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Preservation treatment of fresh raspberries by e-beam irradiation

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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 4 °C. 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.

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 et al., 2010; de Souza et al., 2014; Sariburun et al., 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 et al., 2012; Bowen-Forbes et al., 2010; Diaconeasa et al., 2014; Kula et al., 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 et al., 2019). Concerning berries, the majority of outbreaks associated to them have been caused by viruses, namely norovirus and

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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 et al., 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 et al., 2007). Despite of the general use of sodium hypochlorite and hydrogen peroxide as sanitizers (Artés et al., 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 et al., 2010; Lee et al., 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 et al. (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 et al., 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 hydrolyzable tannins, total flavonoids and total anthocyanins, and chosen for biological assays and for a study of nutritional intervention in humans.

2.2. Irradiation experiments

Irradiation experiments were carried out in a linear electron-beam accelerator (LINAC, adapted from GE Saturne 41) with an energy of 10 MeV located at the ionizing radiation facility IRIS from Centro de Ciências e Tecnologias Nucleares (C2TN) of Instituto Superior Técnico, Universidade de Lisboa.

Fresh raspberries were irradiated in plastic boxes (150 g; one box per dose) at room temperature at doses from 0.5 to 3 kGy at an average dose rate of 0.5 kGy min^{-1} with dose uniformity (DUR) of 1.1. The absorbed dose was estimated using calibrated radiochromic dosimeters FWT-60 (Far West Technology, Inc. Goleta, USA) (Miller, 1983). Three independent irradiation batches were performed per each assay. Non-irradiated samples (0 kGy) were used as control and followed all the experiments.

2.3. Microbial inactivation studies

2.3.1. Natural microbiota

Non-irradiated and irradiated raspberries (25 g) were placed in sterile stomacher bags containing 100 mL of 0.1% Tween 80 physiological solution. Samples (n = 3/dose) were homogenized using a stomacher (Stomacher 3500; Seaward, UK) for 15 min. Serial decimal dilutions were prepared for inoculation in triplicate on Tryptic Soy Agar plates (TSA) for mesophilic microbial counts and Malt Extract Agar (MEA) plates for filamentous fungi counts. Samples were incubated at 30 °C for TSA plates and 28 °C for MEA plates and colony numbers were counted for 7 days. The results were expressed as log colony-forming units per gram of fresh fruit (log CFU/g).

2.3.2. 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. The assay was made in triplicate. For quantification purposes, a calibration plot was performed under the experimental conditions used. Values were expressed as mg per 100 g of raspberries dry weight (dw).

2.4.2. Total phenolic content

The total phenolic content was determined based on Folin-Ciocalteau method (Singleton et al., 1998), in extracts concentrated at 5 mg/mL. The standard curve was calculated using gallic acid (Sigma, St. Louis, US) and the results were expressed as mg of gallic acid equivalents (GAE) per 100 g of raspberries dry weight (dw) (Guerreiro et al., 2016). The assay was carried out in triplicate.

2.4.3. Antioxidant activity

The antioxidant activity was evaluated by two assays based on different mechanisms of action: DPPH radical scavenging activity described by Brand-Williams et al. (1995) with some modifications (Madureira et al., 2019) using EZ Read 2000 Microplate Reader (Biochrom, Cambridge, UK) and Ferric Reducing Antioxidant Power (FRAP) described by Benzie and Strain (1996) using a spectrophotometer (Shimadzu UV 1800, Kyoto, Japan). For FRAP assay, the results were expressed as mmol of ferrous sulfate equivalent (FSE) per 100 g raspberries dry weight (dw). For DPPH method, L-ascorbic acid (*E*-Merck, Darmstadt, Germany) was used as standard compound for the calibration. The antioxidant activity measured by DPPH scavenging activity was expressed as EC50 values (mean ± standard error), which means that higher values correspond to lower antioxidant potential (EC50: extract concentration corresponding to 50% of antioxidant activity). Both assays were made in triplicate.

2.4.4. Cytotoxicity assay - WST-1 proliferation test

Human lung carcinoma epithelial cells (A549, ATCC® CCL-185TM) and human embryonic kidney epithelial cells (293 T, 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 (4 °C) 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 $\alpha = 0.05$.

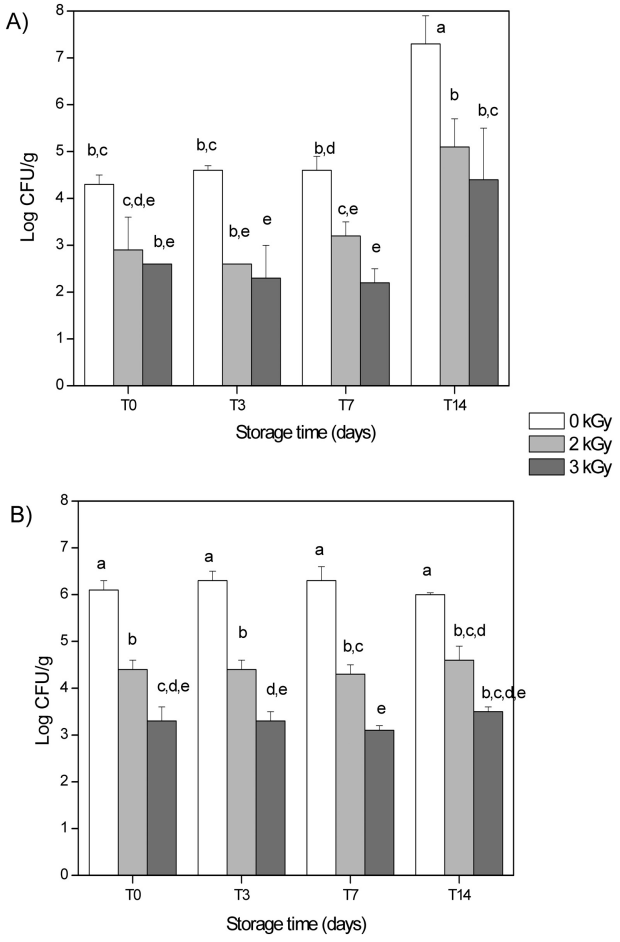

Fig. 1. Natural microbiota counts for non-irradiated (white) and irradiated raspberries (light grey 2 kGy; dark grey 3 kGy) immediately after irradiation (T0) and after 3 (T3), 7 (T7) and 14 (T14) days of refrigerated storage: A) aerobic mesophilic bacterial population, and B) filamentous fungi population.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

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).

3.1. Microbial inactivation

The aerobic mesophilic bacteria and filamentous fungi populations of fresh raspberries were assessed before and after e-beam treatment, immediately after irradiation (T0) and after several periods, namely 3 days (T3), 7 days (T7) and 14 days (T14) of refrigerated storage, in order to evaluate the microbial inactivation and its trend with the treatment and storage. The fresh raspberries indicated an aerobic bacterial mesophilic population of 4.3 ± 0.1 log CFU/g and a filamentous fungi population of 6.1 ± 0.1 log CFU/g (Fig. 1). Previously, an average bioburden between 4 and 6 log CFU/g was reported for fresh raspberries (Baugher & Jaykus, 2016; Cabo Verde et al., 2013; Piechowiak et al., 2019), that supports the obtained results. Nevertheless, the production practices, growth conditions in combination with harvesting and processing, can affect the microbiological quality

of berries at the time of consumption (Oliveira et al., 2019).

With e-beam treatment at 3 kGy (T0) the mesophilic bacterial population of raspberries decreased ($p < 0.05$) 2 log CFU/g and the filamentous fungi reduced ($p < 0.05$) 3 log CFU/g comparatively to non-treated samples (Fig. 1). The e-beam treatment allowed to comply with the Portuguese recommended criteria for fresh fruits and vegetables (bacterial counts at 30 °C < 4 log CFU/g; filamentous fungi < 5 log CFU/g; Santos et al., 2005). In fact, there is no regular monitoring of berries and the current European Union legal framework does not include microbiological criteria applicable for these fruits at the primary production stage (Oliveira et al., 2019).

The bacterial counts of non-treated fruits remained constant ($p > 0.05$) during 7 days of refrigerated storage, but an increase ($p < 0.05$) of 3 log CFU/g was observed at 14 day of storage. Nevertheless, the fungal population remained ($p > 0.05$) at approximately 6 log CFU/g during the 14 days of refrigerated storage (Fig. 1). A statistically significant growth of bacteria up to 7 log CFU/g was cited after 24 h of storage of fresh raspberries at room temperature (Piechowiak et al., 2019). For irradiated raspberries the 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 (Fig. 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 h (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.

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 ($p < 0.05$).

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 et al., 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 (K.L. 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} (K.L. 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 (Te Liao et al., 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 et al., 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 min (Sy, Sy et al., 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 comply with the microbiological criteria. The obtained TP value for non-irradiated fruits was 1092 ± 3 mg GAE/100 g dry weight and, with exception of non-stored irradiated sample (T0, 3 kGy), no significant trend was verified for the 14 days of storage at 4 °C. The irradiation of raspberries at 3 kGy seemed to increase significantly ($p < 0.05$) the phenolic content (1405 ± 75 mg GAE/100 g dry weight) in comparison to control sample. This increase could be related to an improvement of extractability of phenolic compounds with irradiation (Pereira et al., 2015) possibly due to fruit structure

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

Storage time	Dose	DDPH scavenging activity	FRAP	Total Phenolic Content
(days)	(kGy)	(EC_{50} μg/mL)	(mmol FES/100 g dw)	(GAE mg/100 g 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}

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

alterations, and/or to the radiolytic breakage of larger phenolic compounds (e.g. tannins) into smaller ones (P.R. Hussain et al., 2016). Despite of the literature scarcity on the effects of electron-beam radiation on raspberries, Guimarães et al. (2013) observed an increase on phenolic content of raspberries with gamma radiation at 2 kGy and during storage, while Cabo Verde et al. (2013) observed an increase of phenolic content with gamma radiation doses up to 1.5 kGy (T0) with decrease during the storage time. Other preservation technologies tested on raspberries indicated different effects on total phenolic content, namely no effect with chlorophyllin-based photosensitization treatment (Rasiukevičiūtė et al., 2015), or a positive impact (higher level of phenolics) by ozonation process (Piechowiak et al., 2019).

Concerning FRAP assay results, no variation was observed on the antioxidant activity with the refrigerated storage of the raspberries, except for those stored during 14 days (T14, 0 kGy) that presented significantly ($p < 0.05$) higher antioxidant activity. The e-beam treatment significantly ($p < 0.05$) decreased the antioxidant activity by FRAP of non-stored fruits (T0, 3 kGy), but the storage tended to increase ($p < 0.05$) the antioxidant potential of irradiated fruits that presented similar values ($p > 0.05$) to stored controls.

The antioxidant activity of raspberries measured by DPPH scavenging activity, indicated a significant increase ($p < 0.05$) with storage at 4 °C, with higher values for raspberries stored during 14 days. The e-beam treatment pointed out to preserve the antioxidant activity by DPPH of non-stored raspberries (T0). Although it was detected an increase of 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

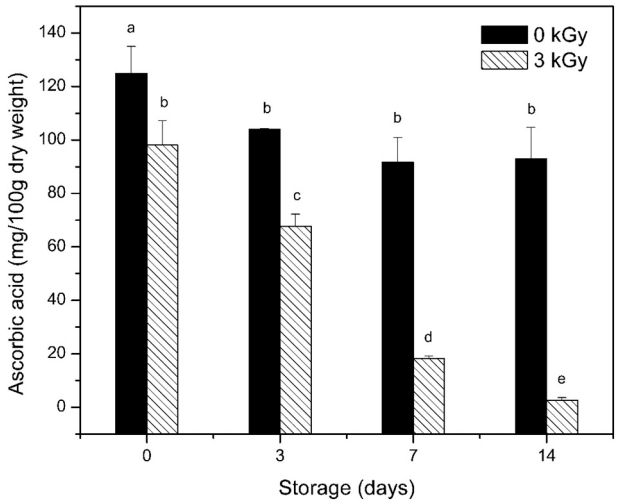

Fig. 2. Effect of electron-beam radiation on ascorbic acid content (mg/100 g of dry weight) of raspberries during the storage. Standard deviation bars correspond to 95% confidence intervals about mean values ($n = 6$; $\alpha = 0.05$). Bars not followed by the same lowercase letter are significantly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

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/100 g of dry weight (Fig. 2). Immediately after irradiation (T0), a significant decrease ($p < 0.05$) in ascorbic acid content was caused by e-beam treatment. This depletion can easily be attributed to its significant capacity to scavenge radical species formed upon water radiolysis that occurs in the fruit medium, in particular the highly reactive hydroxyl radical. Ascorbic acid also manifests its antioxidant activity by a direct protection of other compounds from oxidative degradation (Wong & Kitts, 2001). Both mechanisms result in a (reversible) oxidation of ascorbic acid to dehydroascorbic acid that can be further hydrolyzed and oxidized irreversibly into other products (Deutsch, 2000). During cold storage, ascorbic acid is prone to decrease by enzymatic oxidation. However, the effect on control samples was less pronounced than in treated ones, since after 3 days of storage the amount of ascorbic acid remained similar ($p > 0.05$). The antioxidant activity of ascorbic acid by any of the mechanisms referred to above is expected to last during storage for treated raspberries, and this behaviour can explain the significantly higher depletion observed.

The obtained results are in agreement with those reported by Tezotto-Uliana et al. (2013), which observed a decrease in ascorbic acid levels for non-irradiated and gamma irradiated raspberries during the storage with higher reduction for higher radiation doses. Similar decreasing tendencies of ascorbic acid was observed on raspberries treated by other non-thermal processes and during refrigerated storage (Piechowiak et al., 2019). The degradation of ascorbic acid present in raspberries did not result on a lower antioxidant activity, which could be justified by the oxidation of ascorbic acid to dehydroascorbic acid (a biologically active compound) as observed by P.R. Hussain et al. (2012) for strawberries. It was estimated that ascorbic acid contribute around 20% to the total antioxidant capacity of raspberries (Beekwilder et al., 2005). Dehydroascorbic acid has a recognized physiological role since it can be used by metabolically competent cells, where it is reduced back to ascorbic acid, being also widely accepted that dietary ascorbic acid and dehydroascorbic acid have equivalent bioavailability in humans (Wilson, 2002). In this way, the use of irradiation will not result in a severe loss of nutritional value on raspberries.

3.4. Cytotoxicity assessment of raspberries extracts

Studies have indicated that in raspberry extracts, some individual polyphenols (e.g. anthocyanins, ellagitannins, and ellagic acid) or together with other compounds (e.g. ascorbic acid, carotenoids) with synergetic effects, have anti-proliferative activity against cancer cells in vitro (McDougall et al., 2008). In view of all these, the effects of e-beam treatment on the cytotoxicity of raspberries extracts were evaluated by the WST-1 cell viability assay using two human cells lines, human embryonic kidney 293 (293 T, non-tumor) cell line; and A549 a lung tumor cell line, to assess potential antitumor activity. The obtained results of % of cell viability from the two cell lines exposed to three concentrations of extracts from raspberries non-irradiated, irradiated at 3 kGy, non-stored and stored are presented in Fig. 3. For non-tumorigenic cell line (293 T), the higher extract concentration (400 μg/mL) prompted a significant ($p < 0.05$) inhibitory effect on cell viability, independently of fruit treatment and storage time. The extracts of non-treated and treated fruits at 4 and 40 μg/mL have no significant ($p > 0.05$) effect on cell proliferation, except for the 14 days of storage where all fruits extracts have anti-proliferative activity against 293 T

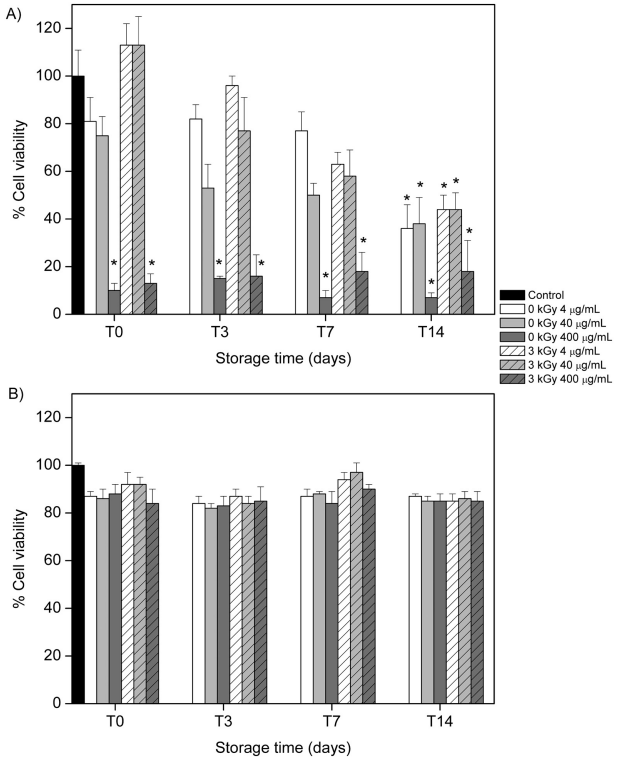

Fig. 3. Cellular viability of A) 293 T and B) A549 cell lines in the presence of different concentrations (4 μg/mL, 40 μg/mL and 400 μg/mL) of raspberries extracts from non-irradiated (0 kGy) and 3 kGy e-beam irradiated samples, immediately after irradiation (T0) and after 3 (T3), 7 (T7) and 14 (T14) 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 $p < 0.05$.

cells (Fig. 3A). Raspberries extracts, at any concentration from any treatment (non-irradiated/irradiated; non-stores/stored), had no effect ($p > 0.05$) on the growth of A549 lung tumor cell line (Fig. 3B), indicating that at the tested conditions the extracts had no in vitro anti-proliferative activity against the tumor cells. Considering the obtained results by WST-1 assay, the extracts at the concentrations of 4 μg/mL and 40 μg/mL from the raspberries irradiated at 3 kGy and stored up to 7 days, had no cytotoxic effect toward the tested cells lines.

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

CRediT authorship contribution 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.

Declaration of competing interest

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.

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