Citação:

Elias, M. I., Madureira, J., Santos, P. M. P., Carolino, M. M., Margaça, F. M. A., & Cabo Verde, S. (2020). Preservation treatment of fresh raspberries by e-beam irradiation. Innovative Food Science & Emerging Technologies, 66, 102487. https://doi.org/10.1016/j.ifset.2020.102487

DOI: https://doi.org/10.1016/j.ifset.2020.102487

Preservation treatment of fresh raspberries by e-beam irradiation

M.I. Elias[a,b], J. Madureira[a,c,d], P.M.P. Santos[a], M.M. Carolino[b], F.M.A. Margaça[a], S. Cabo Verde[a,*]

[a]Centro de Ciências e Tecnologias Nucleares (C^{2}TN), Instituto Superior Técnico, Universidade de Lisboa, E.N. 10 ao km 139.7, 2695-066 Bobadela LRS, Portugal

[b]Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

[c]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5301-253 Bragança, Portugal

[d]Grupo de Investigación en Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno s/n, 37007 Salamanca, Spain

*Corresponding author: sandracv@ctn.tecnico.ulisboa.pt

Abstract

E-beam irradiation was studied as a post-harvest treatment for red raspberries (*Rubus idaeus* L.). Microbial inactivation (natural microbiota and potential pathogenic bacteria) and bioactive properties (phenolic content, vitamin C content and antioxidant activity and cytotoxicity) of these fruits were evaluated before and after irradiation and during storage of 14 days at 4ºC. A reduction of 2 log CFU/g of mesophilic bacteria and 3 log CFU/g on filamentous fungi, and no detection of foodborne inoculated pathogens (3 log CFU/g) was achieved with an e-beam treatment at 3 kGy and during 7 days of refrigerated storage. Regarding bioactive properties, the results suggested that irradiation could preserve the phenolic content and antioxidant activity of raspberries through 7 days of cold storage, even though a decrease of 80% on ascorbic acid concentration was observed. Furthermore, no *in vitro* inhibitory effect on human cells lines was observed for the extracts from e-beam-treated raspberries. The overall results suggested that use of e-beam irradiation as post-harvest treatment of raspberries as an emergent, clean and environmental friendly process to extend the shelf-life of this fruit with safety and preservation of bioactivity.

Industrial Relevance

Red raspberries are known to demonstrate high bioactivity that could be beneficial to human health, but are highly perishable and often associated with foodborne outbreaks, which makes its safety and commercialization a challenge. The use of a terminal control such as irradiation might reduce the burden of disease transmission and extend the quality of fresh red raspberries. The present research indicated that e-beam irradiation can be used as post-harvest treatment of raspberries, guarantying its safety and quality with the add-value of shelf-life extension.

Keywords: raspberries; microbial inactivation; electron-beam treatment; phenolic content; antioxidant activity; cytotoxicity

1. Introduction

Red raspberries (*Rubus idaeus* L.), a small fruit known as the “golden fruit”, are becoming highly appreciated in the world and consumed as fresh and/or processed to juice, jams, confitures and other products or as ingredients for different foods (Teng et al., 2017). In Portugal, the production of high quality red raspberries has been considerably increased in the last years, becoming the second most exported fruit in the country (da Câmara Correia, 2016).

These fruits are known for their antitumoral, antibacterial, anti-inflammatory and antioxidant activities (Bowen-Forbes, Zhang, & Nair, 2010; de Souza et al., 2014; Sariburun, Şahin, Demir, Türkben, & Uylaşer, 2010) due to their content in phenolic compounds such as anthocyanins, ellagitannins, a wide variety of quercetin and kaempferol-based flavonol conjugates, phenolic acids and vitamin C (Bobinait, Viškelis, & Venskutonis, 2012; Bowen-Forbes et al., 2010; Diaconeasa, Florica, Rugină, Lucian & Socaciu, 2014; Kula, Majdan, Głód, & Krauze-Baranowska, 2016; Mullen et al., 2002; Sariburun et al., 2010), among other beneficial nutrients including essential minerals, dietary fibre, potassium and fatty acids.

The contamination of the food supply with pathogens and their persistence, growth, multiplication and/or toxin production has emerged as an important public health concern (Paiva De Sousa, 2008), that also causes industrial economic losses. Fresh fruits and vegetables were considered the number one vehicle of foodborne illnesses, being associated to approximately 200 outbreaks, reported in United States and Europe during 2004-2012 (Callejón et al., 2015). Based on outbreak investigations, the pathogens associated with fruits and vegetables include pathogenic strains of Shiga toxin-producing *Escherichia coli* (STEC), *Salmonella*, *Listeria monocytogenes* and norovirus (Johnson, 2019). These three bacterial pathogens were involved in multistate fresh produce outbreaks from 2010 to 2017 in the United Sates (Carstens, Salazar, & Darkoh, 2019). Concerning berries, the majority of outbreaks associated to them have been caused by

viruses, namely norovirus and hepatitis A, although a *Shigella sonnei* outbreak has also been linked to these fruits (Tavoschi et al., 2015). Berries contamination and cross-contamination can be via equipment, water (irrigation and washing) and particularly via food handlers that have been identified as the main risk factors (EFSA BIOHAZ Panel - EFSA Panel on Biological Hazards, 2014). Raspberries are highly sensitive to the loss of water and susceptible to spoilage, which shortens their period of commercialization. Consequently, extending its shelf-life to improve distribution options, and to increase availability outside of peak production periods is challenging the research on post-harvest preservation technologies (Huynh, Wilson, Eyles, & Stanley, 2019). Currently, the berry industry rely mainly on cold chain management (0–2◦C) and high humidity (90–98%) for maintaining quality (Huynh et al., 2019). Moreover, raspberry is a fruit with an increasing consumption, impelling the berry fruit industry to improve food safety.

There are several methods to reduce and/or eliminate the microbial contamination on whole and fresh-cut produce (Parish et al., 2003). The addition of sanitizers or disinfectants to water washes is one of the most commonly applied strategy to inactivate pathogens on berries. For example, chlorine washes of berries generally yield 1- to 2-log unit reductions in bacteria and viruses (Lukasik et al., 2003; Wei, Zhou, Zhou, & Gong, 2007). Despite of the general use of sodium hypochlorite and hydrogen peroxide as sanitizers (Artés, Gómez, Aguayo, Escalona, & Artés-Hernández, 2009), it is well documented that these compounds can cause irritations in the skin and the respiratory tract and could have an carcinogenic effect. Alternatively, electrolyzed water has been used as a disinfectant in fresh-cut industry (Issa-Zacharia, Kamitani, Muhimbula, & Ndabikunze, 2010; Lee, Hong, & Kim, 2014).

Ionizing radiation is considered an effective technology for microbial inactivation and shelf-life extension. In previous studies, Cabo Verde et al. (2013) showed that gamma radiation at 1.5 kGy could reduce the microbial load on raspberry by 1 log unit without changes in the sensorial quality of the fruit. Regarding the inactivation of enteric virus by gamma radiation in berry fruits, Pimenta, Margaça, & Cabo Verde (2019) reported a 2

log PFU/g (Plaque Forming Units per gram) reduction on murine norovirus type 1 (MuNoV) and human adenovirus type 5 (HAdV) after treatment at 4 kGy. Moreover, the use of gamma radiation at 1 and 2 kGy, associated with cold storage, extended the post-harvest life of fresh raspberries by 8 days (Tezotto-Uliana, Berno, Saji, & Kluge, 2013). In addition, the use of electron-beam irradiation as an environmental friendly and time effective alternative for decontamination, disinfection and disinfestation of fresh fruits has been proposed (Lung et al., 2015; Madureira et al., 2019).

The aim of this work was to evaluate the potential use of the eco-friendly e-beam irradiation as a post-harvest treatment for raspberries through the evaluation of microbial inactivation (natural microbiota and potential pathogenic bacteria) and bioactive activity (phenolic content, vitamin C content and antioxidant activity and cytotoxicity). To our knowledge, there is no study concerning the use of e-beam irradiation as a post-harvest treatment for shelf-life extension of fresh raspberries. Thus, this work can contribute to better understand the potential use of this technology as a treatment process to further increase the safety, quality and economic value of these fruits. One of the major advantage using radiation technologies is that they require a minimal handling of the food item. Consequently, decontamination is achieved without inducing any mechanical damage and the time needed for the product to reach consumers is substantially reduced (Guimarães et al., 2013).

2. Materials and methods

2.1. Sampling

Red raspberries (*Rubus idaeus* L., cv. Amira) of uniform shape size at commercial maturity stage were purchased from a local supermarket in Lisbon, Portugal, and immediately kept at 4 ± 1 °C until analysis. The fruits had no visible mechanical damage or pathogen damage. In a study developed by da Câmara Correia (2016), four cultivars were compared and the cv. Amira showed high levels of total phenolics, total

129 hydrolyzable tannins, total flavonoids and total anthocyanins, and chosen for biological
130 assays and for a study of nutritional intervention in humans.
131
132 **2.2. Irradiation experiments**
133 Irradiation experiments were carried out in a linear electron-beam accelerator (LINAC,
134 adapted from GE Saturne 41) with an energy of 10 MeV located at the ionizing radiation
135 facility IRIS from Centro de Ciências e Tecnologias Nucleares (C2TN) of Instituto
136 Superior Técnico, Universidade de Lisboa.
137 Fresh raspberries were irradiated in plastic boxes (150 g; one box per dose) at room
138 temperature at doses from 0.5 to 3 kGy at an average dose rate of 0.5 kGy min^{-1} with
139 dose uniformity (DUR) of 1.1. The absorbed dose was estimated using calibrated
140 radiochromic dosimeters FWT-60 (Far West Technology, Inc. Goleta, USA) (Miller,
141 1983). Three independent irradiation batches were performed per each assay. Non-
142 irradiated samples (0 kGy) were used as control and followed all the experiments.
143
144 **2.3. Microbial inactivation studies**
145 **2.3.1. Natural microbiota**
146 Non-irradiated and irradiated raspberries (25 g) were placed in sterile stomacher bags
147 containing 100 mL of 0.1% Tween 80 physiological solution. Samples (n = 3/dose) were
148 homogenized using a stomacher (Stomacher 3500; Seaward, UK) for 15 min. Serial
149 decimal dilutions were prepared for inoculation in triplicate on Tryptic Soy Agar plates
150 (TSA) for mesophilic microbial counts and Malt Extract Agar (MEA) plates for filamentous
151 fungi counts. Samples were incubated at 30 °C for TSA plates and 28 °C for MEA plates
152 and colony numbers were counted for 7 days. The results were expressed as log colony-
153 forming units per gram of fresh fruit (log CFU/g).
154
155 **2.3.3. Artificial inoculation with potential foodborne pathogens**

Artificial contamination assays were carried out using three different bacterial strains in separated sets, namely *Salmonella enterica* serotype Typhimurium ATCC 14028, *Escherichia coli* ATCC 8739 and *Listeria monocytogenes* ATCC 19111. To inoculate the raspberries (previously disinfected with 70% ethanol until completely evaporated under a laminar flow cabinet), a droplet of inoculum was deposited on the skin of the fruits (25 g) to obtain approximately 10^3 CFU/g of each bacterium. The fruits were dried in a laminar flow cabinet to allow the attachment of the microorganisms. Bacterial counts of spiked raspberries samples were estimated as described by Madureira et al. (2019). The detection limit of the method was 1 CFU/g. The microbial counts were recorded and expressed as the log CFU/g. D_{10} is defined as the dose (kGy) required to inactivate 90% of a microbial population, or the dose of irradiation needed to produce a 10-fold (1 log) reduction in the population. D_{10} values were estimated by the reciprocal of the slope of the log-linear microbial survival curves.

2.4. Phenolic compounds extraction

Raspberries (18 g) were manually mashed and lyophilized (Heto CD8, Allerod, Denmark) for 72 h and stored until used. The raspberry extracts were prepared by a solid-liquid extraction as previously described (Pinela et al., 2016), using a mixture of ethanol:water (80:20, v/v; 30 mL) as solvent, for 1 h at room temperature.

2.4.1. Ascorbic acid content

Ascorbic acid content was determined by High Performance Liquid Chromatography (HPLC) (Prominence CBM 20-A, Shimadzu, Japan) with UV-DAD detector. The lyophilized extracts (~10 mg) were dissolved in metaphosphoric acid 4.5% (1 mL). All samples were filtered through 0.45-µm nylon filters before analysis. The HPLC column was a Kinetex C18 XB-C18 (5 µm, 250 mm, 4.0 mm) and the detection was made at 245 nm. The mobile phase used was 1.8 mM H_2SO_4 (pH = 2.6) with a flow rate of 0.9 mL min^{-1}. The column temperature was maintained at 35 °C and the injection volume was 10 µL.

184 The assay was made in triplicate. For quantification purposes, a calibration plot was
185 performed under the experimental conditions used. Values were expressed as mg per
186 100 g of raspberries dry weight (dw).
187
188 **2.4.2. Total Phenolic Content**
189 The total phenolic content was determined based on Folin-Ciocalteau method
190 (Singleton, Orthofer, & Lamuela-Raventós, 1998), in extracts concentrated at 5 mg/mL.
191 The standard curve was calculated using gallic acid (Sigma, St. Louis, US) and the
192 results were expressed as mg of gallic acid equivalents (GAE) per 100 g of raspberries
193 dry weight (dw) (Guerreiro et al., 2016). The assay was carried out in triplicate.
194
195 **2.4.3. Antioxidant activity**
196 The antioxidant activity was evaluated by two assays based on different mechanisms of
197 action: DPPH radical scavenging activity described by Brand-Williams, Cuvelier, &
198 Berset (1995) with some modifications (Madureira et al., 2019) using EZ Read 2000
199 Microplate Reader (Biochrom, Cambridge, UK) and Ferric Reducing Antioxidant Power
200 (FRAP) described by Benzie & Strain (1996) using a spectrophotometer (Shimadzu UV
201 1800, Kyoto, Japan). For FRAP assay, the results were expressed as mmol of ferrous
202 sulfate equivalent (FSE) per 100 g raspberries dry weight (dw). For DPPH method, L-
203 ascorbic acid (E-Merck, Darmstadt, Germany) was used as standard compound for the
204 calibration. The antioxidant activity measured by DPPH scavenging activity was
205 expressed as EC50 values (mean ± standard error), which means that higher values
206 correspond to lower antioxidant potential (EC50: extract concentration corresponding to
207 50% of antioxidant activity). Both assays were made in triplicate.
208
209 **2.4.4. Cytotoxicity assay - WST-1 Proliferation test**
210 Human lung carcinoma epithelial cells (A549, ATCC® CCL-185TM) and human
211 embryonic kidney epithelial cells (293T, ATCC® CRL-3616™) were used. Cell viability

after exposition to raspberries extracts (at the concentrations of 4, 40 and 400 µg/mL) was measured using the WST-1 cell proliferation assay based on quantification of mitochondrial activity as an indicator of cytotoxicity based on the protocol described by Madureira et al., 2019. Two independent assays each with three raspberries extracts replicates were performed.

2.5. Storage study

In order to evaluate a potential shelf-life extension of raspberries with e-beam treatment, the previously described assays were performed at different refrigerated (4ºC) storage periods. The microbial inactivation assessments, the vitamin C and phenolic contents, the antioxidant activity and the cytotoxicity of the extracts were carried out after irradiation either immediately (T0; no storage) or followed by different storage periods: 3 days (T3; regular fruit shelf-life), 7 days (T7) and 14 days (T14).

2.6. Data analysis

Origin software version 7.5 (OriginLab Corporation, Northampton, 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 analysed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test with $\alpha = 0.05$.

3. Results and Discussion

As mentioned above, this is the first study applying e-beam radiation to treat and extend the shelf-life of fresh raspberries, being the obtained results important to understand the possible use of this technology in the industry as a post-harvest process of fruits. The applied dose range was selected based on WHO guidelines for fresh fruits shelf-life extension (World Health Organisation & Food and Agriculture Organization of the United Nations, 2000).

240

241 ### 3.1. Microbial inactivation

242 The aerobic mesophilic bacteria and filamentous fungi populations of fresh raspberries
243 were assessed before and after e-beam treatment, immediately after irradiation (T0) and
244 after several periods, namely 3 days (T3), 7 days (T7) and 14 days (T14) of refrigerated
245 storage, in order to evaluate the microbial inactivation and its trend with the treatment
246 and storage. The fresh raspberries indicated an aerobic bacterial mesophilic population
247 of 4.3 ± 0.1 log CFU/g and a filamentous fungi population of 6.1 ± 0.1 log CFU/g (Figure
248 1). Previously, an average bioburden between 4 – 6 log CFU/g was reported for fresh
249 raspberries (Baugher & Jaykus, 2016; Cabo Verde et al., 2013; Piechowiak et al., 2019),
250 that supports the obtained results. Nevertheless, the production practices, growth
251 conditions in combination with harvesting and processing, can affect the microbiological
252 quality of berries at the time of consumption (Oliveira, Rodrigues, & Teixeira, 2019).
253 With e-beam treatment at 3 kGy (T0) the mesophilic bacterial population of raspberries
254 decreased ($p < 0.05$) 2 log CFU/g and the filamentous fungi reduced ($p < 0.05$) 3 log
255 CFU/g comparatively to non-treated samples (Figure 1). The e-beam treatment allowed
256 to comply with the Portuguese recommended criteria for fresh fruits and vegetables
257 (bacterial counts at 30ºC < 4 log CFU/g; filamentous fungi < 5 log CFU/g; Santos,
258 Correia, Cunha, Saraiva, & Novais, 2005). In fact, there is no regular monitoring of
259 berries and the current European Union legal framework does not include microbiological
260 criteria applicable for these fruits at the primary production stage (Oliveira et al., 2019).
261 The bacterial counts of non-treated fruits remained constant ($p > 0.05$) during 7 days of
262 refrigerated storage, but an increase ($p < 0.05$) of 3 log CFU/g was observed at 14 day
263 of storage. Nevertheless, the fungal population remained ($p > 0.05$) at approximately 6
264 log CFU/g during the 14 days of refrigerated storage (Figure 1). A statistically significant
265 growth of bacteria up to 7 log CFU/g was cited after 24 hours of storage of fresh
266 raspberries at room temperature (Piechowiak et al., 2019). For irradiated raspberries the
267 same trend of control samples was observed, the bacterial counts increased ($p < 0.05$)

2 log CFU/g only after 14 days of storage, and the filamentous fungi counts were maintained ($p > 0.05$) for 14 days of storage (Figure 1). After the 14 days of refrigerated storage, the bacterial counts of 3 kGy treated raspberries were similar ($p>0.05$) to the initial counts of non-treated samples (T0), but for fungi the concentration of treated raspberries was always lower ($p < 0.05$) than control (0 kGy). It should be highlighted that the e-beam treatments at 2 kGy and 3 kGy complied with the recommended limits for microbial loads (Santos et al., 2005) through 7 days storage, that were not meet by the non-treated raspberries at any period of analysis.

Previous studies reported one log reduction of microbial load of fresh raspberries after gamma radiation treatment at 1.5 kGy and during 14 days of refrigerated (Cabo Verde et al., 2013). A similar inactivation (approximately 1 log CFU/g) on aerobic mesophilic bacteria and fungi was obtained for fresh raspberries stored at room temperature during 48 h and treated by ozonation with a dose of 8–10 ppm for 30 min every 12 hours (Piechowiak et al., 2019).

Regarding the inactivation of foodborne bacteria, which were artificially inoculated on fruits, the results are presented in Table 1. Different ranges of absorbed doses were used for each microorganism in order to have surviving fractions for the D_{10} values estimation. *Salmonella* Typhimurium on raspberries presented a linear ($R^2 = 0.99$) inactivation kinetics by e-beam irradiation and a D_{10} value of 0.73 ± 0.05 kGy. This bacteria was not detected on fruits treated at 3 kGy for the 14 days of storage (Table 1). The population of *S*. Typhimurium on non-treated raspberries significantly ($p < 0.05$) decreased (<1 log CFU/g) after 3 days of storage, thereafter maintained ($p > 0.05$) its counts until the 14 days (Table 1). On irradiated raspberries, the refrigerated storage indicated a reduction of *S.* Typhimurium counts along the 14 days, suggesting a synergistic effect between storage and irradiation on the inactivation of this bacteria. *Salmonella* is documented to be very sensitive to berry phenolics (Heinonen, 2007), which could be exposed due to raspberries tissue softening during storage (Cabo Verde et al., 2013; Huynh et al., 2019). This synergistic effect between cold storage and gamma

radiation on the delay of the decay of raspberries was mentioned before, pointing out to an extension of the post-harvest life for fruit irradiated at 1.0 and 2.0 kGy by 8 days (Tezotto-Uliana et al., 2013).

E. coli on raspberries also followed a linear inactivation (R^2 = 0.99) by e-beam irradiation with an estimated D_{10} value of 0.72 ± 0.01 kGy. Similarly to *S.* Thyphimurium, on raspberries irradiated at 3 kGy it was not detected the presence of *E. coli* for any period of analysis. Once again, the extended refrigerated storage induced a decrease on bacterial counts (0 kGy T0, T3 and T7, T14; $p<0.05$), more pronounced for irradiated fruits at 1.5 kGy where *E. coli* was not detected on stored samples (Table 1). According to the literature, berry compounds (e.g. complex phenolic polymers such as polymeric tannins) are able to inhibit the growth of this bacteria (Heinonen, 2007). Again, the loss of firmness of raspberries during storage may allow the penetration of surface bacterial contamination to be are exposed to the antimicrobial compounds of this fruit.

Among the foodborne bacteria studied, *Listeria monocytogenes*, was found to be the most radiosensitive to e-beam on raspberries, following a linear (R^2 = 0.99) inactivation kinetics characterized by a D_{10} value of 0.41 ± 0.03 kGy. This microorganism was not detected on raspberries irradiated at 3 kGy (like *S.* Typhimurium and *E. coli*), as well as on all the samples stored at 14 days (Table 1). Nonetheless, the counts reduction was not observed along the 7 days of storage, as it was for *E. coli* and *S.* Typhimurium. As previously reported, *L. monocytogenes* possesses the ability to survive in food matrices at refrigerator temperatures, reaching a steady state that lasts at least up to 8 days (maximum days tested) of storage (Ziegler, Kent, Stephan, & Guldimann, 2019). Moreover, other studies indicated that *Listeria* strains were not affected by berry compounds, with the exception of cranberry (Puupponen-Pimia et al., 2005).

The previous results highlight the efficiency of e-beam as a disinfection process. Based on the estimated D_{10} values, the treatment at 3 kGy is expected to reduce *S.* Typhimurium and *E.coli* by 4 log CFU/g, and *L. monocytogenes* by 8 log CFU/g on post-harvested raspberries.

Other preservation technologies have been studied to guarantee the microbial safety of raspberries. For example, the combined continuous and pressurized ozone treatment indicated to achieve reductions of 3.6 and 3.8 log CFU/g for *Salmonella enterica* and *E. coli* O157:H7, respectively (Bialka & Demirci, 2007). Previous studies indicated that pulsed UV-light treatment on raspberries can reduce *E. coli* O157:H7 by 3.9 log CFU/g at 72 Jcm^{-2}, and *Salmonella* by 3.4 log CFU/g at 59.4 Jcm^{-2} (Bialka & Demirci, 2008). Other study, using UV-C presented that a treatment during 720 s with a total dose of 0.78 Jcm^{-2} can yield a 1.5 log CFU/g reduction of *Listeria monocytogenes* population on the surface of frozen red raspberries (Liao, Syamaladevi, Zhang, Killinger, & Sablani, 2017). The combined treatment of 1% H_2O_2 with water-assisted pulsed light system indicated to reduce *S. enterica* on raspberries by 4 log CFU/g (Huang, Sido, Huang, & Chen, 2015). The preservation treatment of raspberries with gaseous chlorine dioxide presented reductions of 1.5 log CFU/g for *Salmonella enterica* and 2.6 log CFU/g for yeasts and molds, using 8 mg/L of ClO_2 during 120 minutes (Sy, McWatters, & Beuchat, 2005). Comparing the results obtained in the present study with the ones mentioned above, the e-beam treatment at 3 kGy demonstrated similar or higher decontamination (2-3 log CFU/g reduction) and disinfection efficacy (at least 4 log CFU/g reduction), with the benefits of being a single treatment (non-combined) with no chemical/residues and no further manipulations (final treatment that can be performed in the regular packaging system), preventing cross-contamination, and a potential extension of shelf-life up to 7 days for raspberries.

3.2. Phenolic content and antioxidant activity of raspberries extracts

It is recognized that the phenolic compounds contribute to the nutritional and sensory quality of fruits and their antioxidant potential provide health benefits (Shahbaz et al., 2014). The obtained results of total phenolic content (TP) and antioxidant activity of raspberries before and after irradiation and during storage time are presented in Table 2. The bioactivity assessment was only performed at 3 kGy since it was the dose that

352 comply with the microbiological criteria. The obtained TP value for non-irradiated fruits
353 was 1092±3 mg GAE/100g dry weight and, with exception of non-stored irradiated
354 sample (T0, 3 kGy), no significant trend was verified for the 14 days of storage at 4ºC.
355 The irradiation of raspberries at 3 kGy seemed to increase significantly ($p < 0.05$) the
356 phenolic content (1405±75 mg GAE/100g dry weight) in comparison to control sample.
357 This increase could be related to an improvement of extractability of phenolic compounds
358 with irradiation (Pereira et al., 2015) possibly due to fruit structure alterations, and/or to
359 the radiolytic breakage of larger phenolic compounds (e.g. tannins) into smaller ones
360 (Hussain, Suradkar, Javaid, Akram, & Parvez, 2016). Despite of the literature scarcity on
361 the effects of electron-beam radiation on raspberries, Guimarães et al. (2013) observed
362 an increase on phenolic content of raspberries with gamma radiation at 2 kGy and during
363 storage, while Cabo Verde et al. (2013) observed an increase of phenolic content with
364 gamma radiation doses up to 1.5 kGy (T0) with decrease during the storage time. Other
365 preservation technologies tested on raspberries indicated different effects on total
366 phenolic content, namely no effect with chlorophyllin-based photosensitization treatment
367 (Rasiukevičiūtė et al., 2015), or a positive impact (higher level of phenolics) by ozonation
368 process (Piechowiak et al., 2019).
369 Concerning FRAP assay results, no variation was observed on the antioxidant activity
370 with the refrigerated storage of the raspberries, except for those stored during 14 days
371 (T14, 0 kGy) that presented significantly ($p < 0.05$) higher antioxidant activity. The e-
372 beam treatment significantly ($p < 0.05$) decreased the antioxidant activity by FRAP of
373 non-stored fruits (T0, 3 kGy), but the storage tended to increase ($p < 0.05$) the antioxidant
374 potential of irradiated fruits that presented similar values ($p > 0.05$) to stored controls.
375 The antioxidant activity of raspberries measured by DPPH scavenging activity, indicated
376 a significant increase ($p < 0.05$) with storage at 4 ºC, with higher values for raspberries
377 stored during 14 days. The e-beam treatment pointed out to preserve the antioxidant
378 activity by DPPH of non-stored raspberries (T0). Although it was detected an increase of
379 TP on non-stored and irradiated raspberries, it was not reflected on an increase of

antioxidant potential as expected. This fact suggests that new phenolic compounds can be formed upon e-beam treatment that do not necessarily exert their antioxidant activity by single electron transfer, which is the dominant reaction mechanism present in both FRAP and DPPH assays. The total antioxidant activity of raspberries should be considered as a combination of different phytochemicals that can act by additive or synergistic effects. In turn, the storage of e-beam treated fruits induced an increase ($p < 0.05$) of antioxidant activity by DPPH after 7 days, which not corresponded to an increase in TP value. This result could reflect an improvement by irradiation and storage on the extractability of non-phenolic antioxidant compounds.

For raspberries treated by gamma radiation, it was observed an increase of antioxidant activity by FRAP with a dose of 1.5 kGy (T0) and a decrease after 14 days of refrigerated storage (Cabo Verde et al., 2013), but Guimarães et al. (2013) observed an increasing trend on antioxidant activity at a dose of 2 kGy during 12 days refrigerated storage. Other post-harvest preservation technologies also indicated dissimilar effects on antioxidant activity of raspberries, for example, chlorophyllin-based photosensitization treatment had no significant change as measured by DPPH (Rasiukevičiūtė et al., 2015), and ozonation process caused an increase (by DPPH) after treatment and a decrease was detected at 48 h of storage (Piechowiak et al., 2019).

The overall results seemed to indicate that e-beam treatment could guarantee the preservation of phenolic content and antioxidant activity of raspberries during 7 days of cold storage.

3.3. Ascorbic acid content

Ascorbic acid is an important water-soluble and carbohydrate-like nutrient that is very sensitive to both chemical and enzymatic oxidation during food processing and storage, when compared to other nutrients. The amount of ascorbic acid in non-treated raspberries was 125±5 mg/100g of dry weight (Figure 2). Immediately after irradiation (T0), a significant decrease ($p < 0.05$) in ascorbic acid content was caused by e-beam

408 treatment. This depletion can easily be attributed to its significant capacity to scavenge
409 radical species formed upon water radiolysis that occurs in the fruit medium, in particular
410 the highly reactive hydroxyl radical. Ascorbic acid also manifests its antioxidant activity
411 by a direct protection of other compounds from oxidative degradation (Wong & Kitts,
412 2001). Both mechanisms result in a (reversible) oxidation of ascorbic acid to
413 dehydroascorbic acid that can be further hydrolyzed and oxidized irreversibly into other
414 products (Deutsch, 2000). During cold storage, ascorbic acid is prone to decrease by
415 enzymatic oxidation. However, the effect on control samples was less pronounced than
416 in treated ones, since after 3 days of storage the amount of ascorbic acid remained
417 similar ($p>0.05$). The antioxidant activity of ascorbic acid by any of the mechanisms
418 referred to above is expected to last during storage for treated raspberries, and this
419 behaviour can explain the significantly higher depletion observed.
420 The obtained results are in agreement with those reported by Tezotto-Uliana et al.
421 (2013), which observed a decrease in ascorbic acid levels for non-irradiated and gamma
422 irradiated raspberries during the storage with higher reduction for higher radiation doses.
423 Similar decreasing tendencies of ascorbic acid was observed on raspberries treated by
424 other non-thermal processes and during refrigerated storage (Piechowiak et al., 2019).
425 The degradation of ascorbic acid present in raspberries did not result on a lower
426 antioxidant activity, which could be justified by the oxidation of ascorbic acid to
427 dehydroascorbic acid (a biologically active compound) as observed by Hussain, Dar, &
428 Wani (2012) for strawberries. It was estimated that ascorbic acid contribute around 20%
429 to the total antioxidant capacity of raspberries (Beekwilder, Hall, & De Vos, 2005).
430 Dehydroascorbic acid has a recognized physiological role since it can be used by
431 metabolically competent cells, where it is reduced back to ascorbic acid, being also
432 widely accepted that dietary ascorbic acid and dehydroascorbic acid have equivalent
433 bioavailability in humans (Wilson, 2002). In this way, the use of irradiation will not result
434 in a severe loss of nutritional value on raspberries.
435

436 **3.4. Cytotoxicity assessment of raspberries extracts**

437 Studies have indicated that in raspberry extracts, some individual polyphenols (e.g.
438 anthocyanins, ellagitannins, and ellagic acid) or together with other compounds (e.g.
439 ascorbic acid, carotenoids) with synergetic effects, have anti-proliferative activity against
440 cancer cells *in vitro* (McDougall, Ross, Ikeji, & Stewart, 2008). In view of all these, the
441 effects of e-beam treatment on the cytotoxicity of raspberries extracts were evaluated by
442 the WST-1 cell viability assay using two human cells lines, human embryonic kidney 293
443 (293T, non-tumor) cell line; and A549 a lung tumor cell line, to assess potential antitumor
444 activity. The obtained results of % of cell viability from the two cell lines exposed to three
445 concentrations of extracts from raspberries non-irradiated, irradiated at 3 kGy, non-
446 stored and stored are presented in Figure 3. For nontumorigenic cell line (293T), the
447 higher extract concentration (400 μg/mL) prompted a significant ($p < 0.05$) inhibitory
448 effect on cell viability, independently of fruit treatment and storage time. The extracts of
449 non-treated and treated fruits at 4 and 40 μg/mL have no significant ($p > 0.05$) effect on
450 cell proliferation, except for the 14 days of storage where all fruits extracts have anti-
451 proliferative activity against 293T cells (Figure 3A). Raspberries extracts, at any
452 concentration from any treatment (non-irradiated/irradiated; non-stores/stored), had no
453 effect ($p > 0.05$) on the growth of A549 lung tumor cell line (Figure 3B), indicating that at
454 the tested conditions the extracts had no *in vitro* anti-proliferative activity against the
455 tumor cells. Considering the obtained results by WST-1 assay, the extracts at the
456 concentrations of 4 μg/mL and 40 μg/mL from the raspberries irradiated at 3 kGy and
457 stored up to 7 days, had no cytotoxic effect towards the tested cells lines.

458 Previous studies indicated that cell lines of different origins have variable sensitivity in
459 growth toward berry extracts (Seeram et al., 2006), as it was observed in the present
460 study. Nevertheless, to the best of our knowledge none of the cells lines applied was
461 studied before against raspberries extracts, but have demonstrated its applicability to
462 evaluate antitumor activity of extracts from irradiated fruits (Madureira et al., 2019) and
463 the cytotoxicity of plant extracts (Grauzdytė, Pukalskas, Viranaicken, El Kalamouni, &

Venskutonis, 2018). In fact, raspberry extracts have shown to suppress the growth *in vitro* of human colon, prostate, breast, and oral tumor cells (Seeram et al., 2006; Skrovankova, Sumczynski, Mlcek, Jurikova, & Sochor, 2015); thus other cells lines should be used to evaluate the anti-proliferative potential of extracts from e-beam treated raspberries considering the detected increases in phenolic content immediately after irradiation and in antioxidant activity after 7 days of storage.

4. Conclusions

E-beam irradiation was studied as a post-harvest treatment for raspberries through the evaluation of microbial inactivation and bioactivity, namely phenolic content, ascorbic acid content, antioxidant activity and cytotoxicity. The results showed that the treatment at 3 kGy could be used to guarantee the food safety of these fruits, extending the shelf-life up to 7 days of storage. Phenolic content and antioxidant activity of raspberries seemed to be preserved with the treatment although a loss in ascorbic acid amount was detected. Moreover, no cytotoxic effect was observed for the raspberries extracts at lower concentrations irradiated at 3 kGy and stored up to 7 days against the tested tumor and non-tumor cell lines. Further studies using different cell lines need to be performed in order to evaluate the anti-proliferative activity.

ACKNOWLEDGMENTS

This work was developed within the Coordinated Research Project D61024 “Development of New Applications of Machine Generated Food Irradiation Technologies” financed by the International Atomic Energy Agency (IAEA). The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support to C2TN (UID/ Multi/04349/2019) and J. Madureira (SFRH/BD/136506/2018).

Declaration of interest: none

REFERENCES

Artés, F., Gómez, P., Aguayo, E., Escalona, V., & Artés-Hernández, F. (2009). Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities. *Postharvest Biology and Technology*, *51*, 287–296. https://doi.org/10.1016/j.postharvbio.2008.10.003

Baugher, J. L., & Jaykus, L. A. (2016). Natural microbiota of raspberries (Rubus idaeus) and strawberries (Fragaria x ananassa): Microbial survey, bacterial isolation and identification, and biofilm characterization. *Acta Horticulturae*, *1133*, 521–526. https://doi.org/10.17660/ActaHortic.2016.1133.82

Beekwilder, J., Hall, R. D., & De Vos, C. H. R. (2005). Identification and dietary relevance of antioxidants from raspberry. *BioFactors*. https://doi.org/10.1002/biof.5520230404

Benzie, I. F. F., & Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, *239*, 70–76.

Bialka, K. L., & Demirci, A. (2007). Utilization of gaseous ozone for the decontamination of Escherichia coli O157:H7 and Salmonella on raspberries and strawberries. *Journal of Food Protection*, *70*(5), 1093–1098. https://doi.org/10.4315/0362-028X-70.5.1093

Bialka, K. L., & Demirci, A. (2008). Efficacy of pulsed UV-light for the decontamination of Escherichia coli O157:H7 and Salmonella spp. on raspberries and strawberries. *Journal of Food Science*. https://doi.org/10.1111/j.1750-3841.2008.00743.x

Bobinait, R., Viškelis, P., & Venskutonis, P. R. (2012). Variation of total phenolics, anthocyanins, ellagic acid and radical scavenging capacity in various raspberry (Rubus spp.) cultivars. *Food Chemistry*, *132*(3), 1495–1501. https://doi.org/10.1016/j.foodchem.2011.11.137

517 Bowen-Forbes, C. S., Zhang, Y., & Nair, M. G. (2010). Anthocyanin content, antioxidant,
518 anti-inflammatory and anticancer properties of blackberry and raspberry fruits.
519 *Journal of Food Composition and Analysis*, *23*(6), 554–560.
520 https://doi.org/10.1016/j.jfca.2009.08.012

521 Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of Free Radical Method to
522 Evaluate Antioxidant Activity. *LWT - Food Science and Technology*, *28*(1), 25–30.
523 https://doi.org/10.1016/S0023-6438(95)80008-5

524 Cabo Verde, S., Trigo, M. J., Sousa, M. B., Ferreira, A., Ramos, A. C., Nunes, I., …
525 Botelho, M. L. (2013). Effects of gamma radiation on raspberries: Safety and quality
526 issues. *Journal of Toxicology and Environmental Health - Part A: Current Issues*,
527 *76*(4–5), 291–303. https://doi.org/10.1080/15287394.2013.757256

528 Callejón, R. M., Rodríguez-Naranjo, M. I., Ubeda, C., Hornedo-Ortega, R., Garcia-
529 Parrilla, M. C., & Troncoso, A. M. (2015). Reported foodborne outbreaks due to
530 fresh produce in the united states and European Union: Trends and causes.
531 *Foodborne Pathogens and Disease*, *12*(1), 32–38.
532 https://doi.org/10.1089/fpd.2014.1821

533 Carstens, C. K., Salazar, J. K., & Darkoh, C. (2019). Multistate Outbreaks of Foodborne
534 Illness in the United States Associated With Fresh Produce From 2010 to 2017.
535 *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.02667

536 da Câmara Correia, M. M. M. B. (2016). *Caracterização química e avaliação da atividade*
537 *biológica da framboesa (Rubus idaeus L.). Contribuição para o desenvolvimento de*
538 *uma alegação de saúde* (Faculdade de Farmácia, Universidade de Lisboa).
539 https://doi.org/10.22533/at.ed.8521926042

540 de Souza, V. R., Pereira, P. A. P., da Silva, T. L. T., de Oliveira Lima, L. C., Pio, R., &
541 Queiroz, F. (2014). Determination of the bioactive compounds, antioxidant activity
542 and chemical composition of Brazilian blackberry, red raspberry, strawberry,

543 blueberry and sweet cherry fruits. *Food Chemistry*, *156*, 362–368.
544 https://doi.org/10.1016/j.foodchem.2014.01.125

545 Deutsch, J. C. (2000). Dehydroascorbic acid. *Journal of Chromatography A*, *881*(1–2),
546 299–307. https://doi.org/10.1016/S0021-9673(00)00166-7

547 Diaconeasa, Z., Florica, R., Rugină, D., Lucian, C., & Socaciu, C. (2014). HPLC/PDA–
548 ESI/MS Identification of Phenolic Acids, Flavonol Glycosides and Antioxidant
549 Potential in Blueberry, Blackberry, Raspberries and Cranberries. *Journal of Food*
550 *and Nutrition Research*, *2*(11), 781–785. https://doi.org/10.12691/jfnr-2-11-4

551 EFSA BIOHAZ Panel - EFSA Panel on Biological Hazards. (2014). Scientific Opinion on
552 the risk posed by pathogens in food of non-animal origin. Part 2 (Salmonella and
553 Norovirus in berries). *EFSA Journal*, *12*(6), 3706.
554 https://doi.org/10.2903/j.efsa.2014.3706

555 Grauzdytė, D., Pukalskas, A., Viranaicken, W., El Kalamouni, C., & Venskutonis, P. R.
556 (2018). Protective effects of Phyllanthus phillyreifolius extracts against hydrogen
557 peroxide induced oxidative stress in HEK293 cells. *PLOS ONE*, *13*(11), e0207672.
558 https://doi.org/10.1371/journal.pone.0207672

559 Guerreiro, D., Madureira, J., Silva, T., Melo, R., Santos, P. M. P., Ferreira, A., … Cabo
560 Verde, S. (2016). Post-harvest treatment of cherry tomatoes by gamma radiation:
561 Microbial and physicochemical parameters evaluation. *Innovative Food Science*
562 *and Emerging Technologies*, *36*, 1–9. https://doi.org/10.1016/j.ifset.2016.05.008

563 Guimarães, I. C., Menezes, E. G. T., Abreu, P. S. de, Rodrigues, A. C., Borges, P. R. S.,
564 Batista, L. R., … Lima, L. C. de O. (2013). Physicochemical and microbiological
565 quality of raspberries (Rubus idaeus) treated with different doses of gamma
566 irradiation. *Food Science and Technology*, *33*(2), 316–322.
567 https://doi.org/10.1590/s0101-20612013005000040

568 Heinonen, M. (2007). Antioxidant activity and antimicrobial effect of berry phenolics – a
569 Finnish perspective. *Molecular Nutrition & Food Research*, *51*(6), 684–691.
570 https://doi.org/10.1002/mnfr.200700006

571 Huang, Y., Sido, R., Huang, R., & Chen, H. (2015). Application of water-assisted pulsed
572 light treatment to decontaminate raspberries and blueberries from Salmonella.
573 *International Journal of Food Microbiology*, *208*, 43–50.
574 https://doi.org/10.1016/j.ijfoodmicro.2015.05.016

575 Hussain, P. R., Dar, M. A., & Wani, A. M. (2012). Effect of edible coating and gamma
576 irradiation on inhibition of mould growth and quality retention of strawberry during
577 refrigerated storage. *International Journal of Food Science and Technology*, *47*(11),
578 2318–2324. https://doi.org/10.1111/j.1365-2621.2012.03105.x

579 Hussain, P. R., Suradkar, P., Javaid, S., Akram, H., & Parvez, S. (2016). Influence of
580 postharvest gamma irradiation treatment on the content of bioactive compounds
581 and antioxidant activity of fenugreek (Trigonella foenum-graceum L.) and spinach
582 (Spinacia oleracea L.) leaves. *Innovative Food Science and Emerging*
583 *Technologies*, *33*, 268–281. https://doi.org/10.1016/j.ifset.2015.11.017

584 Huynh, N. K., Wilson, M. D., Eyles, A., & Stanley, R. A. (2019). Recent advances in
585 postharvest technologies to extend the shelf life of blueberries (Vaccinium sp.),
586 raspberries (Rubus idaeus L.) and blackberries (Rubus sp.). *Journal of Berry*
587 *Research*, *9*(4), 709–724. https://doi.org/10.3233/JBR-190421

588 Issa-Zacharia, A., Kamitani, Y., Muhimbula, H. S., & Ndabikunze, B. K. (2010). A review
589 of microbiological safety of fruits and vegetables and the introduction of electrolyzed
590 water as an alternative to sodium hypochlorite solution. *African Journal of Food*
591 *Science*, *4*(13), 778–789.

592 Johnson, R. (2019). *Foodborne Illnesses and Outbreaks from Fresh Produce*. Retrieved
593 from www.crs.gov%7C7-5700

Kula, M., Majdan, M., Głód, D., & Krauze-Baranowska, M. (2016). Phenolic composition of fruits from different cultivars of red and black raspberries grown in Poland. *Journal of Food Composition and Analysis*, *52*, 74–82. https://doi.org/10.1016/j.jfca.2016.08.003

Lee, H.-H., Hong, S.-I., & Kim, D. (2014). Microbial reduction efficacy of various disinfection treatments on fresh-cut cabbage. *Food Science & Nutrition*, *2*(5), 585–590. https://doi.org/10.1002/fsn3.138

Liao, Y. Te, Syamaladevi, R. M., Zhang, H., Killinger, K., & Sablani, S. (2017). Inactivation of listeria monocytogenes on frozen red raspberries by using uv-c light. *Journal of Food Protection*, *80*(4), 545–550. https://doi.org/10.4315/0362-028X.JFP-16-245

Lukasik, J., Bradley, M. L., Scott, T. M., Dea, M., Koo, A., Hsu, W.-Y., … Farrah, S. R. (2003). Reduction of Poliovirus 1, Bacteriophages, Salmonella Montevideo, and Escherichia coli O157:H7 on Strawberries by Physical and Disinfectant Washes † ‡. In *Journal of Food Protection* (Vol. 66).

Lung, H.-M., Cheng, Y.-C., Chang, Y.-H., Huang, H.-W., Yang, B. B., & Wang, C.-Y. (2015). Microbial decontamination of food by electron beam irradiation. *Trends in Food Science & Technology*, *44*, 66–78. https://doi.org/10.1016/j.tifs.2015.03.005

Madureira, J., Severino, A., Cojocaru, M., Garofalide, S., Santos, P. M. P., Carolino, M. M., … Cabo Verde, S. (2019). E-beam treatment to guarantee the safety and quality of cherry tomatoes. *Innovative Food Science and Emerging Technologies*, *55*, 57–65. https://doi.org/10.1016/j.ifset.2019.05.013

McDougall, G. J., Ross, H. A., Ikeji, M., & Stewart, D. (2008). Berry extracts exert different antiproliferative effects against cervical and colon cancer cells grown in vitro. *Journal of Agricultural and Food Chemistry*. https://doi.org/10.1021/jf073469n

619 Miller, A. (1983). Dosimetry for Electron Beam Applications. *Citation*, (2401).

620 Mullen, W., Mcginn, J., Lean, M. E. J. J., Maclean, M. R., Gardner, P., Duthie, G. G., …
621 Crozier, A. (2002). Ellagitannins, flavonoids, and other phenolics in red raspberries
622 and their contribution to antioxidant capacity and vasorelaxation properties. *Journal*
623 *of Agricultural and Food Chemistry*, *50*(18), 5191–5196.
624 https://doi.org/10.1021/jf020140n

625 Oliveira, M., Rodrigues, C. M., & Teixeira, P. (2019). Microbiological quality of raw berries
626 and their products: A focus on foodborne pathogens. *Heliyon*, *5*(12).
627 https://doi.org/10.1016/j.heliyon.2019.e02992

628 Paiva De Sousa, C. (2008). The Impact of Food Manufacturing Practices on Food borne
629 Diseases. *BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY*, *51*(4), 815–
630 823.

631 Parish, M. E., Beuchat, L. R., Suslow, T. V, Harris, L. J., Garrett, E. H., Farber, J. N., &
632 Busta, F. F. (2003). Methods to Reduce/ Eliminate Pathogens from Fresh and
633 Fresh-Cut Produce. *Comprehensive Reviews in Food Science and Food Safety*, *2*,
634 161–173.

635 Pereira, E., Barros, L., Dueñas, M., Antonio, A. L., Santos-Buelga, C., & Ferreira, I. C.
636 F. R. (2015). Gamma irradiation improves the extractability of phenolic compounds
637 in Ginkgo biloba L. *Industrial Crops and Products*, *74*, 144–149.
638 https://doi.org/10.1016/j.indcrop.2015.04.039

639 Piechowiak, T., Antos, P., Kosowski, P., Skrobacz, K., Józefczyk, R., & Balawejder, M.
640 (2019). Impact of ozonation process on the microbiological and antioxidant status
641 of raspberries (Rubus ideaeus L.) during storage at room temperature. *Agricultural*
642 *and Food Science*, *28*(1), 35–44. https://doi.org/10.23986/afsci.70291

643 Pimenta, A. I., Margaça, F. M. A., & Cabo Verde, S. (2019). Virucidal activity of gamma

radiation on strawberries and raspberries. *International Journal of Food Microbiology*, *304*, 89–96. https://doi.org/10.1016/j.ijfoodmicro.2019.05.011

Pinela, J., Barreira, J. C. M., Barros, L., Cabo Verde, S., Antonio, A. L., Carvalho, A. M., … Ferreira, I. C. F. R. (2016). Suitability of gamma irradiation for preserving fresh-cut watercress quality during cold storage. *Food Chemistry*, *206*, 50–58. https://doi.org/10.1016/j.foodchem.2016.03.050

Puupponen-Pimia, R., Nohynek, L., Hartmann-Schmidlin, S., Kahkonen, M., Heinonen, M., Maatta-Riihinen, K., … Oksman-Caldentey, K.-M. M. (2005). Berry phenolics selectively inhibit the growth of intestinal pathogens. *Journal of Applied Microbiology*, *98*(4), 991–1000. https://doi.org/10.1111/j.1365-2672.2005.02547.x

Rasiukevičiūtė, N., Valiuškaitė, A., Uselis, N., Buskienė, L., Viškelis, J., & Lukšienė, Ž. (2015). New non-chemical postharvest technologies reducing berry contamination. *Zemdirbyste-Agriculture*, *102*(4), 411–416. https://doi.org/10.13080/z-a.2015.102.052

Santos, M. I., Correia, C., Cunha, M. I. C., Saraiva, M. M., & Novais, M. R. (2005). Valores guia para avaliação da qualidade microbiológica de alimentos prontos a comer preparados em estabelecimentos de restauração. *Revista Da Ordem Dos Farmacêuticos*, *64*, 66–68.

Sariburun, E., Şahin, S., Demir, C., Türkben, C., & Uylaşer, V. (2010). Phenolic content and antioxidant activity of raspberry and blackberry cultivars. *Journal of Food Science*, *75*(4). https://doi.org/10.1111/j.1750-3841.2010.01571.x

Seeram, N. P., Adams, L. S., Zhang, Y., Lee, R., Sand, D., Scheuller, H. S., & Heber, D. (2006). *Blackberry, Black Raspberry, Blueberry, Cranberry, Red Raspberry, and Strawberry Extracts Inhibit Growth and Stimulate Apoptosis of Human Cancer Cells In Vitro*. https://doi.org/10.1021/jf061750g

669 Shahbaz, H. M., Ahn, J.-J., Akram, K., Kim, H.-Y., Parl, E.-J., & Kwon, J.-H. (2014).
670 Chemical and sensory quality of fresh pomegranate fruits exposed to gamma
671 radiation as quarantine treatment. *Food Chemistry*, *145*, 312–318.

672 Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1998). Analysis of total
673 phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu
674 reagent. *Methods in Enzymology*, *299*, 152–178. https://doi.org/10.1016/S0076-
675 6879(99)99017-1

676 Skrovankova, S., Sumczynski, D., Mlcek, J., Jurikova, T., & Sochor, J. Bioactive
677 compounds and antioxidant activity in different types of berries. , 16 International
678 Journal of Molecular Sciences § (2015).

679 Sy, K. V, McWatters, K. H., & Beuchat, L. R. (2005). Efficacy of gaseous chlorine dioxide
680 as a sanitizer for killing Salmonella, yeasts, and molds on blueberries, strawberries,
681 and raspberries. *Journal of Food Protection*, *68*(6), 1165–1175.
682 https://doi.org/10.4315/0362-028X-68.6.1165

683 Tavoschi, L., Severi, E., Niskanen, T., Boelaert, F., Rizzi, V., Liebana, E., … Coulombier,
684 D. (2015). Food-borne diseases associated with frozen berries consumption: a
685 historical perspective, European Union, 1983 to 2013. *Eurosurveillance*, *20*(29).
686 https://doi.org/10.2807/1560-7917.ES2015.20.29.21193

687 Teng, H., Fang, T., Lin, Q., Song, H., Liu, B., & Chen, L. (2017). Red raspberry and its
688 anthocyanins: Bioactivity beyond antioxidant capacity. *Trends in Food Science and*
689 *Technology*, *66*, 153–165. https://doi.org/10.1016/j.tifs.2017.05.015

690 Tezotto-Uliana, J. V., Berno, N. D., Saji, F. R. Q., & Kluge, R. A. (2013). Gamma
691 radiation: An efficient technology to conserve the quality of fresh raspberries.
692 *Scientia Horticulturae*, *164*, 348–352. https://doi.org/10.1016/j.scienta.2013.09.026

693 Van Der Steen, C., Jacxsens, L., Devlieghere, F., & Debevere, J. (2002). Combining high

694 oxygen atmospheres with low oxygen modified atmosphere packaging to improve
695 the keeping quality of strawberries and raspberries. *Postharvest Biology and*
696 *Technology*, *26*(1), 49–58. https://doi.org/10.1016/S0925-5214(02)00005-4

697 Wei, K., Zhou, H., Zhou, T., & Gong, J. (2007). Comparison of aqueous ozone and
698 chlorine as sanitizers in the food processing industry: Impact on fresh agricultural
699 produce quality. *Ozone: Science and Engineering*, *29*(2), 113–120.
700 https://doi.org/10.1080/01919510601186592

701 Wilson, J. X. (2002). The physiological role of dehydroascorbic acid. *FEBS Letters*.
702 https://doi.org/10.1016/S0014-5793(02)03167-8

703 Wong, P. Y. Y., & Kitts, D. D. (2001). Factors influencing ultraviolet and electron beam
704 irradiation-induced free radical damage of ascorbic acid. *Food Chemistry*, *74*(1),
705 75–84. https://doi.org/10.1016/S0308-8146(01)00101-7

706 World Health Organisation, & Food and Agriculture Organization of the United Nations.
707 (2000). FOOD IRRADIATION A technique for preserving and improving the safety
708 of food. In *Food Irradiation*.

709 Ziegler, M., Kent, D., Stephan, R., & Guldimann, C. (2019). Growth potential of Listeria
710 monocytogenes in twelve different types of RTE salads: Impact of food matrix,
711 storage temperature and storage time. *International Journal of Food Microbiology*,
712 *296*, 83–92. https://doi.org/10.1016/j.ijfoodmicro.2019.01.016

713

714

715

716

717

Figures Captions

Figure 1. – Natural microbiota counts for non-irradiated (white) and irradiated raspberries (light grey 2 kGy; dark grey 3 kGy) immediately after irradiation (T0) and after 3 (T3), 7 (T7) and 14 (T14) days of refrigerated storage: A) aerobic mesophilic bacterial population, and B) filamentous fungi population. Standard deviation bars correspond to 95% confidence intervals about mean values (n=18; α=0.05).

Figure 2. Effect of electron-beam radiation on ascorbic acid content (mg/100g of dry weight) of raspberries during the storage. Standard deviation bars correspond to 95% confidence intervals about mean values (n=6; α=0.05). Bars not followed by the same lowercase letter are significantly different ($p < 0.05$).

Figure 3. Cellular viability of A) 293T and B) A549 cell lines in the presence of different concentrations (4 µg/mL, 40 µg/mL and 400 µg/mL) of raspberries extracts from non-irradiated (0 kGy) and 3 kGy e-beam irradiated samples, immediately after irradiation (T0) and after 3 (T3), 7 (T7) and 14 (T14) days of refrigerated storage. Each bar graph represents the mean and 95% confidence interval of six experiments. For each cell line, bars with * indicates a statistically significant difference from control at $p < 0.05$.

745

746

Tables

Table 1. Counts of *Salmonella* Typhimurium, *Escherichia coli* and *Listeria monocytogenes* on non-irradiated (0 kGy) and irradiated (0.5 kGy up to 3.0 kGy) spiked fresh raspberries, immediately after irradiation (T0), after 3 (T3), 7 (T7) and 14 (T14) days of refrigerated storage. The results are presented as the mean ± standard error.

Salmonella Typhimurium log CFU/g					*Escherichia coli* log CFU/g					*Listeria monocytogenes* log CFU/g				
Dose (kGy)	T0	T3	T7	T14	Dose (kGy)	T0	T3	T7	T14	Dose (kGy)	T0	T3	T7	T14
0	3.4 ± 0.1^{a}	2.8 ± 0.1^{b}	2.7 ± 0.2^{b}	2.7 ± 0.1^{b}	0 kGy	3.0 ± 0.1^{a}	3.1 ± 0.1^{a}	2.3 ± 0.1^{b}	2.2 ± 0.1^{b}	0 kGy	3.1 ± 0.1^{a}	2.8 ± 0.1^{a}	3.0 ± 0.1^{a}	ND
0.5	2.7 ± 0.1^{b}	2.3 ± 0.1^{c}	2.1 ± 0.3^{c}	1.6 ± 0.1^{d}	0.5 kGy	2.4 ± 0.2^{b}	1.9 ± 0.3^{b}	1.9 ± 0.3^{b}	1.6 ± 0.3^{b}	0.5 kGy	1.7 ± 0.2^{b}	2.0 ± 0.2^{b}	1.9 ± 0.2^{b}	ND
1.5	1.5 ± 0.2^{d}	0.6 ± 0.1^{e}	0.6 ± 0.1^{e}	ND	1 kGy	1.1 ± 0.3^{c}	ND	ND	ND	0.8 kGy	1.0 ± 0.2^{c}	1.1 ± 0.2^{c}	0.9 ± 0.1^{c}	ND
3	ND	ND	ND	ND	3 kGy	ND	ND	ND	ND	3 kGy	ND	ND	ND	ND

ND - not detected. For the same bacterium, values not followed by the same lowercase letter are significantly different ($p<0.05$).

767 Table 2. Antioxidant activity (DPPH and FRAP assays) and Total Phenolic Content in
768 extracts of non-irradiated and irradiated raspberries analysed immediately after e-beam
769 irradiation and during 14 days of refrigerated storage. The results are presented as the
770 mean ± standard error.

Storage time (days)	Dose (kGy)	DDPH scavenging activity (EC_{50} µg/mL)	FRAP (mmol FES/100g dw)	Total Phenolic Content (GAE mg/100g dw)
0	0	2028±24^{a}	17.5±0.1^{b}	1092±3^{b}
	3	1964±39^{a}	13±1^{c}	1405±75^{a}
3	0	1698±17^{b}	17.2±0.1^{b}	1054±13^{b}
	3	1924±36^{a}	18.3±0.6^{a,b}	1012±87^{b}
7	0	1706±38^{b}	17.8±0.5^{b}	1078±5^{b}
	3	1651±24^{b}	18±1^{a,b}	1099±70^{b}
14	0	1201±12^{d}	21.3±0.1^{a}	1145±23^{a,b}
	3	1401±26^{c}	20.3±0.2^{a,b}	1067±59^{b}

771 Within the column, values not followed by the same lowercase letter are significantly different
772 ($p<0.05$).
773
774
775

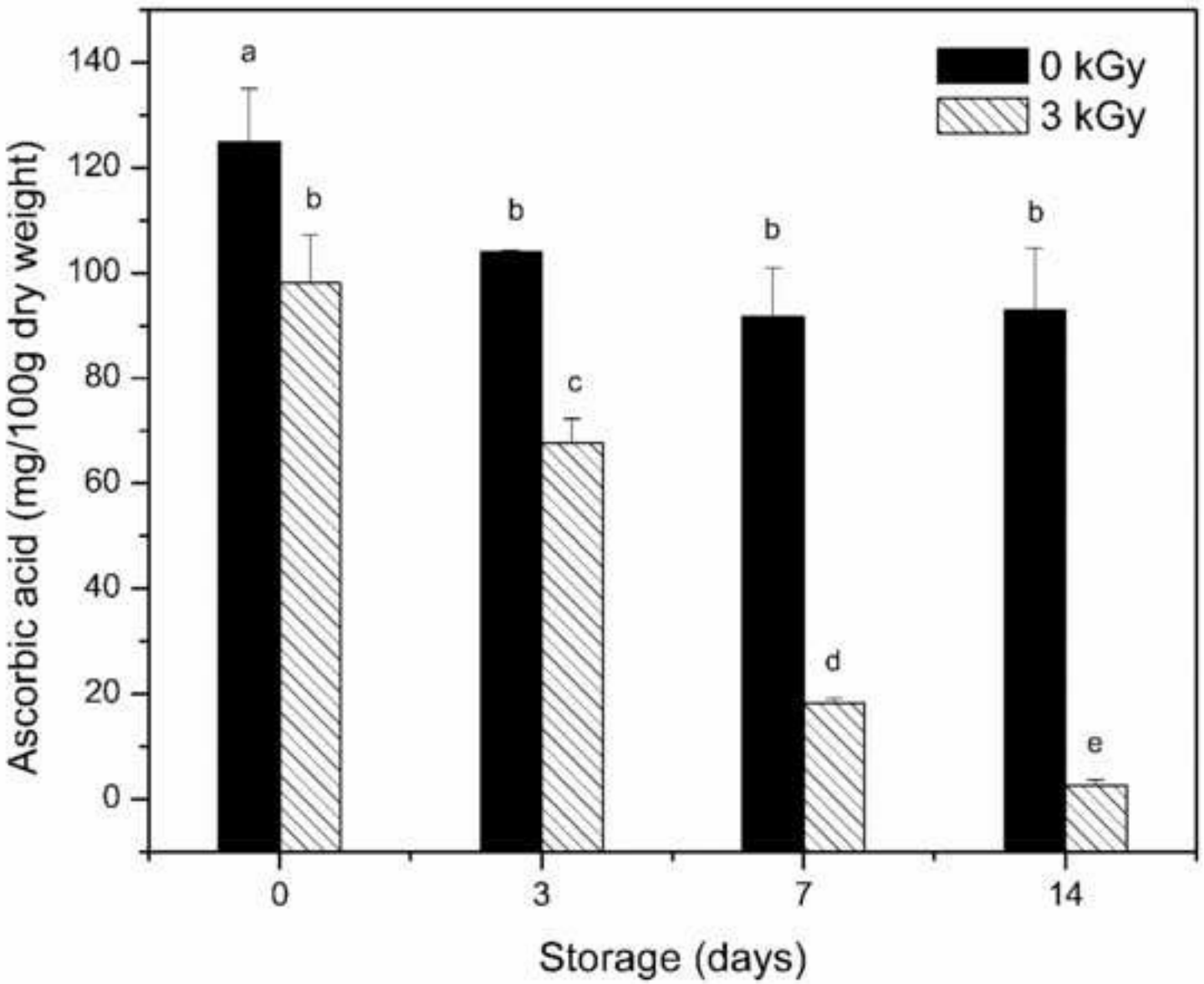


Figure 1

Click here to access/download;Figure;Figure1.tif

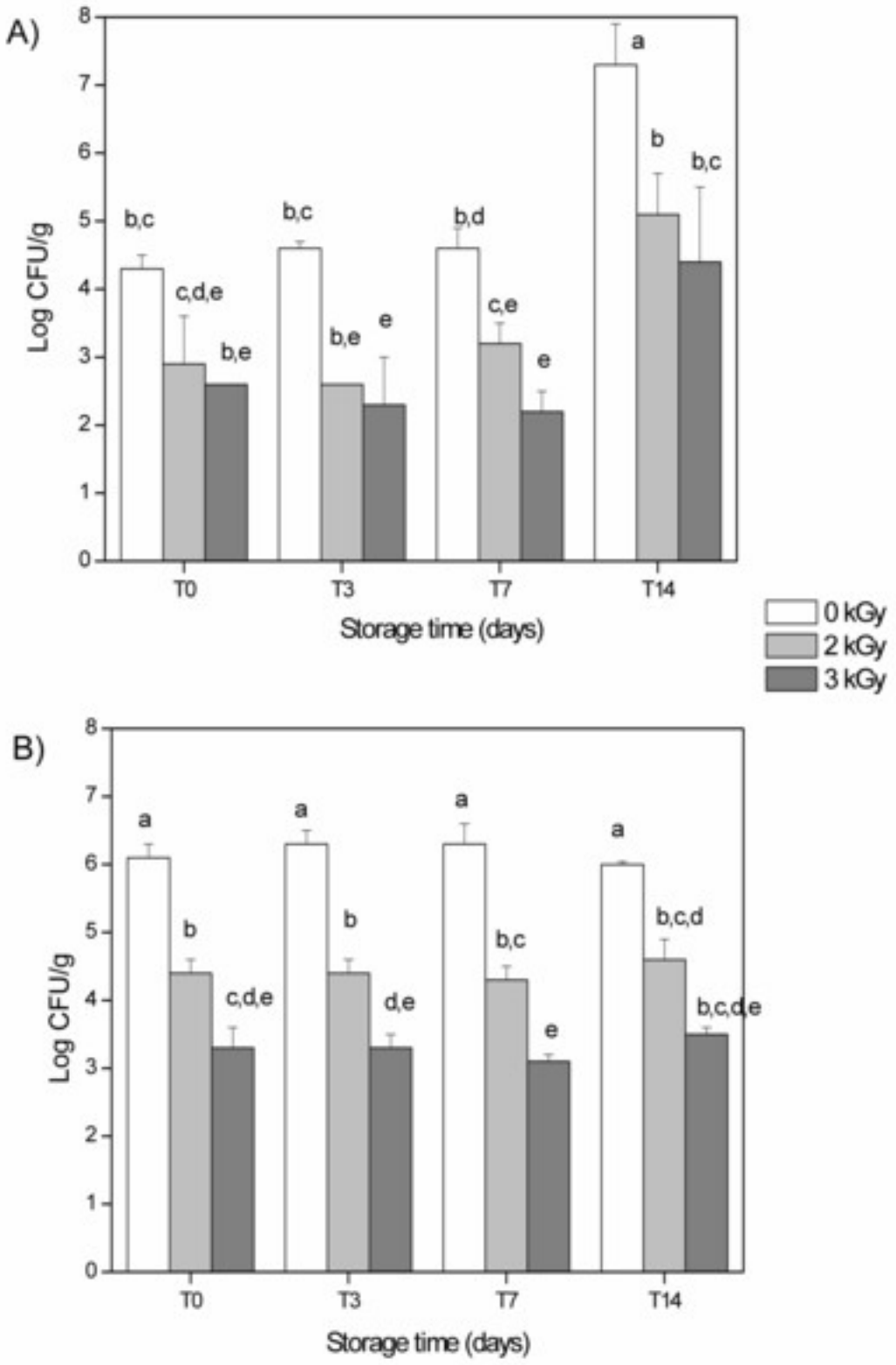
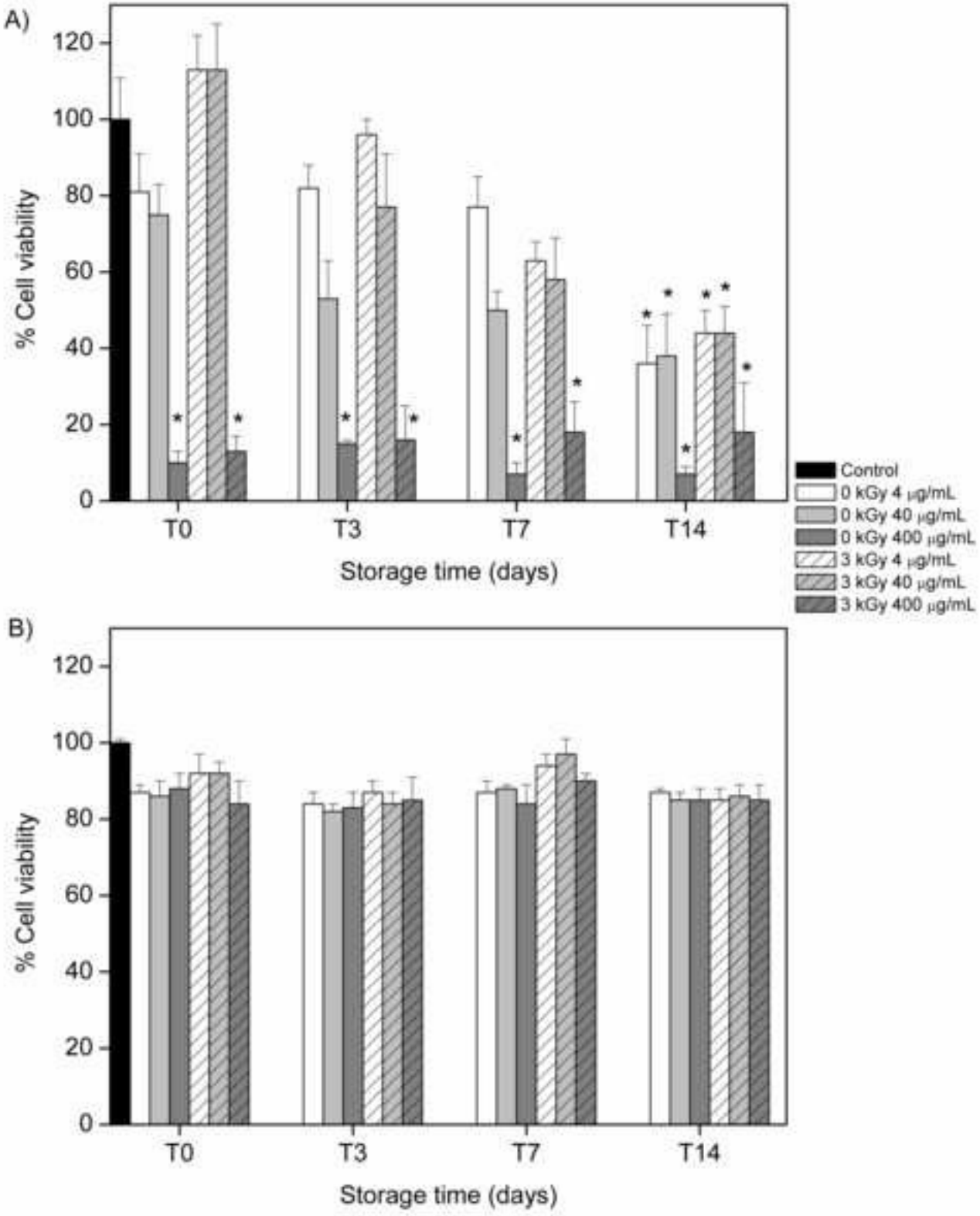


Figure 3

Click here to access/download;Figure;Figure3.tif



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Author statement

Conceptualization and Methodology: Sandra Cabo Verde; **Investigation:** Maria Inês Elias, Joana Madureira; **Writing – Original Draft:** Joana Madureira; **Writing – Review & Editing:** Sandra Cabo Verde, Pedro Santos and Fernanda Margaça; **Supervision:** Sandra Cabo Verde and Maria Manuela Carolino.