria, such as *S. enterica* and *E. coli*, with MIC of 1 mg/mL. Although this MIC was lower than the obtained in this work, the authors related it with the synergetic effect of the anthocyanins and other phenolic compounds present in the extracts and with their extraction conditions using aqueous 70% acetone. In fact, Ma et al. [62] demonstrated the potential of anthocyanins as good growth inhibitors of food pathogens and discussed their possible use to replace antibiotics.

Nevertheless, as far as the authors know, this is the first report concerning the effect of gamma radiation on antimicrobial activity of strawberry extracts. In the present study, no variations were observed in this activity with gamma radiation and/or refrigerated storage of strawberry samples (Table 3), demonstrating that the antimicrobial potential of these extracts can be preserved by gamma radiation and storage at refrigerated temperatures. These results could be used to propose the possibility of using strawberry extracts as natural food preservatives in order to prevent the growth of foodborne microorganisms in fruits.

## Antidiabetic activity

The inhibition of α-glucosidase and α-amylase, enzymes involved in the digestion of carbohydrates, can control postprandial hyperglycaemia and reduce the risk of developing type 2 diabetes. In the last years, the increasing number of diabetes patients encouraged the interest on searching natural α-glucosidase and α-amylase inhibitors from plant sources to replace synthetic agents as acarbose. In this work, the effectiveness of the extracts of non-irradiated and irradiated strawberries in inhibiting α-amylase and α-glucosidase was expressed as $IC_{50}$ values (Table 2). It was evident that gamma radiation promoted a significant ($p < 0.05$) increase in the inhibition of α-amylase of the extracts from non-stored ($IC_{50}$ value of 2444 ± 9 µg/mL) and stored ($IC_{50}$ value of 2288 ± 45 µg/mL) strawberries, with the highest activity for the extracts from irradiated samples stored during 7 days (2 kGy, T7). Concerning α-glucosidase inhibition, different trends were observed. Although gamma radiation treatment suggested an increase in $IC_{50}$ of non-stored samples, meaning a decrease on the activity of these extracts in inhibiting α-glucosidase, an increase in $IC_{50}$ was detected for the non-irradiated samples that were stored during 7 days (2915 ± 60 µg/mL). Comparing to the control samples (T0, 0 kGy), the results indicated that the irradiation at 2 kGy and the storage during 7 days preserved the potential of strawberry extracts to inhibit α-glucosidase enzyme. In fact, the $IC_{50}$ corresponding to α-glucosidase inhibitions of all the studied extracts were higher than those obtained for acarbose ($IC_{50}$ value of 11,000 µg/mL—data not shown), the used standard positive control.

As far as the authors know, this is the first study about the antidiabetic activity of strawberry extracts together with the evaluation of gamma radiation effect on this bioactivity. Nevertheless, there are few studies reporting the antidiabetic activity of strawberry leaves [63] and fruit extracts [64, 65] or isolated and purified compounds from these extracts [5, 65]. McDougall et al. [64] related the inhibition of α-glucosidase to the anthocyanin content of strawberries whereas the inhibition of α-amylase was attributed to the amounts of soluble tannins. Moreover, da Silva Pinto et al. [5] described that the ellagitannins purified from strawberries were better inhibitors for α-amylase than for α-glucosidase.

These findings allowed concluding that the extracts of irradiated and stored strawberries should be further tested to be used as natural and promising antidiabetic agents in controlling hyperglycemia.

## Conclusions

The effects of gamma radiation were assessed on the phenolic profile and bioactive properties of strawberries during refrigerated storage for 7 days at 4 °C. Pelargonidin-3-*O*-glucoside was the most abundant compound in all the extracts, but the strawberries are also rich in other phenolic compounds, such as B-type (epi)catechin dimer, (+)-catechin or acylated methylmyricetin deoxyhexoside. Storage of strawberries increased the extractability of pelargonidin-3-*O*-glucoside and gamma radiation improved the amounts of *p*-coumaric acid hexoside and B-type (epi)catechin dimer. The results demonstrated that gamma radiation and refrigerated storage could preserve the phenolic and ascorbic acid contents of strawberry extracts, as well as it could preserve or improve their antioxidant activity, suggesting the use of these extracts as natural preservatives for food industry. It is important to highlight the high α-glucosidase inhibition of the ethanolic extracts obtained from all the samples, and the enhancement of α-amylase inhibition promoted by gamma radiation at 2 kGy. These findings highlighted the potential of the extracts from irradiated samples to be used as natural and promising antidiabetic agents to control hyperglycaemia. The results obtained in this work can support food industries in developing new functional natural ingredients, and contribute to consumers and food industry awareness regarding the safety and quality of fruits irradiation, increasing their acceptance. Further studies should be performed with individual compounds to understand which compounds could be more effective in triggering these promising results.

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**Data Availability** The data used in this contribution is available upon request.

## Declarations

**Conflicts of interest** The authors declare that they have no conflict of interest.

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