IAEA TECDOC SERIES

IAEA-TECDOC-2008

Development of Electron Beam and X Ray Applications for Food Irradiation

Final Report of a Coordinated Research Project

Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

IAEA
International Atomic Energy Agency

DEVELOPMENT OF ELECTRON BEAM AND X RAY APPLICATIONS FOR FOOD IRRADIATION

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DEVELOPMENT OF ELECTRON BEAM AND X RAY APPLICATIONS FOR FOOD IRRADIATION

FINAL REPORT OF A COORDINATED RESEARCH PROJECT

PREPARED BY THE
JOINT FAO/IAEA CENTRE OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 2022

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Printed by the IAEA in Austria
September 2022

IAEA Library Cataloguing in Publication Data

Names: International Atomic Energy Agency.
Title: Development of electron beam and x ray applications for food irradiation / International Atomic Energy Agency.
Description: Vienna : International Atomic Energy Agency, 2022. | Series: IAEA TECDOC series, ISSN 1011–4289 ; no. 2008 | Includes bibliographical references.
Identifiers: IAEAL 22-01530 | ISBN 978–92–0–137022–8 (paperback : alk. paper) | ISBN 978–92–0–136922–2 (pdf)
Subjects: LCSH: Irradiated foods. | Electron beams — Industrial applications. | Radiography. | Ionizing radiation — Food industry and trade.

FOREWORD

Irradiation by ionizing radiation is one of many different techniques that may be used to process food and improve it for storage, cooking or consumption. The two main advantages of food irradiation are that it does not significantly change a food's temperature and it does not introduce chemicals into the food. In contrast, heating, refrigeration or freezing significantly alters the temperature of food and changes its sensory properties. Chemical treatments can also leave residue of the chemical compounds applied to the food or their breakdown products. An additional advantage is that ionizing radiation is penetrating, and therefore prepackaged food can be irradiated; the packaging protects the food and the food maintains its quality post-treatment.

Food irradiation is used to control spoilage and food-borne pathogenic microorganisms or insect pests without significantly affecting a food's sensory attributes. While it is not necessary to sterilize food completely, doses of ionizing radiation can destroy disease-causing microbes and reduce the risk of food poisoning. Irradiation also destroys organisms that are associated with food decomposition and can therefore inhibit decay, making it possible to keep food for longer, while ensuring a higher level of food safety and quality. Low dosage irradiation is also used to prevent the spread of insects and other pests in shipments of fresh foods such as fruits and vegetables. This low dosage irradiation is a chemical free method of providing phytosanitary security, as ionizing radiation at the correct dosage can prevent pests from developing and reproducing.

More than 70 countries have legislation that allows the use of irradiation for one or more food products. International standards and national authorities list three different modes of ionizing radiation for food irradiation: gamma rays from the radionuclides cobalt-60 (^{60}Co) or caesium-137 (^{137}Cs); X rays generated from machine sources operated at or below an energy level of 7.5 MeV; and electron beams generated from machine sources operated at or below 10 MeV.

It is difficult to accurately measure the quantity of irradiated foods traded each year but it is estimated that more than one million tonnes per year of food and agricultural products are irradiated on a commercial scale worldwide. Most irradiated foods are processed in facilities using gamma radiation from ^{60}Co. Gamma irradiation is a well established technology. However, the amount of ^{60}Co available worldwide is limited, so it is desirable to have other technologies available to irradiate food. Electron accelerators use electricity to generate electron beams and X rays. The effects of these ionizing radiations on food are similar to those of gamma rays. However, the use of electrical machine sources for food irradiation on a commercial scale is not widespread.

The IAEA launched a coordinated research project on the Development of Electron Beam and X ray Applications for Food Irradiation (DEXAFI) in 2015. The project involved coordinated research and development activities on matters that are prerequisites for the practical implementation of processes using electron beams and X rays. The overall aim of the project was to unlock the potential of machine sources for radiation treatment of agricultural and food products. Technological advancements associated with using machines to generate ionizing radiation could provide new devices and applications for food irradiation. Making more use of electron beam and X ray irradiation would add to and complement the commercial capacity to irradiate food that is currently provided by gamma facilities, without increasing the demand for ^{60}Co radioisotope sources.

The coordinated research project was implemented by the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture between 2015 and 2021. The officers responsible for this publication were C. Blackburn and K. Narikawa of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture.

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CONTENTS

6. FOSTERING E-BEAM FOOD IRRADIATION

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Abstract

There is an ever-increasing global demand from consumers for high-quality foods with major emphasis placed on quality and safety attributes. One of the main consumer demands is for minimally processed foods that are highly nutritive but low energy-dense and are natural foods with no or minimal chemical preservatives. Extending the shelf-life of food products, while improving the food safety and quality, will have a positive impact on both the industry and consumers. Food irradiation is emerging as a promising and innovative processing technology in this regard.

The main objective of this research was to investigate and propose fresh irradiated foods that are health promoting, safe and convenient to be treated by electron beam irradiation. One of the purposes of this research is to help foster more wide use of electron beam irradiation especially where it will enhance food safety and quality. To attain these aims, electron beam irradiation parameters were studied in terms of equipment parametrization, as well as, the effects of irradiation on fresh food products through the evaluation of bioactive compounds and also microbial inactivation (natural microbiota and potential pathogenic bacteria). The food products selected for study were cherry tomatoes, raspberries, and mushrooms. These where chosen due to their perishability, nutritional and bioactive compound profile, and socioeconomic importance in the Mediterranean region.

Modelling tools were also applied to simulate high energy electron beam irradiation (10 MeV) of cherry tomatoes and raspberries from the LINAC situated at C2TN (Portugal). An alternative simulation framework, Ensaroot, was also used to test its application in food irradiation studies.

Overall the results of this comprehensive study support the feasibility of electron beam irradiation as a post-harvest treatment of cherry tomatoes (3 kGy), mushrooms (5 kGy) and raspberries (3 kGy). It would guarantee the safety, extend the shelf-life and preserving the bioactive contents of these products.

6.1.INTRODUCTION

Ensuring the security of current and future food supplies is one of the main challenges facing governments globally, this is driven by consumer demand for freshness and variety and the need to feed an increasing world population. There is a need to address issues associated with the supply of safe and healthy food. Fresh fruit and vegetables are important for a healthy and balanced diet; their consumption is encouraged in many countries by health agencies to protect against a range of diseases. According to World Health Organization (WHO), a daily intake of fresh produce rich in phytonutrients at greater than 400 g help develop resistance to certain diseases such as cardiovascular diseases, diabetes and cancer [1].

Food contaminated with pathogenic organisms and the ability of these organisms to persist, grow, multiply and/or produce toxins have emerged as important for public health and that also cause economic losses. Illness associated with food consumption can be due to contaminated irrigation water, organic fertilizers and contaminated soil, as well as, to the contaminated harvesting equipment and post-harvest handling. The human pathogens associated with food illness outbreaks may involve many different microorganisms including viruses (hepatitis A virus, norovirus), bacteria (*Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*) and parasites (*Cryptosporidium parvum*, *Cyclospora cayetanensis*) [2].

The extension of shelf-life is another key factor in making any food product more profitable and commercially available in its best quality for long periods of time, bringing benefits to the consumer, the producer, as well as to the whole food market. Besides the need for extended storage periods, there is also a general trend to develop less severe, and therefore less harmful, food preservation techniques.

There are a variety of methods to reduce the microbial contamination on whole and fresh-cut produce. Various chemical sanitizers have been widely used, such as sodium hypochlorite and hydrogen peroxide. Some chlorine or chlorine-containing compounds, however, are considered carcinogens and can cause irritation to the skin and respiratory tract, and their use to control microbial contamination of food is limited [3].

Better methods of preventing contamination on the farm, and during packing or processing, and the use of a terminal control process, such as irradiation, might reduce the burden of disease transmission and extend the quality of fresh produce. In this sense, there has been extensive research to identify the most suitable technology for fresh food preservation. Irradiation may constitute an alternative technology for fresh food treatments. Non-thermal technologies, like irradiation, have the ability to inactivate microorganisms at ambient or near ambient temperatures, therefore avoiding the deleterious effects that heat has on flavour, colour, and nutrient value of food. Irradiation has the advantage that products are processed in the final packaged stage, reducing the possibility of cross contamination until actual use by the consumer. Reports are available showing that irradiation of food commodities, by gamma rays or electron beams, is effective in overcoming quarantine barriers in international trade, as a mode of decontamination, disinfestation, and improvement of nutritional attributes and shelf-life. Food irradiation technologies are based on three types of ionizing radiations: gamma rays, X rays and electron beams. Each has different characteristics but, from a processing point of view, these can be differentiated as either highly penetrating (gamma radiation and X rays) and low penetrating (electron beams). Other differences are that electron beams can deliver very high dose rates. An electron beam dose rate can be about 100 times higher than gamma radiation, resulting in very short product radiation exposure. These abiotic factors may influence the microbial inactivation and the effectiveness of decontamination/disinfection processes should be investigated. [4]

This study aimed to evaluate the effects of electron beam irradiation on cherry tomatoes, raspberries and mushrooms and to assess the potential use of this technology as a post-harvest treatment for these food products.

6.2.MATERIALS AND METHODS

6.2.1. Sampling

Fresh cherry tomatoes (*Solanum lycopersicum* var. cerasiforme) and raspberries (*Rubus idaeus* L., cv. Amira) were purchased from a local supermarket in Lisbon, Portugal, and immediately kept at 4 ± 1°C until analysis. Portobello mushrooms (*Agaricus bisporus*) were acquired in Bragança (Northeast of Portugal) in a common market. The preparation of the samples is described in each of the following sections according to the specific assay.

6.2.2. Irradiation

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 Instalação de Radiações IonizanteS (IRIS) from Centro de Ciências e Tecnologias Nucleares (C2TN) of IST, Universidade de Lisboa. Fresh cherry tomatoes, raspberries and mushrooms were irradiated in plastic boxes (150 g; one box per dose) at room temperature to doses that ranged from 0.3 up to 5 kGy at an average dose rate of 0.5 kGy/minute, with a dose uniformity (DUR) of 1.1. The absorbed dose was measured using calibrated radiochromic dosimeters FWT-60 (Far West Technology, Inc. Goleta, USA) [5]. Three independent irradiation batches were performed for each assay. Non-irradiated samples (0 kGy) were used as controls and followed all the experiments.

6.2.3. Microbial inactivation studies on cherry tomatoes and raspberries

6.2.3.1. Natural microbiota

Non-irradiated and irradiated cherry tomatoes (25 g) and raspberries (25 g) were placed in sterile stomacher bags containing 100 mL of 0.1% Tween 80 physiological solution. Samples (three samples per radiation dose treatment) were homogenized using a stomacher (Stomacher 3500; Seaward, UK) for 15 minutes. 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 seven days. The results were expressed as log colony forming units per gram of fresh fruit (log CFU/g).

6.2.3.2. 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 fresh fruits (previously disinfected with 70% Ethanol), a droplet of inoculum was deposited on the skin of the fruits (25 g) to obtain approximately 10^3 colony forming units per gram (3 logCFU/g) of each microorganism. The inoculum was left to dry in a laminar flow cabinet to allow the attachment of the microorganisms and placed in sterile stomacher bags for irradiation. Bacterial counts of spiked fruits samples were estimated as described by [6]. The detection limit of the method was 1 CFU/g. The microbial counts were recorded and expressed as log CFU/g.

6.2.4. Bioactive content in fruits

After the irradiation, the fruits were manually mashed and placed in a freezer at -80°C for twelve hours and then freeze dried for seventy-two hours.

For lycopene extraction from cherry tomatoes, a previous described protocol [7] was used with some modifications. Briefly, 1 g of freeze-dried sample was added to a 150 mL flask and stirred for 30 minutes with 20 mL of acetone/n-hexane (1:3 v/v) at room temperature. After this, the extract was filtered through Whatman No. 4 filter paper and the solid residue was extracted twice with additional 20 mL portions of acetone/n-hexane (1:3 v/v). The combined solvent extracts were then evaporated under reduced pressure (Buchi R-210) at 50°C until the solvent was completely removed. The samples were left overnight to dry. The extracts were prepared in triplicate for each sample. The dried samples were dissolved in hexane to obtain samples of 2.5 mg/mL.

The raspberry extracts were prepared by a solid-liquid extraction as previously described [8], using a mixture of ethanol:water (80:20, v/v; 30 mL) as solvent, for one hour at room temperature.

6.2.4.1. Quantification of lycopene content in cherry tomatoes

The lycopene content was analysed by High Performance Liquid Chromatography (HPLC) (Prominence CBM 20-A, Shimadzu, Japan) with UV-DAD detector according to the method described previously [6]. The assay was carried out in triplicate.

6.2.4.2. Total Phenolic Content in raspberry extracts

The total phenolic content was determined based on Folin-Ciocalteau method [9], 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). The assay was carried out in triplicate.

6.2.4.3. Antioxidant activity in fruits extracts

Two assays were used to evaluate the antioxidant activity of fruit extracts, both assays are mechanistically different. One is the α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging method described by Brand-Williams, Cuvelier, and Berset (1995) with some modifications [6], [10] and the second type of assay Ferric Reducing Antioxidant Power (FRAP) described by Benzie and Strain (1996). 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. Both assays were made in triplicate.

6.2.4.4. Raspberry extracts and ascorbic acid content

A method using high performance liquid chromatography (HPLC) (Prominence CBM 20-A, Shimadzu, Japan) with a Ultra-violet Diode-Array detector (UV-DAD was used to determine ascorbic acid (vitamin C) content. Lyophilized raspberry extracts (~10 mg) were added to metaphosphoric acid 4.5% (1 mL). The resulting solutions were filtered (0.45 µm nylon filters) prior to analysis. Sample solutions (10 µL injection volume) were introduced onto a Kinetex C18 XB-C18 (5 µm, 250 mm, 4.0 mm) HPLC column maintained at a temperature of 35°C and ascorbic acid was detected by UV absorbance at 245 nm. The mobile phase used to elute the

sample during HPLC separation was 1.8 mM H_2SO_4 (pH = 2.6) with a flow rate of 0.9 mL per minute. The assays were 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). [10]

6.2.4.5. Cytotoxicity assay in fruits extracts - WST-1 Proliferation test

Human lung carcinoma epithelial cells (A549, ATCC CCL-185) and human embryonic kidney epithelial cells (293T, ATCC CRL-3616) were used. Cell viability after exposition to different concentrations of fruit 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 a previously developed protocol [6]. Two independent assays each with three fruits extracts replicates were performed.

6.2.5. Nutritional value and chemical profile of mushrooms

Carbohydrates, fat, protein, ash and moisture were determined following AOAC procedures [11] as described in [12].

The assessment of chemical composition of mushrooms included the determination of ergoesterol, tocopherols, free sugars, fatty acids, organic acids as detailed in [12].

6.2.6. Storage study

In order to evaluate a potential shelf-life extension of cherry tomatoes, raspberries and mushrooms with electron beam treatment, the previously described assays were performed at different refrigerated (4ºC) storage periods. Cherry tomatoes were assessed immediately after irradiation (no storage; T0), and after 14 days (T14) of storage. For the raspberries, the assays were carried out immediately after irradiation (T0; no storage) or followed by different storage periods: 3 days (T3; regular fruit shelf-life), 7 days (T7) and 14 days (T14). For the mushrooms, the analysis was performed promptly (T0), after for 4 days (T4) and 8 days (T8) of storage.

6.2.7. Modelling

The irradiation of cherry tomatoes and raspberries was simulated using the framework ENSARRoot. This framework is based on ROOT libraries and that allows the use of GEANT4 as a particle and energy transport engine [13].

6.2.8. 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 $\alpha = 0.05$.

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.

6.3.RESULTS AND DISCUSSION

Cherry tomatoes, raspberries and mushrooms 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 technologies.

6.3.1. Microbial inactivation on fruits

The bioburden of cherry tomatoes presented an aerobic bacterial mesophilic population of 5.1 ± 0.1 Log CFU/g and a filamentous fungi population of 2.3 ± 0.1 Log CFU/g. Immediately after irradiation treatment at 3 kGy (T0), the mesophilic microbiota of cherry tomatoes reduced 4 Log CFU/g and the filamentous fungi population was below the detection limit (<1 Log CFU/g), therefore not detected (Table 1). After 14 days of storage (T14), the samples treated with 3 kGy showed significantly ($p<0.05$) reduced bacterial counts as compared to the non-treated samples. Reductions by 4 Log CFU/g, were observed for mesophilic bacteria while filamentous fungi continued to be not detected on stored fruit samples. The effective reduction of cherry tomatoes natural microbiota provided by the electron beam treatment is directly observed in Table 1, comparing the treated samples after 14 days storage with the non-treated samples before storage (T0). The former show even less counts than the latter for the studied microbial populations for the 3 kGy treatment. Even for the lower dose 1.5 kGy the treated samples after 14 days of storage show either less or approximately equal counts than the non-treated sample before storage.

TABLE 1. NATURAL MICROBIOTA COUNTS OF AEROBIC BACTERIAL MESOPHILIC AND FILAMENTOUS FUNGI POPULATIONS FOR NON-IRRADIATED AND IRRADIATED CHERRY TOMATOES IMMEDIATELY AFTER IRRADIATION (T0) AND AFTER 14 DAYS OF REFRIGERATED STORAGE (T14). THE RESULTS ARE PRESENTED AS MEAN ± SD

Dose (kGy)	**Aerobic bacterial mesophilic population (Log CFU/g)**		**Filamentous fungi population (Log CFU/g)**	
	T0	T14	T0	T14
0	**5.1±0.3**	**5.7±0.3**	**2.3±0.3**	**3.9±0.2**
1.5	**2.7±0.3**	**3.6±0.1**	**1.4±0.4**	**2.4±0.3**
3	**1.3±0.3**	**1.8±0.1**	**<1**	**<1**

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. With electron 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 (Table 2). 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 de 14 days of refrigerated storage (Table 2). 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 (Table 2). 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).

In order to evaluate the efficiency of electron beam treatment as a disinfection treatment for cherry tomatoes and raspberries, challenging tests were performed with potential foodborne bacterial pathogens (*Salmonella* Typhimurium, *Escherichia coli*, and *Listeria monocytogenes*). Fruit samples were spiked with individual bacterial suspensions (approximately 10^3 CFU/g) and treated with electron beam at doses of 0.3 kGy up to 3 kGy. The surviving bacterial counts were estimated after irradiation (no storage, T0) and up to 14 days of refrigerated storage (T14). For cherry tomatoes (Table 3), the results pointed out that the bacterial pathogens decrease with the treatment radiation dose being below the detection limit (<1 Log CFU/g), therefore not detected, in samples irradiated at the highest doses. Moreover, results indicate that there are no differences on the inactivation kinetics of the inoculated bacteria between the samples analysed immediately after irradiation and after 14 days of refrigerated storage. There is a general concern that surviving microorganisms to ionizing radiation are able to acquire resistance to antimicrobial agents. The generated data evidenced that for the applied radiation dose range there is no acquired bacterial radio-resistance during the analysed storage time.

TABLE 2. NATURAL MICROBIOTA COUNTS OF AEROBIC MESOPHILIC BACTERIAL AND FILAMENTOUS FUNGI POPULATIONS FOR NON-IRRADIATED AND IRRADIATED RASPBERRIES 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

Dose (kGy)	**Aerobic bacterial mesophilic population (Log CFU/g)**				**Filamentous fungi population (Log CFU/g)**			
	T0	T3	T7	T14	T0	T3	T7	T14
0	**4.3±0.1**	**4.6±0.1**	**4.6±0.1**	**7.2±0.2**	**6.1±0.1**	**6.3±0.1**	**6.3±0.1**	**6.0±0.1**
2	**2.9±0.1**	**2.6±0.1**	**3.2±0.1**	**5.1±0.4**	**4.4±0.1**	**4.4±0.1**	**4.3±0.1**	**4.6±0.1**
3	**2.6±0.1**	**2.3±0.1**	**2.2±0.2**	**4.4±0.4**	**3.3±0.1**	**3.3±0.1**	**3.1±0.1**	**3.5±0.1**

In order to characterize organisms by their radiation sensitivity, the D_{10} value is used. It is defined as the dose required to inactivate 90% of a population or the dose of irradiation needed to produce a 10-fold reduction (1 Log reduction) in the population. All the target bacteria presented an exponential inactivation kinetics that allowed to calculate the D_{10} values presented in the Table 4.

Among the three studied bacteria, *Listeria monocytogenes* presented the lowest radioresistance, which is in accordance with other studies in fresh vegetables at refrigerated temperature[14]. For *E. coli* and *S.* Typhimurium it was observed higher D_{10} values on cherry tomatoes than the ones (0.7 and 0.3 kGy, respectively) reported in similar studies with gamma radiation [15]. This higher radioresistance can be justified by the anoxic conditions generated by electron beam irradiation. The absence of oxygen is an abiotic factor that decreases the lethal effects of ionizing radiation on microbial cells [4].

Regarding the inactivation of foodborne bacteria in raspberries, the results are presented in Table 5. 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 electron 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 5). 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 5). With irradiated raspberries, the refrigerated storage indicated a reduction of *S.* Typhimurium counts up to the 14 days, suggesting a synergistic effect between storage and irradiation on the inactivation of this bacteria.

TABLE 3. COUNTS (LOG CFU/G) OF *SALMONELLA* TYPHIMURIUM, *ESCHERICHIA COLI* AND *LISTERIA MONOCYTOGENS* ON NON-IRRADIATED (0 KGY) AND IRRADIATED (0.5 KGY UP TO 3.0 KGY) SPIKED FRESH CHERRY TOMATOES, IMMEDIATELY AFTER IRRADIATION (T0) AND 14 (T14) DAYS OF REFRIGERATED STORAGE. THE RESULTS ARE PRESENTED AS THE MEAN ± STANDARD ERROR

	Microbial counts (Log CFU/g)			
Storage time	Dose (kGy)	*S.* Thyphimurium	*E. coli*	Dose (kGy)	*L. monocytogenes*
0 days (T0)	0 (control)	**3.4±0.2**	**3.2±0.3**	0 (control)	**3.7±0.1**
	0.5	**2.9±0.2**	**2.8±0.2**	0.3	**2.3±0.1**
	1	**2.3±0.2**	**2.2±0.1**	0.6	**0.71±0.3**
	3	**ND**	**ND**	0.8	**ND**
14 days (T14)	0 (control)	**3.2±0.1**	**3.0±0.2**	0 (control)	**3.2±0.3**
	0.5	**2.7±0.1**	**2.5±0.1**	0.3	**1.8±0.2**
	1	**2.0±0.3**	**2.0±0.1**	0.6	**0.74±0.1**
	3	**ND**	**ND**	0.8	**ND**

ND - not detected.

TABLE 4. D_{10} VALUES OF BACTERIAL STRAINS ARTIFICIALLY INOCULATED ON CHERRY TOMATOES ANALYSED IMMEDIATELY AFTER IRRADIATION (T0) AND AFTER 14 DAYS OF STORAGE (T14). THE RESULTS ARE PRESENTED AS THE MEAN ± STANDARD ERROR

Microorganism	D_{10} value ± standard error (kGy)	
	T0	T14
Salmonella enterica serotype Typhimurium	0.84±0.01[a]	0.84±0.02[a]
Escherichia coli	0.97±0.02[a]	0.98±0.02[a]
Listeria monocytogenes	0.20±0.01[a]	0.24±0.01[a]

Values within a row with similar letters do not differ significantly ($p>0.05$).

Escherichia coli on raspberries also followed a linear inactivation ($R^2 = 0.99$) by electron 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 5). According to the literature, berry compounds are able to inhibit the growth of this bacteria [16]. 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 electron beam on

TABLE 5. COUNTS (LOG CFU/G) OF *SALMONELLA* TYPHIMURIUM, *ESCHERICHIA COLI* AND *LISTERIA MONOCYTOGENS* 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**	**2.8±0.1**	**2.7±0.2**	**2.7±0.1**	0	**3.0±0.1**	**3.1±0.1**	**2.3±0.1**	**2.2±0.1**	0	**3.1±0.1**	**2.8±0.1**	**3.0±0.1**	**ND**
0.5	**2.7±0.1**	**2.3±0.1**	**2.1±0.3**	**1.6±0.1**	0.5	**2.4±0.2**	**1.9±0.3**	**1.9±0.3**	**1.6±0.3**	0.5	**1.7±0.2**	**2.0±0.2**	**1.9±0.2**	**ND**
1.5	**1.5±0.2**	**0.6±0.1**	**0.6±0.1**	**ND**	1	**1.1±0.3**	**ND**	**ND**	**ND**	0.8	**1.0±0.2**	**1.1±0.2**	**0.9±0.1**	**ND**
3	**ND**	**ND**	**ND**	**ND**	3	**ND**	**ND**	**ND**	**ND**	3	**ND**	**ND**	**ND**	**ND**

ND - not detected.

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 5). 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 [17]. Moreover, other studies indicated that *Listeria* strains were not affected by berry compounds, with the exception of cranberry [18]. The previous results highlight the efficiency of electron 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.

6.3.2. Bioactivity of fruits extracts

6.3.2.1. Lycopene content and antioxidant activity in cherry tomatoes extracts

The human intake of lycopene is 85% from tomatoes and tomato products, which is the reason why tomatoes are used in functional food products, and sometimes as functional foods [19]. To assess the feasibility of a food treatment process it is crucial to maintain or improve its quality attributes. As lycopene is the most representative carotenoid in tomatoes, the influence of electron beam treatment and storage on its content and antioxidant activity were assessed by analysed immediately after electron beam irradiation and after 14 days of refrigerated storage (Table 6).

Regarding the impact of electron beam treatment on lycopene content of the samples after irradiation (T0), it was observed that there was no significant ($p>0.05$) variation between the control sample (0 kGy) and the irradiated cherry tomatoes at 3.1 kGy. This result suggests that this treatment could preserve the lycopene content immediately after irradiation. Moreover, for the samples tested immediately after irradiation (T0), the EC50 values indicated no significant difference ($p>0.05$) between non-irradiated and irradiated samples, indicating that the antioxidant activity was preserved after electron beam irradiation. The lycopene content of non-irradiated samples (0 kGy) decreased significantly to less than half ($p<0.05$) after 14 days of refrigerated storage (412 mg/100 g of non-stored sample and 189 mg/100 g sample after 14 days of storage). A decrease in lycopene content during storage at low temperatures (4 and 8°C) was reported in tomatoes cvs. Cappricia and Amoroso and could be influenced by higher moisture content observed in the tomatoes stored at refrigerated temperatures [20]. A pronounced decrease ($p<0.05$) in lycopene content was observed for the irradiated samples stored over 14 days, this reduction in lycopene content was also reflected in the antioxidant activity (increase of EC50 values) of the irradiated cherry tomatoes with 14 days of storage. Nevertheless, the antioxidant activity of lycopene extracts was only significantly lower ($p< 0.05$) than control for the treated fruits at 3.1 kGy and 14 days stored. This could result from the lycopene isomers antioxidant activity induced by higher oxidative stress on the irradiated fruits during storage time. The lower lycopene content can be explained by the production of by-products of lycopene that were not extracted by the applied methodology, consequently the higher EC50 value reflects a lower lycopene concentration in the analysed samples. Nevertheless, this reduction may not necessarily represent a degradation of lycopene by electron beam irradiation, but instead could denote an isomerization or oxidation of this carotenoid enhanced by the combination of irradiation with the storage duration. In fact, other authors verified by HPLC analyses of tomato products extracts, a reduction of (all-E)-lycopene with increasing peaks of lycopene (Z)-isomers following electron beam irradiation [21]. According to these authors, the electron beam treatment increased the antioxidant ability of

tomato products in inhibiting spontaneous and H_2O_2-induced oxidative stress in cultured fibroblasts.

TABLE 6. ANTIOXIDANT ACTIVITY AND LYCOPENE CONTENT IN CHERRY TOMATO EXTRACTS OF NON-IRRADIATED AND IRRADIATED CHERRY TOMATOES RESULTS ARE PRESENTED AS THE MEAN ± STANDARD ERROR

Storage time (days)	Irradiation dose (kGy)	Antioxidant activity* (DPPH scavenging, EC50 µg/mL)	Lycopene content* (mg per 100g sample)
0	0	708±15^{b,d}	412±3^{a}
	1.5	793±10^{b,d}	287±7^{b}
	3.1	685±16^{c,d}	398±4^{a}
14	0	628±15^{c,d}	189±2^{c}
	1.5	861±11^{b}	184±11^{c}
	3.1	1068±21^{a}	105±4^{d}

* Results are presented as the mean ± standard error, note that means within a column with different superscript letters differ significantly ($p<0.05$).

6.3.2.2. Phenolic content, antioxidant activity and ascorbic acid content of raspberries extracts

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 7.

TABLE 7. ANTIOXIDANT ACTIVITY (DPPH AND FRAP ASSAYS) AND TOTAL PHENOLIC CONTENT IN EXTRACTS OF NON-IRRADIATED AND IRRADIATED RASPBERRIES ANALYSED IMMEDIATELY AFTER ELECTRON BEAM IRRADIATION AND DURING 14 DAYS OF REFRIGERATED STORAGE. THE RESULTS ARE PRESENTED AS THE 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}

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

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 possibly due to fruit structure alterations, and/or to the radiolytic breakage of larger phenolic compounds (e.g. tannins) into smaller ones [22].

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 electron 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 ebeam 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 electron 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 electron 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.

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

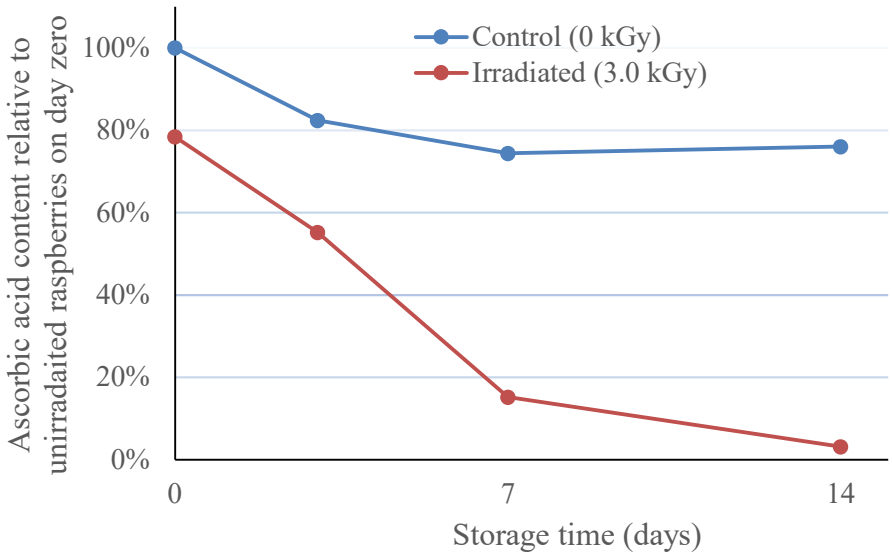

FIG. 1. Effect of electron-beam irradiation on ascorbic acid content of raspberries on days 0, 3, 7 and 14 during storage.

to other nutrients. The amount of ascorbic acid in non-treated raspberries on storage day zero was 125 ± 5 mg/100 g of dry weight. The amount of ascorbic acid on storage days, 0, 3 and 7 are presented in FIG. 1. as a percentage of the initial ascorbic acid content in control samples on day zero.

Immediately after irradiation (day 0), a significant decrease ($p<0.05$) in ascorbic acid content was caused by electron beam treatment and we have presented these results in more detail in ref. [10]. 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 directly protecting other compounds from oxidative degradation [23]. Both mechanisms result in a (reversible) oxidation of ascorbic acid to dehydroascorbic acid that can be further hydrolyzed and oxidized irreversibly into other products [24]. 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 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). It was estimated that ascorbic acid contributed approximately 20% to the total antioxidant capacity of raspberries [25]. Dehydroascorbic acid has a recognized physiological role since it can be used by metabolically competent cells, where it is reduced back to ascorbic acid. It is widely accepted that dietary ascorbic acid and dehydroascorbic acid have equivalent bioavailability in humans [26]. In this way, the use of irradiation will not result in a severe loss of nutritional value on raspberries.

6.3.3. Citotoxicity of fruits extracts

6.3.3.1. Cherry tomatoes extracts

Lycopene is used as food supplement or nutraceutical ingredient in the formulation of food products due to its bioactivity. Considering these applications, the effect of electron beam irradiation in the cytotoxicity of lycopene extracts must be tested. In the present study the cytotoxicity was evaluated by the WST-1 cell viability assay using three human cell lines, namely 293T: Human embryonic kidney; Caco-2: heterogeneous human epithelial colorectal adenocarcinoma – cancer cells; and A549: adenocarcinomic human alveolar basal epithelial cells – lung cancer cells. For non- and irradiated cherry tomatoes analysed immediately after electron beam irradiation (T0), no significant ($p>0.05$) inhibitory activity (cell inhibition<10%) of lycopene extracts at the assayed concentrations was detected on the analysed three cell lines (Fig. 2.) On the contrary, a significant ($p<0.05$) increase (approximately 12%) in the viability of A549 lung cancer cells was observed for lycopene extracts (5 μM, 0.05 μM and 0.005 μM) from non-treated cherry tomatoes (0 kGy T0; Fig. 2b).

After 14 days of refrigerated storage (T14), antiproliferative effects of lycopene extracts were observed on: 293 T cells (cell inhibition between 16 and 53%) for samples of cherry tomatoes irradiated at 1.5 kGy (Fig. 2a); A549 cells (cell inhibition between 29 and 80%) for irradiated samples of cherry tomatoes (Fig. 2b); Caco-2 cells (cell inhibition between 25 and 46%) for non-irradiated samples of cherry tomatoes (Fig. 2c). However, only few T14 samples induced a significant ($p<0.05$) diminution of cells viability comparatively to control, namely the lycopene extracts from cherry tomatoes: treated at 1.5 kGy at a concentration of 0.005 μM on

293 T cells (cell inhibition 53%); irradiated at 1.5 kGy and 3.1 kGy at the concentration of 0.5 μM (cell inhibition 66%) and 0.005 μM (cell inhibition 80%), respectively on A549 cells; and non-treated (0 kGy) at the concentration of 0.005 μM (cell inhibition 46%) on Caco-2 cells. These results express a marked influence of storage time on the cytotoxicity of lycopene extracts as it has also been denoted on lycopene content and antioxidant activity assays, related as mentioned previously with the proposed induced isomerization of lycopene by storage. Among the tested cell lines, lung cancer A549 cells, indicated to be the most sensitive to T14 lycopene extracts, especially from the irradiated cherry tomatoes. Given the fact that lycopene extracts from irradiated cherry tomatoes have no considerable effect on normal cells (293 T cells), while having significant cytotoxic activities towards A549 cells. This highlights the reliability of electron beam irradiation to improve the bioactivity of cherry tomatoes and its potential application as a functional food or improve the regular use of lycopene as a food supplement or nutraceutical ingredient. However, further research should be conducted to clearly assign the bioactive potential for each lycopene by-product.

6.3.3.2. Raspberry extracts

Studies have indicated that in raspberry extracts, some polyphenols (e.g. anthocyanins, ellagitannins, and ellagic acid) either individually or together with other compounds (e.g. ascorbic acid, carotenoids) have anti-proliferative activity against cancer cells in vitro [27] with synergistic effects. Therefore, the effects of electron beam irradiation on raspberry extract cytotoxicity were evaluated using cell viability assays where two human cells lines were used in WST-1 cell viability assays to assess potential antitumor activity. The cell lines were the human embryonic kidney 293 (293 T, non-tumour) cell line and the A549 lung tumour cell line.

The percent cell viability obtained by experiments with the two cell lines exposed to three concentrations of extracts from raspberries (raspberry extracts from non-irradiated, 3 kGy irradiated samples at, non-stored and stored raspberry samples) are presented in detail in ref. [10]. The higher extract concentration of 400 μg/mL was found to have a significant inhibitory effect on cell viability with the nontumorigenic cell line (293 T) and this was independent of storage time and fruit being irradiated or not. Non-irradiated and irradiated fruit extracts at the lower concentrations of 4 and 40 μg/mL had no significant effect on cell (293 T) proliferation immediately after irradiation and up to 7 days of storage. However, extracts stored for 14 days were exceptional as all fruits extracts (non-irradiated and irradiated) were found to have anti-proliferative activity [10]. Raspberries extracts, at any concentration from any treatment (non-irradiated/irradiated; non-stored/stored), were not found to effect cell growth of the A549 lung tumour cell line. We concluded that the extracts had no in vitro antiproliferative activity against tumour cells within our experimental conditions [10].

Previous studies indicate that cell lines of different origins have variable sensitivity in growth toward berry extracts [28], as was observed in our study. Nevertheless, to the best of our knowledge none of the cells lines that we tested were previously studied against raspberry extracts. We have therefore demonstrated its applicability to evaluate antitumor activity of extracts from irradiated fruits [6] and the cytotoxicity of plant extracts [29]. In our view, other cells lines should be used to evaluate the anti-proliferative potential of extracts from electron beam treated raspberries considering the detected increases in phenolic content immediately after irradiation and in antioxidant activity after 7 days of storage.

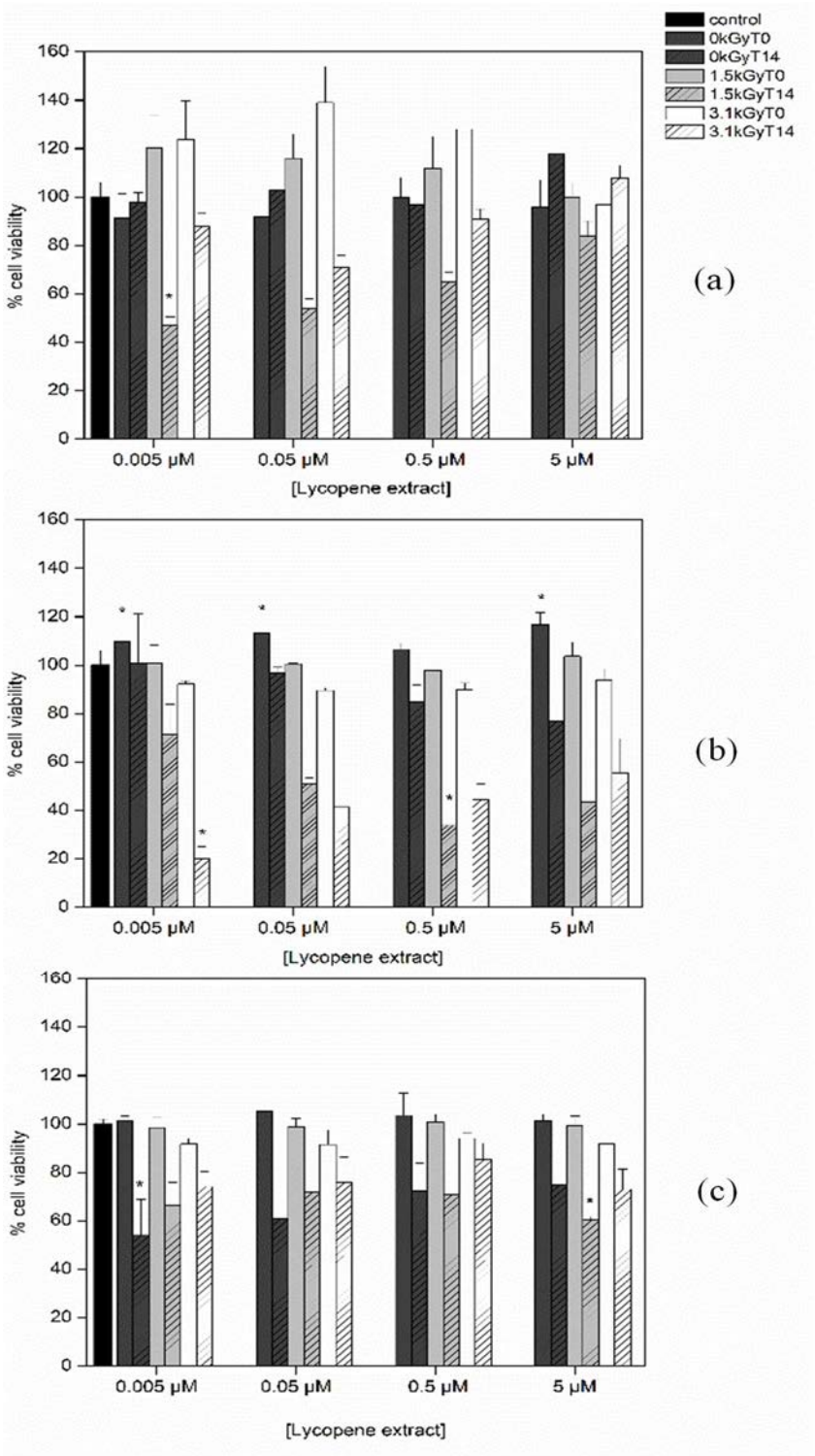

*FIG. 2. Cellular viability of (a) 293t, (b) a549 and (c) caco-2 cell lines in the presence of different concentrations (0.005 µm, 0.05 µm, 0.5 µm, 5 µm) of lycopene extracts from non-irradiated (0 kGy) and e-beam irradiated (1.5 kGy, 3.1 kGy) cherry tomatoes, immediately after irradiation (t0) and after 14 days of refrigerated storage (t14). Each bar graph represents the mean and 95% confidence interval of three separate experiments. For each cell line, bars with * indicates a statistically significant difference from control at $p<0.05$.*

6.3.4. Nutritional value and chemical profile of mushrooms

Fresh mushrooms are highly perishable and therefore it is desirable to apply effective technologies to preserve and protect their chemical composition and nutritional value. Irradiation appears to be an excellent alternative food preservation method that maintains the quality of fresh mushrooms.

The effects of electron beam irradiation and storage time on nutritional parameters (moisture, fat, protein, ash and carbohydrate content) of fresh samples of *Agaricus bisporus* Portobello were evaluated. The measured values were similar for all samples (unirradiated, irradiated and refrigerated storage for up to 8 days). Water was found to be the major component with a moisture content of 89% (Table 8). A high moisture content can amplify the action of ionizing radiation on food components, because primary free radicals (hydroxyl, hydrogen atoms and hydrated electrons) generated directly by the irradiation of water can interact with macromolecules of the food. Therefore, the water content in Portobello mushrooms justified the need to study several different chemical parameters.

On a dry weight (dw) basis, carbohydrates were the main component (64–65 g/100 g dw), followed by protein (23.2–24.5 g/100 g dw), ash (9.2–9.9 g/100 g dw) and fat (1.7–1.8 g/100 g dw). At least one electron beam dose caused a significant change in all nutritional parameters, while storage time only affected protein and carbohydrate content. The only observed overall tendency was the higher protein content in samples irradiated with 5 kGy. All in all, the results indicated that electron beam irradiation does not exert any remarkably negative effect over the nutritional parameters and length of storage time studied (up to 8 days). Our results for Portobello mushroom samples are in general agreement with the results obtained in other mushroom species [30, 31, 32].

The profiles of polar compounds (organic acids and sugars) were also measured for irradiated and stored Portobello mushrooms (Table 9). Organic acid and sugar content are important indicators of reliable preservation conditions, significant differences were observed in all cases except trehalose content ($p=0.051$). Furthermore, several general trends were observed: non-irradiated samples showed a lower malic acid content than irradiated samples (a difference of 0.5 g/100 g dw) and Total organic acid contents were also lower in unirradiated samples (2.7 g/100 g dw) compared to irradiated samples, but higher concentrations of mannitol (38 g/100 g dw) and total sugars (41 g/100 g dw) were found in unirradiated samples as compared to irradiated samples. Portobello mushroom samples irradiated with 2 kGy gave the highest value in quinic acid (1.0 g/100 g dw), which showed the lowest value (0.8 g/100 g dw) in non-stored samples, similarly to malic acid (1.6 g/100 g dw) and total organic acids (3.0 g/100 g dw). The low-extent of changes detected in sugars and organic acids are also in agreement with previous reports describing the effects of irradiation in related mushroom species [31].

Lipophilic compounds were also studied (fatty acids, tocopherols and ergosterol). Fatty acids are also considered as good indicators of suitable shelf-life conditions, while tocopherols and ergosterol are well known for their bioactivity, particularly antioxidant and hypocholesterolemic effects, respectively. These parameters (Table 10) presented also significant differences, except C18:0 and β-tocopherol regarding electron beam irradiation effects, and MUFA, α-tocopherol and β-tocopherol, in regard to storage duration effects. Overall it was possible to conclude that samples irradiated with 1 kGy presented higher percentages of cis-C18:2n−6 (78.9%) and PUFA (79.4%) and lower percentages of SFA (19.5%), while non-irradiated ones showed the lowest content (1.8%) of C20:0. With storage

time, it was only possible to verify that 8 days stored samples showed the lowest percentage of cis-C18:2n−6 (78.1%). The slight differences in lipophilic compounds (which are prone to be oxidized) were previously reported in mushrooms [33] and may result from autoxidation processes, since Portobello samples were not stored in oxygen-free conditions. Since the occurrence of this important phenomenon might affect the sensorial quality of mushrooms, it is worth mentioning, however, that the results obtained herein seem to indicate that lipid oxidation occurred to a minor extent (as indicated by the maintenance of percentages of fatty acids more prone to be oxidized).

All in all, irradiation seems to be a suitable conservation technique, owing to its capacity to maintain the chemical profiles of this mushroom species for extended shelf-life periods.

TABLE 8. PROXIMATE COMPOSITION AND ENERGY VALUE OF *AGARICUS BISPORUS* PORTOBELLO SAMPLES SUBMITTED TO DIFFERENT ELECTRON-BEAM IRRADIATION DOSES AND STORAGE TIMES. THE RESULTS ARE PRESENTED AS MEAN ± SD[1]

		Moisture (g/100 g fw)	Fat (g/100 g dw)	Proteins (g/100 g dw)	Ash (g/100 g dw)	Carbohydrates (g/100 g dw)	Energy (kcal/100 g dw)
EB	0 kGy	90±1	1.8±0.1	24.7±0.5	9.2±0.4	64±1	372±2
	1 kGy	89±1	1.7±0.1	23.6±0.4	9.8±0.4	65±1	369±2
	2 kGy	90±1	1.7±0.1	23.6±0.4	9.8±0.5	65±1	369±3
	5 kGy	89±1	1.8±0.1	24.9±0.5	9.2±0.4	64±1	372±2
	ANOVA *p*-value (n = 27)[2]	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
ST	0 days	89±1	1.8±0.1	24.9±0.5	9.6±0.5	64±1	370±3
	4 days	90±1	1.8±0.1	23.7±0.4	9.4±0.5	65±1	371±3
	8 days	89±1	1.7±0.1	24.0±0.4	9.6±0.2	65±1	370±1
	ANOVA *p*-value (n = 36)[3]	0.518	0.237	<0.001	0.176	<0.001	0.217
EB×ST *p*-value (n = 108)[4]		0.003	<0.001	<0.001	<0.001	<0.001	<0.001

[1]Results are reported as mean values of each irradiation dose, aggregating results from 0, 4 and 8 days, and mean values of ST, combining all irradiation doses. [2] If $p<0.05$, the corresponding parameter presented a significantly different value for at least one dose. [3] If $p<0.05$, the corresponding parameter had a significant difference for at least one of the time intervals. [4] The interaction among factors was significant in all cases; thereby the statistical classification could not be indicated.

TABLE 9. POLAR COMPOUNDS (ORGANIC ACIDS AND SUGARS) OF *AGARICUS BISPORUS* PORTOBELLO SUBMITTED TO DIFFERENT IRRADIATION CONDITIONS AND STORAGE TIMES. THE RESULTS ARE PRESENTED AS MEAN ± SD[1]

		Sugars (g/100 g dw)				Organic acids (g/100 g dw)			
		Fructose	Mannitol	Trehalose	Total	Oxalic acid	Quinic acid	Malic acid	Total
EB	0 kGy	0.8±0.2	38±2	1.8±0.5	41±3	0.5±0.1	0.8±0.1	1.3±0.1	2.7±0.1
	1 kGy	0.7±0.2	33±2	1.5±0.4	35±2	0.6±0.1	0.9±0.1	1.7±0.1	3.2±0.1
	2 kGy	0.6±0.1	31±7	1.5±0.2	33±7	0.6±0.1	1.0±0.1	1.8±0.1	3.4±0.2
	5 kGy	0.7±0.1	34±2	1.7±0.2	36±2	0.6±0.1	0.9±0.1	1.9±0.1	3.4±0.1
	ANOVA *p*-value (n = 27)[2]	<0.001	<0.001	0.051	<0.001	<0.001	<0.001	<0.001	<0.001
ST	0 days	0.7±0.1	36±3	2.0±0.5	39±4	0.6±0.1	0.8±0.1	1.6±0.2	3.0±0.3
	4 days	0.8±0.1	34±1	1.4±0.1	37±1	0.6±0.1	0.9±0.1	1.7±0.2	3.2±0.3
	8 days	0.5±0.1	32±7	1.5±0.3	34±7	0.6±0.1	1.0±0.1	1.7±0.2	3.3±0.3
	ANOVA *p*-value (n = 36)[3]	<0.001	<0.001	<0.001	<0.001	0.022	<0.001	0.063	0.001
EB×ST *p*-value (n = 108)[4]		0.003	<0.001	<0.001	<0.001	<0.001	<0.001	0.011	0.005

[1]Results are reported as mean values of each irradiation dose, aggregating results from 0, 4 and 8 days, and mean values of ST, combining all irradiation doses. [2]If $p<0.05$, the corresponding parameter presented a significantly different value for at least one dose. [3]If $p<0.05$, the corresponding parameter had a significant difference for at least one of the time intervals. [4]The interaction among factors was significant in all cases; thereby the statistical classification could not be indicated.

TABLE 10. LIPOPHILIC COMPOUNDS (FATTY ACIDS, TOCOPHEROLS AND ERGOSTEROL) OF *AGARICUS BISPORUS* PORTOBELLO SUBMITTED TO DIFFERENT IRRADIATION CONDITIONS AND STORAGE TIMES. THE RESULTS ARE PRESENTED AS MEAN ± SD[1]

		Fatty acids (relative percentage)									Tocopherols (μg/100 g dw)		Ergosterol (mg/100 g dw)
		C16:0	C18:0	C18:2n6c	C20:0	C22:0	C24:0	SFA	MUFA	PUFA	α-tocopherol	β-tocopherol	
EB	0 kGy	8.2±0.5	4.1±0.2	78.6±0.2	1.8±0.1	1.5±0.2	1.1±0.1	20.0±0.3	1.0±0.2	79.0±0.2	0.51±0.03	10.2±0.3	216±11
	1 kGy	7.5±0.3	4.2±0.1	78.9±0.4	2.1±0.1	1.5±0.1	1.2±0.1	19.5±0.3	1.0±0.2	79.4±0.4	0.50±0.04	10.0±0.3	226±17
	2 kGy	7.7±0.1	4.1±0.2	78.0±0.5	2.2±0.1	1.6±0.1	1.3±0.1	20.6±0.5	0.9±0.1	78.5±0.5	0.47±0.04	10.1±0.4	233±15
	5 kGy	8.1±0.1	4.1±0.1	78.2±0.2	2.0±0.1	1.5±0.1	1.2±0.1	20.2±0.1	0.9±0.1	78.6±0.2	0.51±0.04	10.1±0.5	238±10
	ANOVA *p*-value (n = 27)[2]	<0.001	0.082	<0.001	<0.001	0.001	<0.001	<0.001	0.026	<0.001	0.002	0.119	<0.001
ST	0 days	8.1±0.5	4.1±0.2	78.5±0.2	1.9±0.2	1.4±0.1	1.1±0.1	20.1±0.3	0.9±0.2	79.0±0.2	0.50±0.04	10.2±0.3	222±18
	4 days	7.8±0.2	4.1±0.1	78.6±0.5	1.9±0.1	1.5±0.1	1.1±0.1	19.9±0.5	1.0±0.1	79.1±0.5	0.50±0.04	10.0±0.4	234±12
	8 days	7.7±0.3	4.2±0.1	78.1±0.5	2.1±0.2	1.6±0.1	1.3±0.1	20.4±0.5	1.0±0.1	78.6±0.5	0.49±0.04	10.1±0.3	229±14
	ANOVA *p*-value (n = 36)[3]	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.428	<0.001	0.317	0.145	0.004
EB×ST *p*-value (n = 108)[4]		0.003	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.087	<0.001

[1]Results are reported as mean values of each irradiation dose, aggregating results from 0, 4 and 8 days, and mean values of ST, combining all irradiation doses. [2]If $p<0.05$, the corresponding parameter presented a significantly different value for at least one GI or EB. [3]If $p<0.05$, the corresponding parameter had a significant difference for at least one of the time intervals. [4]The interaction among factors was significant in all cases; thereby the statistical classification could not be indicated.

6.3.5. Modelling

The electron beam irradiation setup at C2TN was simulated. The simulation cases considered for study related to the irradiation of cherry tomatoes and raspberries with a mono-energetic electron beam. The simulation framework used was ENSARRoot and the simulated setup for the cherry tomatoes is shown in the figure below (Fig. 3). The cherry tomatoes were modelled as water spheres with a diameter of 7 cm.

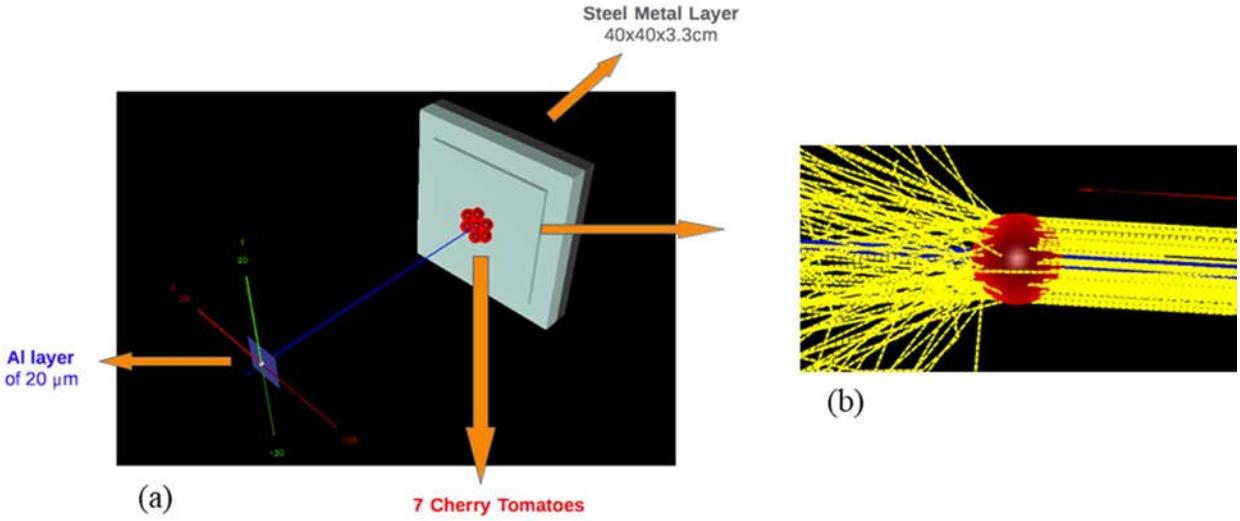

FIG. 3 (a) Simulated setup of cherry tomatoes irradiation using the framework ENSARRoot. (b) Cherry tomatoes exposed to 10 MeV mono-energetic electrons.

The cherry tomato simulation considered exposure to 10 MeV mono-energetic electrons (Fig. 3). As well as indicating the trajectories of beam electrons (shown as yellow trajectories in Fig. 3.), the production of bremsstrahlung photons (blue lines in Fig. 3.) can also be observed to be generated in the model simulation. We studied the dose distribution on the tomatoes, considering two well differentiated regions: the skin (with a thickness of 0.5 cm) and the internal flesh. Furthermore, the deposited dose was also analysed in the front and back halves of the tomatoes (Fig. 4), to verify the homogeneity of the irradiation process. The results are summarized in the Table 11.

TABLE 11. DOSE DISTRIBUTION ON THE CHERRY TOMATOES CONSIDERING DIFFERENTIATED REGIONS: SKIN (WITH A THICKNESS OF 0.5 CM), INTERNAL FLESH, AND FRONT AND BACK HALVES OF THE TOMATOES

	Skin	Flesh	Sum
Front half	3.7%	46.7%	50.4%
Back half	3.4%	46.2%	49.6%
Total	7.1%	92.9%	–

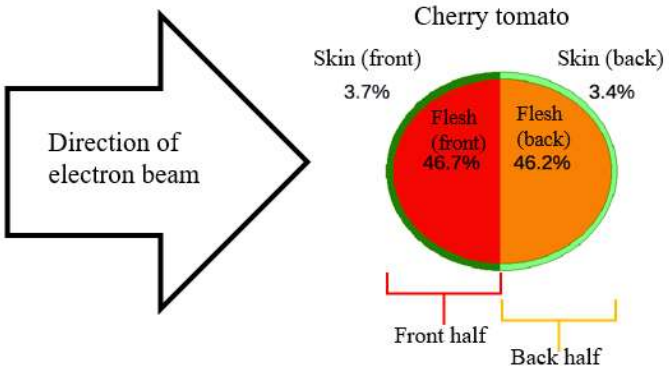

FIG. 4. Modelling of percentage dose distributed in a cherry tomato, considering the skin and the internal flesh (see also Table 11).

The irradiation points out to be quite homogeneous for this proposed geometry, with a rather small fraction of the deposited dose left on the skin of the cherry tomatoes.

For raspberries, a view of the simulated geometry is shown in Fig. 5. The raspberries were simulated as spheres of 5 mm diameter, with a total height of 2.5 cm.

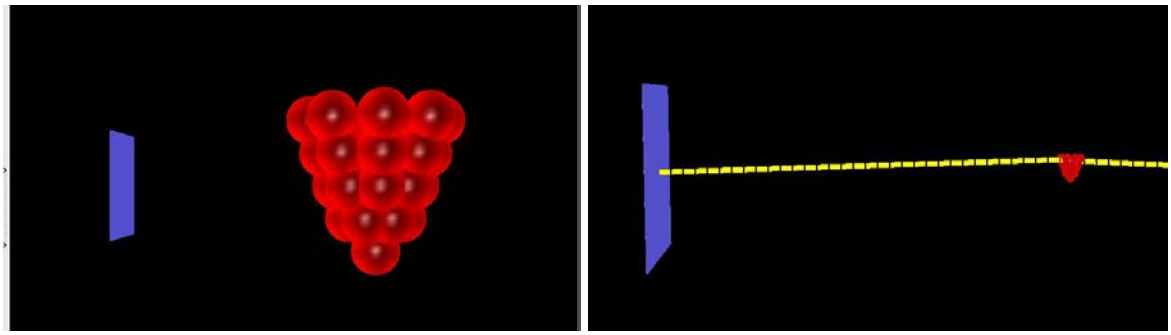

FIG. 5. (a) Left-hand side image is the simulated geometry of raspberries irradiation using the framework ENSARRoot. (b) Right-hand side image is the simulation of raspberries exposed to 10 MeV mono-energetic electrons.

The raspberries were simulated as being exposed to 10 MeV mono-energetic electrons (given by yellow trajectories, schematic view on Fig. 5b). Further studies are needed to evaluate the distribution of the simulated dose as a function of position, and to study the details of the distribution of the deposited energy on each of the individual elements of the fruit.

6.4.CONCLUSION

The present study shows that electron beam treatment at 3 kGy could be used as a disinfection process to guarantee the food safety of cherry tomatoes and raspberries, extending its shelf-life to at least 7 days of storage. This green technology can preserve the bioactivity of these fruits, although in cherry tomatoes a transformation of lycopene (e.g. isomerization, oxidation) was suggested to occur during storage, and a loss in ascorbic acid amount was detected in treated raspberries. Moreover, cytotoxic assays revealed that lycopene extracts from irradiated and stored cherry tomatoes had non-toxic effect against non-cancerous 293T cells, and potential inhibitory activity against A549 cancerous cells. Similarly, 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 antiproliferative activity of raspberries extracts. Regarding Portobello mushrooms, the 5 kGy dose, tended to be associated with higher levels of protein. On the other hand, in what concerns the effect of storage time up to 8 days, it could be verified that electron beam treatment indicated to be effective in maintaining the chemical profiles of Portobello samples, except for quinic acid, grouped organic acids and some particular SFA. Accordingly, this technology might represent effective preservation approaches for Portobello mushrooms.

The future trends of food processing cannot be considered independently of sustainability, eco-friendly, innovation, and advanced technologies. Electron beam treatment might be of interest from a technological perspective, reducing losses from harvest/storage to consumption, and increase cherry tomatoes, raspberries and mushrooms potential for posterior industrial applications.

ACKNOWLEDGEMENTS

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme PT2020 for financial support to CIMO (UID/AGR/00690/2013), C2TN (UID/Multi/04349/2013; UID/Multi/04349/2019). This work was also 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).

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