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Virucidal activity of gamma radiation on strawberries and raspberries

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ABSTRACT

The environmental stability of enteric viruses and resistance to conventional treatments and common disinfectants, leads to their persistence in waters and food, causing serious implications on public health. Among non-thermal treatment methods, ionizing radiation is recognized as a useful and effective mean of disinfection.

The objective of this study was to estimate the inactivation of enteric virus by gamma radiation in raw berry fruits, in order to evaluate the potential of this technology to be applied as a disinfection treatment.

Fresh strawberries and raspberries were inoculated either individually with murine norovirus type 1 (MuNoV; as a human norovirus surrogate) and human adenovirus type 5 (HAdV) or with a viral pool of both viruses, and irradiated in a Co-60 equipment at doses of 1 kGy up to 11 kGy. The infectivity of viral particles of MuNoV and HAdV was assessed by plaque assay using Raw 264.7 and A549 cells, respectively.

A 2 log PFU/g reduction on MuNoV and HAdV titers was obtained after treatment with a dose of 4 kGy for both fruits. However, non-linear inactivation survival curves were obtained for MuNoV and HAdV in fresh fruits, leading to the detection of infective viral particles at a dose of 11 kGy. The irradiation process indicated virucidal potential, although the estimated gamma radiation dose to attain food safety (> 7 kGy) would compromise the preservation of food quality. Nevertheless, the irradiation technology could be an effective virus mitigation tool to treat polluted waters, which are a major vehicle of contamination for fresh produce.

1. Introduction

Gastroenteritis outbreaks due to consumption of contaminated fresh products are still a major food safety burden around the world. It is estimated that 67% of foodborne reports are caused by enteric viral pathogens (Melgaço et al., 2016; Painter et al., 2013). Human enteric viruses like norovirus (NoVs) and adenoviruses (HAdVs) are important players in fresh food-associated outbreaks (Mäde et al., 2013; Maunula et al., 2013). Enteric viruses are primarily transmitted by the ingestion of contaminated water or food. Infected humans can excrete high loads of enteric viruses (i.e. 10^6–10^{11} virus particles per g of stools) for several weeks (Atmar et al., 2008; Rodríguez-Lázaro et al., 2012). Untreated water and sewage or poorly treated wastewaters can re-introduce these viruses in the environment making them available to be transmitted to susceptible individuals continuing the cycle of infection. It is reported that NoVs and HAdVs are persistent in water, highly infectious and extremely resistant to the environmental conditions like temperature or dryness (Bae and Schwab, 2008; Glass et al., 2009). For example, NoV or NoV RNA could persist in some type of waters for 60 to 728 days and in fruits and vegetables for longer than product's shelf life (Cook et al., 2016). This resistance potentiates the survival of NoVs and HAdVs in abiotic surfaces, during repeated freeze-thaw cycles and cold-storage, in the hands of food handlers and attached to surfaces of fruits or exterior re-contamination (De Keuckelaere et al., 2015; Lynch et al., 2009; Maunula et al., 2013; Verhaelen et al., 2013). Other findings support an extremely high chemical resistance of adenoviruses to biocides (Sauerbrei et al., 2007).

Generally, human noroviruses are considered the most common cause of foodborne disease outbreaks (Cook et al., 2016). Adenoviruses are frequently associated with severe gastroenteritis in closed communities, but links to a foodborne source are infrequent (Todd and Grieg, 2015); however human adenovirus have been gaining attention due to its prevalence in rivers, coastal waters, swimming pool waters, and drinking water supplies worldwide (Jiang, 2006). Fresh berries fruits are in general picked, packaged and commercialized in the fresh market without a washing treatment because they deteriorate rapidly. According to the U.S. Food and Drug Administration, washing fresh food by the consumer with water or conventional disinfectants has a limited efficacy in the elimination of foodborne microorganisms. In this scenario, soft berries like strawberries and raspberries are considered high risk vehicles of infection (Jacxsens et al., 2017; Kokkinos et al., 2017). At least 14 berry-related viral outbreaks occurred in Europe between

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1983 and 2013 (Calder et al., 2003; Hjertqvist et al., 2006; Le Guyader et al., 2004; Maunula and von Bonsdorff, 2014), including a multi-country outbreak in 2013 (Bruni et al., 2016), which resulted in a total of 14,600 registered gastroenteritis cases and 18 deaths (Palumbo et al., 2013).

It became crucial to find an effective tool to mitigate enteric viruses from ready-to-eat fruits or from waste-contaminated water. In recent years many conventional treatments has been used and new ones has been studied to reduce the threat of enteric virus contamination and public health risk associated to consumption of soft berries (Gil et al., 2009; Huang et al., 2016; Liu et al., 2015). Nevertheless, all of them face restrictions and limitations in effectively reducing viral contamination. The use of chemical washings, such as chlorine, raise disposal issues of chemical residues, besides its ineffectiveness in viral log reduction (Suslow, 2001). On the other hand, the use of chlorine is not recommended because it may generate the formation of disinfection by-products, such as trihalomethanes, and other potential carcinogenic disinfection by-products (Abbas et al., 2015). Ultraviolet radiation seems to be effective on the killing of pathogens, including viruses, on the surfaces, in water and some fresh produce, being a good alternative to thermal treatments (Guerrero-Beltrán and Barbosa-Cánovas, 2004; Park et al., 2011). Nevertheless, its poor penetrating ability in organic matter and in opaque food surfaces could be a limitation to decontaminate rough food surfaces such as in strawberries and raspberries (Liu et al., 2015).

Among non-thermal methods, gamma radiation appear as a useful and chemical-free process to diminish the viral load of both water sources and fresh foods (Cook et al., 2016; Feng et al., 2011; Pimenta et al., 2016). This technology was suggested to be an effective HAdV mitigation tool to treat sewage polluted waters, which is one of the main vehicle of contamination for minimally processed food products (Pimenta et al., 2016).

The ISO standard 14470:2011 provides state-of-the art requirements for food irradiation, commonly used to improve quality and safety in food processing (International Organization for Standardization, 2011). FDA has approved food irradiation at doses up to 4 kGy for fresh lettuce and spinach, and a maximum dose of 3 kGy for most other fruits and vegetables to inactivate bacterial pathogens and improve the safety of fresh produce (US FDA, 2018). However according to the literature, mainly on norovirus inactivation, a maximum of 2-log reduction on viral load is attained at the approved level for the irradiation of fresh produce (Bidawid et al., 2000; DiCaprio et al., 2016; Espinosa et al., 2012; Feng et al., 2011).

The past epidemiological data point the public health significance of HAdV, based on its environmental persistence, stability, and resistance to treatment processes (Jiang, 2006). The co-presence of NoV and HAdV genomes was detected on surfaces of food service operations, such as in restaurants and canteens, where foodborne gastroenteritis outbreaks were suspected (Maunula et al., 2017). There are several studies detailing the inactivation of human norovirus or its surrogates (Cook et al., 2016; DiCaprio et al., 2016; Feng et al., 2011; Huang et al., 2016; Li et al., 2017; Liu et al., 2015; Park et al., 2011; Praveen et al., 2013; Predmore et al., 2015), but few ones describing the treatment of human adenovirus (Eischeid et al., 2009; Pimenta et al., 2016). This work was carried out to introduce new insights on the inactivation patterns of enteric virus by gamma irradiation in fresh fruits, addressing new aspects as the co-presence of enteric virus in fruits and the potential occurrence in food of human adenovirus considering its environmental persistence.

2. Materials and methods

2.1. Viruses and cell cultures

Human adenovirus type 5 (HAdV-5; ATCC VR-1516) was propagated in confluent monolayers of human lung carcinoma cells A549 (ATCC CCL-185). Murine norovirus type 1 (MuNoV) strain P3 (kindly provided by Christiane E. Wobus at the University of Michigan Medical School, USA) was propagated in confluent monolayers of mouse macrophages Raw 264.7 (ATCC TIB-71). Cells were maintained at 37 °C and 5% CO_2 in Dulbecco's modified Eagle medium (DMEM; Gibco, Life Technologies, Paisley, United Kingdom) supplemented with 1 mM L-glutamine, 10% fetal bovine serum (FBS) (heat inactivated; Gibco, Life Technologies, Carlsbad, CA, USA), 1 U/mL penicillin, 100 g/mL streptomycin, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, and 1 mM HEPES buffer. To prepare HAdV-5 and MuNoV stocks, confluent A549 cells and Raw 264.7 macrophages, respectively, were infected with inoculum containing 10^7 PFU/mL, using a multiplicity of infection (MOI) of 0.1. After 1 h of incubation at 37 °C with mild agitation, the cellular monolayer was washed twice with phosphate-buffered saline (PBS) solution, and supplemented DMEM was added. The viruses were harvested after 7 days and 3 days post infection for HAdV-5 and MuNoV, respectively, by three freeze-thaw cycles at a low centrifugation of 902 $\times g$ (Beckman J2-21M, rotor J20-1) for 30 min at 18 °C. The resulting supernatants were aliquoted and stored at −80 °C.

2.2. Sample preparation

Strawberries and raspberries were purchased from local stores. Approximately 10 g of fresh fruits were weighted and disinfected with a solution of ethanol 70% (v/v) during 15 min and dried at a laminar flow cabinet. One hundred microliters of each viral suspension, containing approximately 10^6 PFU of MuNoV or HAdV, was inoculated randomly on the surface of disinfected fresh fruits with a micropipette. For the viral pool samples, HAdV and MuNoV were inoculated as previously described from a mixed viral suspension containing approximately 10^6 PFU/mL of MuNoV and HAdV at a proportion of 1:1. The prepared fruit samples, after inoculum dried at a laminar flow cabinet, were placed in sterile stomacher bags.

2.3. Irradiation process

The irradiations were performed at room temperature in a Cobalt-60 experimental chamber (Precisa 22; Graviner Manufacturing Company Ltd., United Kingdom; 1971) with an activity of 165 TBq (4.45 kCi) and a dose rate of 1.6 kGy/h located at Campus Tecnológico e Nuclear (Bobadela, Portugal). The dose rate was determined by Fricke dosimetry. Strawberry and raspberry samples (stomacher bags containing 10 g of fresh fruits) were irradiated at gamma radiation doses between 0.9 kGy and 7.6 kGy. The irradiations were performed in separate for each berry fruit. To access the virucidal potential of gamma radiation at higher doses, raspberry samples inoculated with HAdV were irradiated at a dose range between 3.6 kGy and 11.3 kGy. Absorbed doses were measured by routine dosimeters (Batch X; Amber Perspex Harwell, London) with nominal uncertainty limits of about 2.5%. For each set of assayed conditions, one sample was irradiated per gamma radiation dose, and two independent irradiation batches were performed. An average dose uniformity ratio (maximum dose/minimum dose) of 1.1 was achieved. Non-irradiated spiked samples (0 kGy) were kept at room temperature during other samples irradiation and used as controls. After irradiation, samples were stored at 4 °C overnight.

2.4. Viral extraction, concentration and purification

The fruit (strawberries and raspberries) samples were washed with 10 mL of sterile PBS with 0.1% Tween 80 and were homogenized using a stomacher (Stomacher 3500; Seaward, UK) for 15 min. The resulting washing solution was centrifuged for 10,000 $\times g$, at 20 °C for 15 min (Beckman J2-21 M Induction Drive Centrifuge, Ramsey, US) for debris sedimentation. The supernatant was filtered (0.45 μm pore diameter) and placed into an Amicon® Ultra 4 mL filter tube (Merck Millipore)

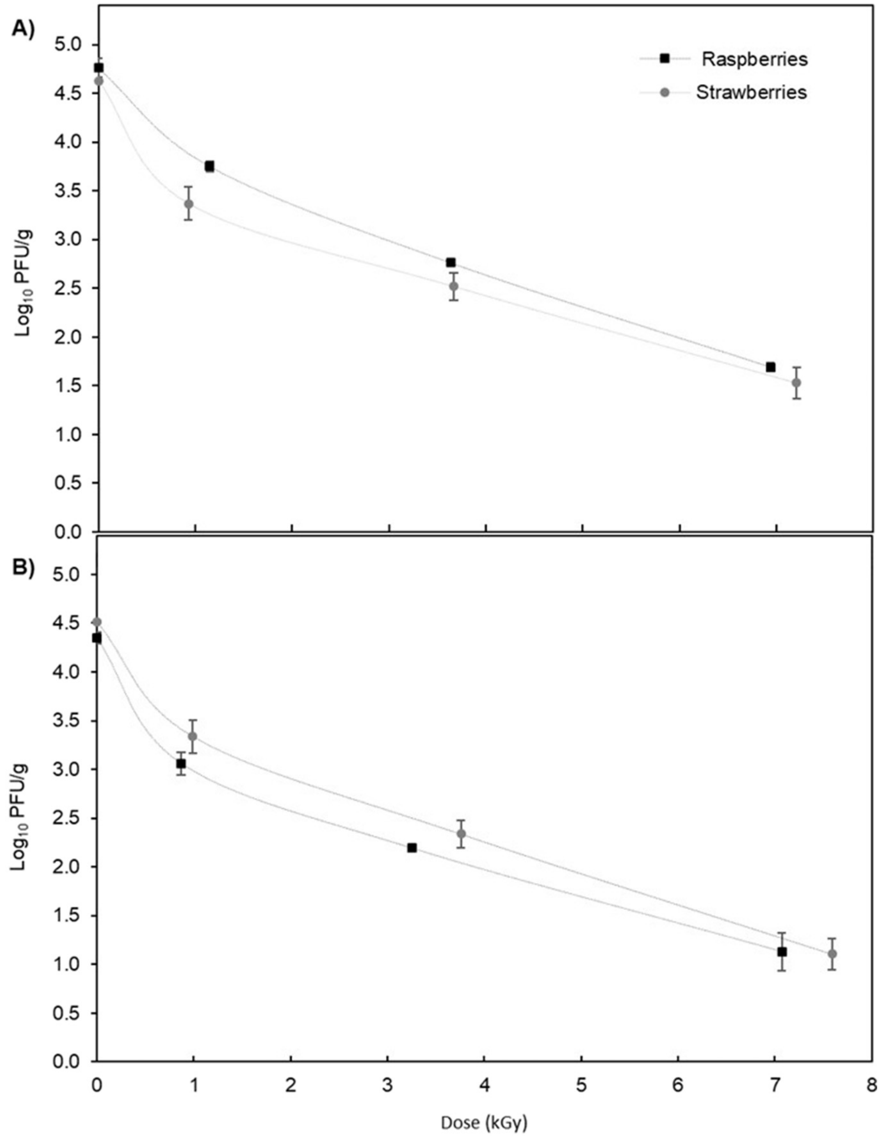

Fig. 1. Survival curves to gamma radiation of (A) human adenovirus type 5 (HAdV) and (B) murine norovirus type 1 (MuNoV) on artificially inoculated fresh strawberries (grey circles) and raspberries (black squares). Error bars correspond to the standard errors about the mean values (n = 6).

and then centrifuged up to 6,500 ×*g* until all the volume passes through the membrane. The Amicon® membrane was washed twice with 3 mL of sterile PBS by centrifugation. The viral particles were recovered in a maximum PBS volume of 1 mL. The purified and concentrated viral samples were used for the plaque assays and viral titer determination.

2.5. Viral plaque assay

Plaque assays were performed in A549 cells and Raw264.7 cells for HAdV-5 and MuNoV, respectively. Briefly, cells were seeded into 60-mm plates at a density of 7.5×10^5 cells per plate. After 24 h of incubation at 37 °C and 5% CO_2, cellular monolayers were infected with 300 μL of 10-fold serial dilutions of HAdV-5 and MuNoV purified suspensions from non-irradiated and irradiated fruit samples. Triplicates were made for each virus suspension sample. After inoculation, plates were incubated for infection for 1 h at 37 °C and 5% CO_2, with mild agitation every 15 min. After removal of the inoculum, cells were overlaid with 3 mL of overlay medium (2× DMEM) with 0.5% agarose (SeaKem ME; Lonza, Rockland, ME, USA). After incubation for 7 days for HAdV-5 or 3 days for MuNoV, a second 1.5 mL overlay of 2× DMEM with 0.5% agarose was added. Viral plaques were subsequently counted after 8 h to 24 h of a third agarose overlay (1.5 mL) with 1% of a neutral red solution (3.3 g/L; Sigma, St. Louis, MO, USA). Virus titer was expressed in PFU per gram of fresh fruit (PFU/g). The detection limit of this plaque assay method is 2 PFU/g.

2.6. Data analysis

The average viral recovery efficiency was estimated based on the determined titers in each performed plaque assay of viral stocks (considered 100% recovery efficiency) and the ones obtained from the viral recovery from inoculated non-irradiated fruit samples (Viral recovery efficiency % = [viral titer sample]/[viral titer stock] ∗ 100). Based on that a correction factor was applied to virus titer (correction factor = 100% efficiency/average viral recovery efficiency %). D10 (measured in kGy), which is the gamma radiation dose required to reduce a virus titer by 1 log, was calculated from the linear regression model of the log of the surviving fractions. Origin software version 7.5 (OriginLab Corporation, Northampton, MA, USA) was used for data analysis. Virus infectivity determined by plaque assay was expressed as the mean log titer plus or minus the standard error. These data were subjected to analysis of variance (ANOVA), and significant differences among the means were determined by Tukey's post hoc test at a P of 0.05 significance level.

Key resources table

Resource	Source	Identifier
CellLine		
A549		
Raw 264.7		
Chemical		
Amino acids		
CO_2		
Cobalt-60		
Ethanol		
HEPES		
L-Glutamine		
Neutral red		
PBS		
Penicillin		
Phosphate-buffered saline		
Sodium pyruvate		
Streptomycin		

3. Results and discussion

In this study, the efficiency of gamma radiation as a process for HAdV and MuNoV inactivation was assessed in both raw raspberry and strawberry samples. The applied method for virus removal from berry fruits presented an average viral recovery efficiency of 82%. Based on this result, a correction factor of 1.2 was applied to all virus titers.

3.1. Inactivation response of HAdV and MuNoV to gamma radiation

The survival of a microorganism when challenged with increasing doses of gamma radiation can be translated into a dose survival curve that represents a logarithmic variation of the surviving fractions in function of the absorbed radiation dose (kGy). Fig. 1 shows the logarithmic viral titer reduction measured by plaque assay of human adenovirus (A) and murine norovirus (B) in function of the applied gamma radiation dose to spiked strawberries and raspberries.

The inactivation of HAdV and MuNoV by gamma radiation in the analysed matrices appeared not to follow an exponential inactivation kinetics, presenting a slightly concave surviving curve that includes a linear phase in the last part of the curve. Previously, the same strain of HAdV presented log-linear inactivation curves in liquid substrates (Pimenta et al., 2016). Inactivation curves showed to depend on virus external factors such as dose, radiation type, temperature and nature of the virus substrate during the irradiation process (Smolko and Lombardo, 2005). This non-linear nature of the inactivation curve is likely to be caused by the complexity of media components, such as berry fruits. The non-linearity of surviving curves of enteric virus to gamma radiation in fruit matrices was previously referred (Bidawid et al., 2000; Feng et al., 2011).

The estimated logarithmic viral titer reduction after irradiation of the inoculated strawberries at an absorbed dose of approximately 3.7 kGy was 2.1 log PFU/g for HAdV and 2.2 log PFU/g for MuNoV. For inoculated raspberries, the viral titer reduction achieved for an absorbed dose of 3.4 kGy was 2.0 log PFU/g for HAdV and 2.2 PFU/g for MuNoV.

Considering the higher applied gamma radiation dose of 7 kGy in fresh fruits, both studied viruses indicated a reduction in their infectious potential of approximately 3 log PFU/g. The virucidal response turned out to be quite similar among strawberries and raspberries for both viruses, and no significant differences ($p > 0.05$) were found between the viral titers from the two different irradiated subtracts at similar doses for each enteric virus.

The obtained data point out a high resistance of both tested viruses to gamma radiation. The high resistance of MuNoV to gamma radiation in fresh produce has been previously described, indicating a 1.3-log reduction in irradiated strawberries at a dose of 2.8 kGy (Feng et al., 2011). Other authors have studied the inactivation by ionizing radiation of human NoV (strain GII.4) and Tulane virus as its surrogate (DiCaprio et al., 2016). The obtained results in that study also highlighted the high radioresistance of both viruses, reporting a maximum of 1-log reduction for both viruses in e-beam irradiated strawberries at 3 kGy (DiCaprio et al., 2016).

The different inactivation responses between studies could be attributed to the dissimilarity of methodologic approaches applied, including virus types, methods to assess its inactivation (culture or molecular methods), and irradiation conditions (temperature, dose rate, gamma radiation or e-beam). Molecular methods could underestimate viral inactivation, since they cannot discriminate between genomic copies detected from an infectious or non-infectious viral particle (Li et al., 2017; Pimenta et al., 2016). The prolonged exposure of virus to radiolysis free radicals may increase virus inactivation (DiCaprio et al., 2016), leading to higher log reductions on virus load. This effect can be explained by differences in dose rate, in gamma irradiation the dose rate is lower corresponding to a longer treatment duration, on the other hand, for e-beam irradiation the dose rate is higher leading to lower exposition times.

For HAdV it is not documented any inactivation response by ionizing radiation on fresh food. Enteric adenoviruses are less implicated in foodborne outbreaks, however they are recognized as important causes of acute diarrhoeal illnesses in sporadic and outbreak settings (Fletcher et al., 2013). Furthermore, a recent survey indicated the presence of HAdV in the irrigation waters and berry fruits in industrial settings (Kokkinos et al., 2017). The application of HAdVs as indicators of human enteric viruses in environmental and food samples has also been suggested due to its simplicity of detection, as well as abundance and stability in the environment (Albinana-Gimenez et al., 2009; Hundesa et al., 2006; Pina et al., 1998; Verhaelen et al., 2012). All these, stress the importance of evaluating inactivation technologies also for this virus.

Other fresh food sanitation methods have been studied and their virucidal efficacy reported. For example, using 40 min of gaseous ozone treatment (6% wt/wt ozone in oxygen) it was verified a 3.3 log reduction in MuNoV viral titer on the surface of fresh strawberries (Predmore et al., 2015). In turn, using a High Hydrostatic Pressure (HHP) treatment at 650 MPa for 2 min at initial sample temperature of 0 °C it was only achieved 1.7 and 2.5 log reductions of GI.1 human norovirus strain on strawberry quarters and raspberries, respectively, using molecular detection methods (Huang et al., 2016). The use of non-thermal technologies such as UV and ultrasound was also investigated for the disinfection of human adenoviruses from fresh food (Birmpa et al., 2016). The authors reported that for the same 30 minute treatment time, the UV treatment at a dose of 3.6 J/cm^2 was more efficient than ultrasound, achieving a log reduction of 2.13, 1.25, and 0.92 for lettuce, strawberries, and cherry tomatoes, respectively. However, in the above mentioned study, the authors verified that the food appearance was impaired after 30 min of treatment with UV and ultrasound (Birmpa et al., 2016). Comparing the above mentioned technologies with the gamma radiation treatment presented in this study, it was verified a similar or increased disinfection capacity for virus in fresh berries, with the advantage of irradiation being a final treatment in which the product can be in its final packaging system and thereafter be delivered directly to market.

3.2. HAdV survival in fresh raspberries at a maximum irradiation dose

As indicated before, non-linear inactivation survival curves were obtained for MuNoV and HAdV in both fresh fruits. Considering that

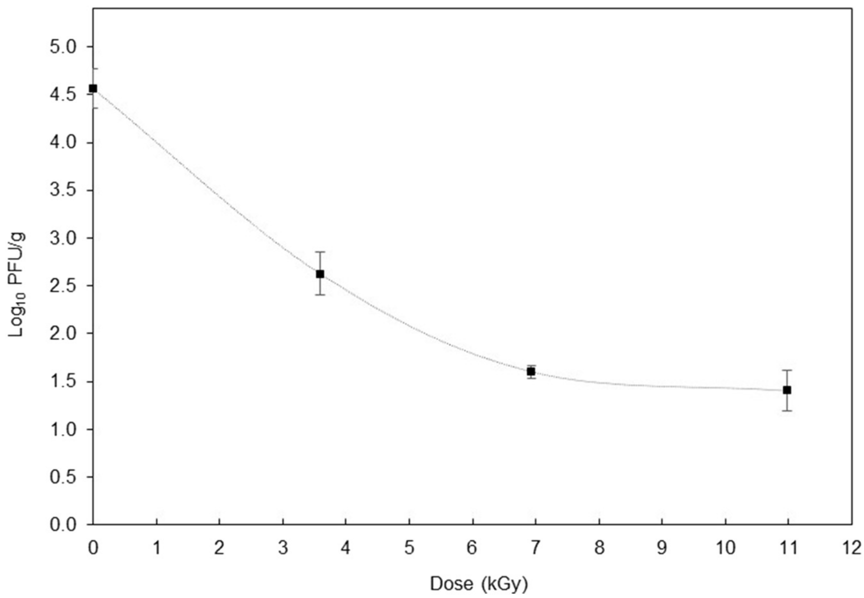

Fig. 2. Survival curve to gamma radiation of human adenovirus type 5 (HAdV) on artificially inoculated fresh raspberries. Error bars correspond to the standard errors about the mean values (n = 8).

HAdV demonstrated to be the most resistant virus to gamma radiation when inoculated on fresh raspberries, as well as to other disinfection treatments (Eischeid et al., 2009), the treatment dose was extended to ≈11 kGy to evaluate the behaviour of the viral particles at higher irradiation doses. As can be verified in Fig. 2, a non-linear inactivation survival curve was obtained with a similar recovery of infectious HAdV particles from 7 kGy to 11 kGy (not significantly different, $p > 0.05$). At higher gamma radiation doses it is notable the persistence of at least 1.4 log PFU/g of recovered infectious viral particles. At the maximum radiation dose of 11 kGy it was achieved a 3.2 log PFU/g reduction on HAdV titer. In fact, the statistical analysis showed significant differences between the viral titer values recovered from non-irradiated samples and those recovered from samples irradiated at the three applied doses ($p < 0.05$).

3.3. Gamma radiation survival assessment of co-existent MuNoV and HAdV in fresh raspberries and strawberries

Considering that enteric viruses persist in the environment mixed together as complex contamination sources, the infectivity of HAdV and MuNoV were evaluated as part of a pooled sample. The results indicated that the radioresistance of HAdV and MuNoV strains in the tested fruits is not influenced by the co-presence of the other virus (Fig. 3). The HAdV and MuNoV survival after an irradiation process with applied doses of approximately 3 kGy and 7 kGy were significantly lower when compared with non-irradiated strawberries or raspberries ($p < 0.05$). Nevertheless, there are no differences between the recovered infectious virus loads after irradiation at 4 kGy and 7 kGy ($p > 0.05$). All together, the results highlighted that it is necessary to apply a radiation dose higher than 4 kGy to achieve an effective viral inactivation in this type of substrates.

In order to characterize organisms by their radiation sensitivity, the D10 value is used, which is defined as the dose required to inactivate 90% of a population or to produce a 10-fold (1 log) reduction in the population. There are several studies detailing the D10 values of different bacteria and fungi in diverse substrates, but the research on the use of ionizing radiation on viruses in or on foods are scarce (Bidawid et al., 2000). In this study, the D10 values for each tested virus individually or in pool in the two food matrices were determined (Table 1) based on the linear phase of virus survival curves (excluding the 0 kGy data point). This approach was made to follow a "worst case scenario" since in that phase of the survival curve the viruses express higher radioresistance (higher D10 values).

The statistical analysis of the estimated D10 values support the previous findings, in which the high radioresistance of MuNoV and HAdV is similar in both type of fresh fruits and is not influenced by the co-existence of the other virus. Other studies have reported the same range of D10 value for MuNoV of 2.55 ± 0.45 kGy when suspended in PBS for E-beam treatment (Praveen et al., 2013). For HAdV, it was not found any reference to D10 value in food substrates. Nevertheless, the radioresistance of HAdV in raspberries seems to be similar to that previously obtained in aqueous matrices with high organic matter content (Pimenta et al., 2016). The load of infectious viral particles at an absorbed dose of approximately 11 kGy (1.4 PFU/g) was comparable to the one obtained before at a similar dose on organic simulated substrates (1.7 PFU/mL) (Pimenta et al., 2016). The efficiency of gamma radiation is reduced by the existence of scavengers on complex substrates, which react with free radicals inducing a radioprotective effect that leads to higher microbial radioresistance (Limoli et al., 2001).

Several studies have confirmed that doses up to 10 kGy do not cause any toxicological hazards or nutritional or microbiological problems in food (Bhat et al., 1999). Nevertheless, it has been also reported that doses higher than 3 kGy could have implication on organoleptic characteristics of fresh fruits like soft berries (Jesus Filho et al., 2018). It was found that the optimal dose range for postharvest treatment of strawberries by irradiation is between 2 kGy and 3 kGy, guarantying a satisfactory decay control, without unduly affecting the fruit quality (International Consultative Group on Food Irradiation et al., 1994). Previous data also indicated that the ideal dose, which does not impair the sensory and physical properties of raspberries is 1.5 kGy (Cabo Verde et al., 2013). Taking these limits into consideration and the virucidal efficiency of gamma radiation estimated in this study (2 log reduction on viral titer at 4 kGy), the treatment radiation doses to attain a safety reduction of enteric virus (> 7 kGy) would compromise the preservation of food quality (< 3 kGy). Probably, food viral contamination could be mitigated upstream in the food chain production. There are numerous surveys that demonstrate that irrigation water used in primary production is an important vehicle of viral contamination for fresh produce, which include fresh berries (Kokkinos et al., 2017). As previously proposed, the irradiation technology can be an effective virus mitigation treatment for polluted waters (Pimenta et al., 2016),

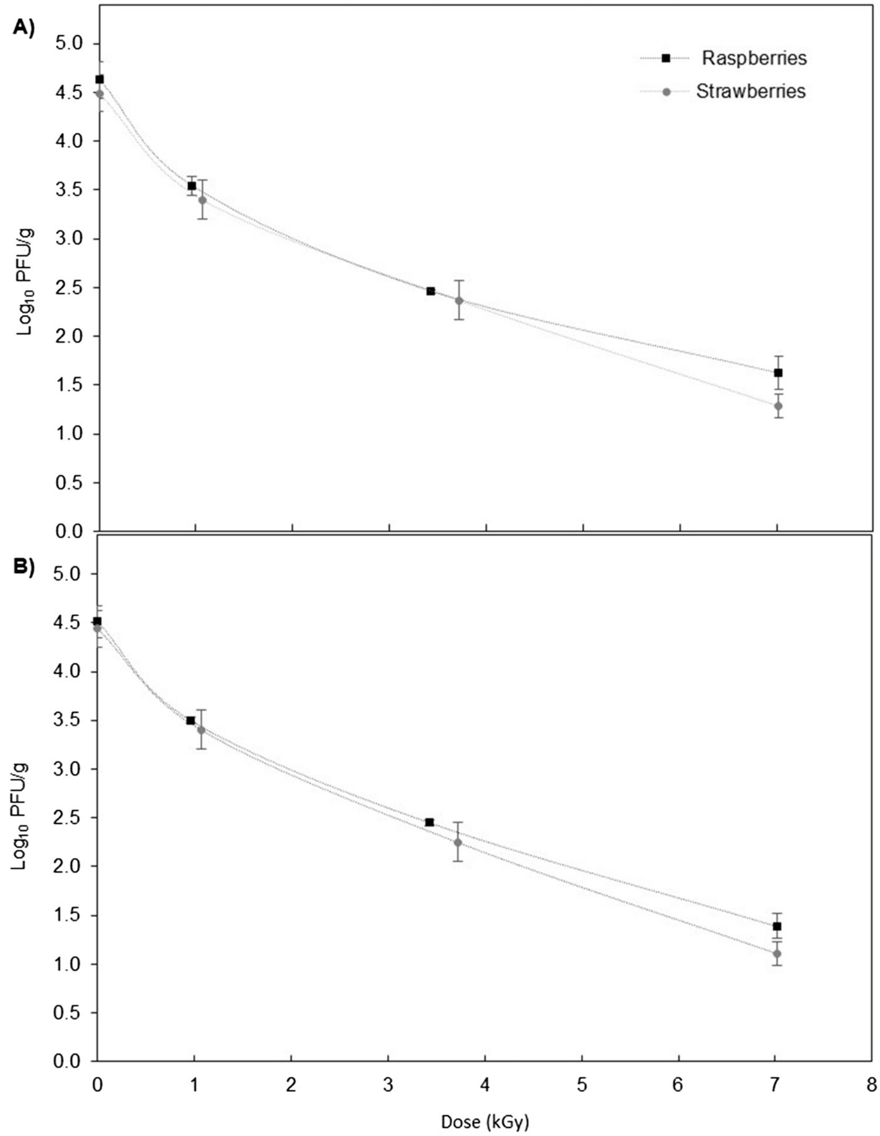

Fig. 3. Survival curves to gamma radiation of human adenovirus type 5 (HAdV) in the presence of murine norovirus type1 (MuNoV) (A) and of MuNoV in the presence of HAdV (B) on two artificially inoculated substrates: fresh strawberries (grey circles) and raspberries (black squares). Error bars correspond to the standard errors about the mean values (n = 8).

Table 1
D10 values (kGy) of human adenovirus (HAdV) and murine norovirus (MuNoV), individually or pooled, in strawberries and raspberries.

Substrate	D10 values ± standard error (kGy)			
	HAdV	HAdV in pool	MuNoV	MuNoV in pool
Strawberries	3.4 ± 0.1[a]	2.8 ± 0.1[a]	3.0 ± 0.1[A]	2.6 ± 0.2[A]
Raspberries	2.8 ± 0.2[a]	3.2 ± 0.6[a]	3.2 ± 0.2[A]	2.9 ± 0.3[A]

For each virus, the D10 values that have the same superscript letter are not considered significantly different ($p > 0.05$).

preventing in this way the contamination for fresh produce by irrigation.

Declaration of Competing Interest

None.

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