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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
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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

SUMMARY

INTRODUCTION

This technical document provides a report of the work undertaken under coordinated research project (CRP) D61024 on the Development of Electron Beam and X ray Applications for Food Irradiation (DEXAFI). The project was developed in 2014 and implemented from 2015 to 2021.

Most irradiated food and agricultural products are processed in facilities using ^{60}Co as the source of ionizing radiation. The amount of ^{60}Co available worldwide is limited and it can be difficult and expensive to supply large amounts of radioisotopes due to security and logistical complexities associated with trans-boundary shipments. Although gamma irradiation is a simple, reliable, and mature technology and will be available for many years to come, alternative irradiation technologies would help complement the available capacity of gamma facilities to irradiate food. Making more use of alternative modes of irradiation would enable food irradiation to be used more widely without increasing the demand for ^{60}Co radioisotope sources. Electron beam and X ray irradiation are alternative technologies that employ electricity to generate ionizing radiation and avoid the procurement, transport, storage, disposal, and safeguard issues associated with radioisotopes.

At the commercial level, radiation processing usually involves an irradiation facility that is a stand-alone service provider. It contracts its irradiation services to others. Irradiation facilities are often multipurpose in that they offer their services for a wide range of different commodities, food and non-food. These facilities are generally located on major transport hubs, or trade routes that serve ports and airports. Each facility is essentially a large warehouse with an irradiator at its centre. In practice, the irradiator is usually a gamma facility, but high energy electron beams or X rays may also be used. In order to stimulate more interest in alternatives to gamma irradiation, the CRP included research into high energy electron beam (up to 10 MeV) and high energy X ray (up to 7.5 MeV) irradiation of foods.

Electron beams are less penetrating than gamma rays and dosimetry and operational parameters need to be carefully considered to ensure that the food product is irradiated correctly. Research to underpin the development of new tools to help establish and validate processing parameters before electron beam irradiation would be helpful. The CRP therefore set out to include research into rapid methods to scan boxed products immediately prior to radiation processing in order to quickly determine if the food package is within specification for correct electron beam irradiation and to calculate and predict the key process parameters such as the location and size of the minimum dose (D_{min}) and maximum dose (D_{max}) plus the dose uniformity ratio ($DUR = D_{max}/D_{min}$) and the dose distribution that would be achieved during subsequent electron beam irradiation.

In addition, the research and development of relatively small machine source irradiators could provide an alternative to the established way that food irradiation is employed. The commercial use of multipurpose irradiation facilities is likely to continue for many years, but adapting irradiation technology that is used in non-food applications may provide methods of irradiating food that can be incorporated in a food factory. Smaller size irradiation units produce relatively low energy beams of electrons or X rays and so do not require heavy shielding to protect workers from the ionizing radiation. In contrast to conventional irradiation, such low energy devices could be used in a food preparation area, food factory or on a food packaging line. Although low energy irradiation (e.g. <300keV electrons) cannot penetrate deeply into food there is scope for new applications. One example is using low energy beams to target the surface

or near surface of food products. This is often where adventitious microbial contamination can be found., The outer volume of food would be treated but it would leave the inner volume un-irradiated. The CRP therefore also investigated the application of low energy irradiation technology for comparison with conventional irradiation.

OBJECTIVE

This publication brings together some of the main results of the research completed under CRP D61024. The overall objective of this publication is to report the novel and practical applications of electron beam and X ray technologies that were developed as part of this CRP in order to facilitate the future use of machine source radiation to irradiate food.

SCOPE

The publication consists of research reports that cover different aspects of using ionizing radiation to treat food and agricultural products. The emphasis is on machine generated electron beam and X ray irradiation. This publication includes both low and high energy electron beam and X ray irradiation of food. It also includes some research reports of gamma irradiation using the radionuclide source (^{60}Co) because several reports compare electron beam and / or X ray irradiation to the more widely used gamma irradiation technique.

STRUCTURE

Research reports are presented under the following general headings:

New Tools to Rapidly Ascertain Treatment Parameters Before Electron Beam Irradiation

The research reports begin with work to develop new tools to rapidly ascertain treatment parameters before electron beam irradiation.

Electron Beam Irradiation

A series of six research reports cover different aspects of electron beam irradiation. It starts with low energy electron beam irradiation (irradiation of the surface and near surface of foods) and then several reports extend this to the use of higher energy electron beams (irradiation of the whole food). The purposes of these electron beam irradiation treatments are to provide food products that are superior to non-irradiated foods in some way. For example, by ensuring food safety, maintaining quality, and extending shelf-life. A number of reports also investigate the use of electron beam irradiation to improve the nutritional value and/or enhance the availability of beneficial bioactive chemical components in food.

X ray Irradiation

X ray irradiation is then covered in a series of five research reports, covering: the development of an improved dosimetry system for low to medium energy (up to 300 keV) X ray irradiators; the use of a low energy (160 keV) X ray cabinet irradiator to provide clean food for immune-compromised hospital patients; the application of high energy (7 MeV) X rays to a fresh strawberry variety; an assessment of activation in food products irradiated with high energy X rays, and; the development of a portable low energy (<200 keV) X ray irradiator for phytosanitary irradiation.

Comparative Studies of Different Modes of Irradiation

Comparative studies of ^{60}Co gamma ray, electron beam and X ray irradiation include research reports on: the choice of electron beam or X ray technology for a phytosanitation program; electron beam and gamma irradiation treatments to prevent post-harvest losses due to plant pathogens affecting fruit products; irradiation as a phytosanitary treatment of fresh produce; the effects of irradiation at two different dose rates (approximately 4 and 330 Gy/minute both from ^{60}Co gamma irradiation) applied as phytosanitary treatments for wax moth and khapra beetle, and; a comparative study of gamma, electron beam and X ray irradiation technologies for a number of different food products.

Dose Intercomparison Exercise

One research report gives the results of dosimetry intercomparison studies that were organized and implemented by Aerial in France and involved most of the CRP participants.

BRIEF REVIEW OF RESEARCH REPORTS

New Tools to Rapidly Ascertain Treatment Parameters Before Electron Beam Irradiation

Research reports 1 and 2 from research institutions in the People's Republic of China and the Socialist Republic of Viet Nam, respectively, focus on two new devices that were developed as pre-irradiation tools to optimize processing by high energy electron beam irradiation. These new tools can be used to quickly assess products as received by the irradiation facility and to check if it is feasible to irradiate products in a specific packing configuration. For example, dose mapping predictions help determine the most efficient means of placing product in a carrier or tote and where to place numerous dosimeters throughout the product load to measure and establish the locations of the minimum and maximum doses. Research report 1, from Tsinghua University in collaboration with Nuctech, People's Republic of China concerns the development and testing of a new X ray device to measure mass thickness. Research report 2 from the Research and Development Center for Radiation Technology, Vietnam Atomic Energy Institute details the development and testing of a new device that uses the attenuation of gamma rays from a ^{137}Cs source to detect mass thickness in products. Both machines scan pre-packaged products presented in the product loading configuration and use measurements of mass thickness to calculate and predict the location and magnitude of D_{min} and D_{max} plus the DUR and the dose distribution that would be achieved during subsequent electron beam irradiation.

Electron Beam Irradiation

Research report 3 from Japan, is a comparative study of the effects of different energies of electrons on bacterial spores and food matrices. The thickness of typical dosimeters can become an issue with irradiation using low-energy electrons (<300 keV). At these low energies there is likely to be a dose gradient across a typical dosimeter that has a thickness measured in millimetres. Different thickness dosimeters may yieled different dose readings when irradiated at the same electron beam. However, correcting all measured doses to the average dose in the first micrometer (the $D\mu$ concept) overcomes this problem. The $D\mu$ concept was used to determine the imparted doses to surface and near surface regions of foods. This approach confirmed that the lethal effects of different energy electron beams on foodborne microorganisms are comparable at energies below 300 keV. Recognizing the importance of dosimetry, the researchers also investigated the development of a dose measurement system for

low energy (80 keV–300 keV) electrons in collaboration with researchers in the Republic of Poland (research report 4).

Research report 4 from the Republic of Poland, also examines the effectiveness of low energy (200–300 keV) electrons to eliminate microbial contamination from dried whole-spice samples (bay leaves, onion flakes, white pepper corns, black pepper corns and allspice grains). Collaborative work with researchers in Japan (research report 3) investigated the development of a dose measurement system for low energy electrons.

Research report 5 from the People's Republic of China concerns the use of higher energy (2 MeV) electron beam irradiation of mango fruits and investigated the use of electron beam irradiation to preserve the fruit and extend its storage life by delaying ripening.

Research report 6, from Portugal investigated the use of high energy (10 MeV) electron beam irradiation to provide fresh foods such as cherry tomatoes, raspberries, and mushrooms, with enhanced nutrient or bioactive chemical components that are safe and convenient.

Research from the Kingdom of Thailand is presented in research report 7. This involved four different types of pickled products (pickled ginger, mixed pickled vegetables, pickled shredded sweet-salted white radish, and fermented bamboo shoots) and working with commercial enterprises to develop electron beam-based processes that would result in irradiated products superior to their traditional counterpart and with a longer shelf-life.

Research report 8, from the Republic of Indonesia, investigated using electron beam irradiation to improve the nutritional quality of soy flour. The research investigated soy (Glycine max L.) flour and the influence of irradiation using two different electron beams (1.5 MeV and 10 MeV) at different doses on the anti-nutrient compound phytic acid, and beneficial chemical components (isoflavones).

X ray Irradiation

Research report 9, from France provides information on establishing improved dosimetry systems for low to medium energy (up to 300 keV) X ray irradiators. Alanine / electron paramagnetic resonance dosimetry systems are established for reference, transfer and routine dosimetry using high energy (MeV) beams. Alanine's relative response to kilovoltage X rays, compared to a ^{60}Co reference quality gamma beam, was studied in order to determine correction factors that could be applied to alanine's response when calibrated with ^{60}Co gamma radiation and irradiated with low to medium energy X rays.

Research report 10, from the Republic of Korea, concerns the use of a low energy (160 keV) X ray cabinet irradiator to provide clean food for immune-compromised hospital patients. Some cancer patients need to ensure that their food conforms to a very high standards of hygiene because they are more vulnerable to infection. This means that some foods, such as fresh cut vegetables, are excluded from their diet. This research concerns microbial analysis and sensory survey tests of X ray irradiated fresh cut vegetables to determine if these irradiated foods are suitable for cancer patients.

Research report 11, also from the Republic of Korea, concerns the application of high energy (7 MeV) X rays to a variety of strawberries produced in the Republic of Korea. It is a part of a wider body of research into X ray irradiation as a post-harvest phytosanitary treatment for fresh agricultural products. The research presented in this publication focuses on an evaluation

of the effects of irradiation (0–1 kGy) on key quality parameters and sensory characteristics of "Maehyang" strawberries during storage at 15°C for 9 days.

Research report 12, from France, involves an assessment of activation in food products irradiated with high energy X rays. Advances in Monte Carlo simulation and activation calculations have enabled the development of a reliable and precise numerical tool for calculating the activation of radioactivity in food products. This numerical tool, after careful experimental validation (including neutron measurements), can be used to prove that the induced activity in food irradiated with 7 MeV X rays is not significant in comparison to natural radioactivity already present in food.

Research report 13, from the USA, is on the development of a low energy (<200 keV) X ray irradiator for phytosanitary irradiation. The X ray irradiator is being constructed in a modified standard 6 m shipping container and will be capable of treating the full width of fruit boxes of various sizes used by the sweet cherry industry.

Comparative Studies of Different Modes of Irradiation

Research report 14, from the USA, aims to address the question "Which do you choose for a phytosanitation program: electron beam or X ray technology?". It compares the practical and economic factors of electron beam and X ray irradiation for commercial use as phytosanitary treatments against regulated pests.

Research report 15, from the Socialist Republic of Viet Nam, is on the development of irradiation treatments to prevent post-harvest losses due to plant pathogens affecting fruit exports from the country. Electron beam irradiation (10 MeV) and gamma irradiation (^{60}Co) of green pomelo, king orange, star apple and custard apple fruit were compared as methods to control various fruit disease pathogens in the dose range that is generally used for phytosanitary treatments. Electron beam and gamma ray irradiation were found to give similar results. Both could be used to treat star apple, custard apple and king orange as a phytosanitary treatment and prevent post-harvest losses due to various plant pathogens.

Research report 16, from the Arab Republic of Egypt, concerns irradiation as a phytosanitary treatment of fresh produce and stored products. Investigations were undertaken using gamma (^{60}Co), electron beam and X ray irradiation of various development stages of Peach Fruit Fly, Cowpea Seed Weevil, Rice Moth and Fig Moth. Research also evaluated the impact of the effective phytosanitary irradiation dose on the quality attributes of their host commodities.

Research from Syria is presented in research report 17. One of the main differences between electron beam and gamma irradiation is the rate at which the radiation dose is delivered. Electron beams have much higher dose rates than gamma irradiation. The effects of two different dose rates (approximately 4 and 330 Gy/minute both from ^{60}Co gamma irradiation) were studied to see if there was a dose rate effect for the phytosanitary irradiation of wax moth and khapra beetle.

Research report 18, from the Islamic Republic of Pakistan, is a comparative study of gamma, electron beam and X ray irradiation technologies. An irradiated, long shelf-life beef keema meal (spicy meat dish) was developed as a ready to eat meal in a pouch. The use of irradiation to preserve persimmon fruits and reduce astringency was also studied, as was irradiation for sprout inhibition in vegetables such as potato, ginger and garlic. The three different modes of irradiation were compared in the analysis of the results of these studies.

Dose Intercomparison Exercise

Research report 19 presents the results of dosimetry intercomparison studies. This work was coordinated and implemented by Aerial in France. CRP participants voluntarily participated in irradiation and dose measurement inter comparison studies over the course of the project. Two performance indicators were evaluated. The first was the ability of participants to meet pre-set dose values and the second was their ability to measure doses applied.

ANALYSIS OF COORDINATED RESEARCH PROJECT OUTPUTS

The research results achieved at the close of the CRP in 2021 are analysed below in terms of the nine expected research outputs that were agreed at the start of the project in 2015.

Output 1. Publications and presentations on new concepts of electron beam and X ray machines customized for food irradiation, especially in-line in existing food manufacturing facilities.

This output was met by the following:

- Research reports 3 and 4, low energy electron beam irradiation of foods such as spices (irradiation using <300 keV electrons);
- Research report 10: cabinet X ray food irradiation e.g. “clean-food” for the immune-compromised in hospitals (low energy X ray irradiation of 160 kV energy);
- Research report 13: Cabinet X ray irradiation system for phytosanitary irradiation (low energy X ray of 160 kV).

There is renewed interest in developing and using low energy beam devices. During this CRP a commercial company (Bühler) has developed a device (the Laatu machine) that uses a free flow system to pass dried ingredients through a beam of low energy electrons to ensure that microbiological contamination on dried products can be controlled and maintained within acceptable levels. A representative of Bühler attended the second research coordination meeting in France and made a presentation. The Laatu is being used and tested commercially. There is growing interest in the industry to use low energy electron beams in food industry settings, as is shown by the food engineering group Bühler and its in-line electron beam surface decontamination unit.

At the final research coordination meeting of the CRP in 2021, participants commented that they have also noticed that there are other companies now working on low energy X ray. In the presentation on a low energy X ray irradiation unit for phytosanitary irradiation being developed in the USA (Peter Follet) it was stated that several potential end users of such a device have already been identified. There seems to be a commercial opportunity for low energy X ray irradiators. Over the period of the CRP, industry has made improved devices and new irradiation machines are now marketing. Independently from the CRP, industry has developed new sources (e.g. panel lamps) and machines (e.g. the Stellarray low energy X ray irradiator and the EXEDE low energy X ray food irradiator).

Output 2. Improved dosimetry protocols for low energy X ray food irradiation, and validation of existing dosimetry.

This output was met by the following:

- Research reports 3 and 4, on low energy electron beams adapted existing dosimetry system and measurement procedures for the determination of irradiation dose in terms of

surface dose and depth-dose profiles and proved the applicability of existing tools for dose determination.

- Research report 9: X ray dosimetry, systems for low energy applications to food (DOLOX);
- Research report 13: low energy level X ray dosimetry (this involved working with low energy X ray to do practical work on dosimetry using a correction factor to apply to alanine when used with low energy X ray);
- Research report 13: dosimetry of the cabinet X ray (dose mapping considered low energy effects in dosimeters);

Output 3. New imaging tools to support product dose mapping for heterogenous products

This was achieved. New technology was developed to quickly determine mass thickness of items before irradiation processing. These novel systems can predict D_{min}, D_{max}, DUR and dose distributions. They can be used as dose mapping tools. These technical developments are leading to new methods for dosimetry validation. The two new systems are:

- Research report 1: a prototype X ray system was designed, built, refined, and tested in an irradiation facility; a commercial product will result from this technical work;
- Research report 2: a prototype gamma ray detection system was designed, constructed, refined, and tested in an irradiation facility. It is in use in a commercial facility in the Socialist Republic of Viet Nam.

These systems have been brought to the attention of the radiation processing community at several major conferences, including the International Meeting of Radiation Processors (IMRP) and the International Phytosanitary Irradiation Forum. Recently, the NUCTECH has been invited to present on their system to a meeting of The Panel on Gamma and Electron Irradiation at their next meeting, which indicates that this approach is of interest to the radiation processing industry. A commercial product from NUCTECH is ready for market. As regards the similar system developed and built in the Socialist Republic of Viet Nam, the gamma ray detection system is used in VINAGAMMA and their electron beam irradiation facility and is proving to be a valuable tool but the acquisition time could be improved by speeding up the scanning system.

Output 4. Publications detailing examples of irradiation by electron beam or X ray of specific categories of food with pre- and post-irradiation requirements. This was achieved and at the time of writing, some manuscripts are in preparation for publication in scientific journals.

This output was achieved. The main outputs were produced by the CRP participants in the People's Republic of China, Republic of Indonesia, Republic of Korea, Islamic Republic of Pakistan, Portugal, Kingdom of Thailand, the Socialist Republic of Viet Nam and USA:

- People's Republic of China (research report 5), electron beam irradiation of Sichuan pickles and fresh fruits. Pickled carrot and lettuce irradiated to 3 kGy combined with other methods both had a better texture and longer shelf life in comparison to the control samples. Xiaotaimang Mango irradiated to 0.5 kGy and 1.0 kGy could maintain a good appearance quality, have a delayed brown rate, reduced the rot rate and weight loss rate. The irradiation treatments inhibited the ripening and yellowing of the mango, and maintained the colour and brightness;

- Republic of Indonesia (research report 8), soy flour (two different varieties of soy were used and the novel application was investigating the use of ionizing radiation as a means of increasing the nutritional quality of the soy flour);
- Republic of Korea (research reports 10 and 11), low energy X ray (fresh cut carrot, green pepper, cherry tomatoes, paprika) and the high energy X ray irradiation of 'Maehyang' strawberries;
- Islamic Republic of Pakistan (research report 18), comparison of X ray, electron beam and gamma irradiation of (i) beef keema a ready to eat meal (long life storage and assured food safety), (ii) persimmon fruit (novel application to improve the quality by making the fruit less astringent), (iii) 'Cardinal' potatoes plus onions, garlic and ginger (sprout inhibition);
- Portugal (research report 6), electron beam irradiation studies plus simulation modelling of energy deposition, of cherry tomatoes, raspberries, mushrooms, and collaboration with researchers in Tunisa interested in irradiated strawberries. A novel use of irradiation to improve some properties of the foods studied, to help preserve but also increase extractability of bioactive compounds (e.g. irradiation of strawberries to increase anti diabetic and anti-oxidant properties, irradiation of cherry tomatoes and raspberries to also improve the availability of bioactive compounds, and the irradiation of mushrooms);
- Kingdom of Thailand (research report 7), electron beam irradiation of pickled products (ginger, mixed vegetables, sweet salted white radish) fermented products (bamboo shoots) and other products such as dry shrimp, green and yellow mango irradiated with both X ray and gamma radiation. The CRP participant from the Kingdom of Thailand also won an industry award for the pickled ginger work;
- USA (research report 14), the report in this publication concerns the choice between electron beam and X ray irradiation for phytosanitation, but other work during the course of the CRP included fundamental research into electron beam irradiation of fresh fruit and the metabolomic profile of foodborne pathogens;
- The Socialist Republic of Viet Nam (research report 15), phytosanitary irradiation (up to 1 kGy) tolerance of green pomelo, king oranges, purple star apple, custard apple.

In addition to specific categories of food, there are valuable outputs for phytosanitary treatments (comparison of electron beam, X ray, and gamma irradiation):

- Arab Republic of Egypt (research report 16), on the gamma, electron beam and X ray phytosanitary treatments against Peach Fruit Fly (*Bactrocera zonata*), Cowpea Seed Weevil (*Callosobruch maculatus*), Rice Moth (*Corcyra cephalonica*) and Fig Moth (*Ephestia cautella*);
- Syria (research report 17), phytosanitary treatments against wax moth (*Galleria mellonella* L.) and Khapra beetle (*Trogoderma granarium* n), examined the effects of low and high dose rates of approximately 4 Gy/min and 330 Gy/minute (gamma dose rates because the electron beam facility could not be used);
- Viet Nam (research report 15), electron beam and gamma irradiation at typical generic phytosanitary irradiation treatment doses for the prevention of food lossess due to plant disease infection by *Xanthomonas* sp, *Phyllosticta citriasiana, Colletotrichum gloeosporioides,* and *Lasiodiplodia theobromae.*

Also, France (Aerial) and the USA (Texas A&M University) have worked directly with industry to assist in the commercial development of food irradiation. For example, by deleveloping a framework for cost-benefit analysis of machine source irradiation and rapid laboratory tests to confirm the efficacy of electron beam processing.

In addition, the International Irradiation Association, iia (*sic*) will publish a guide targeted at those interested in purchasing an electron beam accelerator. Increasing interest in using electron beam and X rays in place of gamma irradiation has created the need for a guide to provide the fundamental and important information on this technology so that any prospective buyer can be equipped to ask the right questions and make an appropriate specification when negotiating to purchase an accelerator. The iia (*sic*) guide has been produced in collaboration with equipment suppliers, large users and major contractors plus experts from Aerial. The first edition is focused on medical device sterilization, but it would also be useful for food applications too. The iia (*sic*) is interested in producing a guide for food applications.

Documents produced as part of an associated IAEA technical cooperation project in the Asia and Pacific region have been drafted to give information on gamma rays, electron beams and X rays as can be used for food irradiation and also the benefits of moving from 5 MeV X rays to 7.5 MeV X rays. These two provide guidance and help for potential users of these technologies and will help generate more widespread adoption of electron beam and X ray irradiation.

Output 5. Best Dosimetry Practice document for low energy electron beam and X ray applications.

This output was achieved. Training on practical aspects of dosimetry was undertaken at the second RCM hosted by the Aerial technology resource centre in France. This included practical exercises where participants received hands-on training and experience using the equipment and facilities at the Aerial laboratories. Training was supplemented by two interlaboratory exercises where proficiency in delivering dose and measuring dose was tested (research report 19). A presentation and paper on the first inter-comparison exercise was provided at the first IAEA International Conference on Applications of Radiation Science and Technology (ICARST-2017). This interlaboratory exercise provided the basis for one-to-one discussions on individual dosimetry practices, research irradiation protocols and the need for any changes. Aerial provided advice and guidance to participants as required and when asked. These activities help to reinforce the importance of sample preparation, dose uniformity in samples and treatment protocols when it comes to irradiation, especially for electron beam processing.

Output 6. Research nuclear data and Monte Carlo simulation framework clarifying the effect of X ray above the threshold 5.0 MeV on induced radioactivity in food (with a view to support the revision of the Codex Alimentarius General Standard on Irradiated Food).

The above output was met, the research project by the Institut Pluridisciplinaire Hubert Curien in France, in collaboration with Aerial (research report 9), investigated the issue of activation of radioactivity by irradiation with different energy X rays. The maximum energy of an X ray is related to the maximum kinetic energy of the electron beam that is used to produce the X ray emission. The higher the kinetic energy of the electron beam, the more efficient the process (X ray production and food treatment), but it is essential to consider the possibility of photonuclear activation which can lead to the production of radioactive nuclei inside the irradiated material. International food standards give a maximum X ray energy of 5 MeV for food irradiation. However, some countries allow 7.5 MeV X rays. Research to date has concluded that food irradiated with X rays up to 7.5 MeV is safe and efficacious. Any induced radioactivity is short lived, and the levels are very low, well below natural radioactivity levels in non-irradiated food. Levels are so low that simulation methods are needed to estimate activation levels as the radioactivity is difficult to measure. The major finding is that modelling can predict levels of induced radioactivity and this research has produced a method to validate

the simulations. Experimental validation of the simulations was undertaken at two high energy X ray irradiation sources (STERIS in Switzerland and FEERIX at Aerial in France).

The IAEA has formally asked the secreatariat to the Codex Alimantarius Committee on Food Hygiene and the secretariat of the International Plant Protection Convention for their international food and phytosanitary standards respectively to be revised to increase the maximum energy for the X ray irradiation of food from 5 to 7.5 MeV. The secretariat of the Codex Alimentarius Committee on Food Hygiene has agreed to consider this as a potential future work item.

The International Standards for Phytosanitary Measures (ISPM) of the International Plant Protection Convention also give a maximum X ray irradiation energy of 5 MeV for phytosanitary irradiation treatments. The relevant standard is being revised and has also been redrafted to increase the maximum energy for the X ray irradiation of food from 5 to 7.5 MeV (ISPM 18 gives guidelines for the use of irradiation as a phytosanitary measure but it is being rewritten and it is intented that the revised version will provide international requirements for the use of irradiation as a phytosanitary measure).

Output 7. Case studies on pilot scale testing and full-scale demonstration of electron beam and X ray irradiation of food in collaboration with food industry. This has also been successfully achieved. In addressing output 7, many initiatives were also taken forward under output 4 (i.e. examples of irradiation by electron beam or X ray of specific categories of food).

The participating institutions mainly contributed to output 7 by providing demonstrations of electron beam and X ray irradiation (the People's Republic of China, France-Aerial, Portugal, the Islamic Republic of Pakistan, the Kingdom of Thailand, the Socialist Republic of Viet Nam, USA). For example, the Sichuan Institute of Atomic Energy in the People's Republic of China worked in collaboration with the pickle industry for the electron beam irradiation of different products, Aerial in France has demonstrated that high energy X rays from a Rhodotron (FEERIX) can deliver the appropriate range of doses necessary for phytosanitary irradiation treatments using pallets of fruits. The Centre for Nuclear Sciences and Technologies (C2TN) in Portugal worked with the mushroom industry, the Nuclear Institute for Food and Agriculture (NIFA) in the Islamic Republic of Pakistan has worked with range of stakeholders to develop specific irradiated food products, the Thailand Institute of Nuclear Technology worked with small and medium sized enterprises and also won an award for developing an irradiated ginger pickle product, the Research and Development Center for Radiation Technology in the Socialist Republic of Viet Nam is working with the food and fruit industry to commercialize the irradiation of different foods.

Output 8. Material to support workshops and short courses on electron beam and X ray irradiation applied to food.

The above output was met as materials were developed and used for workshops and courses. Dosimetry material, practical exercises, and course materials were provided during the 3rd RCM. Most participants produced material on food irradiation for conferences and seminars: Indonesia recently produced an infographic (a short animation), Aerial has developed remote training material for dosimetry (videos) and also organized and implemented an international symposium on food irradiation that was held online as a virtual event (the first International Food Irradiation Symposium, 9–11 March 2021). The Thailand Institute of Nuclear Technology is one of many participating organizations that provided lectures and seminars on food irradiation for various different groups nationally and internationally. The IAEA Collaborating Center for Electron Beam Technology, Texas A&M University, has produced case studies, for

example on irradiated fresh mangoes exported from Mexico to USA. As mentioned under Output 7, The IAEA Collaborating Center at Aerial in France has produced a demonstration on the use of a Rhodotron for X ray irradiation of fruits to the doses required in phytosanitary treatments. These products and information resources are being used to illustrate and demonstrate the use of this technology.

Output 9. Research and technical papers published in scientific journals and development of information packages e.g. internet content and online data packages / courses.

The above output was achieved. The CRP participants were encouraged to publish their research findings in scientific journals. The CRP participants did publish their research findings and, at the close of the CRP, several manuscripts are in an advanced stage of production for submission to various scientific journals. All participants have also provided articles for this publication.

The following are general conclusions at the close of this CRP:

(a) This CRP was successful in that it established an international collaborative network that worked together to undertake research that will facilitate the implementation of practical techniques to irradiate food and agricultural products using electron beam and X ray radiation;

(b) Low energy electron beam and X ray applications were proposed as new concepts for surface and near surface irradiation. These low energy devices can be easily integrated into existing food processing lines. There is commercial interest and industry is producing new types of machines that can be used in food applications (e.g. the Laatu system of Bühler);

(c) Several fresh and pre-packaged irradiated food products have been developed. These products have improved food safety, preservation and convenience through the use of electron beam and X ray irradiation. These products were developed with extensive tests such as nutritional composition, sensory evaluations and the determination of the lethality of irradiation against food-borne microorganisms or insect pests. A number of studies also investigated food irradiation as a means of improving the nutrient content of foods, either by increasing the extractability or nutritional availability of bioactive compounds or by reducing concentrations of anti-nutrients;

(d) Research under this CRP used modern techniques to simulate and re-assessed the irradiation of food with high energy X rays. These studies were used to determine if components in food became activated with significant levels of induced radioactivity after irradiation with X rays that span a broad range of energies ranging from up to 5 MeV to up to 7.5 MeV. Induced radioactivity was not found to be significant. A major finding is that simulation modelling can adequately predict activation levels for a given food composition. The methodology produced in this research was validated by experiments at two high energy X ray irradiation sources;

(e) Dosimetry methods and tools were developed. Research has improved the understanding of alanine dosimeters and their use to measure low energy X ray irradiation. This research is fundamental to the future development of dosimeters that can accurately and precisely measure doses imparted by low energy (<300 keV) X rays. Research has studied and characterized low energy electron beam irradiation and the measurement of surface and subsurface dose. Two new devices were developed as pre-irradiation treatment tools to facilitate correct electron beam irradiation processing. These new devices can measure

mass thickness (a key parameter for electron beam irradiation) and also predict the magnitude and location of maximum dose and minimum dose in a process load. They can also give a predicted dose uniformity ratio and dose distribution profiles for real products;

(f) Comparative studies have investigated the three modes of ionizing radiation (gamma, electron beam and X ray) available as technological options for food and phytosanitary irradiation. Data has been produced to show that electron beam and X ray irradiation are useful techniques and enable users to make an informed decision when adopting the technology. It was pointed out that in those comparative studies, the mastering of the irradiation treatment parameters and geometries as well as the design of experiments are of prime importance to produce scientific relevant data.

New Tools to Rapidly Ascertain Treatment Parameters Before Electron Beam Irradiation

1. CONCEPT DEVELOPMENT OF X RAY MASS THICKNESS DETECTION FOR IRRADIATED ITEMS UPON ELECTRON BEAM IRRADIATION PROCESSING

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Abstract

It is well known that penetrability of an electron beam is limited. The mass thickness (mass thickness = density × thickness) is a critical factor to control the quality of electron beam (EB) irradiation processing. We developed a two-dimensional X ray detection technology to quickly measure the mass thickness of items. This makes it possible to quickly determine whether packaged items in a loading configuration will be able to be irradiated correctly. The mass thickness data can be used to predict the EB dose distribution and therefore dose mapping can be calculated prior to EB irradiation. We also developed a user-oriented service software system for X ray detection to make the technology better serve EB radiation processing. A variety of materials, products and pilot scale experiments proved that the system operated reliably, and the results have great significance for electron beam irradiation processing. The main functions of the X ray detection system include: (1) the production of a two dimensional image of the item to show the measured mass thickness distribution as well as the predicted dose distribution and dose uniformity ratio (DUR) if the product was to be irradiated by EB; (2) to generate data as an output. Mass thickness can be measured across a product and these data can be produced as data files; (3) to generate predictions of dosimetry parameters. Computer models can use the mass thickness data to predict and report EB dosimetery parameters such as dose distribution, the locations and size of the maximum dose and the minimum dose within a product load and the DUR. The method is quick and reliable and can be used to visualize and optimize the loading configuration before using an EB for the performance qualification of the actual product. They may also aid validation studies by indicating where to place dosimetrs in order to measure the maximim and minimum doses of radiation received by the product.

1.1.INTRODUCTION

There are many uses of EBs and they are used in many different applications including food irradiation, medical product sterilization, quarantine treatments, chemical and material industries and so on. The market share of EB irradiation is growing. Accelerators are widely used because EB irradiation has many advantages, such as a high processing speed, dose adjustment flexibility, no radioactive waste and the like. However, it is well known that the weak penetrability of EB limits its application. As it penetrates through a product the intensity of the EB is attenuated due to interactions between the energetic electrons and atoms in the product. The optimal range (R_{opt}) of an EB in a product is limited. For example, a one-side irradiation using a 10 MeV EB (water, DUR ≤ 1.5) has an R_{opt} of approximately 3.8 cm; for two-sided irradiation (water, DUR ≤ 1.5), R_{opt} is ca. 9 cm. The penetrability of an EB is very

sensitive to the mass thickness of irradiated item (wherein the mass thickness of the item = density of the item × thickness of the item). In radiation processing, performance qualification is necessary to determine dose delivery characteristics to a specific product in a defined load configuration. The mass thickness of the process load (the product as presented to the EB during irradiation) must be within a certain range in order for it to meet performance qualification acceptance criteria in terms of minimum and maximum dose, and the ability to meet a specific dose range requirement. Therefore, it is very important to know how the dose imparted by the EB is distributed inside a product. So, the mass thickness is a critical factor to control the quality of EB irradiation processing. The ability to accurately measure mass thickness and therefore predict dose distribution in a product is helpful for process control. Although it is relatively easy to measure mass thickness of uniform density items; in practical application of radiation processing it is difficult to measure mass thickness for typical products because their density is usually non-uniform.

It is well known that the intensity of X rays will decrease while passing through product. The X ray imaging technique has been widely used in security inspection systems (Fig. 1). We adopted the principles and technology of X ray imaging [1, 2] to innovate and develop new technology that could quickly measure the mass thickness of items. An algorithm was established to convert measurements of X ray intensity into mass thickness. Experiments using standard products were used to generate data that could be used to develop the algorithm and related software. A machine that makes X ray mass thickness measurements was developed by integrating X ray imaging equipment with the algorithm and related software. Scanning with X rays could therefore be used to help determine whether a loading configuration (size and location of items within a package) would be capable of being irradiated by an EB, for example would the electrons pass through the entire volume of the product and could the necessary minimum dose and maximum dose be delivered by an electron beam. X ray mass thickness detection is the core technology of this detection technique.

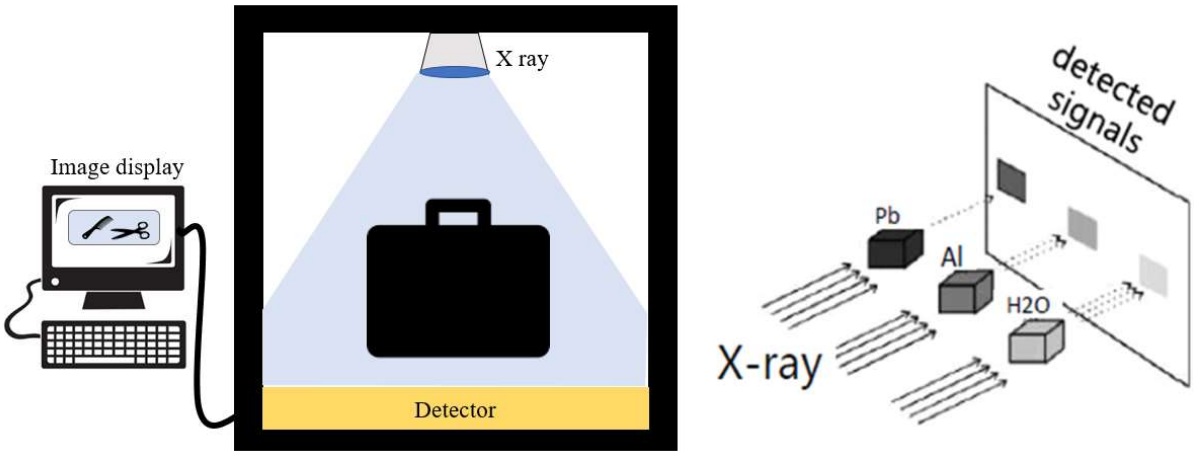


FIG. 1. X ray *imaging technique.*

Though X ray and EB propagate differently, this technology could be a step forward in predictive dosimetry. Predictive dosimetry based on mass thickness measurement could support the formal process of validation and formulating processing schemes such as loading configurations. Integrating the X ray mass thickness measurement device within a 10 MeV EB irradiation facility would enable the rapid and efficient monitoring of the mass thickness and therefore dose distribution within products, to guarantee the quality of the electron beam irradiation process as applied at that facility.

1.2.MATERIALS AND METHODS

1.2.1. Materials

A model that could be used as an algorithm to establish mass thickness from X ray attenuation was developed from X ray experiments with different representative materials. The materials used in these experiments included polyethylene sheets (Fig. 2a and Table 1), aluminum sheets (Fig. 2b and Table 2), polymethyl methacrylate (PMMA) sheets with two kinds of thickness (Fig. 2c plus Table 3 and Fig. 2d plus Table 4), and so called “step blocks” of sheets of PMMA (Fig. 2e) and of aluminum (Fig. 2f).

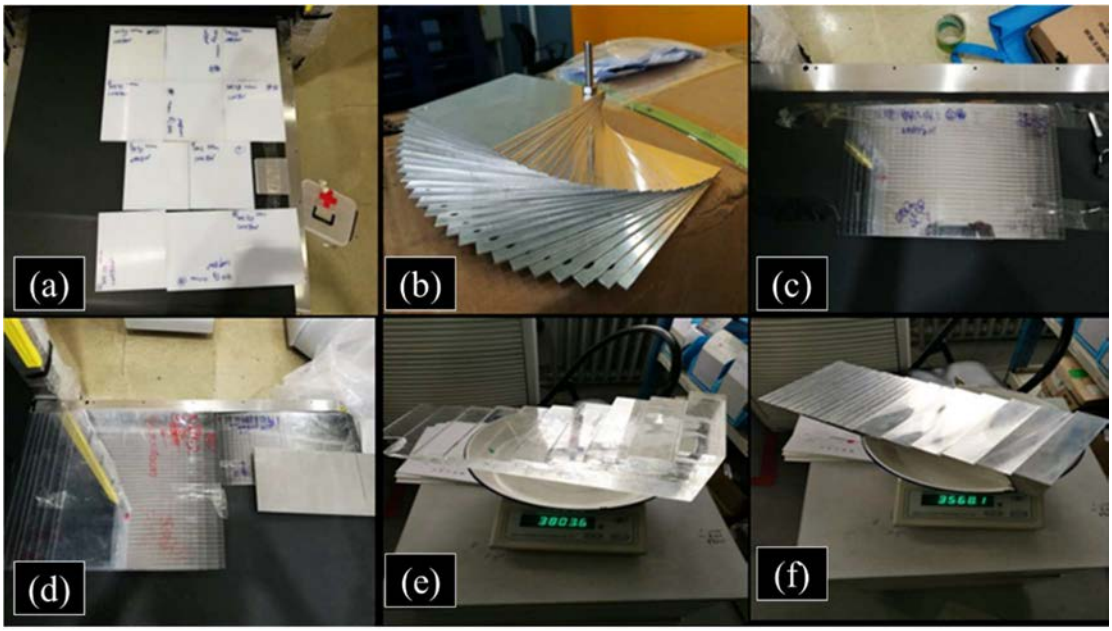


FIG. 2. Test materials (a) polyethylene sheet, (b) aluminium sheet, (c) polymethyl methacrylate (PMMA) sheet, (d) PMMA sheet, (e) step block of PMMA, (f) step block aluminium.

TABLE 1. POLYETHYLENE SHEET (A)

Code	Material	Length(mm)	Width (mm)	Thickness (mm)	Nominal mass thickness (g/cm2)
A	Polyethylene sheet	200	200	1	1

TABLE 2. ALUMINUM SHEET (B)

Code	Length (cm)	Width (cm)	Thickness (mm)	Mass(g)	Density(g/cm^3)	Nominal mass thickness(g/cm^2)
1-1	10±0.2	10±0.2	1.10	29.40	2.67	0.2940
1-2	10±0.2	10±0.2	1.10	29.40	2.67	0.2940
1-3	10±0.2	10±0.2	1.11	29.66	2.67	0.2966
1-4	10±0.2	10±0.2	1.11	29.73	2.68	0.2973
1-5	10±0.2	10±0.2	1.11	29.65	2.67	0.2965
1-6	10±0.2	10±0.2	1.11	29.64	2.67	0.2964
1-7	10±0.2	10±0.2	1.11	29.75	2.68	0.2975
1-8	10±0.2	10±0.2	1.11	29.64	2.67	0.2964
1-9	10±0.2	10±0.2	1.11	29.65	2.67	0.2965
1-10	10±0.2	10±0.2	1.11	29.69	2.67	0.2969
1-11	10±0.2	10±0.2	1.11	29.65	2.67	0.2965
1-12	10±0.2	10±0.2	1.11	29.70	2.68	0.2970
1-13	10±0.2	10±0.2	1.11	29.72	2.68	0.2972
1-14	10±0.2	10±0.2	1.11	29.66	2.67	0.2966
1-15	10±0.2	10±0.2	1.11	29.67	2.67	0.2967
1-16	10±0.2	10±0.2	1.11	29.64	2.67	0.2964
1-17	10±0.2	10±0.2	1.11	29.67	2.67	0.2967
1-18	10±0.2	10±0.2	1.10	29.42	2.67	0.2942
1-19	10±0.2	10±0.2	1.11	29.70	2.68	0.2970
1-20	10±0.2	10±0.2	1.11	29.66	2.67	0.2966
1-21	10±0.2	10±0.2	1.11	29.66	2.67	0.2966
1-22	10±0.2	10±0.2	1.11	29.68	2.67	0.2968
1-23	10±0.2	10±0.2	1.11	29.70	2.68	0.2970
1-24	10±0.2	10±0.2	1.11	29.64	2.67	0.2964
1-25	10±0.2	10±0.2	1.11	29.78	2.68	0.2978
1-26	10±0.2	10±0.2	1.11	29.67	2.67	0.2967

TABLE 3. PMMA SHEET(C)

Code	Length (mm)	Width (mm)	Thickness (mm)	Mass(g)	Density(g/cm^3)	Nominal mass thickness (g/cm^2)
1	20.1	20.1	1.00	47.9	1.19	0.119
2	20.1	20.1	1.00	47.6	1.18	0.118
3	20.1	20.1	0.98	46.4	1.17	0.115
4	20.1	20.1	0.97	46.2	1.18	0.114
5	20.1	20.1	0.97	46.1	1.18	0.114
6	20.1	20.1	0.99	48.3	1.21	0.120
7	20.1	20.1	0.99	48.6	1.22	0.120
8	20.1	20.1	1.00	47.6	1.18	0.118
9	20.1	20.1	0.97	46.2	1.18	0.114
10	20.1	20.1	0.96	45.9	1.18	0.114
11	20.1	20.1	0.96	45.9	1.18	0.114
12	20.1	20.1	0.97	46.3	1.18	0.115
13	20.1	20.1	0.99	46.5	1.16	0.115
14	20.1	20.1	0.99	47.1	1.18	0.117
15	20.1	20.1	0.97	47.1	1.20	0.117
16	20.1	20.1	0.99	47.3	1.18	0.117

TABLE 4. PMMA SHEET (D)

Code	Length (cm)	Width (cm)	Mass (g)	Density (g/cm^3)	Nominal mass thickness (g/cm^2)
D	40	40	2	1.15	0.23

1.2.1. Methods

1.2.1.1. Mass thickness detection equipment

In consideration of the practicability and safety of use, a commercially available X ray security scanner (Fig. 3) was used as the basis for the X ray mass thickness detection system. This equipment provides the following key technical indexes:

(a) It is developed to scan baggage items and can accommodate packages of the size generally used as process loads in EB facilities;
(b) Items are transported through the device stably and consistently;
(c) The X ray emitter is capable of penetrating items with depth (thickness) typical of process loads in EB facilities;
(d) It has the ability to image items with a good degree of resolution across the process load;
(e) It is designed to be safe in terms of radiation protection and operators.

The parameters of this equipment that were used in mass thickness detection experiments are given in Table 5.

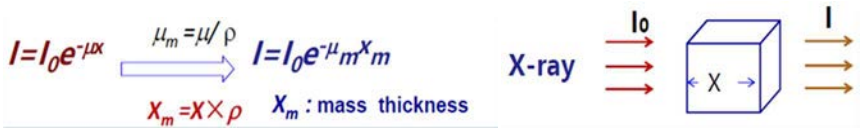
FIG. 3. X ray *mass thickness detection system.*

TABLE 5. EQUIPMENT PARAMETERS

Parameter	Size
Channel size	1010mm (wide)×1010mm (height)
Transfer speed	0.2m/s
Conveyor height	315mm
Maximum load	200 kg (220v single phase)
Shred resolution	diameter 0.08mm metal wire
Penetrating power	38mm
Anode voltage	160kV
Anode current	0.7mA
X ray conversion device	Lpd array
Energy	Maximum 160 keV

1.2.2.2. Build mass thickness measurement theory analysis and algorithm

When an X ray beam penetrates through an object, the degree of the attenuation is related to the mass thickness of the path taken through the object. So, X ray detection can accurately estimate mass thickness at a measurement point. According to the X ray energy equivalence (Equation 1), we can build an algorithm model of X ray mass thickness measurement.



$$I = I_0 e^{-\mu_m x_m} \quad (1)$$

Therefore $$\ln (I_0/I) \propto x_m \quad (2)$$

Where:

I_0 = intensity of the incident X ray beam;
I = intensity of the X ray beam transmitted through the material (observed at detector);
μ_m = mass attenuation coefficient, and;
x_m = mass thickness.

The quantity $\ln(I_0/I)$ is directly proportional to mass thickness (Equation 2). Analyzing this linear relationship by taking the results of experiments using materials of a known mass thickness as and recording the intensity of X rays observed at the detector, enables the constant of proportionality to be calculated. Therefore, an algorithm can be coded to give mass thickness directly from transformed quantitative data of I_0/I.

To scan and measure across a whole product, the item was divided in many units parallel to the incident X ray bam (which is also the direction of the EB incidence). In this way the scan of the item was composed by many columns. Each column is used to take a measurement of mass

thickness. The minimum size (the size can be adjusted) of each column was set as 1.5 mm by 1.5 mm (Fig. 4). So, a series of mass thickness data could be obtained at this resolution. For example, with an item measuring 40cm (length) by 30cm(width), 53 000 data points would be obtained as mass thickness data.

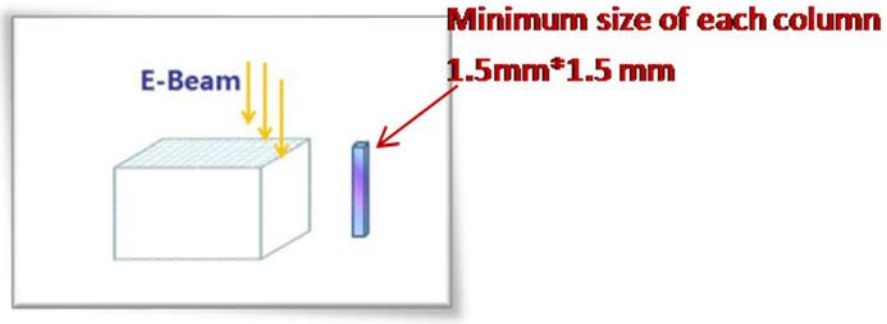


FIG. 4. Size of each column.

1.2.2.3. Mass thickness detection experiments and algorithm optimization

Experiments to optimize the algorithm for obtaining mass thickness involved directly measuring the mass thickness of the test modules (Fig. 5) selected as test materials in section 2.1, collecting and analyzing the X ray intensity test data and calculating the error, then modifying the algorithm and testing its accuracy once again according to repeat experiments. The error associated with mass thickness determinations was reduced to an acceptable range by this approach to algorithm optimization.

FIG. 5. Experiments process.

1.2.2.4. Build theoretical model of electron beam irradiation dose prediction

In order to predict EB dose distributions it was necessary to establish a theoretical model that could be used to predict dose from mass thickness data. The model was constructed from measured mass thickness distribution data, Monte Carlo Simulation Calculations and the surface normalization processing data. The software of the X ray scanner could then be used to predict EB maximum dose, EB minimum dose and EB dose uniformity in the measured object. These predictions rely on X ray measured mass thickness distribution data for the scanned item.

(1) Method

Data used to predict dose distribution (including single irradiation and two-sided irradiation) of electron beam with energy between 1~20MeVwere obtained by simulation calculation. Therefore, the theoretical dose distribution data is established. Finally, through comparison with the theoretical dose distribution and surface normalization processing data, a set of dose data is obtained, which includes the maximum dose, minimum dose, the degree of uniformity and so on.

(2) Visualization of measured objects and the dose distribution prediction

(a) After scanning, a colour image of the measured subject is generated by the software. The image contains the mass thickness data of the measured object. Colour images are made up of 5 colours, each of which represents a different mass thickness range, and for the each colour the darker the colour, the greater the mass thickness (Fig. 6).

(b) Through the mass thickness aggregate data, the system can predict the relative dose at different locations, the maximum dose, the minimum dose and the dose uniformity of each mass thickness module. We mathematically counted the relative dose data of the scanned product and obtained a statistical chart of the dose distribution, as shown in Fig. 6.

(c) Output the predictive data of dose distribution. The mass thickness detection system can record each mass thickness data point (for each measurement column) and the predicted EB maximum, minimum dose and dose uniformity information of its corresponding single-side, two-side irradiation mode. And then all the mass thickness data and their corresponding dose distribution information are exported to an excel table for subsequent analysis of the predicted dose distribution.

1.2.2.5. Software solutions

Software was written that is tailor made to the requirements of this mass thickness detection device and X ray equipment. We developed specific software for the analysis of mass thickness and dose distribution, according to the data collection scheme illustrated in Fig. 6. The software presents an image of the scanned item in real time, as it collects and displays the mass thickness data and the predicted EB dose distribution information generated from the Monte Carlo simulation that converts mass thickness measurements to predicted EB dose. The software can also export the final conclusion as data files.

The software interface has three parts: (1) image display area, (2) chart area (predicted EB dose distribution of the product), (3) calculation allocation and conclusion area. The function of the software is described as follows.

(1) Imaging mass thickness distribution with corresponding irradiation process information

The software can display the current X ray scan as an image according to colour coded values and therefore present the mass thickness distribution information as a colour image (Fig. 6). The mechanism for allocating the colour partitioning is related to the irradiation process.

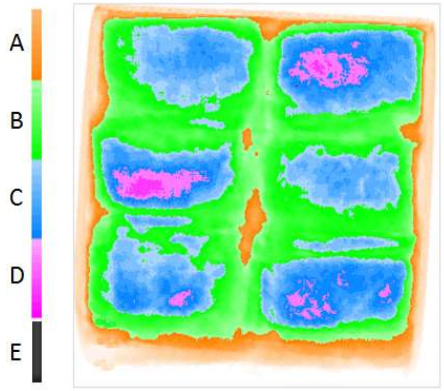


FIG. 6. Image display interface where colours labelled A to E represent the DUR zones in Fig. 7.

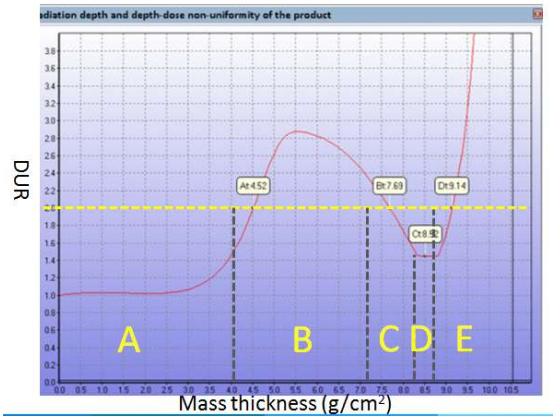


FIG. 7. DUR curve with mass thickness of 10MeV EB.

Fig. 7 is the curve for DUR plotted against mass thickness. The yellow line represents the degree of DUR set by the user (in this case 2.0). According the DUR setting we divide the curve into several regions:

- A: relatively thin with a low degree of DUR;
- B: penetrated by double-side irradiation by 10M EB, but DUR is high;
- C: the optimal thickness area;
- D: DUR increase but acceptable;
- E: too thick to be penetrated.

The image is marked with different colours according to the above principles, the colour represents the ABCDE respectively. At the same time, the proportion of each region to the whole product is given in the analysis conclusion area which is a more accurate display of the DUR distribution.

Besides, with the function of data resolution adjustment, the minimum column surface area is 2 mm^2 of the software, which is the highest resolution. For example, for a 40 cm (L) by 30 cm (W) project, 53 000 data will be obtained. We can use software to adjust the resolution. The surface area of the column can reach to 1 cm^2, which is equivalent to the area of B3, FWT, CTA and other dosimeters. The software can achieve the zoom function and scale function of perspective view, so that the user can compare and analyze the picture with the actual product more conveniently.

(2) Dose distribution of EB irradiation; Mark the spots where dose extremes are predicted

The statistical analysis of dose data in the product can be obtained, including not only the dose range, but also the proportion of each dose range. And display the dose distribution (the surface dose is set to 1) of the product in the software interface (Fig. 8).

The software also has the gray-scale mode display. When the grayscale mode is selected, the software can calculate the maximum and minimum dose and their positions in the product, mark and displays the spots in the software interface (Fig. 9).

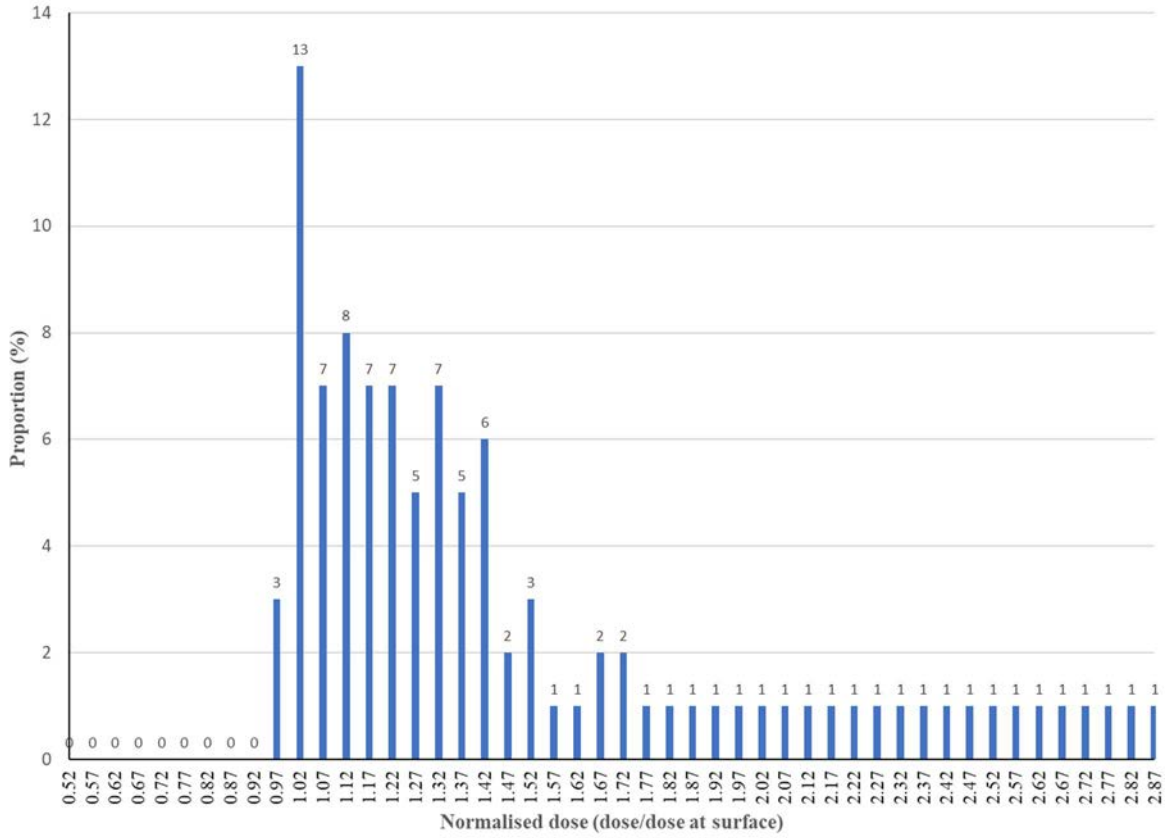


FIG. 8. Statistical analysis of dose distribution.

(3) Available for multi-energy to select, single-side or double-side irradiation

In this system, the energy of EB can be selected (from 1-20MeV, even higher energy is no problem). The results of the energy we selected will be given. Double-side or single-side irradiation mode can be selected, and the corresponding analysis will be given.

(4) Export the data and result

The mass thickness detection system can record each mass thickness data point and the predicted EB maximum, minimum dose and dose uniformity information of its corresponding single-side, double-side irradiation mode. And then all the data and result are exported to an excel table for subsequent analysis of the dose distribution state.

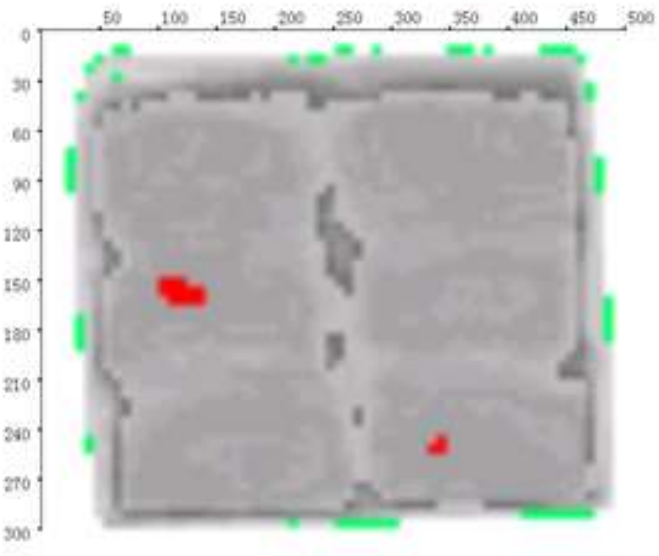


FIG. 9. Grayscale mode-display of the dose extremes.

1.3.RESULTS AND DISCUSSION

Different materials and products such as homogeneous materials (water, millet, etc.), actual products (medical products, pet food, etc.) and some pilot products were used to test and verify the device and its ability to measure collect thickness measurements and dose distribution predictions results. It also served as a pilot study to test the operational stability of the whole system.

1.3.1. Verification and analysis of X ray mass thickness detection

1.3.1.1. Homogeneous material-water

Water is an homogeneous density material commonly used in irradiation processing. We use water of 9 cm depth to analyse the results of X ray mass thickness detection. The water sample was presented in a plastic container which is very thin, and the influence of the plastic can be neglected. The results of the X ray mass thickness measurement test are shown in Fig. 10 with two data locations highlighted. Some of the location give a mass thickness measurement value of 9.0 g/cm^2 and some locations give 8.75 g/cm^2, and the total is about 9 g/cm^2. It is in good agreement with the actual value.

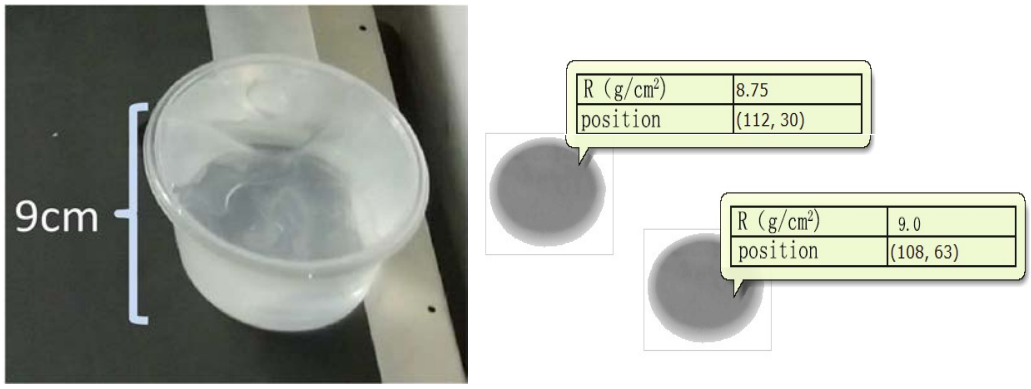


FIG. 10. Mass-thickness for water of 9 cm depth.

1.3.1.2. Homogeneous material-millet

Vacuum packed millet with different thicknesses was tested. The bulk density of millet is generally recognized as 0.92 g/cm^3.The test results are shown in Fig. 11 to Fig. 13 and Table 5.

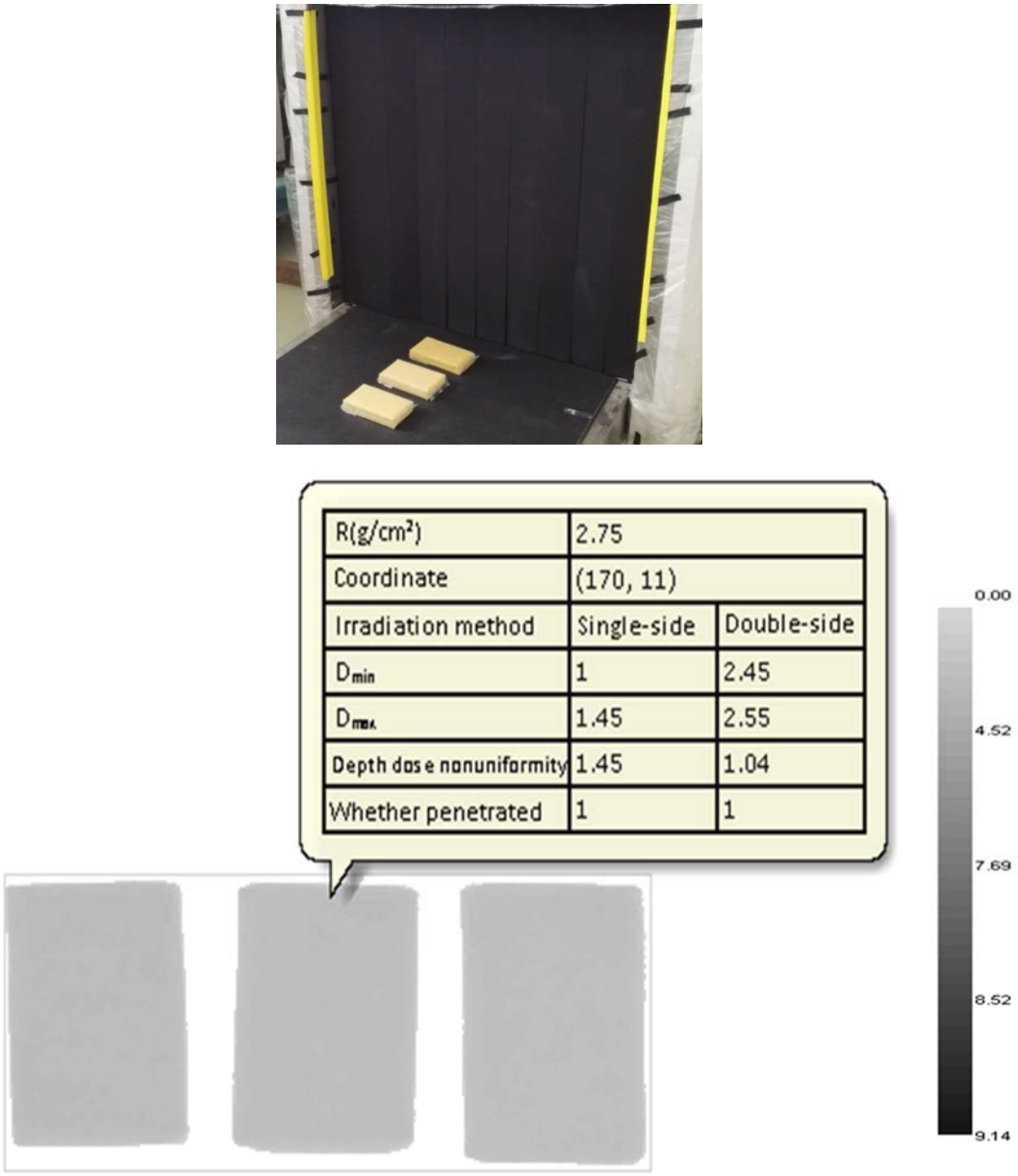


FIG. 11. Mass-thickness for millet of 3 cm (The darker the colour of the scanned image in the figure, the greater the mass thickness calculated) Using a density of 0.92 g/cm^3 for the millet the mass thickness is 3 × 0.92 = 2.76 g/cm^2).

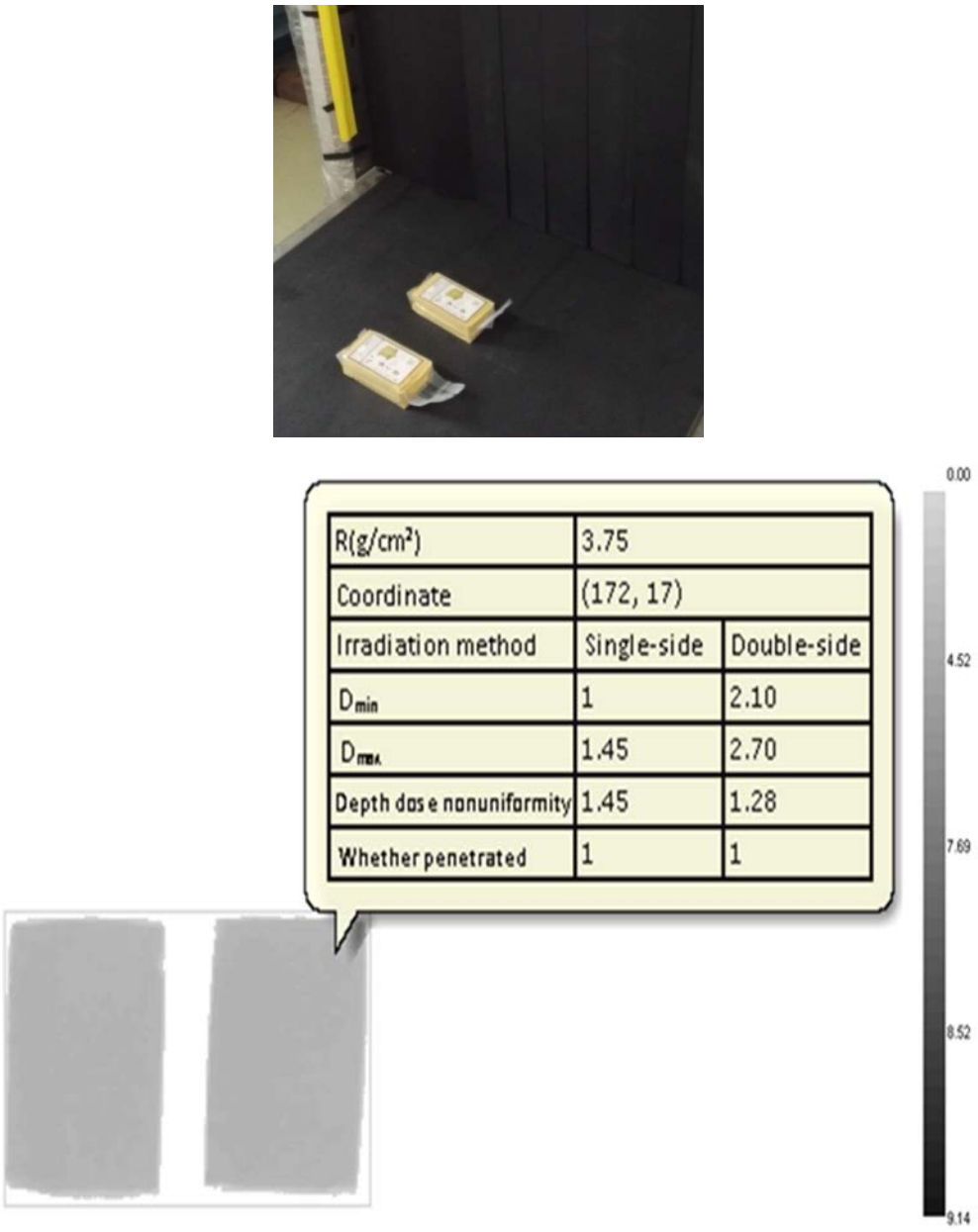


FIG. 12. Mass-thickness for millet of 4 cm depth.

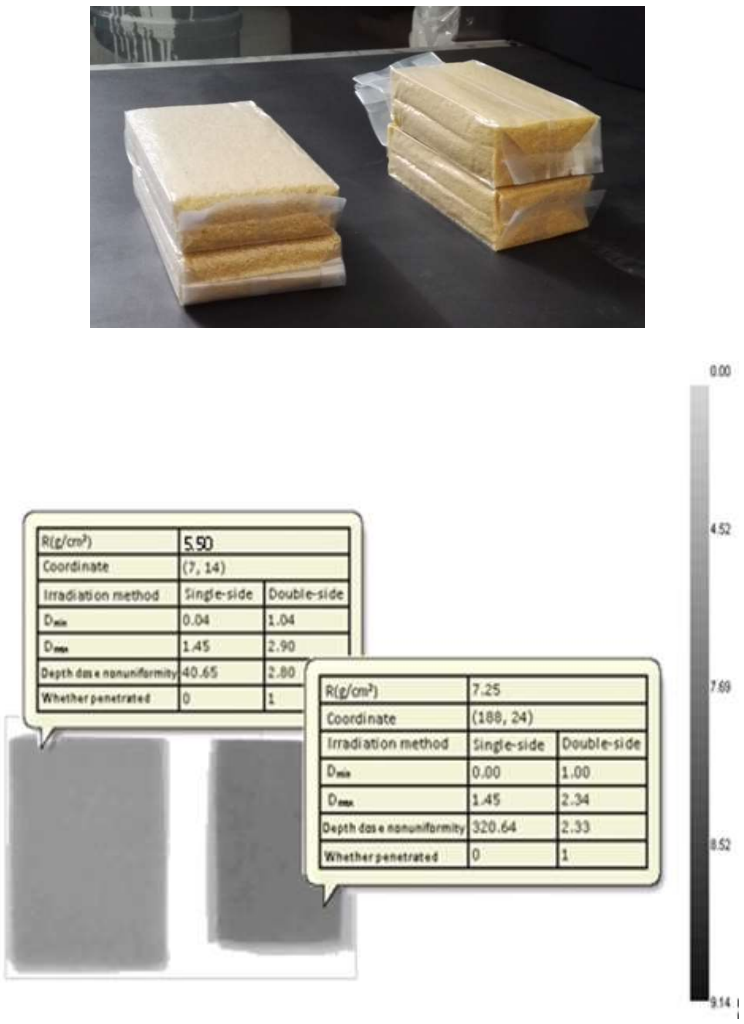


FIG. 13. Mass-thickness measurement of millet samples. On the left is a stack of two 3 cm depth samples with a total depth of 6 cm (6×0.92=5.52 g/cm^2) and on the right is a stack of two 4 cm depth samples with a total depth of 8 cm (8×0.92=7.36 g/cm^2).

Comparison and analysis the mass thickness detection data and actual mass thickness of simulating material (Table 6), and the error is less than 2%. We can conclude that the detection result of mass thickness equipment is very reliable.

TABLE 6. SUMMARY OF MILLET TEST RESULTS

Thickness	Density (g/cm^3)	Actual Mass thickness (g/cm^2)	Detected Mass thickness (g/cm^2)	Deviation
3cm	0.92	2.76	2.75	0.4%
4cm		3.68	3.75	1.9%
6cm		5.52	5.5	0.4%
8cm		7.36	7.25	1.5%

1.3.1.3. Summary

From the test results of the simulating material, small deviations prove the reliability of mass thickness detection technology.

1.3.2. Verification and analysis of X ray dose distribution prediction

We carried out mass thickness detection for homogeneous material and some specific packaged products and predicted the dose distribution of products according to the mass thickness test results. The electron beam irradiation dose distribution was also measured using dosimetry, and the test results were compared and analysed to evaluate the reliability of mass thickness test results.

Taking experiments of homogeneous material such as millet and some packaged products such as frozen shrimp and potato starch as example, the analysis results are shown below.

1.3.2.1. Homogeneous material-millet

(1) Dose distribution prediction

The millet was packed in a 9 cm depth box, and its mass thickness was measured and the dose distribution was predicted.

As shown in Fig. 14, the mass thickness test results are $8.0g/cm^2$, there is a slight difference with the actual mass thickness of $8.28g/cm^2$. By preliminary analysis, we think the result of detection is lower than the actual mass thickness is due to the large porosity of millet in the box.

According to the mass thickness equipment detection result ($8.0g/cm^2$), we predict the depth-dose distribution of millet, the result is shown in Fig. 15.

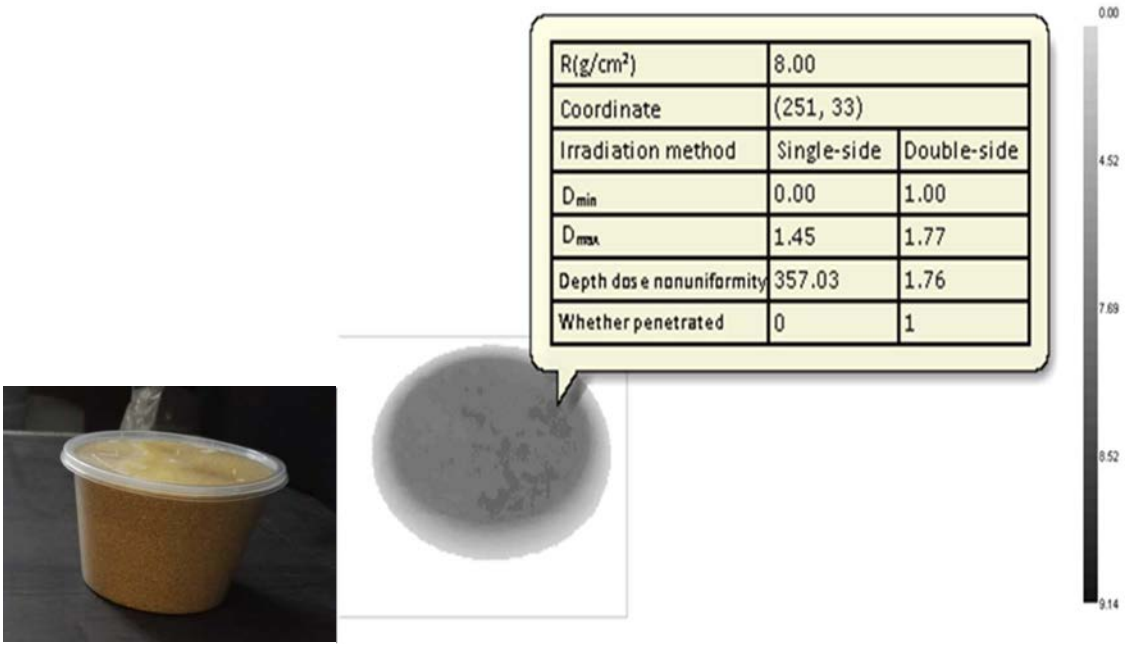


FIG. 14. Mass-thickness for millet of 9 cm depth.

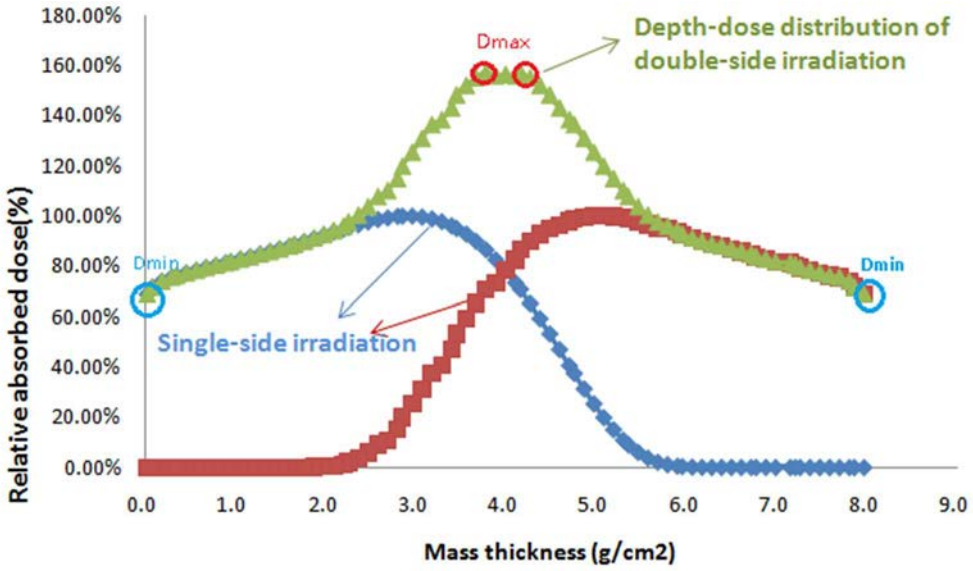


FIG. 15. Predicted depth-dose distributions for millet of 8.0g/cm^2.

(2) Actual dose distribution measurement, comparison and analysis

Dose mapping measurement is as follows: a dosimeter is fixed on a belt with the interval of 1 cm (Fig. 16), placed in millet and irradiated by 10MeV EB from one side.

FIG. 16. Dosimeters deployment for millet.

After single-side irradiation, dose values at different depths within the millet are shown in Table 7. With the depth as the abscissa, the relative dose value as vertical axis, the actual depth-dose distribution is obtained (see Fig. 17). By comparing the actual depth-dose distribution chart (red curve in the figure) with the predicted depth-dose distribution chart (blue curve in the figure), it can be seen that the consistency of the two is quite good.

Therefore, it can be concluded that: for the simulating material like millet, the dose distribution predicted by the mass thickness equipment is reliable and can be used to analyze the actual dose distribution inside the simulating material.

TABLE 7. ACTUAL DOSE FOR MILLET OF SINGLE-SIDE IRRADIATION

Depth /cm	Dose/kGy
0	6.1
1	7
2	7.7
3	8.1
4	6
5	2.4
6	0.35
7	0.06
8	0.04
9	0.02

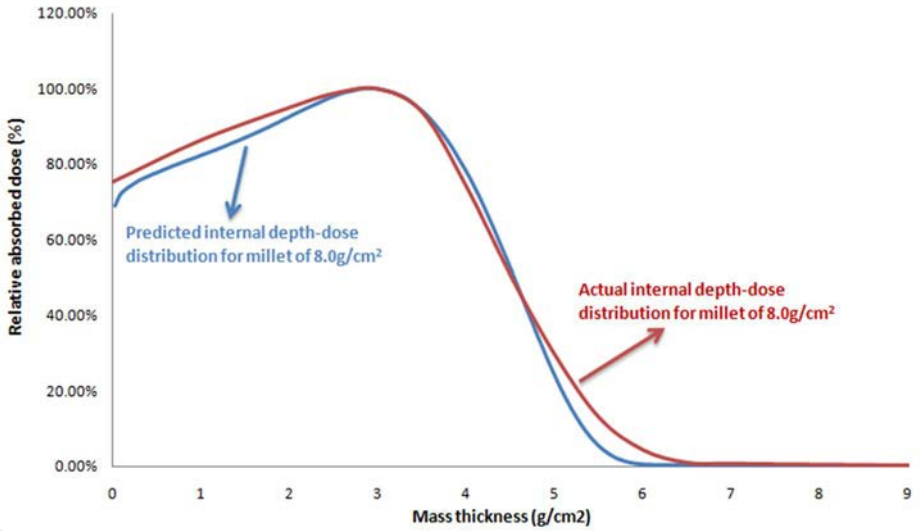


FIG. 17. Comparison of predicted (blue) and measure (red) depth-dose distributions.

1.3.2.2. Products-frozen shrimp

(1) Mass thickness detection data and dose prediction

Four bags of frozen shrimps were stacked up for X ray mass thickness measurement and the value was 8.5g/cm^2.The depth-dose distribution is predicted by the mass thickness value of 8.5g/cm^2. The result is shown in Fig. 18.

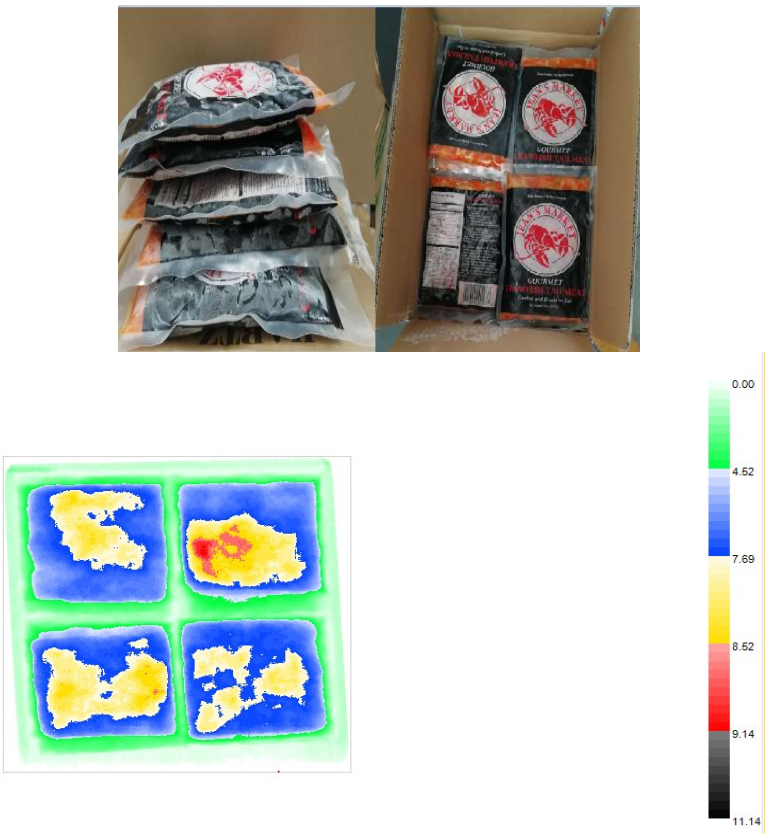


FIG. 18. Frozen shrimps and mass-thickness detection image.

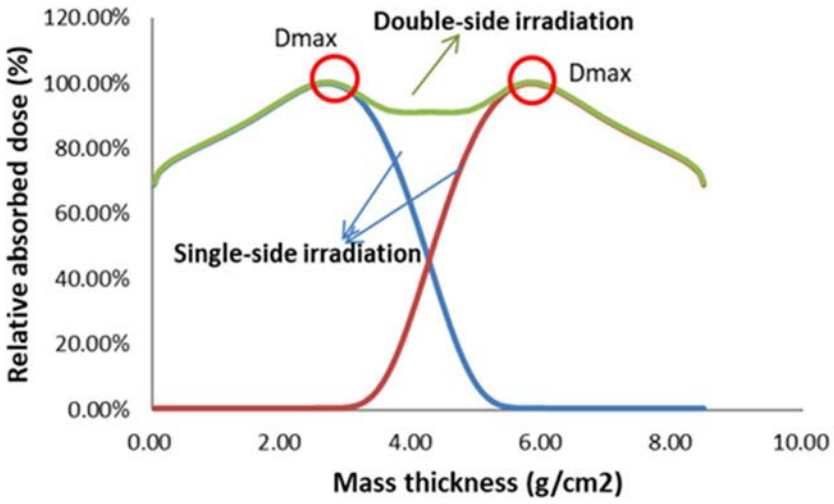


FIG. 19. Predicted depth-dose distributions of 8.5 g/cm^2.

Therefore, for e-beam of 10MeV, according to the mass thickness test results, the irradiation dose profiles for both single-side and double-side are predicted, as shown in Fig. 19. For single-side irradiation, the dose increases and then decreases from the top surface to the lower surface. The maximum dose is within the product, and the minimum dose is on the lower surface of the product. For double-side irradiation of 8.5g/cm^2, absorbed dose is the result of overlap of twice. The internal dose distribution is shown in Fig. 19. As the maximum dose is inside the product and the minimum dose is in surface. And the depth dose uniformity for double-side is 1.5.

(2) Actual dose distribution measurement, comparison and analysis

Dosimeters are placed inside and on the surface center of frozen shrimp (Fig. 20) and the absorbed dose are tested after electron beam irradiation. The position of the dosimeter corresponds to the mass thickness prediction of the position of 8.5 g/cm^2. The test data are shown in Table 8.

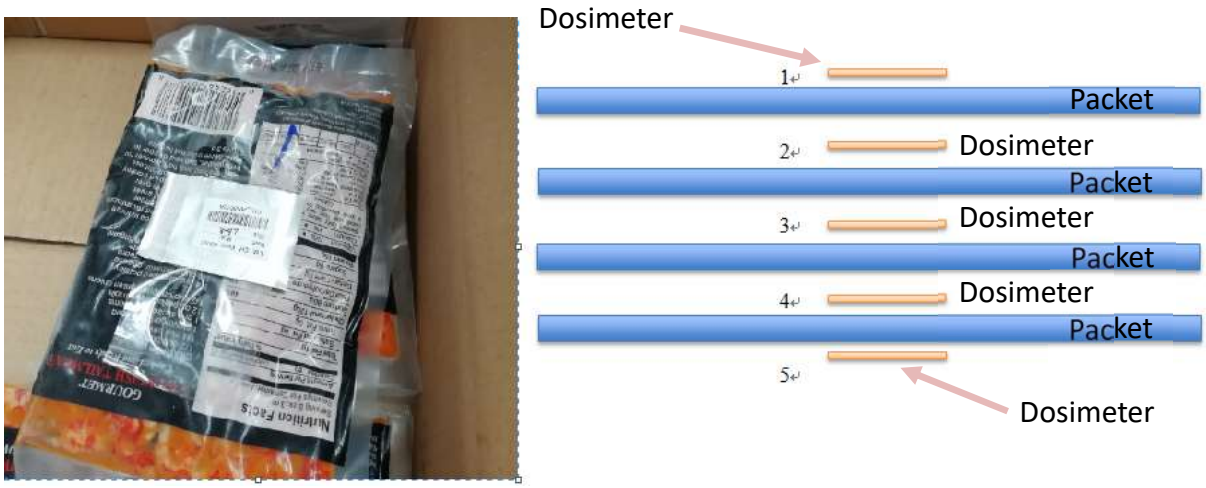


FIG. 20. Dosimeters deployment for frozen shrimps.

TABLE 8. ACTUAL DOSE FOR FROZEN SHRIMPS OF DOUBLE-SIDE IRRADIATION

No.	Dose/kGy	DUR
1	4.2	
2	5.1	
3	4.2	1.21
4	5.1	
5	4.4	

As shown in Table 8, the maximum dose of frozen shrimp is 5.1kGy, the minimum dose is 4.2 kGy, and the measured depth-dose uniformity for double-side is 1.21, which is a little different to that predicted by the mass thickness test result of 1.5. The difference may be mainly because the actual measured dosimeter distribution interval is larger and the maximum dose value is not obtained.

Dose values at different mass thickness within the frozen shrimp are shown in Table 8. Using the mass thickness as the abscissa, the relative dose value as vertical axis, the actual depth-dose distribution is obtained (see Fig. 21). By comparing the actual depth-dose distribution chart (blue curve in the figure) with the predicted depth-dose distribution chart (red curve in the figure), we find that the distribution trends of two curves are basically coincident.

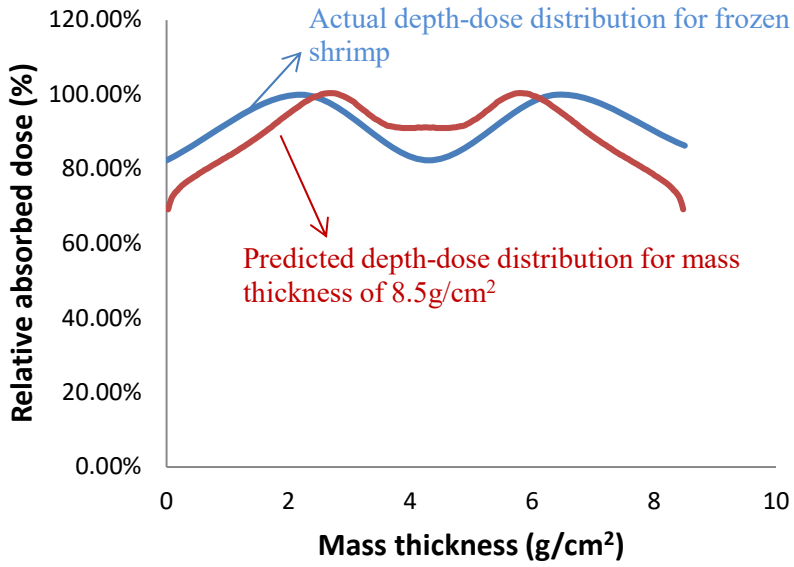


FIG. 21. Comparison of actual and predicted depth-dose distribution for boxed frozen shrimp.

1.3.2.3. Products-potato starch

(1) Mass thickness detection data and dose prediction

Four bags of potato starch were stacked up for X ray mass thickness measurement and the value was 9.0g/cm^2.The depth-dose distribution is predicted by the mass thickness value of 9.0g/cm^2. The result is shown in Fig. 22.

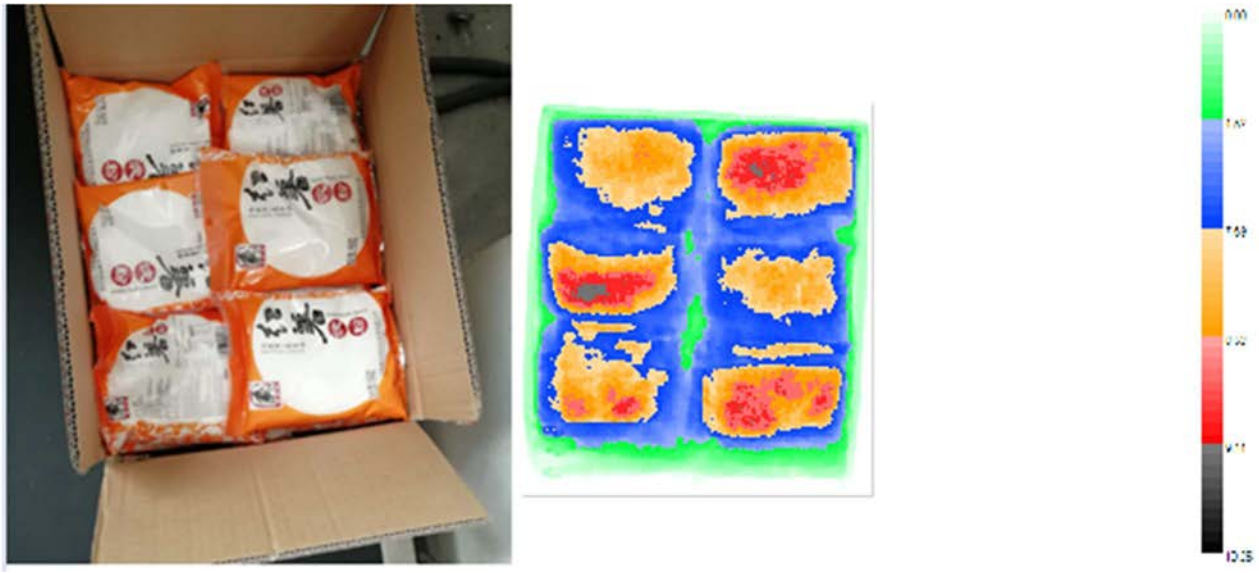


FIG. 22. Mass-thickness detection output image of potato starch.

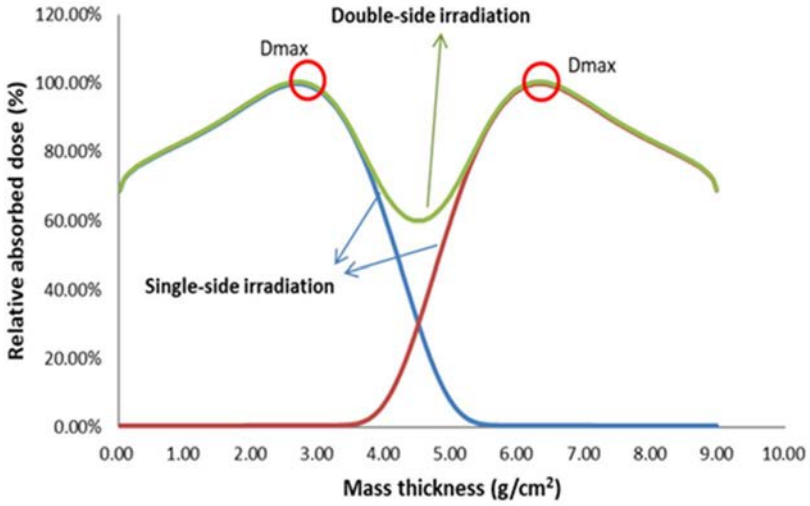


FIG. 23. Predicted depth-dose distributions for 9.0g/cm^2.

For single-side irradiation, the dose increases and then decreases from the top surface to the lower surface. The maximum dose is within the product, and the minimum dose is on the lower surface of the product. For double-side irradiation of 9.0g/cm^2, absorbed dose is the result of overlap of twice. The internal dose distribution is shown in Fig. 23. As the maximum dose is inside the product and the minimum dose is in surface. And the depth dose non-uniformity for double-side is 1.45.

(2) Actual dose distribution measurement, comparison and analysis

Dosimeters are placed inside and on the surface center of potato starch (Fig. 24) and the absorbed dose are tested after electron beam irradiation. The position of the dosimeter corresponds to the mass thickness prediction of the position of 9.0g/cm^2. The test data are shown in Table 9.

FIG. 24. Dosimeters deployment for potato starch.

For the value of DUR, the measured value for double-side is 1.2, which is a little different to that predicted by the mass thickness test result of 1.45. The difference may be mainly because the actual measured dosimeter distribution interval is larger and the maximum dose value is not obtained. By comparing the actual depth-dose distribution chart (Fig. 25, blue curve in the figure) with the predicted depth-dose distribution chart (red curve in the figure), it can be found that the distribution trends of two curves are basically coincident.

TABLE 9. ACTUAL DOSE FOR POTATO STARCH OF DOUBLE-SIDE IRRADIATION

No.	Dose/kGy	DUR
1	4.7	1.2
2	5.3	
3	4.4	
4	4.4	
5	5.1	
6	4.7	

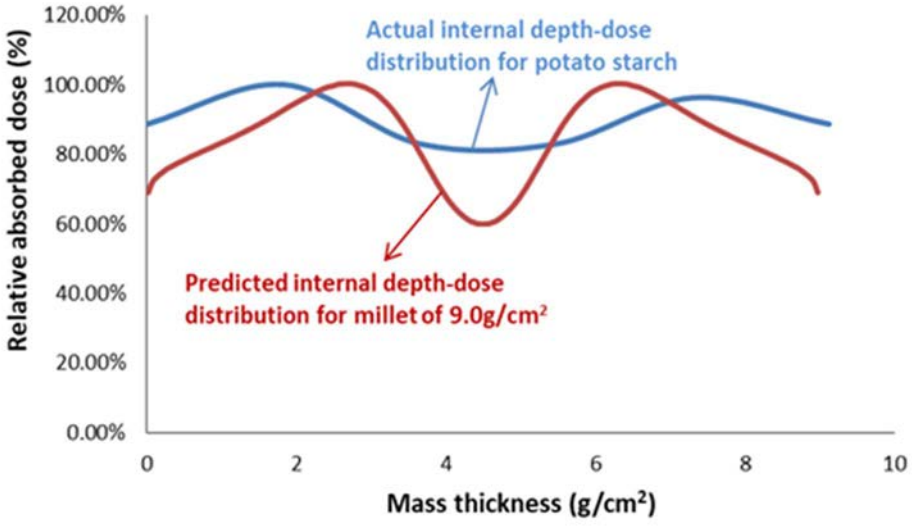


FIG. 25. Comparison of depth-dose distribution.

1.3.2.4. Summary

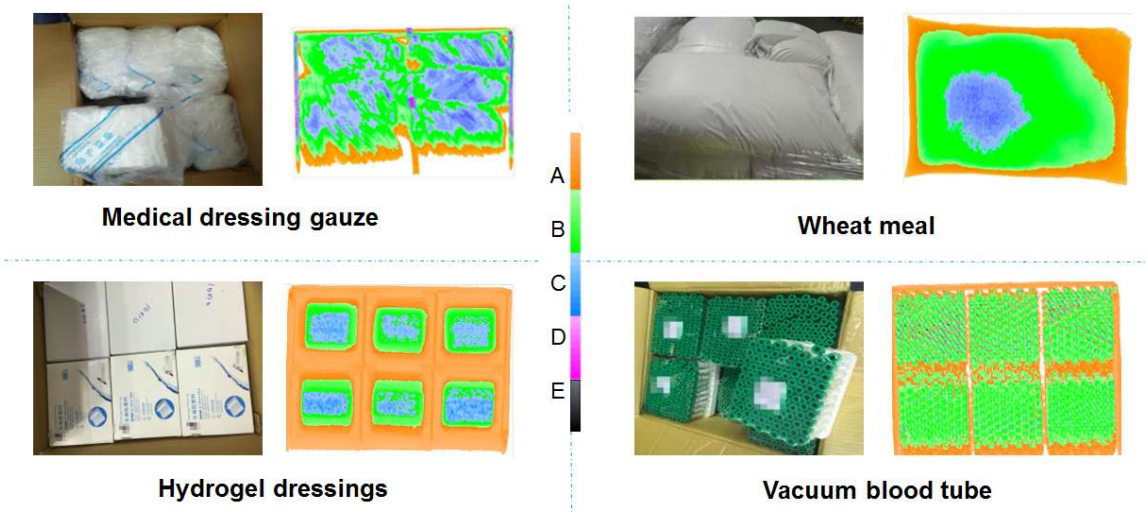
(1) By comparing and analyzing the dose distribution predicted by the detected mass thickness of millet and the measured dose distribution of electron beam, it can be concluded that: for the homogeneous material like millet, the dose distribution predicted by the mass thickness equipment is reliable and can be used to analyze the actual dose distribution inside the homogeneous material.

(2) Through the experiment result of actual products, we find that errors exist between the measured dose distribution and dose prediction from mass thickness detection. In view of this, we focused on analyzing the errors caused by special shape, uniformity and internal dosimeter arrangement. It was proved that the error was mainly due to the placement of the dosimeter in the product. And the actual electron beam dose test method has been optimized. So that the mass thickness equipment can be better applied to most of the products on the market to detect mass thickness and predict dose distribution.

1.3.3. Verification and analysis of pilot-scale study EB factory

We carried out pilot-scale study in EB factory to investigate the function and practicability of the mass thickness detection equipment. The equipment has been tested for more than two years at the EB factory.More than 500 kinds of products with different packages were tested and more than 1500 scanned images containing quality and thickness data were collected. Some of theresults are shown in Fig. 27. And the pilot test proves that the quality and thickness testing equipment works stably and is suitable for all kinds of container testing at EB factory.

FIG. 26. Pilot-scale studies in EB factory.



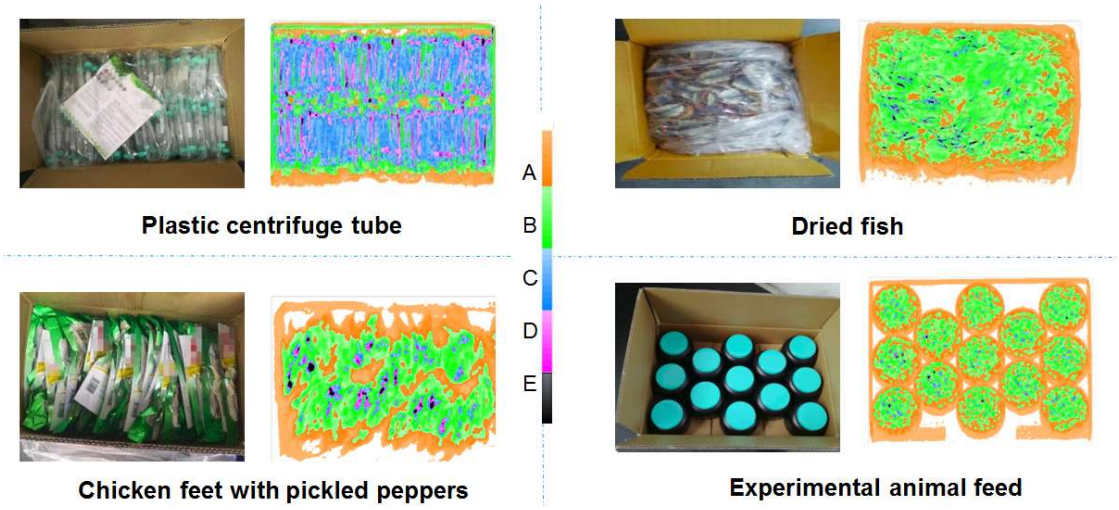


FIG. 27. Test results (see picture captions).

The pilot-scale study shows that the system can apply to all kinds of actual products and support actual processing in a commercial facility. In summary the new device can be used for dithe folloing different applications within an EB irradiation facility

(a) Rapid screening.

Through the analyzer, the mass thickness can be obtained and imaged quickly. It is easy to screen the product and identify any black coloured areas (EB irradiation would be out of range) and judge if the products is able to be irradiated by EB in this configuration. 28).

FIG. 28. Some typical products.

(b) As a dose mapping guide.

Using the X ray machine to predict EB doses can be helpful to guide dose mapping measurements, especially for non-uniform products. The dose prediction can be used to show which are the critical parts of the process load and where to focus attention and the areas where to concentrate dosimeter placement (Fig. 29).

FIG. 29. Some typical products.

(c) As a guide to establishing the loading configuration mode.

The system can quickly check the product as packed in different loading configurations and determine the most appropriate loading configurtion mode: e.g. the orientation (direction) that the package should be passed throug the EB, of the products packed together in a box need to be packed in a different, if boxed items need to be removed from the boxes and irradiated in different packing configuaration, etc. For example in Fig. 30 large boxes containing stacks of several smaller boxes of packaged dog food products were tested but the analysis showed that the larger box would need to be unpacked as only one mode was feasible an this was for the smaller boxes of product to be irradiated as as single layer. For the bottle caps, it's evident that some parts of the product as packed would not be correctly irradiated and the packaging / loading configuration needs to be adjusted.

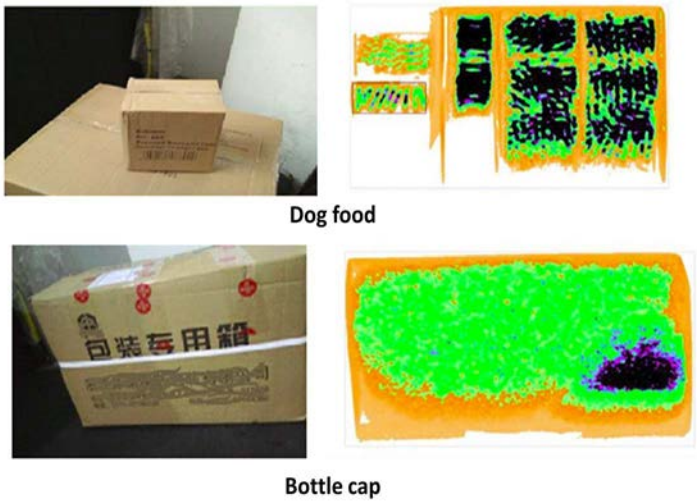


FIG. 30. The typical products.

(d) As a training tool.

The visual display is relatively intuative and easy to understand. It is conducive to communicating with people and demonstrating the importance of how products are packed and presented to the EB. The device can be used to make quick demonstrations with actual products and communicate the results to non-specialists.

1.4.CONCLUSIONS AND DISCUSSION

The X ray system can be used to guide EB irradiation processing. The X ray mass thickness measurements enable an efficient dose distribution prediction system. The machine comprises X ray scanning equipment, software and analysis methods. The algorithms and design of the device are optimized so that it can be used as a tool to guide judgments and decisions that are important in electron beam irradiation processing. A comprehensive characterization study was undertaken to test and to evaluate and thus to optimize the system for use in a commercial scale EB facility. The conclusions are as below:

(1) The homogeneous material tests using different thicknesses of materials such as water and millet proved that the X ray mass thickness measurement data for homogeneous materials are consistent with actual measured results;

(2) For actual products, the dose distribution predicted by mass thickness detection were in good agreement with the measured dose distribution results for EB irradiation, but there is a certain error, or discrepancy. Differences may be due to actual dosimetry measurements taking doses in a number of discrete locations to derive the dose distribution whereas the modelling predicts dose in the whole volume of product to a higher degree of resolution;

(3) The pilot-scale study in the EB factory shows that the system can be used in a variety of different ways and has a great guiding significance as a tool to set up and plan a process for efficiently and correctly irradiation products. These tests also showed that the system is stable in a commercial setting and can effectively improve the efficiency of the EB facility.

During the pilot test phase of this project, we found a phenomenon that cannot be ignored. For some product, there is naturally stacking, and the new system indicates a small proportion of super large mass thickness. The overall mass thickness of the product is not large; but there are a few areas marked black (see Fig. 31). These black areas are small but indicate that the electron beam may not be able to penetrate through these parts of the product. This may be an artifact of the system, but it could be a real phenomenon. There is a small but finite probability that discrete areas of super large mass thickness are caused by product stacking. This would be hard to avoid in multi-layered products.

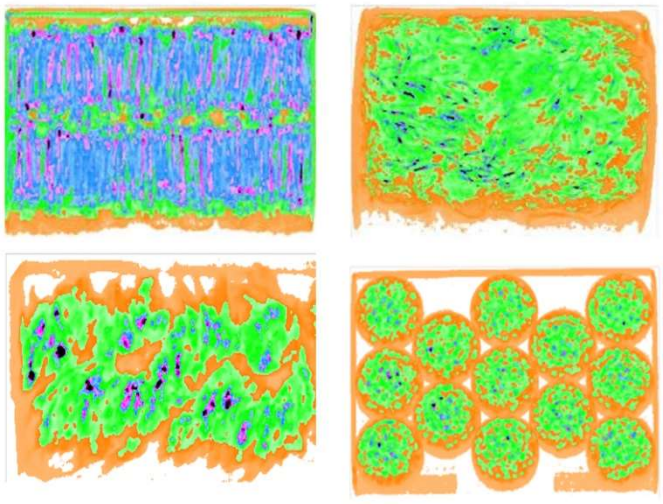


FIG. 31. The products with a small proportion of super large mass thickness.

Detecting areas of super mass thickness would be challenging for conventional dose measurements using dosimeters placed throughout the product, such as in traditional dose mapping. The stacking of items within a product load resulting in super-mass thickness may be random and unusual, affecting only a few locations in the product. Conventional dose mapping may miss these locations because the dosimeters do not measure the dose at every point in the product. The areas marked black by the system are very small, for example, the area of a data point is only $2mm^2$, and the dosimeter is usually 1 cm^2. It is therefore possible that the dosimeter cannot detect at this level of resolution even if a dosimeter was placed in the location. So, identifying these "black-areas" is likely to be impossible for dose mapping using the traditional and standard method. If the traditional dose mapping approach cannot find such small black spots, it could be that irradiation processing in practice can tolerate a certain dose limited error.

The degree of resolution in two dimensions and the accuracy of this method of using X rays to calculate mass thickness is beyond the scope of traditional dose mapping. For this new technology, we might need to establish better standards or guides in future to ensure that an electron beam can fully penetrate through a process load and each and every part of the product can receive a dose that is within the desired specification for the treatment. This new method can quantitatively analyze the whole range of dose distribution throughout the product and not just at discrete locations. If this phenomenon is real, a risked based approach may be necessary to deal with situations where a small proportion of product could receive a treatment dose beyond the dose limit. A method based on proportional statistics and risk assessment may complement this new method of using s for mass thickness detection and algorithms for simulation of electron beam dose distribution. However, calculating an acceptable degree of risk would require a great deal of data and thorough statistical analyses.

ACKNOWLEDGEMENTS

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2. DEVELOPMENT OF A METHOD TO ASSESS THE FEASIBILITY OF IRRADIATING FOOD PRODUCTS WITHIN A GIVEN DOSE RANGE BY ELECTRON BEAM

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Abstract

The application of ionizing radiation (gamma, electron beams, X rays) as a sanitary or phytosanitary treatment for food and its commodities has expanded rapidly in recent years. The use of electron beam (EB) facilities has also increased but relatively slowly because the penetrability of EB is limited. Since 2012, the EB accelerator UERL-10-15S2 (10 MeV, 15 kW, Corad, (Russian Federation) has been operated for research and commercial purposes at VINAGAMMA Center (Socialist Republic of Viet Nam). Besides the sterilization of medical products, some agricultural products such as dehydrated vegetables (onion cut-leaves, carrots, pumpkins etc.) with low densities have been treated to eliminate bacterial and fungal contamination. Other agricultural products such as frozen fruits, dried and frozen seafood and cut flowers differ in densities, packaging configurations and irradiation dose requirements but would also benefit from EB irradiation due to the fast delivery of the treatment. To help support the application of EB to a wide range of different products, a device to measure density was designed and developed to analyze process loads quickly, before EB treatment. This device was developed to also predict the EB dose distribution throughout products with different packages and packing configurations, and to also calculate the maximum and minimum doses (D_{max} and D_{min}) and the dose uniformity ratio (DUR, D_{max}/D_{min}) of cartons of products. The data obtained by using this device scan a wide range of foods are useful to ensure and demonstrate efficient and safe applications of EB processing to improve the quality of food and agricultural products.

2.1.INTRODUCTION

The application of ionizing radiation (gamma, electron beams, s) as a treatment method to preserve food and food commodities has expanded rapidly world-wide in recent years [1]. However, food irradiation has developed in many countries largely through the use of gamma irradiation at multi-purpose ^{60}Co irradiators [2]. The use of electron beam (EB) facilities on a has also increased with time, but slowly in comparison to gamma irradiators, mainly due to the low penetration of EBs through packages with high densities and a high cost of irradiation [3–6]. The use of machine sources to generate EBs and s can overcome and avoid concerns related to radioactive isotope sources such as ^{60}Co used to produce gamma rays and can be as effective for radiation processing [7]. An EB accelerator (10 MeV, 15 kW) has been operated for research and commercial purposes at VINAGAMMA Center in the Socialist Republic of Viet Nam since 2012. EB radiation processing is quite easy for low density products. However, the ability of an EB of a specific energy to penetrate through products is dependent on both the density and thickness of the item. In this regard, mass thickness (mass per unit area) is a critical factor to control the quality of EB irradiation.

In this work, a density detector device was designed, manufactured, and operated effectively to detect and determine mass thickness and predict EB dose distribution in cartons of products as

well as predict D_{max}, D_{min} and DUR. The device is being used in the EB facility and is helping to improve the quality assurance of electron beam irradiation at the VINAGAMMA Center.

2.2.MATERIALS AND METHODS

2.2.1. Materials

2.2.1.1. Sample preparation

Real products are packaged in a variety of shapes and sizes included medical products, frozen and dried seafood, spices and pet chews supplied by customers were used for experiments.

2.2.1.2. Equipment

— Irradiation facility:

- Electron beam accelerator UERL-10-15S2;
- 10 MeV energy;
- 15 kW power, supplied by CORAD Co. Ltd., Russian Federation, at the Research and Development Center for Radiation Technology (VINAGAMMA).

— The device system:

- NaI scintillation detector (England) at 30 cm as referent counter by 20,097 count/second;
- ^{137}Cs (0.5 mCi) gamma radiation source (Model CDC.P4 supply by Eckert&Ziegler);
- Controlling software Arduino MEGA-2560;
- CPU and electronic circuit boards.

2.2.2. Method

2.2.2.1. Principle of operation

For a narrow beam of mono-energetic photons, the change in X ray or gamma ray beam intensity at some distance in a material can be expressed in the form of an equation as follows (Equation 1):

$$I=I_0 .e^{-\mu x} \quad (1)$$

Where:

I = the intensity of photons transmitted across some distance x;

I_0 = the incident intensity of photons;

μ = the linear attenuation coefficient;

x = distance traveled.

The main objective of this work is to carry out the studies aiming at design and construction of a prototype density measuring device using and ^{137}Cs gamma source and scintillation detector to determine mass thickness and therefore predicting the dose absorbed in EB irradiated products.

The radiation source and the detector are held by mechanical holders so that as much of the emitted gamma radiation as possible can reach the surface of the detector having passed directly through the product carton. The gamma detector is placed on one side of the product and the radiation source in its holder is placed on the opposite side of the cartons (Fig. 1). The radiation beam is focused to the detector's surface using a lead collimator. The number of gamma photons

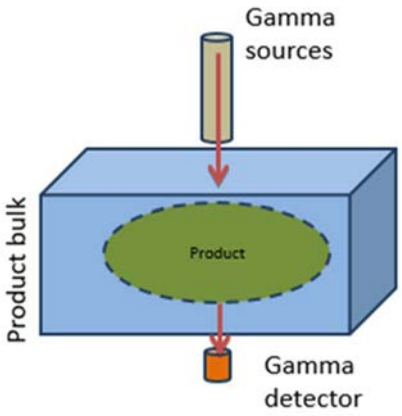

FIG. 1. Experiment layout for areal density detection system via gamma attenuation.

(ray intensity) that reach the detector depends on the distance of separation and the product between source and detector (i.e. the materials inside the carton box, the size of the cartons and the densities). As the density of the material in the carton changes, the amount of radiation reaching the detector is changed. The greater the density of the material, the lower the radiation field measured at the detector, the lower the density of the material, the higher the radiation field at the detector.

A high voltage (HV) is applied to the detector and a high electrical current in the detector signals high intensity gamma ray. The density of the materials in cartons is inversely proportional to signal strength gathered by the electronic device of the gamma detector. The electronic device is based on microprocessor MEGA-2560 (Arduino). It is programmed to calibrate using experimental data (standard test material) and it will then measure densities at specific points across a carton. Readings are automatically calculated as the areal density (mass thickness). The maximum number of measuring points across a carton is 400 (20 x 20).

A keypad and LCD display or an external computer and USB link can be used to make the calibration and set the measuring points.

2.3.RESULTS AND DISCUSSION

2.3.1. Practical examples of mass thickness measurements with different product types

2.3.1.1. Medical products

In this section, we present two kinds of medical products that were chosen to test the device. One product was a type of spatula and the other was a bottle of eye drops. In both cases, many products were packed inside a standard carton box. A carton box of Spatula product had a gross weight of 2.4 kg, and dimension of 18 x 31.5 x 22 cm. The calculated average areal density is 3.46 g/cm^2. The carton of eye drop bottles was 8.4 kg in gross weight and 48 x 48 x 48 cm in dimensions and therefore a calculated average areal density is 3.64 g/cm^2.

When using the density detection device (DDD), the areal density distribution of products which is recorded and shown as in Fig. 2 for the spatula product and Fig. 3 for the eye drop product. The actual DDD measurement of areal density of product is quite different compared with the calculation via weight and product carton size. For the spatula product an array of 8 x 4 measuring points across the top of the carton box measured 32 values of mass thickness (areal density that ranged from 4.34 g/cm^2 to 7.31 g/cm^2. For the carton box of bottled eye drops, the DDD measured an array of 8 x 4 measuring points across the top of the carton box to measure 32 values of mass thickness (areal density) and these data ranged from 4.50 g/cm^2 to 7.01 g/cm^2.

FIG. 2. Areal density distribution of a carton of spatula product by using DDD.

FIG. 3. Areal density distribution of a carton of eye drop product by using DDD.

2.3.1.2. Frozen food and dried spices

In this example we consider a frozen food and a dried food. Ahi tuna product (frozen seafood) and garlic powder are two products that are irradiated on a commercial scale in large amounts at VINAGAMMA. Approximately 200 tons of frozen Ahi tuna are irradiated per year and more than 20 tons of garlic powder are irradiated per year. The frozen tuna blocks have an irregular and complex packing pattern (Fig. 4a). In contrast, the garlic powder is a relatively homogenous powdered products, that is packed relatively uniformly in each carton (Fig. 4b). This type of product makes the QA process differently in identity product areal density.

(a) *(b)*

FIG. 4. Frozen, packaged Ahi tuna (a) and packaged dried garlic powder (b) products in box cartons as packaged for irradiation treatment.

The Ahi tuna is packaged in cardboard boxes, the cartons had dimensions of 34.5 x 24.5 x 12.5 cm and in the case the gross weight was 5.0 kg. The garlic powder has packed in box cartons with dimensions of 38.5 x 48.5 x 12.5 cm and a gross weight of 13.0 kg. The calculated average areal density for Ahi tuna and garlic powder is 6.0 g/cm^2 and 6.9 g/cm^2, respectively. But the distribution of areal density as measured at discrete locations across the product is quite different compared with the calculation via weight and product carton size (Fig. 5).

(a) *(b)*

FIG. 5. Areal density distribution of Ahi tuna(a) and garlic powder(b) by using DDD.

2.3.1.3. Dried seafood products

Many dried marine foods (fish and squid etc.) are irradiate on a commercial scale. In this example a dried fish (dried yellow stripe trevally) product was measured, one was the dried fish as a semi-finished product and the other was the same dried fish in its final finished product form (Fig. 6). For semi-finished dried yellow stripe trevally products, the average aerial density from the with and carton dimensions is equal to a mass density of 6.3 g/cm^2, but its measured average density is 9.51 g/cm^2 by using DDD (Fig. 7a). For the finished dried yellow stripe trevally products, the calculated density is 4.9 g/cm^2, but an average mass density of 7.61 g/cm^2 was measured by the DDD (Fig. 7b).

FIG. 6. Dried yellow stripe trevally as semi-finished (left) and finished (right) products.

(a) (b)

FIG. 7. Areal density of dried yellow stripe trevally in semi-finished (a) and (b) finished product.

2.3.1.4. Pet chews

A pet chew product is irradiated in large amounts on a commercial scale (more than a thousand tons per year). The required dose varies but ranges from a minimum dose of 4.6 kGy to 12 kGy. The product is produced in a variety of different packaging, and it is not easy to determine the dose distribution for each different packaged product. The DDD measured average mass thickness (10.62 g/cm^2) to the calculated average mass thickness (7.7 g/cm^2) because of the non-uniform density of products inside each box (Fig. 8).

FIG. 8. Pet chews product and measured areal density.

2.3.2. The density detector for rapidly predicting dose distribution inside product irradiated by a 10 MeV electron beam

A large variety of different products are processed at EB facilities. As can be seen in the previous section, mass thickness can vary in different products. Products are packed differently, and they can be boxed in different packaging configurations inside box cartons. These factors, and the relatively short penetration of 10 MeV electron beams, means that it is complicated to evaluate if a specific product in a certain type of packaging and boxed in cartons in a specific configuration will be suitable for EB processing. In practice, quality control (QC) staff at the EB facility can undertake dose mapping for each different product and packing configurations. Normally, health-care products are arranged uniformly inside cartons, and lighter in weight, the procedure to make dose mapping is not as difficult as for dense non-uniform products. The dose mapping procedure can be complicated for food products (such as dried or frozen foods, spices, and others) because of the way the product is packaged both in weight and arrangement. For a huge quantity of different food products, dose mapping using dosimeters placed at different positions inside the carton box is very difficult, for example to obtain exact results for the absorbed dose (such as the location and size of D_{max} and D_{min}). The density detection device could help QC staff by measuring areal densities with high accuracy and by being able to predict EB dose distributions by combining the point measurements of aerial density (mass thickness) with estimated depth dose profiles for the 10 MeV electron beam at these locations.

To predict dose distributions that would be imparted by a 10 MeV electron beam, the prototype device was improved to increase its accuracy. These improvements were to collimate the gamma source and detector for a narrower measurement beam, and to measure continuously instead of taking a series of point measurements of gamma ray intensity. The system is running well in a commercial and research facility. It is being used to measure mass density of product cartons before EB irradiation and predict dose distributions for EB irradiation (Fig. 9).

FIG. 9. Density detector device: the product in a standard box carton is passed through the gamma beam to measure mass thickness and predict the dose distributions that would result from electron beam irradiation.

2.3.2.1. Example of mass thickness calibration

We chose a test material (medium-density fiber board, MDF) with a similar density to typical irradiated products. The MDF board is available in standard thicknesses and boards could be layered, one on top of another to measure the linear attenuation of the gamma beam. Each layer of MDF had an areal density of 1.12 g/cm^2. For each measurement we increase the number of layers of MDF to increase the areal density in a step wise fashion. Readings of gamma beam intensity (detector counts) were taken. The path taken by the measuring gamma beam is the same as the path that the EB would take through the box carton during irradiation treatment. A series of results of gamma counted numbers (N) versus areal density (mass thickness) of MDF are given in Table 1.

TABLE 1. COUNTED NUMBERS VERSUS DENSITY BY USING MDF LAYERS

No. of MDF layers	Detector reading (N)	Ln(N)	Areal density (g/cm^2)
0	20097	9.90	0.00
1	18979	9.85	1.12
2	18067	9.80	2.24
3	17250	9.75	3.36
4	16159	9.69	4.48
5	15313	9.63	5.60
6	14337	9.57	6.71
7	13634	9.52	7.83
8	12667	9.44	8.95
9	12017	9.39	10.07
10	11135	9.317	11.19

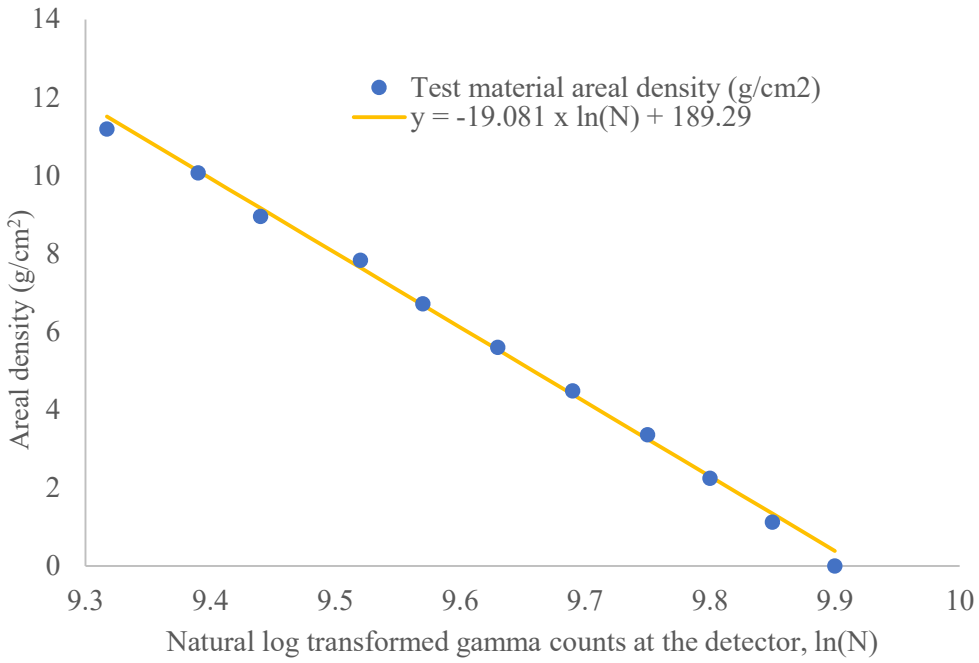

FIG. 10. Plot of gamma intensity at the detector against areal density.

From equation (1), μ is found out by following (Equation 2):

$$\mu = (1/30).\ \ln(I_0/I) \quad (2)$$

But a simpler approach is to correlate areal density (mass thickness) and the gamma count reading (N) at the detector below the test material by plotting ln(N) against aerial density (Fig. 10). Linear regression yields the following equation (Equation 3) for the relationship between areal density (ρ) and natural log transformed reading at the gamma detector (ln(N)):

$$\rho\ [g/cm^2] = -19.081 * \ln(N) + 189.29 \text{ with } R_2 = 0.997 \quad (3)$$

The device system is in use to measure density of product cartons before e-beam irradiation. It supports the operation of the EB facility, and the device helps to enhance the quality assurance programme for electron beam irradiation at the VINAGAMMA Center.

The detector and system for moving the product through the beam communicate with personal computer via a Bluetooth connection. Data are recorded by software written in Visual Studio C#. Figure 11 shows the Density Measurement Interface. This software interface allows the user to select the number of measurement points, the measurement duration for each point and to extract data (e.g. as an Excel file) for further calculations.

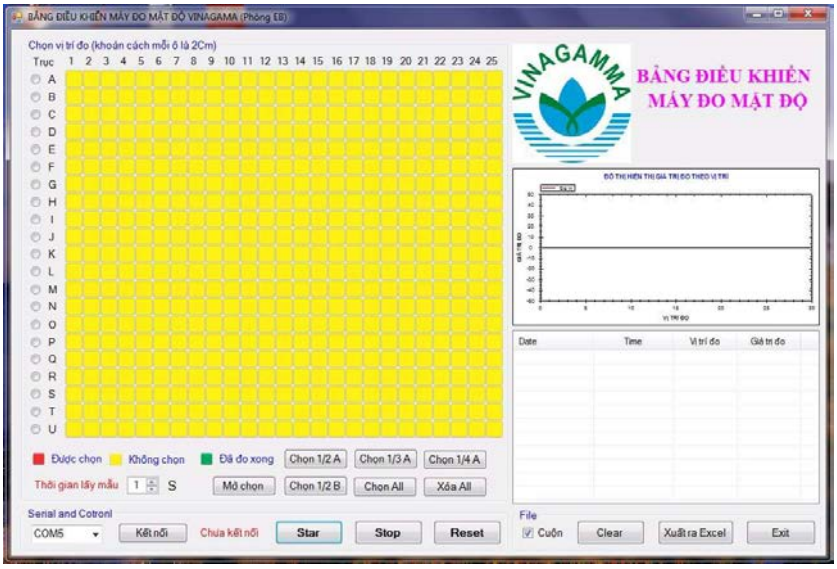

FIG. 11. Density Measurement Interface.

The interface has a "dose calculator layer" that will use the areal thickness measurements to estimate the depth dose distribution (predict EB dose distributions). This dose calculator layer will estimate the suitable speed for the conveyer to pass product through the EB, it can also predict dose distributions from single and double sided EB irradiation and predicts DUR for the irradiated product (Fig. 12).

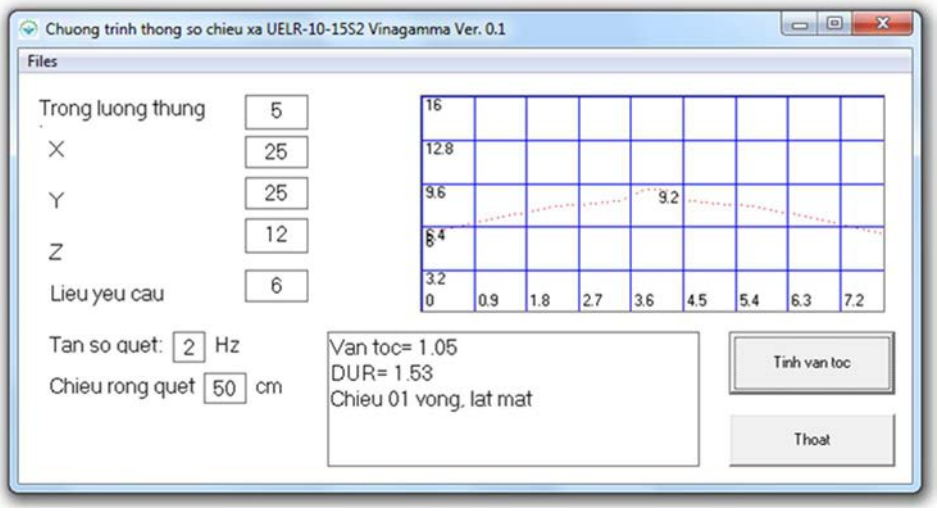

FIG. 12. Dose calculation windows.

The system has been calibrated using suitable phantoms and tested inside an EB facility, using real products. The products chosen for these tests were typical healthcare products, foods, spices, etc. The results calculated by the system were in good agreement with conventional dose mapping using B3 dosimeters at an accuracy of 89%–95% (Table 2).

TABLE 2. CALCULATED RESULTS OF EB IRRADITION PARAMETERS

Customer Code	Product	Required Dose (kGy)	Conveyor Speed (M/S)	DUR	Accuracy compared to actual dose mapping
MIDA	Petri dish	6–7	1.35	1.54	0.95
ROH	Bottle	17.5	0.55	1.32	0.90
HERC	Cap	15	0.65	1.57	0.92
NAMP	Dried green onion	3.5	3.20	1.20	0.94
BASF	Dried anchovy	7	1.40	1.82	0.92
THACON	Shrimp powder	7	1.35	1.41	0.89

2.4.CONCLUSIONS

The density detector device has been designed, manufactured, and operated effectively to detect and determine parameters for EB processing (including dose distribution in boxed cartons of products as well as D_{min}, D_{max}). This device can be used before EB irradiation to quickly evaluate required doses and DURs. It can probe for process loads. The device is a valuable tool to probe different possibilities for irradiating products in different packing configurations and orientations and with different characteristics. It can be used to guide dose mapping of real products and actual measurements of dose.

ACKNOWLEDGEMENTS

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Electron Beam Irradiation

3. COMPARATIVE STUDY OF THE EFFECTS OF DIFFERENT ENERGIES OF ELECTRONS ON BACTERIAL SPORES AND FOOD MATRIXES

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Abstract

We have set up a dosimetry system for low-energy electron beam (EB) in situ by simultaneously irradiating transfer dosimeters (alanine dosimetry system) and routine dosimeters (film dosimeters, B3 and FWT-60 film dosimetry systems) by 10 MeV EB and making a dose-response curve at NFRI. In addition, to have reliable dosimetry methods for low energy EB, we obtained a depth-dose profile of low energy EB (80 keV, 150 keV, 250 keV) with stacked B3 film dosimeters. The depth-dose profile was used to determine the dose at the upper most surface in the first micrometer of the absorbing medium ($D\mu$). The *Bacillus pumilus* spore-inactivation curve fitted and adjusted by the $D\mu$ value gave a decimal reduction dose (D_{10} value) of 1.69 kGy for *B. pumilus* spore inactivation, which was in a good agreement with earlier studies (1.65 kGy). Finally, we have irradiated real food materials (black pepper, white pepper, and allspice) with low energy EB (300 keV). The logarithmic reduction of the bacteria number was more than 2 for black pepper and no colonies were observed for the irradiated white pepper and allspice (detection limit=50 CFU/g, the number of bacteria in non-irradiated black pepper, white pepper, and all spice was 2.9×10^6, 7.5×10^2 and 7.9×10^5, [CFU/g], respectively). The black pepper was contaminated with *Cronobacter sakazakii*, a radiation resistant bacterium, which resulted in the low-level decontamination by irradiation. Comparable amounts of *C. sakazakii* cells were also found in the black pepper irradiated by high energy EB (10 MeV). The advantage of spice irradiation by low-energy electron, in addition to the lower effect on food ingredients, is that low energy e-beam machines do not require thick shielding and can be safely applied for in-line irradiation. Thus, low-energy EB machine can eliminate the necessity of transportation of a large number of products to an irradiation facility. In conclusion, despite its low penetration ability, the low-energy EB is an alternative technology for the currently employed highly penetrating ionizing radiation to eliminate microbial contamination of spices. This study involved some collaboration with the Institute of Nuclear Chemistry and Technology, Warsaw, Republic of Poland.

3.1.INTRODUCTION

Food irradiation can be used to eliminate harmful microorganisms, insects, fungi, and other pests and may reduce the need to use hazardous pesticides, fumigants, and preservatives [1]. Irradiation at relatively lower dose levels also extends the shelf-life of food and can be used to control insects [2].

Recently, irradiation by low-energy electron beams (EB) of less than 300 keV have attracted attention. A low energy EB of 300 keV or less has a limited penetration ability and potentially can decontaminate the surface of various kinds of cereals and grains with minimal quality deterioration inside the food. Besides the minimal quality deterioration, the low energy EB have some additional advantages for industrial applications: They are generated by electricity (machine-source not radionuclide source) and can be easily installed in the existing food processing equipment at a relatively low cost. Furthermore, heavy shielding of the irradiation

chamber is not required for the installed irradiator because of the limited penetration ability of the low energy electrons. However, the dosimetry of the low energy EB is more difficult than that of high energy EB and gamma ray irradiation due to the poor penetration ability.

In the present study supported by IAEA (CRP D-61024, DEXAFI 2015-2020), we tried to develop a reliable dosimetry system for low energy EB as a first step. In addition, we measured a range of depth-dose profile for low energy EBs of different energies (80 keV, 150 keV, 250 keV). Depth-dose profiles were determined using stacked B3 film dosimeters and in collaboration with researchers in the Republic of Poland. The $D\mu$ approach was used, where the dose in the first micrometer of an absorbing medium is determined, and which was recently proposed for the surface dose estimation [3]. Finally, we have irradiated real food materials by low energy EB (300 keV) to confirm the inactivation of bacteria on the surface, and determined bacteria species detected in the materials with or without irradiation in collaboration with researchers in the Republic of Poland. Detailed research results have been published [4] and we have also undertaken several activities to disseminate the knowledge and technology of low energy EB to food industries.

3.2.MATERIALS AND METHODS

3.2.1. Dosimeters

We used an alanine dosimeter (Kodak BioMax) and film dosimeters, B3 (GEX corporation) and FWT-60 (Far West Tech.). Dosimeters were kept in tightly sealed packaging until their use and doses were read out from the dosimeter within 48 hours after irradiation. The B3 film dosimeters were heated at 58.5 ± 0.5 ^{o}C for 18 min to complete the colour development.

Optical density (OD) of B3 film (OD550 nm) and FWT-60 film (OD605 nm) were measured by a UV-Vis spectrophotometer (Shimadzu UV-1700) with a holder to put the film in the correct position. The thickness of the films was measured by a thickness gauge (Mitutoyo VL-50-B). Measurements were duplicated.

Electron spin resonance (ESR) (EMX-plus, Bruker BioSpin K.K.) signals from the alanine film dosimeter were recorded by setting it together with manganese marker in the holder. Normalized ESR signal amplitude of alanine to the manganese marker was determined. ESR measurements were duplicated, and the calibration curve was created by using ESR analyzing software (ESR-A, Bruker BioSpin K.K.).

3.2.2. Irradiation

The following electron beam irradiators and gamma-irradiators were used in this research:

(a) Food Research Institute, National Agriculture and Food Research Organization (NFRI/NARO)
- — Electron Beam Accelerator (Model EC250/15/10 mA, Iwasaki Electric, Co. Ltd.)
- — Gamma Cell 220 (Nordion Co. Ltd.)

(b) Eye Electron Beam Co., Ltd., Iwasaki Electric Co., Ltd.
- — Electron beam irradiator (EC 300/30/30 mA, Iwasaki Electric Co., Ltd.)

(c) Nuclear Fuel Industries, Ltd.
- — Electron Beam Accelerator (10 MeV, Rhodotron)

(d) Takasaki Advanced Radiation Research Institute
- — Cobalt 60 Radiation Building, Cobalt Source (JAEA, Takasaki)

Spice samples were irradiated with an electron beam of energy 10 MeV, using accelerator Electronica 10-10, and with an electron beam of energy 300 keV, using accelerator ILU 6 in the Republic of Poland [4].

3.2.3. Microbiological analysis

Colony counting, strain isolation and species identification, and other microbiological analyses employed in this study were performed as described previously [4].

3.3.RESULTS AND DISCUSSION

3.3.1. Setting up a dosimetry system for low-energy EB irradiation

We developed a dosimetry procedure suitable for low-energy electron beams according to ISO/ASTM 51261 (Fig. 1). First, we set up a calibration procedure for the dosimetry system for electron beam irradiation at the Food Research Institute, National Agriculture and Food Research Organization (NFRI/NARO). As shown in Fig. 1 the value of the absorbed dose is traceable to the National and International Standard.

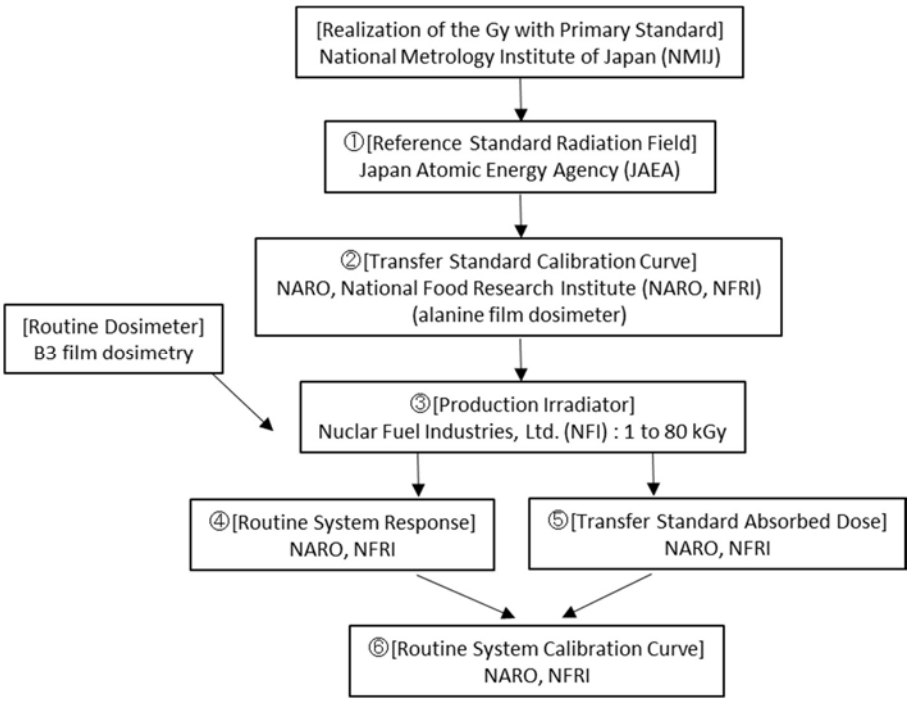

FIG. 1. Calibration procedure of the dosimetry system at NFRI/NARO, Japan and value traceability to the national/international standard.
*JAEA: promising accredited standard dosimetry laboratory for radiation processing
**NPL: National Physical Laboratory

The Japan Atomic Energy Agency -Takasaki Advanced Radiation Research Institute (hereafter, JAEA-Takasaki) possesses a ^{60}Co gamma ray irradiation field that is defined as the Reference Standard Radiation Field (Fig. 1, ①) where the absorbed dose is traceable to the national standards established by the National Metrology Institute of Japan (NMIJ). JAEA-Takasaki approves the uncertainty of the irradiation in the Reference Standard Radiation Field. In NFRI/NARO, ESR signals from a transfer dosimeter (alanine film dosimeter) irradiated at the Reference Standard Radiation Field are measured, and then the transfer standard calibration curve is created from the measured values.

Routine dosimeters (B3 and FWT-60 films) were calibrated by irradiating them simultaneously with the transfer alanine dosimeter and comparing the transfer standard dose and the response (ΔOD/mm) from the routine dosimeters. Electron accelerators at JAEA-Takasaki (for doses from 10 to 80 kGy) and the Nuclear Fuel Industry, Ltd. (NFI, from 1 to 10 kGy) were used for calibration of the routine B3 and FWT-60 film dosimeter. Details of the procedure and apparatus used for the dose calibration are described below. Numbers ① to ⑥ of the heading lines correspond to descriptions ① to ⑥ in Fig. 1.

3.3.1.1. Transfer dosimeter calibration (Alanine film dosimetry system)

① Calibration of the Reference Standard Radiation Field (Co-60 radiation field) of JAEA
The position of the dosimeter in the radiation field at JAEA is adjusted with high precision by an automatic jig. A plane-parallel ionization chamber is used as a reference dosimeter (uncertainty, ±1.0%) that is calibrated and traceable to the NMIJ primary standard. The reference ionization chamber measures the dose rate, and the phantom is irradiated at the same position as the reference dosimeter. The Co-60 irradiation field is calibrated in a range from 7 Gy h^{-1} to 200 Gy h^{-1}, and JAEA approves the uncertainty.

② Preparation of the transfer standard calibration curve
The alanine film dosimeter in a phantom is irradiated at the JAEA irradiation field (uncertainty ±3.0%) in increments of 5-dose points from 1 to 5 kGy and at the same dose increment from 10 to 80 kGy. The ESR signal from the alanine dosimeter (transfer dosimeter) is read out at NFRI, and a calibration curve is prepared using an ESR analyzing software (Fig. 2).

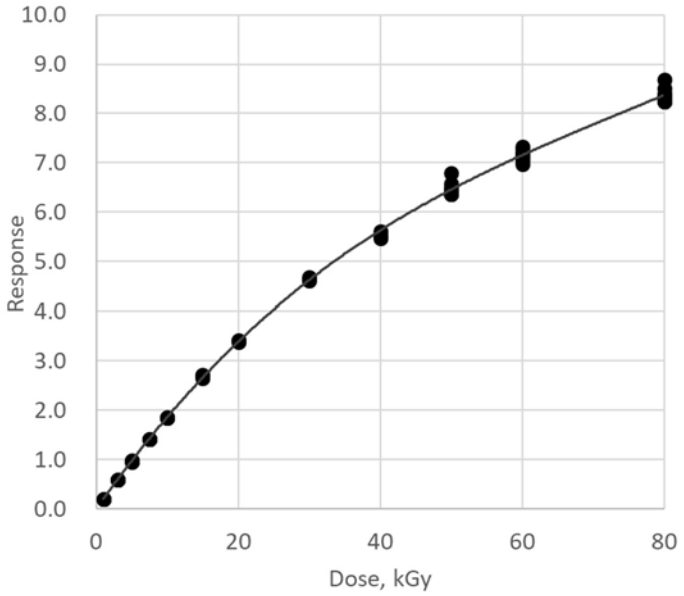

FIG. 2. The ESR signal response from the gamma irradiated alanine dosimeter.

3.3.1.2. Routine dosimeter calibration for electron beam irradiation (radiochromic film dosimetry system) (Fig. 3 and Fig. 4)

③ Electron beam irradiation of routine and transfer dosimeters
B3 and FWT-60 films (routine dosimeter) and the alanine film dosimeter (transfer dosimeter) are simultaneously irradiated by 10 MeV EB in an electron accelerator (NFI, Rhodotron). The phantoms and dosimeters are stacked at regular intervals and irradiated in increments of 5-dose

points from 1 to 5 kGy and 5-dose points from 10 to 80 kGy considering the uniformity of the dose.

④ **Reading of the routine dosimeter**
The optical density of the irradiated routine dosimeters is measured by a UV-Vis spectrophotometer, and the values of the specific net absorbance, ΔOD605 nm/mm or ΔOD550 nm/mm, are deduced from the thickness of the films.

⑤ **Transfer standard absorbed dose**
The absorbed doses are deduced from the EPR signal of the alanine dosimeter (transfer dosimeter) and the calibration curve created by procedure ②.

⑥ **Routine system calibration curve**
The calibration curve for the B3 and FWT-60 film routine dosimeter is created from the doses estimated by the alanine film transfer dosimeter and the ΔOD550nm/mm value for the irradiated B3 and FWT-60 films. Irradiation of product materials and the routine dosimeter is performed simultaneously, and the absorbed dose is estimated from the calibration curve. Extended uncertainty of the absorbed dose is determined with the uncertainties of the OD value, the thickness of the B3 and FWT-60 films, and curve fitting.

3.3.1.3. Intercomparison

The performance of the dosimetry system was evaluated by Intercomparison: The uncertainty associated with doses measured by our routine dosimeter (FWT-60) was calculated, and alanine dosimeters provided by Aerial (a secondary standards laboratory) were irradiated simultaneously and sent to Aerial, the accredited standard dose dosimetry laboratory, to determine variations between target dose and applied dose as well as between measured dose and applied dose (Table 1). The results were evaluated from Z-score, which was acceptable (Table 1).

TABLE 1. SUMMARY OF INTERCOMPARISON RESULTS

Target dose	**Applied dose**		**Measured dose**			**Z-score**
kGy	kGy* (Ananine: reference dosimeter)	Uncert. (*k*=2)	kGy** (FWT-60: routine dosimeter)	Uncert. (*k*=2)	% Deviation of routine dosimeter response	Dimensionless
1	1.02	0.03	1.04	0.08	-1.9%	-0.4
5	5.03	0.18	5.14	0.38	-2.2%	-0.5
10	10.00	0.36	10.10	0.75	-1.0%	-0.2

* 10-MeV EB irradiation by NFI, readout by Aerial
** readout by NFRI

Dose level (kGy)	Dose level average, D_{ave} (kGy)	Standard deviation (s)	Relative Variance (s^2/D_{ave})		Number of re (n)
1.0	1.06	0.030838432	9.2872E-07		3
3.1	3.14	0.107024476	0.001161849		5
5.2	5.16	0.053001041	0.000105501	U_{Re}=1.76	5
10.5	10.3	0.049221826	2.26295E-05	U_{CF}=1.58	5
15.7	16.0	0.064352894	1.61705E-05		5
21.1	20.9	0.321659805	0.000236341		5
32.2	32.2	0.205642385	4.07318E-05		5

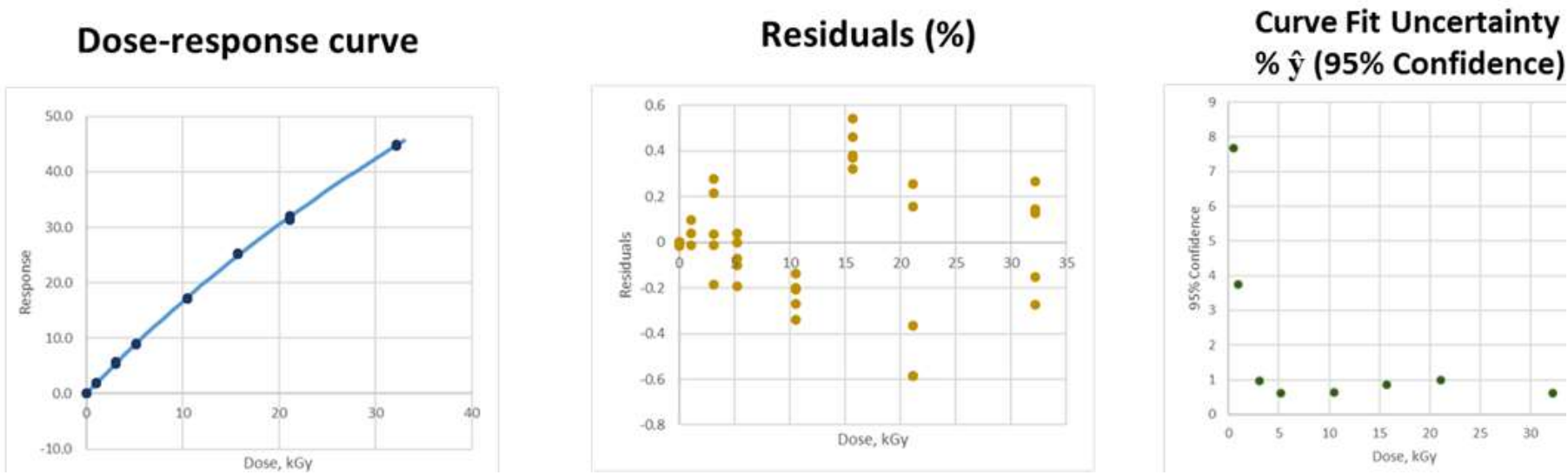

FIG. 3. Dose calibrations and estimates of the component of measurement repeatability of FWT-60 film (FWT-60 films were

Dose Target (kGy)	Routine dosimeter dose (kGy)	Transfer Standard dose (d_t)	Relative Variance (s^2/d_t^2)	Number of replicate (n)
1	1.03 1.05 1.06 0.94 1.09	1.04	0.002370	5
5	5.20 5.22 5.14 5.22 5.08 5.00	5.20	0.000363	6
10	9.90 10.0 10.0 10.0 10.0 9.9	10.4	0.000965	6

u_{CV}=3.2

u_{TM}=1.00 (Calibration verification thickness, Mitsutoyo thickness gauge)
u_{DOSE}=2.00 (uncertainty of irradiation field, JAEA and NFI)
u_{lab} (uncertainty of the transfer standard temperature correction), NA
u_{GP} (positioning or dose gradient), NA
u_{IN} was calculated from the formula:

$$u^2_{CV}=(\sqrt{(u^2_{Re}+u^2_{CF}+u^2_{TM})^2}+=(\sqrt{(u^2_{IN}+u^2_{Lab}+u^2_{GP})^2}$$

$$u_{ov}=\sqrt{\Sigma u_i^2}$$
$$=3.71\ (k=1)$$

$$U_{ex}=2(u_{ov})=7.4\,\%\ (\text{at } 2\sigma)$$

FIG 4. Calibration verification test results.

3.3.2. Spore inactivation by irradiation of low energy electron beam

Spores of *Bacillus pumilus* (ATCC27142 strain) were purified, serially diluted, and the cell-number-adjusted spore suspension was subjected to suction filtration with a membrane filter (Fig. 5). The spores, dispersed on the filter surface, were irradiated by EB of different energies (80, 150, and 250 keV). The *Bacillus pumilus* spores were irradiated under atmospheric conditions. Post irradiation, the surviving spores were cultured on tryptic soy agar medium for colony counting. The irradiation dose was monitored with an alanine film dosimeter.

A plot of the viable spore count versus the apparent dose measured by the alanine dosimeter is shown in Fig. 6. The spore inactivation curve shows a straight line, and there is a linear relationship between the dose and spore inactivation. These results mean that the EB machine worked as expected and that the electron beam was sprayed on the spores on filters uniformly and reproducibly. These results also indicate that the alanine dosimeter alone is not capable of providing the correct dose absorbed by the spores. In other words, the apparent dose (D_{app}) measured by alanine dosimeter was underestimated for low energy EB and K constant value (dose × conveying speed ÷ beam current) which is usually constant for each machine) varied energy-by-energy in this case (Fig. 7).

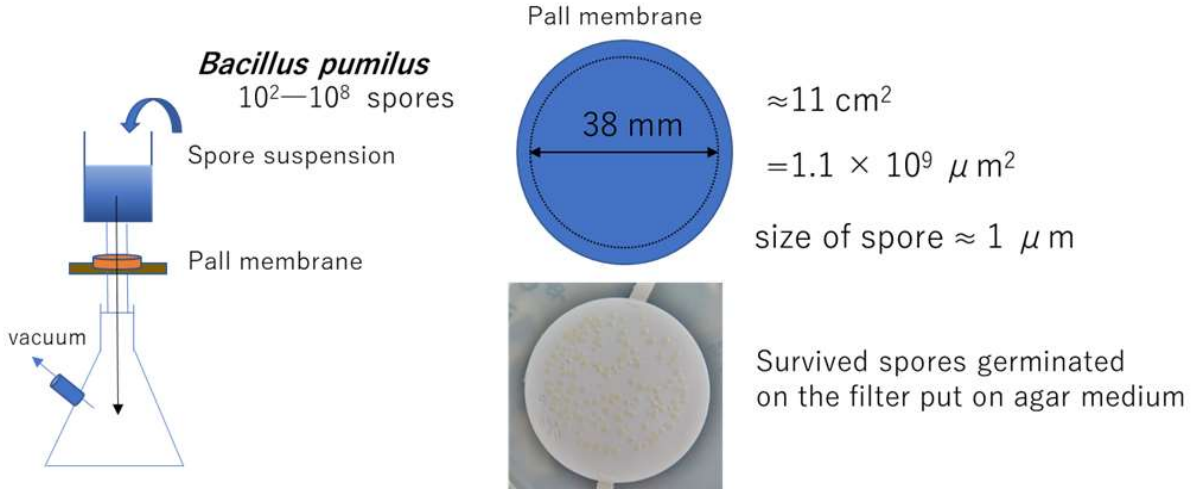

FIG. 5. Preparation of spore samples on the filter by suction filtration (left panel) and photo of colonies grown on the filter (right panel).

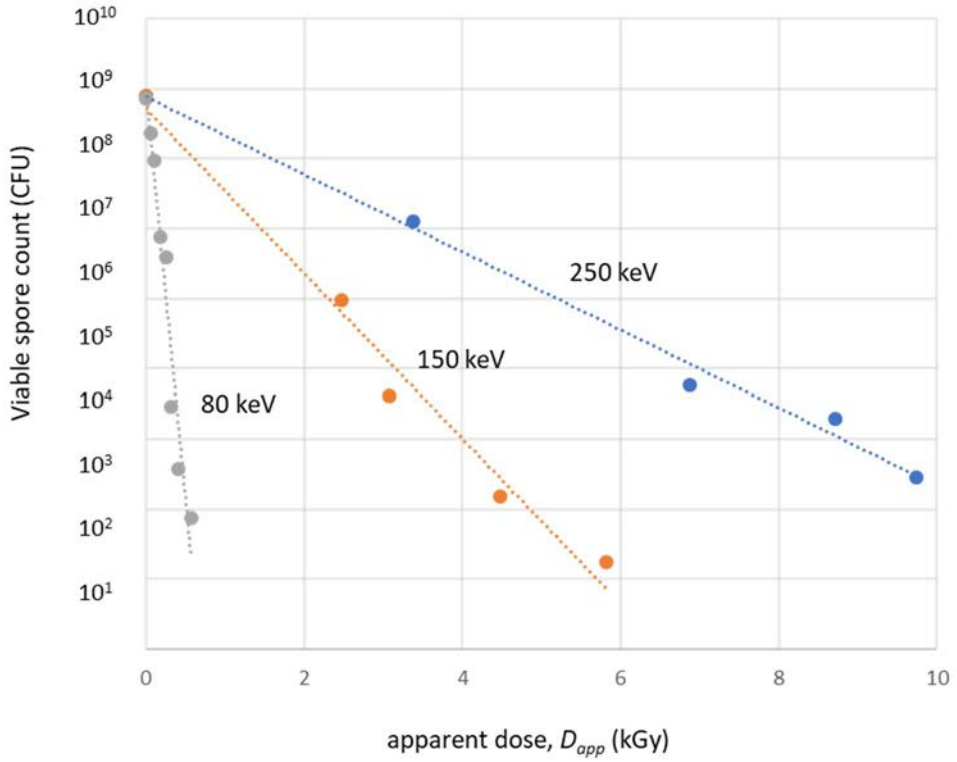

FIG. 6. The viable spore count and apparent dose (D_{app}) measured by alanine dosimeter.

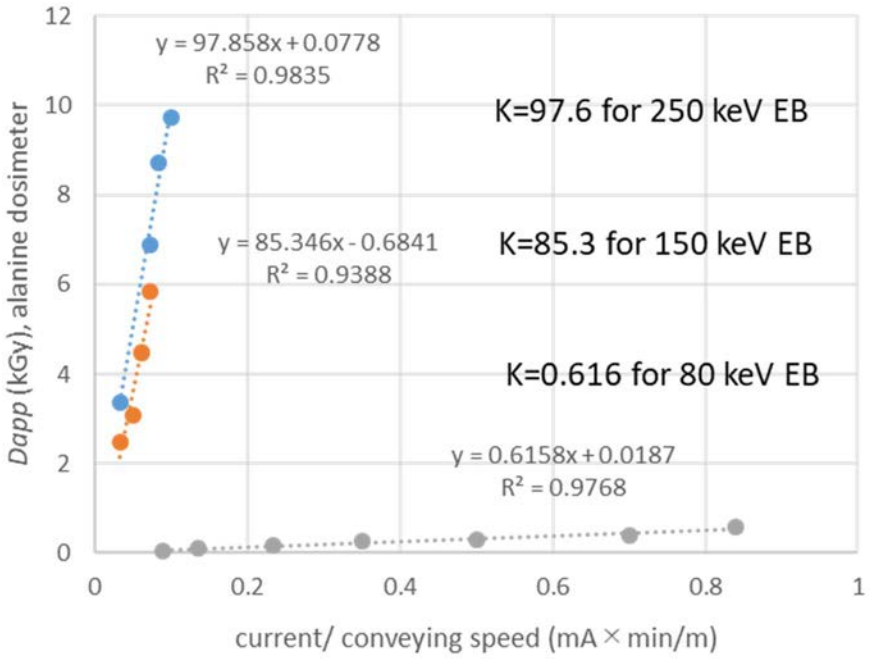

FIG. 7. K value observed for a low energy EB irradiator (EC 300/30/30 mA, Iwasaki Electric Co., Ltd.).

3.3.3. Depth-dose profile determination to estimate $D\mu$ for low energy EB dosimetry

The poor penetration ability of a low-energy EB makes a precise dose measurement difficult due to the dose gradient created across the thickness of the film dosimeter [5]. Apparent doses measured for low energy EB may differ dosimeter-by-dosimeter with different thicknesses. The $D\mu$ approach [5] was proposed to solve this problem.

J. Helt-Hansen *et al*. reported a method of calculating the top surface dose ($D\mu$) by estimating the dose distribution in the depth direction of a film dosimeter (depth-dose profile) [6]. Based on this report, we obtained the $D\mu$ value from the depth-dose distribution in the dosimeters by three-step data processing (Fig. 10 section 3.3.3.2.). In this process, the inverse operation of the dose-response curve of the dosimeter obtained in section 3.1.2 (Fig. 3) used to determine the apparent doses of the sub-layers, and D_{app} was deduced by summing the response of each 1-μm sub layer. $D\mu$ was determined from D_{app} and function of the depth-dose distribution as described [5].

3.3.3.1. Depth-Dose profile of the B3 film dosimeter

The depth-dose curve was obtained in collaboration with researchers at the Institute of Nuclear Chemistry and Technology in the Republic of Poland. As shown in Fig. 8, the laboratory in Poland sent us stacked B3 films. We irradiated them using our machine and sent them back to Poland. Irradiating films with a 150 keV EB caused colour development up to the 7th layer of stacked film, and doses in each film were able to be determined.

FIG. 8. Photo of the B3 films irradiated by 150-keV electron beam. The films shielded were shipped par avion (blank control film simultaneously shipped showed no colour development).

Fig. 9 shows the depth–dose curve. Titanium and the air gap were considered in the thickness (x-axis). The y-axis indicates the absorbed doses. A build-up of the dose was observed for the 250 keV EB. For 80 keV, only the first film developed colour. Probably, 80-keV electrons were unable to pass through the first B3 film. We determined $D\mu$ for the 150-keV electron beam as an example (next section).

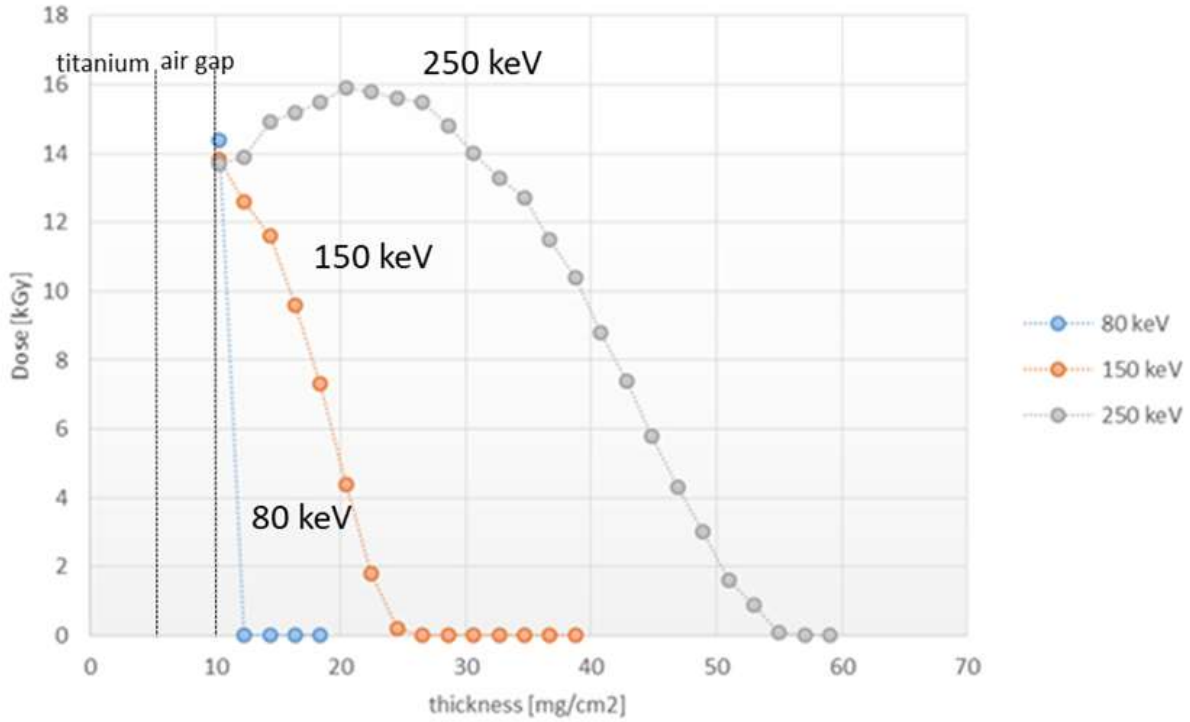

FIG. 9. Depth-dose profile in the stack of B3 films irradiated with low energy EB of energies 80, 150 and 250 keV.

3.3.3.2. Determination of Dμ

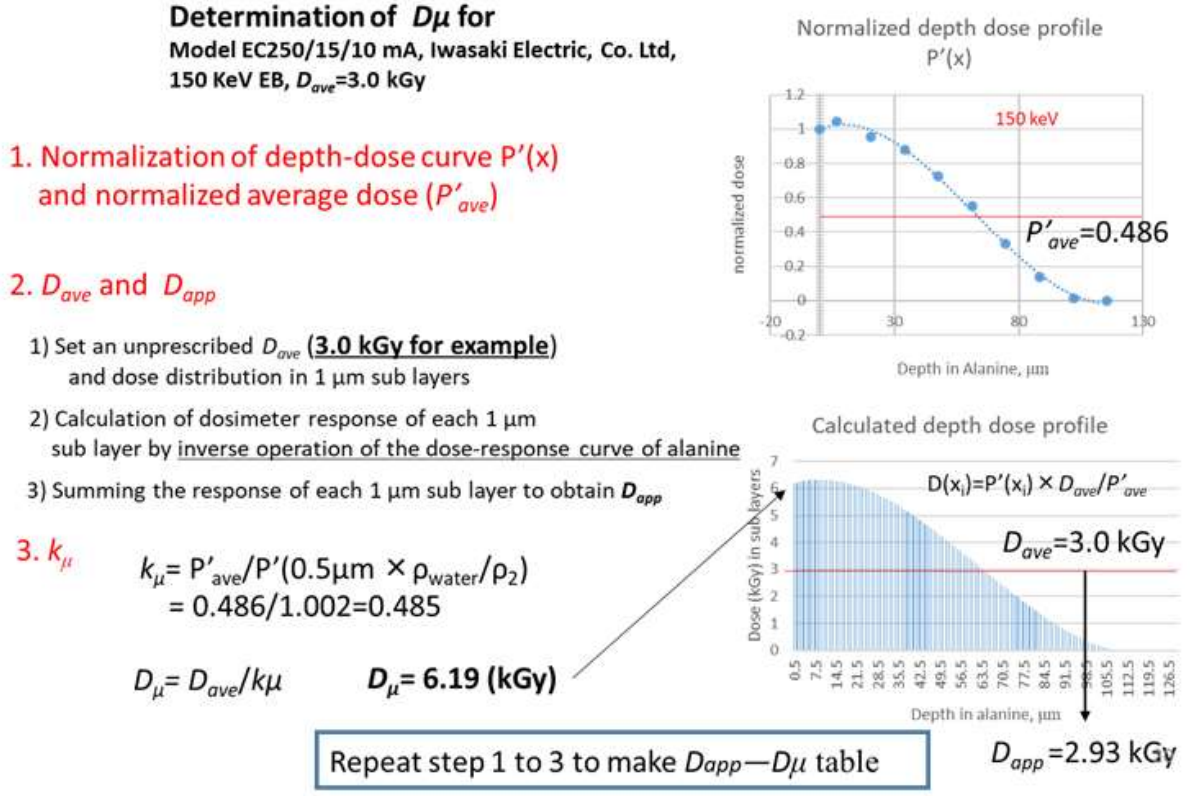

FIG. 10. Depth-dose profile and Dμ estimation for 150-keV EB with Electron Beam Accelerator (Model EC250/15/10 mA, Iwasaki Electric, Co. Ltd). Density of alanine dosimeter (ρ_2) is 1.482.

Fig. 10 shows how we determined the $D\mu$ for 150-keV EB with the Iwasaki machine. From the normalized polynomial of the depth-dose curve equation, the average dose, P_{ave}, was calculated to be 0.486. Next, we hypothesized an average dose of 3 kGy and determined the dose distribution in the 1-μm sub-layers. The doses in the sub-layers were converted to a dosimeter response based on the dosimeter's dose-response curve (Fig. 3), and the dosimeter response of the sub-layers was combined to give a total response of the dosimeter and the apparent dose. In this case, if the average dose was 3 kGy, the apparent dose was 2.93 kGy. Values of $k\mu$ and $D\mu$ were determined to be 0.485 and 6.19 hGy, according to ISO/ATSM 51818 [5]. The apparent dose-$D\mu$ correlation was tabulated by repeating steps 1 to 3.

3.3.3.3. The spore inactivation curve and the D_{10} *value*

The spore inactivation curve was plotted to the $D\mu$ values (Fig. 11), and the D_{10}, the dose that inactivates 90% of the spores, was determined to be 1.69 kGy. Tallentire reported a D_{10} value of 1.65 kGy for *B. pumilus* in the low-energy EB irradiation [6]. There was no significant difference between our results and the published data considering the uncertainty in dose measurements (7.4% for dosimetry system, Fig. 4) and colony counting.

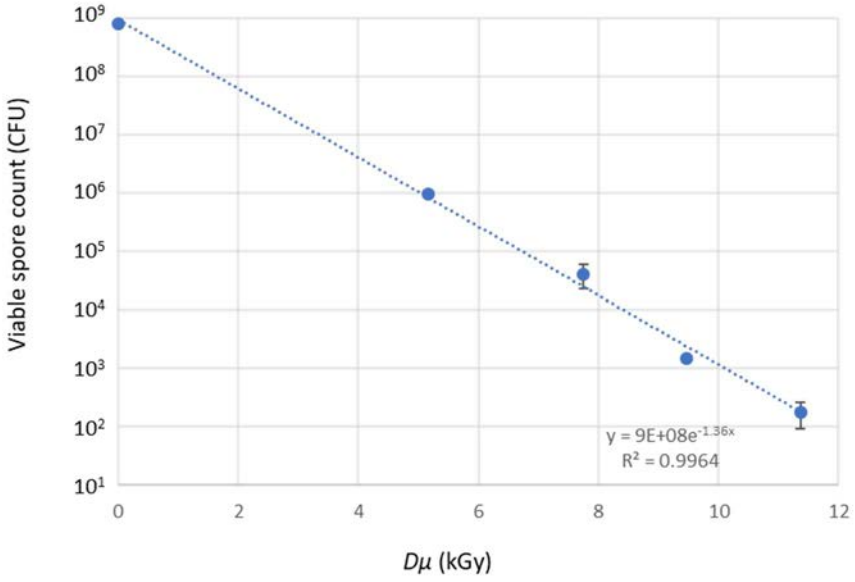

FIG. 11. B. pumilus spore inactivation by 150-keV EB.

3.3.4. Inactivation of bacteria on the surface of dry food by low-energy EB and species determination [4]

3.3.4.1. Irradiation

We irradiated real food materials by low energy EB (300 keV) to confirm the inactivation of bacteria on the surface and determine the bacteria species found in the materials with or without irradiation, in collaboration with researchers at the Institute of Nuclear Chemistry and Technology in the Republic of Poland. Black pepper, white pepper, and allspice were irradiated by EB in the Republic of Poland [4]. For microbiological analysis, the spice samples (Fig. 12) were irradiated using a low energy electron beam for 5 min, with a dose rate of about 1.3 $kGy \cdot min^{-1}$, determined as the average dose measured for the first layer of rolled B3 foil.

FIG. 12. The spice samples (left: black pepper, centre: white pepper, right: allspice) sent from Poland.

3.3.4.2. Microbial effectiveness

Fig. 13 shows the total aerobic bacteria counts for the samples before and after irradiation.

The control samples of black pepper and allspice had high counts of spore-forming bacteria, however white pepper had much less. We considered that these bacteria are spore form, because the significant viable count of all sample was still observed after heating them for 30 min at 80°C. After irradiation with low energy EB (300 keV), the logarithmic reduction of the bacteria number was more than 2 for black pepper and no colony was observed for the irradiated white pepper and allspice.

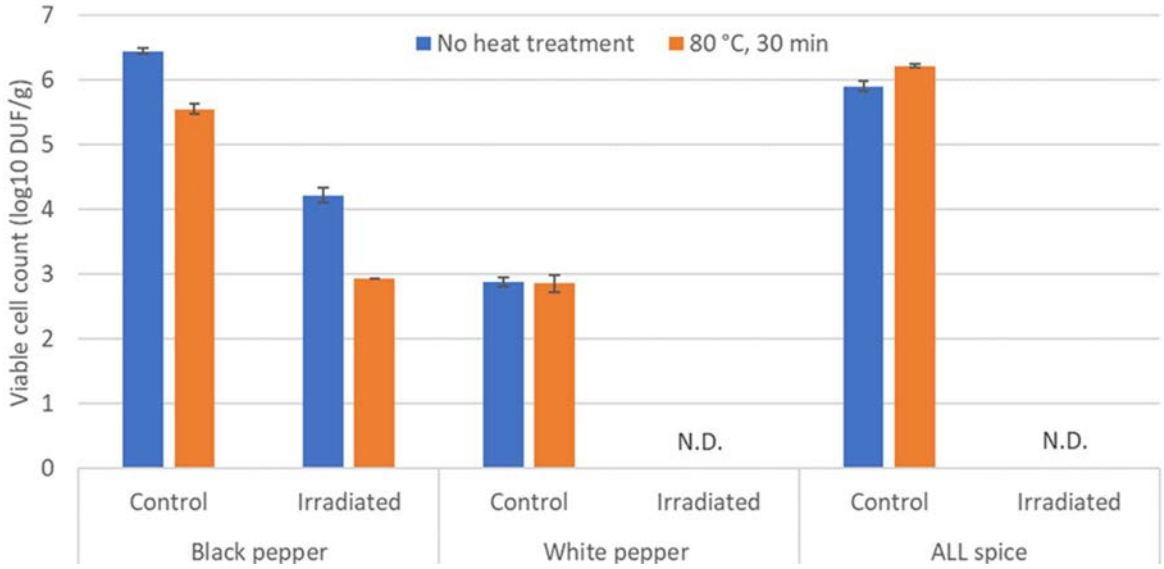

FIG. 13. Viable cell counts of the black pepper, white pepper, and allspice. N.D.: not detected (<0.5 × 10^2, CFU/g).

3.3.4.3. Microbial species contaminating spices

Fig. 14 shows the bacterial species adhering to control and irradiated samples analysed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectroscopy (MALDI Bio Typer, Bruker) [7] and a phylogenetic analysis of the 16S rDNA gene nucleotide sequence.

The microbial flora of the white pepper and allspice was dominated by spore or endospore-forming bacilli. Indeed, all tested colonies were identified as *Bacillus*, *Lysinobacillus*, *Paenibacillus*, *Rummeliibacillus*, and *Brevibacillus*. In the black pepper sample

twelve species of bacteria were found, and the most of these belong to the *Bacillus* spp. Characteristically, *Cronobacter Sakazakii* was confirmed to be in the control and irradiated black pepper as well. *C. sakazakii* has been reported to occur in dried food [8–9]. The D_{10} values of 0.82–1.95 kGy were reported for *C. sakazakii* with gamma-ray [10]. However, the D_{10} value of this bacterium was reported to exceed 4 kGy during drying in powdered milk formula for both gamma and electron beams [10–11].

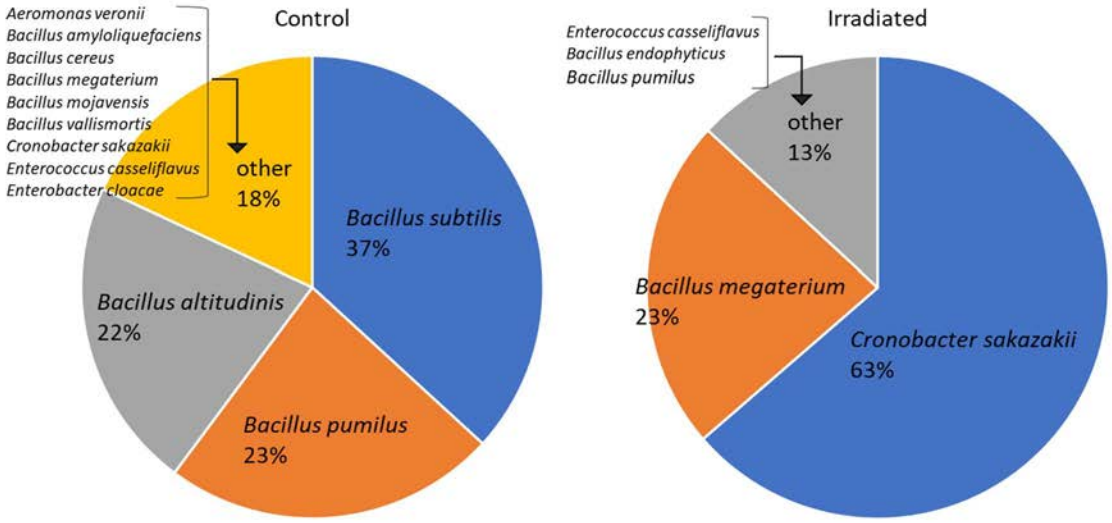

FIG. 14. The bacteria species found in black pepper. A total of 60 colonies were randomly picked up.

The black pepper was irradiated by high energy EB (10 MeV, 6.6 ± 0.4 kGy) and gamma-ray (5 kGy) from 60-Co, which resulted in reduction of the bacteria number (CFU/g) by approximately 3 log10. The survived species was mainly occupied by the *C. sakazakii* in both irradiations with high energy EB and gamma-ray [4], and unpublished results] as in the case of low energy EB irradiation [4].

3.3.5. Developmental works

During CRP-D61024 outreach activities were performed as listed below:

— 2015 December 9
Lecture and seminar, "Radioactivity and environmental measurements", Prof Abdel-Mjid NOURREDDINE (CNRS-Univeristé de Strasbourg), at NFRI/NARO and National Metrology Institute of Japan (NMIJ-AIST);

— 2016 January 18
Meeting for discussion at Nuclear Fuel Industry Ltd., (NFI), "Dosimetry of EB and facilities for it";

— 2016 October 14
New Practical Arrangements Between the FOA-IAEA Joint Division and the National Agriculture and Food Research Organization (NARO) of Japan, at Vienna, Austria;

— 2017 November 16
Lecture and seminar, “Phytosanitary Irradiation of Fresh Produces in the U.S.”, Dr Peter Follett (USDA-ARS), at Japanese Research Association for Food Irradiation;

— 2018 November 20
Lecture and seminar, "Global progress of food irradiation and perspectives", Dr Yves M. Henon (International Irradiation Association), in 17th Radiation Process Symposium of Japanese Research Association for Food Irradiation;

— 2020 January 22
Lecture, "IAEA Research Project about Low Energy Beam", Dr Setsuko Todoriki (National Agriculture and Food Research Organization), at annual meeting of Japanese Research Association for Food Irradiation.

3.4.CONCLUSIONS

An electron beam of energy of 300 keV or lower has the potential to decontaminate various types of food products. We established an *in situ* dosimetry system for the low-energy electron beam (EB) treatments. This involved simultaneous irradiation of the transfer dosimeter (alanine dosimeter, Kodak BioMax) and the routine dosimeter (film dosimeters, B3 GEX corporation) with 10 MeV EB and a calibration of the dose-response curve at NFRI. Additionally, to have reliable dosimetry methods for low-energy EB, we obtained a depth-dose profile of low energy EB (80 keV, 150 keV, 250 keV) with stacked B3 film dosimeters. The depth-dose profile was used to determine the $D\mu$ value, the dose at the uppermost surface. The radiation inactivation of *Bacillus pumilus* was used as a check on dosimetry. The *Bacillus pumilus* spore-inactivation curve fitted and adjusted by the $D\mu$ value gave a D_{10} value of 1.69 (kGy) for spore inactivation, which was in a good agreement 1.65 kGy reported in earlier studies [3].

We irradiated real food materials with low energy EB (300 keV) to confirm the inactivation of bacteria on the surface and determined the bacteria species detected in the materials with or without irradiation in collaboration with researchers at the Institute of Nuclear Chemistry and Technology in Poland. In these experiments, the microbial flora of spice samples were dominated by spore or endospore-forming bacilli. Spice samples were decontaminated by both the low energy (300 keV) and the high energy (10 MeV) electron beams, which suggests that the majority of bacteria that contaminated the spices were located on or near the surface of the spice grains. The log reduction of contaminated bacteria by the low energy EB (300 keV, 5 minutes irradiation) corresponded to a dose of approximately 6 kGy with the high energy EB (10 MeV) under the conditions employed. The presence of a relatively radiation-tolerant microbe (*Cronobacter sakazakii*) caused the lowest reduction of microbial contamination observed in black pepper samples. The *Cronobacter sakazakii* was resistant to radiation, regardless of the energy of the EB.

The advantage of low-energy EB spice irradiation, in addition to the treatment having least effect on the bulk food (the volume below the surface), is that low energy EB machines do not require thick shielding and can be used for in-line irradiation as part of a packing or production process in a factory. Using low-energy EB machines to irradiate food in a factory would eliminate the need to transport food products from the factory to a conventional irradiation facility. Despite its low penetration ability, the low-energy EB is an alternative technology to the currently employed highly penetrating ionizing radiation used to eliminate microbial contamination on spices.

ACKNOWLEDGEMENTS

We would like to thank Eye Electron Beam co., Ltd., Iwasaki Electric co., Ltd. and Nuclear Fuel Industries, Ltd. for technical assistance with the experiments. We also thank Dr Seito and Dr Kojima of Takasaki Advanced Radiation Research Institute for lending their expertise on the application of dosimetry system techniques.

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4. SURFACE TREATMENT OF FOOD BY LOW ENERGY ELECTRONS IN VIEW OF THEIR MICROBIAL DECONTAMINATION

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Warsaw, Republic of Poland

Abstract

New approaches for the radiation processing of food and agricultural products use the limited penetration of electrons of energy below 300 keV. The aim of the project was to examine the effectiveness of low energy electron beam (LEEB) irradiation to eliminate microbial contamination from the surfaces of food products. The energies of electrons used in our experiments allowed the beam to penetrate to a depth no greater than 400 μm in the food products that we studied. For tested samples the LEEB effectively eliminated microbial contamination, and the decrease in number of microorganisms depended on the energy of electron beam, the absorbed dose on the surface and the microbial species naturally inhabiting tested samples. This study involved some collaboration with the Food Research Institute, National Agriculture and Food Research Organization, Ibaraki, Japan.

4.1.INTRODUCTION

Spices are often contaminated with high levels of bacteria, molds and yeasts originating from microflora found in the plant's environment, namely soil, water and air. If untreated, the affected herbs and spices could cross contaminate food and result in spoilage of the products, or when contaminated with pathogenic organisms may also cause serious food-borne illnesses. Many processing techniques have been developed to control food spoilage and raise food safety. Traditional control methods such as fermentation, salting and drying have been supplemented with pasteurization (by heat), canning, freezing, refrigeration, chemical preservatives, pressure, microwave, ultrasonic and ionising radiation treatments. The sources of ionizing radiation approved for food irradiation listed in the Codex Alimentarius General Standard for Irradiated Foods are: (1) gamma rays from radionuclides ^{60}Co or ^{137}Cs; (2) X rays generated from machine sources operated at or below an energy level of 5 MeV and (3) electrons generated from machine sources operated at or below an energy level of 10 MeV [1].

A new approach to radiation processing of food and agricultural products uses the limited penetration of electrons having energy below 300 keV for dried food products like spices, nuts or cereals with surface microbial contamination. These kinds of products may be contaminated with adventitious pathogenic microorganisms, originating from post-harvest processing and if untreated, this contamination could pose a significant food safety risk when these ingredients are used to prepare foods such as ready-to-eat meals.

The aim of this work was to investigate the possibility of using low electron beam (LEEB) irradiation of spices in order to significantly reduce levels of microbial contamination.

4.2.MATERIALS AND METHODS

4.2.1. Materials

The dried spices used in the experiments were: black pepper, white pepper, allspice, dried onion flakes and bay leaves. All samples were naturally contaminated with microorganisms. The

spices selected for study are characterized by different densities and different internal structures, which would influence the range that electrons could penetrate into the product. The density was determined by immersion in ethanol (Archimedes principle) using laboratory scales set up for density determination. The structure of samples was observed using a digital microscope connected to a computer (USB microscope).

4.2.2. Irradiation

For irradiation with electrons having energy below 300 keV the accelerator ILU 6 was used. Accelerator ILU 6 is a resonant type machine which nominal operating range of the beam covers the energy range from 500 keV to 2 MeV. Lower electron energies were achieved by reducing the accelerating field strength. Modifications to the pulse power supply system, and the arrangement of the electron gun and beam sweep system of the ILU-6 accelerator enabled it to emit electron beams with energies below 300 keV [2]. For high energy electron beam irradiation, a 10 MeV accelerator (Elektronika 10-10) was used.

The LEEB irradiation of loose spice samples were performed in a rotating metal-basket cylinder, as shown in Fig. 1. The rotating drum has a diameter of 7 cm and a length of 8.5 cm, it was rotated at a constant speed of 150 rpm. This set up was used to present the different surfaces of the spices to the LEEB. It enabled different sized samples to be accommodated and the rotation during irradiation enabled the dose to be delivered and distributed more uniformly on the surface of each food item. The dose applied to the food sample was control by controlling the time duration of irradiation.

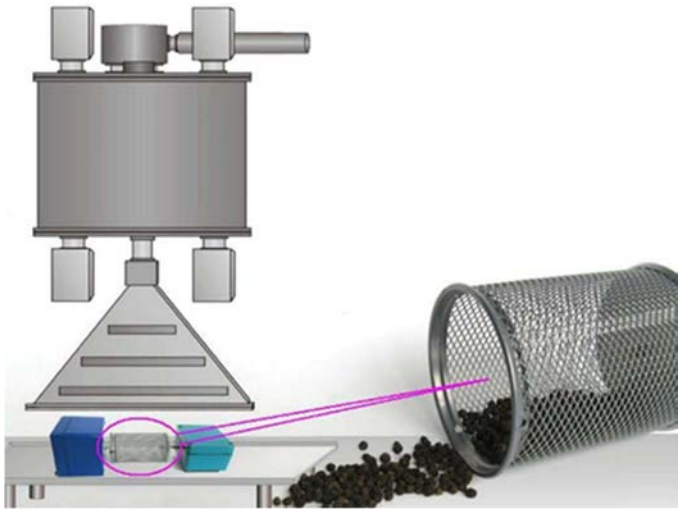


FIG. 1. Diagram of irradiation of loose materials in rotating drum using ILU-6 accelerator.

4.2.3. Dose measurements

Electron beams with an energy below 300 keV do not penetrate deeply into materials. For example, their penetration depth is comparable to the thickness of most conventional dosimeters. The dose gradients within dosimeters can result in significant differences in doses indicated by the dosimeter and the actual surface dose. Thus, the most appropriate dosimeters for low energy electron beam dose measurements are thin films or foils.

In the Institute of Nuclear Chemistry and Technology (INCT), B3 dosimetric foil (from the Riso High Dose Reference Laboratory, Denmark) was used for the dose measurements when irradiating with the LEEB. This dosimeter is a 18 μm thick film and can be used to measure

doses delivered by low energy electron irradiation by using a calibration function that has been established using high energy electron irradiation [3]. The degree of radiation induced colour change in the B3 films (dose readings) were measured using a flatbed scanner and RisoScan software.

Mode RTL software was used to simulate depth dose distributions.

4.2.4. EPR measurements

Continuous wave X-band electron paramagnetic resonance (EPR) measurements were carried out in thin-wall Suprasil tubes at room temperature 1hour after irradiation on a Bruker EMXplus spectrometer. A wide range of microwave powers and modulation amplitudes were tested in order to optimize the detection conditions. We applied a modulation frequency and amplitude of 100 kHz and 0.1 mT, respectively, and a microwave power of 1 mW. For absolute g value determination, a calibration using DPPH (diphenylpicrylhydrazyl) at 0.1 mW (g=2.0036) was performed. Spectral intensities were normalized against sample mass (in the range 10-50 mg).

4.2.5. Microbial analysis

Food samples were tested to determine the reduction in total counts of aerobic bacteria after irradiation with either low or high energy electron beams. After irradiation, 10 g of each spice sample was taken and sealed in a sterile plastic bag containing 90 ml of Ringer solution and homogenized in a stomacher. A series of sequential dilutions were prepared from the homogenized suspension. These dilutions were used to reduce a dense culture of cells to a more usable concentration. Casein-Peptone Dextrose Yeast Agar was used as the culture medium and bacterial growth was observed after 72 hours of incubation at 30°C. The total counts of colony forming units (CFU) of aerobic bacteria were transformed into log10 values (log_{10}CFU) and presented as average from two replicates. MALDI-TOF mass spectroscopy (MALDI Bio Typer, Bruker) was used for species determination in naturally contaminated spices before and after irradiation. Microflora determination and analyses were performed at the National Food Research Institute (NFRI), Japan.

4.3.RESULTS AND DISCUSSION

4.3.1. Characteristic of low energy electron beam

Low energy electron beam penetration ability is influenced by thickness of Ti foil in the accelerator window, the air gap distance between the accelerator window and the irradiated samples or any other layer which can absorb the energy of the beam before it reaches its intended target. Therefore, the actual penetration depth of the electron beam depends on the conditions under which the irradiation took place. For a specific irradiation condition, the penetration depth characteristics can be determined either using mathematical modelling or experimentally by irradiating stacks of thin dosimetric foil.

Mathematical modelling is one of the tools that can be useful in characterization of penetration ability of LEEB. Fig. 2 shows simulated and measured (B3 film) depth-dose curves for a 150 keV electron beam. As shown in Fig. 2. for 150 keV electron beam at the National Food Research Institute, NFRI, Japan (Accelerator type: Model EC/15/250 mA, Ser No. LB4036, Iwasaki Co, Ltd, Japan), the depth dose curve measured using stacks of B3 dosimetric foil, can be obtained and the results of depth-dose simulations can be generated assuming the spread of the electrons kinetic energy withing the beam, in this case an energy spread of 12 percent gives a good fit to the actual measurements using B3 film.

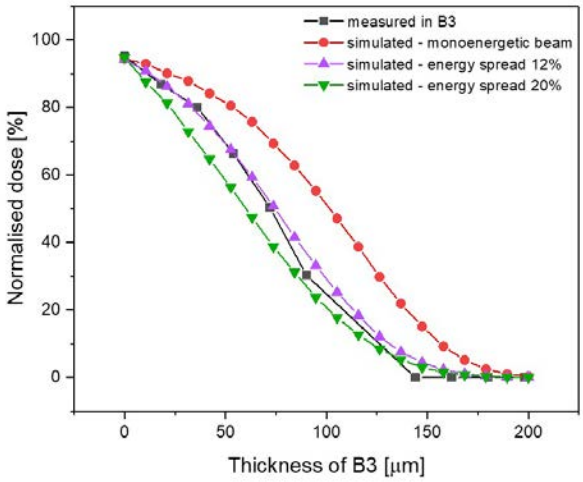


FIG. 2. Measured and simulated depth dose curves for 150 keV electron beam in B3 dosimetric foil.

In the INCT the ILU-6 accelerator was used for irradiation experiments, it has the built-in instrument for indicating the accelerating voltage (kinetic energy of the beam). The actual energy of the electron beam that the material is exposed to, was lower than the instrument read-out because of absorption in of the accelerator extraction window (50 μm Ti foil), and in the air gap between the window and the irradiated samples (approximately 10 cm). The ability of the low energy electron beam to penetrate material can be visualized by the darkening of PVC film when it is irradiated, the darker the colour the higher the imparted dose. The depth of the "irradiation layer" can be observed directly in a cross-section of irradiated PVC film (density of 1.4 mg/cm^3) as shown in Fig. 3.

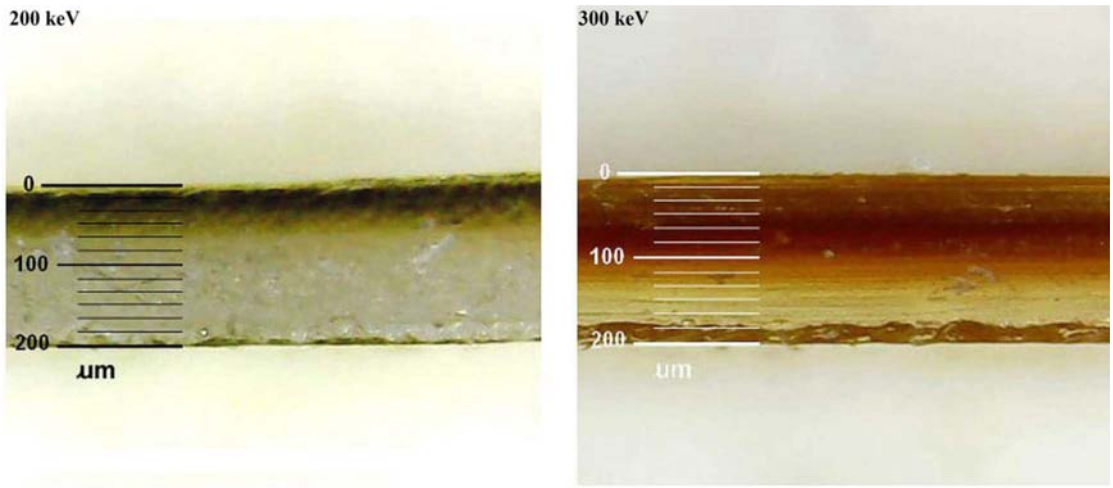


FIG. 3. Visualization of the irradiated layer thickness, observed as darkening in a photograph of the cross-section of PVC film that had been exposed to the electron beam.

Under the experimental conditions, a significant amount of energy was absorbed on the surface of rotating drum during spice sample irradiation, as shown in Fig. 4. Depth doses curves were measured for 200 and 300 keV LEEB irradiation both inside and outside of the drum, determined at the same distance from accelerator window.

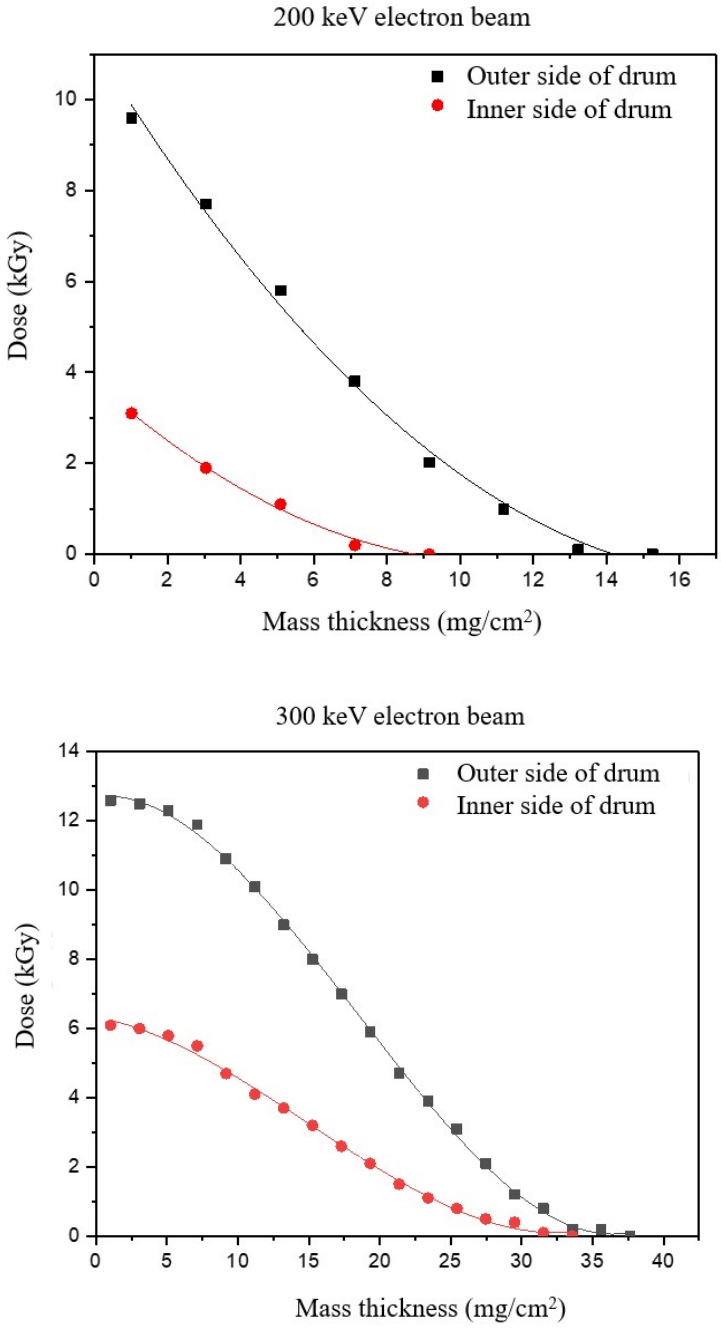


FIG. 4. Depth dose curves of 200 and 300 keV electron beam measured using B3 dosimetric foil.

The significant parameter influencing the LEEB depth dose curve is the geometry of the irradiated samples. In Fig. 5. the depth dose curves determined for “flat” (a flat stack of B3 films) and “round” (a cylinder of rolled B3 films) samples irradiated in the rotating drum.

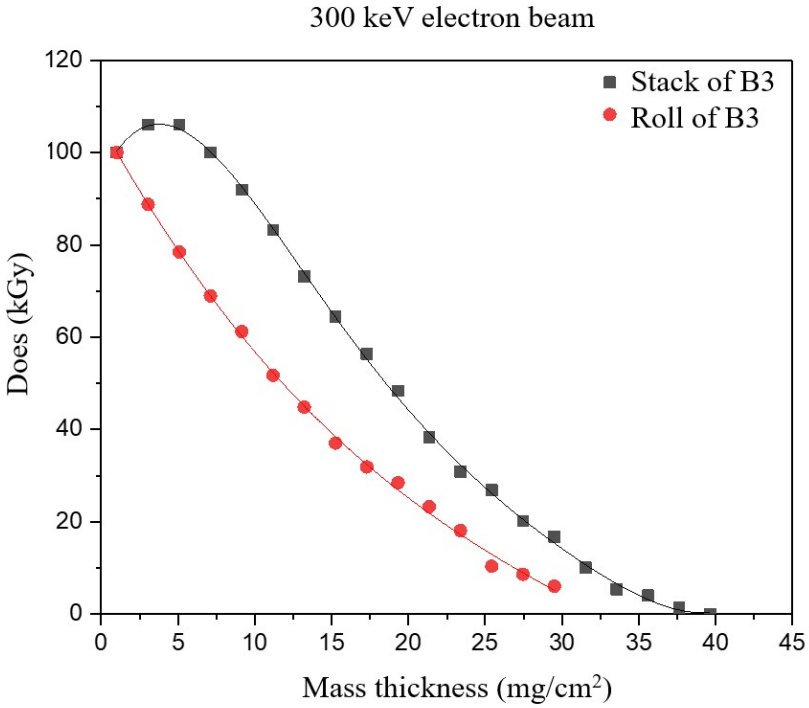


FIG. 5. Depth dose distribution of low energy electron beam determined for flat and round samples under the experimental conditions.

4.3.2. Range of low energy electron beam in food products

The penetration ability of LEEB in food products cannot be measured using traditional dosimetric systems. It can be simulated using mathematical modelling or estimated based on depth dose profiles obtained using film dosimeters. Also, a number of different analytical methods have been tested as methods to directly estimate the depth of low energy electron beam penetration in food products, for example based on viscosity [4] or Electron Paramagnetic Resonance spectroscopy (EPR).

Depending on the density and structure of irradiated food products LEEB can penetrate different depths of food products. Food samples that differed in structure were selected for the experiments and these were dried bay leaves, dried onion flakes, whole white pepper corns and whole black pepper corns and allspice as shown in Fig. 6.

Dried bay leaves and dried onion flakes are homogenous but have different average densities, 0.7 and 1.2 g/cm^3 respectively. Black pepper corns, white pepper corns and allspice grains have a heterogeneous structure, which can be observed in the cross section of the grain (Fig. 6). In tested grains two main layers were observed. The external layer characterized with density of about 0.9 g/cm^3 was from 1 mm in allspice, 200 to 500 μm thick in the black pepper while in the case of the white pepper it was less than 100 μm thick. The internal layer of grains was characterized by density of about 1.5 g/cm^3.

FIG. 6. Microscopic images of the structures of analysed spices.

Mode RTL software was used to simulate depth dose distribution in food samples irradiated with electron beam of energy 200 and 300 keV as show in Fig. 7. and Fig. 8.

According to simulated depth dose distributions electron beam of both tested energies (200 and 300 keV) could penetrate the entire thickness of only the least dense bay leaves. For other tested food samples, the energies of the beam were sufficient to penetrate only limited thickness of the product. For heterogeneous structure products such as pepper and allspice samples it was observed that a 200 keV beam can penetrate the surface layer of not more than 200 μm thick. This means that a 200 keV electron beam will be almost completely absorbed in the external layer of black and white peppercorns. Electron beams with an energy 300 keV can penetrate the external layer of white pepper but will absorbed in its inner part.

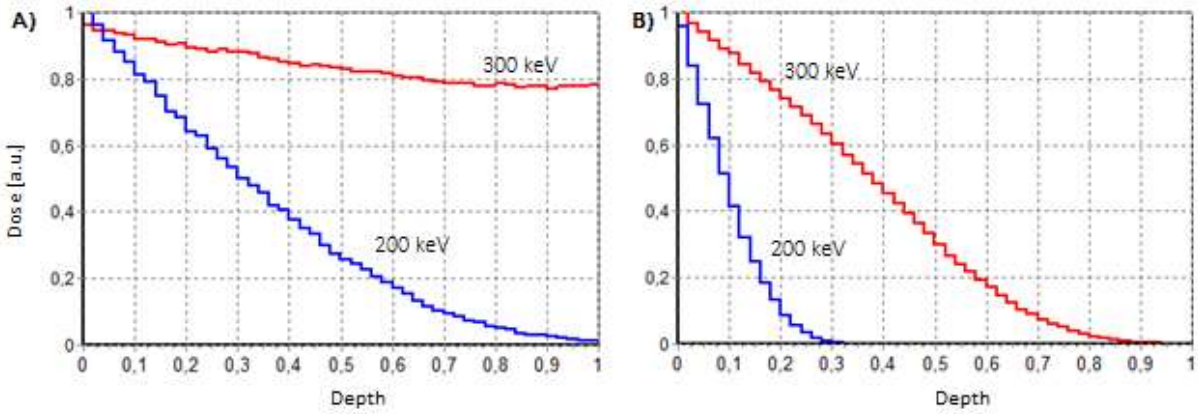


FIG. 7. Simulated depth dose distribution in A) bay leaves (x: 1 = 0,025cm; y: 1 = 5 kGy) and B) dried onion flakes (x: 1 = 0,038 cm; y: 1 = 5 kGy) irradiated with electrons having energy 300 keV and 200 keV.

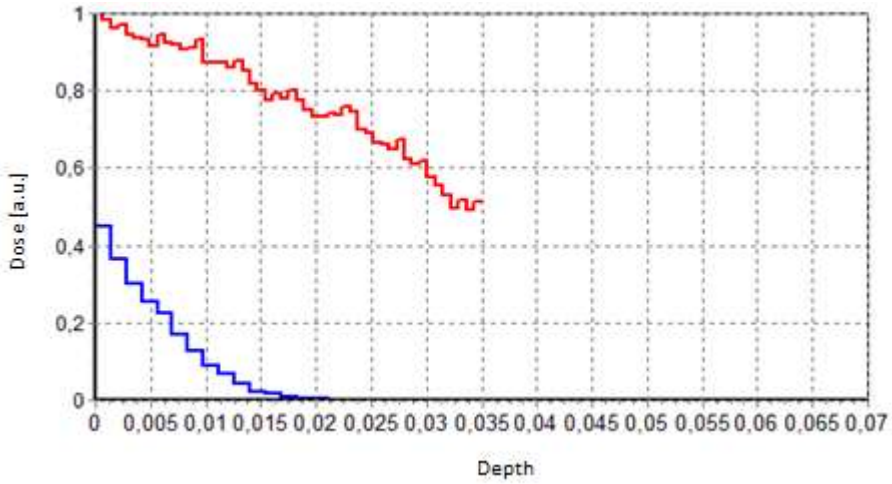


FIG. 8. Simulated depth dose distribution in surface layer of pepper and allspice grains irradiated with electrons having energy 300 keV (red line) and 200 keV (blue line) (x: cm; y: 1 = 5 kGy).

To confirm the results obtained from mathematical modelling, experiments using EPR spectroscopy were performed. Ionizing radiation creates radicals and some of these are stable in treated foods. Therefore, EPR spectrometry can be applied to evaluate the number of stable radicals in a sample and this can be used to give information on the penetration of electrons in food samples. Stable radicals can be created under influence of ionizing radiation especially in foods containing cellulose or crystalline sugars and the EPR signal depends on the composition of irradiated sample [5].

The EPR signal of irradiated onion was unstable at room temperature at it was not registered. The registered EPR signal of bay leaves (Fig. 9) was a low intensity singlet, the intensity of the EPR spectrum increased with increasing electron beam energy.

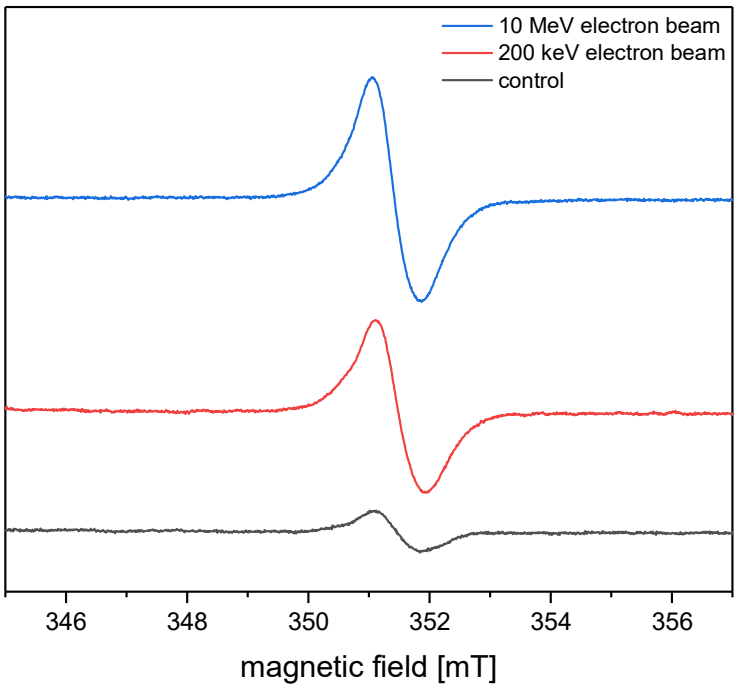
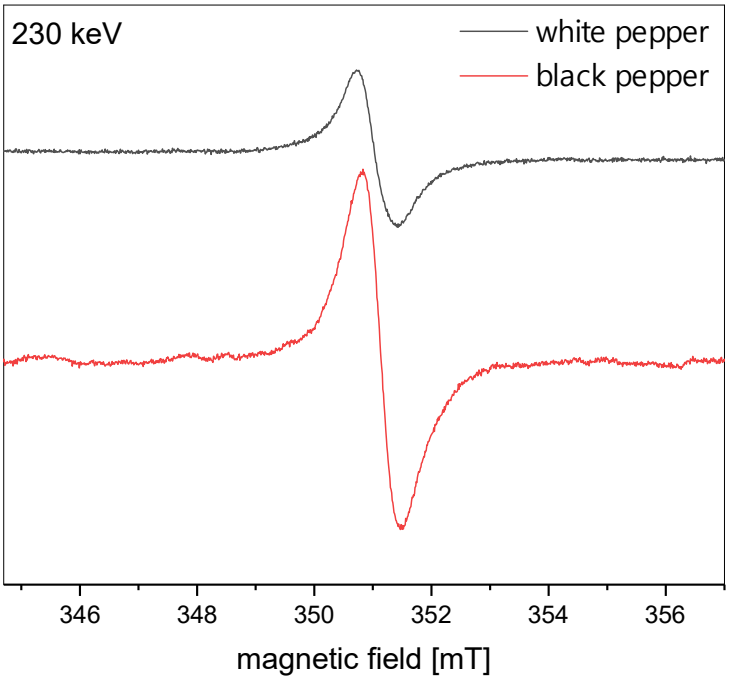


FIG. 9. EPR spectra of bay leaves irradiated with low and high enegy electron beam.

For pepper grains low intensity singlet was registered in unirradiated samples. The signal intensity increases for samples irradiated with both 230 and 300 keV electron beam, as shown in Fig. 10.

For samples irradiated with 300 keV the increase in signal intensity was observed for both white and black pepper. Whereas the EPR signal recorded for white and black pepper was the same when irradiating using electrons of energy 230 keV, the differences were observed when irradiated with 300 keV electron beam. The shape of the spectrum recorded for black pepper remains the same but additional signal was observed for white pepper samples, as shown in Fig. 10. The layers in grains are different in structure and also in composition. The external layer contains mostly dietary fibers while the inner part is composed mostly of starch, which caused that upon irradiation additional signal can be observed due to presence of radicals induced in inner part of pepper grain.



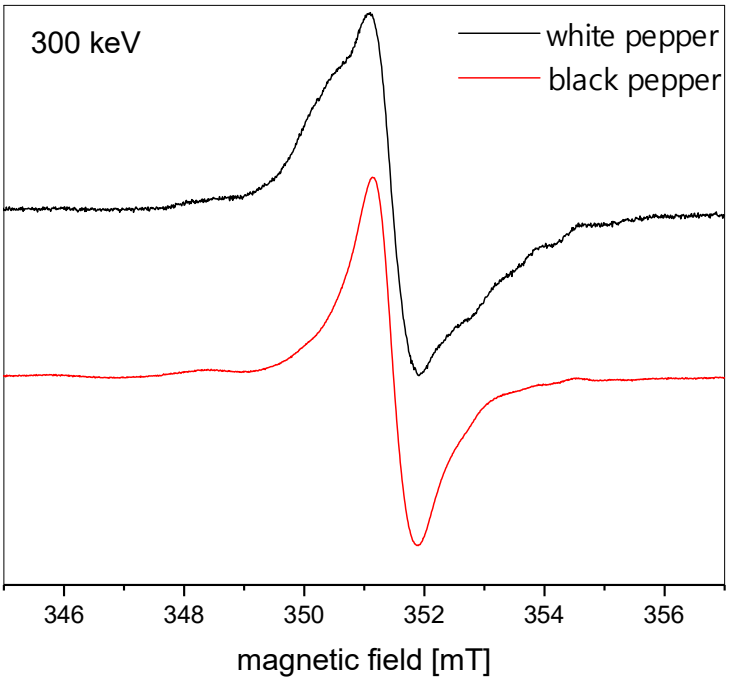


FIG. 10. EPR spectra of black and white peppercorns irradiated with 230 and 300 keV electron beams.

4.3.3. Microbial effectiveness

The effectiveness of the LEEB microbial decontamination process was tested on selected food samples irradiated with the low and high energy electron beams. The results of the microbiological analysis for samples irradiated with high energy (10 MeV) electrons are presented in Table 1.

TABLE 1. NUMBER OF AEROBIC BACTERIA IN FOOD SAMPLES IRRADIATED BY HIGH ENERGY ELECTRON BEAMS

	Total aerobic plate counts (log_{10}CFU/g)		
Dose* (kGy)	Black peppercorns	Dried onion flakes	Dried bay leaves
0 (Control)	**6.21±0.13**	**5.51±0.05**	**5.60±0.08**
2.5	**5.43±0.18**	**4.16±0.06**	**2.40±0.27**
5	**4.66±0.05**	**3.09±0.04**	**<10**
10	**2.82±0.03**	**<10**	**<1**

* Imparted by irradiation with a 10 MeV electron beam.

The effectiveness of low energy electron beam irradiation at reducing levels of microbial contamination can be assessed by comparison to results obtained for microbial decontamination with high energy electron beams. For onion flakes and bay leaves, low energy electron beam irradiation for a period of 6 minutes was observed to be comparable to microbial reduction with high energy (10 MeV) electron beam irradiation at treatment doses of 1 and 2 kGy respectively. With both low and high energy beam processes the most effective microbial reduction was observed for irradiated bay leaves and the lowest for black pepper [6]. This is probably due to differences in the microbiological flora associated with these products. The sources of microbiological contamination of spices are air, soil as well as the production process. Foods were not inoculated with microorganisms in the laboratory, the experiments investigated samples as received at the laboratory, which means that the different foods were contaminated with different microorganisms that had different radiosensitivities. Most often, the spice samples were found to be contaminated with *Bacillus* and *Clostridium* species as well as moulds and fungi. Pathogenic bacteria such as *Salmonella* species, *Shigella*, *Escherichia coli* and *Staphylococcus aureus* can also occasionally contaminate food products of plant origin. A product contaminated with higher concentrations of more radiation resistant microorganisms would need higher irradiation doses to reduce the total microbiological load than another product contaminated with lower concentrations of radiation sensitive microorganisms.

For the spice samples tested in experiments, the microbial flora of black pepper sample was found to be more diverse than other tested spices and was dominated by spore forming *Bacillus* sp. (in total 93.2%), and non-spore forming species such as *Aeromonas veronii*, *Cronobacter sakazakii*, and *Enterobacter cloacae* were also identified (Table 2). To compare the decontamination of black pepper with both low and high energy electron beam microbial flora of irradiated samples was analysed. It was revealed that *C. sakazakii* was the most abundant species that survived the low energy e-beam irradiation (Table 2). Comparing the total viable count and the fraction identified as *C. sakazakii* before and after the irradiation, it was implied that the viability of *C. sakazakii* was not affected by the irradiation under the conditions employed in this study [7]. The D_{10} values reported for *E. sakazakii* in powdered weaning food irradiated with e-beam was 4.83 kGy [8]. The black pepper sample exposed to high energy e-beam irradiation confirm the resistance of *C. sakazakii* against ionizing irradiation.

TABLE 2. DOMINANT BACTERIA SPECIES FOUND IN BLACK PEPPER IRRADIATED BY LOW ENERGY ELECTRON BEAM (300 keV, 5 minutes)

Unirradiated black peppercorns (control samples)	%*	Irradiated black peppercorns	%
Bacillus subtilis	36.7	*Cronobacter sakazakii*	63.3
Bacillus pumilus	23.3	*Bacillus megaterium*	23.3
Bacillus altitudinis	21.7	*Bacillus pumilus*	6.7
Bacillus vallismortis	5.0	*Bacillus endophyticus*	3.3
Aeromonas veronii	1.7	*Enterococcus casseliflavus*	3.3
Bacillus amyloliquefaciens	1.7	–	–
Bacillus cereus	1.7	–	–
Bacillus megaterium	1.7	–	–
Bacillus mojavensis	1.7	–	–
Cronobacter sakazakii	1.7	–	–
Enterobacter cloacae	1.7	–	–
Enterococcus casseliflavus	1.7	–	–

*A total of 60 colonies were selected at random. Note that the sum of percentage values may not add to 100 due to the values being rounded to one decimal place.

The black peppercorn samples were analysed to compare the effectiveness of microbial decontamination process when treated with electron beam having different energies (200, 230 and 300keV) and high energy (9 MeV) electron beam irradiation. The samples were irradiated with the same surface dose determined as the average dose measured from the one layer of B3 dosimetric foil. The higher the energy of the beam, the more effective was the decontamination (Fig. 11). This suggests that microorganisms were present throughout the black peppercorns and not only at the surface and near surface layers. Microorganisms were most likely inhabiting layers deep from the surface of the peppercorn and the lower energy electrons could not reach some of these microbes because they were unable to fully penetrate to that depth [9].

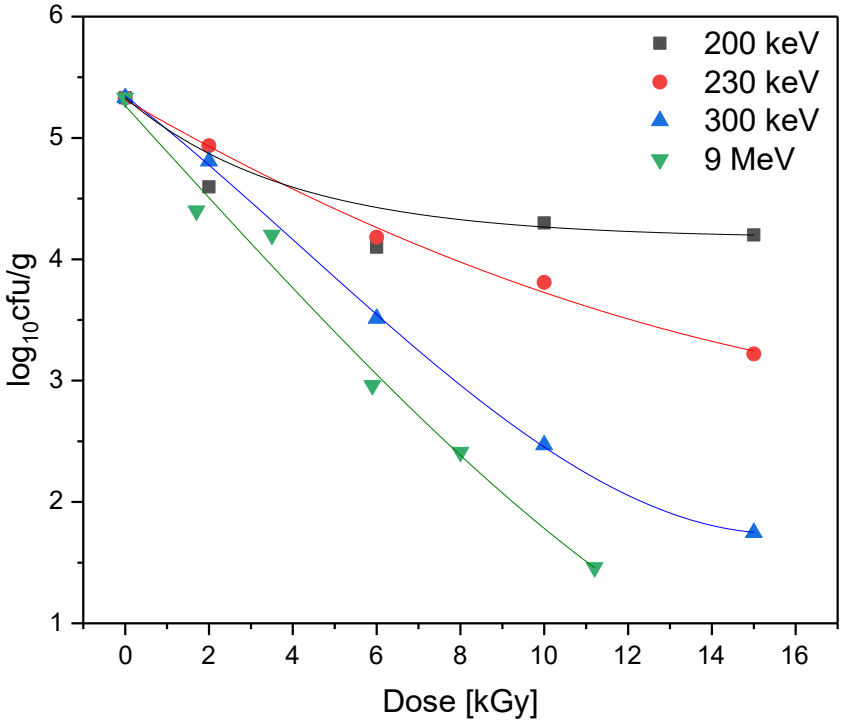


FIG. 11. Effectiveness of microbial decontamination of black peppercorns with different energy electron beam irradiation treatments.

4.4.CONCLUSION

Electron beams with energies of up to 300 keV can be used to eliminate viable microbes present in a range of different dried foods. The effectiveness of low energy electron beams to eliminate microbial contamination was shown by comparing the efficiency of low energy beam treatments to conventional high energy electron beam irradiation. To achieve high efficiency of microbial decontamination with low energy electrons, the process must be controlled to ensure that a uniform dose is delivered to the surface of food samples and that the energy of the electron beam is sufficient to penetrate to an appropriate depth and reach the organisms that may contaminate layers below the food surface.

The use of electron beams with energies of up to 300 keV as food treatments can be an alternative to conventional gamma irradiation using ^{60}Co sources and high energy electron beam irradiation. The advantage of such a solution is that low energy electron beam machines do not require extensive shielding (e.g. thick concrete walls) to attenuate radiation and can be installed as part of a production or food packing line in a factory.

ACKNOWLEDGEMENTS

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5. EFFECT OF ELECTRON BEAM IRRADIATION ON PRESERVATION OF MANGO

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Abstract

Our research investigated electron beam irradiation of mango fruits. The effects of irradiation on nutritional content, appearance and preservation were studied. Mango samples were irradiated to doses of 0.5 kGy, 1.0 kGy, 1.5 kGy and 2.0 kGy. The rate of fruit browning, decay (rot rate), weight loss and the impact on various nutritional components were evaluated during storage at room temperature. Experimental results showed that there were no significant effects of irradiation on the hardness, soluble solids, ascorbic acid content, total acid and reducing sugar content as compared with the control group of unirradiated mango samples. However, the appearance, browning rate and rot rate was altered to some extent by irradiation. Weight loss and mango colour also changed to some extent after irradiation. Our results indicate that with low dose treatments (0.5 and 1.0 kGy) the appearance of the fruit was improved, and the rot rate and weight loss reduced, fruit ripening was inhibited, and its colour and brightness were also be maintained. However, higher radiation dose treatments (1.5 kGy and 2.0 kGy) were found to accelerate fruit browning and rotting and lower the appearance quality, especially at the highest radiation dose studied (2.0 kGy). In conclusion, the optimal radiation treatment dose for mango preservation was found to be in the range 0.5–1.0 kGy.

5.1.INTRODUCTION

Mango is a subtropical fruit rich in sugars, proteins, crude fibers, vitamins, phenols, and a small amount of calcium, iron, phosphorus, and other minerals. According to some research, it also has anti-cancer properties, preventing hypertension, atherosclerosis, constipation and other effects [1, 2] with long-term consumption. Mango is a respiration climacteric fruit, and it becomes yellow, soft, and perishable due to vigorous metabolism after harvest. Mango fruits do not store well at ambient temperatures and thus fresh mangoes are highly prized for eating and of significant economic value, the value of the fruits decrease greatly with storage [3]. Therefore, postharvest technologies that can improve storage and preserve mango fruits, to suppress the browning and rot rate of the product, maintain the fruits quality, and extend its shelf life are important.

At present, the preservation of fruits and vegetables mainly relies on technologies such as low-temperatures, film coatings, modified atmosphere packaging, and chemical additives as physical, chemical, or biological methods to inhibit the growth of microorganisms, slow the respiration rates of fruits and vegetables and generally extend shelf life. However, the above methods have a number of disadvantages in that they require expensive technical equipment, have a high energy consumption, are not always the most practicable, may leave toxic residues, are not work efficient and make it difficult to industrialize commercial production. It is necessary to develop preservation technologies for fruits and vegetables that are safer, more environmentally friendly, low carbon and energy saving as well as convenient to operate and that can be easily incorporated into industrial production [4].

Electron beam irradiation is a new processing technology that uses an accelerator to produce a beam of electrons to treat foods or other products. Electron beam irradiation can be used as a preservation technology to control microorganisms and storage pests, delay senescence and inhibiting germination [5]. Gamma rays emitted by ^{60}Co radio isotope sources can also be used to irradiated foods. Compared with gamma irradiation, electron beams are generated by machines and have the advantages of delivering uniform radiation dose, high beam utilization rate, short processing time, high work efficiency, strong controllability, and avoid issues related to radioactive sources [6, 7]. As electron accelerator technology has improved, electron beam irradiation offers considerable advantages in terms of processing capacity and economic benefits. New electron beam facilities are becoming more popular for commercial scale radiation processing [8].

Many researchers have produced reports on the application of electron beams for the storage and preservation of fruits and vegetables. Chen Zhijun [9] found that the colour change of stored grapes after 2.1 kGy irradiation was less than unirradiated grapes. Zhou Jiaren [10] reported that the browning of fresh-cut cantaloupe (sweet melon) could be delayed by electron beam irradiation. Zhou Huijuan [11] also found that electron beam irradiation with 0.5–1.0 kGy could reduce the loss of titratable acid, soluble solids and vitamin C content of kiwifruit. There are very few published studies on the application of electron beam irradiation to the preservation of mango. The main purpose of our research is to study the effects of electron beam irradiation on the nutritional components and the appearance of Xiaotainong mango after exposure to different doses of electron beam radiation, and to select a suitable irradiation dose for mango preservation.

5.2.MATERIALS AND METHODS

5.2.1. Materials and instruments

Materials: Xiaotainong mango, purchased from Wal-Mart Supermarket. Fruits with uniform size, consistent maturity (level eight maturity), no mechanical damage, and no pests.

Irradiator: industrial electron accelerator (2 MeV, 20 kW).

5.2.2. Experimental methods

Mango fruits were purchased from a supermarket. Fruits were selected, and packed in the laboratory, then treated by electron beam in the irradiation engineering center of the institute. The irradiation doses imparted to the fruit samples were set to 0.5, 1.0, 1.5, and 2.0 kGy. After irradiation, the samples were stored at room temperature (20–25℃) the relative humidity was 78%. The storage period was set to 9 days and the appearance quality was tested every 3 days, and nutritional quality indicators were tested every 4 days.

5.2.2.1. Determination of brown and rot rate index

The state of browning and rotting of the skin of mango fruits were classified according to reference [12]. The rate of browning and rotting was therefore expressed according to an index related to the area of damaged skin (Table 1). According to this scale, mango fruit would lose its commercial value when the damaged skin area was greater than 10% (grades 2 to 4 in Table 1). There were 15 samples per group, with three replicate samples for each treatment.

5.2.2.2. Hardness measurement

The hardness of the sample was measured with a TMS-Pro texture analyzer (Inductive element range 25 N, TMS10 mm needle probe, puncture distance 5 mm). Each sample was tested at five different locations, five replicates per sample. The percent of browning and rotting fruits was calculated as the number of browning and rotting fruits divided by the total number of fruits in the sample group.

TABLE 1. CLASSIFICATION CRITERIA FOR SKIN ROTTING OF MANGO FRUIT

Rotting Grade	Fruit skin damage
0	Bright skin, no lesions
1	Skin damage area <10%
2	Skin damage area 10%–30%
3	Skin damage area 30%–50%
4	Skin damage area >50%

5.2.2.3. Weight loss rate measurement

Samples were weighed (5 samples per group, 3 replications), then the weight loss rate was calculated according to the following formula (Equation 1).

$$\text{Weight loss (\%) at time t} = \frac{\text{Initial weight} - \text{weight at time t}}{\text{Initial weight}} \times 100 \quad (1)$$

5.2.2.4. Colour measurement

The colour of the sample was measured with Colour Difference Meter, and fruits (10 samples in each group) were measured every 3 days, repeated 3 times. L*, a*, and b* are three-dimensional rectangular colour space parameters, L* is the brightness axis parameter (brightness) that “0” is black and “100” is white; a* is red / green axis parameters that positive values are red, negative values Green, and “0” is neutral colour; b* is the blue / yellow axis parameter that positive value is yellow, negative value is blue, and “0” is neutral colour [13].

5.2.2.5. Determination of soluble solids, ascorbic acid, total acid and reducing sugar

A hand-held refractometer was used to determine the content of soluble solids [14], high-performance liquid chromatography was used to determine the ascorbic acid content [15], acid-base titration was used to determine the total acid content [16], and direct titration was used to determine the content of reducing sugars [17].

5.2.2.6. Statistical analysis of data

Measurements were taken as three replicates. The data were analysed by SPSS17.0 software, including the mean, standard error, Duncan’s multiple difference significance analysis and correlation analysis. A p value of greater than 0.05 was not statistically significant.

5.3.RESULTS AND DISCUSSION

5.3.1. Effect of irradiation on appearance quality of mango during storage

Appearance is the most intuitive indicator of fruit freshness; it directly affects the consumer's willingness to purchase the product. As shown in Fig. 1, small brown spots appeared in the 1.5 kGy and 2.0 kGy irradiated sample groups on the 3rd day, and other groups were in good condition. On the 6th day, dark spots appeared in the control group (CK), and the brown spot area of the 1.5 kGy irradiated group reached 30%. In the 2.0 kGy treatment group, it reached 45%, while the colour of the skin remained good (no obvious brown spots appeared) on the 0.5 kGy and 1.0 kGy treatment groups. On the ninth day, the dark spot area of the CK group had reached half of the surface area of the sample, and browning had occurred in almost all fruit samples in the 2.0 kGy irradiated group of sample fruits, while most of the 0.5 kGy and 1.0 kGy irradiation groups just had small areas of browning, maintaining good appearance quality. The appearance quality results showed that irradiation could reduce the appearance damage of fruit skin, but high-dose irradiation could cause skin browning.

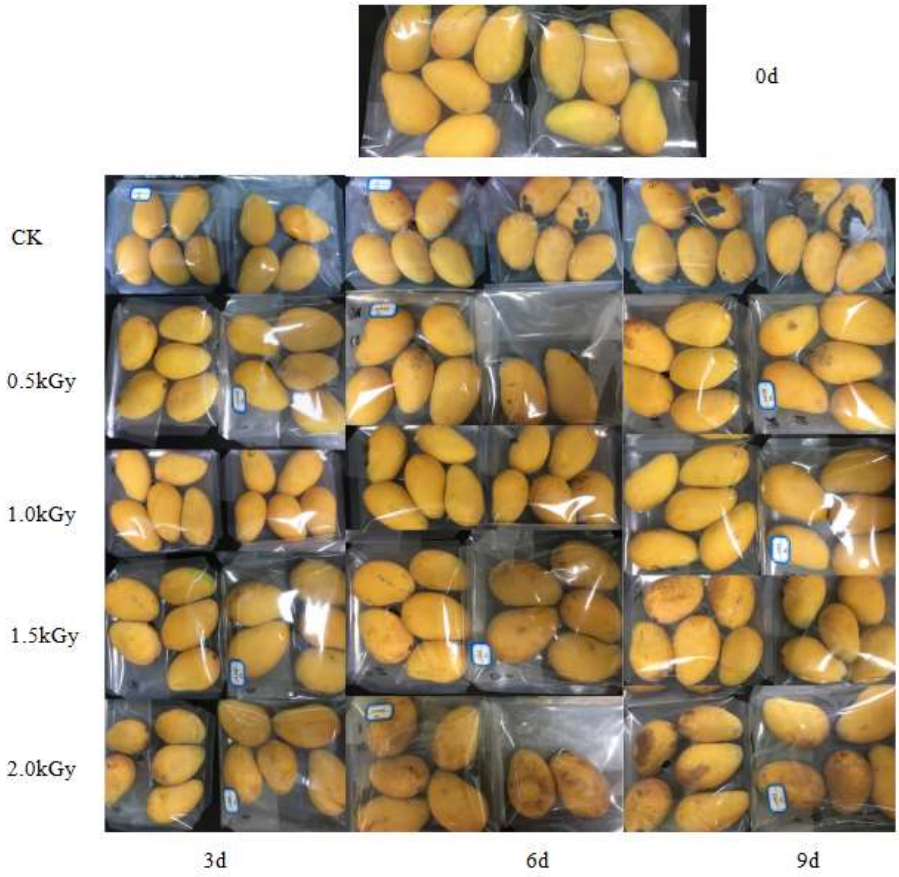

FIG. 1. Changes on appearance of mango during storage.

5.3.2. Effect of irradiation on brown and rot rate of mango during storage

Fruit browning not only directly reduces its quality, but also causes decay and deterioration, that shortens the shelf life of the product. As shown in Fig. 2, on the 3rd day, browning occurred in the 1.5 kGy and 2.0 kGy irradiated groups, and the brown rate reached 20% and 25%, respectively. On the 6th day, browning and rotting occurred in the CK, 0.5 kGy and 1.0 kGy irradiation groups, while the brown and rot rate was significantly lower in the samples treated with 0.5 kGy and 1.0 kGy irradiation than in the control group (p<0.05). On the 9th day, the

brown rate of the CK group had reached 45%, the 1.5 kGy and 2.0 kGy irradiation groups were 45% and 70%, and the 0.5 kGy and 1.0 kGy irradiation treatments were 15%. The results showed that high-dose irradiation increased the browning degree and rotting of fruits, and 0.5–1.0 kGy irradiation had the best effect. Long Mingxiu also reported that high-dose irradiation to a dose of 2.0 kGy caused certain damage to fruit tissue structure, and accelerated fruit senescence and quality deterioration [18].

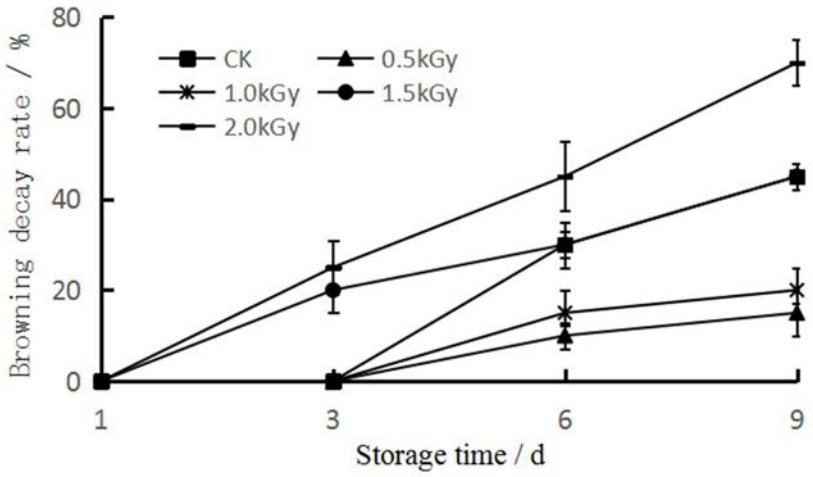

FIG. 2. Effect of irradiation on browning and rot rate of mango against storage days (d).

5.3.3. Effect of irradiation on hardness of mango during storage

Hardness is one of the characteristics to measure the ripening and aging process of fruit. The decrease of fruit hardness is mainly due to the hydrolysis of protopectin by various hydrolytic enzymes [19]. As shown in Fig. 3, the hardness of mango after irradiation was significantly lower than that of the control group ($p<0.05$), indicating that the hardness of fruits by irradiation reduced slightly to a certain extent.

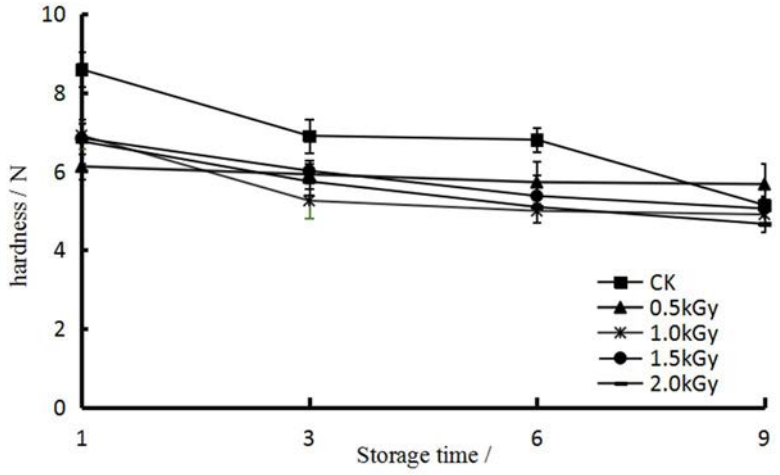

FIG. 3. Effect of irradiation dose of on hardness against storage time in days.

However, during storage, the hardness of the control samples decreased significantly, while the hardness of the samples in the irradiated groups decreased slowly, and the hardness of the control group dropped sharply on the 6th day, because the samples were close to the end of the storage period. During the whole storage period, the hardness change of the sample group treated with 0.5 kGy irradiation was the smallest, which indicated that the irradiation had the smallest effect on the fruit hardness and the fruit could maintain its original quality, thus the hardness of the samples between the irradiation groups were not significantly different (p>0.05).

5.3.4. Effect of irradiation on weight loss rate of mango during storage

Once mango is picked, nutrient content loss occurs because the fruit respires. Loss of water from the fruit is one of the main reasons for the loss of fruit quality post-harvest and during mango storage [20]. As shown in Fig. 4, weight loss increases with storage time for all mango samples. On the 3rd day, the weight loss rates of the irradiation samples were lower than the control samples. The weight loss rate of mango treated with 0.5 kGy and 1.0 kGy was significantly lower than other irradiation groups (p<0.05). The weight loss rate of the control group reached 4.74% on 9th day and was significantly higher than the irradiation groups (p<0.05). Low-dose irradiation effectively reduced post-harvest ripening of fruits and delayed the dehydration and shrinkage of fruits, which is consistent with other research findings [21].

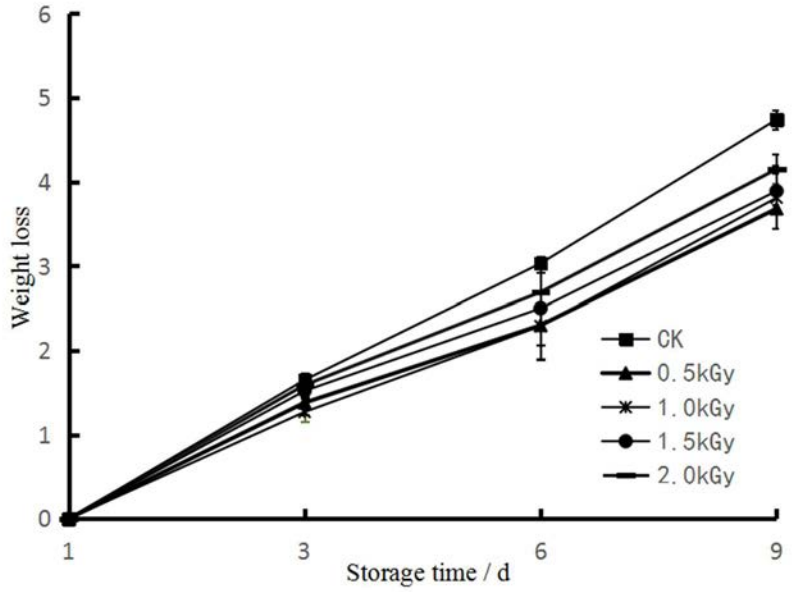

FIG. 4. Effect irradiation on weight loss rate of mango.

5.3.5. Effect of irradiation on mango colour during storage

As a basic physical property, colour is an important index for evaluating the quality of fruit, which greatly affects the consumer's purchase choice. As shown in Table 2, the L* value of the 1.5 kGy and 2.0 kGy irradiated samples group on the 1st day all were significantly higher than the other groups (p<0.05); on the 3th day, the L* value of the control group was significantly lower than the irradiated groups (p<0.01); at the 6th day, the L* value of the 0.5 kGy irradiation group was higher than other treatment groups, and there was a significant difference (p<0.01); at the 9th day, the L* value of 0.5 kGy and 1.0 kGy irradiation groups was higher than other treatment groups, and the samples treated with 2.0 kGy irradiation had the lowest L* value.

TABLE 2. EFFECT OF IRRADIATION ON L* VALUE OF COLOUR

Dose (kGy)	L*			
	Storage time			
	1 day	3 days	6 days	9 days
0 (control)	**66.71±0.75b**	**65.02±1.21B**	**63.50±0.32B**	**63.18±0.33B**
0.5	**67.19±1.16b**	**66.54±0.42A**	**65.56±0.68A**	**65.09±0.50A**
1.0	**67.25±0.79b**	**67.14±0.69A**	**63.95±1.17B**	**65.13±0.61A**
1.5	**68.20±0.55a**	**66.09±0.56A**	**64.24±0.96B**	**63.24±1.77B**
2.0	**68.57±0.51a**	**66.44±0.50A**	**63.15±0.69B**	**59.77±0.93C**

Note: Different lowercase letters in the same column indicate a significant difference (p<0.05), different capital letters in the same column indicate a significant difference (p<0.01), the same below.

The a* value (red / green axis parameter) and b* value (yellow / blue axis parameter) were indicated in Table 3 and 4. The a* value of the control group was significantly higher than the irradiated groups (p<0.01) from the 3rd day during storage, indicating that the colour of the control samples changed from green to red and gradually matured. The appearance and colour of fruit skin in control samples gradually turned yellow, while the a* value of the irradiation group sample remained relatively stable, indicating that the yellowing process was slow than CK. The b* values of 1.5 kGy and 2.0 kGy irradiation treatment groups were significantly lower than other irradiation treatment groups (p<0.01) from the 3rd day, and the browning had begun.

The above results indicated that the colour and brightness of mango samples tended to darken during storage, as the fruits began to mature, yellowness decreased, and the skin gradually became browner. Irradiation treatment to doses of 1.5 kGy and 2.0 kGy reduced the skin's brightness, and also accelerated the yellowing and brown rate of mango. Xu Yun also reported that when the irradiation dose was less than 0.85 kGy, it could inhibit the ripening and yellowing of mango, high-dose irradiation accelerated browning [21].

TABLE 3. EFFECT OF IRRADIATION DOSE OF ON A* VALUE

Dose (kGy)	a*			
	Storage time			
	1 day	3 days	6 days	9 days
0 (control)	**12.69±0.52AB**	**14.88±1.15A**	**16.81±0.70A**	**18.44±0.47A**
0.5	**11.81±1.11B**	**13.29±0.42B**	**14.32±0.76C**	**14.07±0.45BC**
1.0	**13.12±0.40A**	**13.34±0.67B**	**15.19±0.50B**	**14.54±0.90B**
1.5	**11.82±0.70B**	**14.35±0.34A**	**14.12±0.53CD**	**14.54±0.88B**
2.0	**12.16±0.65AB**	**13.01±0.51B**	**13.38±0.49D**	**13.24±0.44C**

Note: Different lowercase letters in the same column indicate a significant difference (p<0.05), different capital letters in the same column indicate a significant difference (p<0.01), the same below.

TABLE 4. EFFECT OF IRRADIATION DOSE ON B* VALUE

Dose (kGy)	b*			
	Storage time			
	1 day	3 days	6 days	9 days
0 (control)	**48.24±0.96c**	**52.94±0.86A**	**53.23±0.44A**	**52.61±0.38A**
0.5	**49.56±0.98b**	**50.04±1.45C**	**53.41±1.24A**	**52.77±0.77A**
1.0	**50.74±0.88a**	**52.08±0.63AB**	**52.77±1.00A**	**53.50±0.92A**
1.5	**49.30±0.76b**	**51.47±0.37B**	**51.14±1.10B**	**49.84±1.08B**
2.0	**49.60±0.83b**	**48.52±0.73D**	**47.43±1.47C**	**46.27±1.50C**

Note: Different lowercase letters in the same column indicate a significant difference (p<0.05), different capital letters in the same column indicate a significant difference (p<0.01), the same below.

5.3.6. Effect of irradiation on total acid and reducing sugar content of mango during storage

The total acid in fruits is composed of a variety of organic acids, which is an important factor affecting the flavour and storage quality of fruits. As shown in Fig. 5-A, the total acid contents in the irradiated mango samples (1.13 g / kg, 0.76 g / kg, 0.99 g / kg, 0.96 g / kg at the 0.5, 1.0, 1.5, 2.0 kGy) were lower than the control group (1.57 g / kg). The total acid content in the mango sample decreased by the storage time, and the content of the control group decreased significantly (p<0.05). There was no significant difference in total acid content between control group and irradiated groups after 3 days. It showed that the irradiation treatment can reduce the acidity of the fruit to a certain extent, improve the taste. During storage, irradiation had no significant effect on the total acid content of mango (p>0.05).

The reducing sugar content reflects the maturity of the fruit. The reducing sugar content of the fruit during the initial storage period increases with the fruit matures. After the fruit is fully mature, the sugar is decomposed and consumed, then the content shows a downward trend. As shown in Fig. 5-B, the reducing sugar content of the irradiation group increased at the beginning, then decreased during the storage, while the content of the control group decreased in all storage period, which indicated that the irradiation treatment could delayed the post-ripeness of Xiaotainong mango. On the 4th day, there was no significant difference in the reducing sugar content between the irradiation treatment and the control, and on the 8th day, the reducing sugar content in the control group was 4.36 g/100 g, significantly lower than irradiation groups (p<0.05), and the content in the irradiation group at 2.0 kGy irradiation was 4.70 g/100 g, significantly lower than other irradiation groups (p<0.05). This indicated that irradiation by the dose of 0.5 kGy–1.5 kGy could delay the reduction of reducing sugar content in mango.

5.3.7. Effect of irradiation on ascorbic acid and soluble solids content of mango during storage

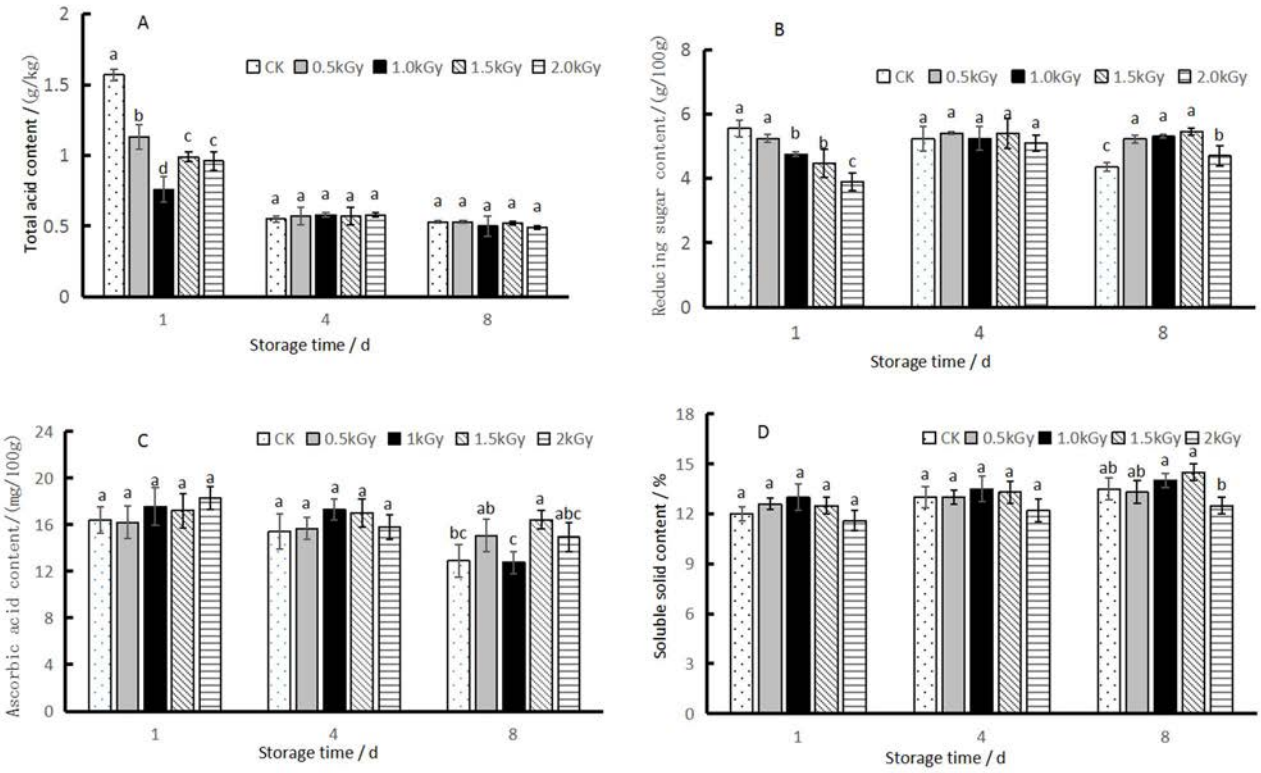

FIG. 5. Effect of different irradiation doses on nutritional quality.

Ascorbic acid is one of the main nutritional physicochemical properties of mango. It plays an important role in improving the antioxidant, anti-browning and anti-aging capabilities of fruits. It is easily oxidized and decomposed by ascorbic oxidase during storage and transportation [23]. As shown in Fig. 5-C, the ascorbic acid content gradually decreased with storage time. On the 4th day, the ascorbic acid content of the irradiated samples at 0.5 kGy, 1.0 kGy, 1.5 kGy, and 2.0 kGy (respectively 15.67 mg/100 g, 17.3 mg/100 g, 17 mg/100 g, 15.8 mg/100 g) were higher than the control group (15.4 mg/100 g). On the 8th day, 0.5 kGy and 1.5 kGy of mango ascorbic acid (15.07 mg/100 g, 16.43 mg/100 g respectively) were significantly higher than the control group (12.9 mg/100 g); the ascorbic acid content of the control group decreased significantly during the entire storage period, while its content was more stable in the irradiation treatment group on the 8th day (except the 1.0 kGy irradiation group), the irradiation treatment could delay the decline of ascorbic acid content in mango during storage, and maintain good taste and flavour and nutritional quality of the fruit. The study by Lei Qing [24] also showed that Electron beam irradiation could delay the decrease of ascorbic acid and reducing sugar content in strawberry.

Soluble solids content is an important indicator of fruit storability and nutritional value [25]. As shown in Fig. 5-D, the content of soluble solids gradually increases as the storage period prolongs. This is because picked mangoes are not yet fully mature, and after ripening during storage, some organic matters are decomposed and converted into sugars, causing soluble sugars to gradually increase and sweeten the taste. During the whole storage, the soluble solids content in the irradiation group was slightly higher than the control group (except the treatment group by 2.0 kGy), but there was no significant difference between the groups (p>0.05), indicating that the irradiation treatment had no effect on the soluble solid content of mango.

5.4.CONCLUSION

In general, when irradiated samples were compared with the control samples, irradiation to doses of 0.5 kGy, 1.0 kGy, 1.5 kGy, and 2.0 kGy had no significant effect on mango hardness, soluble solids, ascorbic acid, and total solids content during storage. Irradiation did not obviously reduce the nutritional content of fruits. However, electron beam irradiation did effect mango appearance, browning and rotting rate, weight loss rate and colour. Irradiation to doses of 0.5 kGy and 1.0 kGy resulted in mango fruits that maintained a good appearance, had delayed browning, and a reduced rate of rotting and weight loss, ripening and yellowing was also inhibited, colour and brightness were maintained. However, 1.5 kGy and 2.0 kGy irradiated mango were found to have accelerated browning and decay to a certain extent, quality (appearance) and colour reduced. Therefore, the optimal irradiation dose for mango fresh-keeping treatment with electron beam irradiation is 0.5–1.0 kGy.

ACKNOWLEDGEMENTS

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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

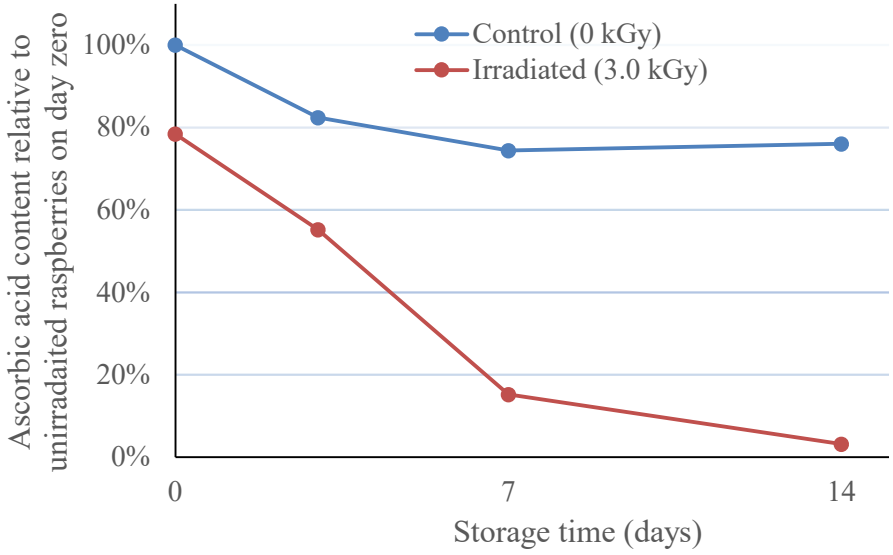

FIG. 1. Effect of electron-beam irradiation on ascorbic acid content of raspberries on days 0, 3, 7 and 14 during storage.

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.

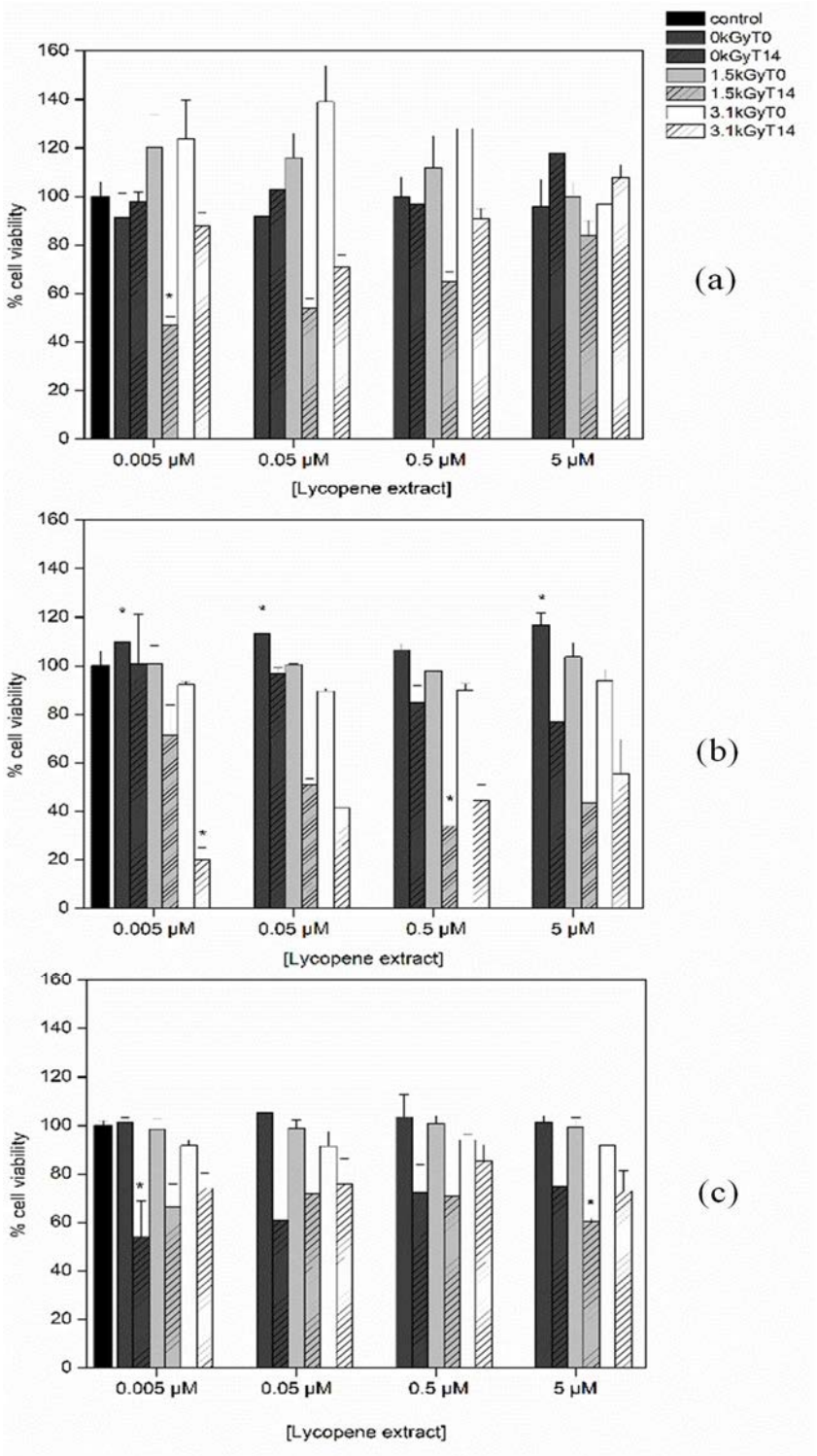

*FIG. 2. Cellular viability of (a) 293t, (b) a549 and (c) caco-2 cell lines in the presence of different concentrations (0.005 µm, 0.05 µm, 0.5 µm, 5 µm) of lycopene extracts from non-irradiated (0 kGy) and e-beam irradiated (1.5 kGy, 3.1 kGy) cherry tomatoes, immediately after irradiation (t0) and after 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.

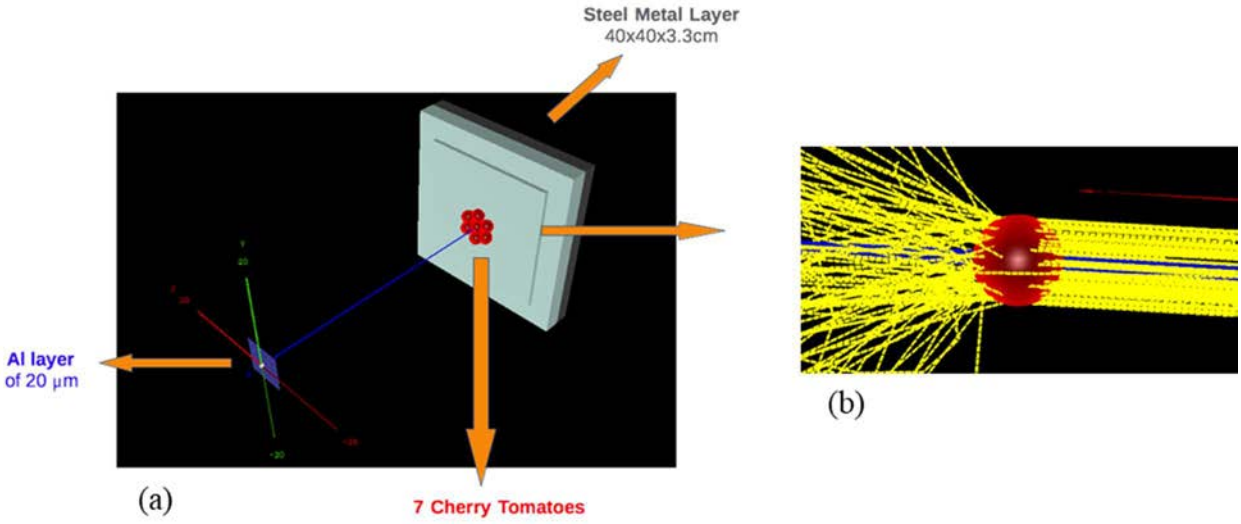

FIG. 3 (a) Simulated setup of cherry tomatoes irradiation using the framework ENSARRoot. (b) Cherry tomatoes exposed to 10 MeV mono-energetic electrons.

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%	–

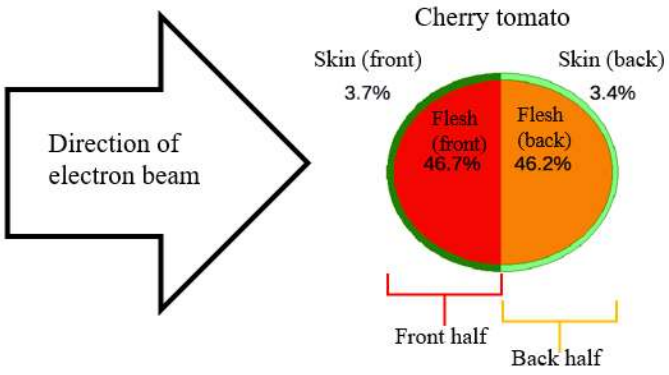

FIG. 4. Modelling of percentage dose distributed in a cherry tomato, considering the skin and the internal flesh (see also Table 11).

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.

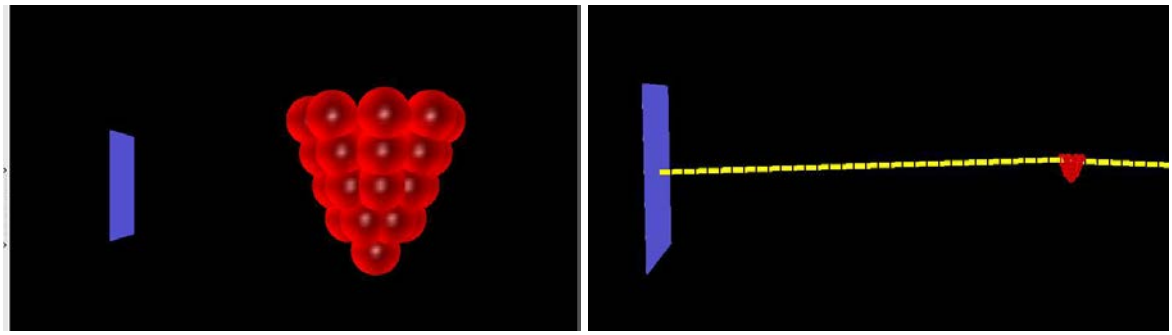

FIG. 5. (a) Left-hand side image is the simulated geometry of raspberries irradiation using the framework ENSARRoot. (b) Right-hand side image is the simulation of raspberries exposed to 10 MeV mono-energetic electrons.

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.

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7. DEVELOPMENT OF AN IRRADIATION PROCESS USING ELECTRON BEAM FOR NOVEL PICKLED AND FERMENTED FOOD PRODUCTS WITH AN EXTENDED SHELF-LIFE

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Abstract

Pickled food products such as vegetables are popular side dishes and appetizers. The pickled products currently available have several limitations including limited shelf-life at room temperature, also the excessive heating processes (blanching, boiling) to which they are submitted affects sensory qualities such as flavour and crunchiness. In addition, the necessary restraint on heating may lead to insufficient microbial decontamination and may result in the product spoiling too rapidly or being microbiologically unsafe. Irradiation with ionizing radiation appears to be an ideal alternative to mild heat treatments and could lead better products in terms of sensory quality and shelf-life. The objective of the project is to develop a process based on electron beam irradiation of types of pickled and fermented food products that would give a superior product when compared to their traditional counterpart and have an extended shelf-life. Several different types of pickled vegetable products were selected: pickled ginger, mixed pickled vegetables, pickled shredded sweet-salted white radish, and fermented bamboo shoots. These were packaged in partial vacuum-sealed laminated plastic bags and electron beam irradiated at 2–10 kGy. Samples were subjected to microbiological investigations. Electron beam irradiation to at least 4–6 kGy was found to improve the microbial quality and extend the shelf-life of pickled vegetable products. Slight to moderate discoloration of pickled vegetables after irradiation were commonly observed and the discoloration increased with dose. The darkening of some pickled vegetables during ambient storage for three months failed to pass a sensory analysis while the microbial qualities were still maintained. The results of this study would reassure those who study the potential for irradiation using electron beam as a commercial application to produce novel pickled and fermented food products with an extended shelf-life.

7.1. INTRODUCTION

There is a growing demand for food that is both healthy and convenient, with food safety also being an implicit demand. Heating is in general one of the most common technologies used to ensure food safety but it induces changes in food flavour and texture. To obtain a pickled vegetable product that is acceptable, heating has to be limited, and this means that heat-resistant microorganisms, some of which can be pathogenic, may survive the process. Some recontamination may also occur after the heat process, during subsequent production steps. This microflora will develop during storage and shorten the shelf-life of pickled vegetables. Irradiation has advantages that could be beneficial for this type of product: it can be applied to the packaged product at the very final stage of manufacturing, preventing any recontamination. Irradiation will inactivate all organisms still present at this stage thus leading to an extension of

shelf life. The effects of irradiation on texture and taste are generally much less than heating. Consequently, irradiation would appear to be an ideal alternative to mild heat treatments and its use could lead to superior pickled vegetable products in terms of sensory quality and shelf-life.

Pickled vegetables and fruits are prepared from whole or sliced vegetables or fruits. The production process involves simmering vegetables or fruits in a sweet-sour syrup containing a seasoning blend (with or without seeds, spices, aromatic herbs and/or condiments) or steeped in brine without heating to create a crisp and firm texture. The lack of heating process to destroy the yeasts, molds and bacteria cause food spoilage rapidly. Furthermore, the use of chemical preservatives is limited according to food safety requirements and also the effect on flavour. Though a large amount of information is available on irradiated meats and marine products, there are few reports on irradiated pickled fruits and vegetables. It is reported that a combination of heating and 20 kGy of gamma irradiation was able to sterilize kimchi, a Korean fermented vegetable product, regardless of irradiation temperature [1]. In addition, a deep-freezing process prior to high dose gamma irradiation was able to improve the texture (hardness) of Kimchi, sterilized as a space-food and maintain its shelf-life for 30 days storage at 35^{o}C relative to non-irradiated counterpart [2]. Recently, a salted pickled vegetable sterilization processing method using gamma irradiation has been patented in the People's Republic of China. It was shown that 3–4 kGy can greatly reduce the amounts of microbial contaminated while maintaining the food products organoleptic characteristics and extending the shelf life to 12 months [3].

Although consumer acceptance of irradiated foods has increased considerably over the last two decades, there is still some reluctance to accept irradiated foods. One of the factors that may concern people is that food irradiation must make use of radioactive materials. This is one of the misconceptions about food irradiation. Those who oppose food irradiation are generally unaware of the fact that food irradiation can also be carried out in facilities that use non-radioactive sources, such as high-energy electrons or X rays [4]. It is anticipated that consumers are more willing to accept irradiated food if these foods are treated with electron beams or X rays and have a more informed choice of irradiated products. The overall objective of the work we present here was to develop an electron beam based process for types of pickled food products that would be superior to their traditional non-irradiated counterparts.

7.1.1. Effect of electron beam irradiation on the microbial quality and shelf-life extension of some pickled and fermented vegetable products

Pickling and fermenting are both ancient food preservation techniques. Fruit and vegetable pickles are fundamentally products that have been prepared or preserved in a brine (salt or salty water) or an acidic solution (vinegar or lemon juice). The high osmotic potential and/or low pH of the pickle will slow microbial growth. In contrast, fermented foods are preserved by the action of "good microorganisms" such as lactic acid bacteria that transform the food product by using sugar and carbohydrates presented in the food as carbon sources. The bacteria change the sugars and carbohydrates into other substances, such as acids, carbon dioxide, and alcohol. The acid produced by this fermentation then preserves the foods and many fermented foods taste very acidic. Once fermentation is complete, the food product has a low pH and therefore further microbial growth is inhibited. Therefore, some pickles are fermented while some fermented vegetables are pickled but not all. Food acidity can be defined according to pH. Acid foods are foods with a pH of 4.6 or lower while a low-acid food is any food (other than alcoholic beverages) with a finished equilibrium pH greater than 4.6. To control the microbial quality in commercialized products, producers in some countries prepare pickled vegetables and

fermented vegetables following the international food standards such as CODEX [5–6], and national standards [7–9]. The products that are the subject of this study were chosen to represent a diverse range of both pickled and fermented vegetables that are easy-to-find in daily life.

7.1.1.1. Shelf-life extension of pickled ginger using electron beam and packaging techniques.

Pickled Ginger is one of the most popular side dishes that can be eaten with various kinds of Asian foods such as sausage, roast duck, sushi, curry rice and salad. It is a vinegar-based pickle that can be easily made at home within 48 hours or bought from supermarkets. Young ginger (6–8 months) and lime juice in the pickling brine give a mild spicy, sweet and sour taste and the prepared product has a distinctive natural pink colour (artificial colour additives are prohibited). Although pickled ginger is an acid food, it can easily spoil because microbial contamination can occur during preparation. Under conventional handling conditions, the preservative-free pickled ginger can only be kept for a few days at room temperature or stored in the refrigerator for a couple of weeks. Pickled ginger was selected for study as a potential new type of irradiated food product because it is widely used in Thailand, the ginger rhizome (*Zingiber officinale*) is available at local markets in all seasons. Therefore, we decided to investigate the effect of electron beam irradiation on the microbial quality and shelf stability of pickled ginger during storage at different temperatures.

7.1.1.2. Shelf-life extension of mixed pickled vegetables using electron beam and packaging techniques.

Mixed pickled vegetables refer to a product prepared from a mixture of two or more types of vegetables, available in different recipes from different countries. It is prepared as a ready-to-eat food that can be served as a condiment with bread or sandwiches of all types, eaten with rice, stewed pork leg or side dishes. The products currently available in Thailand are usually canned for long-term storage or have preservatives added. Canned mixed pickled vegetables have a high sodium content, and this is a concern for many consumers. Our study included mixed pickled vegetable products for similar reasons to including pickled ginger. We investigated the shelf-life stability of electron beam irradiated mixed pickled vegetables (pickled Chinese white radish, ginger, and red chili).

7.1.1.3. Shelf-life extension of pickled shredded sweet-salted white radish using electron beam and packaging techniques.

White radish is also known as daikon or Japanese radish or Chinese radish (*Raphanus sativus* subsp. Longipinnatus). Pickled white radish is used as a cooking ingredient in various types of dishes. It can be preserved as a salted product that is packed as a salted product (a solid product, without a liquid medium) and can be stored at room temperature for over year. Another type of white radish product is sweet-salted white radish which requires other condiments and brown sugar to be added during the process. In commercial production, some chemical additives and preservatives can be allowed to be used (e.g. calcium chloride, benzoic acid and sorbic acid). When preservatives are not used, there is a risk that the microbial load in the products can continue fermentation and produce gas leading to swollen bag and a shortened shelf-life. In this study, we collaborated with a food manufacturer to improve a pickled vegetable product that is already commercially available. The microbial quality of pickled shredded sweet-salted white radish without preservatives was investigated to determine the effectiveness of electron beam irradiation.

7.1.1.4. Shelf-life extension of fermented bamboo shoots using electron beam and packaging techniques.

Fermented bamboo shoots (also known as sour bamboo shoots) is a salt-based fermented product of young bamboo shoots. It is commonly used as a cooking ingredient in various Asian dished. It is prepared fresh in the home, but commercial products are becoming increasingly popular for convenience and fermented bamboo shoots are available as a canned product for long-term storage. Many consumers are concerned about the sulfite content of canned products and the use of bleaching agents. In addition, several outbreaks of botulism have been attributed to poor hygiene during domestic preparation of fermented bamboo shoots these have occurred from time to time because anaerobic fermentation is required. In this study, the microbial quality and shelf storage were determined after fermented bamboo shoots were exposed the electron beams. Microbial challenge test used *Clotridium sporogenes* as a surrogate organism for *Clostridium botulinum* in order to evaluate the effectiveness of the irradiation process at preventing the growth of this toxin producing bacteria.

7.2.MATERIALS AND METHODS

7.2.1. Preparation of samples

Pickled ginger: Initial work tested five different pickling recipes using each of the pickling solutions plus thin slices of young ginger and pickling for 5–7 days in glass jars before tasting. The most favorite recipe (salt 2.3%, sugar 55.4%, vinegar 21.8%, lime juice 5.7%, water 14.8%) was selected to produce pickled ginger for use in further experiments. The pickled ginger product had a yellowish-light pink colour with the pH of approximately 3.2 and a sugar content of 40^{o} Brix.

Mixed pickled vegetables: Fresh Chinese white radish, ginger, and red chilli were cleaned, chopped and separately pickled in liquid medium before mixing to taste.

Pickled shredded sweet-salted white radish: A commercially vacuum-sealed packed shredded sweet-salted white radish (no added preservatives) were supplied by a local manufacturer. The product is dark yellow to golden brown in colour and had an intermediate water content of 50%–60%.

Fermented bamboo shoots: The samples were prepared by small and micro community enterprises (SME) using shredded bamboo shoots, anaerobically fermented in brine without added preservatives or bleaching agents. Natural lactic acid bacteria were generated during fermentation and prevented the growth of spoilage bacteria and would have also inhibited the growth of any adventitious pathogenic bacteria associated with the bamboo shoots. The bamboo shoots were fermented in a closed container, the fermentation took 4 weeks to mature and the products appear naturally off-white to faint yellow in colour with a unique smell.

Samples of pickled vegetable products (100 g) were packed under a partial vacuum in PET/LL bags and stored at refrigerated temperatures (4 to 10^{o}C) before being irradiated.

7.2.2. Electron beam irradiation

Irradiation was performed at room temperature at Thai Irradiation Center. One-sided irradiation treatments were applied to samples having 20–35 mm thickness. Samples were irradiated to doses in the range 2 kGy to 10 kGy using a high energy (8 to 10 MeV) electron accelerator (Mevex Corp Ltd. MB 20–16, Canada). A B3 radiochromic film (GEX Corporation, US) was

used as the dosimetry system. After irradiation, the samples were stored at ambient temperature until analysed. Non-irradiated samples from the same batch were used as controls.

7.2.3. Microbial analyses and storage studies

Total plate counts were determined using methods of FDA BAM (2001) chapter 3 [10]. Enumeration of total yeasts and molds were performed according to AOAC (2019) 997.02 [11]. Counts of *Escherichia coli*, *Staphylococcus aureus*, *Clostridium* spp, and lactic acid bacteria were obtained following the reference method guidelines [12, 13, 14, 15].

Shelf-life extension studies at 15, 30, 45, 60, 90, and 180 days after irradiation were determined at ambient temperatures (30^{o}C to 35^{o}C) unless otherwise specified.

7.2.4. Microbial challenge test

Clostridium sporogenes (DMST 15282) stock culture in gelatin discs were obtained from The Department of Medical Sciences Thailand (DMST). Endospore suspensions were prepared, and purification as described by Yang, 2009 [16]. The spore test sample was enumerated in duplicate using a hemocytometer. Prior to irradiation, one-milliliter aliquots of spore suspension were inoculated into each of triplicate sterilized laminate bags (35 mm by 35 mm) or put directly into five grams of fermented bamboo shoot samples to yield a single inoculum of 10^9 spores/gram. The bags were partial vacuum-sealed and stored at 4^{o}C until irradiated. The inoculated samples were exposed to target doses of 2, 4, 6 and 8 kGy. The surviving organisms were determined using Reinforced Clostridial Agar (Difco Co.) and incubated anaerobically for 72 h. The surviving fraction of spores (N) was determined as N/N_0, where N_0 is the viable count of the non-irradiated control sample.

7.2.5. Physico-chemical properties evaluation

Colour difference evaluations (Konica Minolta BT-10 Plus baking contrast meter) were conducted using L*a*b* Coordinates.

7.2.6. Sensory evaluation

Sensory evaluations were determined for each type of pickled vegetables by experienced panelists using a 9-point Hedonic Scale. Five descriptors were employed to evaluate the quality in terms of colour, flavour, texture, taste, and overall acceptance (unless otherwise specified).

7.2.7. Statistical analyses

The samples were analyzed in triplicate, and all results are expressed as mean ± one standard deviation (SD). Statistical analyses to determine the differences between means were performed using one-way ANOVA, followed by a post-hoc Tukey test or Student's t test. $p<0.05$ was considered as statistically significant.

7.3. RESULTS AND DISCUSSION

7.3.1. Irradiated pickled ginger

The results of the microbial cell count on storage day 0 (immediately after electron beam irradiation) are presented in Table 1. Non-irradiated (0 kGy) control samples were found to have a total microbial load of 1.82 $\log_{10}$CFU/g. Where microorganisms were detected, this

initial microbial load was decreased by increasing doses of electron beam radiation. Pickled ginger samples irradiated at 2 and 4 kGy were observed to have similar higher organoleptic evaluation scores and a treatment dose of 4 kGy was selected for the shelf-life studies (for storage at 4, 25, and 35ºC) because this appeared to be the optimum dose, receiving good scores in sensory tests and efficient at removing microbial contamination, including *Staphylococcus aureus* and *Bacillus cereus*. It was noticed that no difference in physico-chemical properties among irradiation doses were detected, except the fading of the naturally pink colour of the products after irradiation was observed as shown in Fig. 1.

TABLE 1. EFFECTS OF ELECTRON BEAM IRRADIATION ON THE MICOBIOLOGICAL QUALITY OF PICKLED GINGER

Dose (kGy)	Total Bacteria*	*Bacillus cereus**	*Staphylococcus Aureus**	*Listeria Monocytogenes**	*Clostridium Perfringens**	Lactic Acid bact.*	Yeast & Moulds*
0	**$1.82^{a}\pm0.07$**	**$1.46^{a}\pm0.15$**	**$1.10^{a}\pm0.17$**	**nd**	**nd**	**nd**	**$1.30^{a}\pm0.30$**
2	**$1.20^{b}\pm0.17$**	**nd**	**$0.77^{a}\pm0.68$**	**nd**	**nd**	**nd**	**nd**
4	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
6	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
8	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
10	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**

* Viable cell counts are in units of $\log_{10}$CFU/g, **nd** is "not detected".
[a-b]values with different letters within a column differ significantly ($p<0.05$)

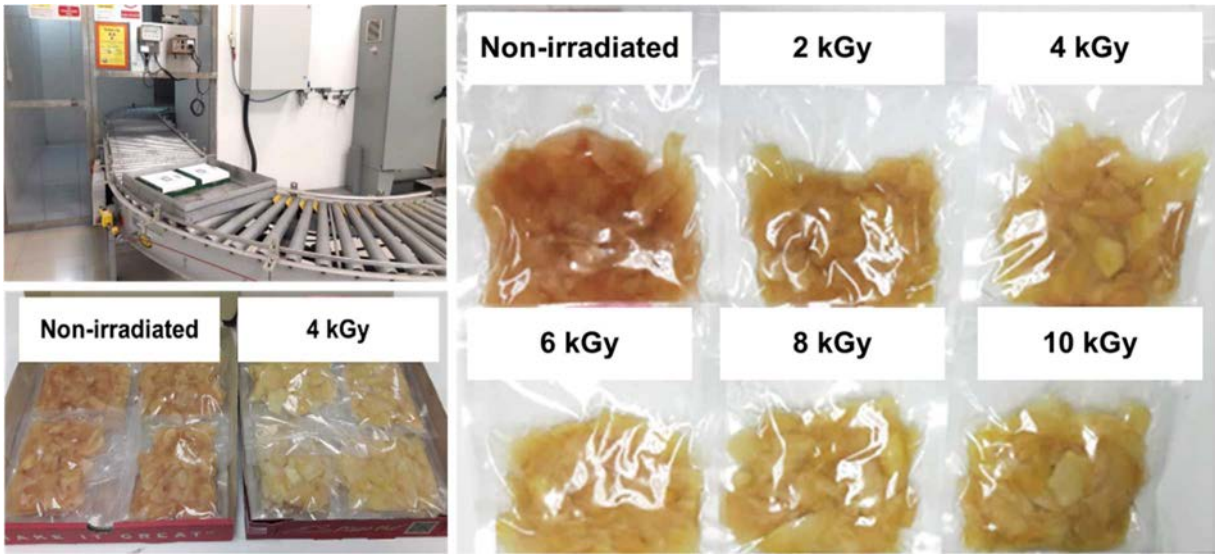

FIG. 1. Pink colour fading of the pickled ginger product was observed immediately after electron beam irradiation.

Microbiological results for the storage studies are given in Table 2. During the storage studies, non-irradiated samples were observed to have detectable levels of microbial contamination throughout the shelf life study period, particularly at temperatures of 25 and 35ºC. After 60 days, yeasts and moulds were found in non-irradiated samples stored at all the storage temperatures investigated, also these samples did not pass the sensory evaluation (data not shown) due to loss of texture (crunchiness) and colour (darkening). However, the 4 kGy irradiated pickled ginger samples, did not show detectable levels of microorganisms for up to 90 days of storage where microbial contamination was detected on irradiated samples stored at 25 and 35ºC but were not observed at 4ºC. However, after 3 months, all irradiated products

failed to pass all physiochemical and sensory evaluation. These finding suggested that electron beam irradiation demonstrates a potential to be used with pickled vegetables in maintaining microbial safety and extending the shelf-life superior to its counterpart for at least two months.

7.3.2. Mixed pickled vegetables

The results obtained for mixed pickled vegetable samples were similar to those for pickled ginger samples. Microbiological contamination of mixed pickled vegetables irradiated by electron beam (2–8 kGy) were significantly reduced relative to non-irradiated samples (both with and without added preservatives). The organisms detected in these samples were Lactic acid bacteria plus *Bacillus cereus*, *Staphylococcus aureus*, and *Listeria monocytogenes*. Interestingly, the colour fading of the mixed pickled vegetable products was also observed immediately after the samples were exposed to the electron beam (Fig. 2.). Significant changes in the hue angle (H^o) were detected in the radish components of the irradiated mixed pickled products. Shelf-life stability tests were conducted at both ambient temperatures and a higher temperature that would accelerate microbial growth (45°C) using non-irradiated samples and 6 kGy irradiated samples. Non-irradiated samples with and without added preservatives were used as controls. The irradiated samples maintained their low microbiological counts for 6 months at both ambient and 45°C. However, a darkening in the colour of these irradiated

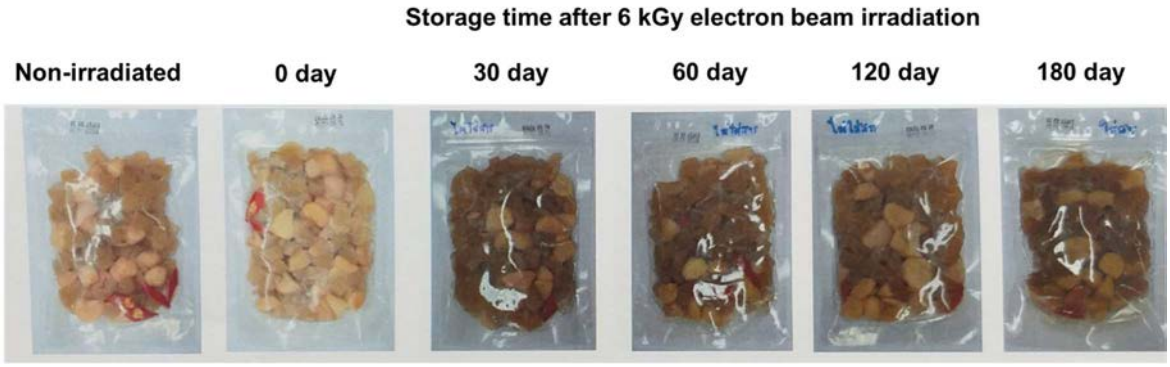

FIG. 2. Colour change of the irradiated mixed pickled vegetable. An initial colour fading was observed immediately after 6 kGy electron beam irradiation and the product darkened with prolonged storage.

samples was observed to start after one month of storage at both ambient temperature and 45°C. The darkening was due to the significantly change of hue angle (H^o) from both the ginger and radish components of the mixed pickled vegetable product, a change in b* value (yellow/blue coordinate) was not detected (data not shown).

TABLE 2. PICKLED GINGER: SHELF-LIFE STORAGE TESTS AND MICROBIAL COUNTS OF UNIRRADIATED (CONTROL) SAMPLES AND ELECTRON BEAM IRRADIATED SAMPLES

	Microbial Counts*								
	7 days storage			28 days storage			90 days storage		
	Storage temperature (°C)			Storage temperature (°C)			Storage temperature (°C)		
	4	25	35	4	25	35	4	25	35
Non-irradiated samples (0 kGy)									
Total bacterial counts	**nd**	**1.65±0.31**	**1.72±0.07**	**nd**	**2.15±0.22**	**2.49±0.04**	**4.60±0.56**	**5.46±0.16**	**6.57±0.20**
Bacillus cereus	**nd**	**nd**	**nd**	**nd**	**nd**	**1.82±0.07**	**nd**	**2.26±0.27**	**1.82±0.07**
Staphylococcus aureus	**nd**	**nd**	**nd**	**nd**	**3.02±0.03**	**3.05±0.04**	**2.50±0.24**	**4.01±0.02**	**4.19±0.12**
Listeria monocytogenes	**nd**	**nd**	**nd**	**detected**	**nd**	**nd**	**nd**	**nd**	**nd**
Yeast and Moulds	**nd**	**nd**	**nd**	**nd**	**nd**	**1.46±0.15**	**1.80±0.12**	**1.67±0.25**	**2.40±0.09**
Clostridium perfringens	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
Lactic acid bacteria	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
Salmonella	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
E. coli (log_{10}MPN/g)	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
Irradiated samples (4 kGy)									
Total bacterial counts	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**2.33±0.19**	**4.52±0.19**
Bacillus cereus	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
Staphylococcus aureus	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
Listeria monocytogenes	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
Yeast and Moulds	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
Clostridium perfringens	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
Lactic acid bacteria	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
Salmonella	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
E. coli (log_{10}MPN/g)	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**

* Viable cell numbers are in units of log_{10} CFU/g, except for *E. coli* which are in units of log most probable number per gram (log_{10}MPN/g), **nd**: not detected.

7.3.3. Pickled shredded sweet-salted white radish

Similar results were obtained when prepackaged (preservative-free) shredded sweet-salted radish from SMEs were irradiated using electron beam radiation (Fig. 3.). This preserved radish product is an intermediate moisture content food with a water content ranging from 40–60% and bloating of the plastic packaging is a clear indication that microorganisms are present. Non-irradiated samples had bloated packaging after a few days of storage. After irradiation, colour fading of the product was observed, the degree of fading increased with increasing doses (3 kGy and 8 kGy), from golden brown to pale golden brown, relative to the non-irradiated control samples. Though the samples failed to pass the sensory evaluation at 15 days of storage at room temperature, the microbial quality of 6 kGy irradiated samples was maintained for at least 30 days of storage. These finding suggested that electron beam irradiation has a potential for use with pickled vegetables and maintaining microbial safety, plus extending product shelf-life which is far superior to the non-irradiated product. However, discoloration is a primary hurdle that would need to be addressed for a commercial product.

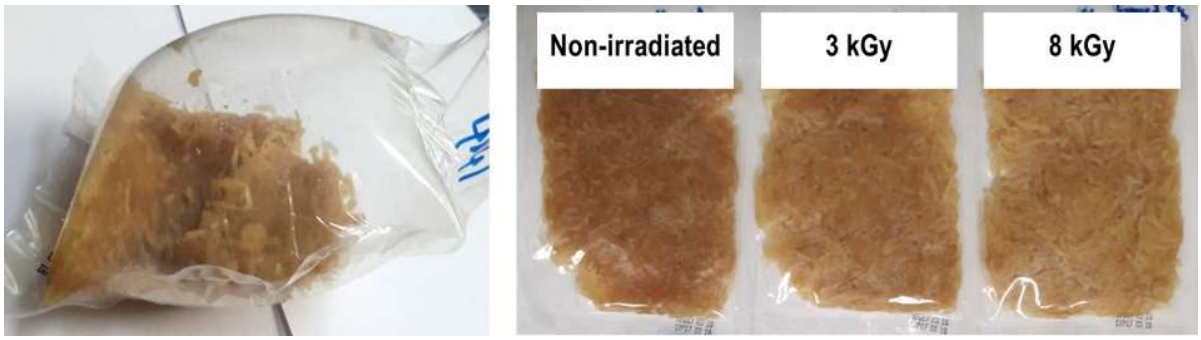

FIG. 3. Pickled shredded sweet-salted white radish. Bag of preservative-free unirradiated sample was swollen after a few days (left) and the observed colour fading of irradiated pickled shredded sweet-salted white radish after electron beam irradiation (right).

7.3.4. Fermented bamboo shoots

Electron beam irradiation was found to effectively remove the microbial contamination in fermented bamboo shoots in a dose-dependent manner (Fig. 4). At decrease of at least 4 log cycles of total microbial counts were observed at a dose of 4 kGy. However, a slight change in colour was immediately noticed after irradiation and the colour changed gradually with storage (Fig. 5). The change in colour became more obviously after 90 days of storage and the irradiated product failed to pass a sensory analysis, however the microbial quality was maintained (data not shown).

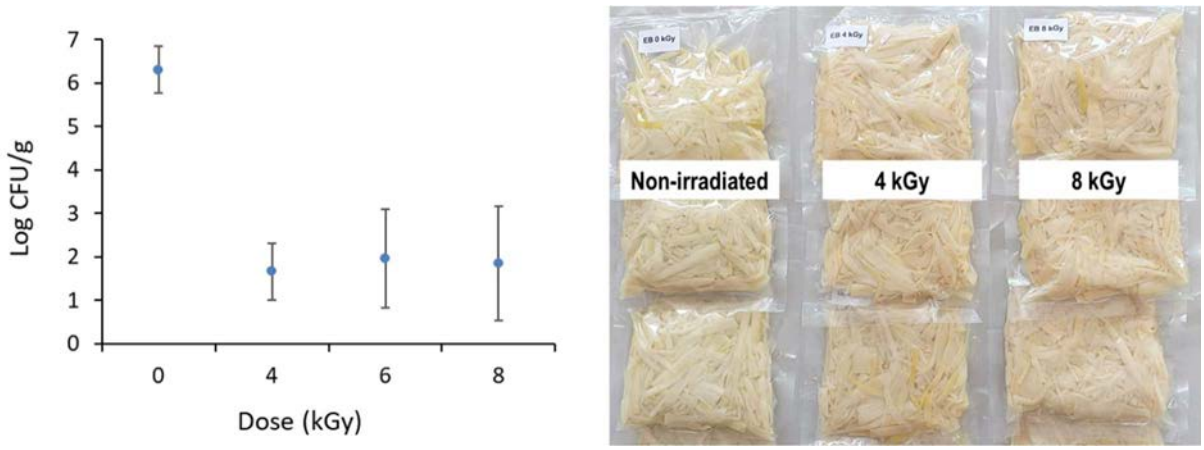

FIG. 4. Fermented bamboo shoot product and the effect of electron beam irradiation on microbial contamination (left) plus the observed change in colour after irradiation (right).

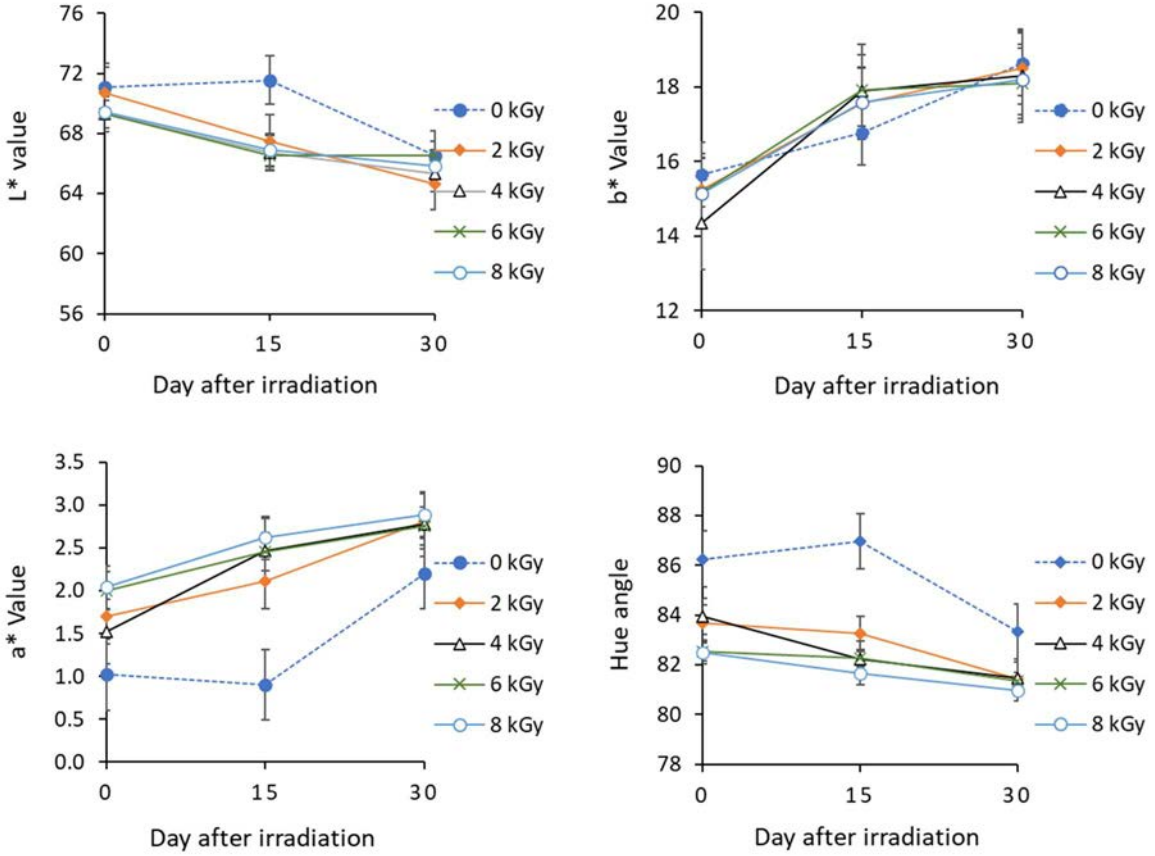

FIG. 5. Discoloration in irradiated fermented bamboo shoots were observed during storage.

Dose response curves were generated from the results of experiments into the effects of electron beam irradiation of *C. sporogenes* in order to measure the response of test organism inoculated into the food product and also in distilled water. These were used to calculate the D_{10} values presented on Table 3.

TABLE 3. D_{10} VALUES CALCULATED FOR REPLICATE EXPERIMENTS OF THE *C. SPOROGENES* (DMST 15282) RESPONSE TO ELECTRON BEAM IRRADIATION

Spores directly added into food matrix D_{10} (kGy)	Spores in distilled water D_{10} (kGy)
1.52	2.36
1.50	2.51

Experiment showed that the D_{10} value of *C. sporogenes* (DMST 15282) spores prepared in distilled water (D_{10} of approximately 2.44 kGy) were slightly higher than the D_{10} values of spores that were directly added into food matrix (D_{10} value of approximately 1.51 kGy). The difference between two values for the replicate experiments is not statistically significant. Estimation of radiation D_{10} value of spores of of *C. sporogenes* (DMST 15282) is shown in Fig. 6. Although the inactivation of microorganisms by ionizing radiation generally follow an exponential curve as seen in spores prepared in distilled water, spores that were directly added into fermented bamboo shoots displayed an initial sigmoid inactivation response curve with a short shoulder at lower doses (2–4 kGy) before the exponential inactivation commences. This shoulder may be due to the repair mechanism of the organism with a proportion of the organisms DNA requiring multiple hits to be inactivated [17].

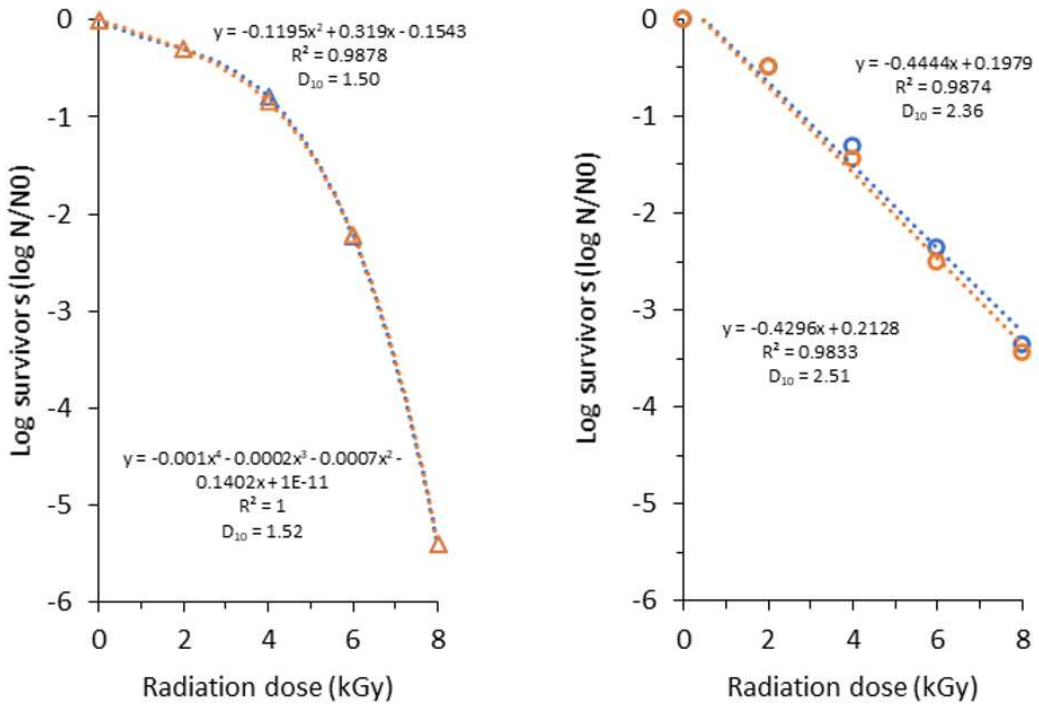

FIG. 6. Effect of electron beam irradiation on the survival of C. sporogenes spores in the fermented bamboo shoots matrix (left) and in distilled water (right). Data were from two separated experiments.

7.4.CONCLUSION

A study was conducted to investigate the irradiation of various pickled and fermented vegetable products using electron beam irradiation for microbial decontamination and to ensure food safety. Although a slight to moderate discoloration of pickled vegetables after irradiation was generally observed, it was shown that hygienic, safe and convenient pickled and fermented vegetable products can be prepared using electron beam irradiation. The effectiveness of electron beam irradiation to reduce the microbial load in preservative-free pickled vegetables and fermented vegetables allowed the products to be kept longer at ambient temperatures. The darkening observed in some irradiated pickled vegetable products during storage for 90 days failed to pass a sensory panel test in terms of acceptable colour. But, the microbial qualities were still maintained. The undesirable discoloration effects of irradiation need to be further investigated and improved. The successful development of an irradiation process using electron beam irradiation as a process to produce novel pickled and fermented vegetable products with an extended shelf-life is a beneficial, non-thermal, process that can be applied to a wider range of different food products.

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8. EFFECT OF DOSE VARIATION, FRACTIONATION, AND EXPOSURE TIME FROM HIGH ENERGY ELECTRON BEAM (10 MEV) ON PHYTIC ACID, COLOUR (WHITENESS INDEX), AND THE ISOFLAVONES CONTENTS OF SOY (*GLYCINE MAX* L.) FLOUR

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Abstract

Our research investigated the influence of Electron Beam (EB) irradiation on flour made from two different varieties of soybean (*Glycine max* L.). Different doses of EB radiation were applied and the effects on soy flour colour, phytic acid content and isoflavones (daidzein and genistein) were measured. The purpose of this research was to study the effects of both radiation dose and different methods of applying the EB irradiation process. Soy flour colour (Whiteness Index) was measured as a quality indicator. Phytic acid content was determined because this is an anti-nutrient compound and irradiation may be able to reduce its content in the flour. Isoflavones are bioactive chemicals that have a nutritional role and are associated with promoting health. The isoflavones were investigated because irradiation may influence their chemical form in soy flour and promote bioavailability. Radiation processing parameters such as the total dose imparted and the dose fractionation technique of delivering the ionizing radiation beam were controlled. A high energy (10 MeV) EB accelerator was used to deliver fractionated doses (the dose targeted for one fraction was 5 kGy) and samples were either irradiated from one side to the largest face of the packaged sample (one sided irradiation) or were irradiated from two opposite sides (double-side irradiation). Results showed that EB irradiation to increasing doses of radiation causes the colour and phytic acid content of soy flour to change. These changes were analysed according to a simple regression model. One-sided irradiation (one times process) was observed to be more effective at destroying phytic acid than the same dose delivered by two-sided irradiation (two times process). One- and two-sided irradiation had less effect on the colour of the soy flour, the flour darkens slightly with increasing dose, but at a much-reduced rate when compared to the change on phytic acid content. Radiation processing effects on isoflavones as a function of radiation doses showed a different pattern. Our research findings suggest that irradiation to doses in the range 5.8 kGy to 20 kGy appears to promote increased levels of daidzein and genistein isoflavones ("free-daidzein" and "free-genistein") but concentrations level or reduce at higher doses. This indicates that the applied treatment dose could potentially be optimized in terms of maintaining colour, reducing phytic acid content and enhancing the bioavailability of isoflavones.

8.1.INTRODUCTION

In the Republic of Indonesia, soybean (*Glycine max* L.) is the primary source of vegetable protein and has an essential amino acid profile that is superior to other legumes [1]. Soybeans contain natural anti-nutritional compounds such as phytic acid and antitrypsin that can reduce the nutritional value of soy food products. Phytic acid can bind minerals, such as calcium, zinc, iron, and magnesium. Consumption of foods with high phytic acid content can cause mineral deficiencies. Soybeans are usually rich in minerals such as iron, an essential ingredient for preventing anemia. However, soybean seeds are also the primary source of isoflavone, mainly in the form of β-glucoside and its aglycone (free from glucose molecule) that consist of daidzein, genistein, and glycitein [2,3,4]. Many studies on the potential health benefits of soybean isoflavones have been published and have associated these compounds with anti-carcinogenicity, antioxidant activity, and the ability to prevent or inhibit heart attacks, osteoporosis, and menopause symptoms [2,5,6]. There are two types of local soybeans grown in the Republic of Indonesia, the genotype of black (Mutiara) and yellow (Mitani) soybeans. The black soybean genotype is potentially a good functional food because it contains essential amino acids, vitamin E, saponins, and is rich in antioxidants such as flavonoids, isoflavones, and anthocyanins. It also contains tannins four times higher than the genotype of yellow soybeans [7].

Research has indicated that ionizing radiation can be used to reduce levels of anti-nutritional compounds, such as phytic acid in soybeans [8,9]. It has also been shown that the irradiation of Soybean to high doses of up to 30 kGy will reduce cooking time and improve the functional properties of protein isolates [10]. The results of our previous research [11] showed that gamma irradiation at different dose rates and exposure times can cause changes in concentrations of isoflavones in soybean. Gamma irradiation at low dose rates produces more free-daidzein and free-genistein than gamma irradiation to the same dose but irradiated at higher dose rates. Also, irradiation at higher dose rates is more effective at destroying anti-nutritional compounds as compared to that of the irradiation process at the lower dose rate [12]. Furthermore, preliminary experiments indicated that one-sided and two-sided electron beam irradiation of individual packets of soybean flour to doses of 5 to 15 kGy may potentially promote increased levels of free-daidzein. Consequently, the selection of radiation dose and the method of delivery of the dose (e.g. dose fractionation) may be useful as a tool to control isoflavone content, especially free-daidzein [13].

Irradiation with ionizing radiation can be used to improve the content of certain chemical compounds in foods, enhancing, among other things, the bioactive properties, and antioxidant activity of food products [14, 15]. The isoflavone content of soybean varies as affected by the degree of processing and also depends on the type (variety) of soybeans [16, 17, 18]. Furthermore, Park, *et al.* [19] reported that soy germ isoflavone content was enhanced in EB irradiated soybean (15 kGy) as there is an increase of aglycone form and in isoflavone totals as compare to non-irradiated soybean. Variyar, *et al.* [20] reported that isoflavones of soybean irradiated with gamma irradiation at dose levels of 0.5 to 5 kGy is mostly in the form of aglycones. Irradiation may attack the glycosidic bond of soy isoflavone glycosides (genistein, daidzein, and genistin) to produce aglycones. However, doses of gamma radiation of up to 10 kGy did not affect the total isoflavone content of soybean [21].

Phytic acid in sorghum is reduced to lower levels by radiation processing, EB irradiation to doses of 10, 15, 20, 25, and 30 kGy have been shown to reduced levels of phytic acid by 39%; 49%, 66%; 79%; and 90% respectively [22]. Treatment with EB irradiation to doses of 30 and 45 kGy in soybean flour [23] and canola flour respectively have been shown to eliminate whole

phytic acid [24]. The irradiation of chickpea flour to doses of 2.5 to 10 kGy decreased phytic acid content by 10.2 to 18.2% [25]. Canola seeds irradiated with doses of 30 and 45 kGy [26] and the seeds of Mucuna pruiens (velvet bean) irradiated to doses of 15 to 30 kGy will eliminate phytic acid [27]. Irradiation with a dose of 5 to 10 kGy is reported to lower the phytic acid content in different types of beans (pea, cowpea, lentils, kidney beans, and chickpeas) to about 6.5%–32.7% and it is estimated that an irradiation dose at 34.9–59.7 kGy would eliminate phytic acid in these food products [28]. Cooking (heat treatment) was not found to lower the phytic acid content in the making of sorghum porridge, but a combination of cooking and irradiation (10 kGy) causes a decrease in sorghum porridge phytic acid by 40% [29]. However, the phytic acid content of two varieties of Brazil nuts soaked in water for 10 hours, then heated to 100 °C and irradiated to doses of 0.5 to 10 kGy was not found to decrease [30]. Gamma irradiation (2 kGy at a dose rate of 20 Gy/minute) combined with the soaking and cooking the seeds and flour from two varieties of barley was not found to damage phytic acid after 30 and 60 days of storage [31].

The radiation dose is the amount of radiation energy absorbed by the material [32, 33]. It is the critical factor in food irradiation and determines the adequacy of the process according to its intended purpose. The radiation dose delivered to a food depends on the time of exposure because dose is the product of the relatively constant dose rate (e.g. kGy/hour) and the duration of exposure (hours). Each type of food requires a specific irradiation dose (time of exposure) according to the intended purpose of the irradiation treatment. In general, research publications on the radiation processing of food products tend to focus on the effect of different radiation doses to changes in nutrient content and product quality. Therefore, this research aimed to study the effect of irradiation dose on the effectiveness of irradiation. The result may be used to optimize the process to promote isoflavones, suppress levels of phytic acid with least effect on other product quality attributes (such as colour). Irradiation techniques such as one-sided irradiation (one times process) and two-sided irradiation (two times process) from two opposite sides were used vary the dose-delivery because it may minimize radiation damage in the sample. The ability to use one-sided and two-sided irradiation is associated with factors related to the ability of the electrons to penetrate and the limitations of thick samples and the energy of Electron Beam Machine (EBM) equipment.

In particular, this research aims to study the effects of combination treatment between radiation dose and the technique of delivering EB irradiation on the effectiveness of the irradiation process to improve soy flour, e.g. decrease in anti-nutritional compounds, colour (Whiteness Index), and enhance isoflavones during the radiation process. Different irradiation process parameters (dose-delivery variation, fractionation, and the time of exposure to a 10 MeV EB) were used to investigate if irradiation to the same dose will result in different soy flour product quality.

Specifically, this research evaluates the influence of radiation dose fractionation (kGy/fraction) when applied to samples of soyflour as one-sided (one times process) or two-sided (two times processes) irradiation in the dose range from 0 to 58 kGy. Changes in anti-nutritional compounds, colour, and isoflavones content will be measured in the irradiated soy flour.

8.2.MATERIALS AND METHODS

8.2.1. MATERIALS

Two varieties of Soybean (*Glycine max* L.), i.e., Mitani variety (Genotype of yellow soybean) and Mutiara variety (Genotype of black soybean) obtained from the Plant Breeding Section,

CIRA, BATAN. Dose measurments were made with B3WINDOSE^{TM} Dosimeters Gex Corporation USA. Isoflavones and phytic acid standard reagents (analytical grade) were purchased from Sigma Chemical Co.

8.2.2. Equipment

Samples were irradiated by a 10 MeV EB at the GEMS Irradiation Centre of the Thai Institute of Nuclear Technology in Bangkok, Thailand. Spectrophotometer reader, Spectro UV-2450 Shimadzu, Shimadzu liquid chromatograph equipped with LC 20 AD type pump, SPD 20A type UV detector and RF 10AXL type, Chromameter 200b Minolta Ltd., Water bath (Napco models 220A, USA), Centrifuge (IEC Centra 8 Centrifuge, USA), Magnetic stirrer (Velp Scientifica type of Ate, Italy).

8.2.3. Sample preparation

8.2.3.1. Sample Preparation

Samples were prepared in triplicates [34, 35]. Soy flour (72.33 g), was packed in polyethylene plastic packaging. Each packaged sample was in the form of a thin sheet rectangle (18 cm by 12 cm) with a mass thickness of 3 g/cm^2.

Samples were passed through the irradiation zone on trays (60 cm x 60 cm). The samples were placed at predetermined positions on each standard tray such that a one pass through the irradiation zone was equivalent to a dose fraction of 5 kGy. This dose fraction was established in a preliminary study where four locations (Fig. 1) in the sample tray (and therefore in the irradiation zone) were identified as receiving the same dose (positions 1, 6, 11 and 12 on the sample tray). The samples were placed in the irradiation area (The dry tray (60 x 60) cm) with different locations; to get irradiation treatment with frequency (1 (one) fraction = 5 kGy). The frequency of fraction used was previously determined at a preliminary study. Fig. 1, four (4) locations in the irradiation area have been identified to have the same dose of position no. 1, 6, 11 and 12.

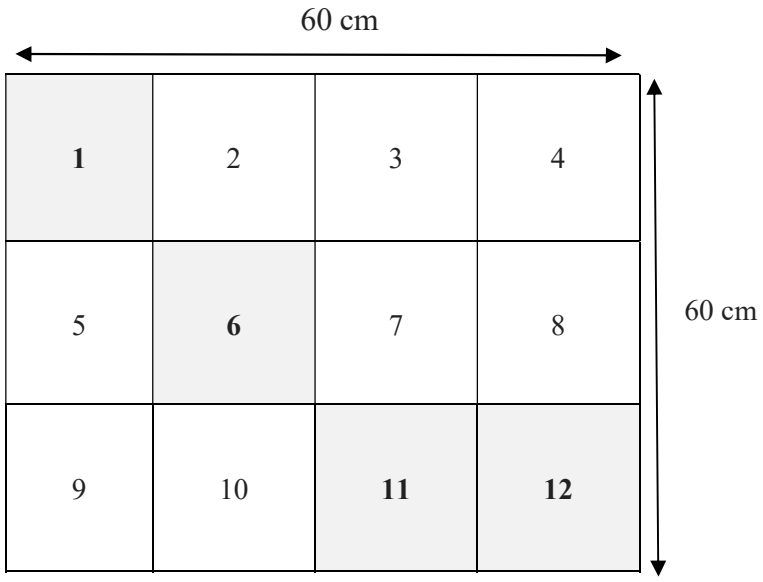


FIG. 1. Top view of sample tray and the placement positions of bagged soy flour samples. Four locations (1, 6, 11 and 12). on the sample tray were used for electron beam irradiation treatments of flat packets of soy flour.

8.2.3.2. Experimental Design

The irradiation process was conducted by using a 10 MeV EB. The dose-variation technique for one-sided irradiation involved delivering 5 kGy dose fractions to the top surface of the sample in each pass through the irradiation zone (one times process). The dose-variation technique for two-sided irradiation involved delivering 10 kGy dose for each two sided treatment, 5 kGy to the top surface and 5 kGy to the bottom surface (two times processes).

One-sided Treatment

Control samples were not irradiated (0 kGy), irradiated samples were exposed to multiple dose fractions of 5 kGy, with each dose fraction being delivered to the top surface of the sample. Samples were irradiated to 5, 10, 15, 20, 25, 30, 40 and 50 kGy corresponding to 1, 2, 3, 4, 5, 6, 8 and 10 passes through the irradiation zone with each pass delivering a dose of 5 kGy (Fig. 2).

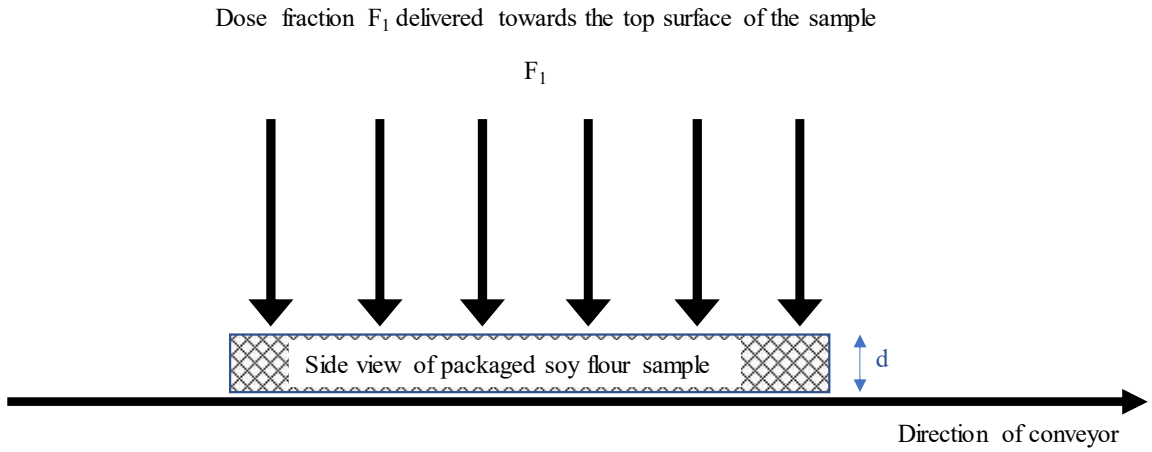


FIG. 2. One-sided irradiation, d is the thickness of the packet of soy flour and F_1 is the dose fraction. Imparted by the electron beam as it is shone onto the top surface of the sample and passes through the soy flour, perpendicular to the direction of travel through the irradiation zone.

Two-sided treatments

Control samples were not irradiated (0 kGy), irradiated samples were exposed to multiple dose fractions of 5 kGy, with each two sided treatment involving two dose fractions: 5 kGy from to the top surface of the sample and 5 kGy to the bottom surface of the sample and therefore 10 kGy in total for each cycle of double sided treatments (Fig. 3). Samples were irradiated to 10 (1 cycle), 20 kGy (four dose fractions, two to the top surface and two to the bottom surface), 30 kGy (six dose fractions, three to the top surface and three to the bottom), 40 kGy (8 dose fractions, four to the top surface and four to the bottom) and 50 kGy (ten dose fractions, five to the top surface and five to the bottom).

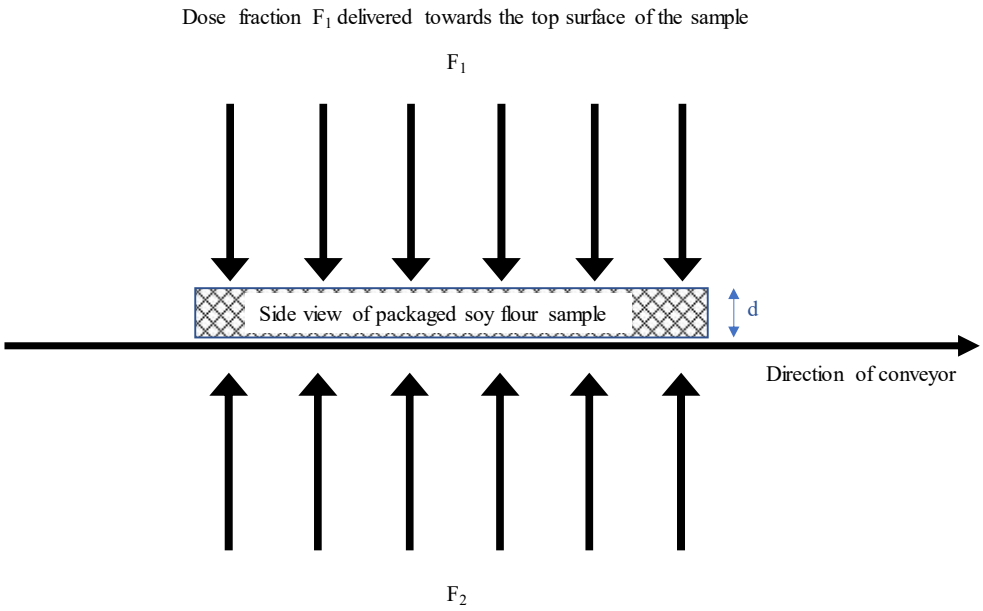


FIG. 3. Two-sided irradiation, d is the thickness of the packet of soy flour and the sample is irradiated by dose fraction F_1 from to the top side and then F_2 to the bottom side (the packet is passed through the irradiation zone, flipped by 180^o and passed a second time through the irradiation zone).

8.2.4. Radiation processing of electron beam

Initial experiments characterized the radiation process using a 10 MeV EB source radiation. The outputs included mapping dose distribution, measuring sample density and thickness, determining the irradiation technique (irradiated position on the tray, and the radiation dose determined from dosimetry). Radiation dose measurement was done using B3WINDOSE Dosimeters (Gex Corporation USA) and a spectrophotometer reader [36]. Results of measurements of the radiation dose received by one-sided and two-sided EB treatments are provided in Table 1. Condition of EBM: The temperature has been measured before irradiation at the positions of soy flour bags placed on the dry tray for EB treatment. The EBM sets up to the energy 10 MeV, PRF 120, conveyor speed 0.290 m/min, frequency of irradiated technique is 5 kGy/fraction, and temperature conditions were 30.90º C (before irradiation 29.90ºC). The samples were irradiated one-sided and two-sided at the beam currents and the gap of the windows-target surface was 20 cm [37].

8.2.5. Irradiation

The sample was placed in the irradiation area with different locations to get irradiation treatment with specific dose. Irradiation treatments were done at room temperature (28 ± 2)°C for each of the samples with the frequency of irradiation fraction (5 kGy/fraction) ranging from 1 to 10 times; depending on its radiation doses.

TABLE 1. RESULTS OF RADIATION DOSE RECEIVED ONE-SIDED AND TWO-SIDED OF EB TREATMENT WAS BY USING B3WINDOSE™ DOSIMETERS[a)]

Required dose (kGy)	Radiation dose	
	One-sided of EB treatment (kGy)	Two-sided of EB treatment (kGy)
5	5.80±0.71[b)]	–
10	11.60±1.41	11.50±0.57 [a)]
15	17.40±2.12	–
20	23.20±2.83	23.00±1.13
25	29.00±3.54	–
30	34.80±4.24	34.50±1.70
40	46.40±5.66	46.00±2.26
50	58.00±7.07	57.50±2.83

[a)] Dose uniformity: One-sided (1.19) and Two-sided (1.07), [b)] Average ± Stdev (kGy)

8.2.6. Determination of phytic acid

The method used to determine phytic acid is reported in the literature [38], Milled aliquotes of 5 g, were taken and extracted with 100 ml of 2.4% HCl. The resulting extracts were centrifuged and the supernatant prepared as 3 ml samples and blanks, to which was added 1 ml Wade reagent. The supernatant was analysed for phytic acid with a spectrophotometer the using absorbance 500 nm.

8.2.7. Determination of colour (white index)

Colour measurements were undertaken according to the Hunter *L, a, b* scale using a Chromameter 200b [39]. The scale is based on the perception of color as a series of three oppositions: black (L=0) to white (*L*=1), red (+*a*) to green (-*a*), and yellow (+*b*) to blue (-*b*). The colour values were expressed as Whiteness Index (WI), as defined below (Equation 1).

$$WI = 100 - \sqrt{L^2 - (a-b)^2} \quad (1)$$

8.2.8. Determination of contents of isoflavone (daidzein and genistein) on irradiated soy flour

Isoflavone concentration was determined according to the method of Wang *et al*. [40]. Isoflavone standard reagent (daidzein, genistein) was purchased from Sigma Chemical Co., Other analytical grade chemical reagents were from different suppliers (methanol HPLC, chloride acid, ammonium acetate from Merck, acetonitrile and water solvent from JT. Baker). The two grams of ground sample was extracted with hexane for 6 hours to remove fat. The isoflavone content of the non-fat sample was analysed by HPLC (the mobile phase was a methanol: ammonium acetate mixture at a flow rate of 1.0 ml/min through a Agilent XDB-C18 column) with a UV detector at a wavelength of 254 nm, and the excitation fluorescence and emission wavelengths used were 365 nm and 418 nm.

8.2.9. Data analysis

Changes in the content of phytic acid and colour (Whiteness Index) of soy flour were tabulated and analysed according to a simple regression model. In general, it is assumed that the rate of change of reactants to products may be described by a simple equation [41, 42]:

$$\text{Reactant} \xrightarrow{k} \text{Products} \quad (2)$$

If ***t*** is the time and n is the reaction order, then the rate of change of reactants into products is as follows:

$$\frac{\delta\,[\text{reactant}]}{\delta\,t} = -k\,[reactant]^n \quad (3)$$

Where: reactant can be either phytic acid concentration or colour (Whiteness Index), and ***k*** is the rate constant (Equation 2), for n = 1, then equation 3 is integrated, resulting in equation 4.

$$Ln\frac{[\text{reactant}]_t}{[\text{reactant}]_o} = -\,k\,t \quad (4)$$

Equation 4 shows that a plot of the natural log of the measured value of reactant at dose fraction ***t***, normalized by the measured value of the reactant in the unirradiated samples (Ln $[\text{reactant}]_t$ / Ln $[\text{reactant}]_0$) against ***t*** the frequency of irradiation fraction (ranging from 1 to 10 fractions each of 5 kGy) would give a straight line with a slope of – ***k***, the rate constant for that specific reactant.

8.3.RESULTS AND DISCUSSION

8.3.1. Results

Changes in physico-chemical properties of soy flour during EB radiation processing were measured. The properties observed in this study are phytic acid content, colour (Whiteness Index), and changes to the concentration of isoflavones. Radiation processing used a high energy EB and samples were irradiated to doses of 5.8 up to 58 kGy (control samples were not irradiated).

8.3.1.1. Anti-Nutritional Compound (Phytic Acid) of Soy Flour

The anti-nutritional compound observed in this study is phytic acid. Tables 2 and 3 show that the initial concentration of phytic acid in the genotype of yellow and black soy flour, control (non-irradiated) is 122.65 mg/g and 98.15 mg/g, respectively. Previous research reported that the phytic acid content of soybean seeds from the yellow genotype (Mitani) was 14.98 mg/g Tanhindarto *et. al.* [12] and Kumar *et.al.* [43] also reported that the phytic acid content of soybean ranged from 9.2 to 16.7 mg/g. The difference in phytic acid contents among soybeans is probably due to differences in the varieties of soy studied, as well as natural variations associated with planting location, growing conditions and harvesting time.

In general, this study showed that EB irradiation degraded phytic acid in both genotypes of yellow and black soy flour. The effect of changes in phytic acid contents in soy flour after radiation process is presented in Fig. 4. These results show that EB irradiation decreases phytic

acid content. A higher rate of decrease of phytic acid content was observed to occur with the one-sided irradiation process than with the two-sided. The changes of phytic acid concentration after both one and two-sided EB irradiation can be compared in Fig.4. where data are plotted according to a simple regression equation (Equation 3) and the slope of the plot gives the rate constant (***k***) for phytic acid loss with one-sided and two-sided irradiation.

From Table 4, it can be seen that at the same radiation dose, the ***k*** value for the degradation of phytic acid content Mutiara variety was greater than the ***k*** value for the degradation of Mitani variety. This indicates that phytic acid in the soy flour from the Mitani variety has a higher resistance to radiation than soy flour from the Mutiara variety.

Phytic acid content declines as a function of radiation dose (Fig. 5). The changes in the phytic acid content of one-sided EB irradiated flour from the Mitani variety (Fig. 5A) are lower than with the two-sided irradiation process. This indicates that the method of delivering the radiation dose (one-sided irradiation or two-sided irradiation) may be an important factor.

In general, this study shows that the process by EBM irradiation of both black (Mutiara) and yellow (Mitani) soy flour can degrade phytic acid. The results of other studies using gamma radiation indicate that phytic acid is converted to inositol and inositol phosphate [25, 28] as the ionizing radiation breaks the ring structure of phytic acid [29].

8.3.1.2. Colour of soy flour

Hunter's colour notation system is characterized by three parameters: L, a, and b. In Tables 5 and 6, the Genotype yellow (Mitani) and black (Mutiara) soy flour colour analysis are expressed as Whiteness Index (WI). The results of this study showed that radiation processing EB causes changes in the brightness of the soy flour. Our experimental results indicate that irradiation can cause browning and lower the brightness or whiteness index. This is also observed with gamma irradiation of some food products, for example gamma irradiated cashew nuts [44] and Atomica IV rice flour [45].

The initial whiteness index of control (non-irradiated) samples of genotype yellow and black soy flour were 51.10 (Table 5) and 52.19 (Table 6) respectively. Previous research reported that the whiteness index of soybean seeds from the genotype Mitani was 54.9 (TANHINDARTO et. al.) [12]. The radiation processes in both genotype yellow and black soy flour of whiteness index showed that the same pattern of decline after irradiated one-sided and two-sided in the range of 5.8 up to 58 kGy. The changes in the whiteness index after EB irradiation of soy flour can be seen in Fig. 6. In general, there is a similar pattern to the degradation of the whiteness index of soy flour both on the one-sided and two-sided surface. The pattern of changes in brightness as a result of irradiation using an EB radiation source can be explained by a first-order reaction model (Fig. 6); with a constant value of the rate of change (***k***) at the irradiation treatment EB is presented in (Table 7).

Soy flour brightness (colour) decreases with increasing doses of radiation for both the one-sided (one times process) and two-sided (two-times process) irradiation processes. This is a similar pattern to the that observed for changes in phytic acid content (Fig. 4). However, colour change as a function of radiation dose (Fig. 7) shows a different pattern from the pattern of changes in phytic acid contents (Fig. 5). In general, there is a tendency that irradiation at two-sided (two-times process) will cause smaller changes in colour brightness. This is an indication because irradiation cannot cause a reaction of carbonyl compounds from sugar to react with amino groups to form brown compounds.

TABLE 2. RESULTS OF ANALYSIS CHANGE IN PHYTIC ACID CONTENTS SOY FLOUR DURING ELECTRON BEAM RADIATION PROCESSING (MITANI VARIETY, GENOTYPE OF YELLOW SOYBEAN)

	One-sided EB irradiation								
Radiation dose (kGy)	**0**	**5.8**	**11.6**	**17.4**	**23.2**	**29.0**	**34.80**	**46.40**	**58.0**
Phytic acid (mg/g)	**98.15±1.83**	**83.01±1.35**	**82.78±5.30**	**76.59±1.91**	**74.68±0.37**	**63.15±2.87**	**53.19±0.94**	**21.49±0.40**	**18.50±0.75**
	Two-sided EB irradiation								
Radiation dose (kGy)	0		11.5		23.0		34.5	46.0	57.5
Phytic acid (mg/g)	98.15±1.83		88.31±0.35		86.24±1.71		74.09±1.33	66.47±0.92	45.21±0.54

TABLE 3. RESULTS OF ANALYSIS CHANGES IN PHYTIC ACID CONTENTS SOY FLOUR DURING ELECTRON BEAM RADIATION PROCESSING (MUTIARA VARIETY, GENOTYPE OF BLACK SOYBEAN)

	One-sided EB irradiation								
Radiation dose (kGy)	**0**	**5.8**	**11.6**	**17.4**	**23.2**	**29.0**	**34.80**	**46.40**	**58.0**
Phytic acid (mg/g)	**122.65± 2.83**	**80.76± 1.66**	**74.21±2.12**	**65.49±1.14**	**32.68±3.74**	**20.35±1.91**	**14.94±2.50**	**6.18±2.91**	**3.63±3.85**
	Two-sided EB irradiation								
Radiation dose (kGy)	0		11.5		23.0		34.5	46.0	57.5
Phytic acid (mg/g)	122.65±2.83		63.85±2.70		27.60±0.60		24.16±7.05	13.12±2.70	11.44±5.91

TABLE 4. THE *K* AND R^2 VALUES OF THE CHANGE IN PHYTIC ACID CONTENT OF SOY FLOUR MADE FROM TWO VARIETIES OF SOYBEAN DURING THE ELECTRON BEAM RADIATION PROCESS AT THE EB IRRADIATION TREATMENT

Variety	EB irradiation treatment	***k*** value [a] (dose fraction^{-1})	r^2 value
Mitani	One-sided	0.1454	0.9202
	Two-sided	0.0604	0.9332
Mutiara	One-sided	0.3502	0.9916
	Two-sided	0.2665	0.9816

[a] The ***k*** value is per 5 kGy dose fraction

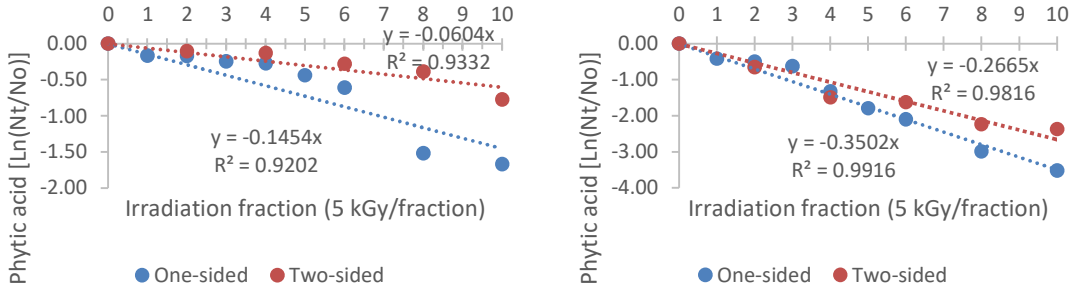


FIG. 4. The changes of the phytic acid content of Mitani (a) and Mutiara (b) soy flour that the irradiated one-sided and two-sided EB during the radiation dose.

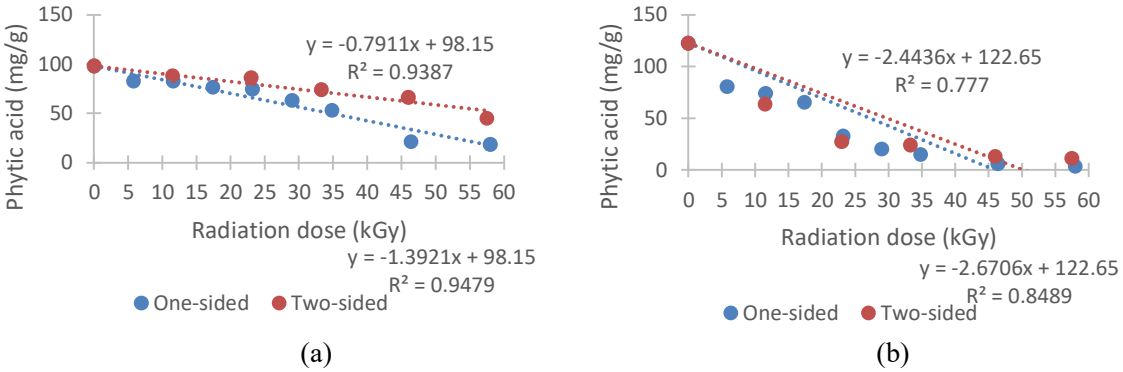


FIG. 5. The changes of phytic acid content of Mitani (a) and Mutiara (b) soy flour as a function of the radiation dose.

TABLE 5. RESULTS OF ANALYSIS CHANGE IN COLOUR (WHITENESS INDEX) SOY FLOUR DURING ELECTRON BEAM RADIATION PROCESSING (MITANI VARIETY, GENOTYPE OF YELLOW SOYBEAN)

	One-sided EB Radiation dose (kGy)								
	0	**5.8**	**11.6**	**17.4**	**23.2**	**29.0**	**34.80**	**46.40**	**58.0**
Colour (Whiteness index)	**51.10±0.20**	**50.97±0.18**	**50.79±0.45**	**50.66±0.30**	**50.11±0.08**	**50.00±0.30**	**49.70±0.17**	**49.37±0.10**	**48.71±0.28**
	Two-sided EB Radiation dose (kGy)								
	0		11.5		23.0		34.5	46.0	57.5
Colour (Whiteness index)	51.10±0.20		50.97±0.18		50.79±0.45		50.66±0.30	50.11±0.08	50.00±0.30

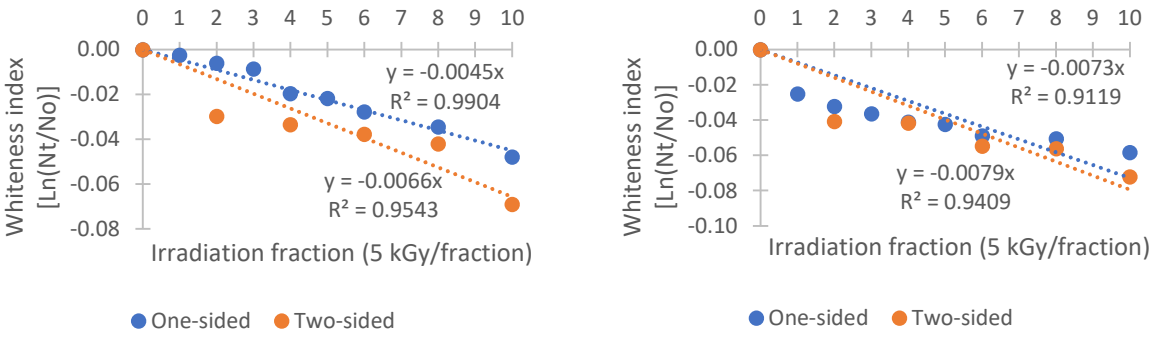
TABLE 6. RESULTS OF ANALYSIS CHANGE IN COLOUR (WHITENESS INDEX) SOY FLOUR DURING ELECTRON BEAM RADIATION PROCESSING (MUTIARA VARIETY, GENOTYPE OF BLACK SOYBEAN)

	One-sided EB Radiation dose (kGy)								
	0	**5.8**	**11.6**	**17.4**	**23.2**	**29.0**	**34.80**	**46.40**	**58.0**
Colour (Whiteness index)	**52.19±0.14**	**50.90±0.35**	**50.54±0.30**	**50.33±0.27**	**50.09±0.28**	**50.03±0.45**	**49.70±0.10**	**49.62±0.21**	**48.56±1.84**
	Two-sided EB Radiation dose (kGy)								
	0		11.5		23.0		34.5	46.0	57.5
Colour (Whiteness index)	52.19±0.14		50.90±0.35		50.54±0.30		50.33±0.27	50.09±0.28	50.03±0.45

TABLE 7. THE *K* AND R^2 VALUES OF THE CHANGE IN COLOUR (WHITENESS INDEX) DURING THE ELECTRON BEAM RADIATION PROCESS AT THE EB IRRADIATION TREATMENT

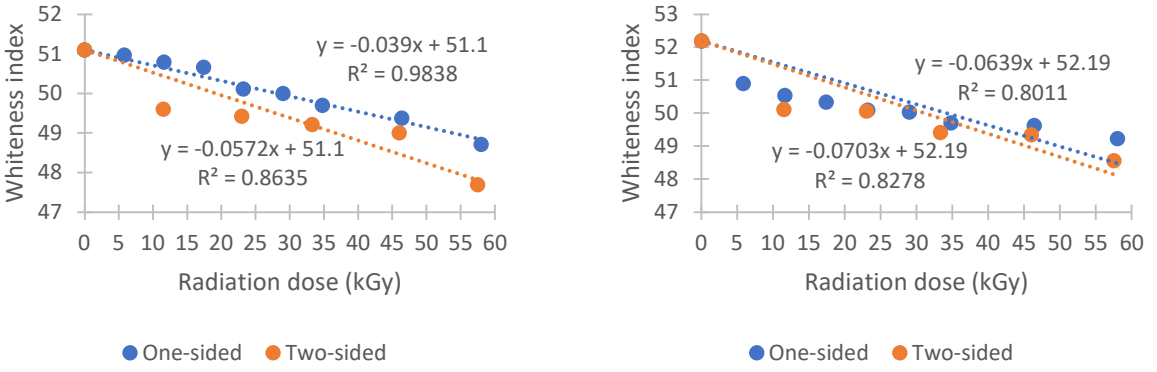
Variety	EB irradiation treatment	***k*** value [a] (dose fraction^{-1})	r^2 value
Mitani	One-sided	0.0045	0.9904
	Two-sided	0.0066	0.9543
Mutiara	One-sided	0.0073	0.9119
	Two-sided	0.0079	0.9409

[a] The k value is per 5 kGy dose fraction.



(a) (b)

FIG. 6. The change in whiteness index of flour made from (a) Mitani and (b) Mutiara soybean with one-sided and two-sided EB irradiation delivered as dose fractions.



(a) (b)

FIG. 7. Change in the whiteness index of flour made from (a) Mitani and (b) Mutiara soybean as a function of the total radiation dose.

8.3.1.3. Isoflavones contents of soy flour

High energy EB irradiation resulted in changes to free-isoflavones. The free-daidzein and free-genistein content of unirradiated (control) and irradiated samples of flour made from two

varieties of soy are given in Tables 8, 9, 10 and 11. These data include the results for radiation processed samples using one-sided and two-sided irradiation to doses ranging from 5.8 to 58 kGy.

The initial content of isoflavones in unirradiated Mitani variety soy flour samples used in this study were 11.58 μg/g and 35.12 μg/g for daidzein and genistein respectively. Unirradiated samples of soy flour made from the Mutiara variety of soy were found to contain 12.48 μg/g and 33.98 μg/g of daidzein and genistein respectively. Our previous research reported that free daidzein and genistein contents in the same variety soybean of Mitani were 81.39 μg/g and 62.27 μg/g, [11] and variety soybean of Mutiara were 13.99 μg/g and 54.80 μg/g, respectively [13]. Wang et al. [40] reported that free daidzein contents in soybean were 25.6 μg/g and for free genistein were 28.4 μg/g. Differences in reported soybean isoflavone content is probably due to differences associated with the varieties, planting location, and harvesting time.

The effect of EB irradiation on isoflavones in soy flour are presented in Fig. 8. The results indicate that dose fractions delivered by one-side EB irradiation have a different pattern of changes for isoflavones content than dose fractions delivered by two-side EB irradiation. In general, there is an increase of daidzein and genistein concentration (Fig. 8), especially, at the beginning of radiation processing when the first few dose fractions were delivered. Furthermore, a higher increase of daidzein and genistein content occurs in the irradiation process with one-sided irradiation than with two-sided irradiation. However, one-sided and two-sided irradiation under the conditions used in this study show significant differences for the production of free daidzein and free genistein in flour made from both Mitani (Genotype Yellow Soybean) and Mutiara (Genotype Black Soybean) varieties after the samples were irradiated with 3 or 4 dose fractions of 5 kGy (15 to 20 kGy).

Fig. 9 showed how isoflavone changes as a function of radiation dose. Soy flour irradiated at the absorbed dose has a higher concentration of free daidzein and free genistein as compare to those irradiated at one-sided. Soy flour irradiated from one-sided showed an increase in free daidzein as compare to samples of flour irradiated to the same dose but with the dose fractions delivered on two opposites sides of the sample (Fig. 9a and 9b). A similar pattern was also observed for changes in free genistein (Fig. 9d) for flour derived from the Mutiara variety of soy. In general, there is a strong indication, especially at the beginning of radiation up to the dose level 5.8 up to 20 kGy, that one-sided irradiation treatment results in higher concentrations of free daidzein and free genistein as compared to that of the two-sided irradiation process. Fig. 9c shows patterns of concentration change of free genistein as a function of radiation dose. No significant reduction of the free-genistein isoflavone was observed, but there is an indication that levels of free-genistein were slightly increased by an irradiation dose of 10 kGy.

Our results (Fig. 9) suggest that one-sided and two-sided irradiation decompose the glycoside isoflavone into aglycone. A similar phenomenon was reported by Kao *et al*. [46] at which soaking of soybean at the higher temperatures would cause more decomposition of acetyl-glucoside and glucose- and malonyl-glucoside isoflavone into its aglycone. The isoflavone increase was also reported by Variayar *et al*. [20], where research showed that gamma irradiation (0.5–5 kGy) of soybean would potentially increase the aglycone isoflavone. In general, Fig. 9 also show that irradiated on both techniques (one- and two-sided) at a certain dose level had increased free isoflavone concentrations.

TABLE 8. RESULTS OF ANALYSIS CHANGES IN CONTENTS OF ISOFLAVONES (DAIDZEIN) SOY FLOUR DURING ELECTRON BEAM RADIATION PROCESSING (GENOTYPE OF YELLOW SOYBEAN)

One-sided EB	Radiation dose (kGy)								
	0	5.8	11.6	17.4	23.2	29.0	34.80	46.40	58.0
Daidzein (ug/g)	11.58±0.31	15.21±0.14	34.58±0.45	39.22±0.75	23.79±0.77	24.54±1.07	22.75±0.39	20.85±1.51	19.87±0.23
Two-sided EB	Radiation dose (kGy)								
	0		11.5		23.0		34.5	46.0	57.5
Aidzein (ug/g)	11.58±0.31		33.32±1.08		25.59±0.64		14.51±1.61	10.95±0.26	8.17±0.78

TABLE 9. RESULTS OF ANALYSIS CHANGES IN CONTENTS OF ISOFLAVONES (GENISTEIN) SOY FLOUR DURING ELECTRON BEAM RADIATION PROCESSING (GENOTYPE OF YELLOW SOYBEAN)

One-sided EB	Radiation dose (kGy)								
	0	5.8	11.6	17.4	23.2	29.0	34.80	46.40	58.0
Genistein (ug/g)	35.12±0.14	30.78±2.61	50.41±0.50	31.21±0.17	31.81±0.99	35.62±3.11	35.39±2.27	37.96±0.14	33.69±0.34
Two-sided EB	Radiation dose (kGy)								
	0		11.5		23.0		34.5	46.0	57.5
Genistein (ug/g)	35.12 ±0.14		46.95±0.82		37.87±0.09		36.40±2.99	33.91±0.30	27.94±0.62

TABLE 10. RESULTS OF ANALYSIS CHANGES IN CONTENTS OF ISOFLAVONES (DAIDZEIN) SOY FLOUR DURING ELECTRON BEAM RADIATION PROCESSING (GENOTYPE OF BLACK SOYBEAN)

One-sided EB	Radiation dose (kGy)								
	0	5.8	11.6	17.4	23.2	29.0	34.80	46.40	58.0
Daidzein (ug/g)	12.48±0.24	21.70±0.61	25.37±1.52	41.72 ±0.45	55.06±0.67	41.24±0.53	51.87±0.91	49.50±0.84	41.24±0.28
Two-sided EB	Radiation dose (kGy)								
	0		11.5		23.0		34.5	46.0	57.5
Daidzein (ug/g)	12.48±0.24		23.10±0.39		20.22±0.75		19.81±0.60	15.50±0.28	10.40 ±0.24

TABLE 11. RESULTS OF ANALYSIS CHANGES IN CONTENTS OF ISOFLAVONES (GENISTEIN) SOY FLOUR DURING ELECTRON BEAM RADIATION PROCESSING (GENOTYPE OF BLACK SOYBEAN)

One-sided EB	Radiation dose (kGy)								
	0	5.8	11.6	17.4	23.2	29.0	34.80	46.40	58.0
Genistein (ug/g)	33.98±0.39	42.77±0.50	52.96±1.41	53.38±1.81	82.32±0.98	79.46±0.84	87.80±0.95	72.12±1.17	64.30±2.78
Two-sided EB	Radiation dose (kGy)								
	0		11.5		23.0		34.5	46.0	57.5
Genistein (ug/g)	33.98±0.39		64.84±2.73		53.58±2.25		42.30±0.36	39.65±0.81	35.80±2.73

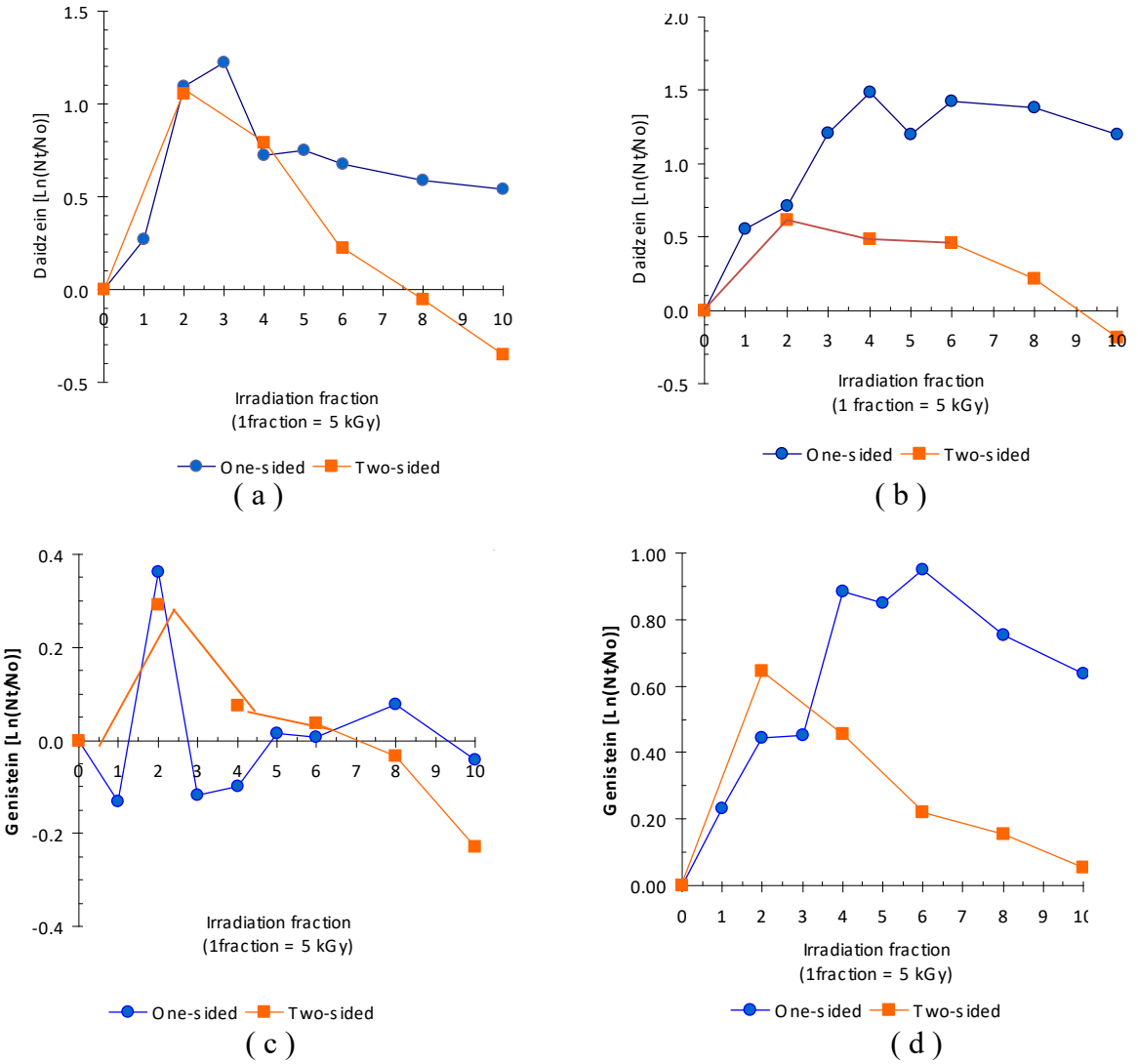


FIG. 8. Changes of isoflavones in flour made from Mitani and Mutiara soybean following one-sided EB irradiation and two-sided EB irradiation: (a) the daidzein isoflavone (Mitani variety soybean); (b) daidzein (Mutiara variety soybean); (c) the genistein isoflavone (Mitani variety soybean); and (d) genistein (Mutiara variety soybean).

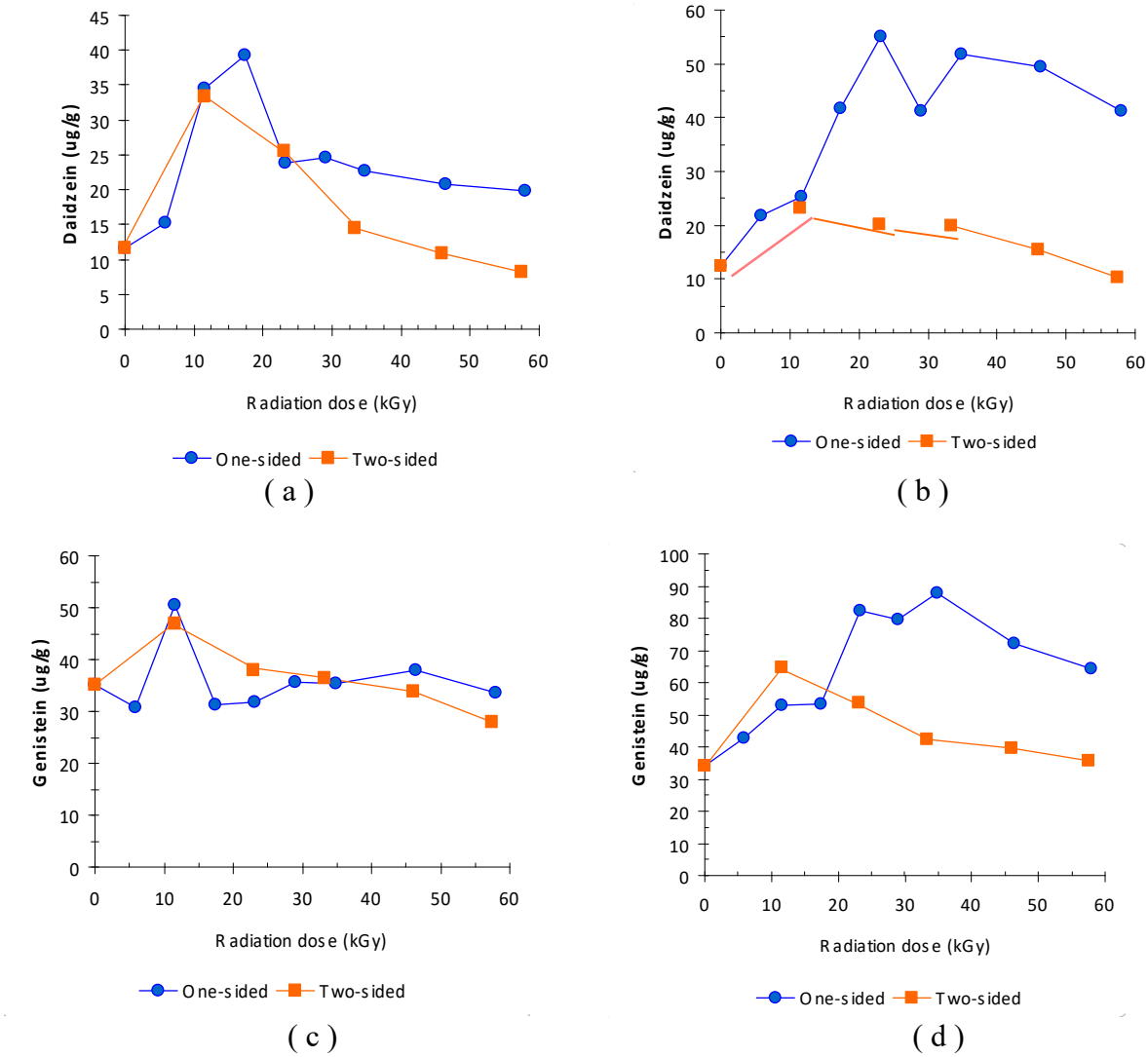


FIG. 9. The change in content of daidzen and genistein isoflavones for soy flour made from the Mitani and Mutiara varieties of soybean as a function of radiation dose: (a) is daidzein (Mitani variety); (b) daidzein (Mutiara varieity); (c) genistein (Mitani variety); (d) genistein (Mutiara variety).

8.3.2. Discussion

Anti-Nutritional Compound (Phytic Acid), Colour (Whiteness Index), and the Isoflavones Contents of Soy Flour.

Irradiation can be applied as a treatment to reduce the content of anti-nutritional compounds and improve the functional properties of food products such as seeds and nuts [8, 9, 28]. Gamma irradiation at a dose of 30 kGy has been used to improve soybean functional properties, such as solubility, emulsion activity, foam stability in protein isolates, and increasing yield [10].

In this study, changes in the content of certain chemical components due to the irradiation process was found to be highly dependent on EB radiation dose. The relationship between radiation dose and changes in the content of certain chemical compounds has been widely reported.

In general, the concentration of certain food components will change during the radiation process, while the rate of change (***k*** value) is influenced by the method of dose fractionation used (e.g. one-sided or two-sided irradiation). From the data presented in Tables 4 and 7, the

relationship between the one-sided irradiation (one times process) and two-sided irradiation (two-times process) and the rate of change ***k*** (expressed as ***k*** values) for phytic acid content and brightness (colour) of soy flour. It can be seen that the ***k*** value for the change in brightness (Whiteness Index) is much lower than the ***k*** value for change in phytic acid (anti-nutrient) content. These results indicate the potential for selecting the optimum irradiation processing conditions (dose, one-sided or two-sided irradiation) in order to maximize the reduction of anti-nutritional compounds such as phytic acid whilst minimising to loss in colour (whiteness index).

The ***k*** values for colour change (Whiteness Index) brought about by irradiation presented in Table 7 are smaller than the ***k*** values for phytic acid reduction given in Table 4. Indicating that irradiation has less impact on colour change than on reduction in phytic acid. Also, the differences in k values for one sided and two-sided irradiation in Table 4 indicate that reduction in phytic acid content is more sensitive to delivering dose fractions from one side only or from two sides. One sided irradiation seems to have been more efficient than two-sided irradiation at reducing phytic acid content (especially in flour made from the Mitani variety of soy). This information will be useful for optimizing the radiation process, by selecting a combination of one-sided or two-sided irradiation treatments and the irradiation faction that would give an optimum reduction in phytic acid content (an intended purpose of the treatment) yet minimum reduction in the Whiteness Index of the flour (a change in a quality parameter that is not desired). In practice, it shows that the irradiation of the one-sided or two-sided surfaces and ***k***-value are important parameters of change during irradiation and both must be considered in the design of the radiation process.

Each food component behaves differently due to the effect of irradiation, so it is very useful to know the pattern of changes in quality due to irradiation. In general, changes in isoflavones due to irradiation doses can degrade free isoflavones in soybeans. Irradiation can break the chemical structure of isoflavones in the form of malonyl glucosides into aglycones through decarboxylation, de-esterification, and hydrolysis reactions. However, this breakdown process will depend on the radiation dose used to break down the chemical structure. This breakdown also occurs when soybeans undergo processing during processing, such as heating [47].

From the results of the study, the pattern of changes in free isoflavones (daidzein and genistein) between differences in irradiation dose, fractionation, and time of exposure in EB irradiation showed the same pattern of changes. At the beginning of irradiation, the free isoflavone content at low doses will increase if 1 (one) fraction is irradiated (1 fraction = 5 kGy), then the free isoflavone content decreases in the irradiation range up to 4 times fraction and then followed by a constant pattern and tended to decrease.

Although the results for samples of flour produced from the soy variety Mitani showed that levels of the genistein isoflavone tended to remain constant with increasing radiation doses (Fig, 9c). The general pattern of change was that the levels of free isoflavones (daidzein and genistein) initially increased as a function of radiation dose but leveled off or reduced at high doses (Fig. 9a, 9b and 9d.). Several research studies on the irradiation of polyphenol compounds have shown that irradiation treatment of up to 8 kGy on soybeans from 5 different varieties show an increase in total phenol content [48]. Bhat *et al.* [27] reported that *Mucuna pruriens* seeds irradiated at a dose of 2.5 to 30 kGy experienced a significant increase in phenols. The results of studies of various phytochemicals and antioxidant-producing plants showed that phenolic compounds increased due to irradiation [49].

Our results indicate that the selection of radiation dose for treatment is a significant factor when developing a functionalized food product. The experiments indicated that dose fractionation and applying doses using a one-sided irradiation process was more effective in destroying anti-nutritional compounds (phytic acid) as compared to that of the two-sided irradiation process. There is a good indication that irradiation of soy flour with dose fractions of approximately 5 kGy to total irradiation treatment doses of up to 20 kGy may potentially be used to promote free-daidzein and free-genistein content and remove phytic acid. Besides the radiation dosage, altering the irradiation technique, (e.g. one-sided or two sided irradiation) may be an important factor in the radiation process. Consequently, irradiation can be used as a tool to control levels of free isoflavones in foods like soy flour, especially concerning free daidzein and free-genistein.

8.4.CONCLUSIONS

The results presented in this research report concerns the use of EB radiation processing (one-sided and two-sided) of Mitani and Mutiara varieties of soy flour with radiation doses from 5.8 kGy to 58 kGy. This research can be concluded as follows:

1. Irradiation can reduce anti-nutritional compounds (phytic acid) but modify other quality characteristics of soy flour, such as whiteness;
2. Besides doses, the irradiation techniques (one-sided and two-sided irradiation) may be important factors to control and optimize the radiation process by using EB irradiation;
3. Irradiation delivered as a one-sided treatment seems to reduce phytic acid content more effectively than two-sided irradiation under our experimental conditions and provided better colour brightness than two-sided irradiation;
4. There is a good indication that irradiation of soy flour with the dose range from 5 to 20 kGy may potentially be used to produce soy flour that is a functional food product by promoting levels of free-daidzein and genistein and reducing levels of phytic acid.

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X Ray Irradiation

9. KILO-VOLTAGE X RAY DOSIMETRY USING ALANINE/EPR DOSIMETRY SYSTEMS

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Abstract

Nowadays, low to medium energy X ray irradiators are starting to replace irradiators that use radioactive sources, mainly in the fields of blood irradiations, Sterile Insect Technique and food irradiation. A dosimeter is placed on the irradiated product to ensure that the desired dose is delivered to the product. One of the dosimetry systems that is widely used in radiation processing is the alanine / electron paramagnetic resonance spectroscopy (EPR) dosimetry system. Alanine is considered as water equivalent, from a dosimetric point of view for photon energies that are higher than 200 keV. However, it loses its water equivalency for lower photon energies. This work presents the use of alanine dosimetry for the control and validation of irradiation processes performed with low to medium energy X rays, as well as different methods developed to determine corrective factors to be applied to the response of this dosimeter. These methods are based on experimental measurements, Monte Carlo simulations and analytical calculations.

9.1.INTRODUCTION

Low to medium energy X ray (up to 300 keV) irradiators are starting to replace radionuclide source irradiators that use gamma emitting radioactive sources such as cesium (^{137}Cs) or cobalt (^{60}Co) [1, 2], mainly in the fields of blood irradiations [3], Sterile Insect Technique (SIT) [4] and food irradiation [5, 6]. This drift towards low energy X ray generators is mainly driven by the difficulty of acquiring and transporting radioactive sources, as well as the reassuring radiation safety advantages that self-shielded X ray irradiators provide to its operators.

Alanine/EPR (Electron Paramagnetic Resonance) dosimetry systems are used for reference, transfer and routine dosimetry. Their very stable and reproducible measurement insures a low uncertainty on absorbed dose determination. Yet, for low to medium energy X rays, it has been reported in many studies [7–13] that alanine's response with respect to water is lower than unity in contrast to photon energies higher than 200 keV.

Khoury *et al* [9] determined the response of Aerial's alanine dosimeters irradiated with a 125 kV X ray spectrum, with respect to dosimeters irradiated with ^{60}Co gamma rays (average photon energy of 1.25 MeV). Results showed that the ratio of alanine's response to ^{60}Co with respect to X rays is equal to 1.2.

Anton *et al* [7] carried out Monte Carlo calculations as well as experimental measurements, in order to study alanine's relative response to medium energy X rays, with tube potentials ranging from 30 to 280 kV. Their work showed that alanine's response drops to 64% for an irradiation at 30 kV, compared to a 100% response in the case of ^{60}Co beam.

Waldeland *et al* [11, 12] studied the dose to water energy dependence of alanine dosimeters. Monte Carlo and experimental measurements were carried out with tube voltages ranging from 50 to 200 kV. Experimental measurements showed that alanine's response in the case of X ray irradiations with respect to ^{60}Co beams varied from 0.68 to 0.9 at 50 kV and 200 kV respectively.

This work aims to develop three different methods to study the alanine dosimeter's relative response to kilo-voltage X rays, compared to ^{60}Co gamma rays. If the EPR dosimetry system is calibrated with high energy photons (gamma or X rays) or electron beams, absorbed dose to water measurements with alanine dosimeters could be underestimated when used for kilo-voltage (kV) X ray irradiations.

9.2.ADOPTED APPROACHES

X ray tubes generate a continuum of X rays having different energies; thus, it is more complicated to determine a correction factor that could be applied to the alanine's response based of mass-energy absorption coefficients, compared to irradiations with mono-energetic photon irradiations. However, in this work, one of the studied methods that determines alanine's relative response to kV X rays compared to ^{60}Co is based on mass-energy absorption coefficients.

To adapt the use of Aerial's alanine/EPR dosimetry system for kV X ray applications, the relative response of Aerial's alanine dosimeters needed to be well characterized, in order to determine correction factors that can be applied to the alanine's EPR response for a precise absorbed dose to water measurement. Thus, three different methods were studied in this work to determine Aerial's alanine dosimeter response to different kV X ray beam qualities, compared to ^{60}Co reference beam quality. Fig. 1 shows a diagram that summarizes the logic that was adopted in each method to determine the relative response of alanine dosimeters to kV X ray beam qualities Q compared to a reference beam quality Q_0.

The first method is based on direct experimental measurements, the second method is based on Monte Carlo calculations and the third method, which brings all the novelty to this work, involved analytical calculations of the alanine dosimeters relative response to kV X rays, with results that were found to be in a good agreement with Monte Carlo simulations, yet, the analytical calculations are much faster (few seconds) than running time for Monte Carlo simulations (few hours).

9.2.1. Experimental measurements

The alanine's relative response is defined as the ratio of the dosimeter's EPR response per unit of absorbed dose to water, for a specific X ray beam quality compared to the same ratio for a ^{60}Co reference beam quality. It is expressed as such (Equation 1):

$$f_{exp}^{Q,Q_0} = \frac{(r/D_w)^Q}{(r/D_w)^{Q_0}} \quad (1)$$

where f_{exp} is the relative response of the alanine dosimeter to an X ray beam quality Q compared to a reference beam quality Q_0, r is the dosimeter's EPR response and D_w is the absorbed dose to water delivered to the alanine dosimeter. The value of the delivered absorbed dose to water is determined by ion chamber measurements.

Alanine reference dosimeters, as normally used for the calibration of Aerial's alanine/EPR dosimetry system, were used to establish the calibration curve for ^{60}Co reference beam quality. The reference dosimeters were irradiated at the National Physical Laboratory (NPL, Teddington, UK).

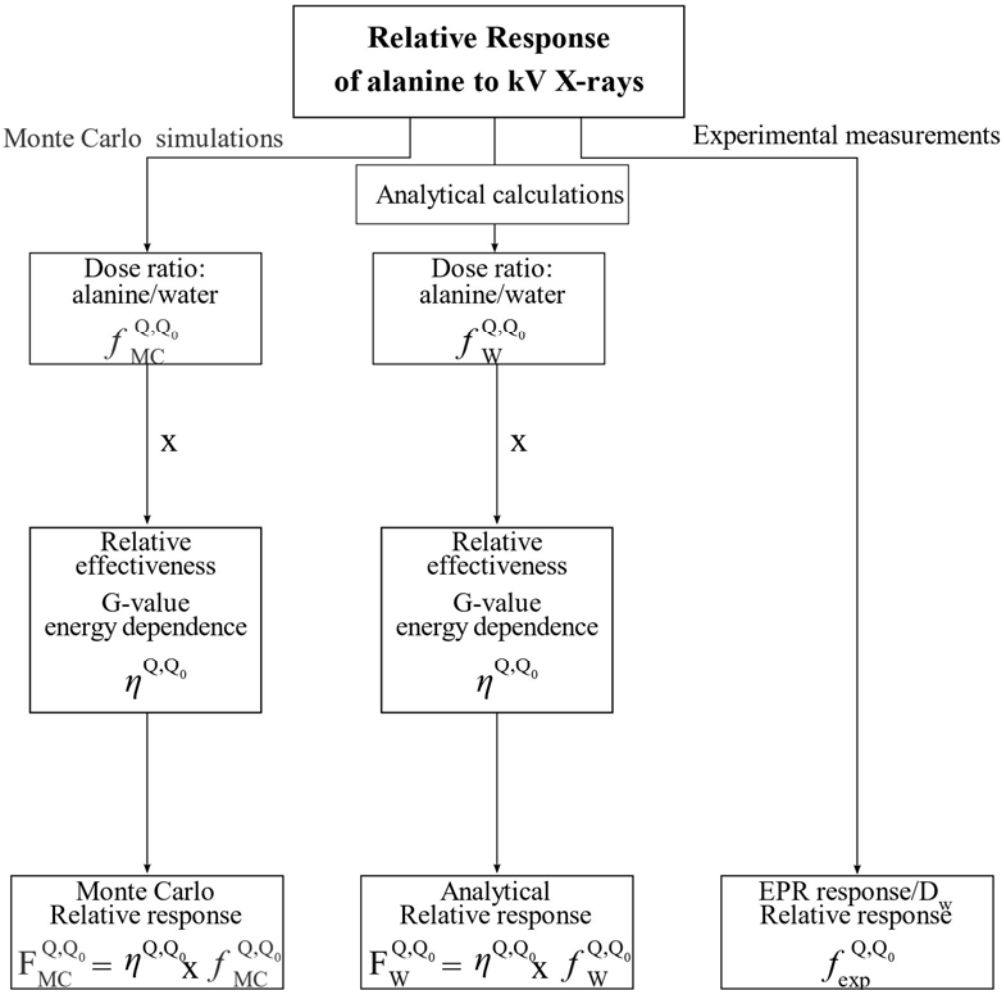

FIG. 1. Diagram of the three studied methods to determine the relative response of alanine to kv X rays.

9.2.1.1. Alanine dosimeters and EPR readout

Aerial's commercial alanine dosimeters (Lot 09/11) were used in this work. Alanine pellets have a 4 mm diameter, a thickness of 2.35 mm and an average mass of 36.05 ± 0.05 mg, with a chemical composition of 91.63% of pure L-α-alanine, 6.37% of EUDRAGIT NE 30D and 2% MYVATEX.

EPR readout was performed using a Freiberg Instruments Magnettech Miniscope MS5000 spectrometer (Freiberg, Germany) using the following parameters: magnetic field sweep width of 2 mT, sweep time of 5 seconds, modulation amplitude and frequency of 0.7 mT and 100 kHz respectively, microwave power and frequency of 10 mW and 9.253 GHz respectively. Measured spectra were taken as input in the aerede software, developed and commercialized by Aerial, in order to correct the dosimeter's response with irradiation temperature.

9.2.1.2. X ray irradiations

Two sets of irradiations were carried out in this study. The first set was performed at Aerial where the absorbed dose to water was measured by a calibrated (D_w calibration for kV X rays) PTW 30013 Farmer ion chamber. The second set of irradiations was carried out at NPL, where a calibrated (air kerma ka calibration for kV X rays) PTW 30012 Farmer ion chamber was used to measure absorbed dose to water. Table 1 lists the beam qualities that were used in this study. For each beam quality, high voltage (HV) and external added filtration are listed, as well as two different beam specifiers: first half value layer (HVL_1) in aluminum, and the beam's effective energy (E_{eff}) in aluminum.

TABLE 1. LIST OF USED X RAY BEAM QUALITIES

Irradiation site	High Voltage (kV)	Added external filtration material (mm)					*HVL1* [(a)] (mm)	E_{eff} [(b)] (keV)
		Al	Cu	Sn	Solid Water	PMMA		
Aerial	50	2.39	0	0	0	5	1.81	27.5
Aerial	70	2.88	0	0	0	5	2.65	31.9
Aerial	90	3.35	0	0	0	5	3.64	36.3
Aerial	100	1.43	0	0	0	5	2.57	31.5
Aerial	100	3.84	0	0	0	5	4.32	39.2
Aerial	100	4.95	0	0	0	5	4.93	41.7
Aerial	90	0.96	0	0	0	0	1.52	25.8
Aerial	100	1.43	0	0	0	0	2.18	29.5
Aerial	100	3.84	0	0	0	0	4.06	38.1
NPL	135	1.2	0.27	0	20	0	9.01	58.9
NPL	280	1	0.26	1.5	20	0	19.6	168

[(a)] First half value layer in aluminium
[(b)] The effective energy in aluminium

The effective energy of the X ray spectrum is considered as the quality of the X ray beams, according to the recommendations of the International Atomic Energy Agency (IAEA) according to Andreo *et al* [14]. This quantity is defined as the energy of a monoenergetic beam of photons having the same half-attenuation layer (HVL_1) of a polyenergetic spectrum. Values of beam quality specifiers were calculated using SpekCalc [15], based on the properties of the X ray tubes.

For irradiations carried out at Aerial, an uncertainty budget of 2.9% (k=1) was established, whilst the established uncertainty budget for irradiations carried out at NPL was equal to 2.38% (k=1). These budgets accounted for uncertainties concerning alanine/EPR measurements as well as the absorbed dose to water measurements using the calibrated ion chambers.

9.2.2. Monte Carlo simulations

The MCNPX Monte Carlo simulation code (version 2.7) was used to calculate the alanine dosimeter's relative response for the beam qualities listed in Table 1. The true irradiation

geometries (for both Aerial and NPL irradiation sets) were reproduced in the simulation model in order to have comparable results with the experimental measurements.

This method makes it possible to accurately reproduce the irradiation geometry, then generate events (representing the X rays) and finally model the interaction of the X rays with a sensitive volume: the alanine dosimeter, and an equivalent volume of water material. The determined factor is the following (Equation 2):

$$f_{MC}^{Q,Q_0} = \frac{\left(\frac{D_{dos}}{D_w}\right)^{Q}}{\left(\frac{D_{dos}}{D_w}\right)^{Q_0}} \quad (2)$$

where f_{MC} is the alanine to water dose ratio, for an X ray beam quality Q relatively to a reference beam quality Q_0, determined by Monte Carlo simulations and D_{dos} is the absorbed dose to the dosimeter. For ^{60}Co reference beam irradiations, a punctual ^{60}Co gamma source was modelled emitting gamma rays isotropically. Alanine and water volumes were placed in the same configuration as the one reproducing X ray irradiations carried out at NPL, for ^{60}Co reference beam irradiations. For each simulation, 10^9 primary photons were generated in order to obtain a low statistical uncertainty on calculated doses. The standard uncertainty for the calculation of the f_{MC} factor is estimated to be equal to 1.4% (k=1).

Monte Carlo simulations modelled only the physical interaction of X rays with alanine and water materials, which leads to the determination of a physical absorbed dose. Yet, the free radical generation processes in alanine are not modelled in these simulations, thus, an additional factor (the relative effectiveness of alanine) needs to be determined more precisely, and then applied to results obtained with simulations. The simulation run-time varied from half an hour up to two hours depending on the simulation geometry and the beam quality studied. A PC with an Intel Xeon E5-2620 V4 processor (8 cores, 16 threads, 2.1 GHz base frequency) was used to run these simulations.

9.2.3. Analytical calculations

This method relies on the calculation of the ratio of absorbed dose in alanine dosimeter to absorbed dose in water, for a specific X ray spectrum, compared to the same ratio for a reference beam quality irradiation. This calculation is based on mass-energy absorption coefficients tabulated by the U.S. National Institute of Standards and Technology (NIST) [16]. Energy spectra of all beam qualities, listed in Table 1, were calculated by SpekCalc [15]. Each spectrum was weighted by mass-energy absorption coefficients given by NIST [16].

The following three equations represent the alanine to water dose ratio calculated for an X ray beam quality Q (Equation 3), a ^{60}Co reference beam quality Q_0 (Equation 4) and the final calculated factor $f_{Q;Q0}$ (Equation 5):

$$f_W^Q = \frac{\int_0^{E_{max}} \left(\frac{\mu_{en}(E)}{\rho}\right)_{dos} . E . \phi(E) . dE}{\int_0^{E_{max}} \left(\frac{\mu_{en}(E)}{\rho}\right)_{w} . E . \phi(E) . dE} \times \frac{\left(e^{-\bar{\mu}_{att}^{Q} . x}\right)_{dos}}{\left(e^{-\bar{\mu}_{att}^{Q} . x}\right)_{w}} \quad (3)$$

$$f_W^{Q_0} = \frac{\left(\frac{\mu_{en}(^{60}Co)}{\rho}\right)_{dos}}{\left(\frac{\mu_{en}(^{60}Co)}{\rho}\right)_{w}} \times \frac{\left(e^{-\mu_{att}(^{60}Co) . x}\right)_{dos}}{\left(e^{-\mu_{att}(^{60}Co) . x}\right)_{w}} \quad (4)$$

$$f_W^{Q,Q_0} = \frac{f_W^Q}{f_W^{Q_0}} \quad (5)$$

where E_{max} is the maximum energy of the X ray spectrum, $\mu_{en}(E)/\rho$ are the mass-energy absorption coefficients given by NIST at the photon energy E for the dosimeter or water materials, $\emptyset(E)$ is the photon spatial fluence value at the photon energy E, μ_{att} is the average attenuation coefficient of the X ray spectrum of quality Q and x is the dosimeter thickness.

To better simulate the photon interaction probability in the dosimeter and water volumes, the contribution of photon attenuation in 2.35 mm of dosimeter or water thickness was considered, by adding the second fraction present in the first two equations. This factor calculates the ratio of the attenuation percentage of incident photons in the dosimeter and water material, thus better representing the interaction probability of photons in these media. The standard uncertainty for the calculation of the f_W factor is equal to 2.1% (k=1).

A C++ code was developed during this work to analytically calculate the f_W factor. The code takes as entry the energy distribution spectra calculated by SpekCalc for each X ray beam quality. Each X ray energy distribution is then weighted by calculated mass-energy absorption and attenuation coefficients, for both dosimeter and water materials. After that, the same f_W factor is calculated for a ^{60}Co source, and finally the dose ratio of the dosimeter to water is calculated for each X ray beam quality. The code was executed on the same processor as the one used for Monte Carlo simulations. The execution times did not surpass 5 seconds per beam quality. This method is much more time efficient compared to Monte Carlo simulations and does not require the modelling of the irradiation geometry.

On the other hand, this analytical calculation method, like Monte Carlo simulations, represents only the physical interactions and energy depositions of incident X rays in matter. The free radical creation in alanine is not considered in this approach, thus, the relative effectiveness of alanine to X rays needs to be taken into account in order to have comparable results with experimentally measured alanine relative responses.

9.2.4. The relative effectiveness of alanine

The main purpose of this part of the study is to separate the alanine's relative response into two terms: a first term that depends only on the EPR response of the dosimeter, per unit of absorbed dose to the dosimeter, and a second term that depends only on energy deposition differences between dosimeter and water material, in other words, the absorbed physical dose to the dosimeter or water material. This approach can be mathematically represented by this relation (Equation 6):

$$\frac{(r/D_w)^Q}{(r/D_w)^{Q_0}} = \frac{(r/D_{dos})^Q}{(r/D_{dos})^{Q_0}} \times \frac{(D_{dos}/D_w)^Q}{(D_{dos}/D_w)^{Q_0}} \quad (6)$$

The left-hand side of this equation represents the relative response of alanine to kV X rays of quality Q compared to a Q_0 reference beam quality and is equal to the f_{exp} factor that is experimentally determined in this work. The right-hand side of this equation can be divided into two terms: the first term represents the relative effectiveness of the alanine dosimeter, and the second term is the ratio of absorbed dose in the dosimeter to water, for an X ray beam quality Q compared to a Q_0 reference beam quality. This approach was also adopted by different studies [7, 8, 12, 15, 16]. The last equation (Equation 6) can thus be expressed as follows (Equation 7):

$$f_{exp}^{Q,Q_0} = \eta^{Q,Q_0} \cdot f_{MC}^{Q,Q_0} \quad (7)$$

where f_{exp} is the relative response of alanine to kV X ray beam qualities, f_{MC} is the alanine to water dose ratio for X-rays compared to ^{60}Co determined by Monte Carlo simulations, and η is the relative effectiveness of alanine to kV X rays compared to ^{60}Co gamma rays.

In order to study the energy dependence of the relative effectiveness to kV X rays of Aerial's alanine dosimeters, three different X ray beam qualities were chosen. The effective energies ranged from 19.1 to 50 keV and the first *HVL*s ranged from 0.65 up to 6.83 mm of aluminium.

For each X ray beam quality, 16 alanine dosimeters (lot 09/11) were placed in a polyethylene holder and irradiated at the same time. The distance between two adjacent pellets is 5 mm, from center to center. The holder was placed in a homogeneous dose zone (dose homogeneity of ±2%). For each X ray beam quality, the delivered absorbed dose to water was estimated using the PTW 30013 Farmer ion chamber. An absorbed dose to water of 100 Gy was delivered to all dosimeters for all three X ray beam qualities. This was done in order to exclude any effect that could arise from differences in delivered doses. The relative effectiveness of alanine dosimeter was considered to be equal to unity for ^{60}Co gamma rays. Alanine reference dosimeters, that were irradiated with ^{60}Co gamma rays, were used to determine the relative effectiveness of alanine for the different X ray qualities Q compared to ^{60}Co reference beam quality Q_0.

9.3.RESULTS AND DISCUSSION

9.3.1. The relative effectiveness of alanine dosimeters

Results showed that the relative effectiveness of alanine dosimeter ranged from 0.912 up to unity for effective energies from 19.1 keV up to ^{60}Co gamma rays (1250 keV on average). The results obtained, as well as literature data are plotted in Fig. 2.

The obtained results agree with published data. However, even by taking account of all uncertainties and variabilities, one can notice that general tendencies are different between the results of cited works. This can be caused mainly by the differences in the chemical composition of studied dosimeters, where Olko *et al* [17] used experimental data published by Regulla and Defner [19] which were obtained by irradiating dosimeters containing 90% alanine and 10% paraffin, Anton and Büermann [7] and Hjørringgaard *et al* [8] used dosimeters manufactured by Harwell (UK) containing 91% alanine and 9% paraffin wax and Waldeland *et al* [12] used dosimeters that were purchased from Gamma Service Produktbestrahlung GmbH (Germany) that contain 96% alanine and 4% of unknown binder.

Other reasons leading to differences between results are the adopted formalism and method for the determination of the dosimeter's relative effectiveness, where Olko *et al* [17] and Anton and Büermann [7] obtained their results using experimental measurements in parallel with Olko's one hit detector model whereas experimental measurements accompanied by Monte Carlo simulations were used in this work as well as in the work of Waldeland *et al* [12] and Hjørringgaard *et al* [8].

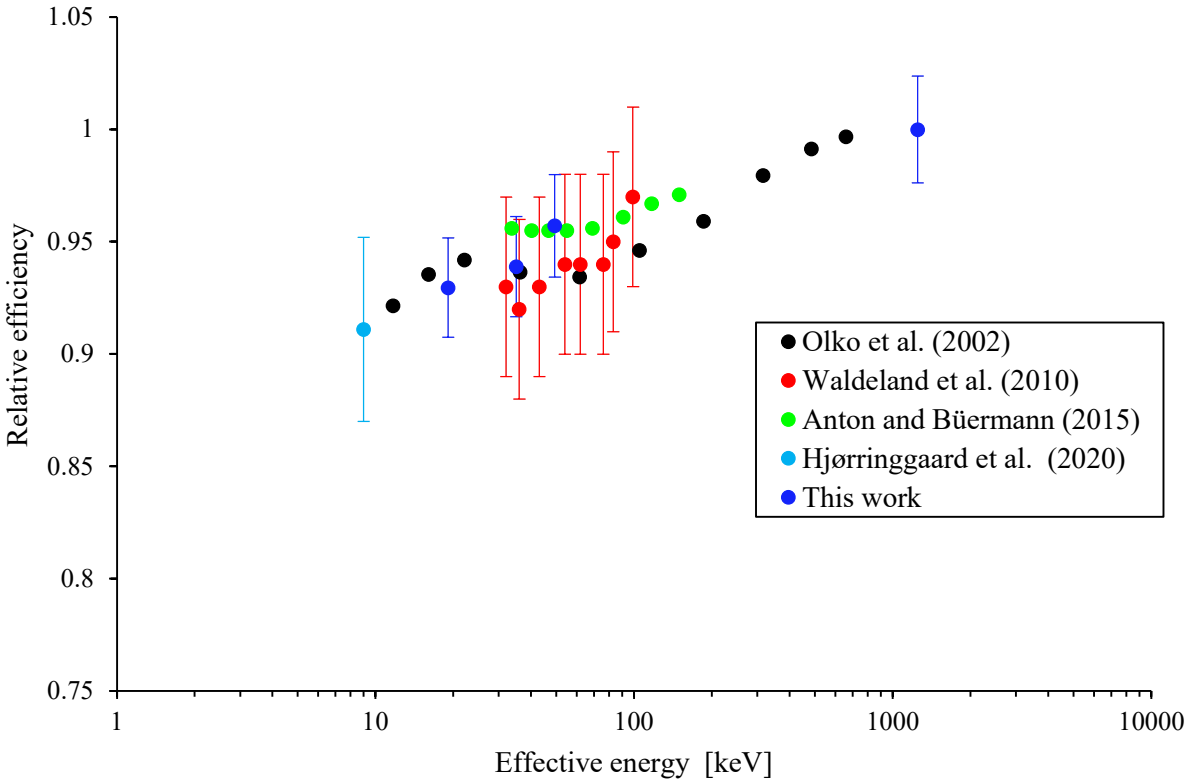

FIG. 2. Comparison of the measured relative effectiveness of alanine dosimeters with published data.

The standard uncertainty (k=1) on the determined relative effectiveness values is found to be equal to 2.4%. Obtained results were then fitted using a mathematical model in order to estimate the relative effectiveness of alanine dosimeters irradiated with X ray beam qualities that are listed in Table 1. The fit uncertainty was equal to 0.3%. The estimated η values where then applied to the f_{MC} and f_W factors in order to have a better comparison with the experimentally determined relative responses of alanine (f_{exp}).

9.3.2. The relative response of alanine to kV X rays

The experimentally measured results of the relative response of alanine dosimeters, as well as the ones determined by Monte Carlo (MC) simulations and analytical calculations are shown in Fig. 3. Results obtained by simulations and calculations have been updated with values of the relative effectiveness that were previously determined, in order to have a better comparison with experimental measurements.

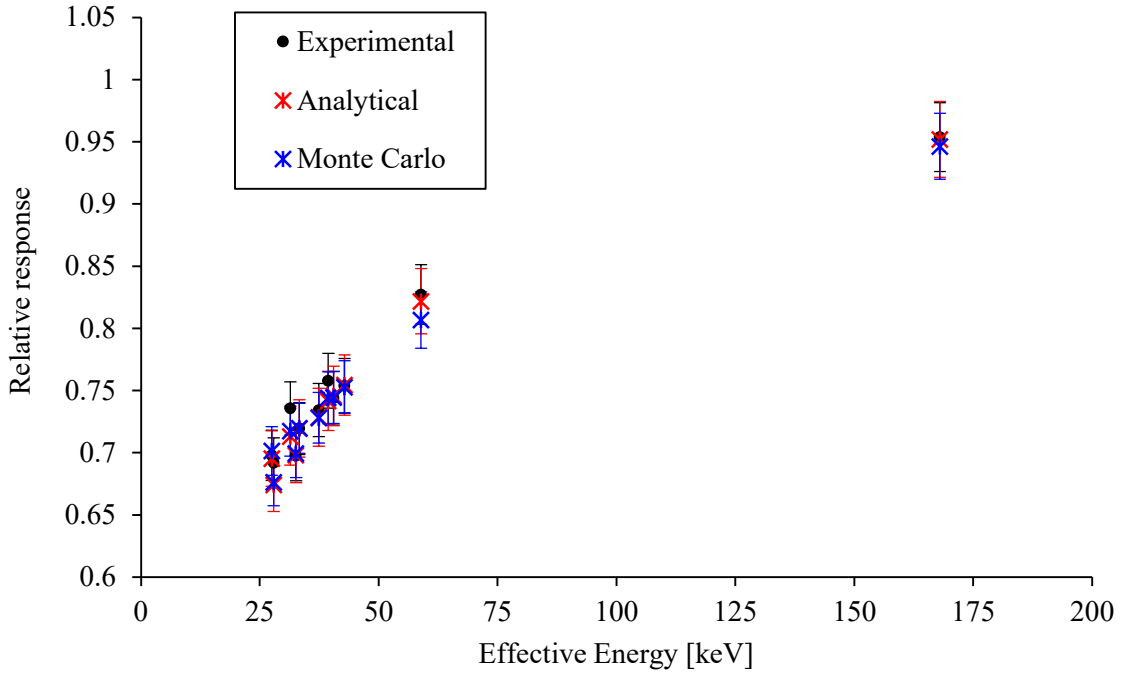

FIG.3. Relative responses of alanine dosimeters to kV X rays obtained by experimental measurements, Monte Carlo (MC) simulations and analytical calculations.

An uncertainty budget of 2.8% (k=1) was established for relative responses determined by Monte Carlo simulations, taking into account the uncertainties on the determination of the relative effectiveness of alanine dosimeters. For relative responses determined by analytical calculations, the uncertainty budget is equal to 3.2% (k=1). The average variation coefficient of the results obtained by all three methods is found to be equal to 0.7%.

The results presented in Fig. 3 show that the use of Monte Carlo simulations, as well as analytical calculations, that take into account the energy dependence of the free radical creation yields of alanine radicals, are suitable to determine the relative response of alanine dosimeters to kV X rays compared to ^{60}Co reference beam quality. Our results also confirmed that the use of analytical calculations for this purpose is justified, as this analytical calculation method allowed us to obtain results that are in good agreement with experimental measurements. It is clear that analytical calculations are able to obtain results, with comparable uncertainties, in a much shorter calculation time (few seconds), compared to Monte Carlo simulations (few hours). The results presented in Fig. 3 are also tabulated below (Table 2).

TABLE 2. RELATIVE RESPONSE OF ALANINE DOSIMETERS DETERMINED BY THREE DIFFERENT METHODS AS WELL AS UNCERTAINTIES (K=1)

Effective energy of X ray spectrum	Experimental measurement		Monte Carlo simulation		Analytical calculation	
E_{eff} (keV)	f_{exp}	$u f_{exp}$	f_{MC}	$u f_{MC}$	f_W	$u f_W$
27.5	0.698	0.020	0.702	0.020	0.696	0.022
27.9	0.692	0.020	0.677	0.019	0.674	0.022
31.4	0.736	0.021	0.718	0.020	0.713	0.023
32.6	0.698	0.020	0.700	0.020	0.698	0.022
33.3	0.720	0.021	0.720	0.020	0.720	0.023
37.4	0.734	0.021	0.728	0.020	0.729	0.023
39.4	0.758	0.022	0.744	0.021	0.742	0.024
40.5	0.744	0.022	0.745	0.021	0.746	0.024
42.8	0.754	0.022	0.753	0.021	0.755	0.024
58.9	0.827	0.024	0.807	0.023	0.822	0.026
168	0.954	0.028	0.947	0.027	0.952	0.030

f is the relative response of the alanine dosimeter and *uf* is the associated uncertainty to one standard deviation

In order to correct the alanine dosimeter response, when irradiated with kV X rays and measured with a ^{60}Co calibrated EPR dosimetry system, one has to determine the effective energy of the used X ray beam quality, and then based on results listed in Table 2, a correction factor (Equation 8) can be determined and applied as such (Equation 9):

$$k_{corr}^{Q,Q0} = \frac{1}{f^{Q,Q0}} \tag{8}$$

$$D_w^{Q0} = k_{corr}^{Q,Q0} \times D_w^Q \tag{9}$$

where k_{corr} is the correction factor to be applied to the absorbed dose to water ($D^Q{}_w$) measured by alanine for a kV X ray quality Q and $D^{Q0}{}_w$ is the corrected absorbed dose to water which is traceable to the reference beam quality Q_0. One should note that this method can be applied for irradiations at doses lower than 10 kGy, while at higher doses, the correction factor should be firstly applied to the dosimeter's response, due to the non-linearity of the latter versus absorbed dose to water [18], thus, absorbed dose to water can be then correctly estimated using the EPR system's calibration curve.

9.4.CONCLUSIONS

The relative response of alanine dosimeters to different kilo-voltage X ray beam qualities was determined relative to ^{60}Co gamma ray reference beam quality. This study determined the relative response of alanine dosimeters using three different methods: experimental measurements, Monte Carlo simulations and analytical calculations. The results obtained should be used to determine and apply correction factors to absorbed dose to water measurements undertaken with alanine/EPR dosimetry systems that are calibrated in a ^{60}Co

gamma ray reference beam quality, whilst alanine dosimeters are irradiated with kV energy X rays.

The results of the three studied methods were found to be in good agreement, with an average variation coefficient of 1.3% over the studied effective energy range (27.5–168 keV). The novelty of this work resides in the determination of the relative response of alanine dosimeters using analytical calculations based on the weighting of X ray energy spectra by mass-energy absorption coefficients tabulated by NIST. This method proved to be reliable, efficient and rapid. The results obtained by analytical calculations were in good agreement with experimental results (average deviation of 2.4%), and the calculation time is of the order of a few seconds whereas Monte-Carlo simulations are more time consuming and could take a few hours to determine results that are very close to ones obtained by analytical calculations (average deviation of 0.7%).

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10. MICROBIAL ANALYSIS AND SENSORY SURVEY TEST OF X RAY IRRADIATED FRESH CUT VEGETABLES FOR CANCER PATIENT'S FOOD

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Abstract

The microbiological and sensory properties of fresh-cut vegetables (e.g. carrot, green peppers, cherry tomatoes, and paprika) after X ray irradiation were evaluated to verify if they would be suitable as safe foods for cancer patients with suppressed immunity. Total concentrations of aerobic bacteria in non-irradiated samples, except for cherry tomatoes, were 1.63–3.34 log CFU/g. A sterilizing dose of 0.4 kGy was microbiologically acceptable for fresh cut carrots and green peppers whereas the dose of 0.2 kGy was microbiologically acceptable for both cherry tomatoes and paprika. Therefore, a dose of 0.4 kGy was tentatively determined as the minimum allowable dose for sterilization of these fresh-cut vegetables. With respect to the sensory qualities, both texture and overall acceptance taste panel scores gradually decreased as the irradiation dose increased ($p<0.05$). However, irradiated samples scored higher than 5.0 points on a 7-point hedonic scale, indicating a good sensory quality. In a survey of hospitalized cancer patients (n = 50), the average scores for the overall acceptance of the fresh-cut vegetables sterilized with the dose of 0.4 kGy were higher than 4.0 points on a 5-point hedonic scale. In conclusion, this study strongly suggests that the fresh-cut vegetables sterilized by X ray can be served to hospitalized cancer patient as hygienically safe foods with acceptable sensory properties. A relatively small cabinet X ray system was used as this may be convenient in a hospital setting. Fresh food products could be X ray treated on-site before being served to patients.

10.1. INTRODUCTION

Cancer patients are gradually increasing due to the development of medical diagnosis technology and the increase of elderly population [1]. The patients receive well known conventional anticancer therapies such as radiotherapy and chemotherapy for their disease, but these medical treatments cause a significant decrease in their appetite and immunity. A decrease in appetite may lead to malnutrition of cancer patients, which is one of the main reasons for disrupting their cancer treatment process or increasing their mortality [2]. Therefore, a variety of foods to meet the nutrition needs of cancer patients is very important. However, the food items that cancer patients generally consume are very limited compared to those of normal individuals. This is because cancer patients with decreased immunity are more likely than normal individuals to suffer complications due to intra-intestinal infection with bacteria present in foods when ingesting non-sterile foods [3].

Recent economic growth in the Republic of Korea is changing individual dietary patterns. Accordingly, the market size of convenience foods such as fresh-cut vegetables, which have

been minimally processed by washing, peeling or cutting, is also increasing [4]. However, fresh-cut vegetables are vulnerable to exposure to harmful food poisoning bacteria derived from the soil since they undergo simple hygienic processes such as washing. In addition, these agricultural foods may have low hygienic stability due to the possibility of adventitious, secondary contamination by workers and cross contamination by contact between foods during commercial manufacturing processes, transport and storage. Therefore, it is necessary to apply food hygiene technology that can not only maintain the inherent taste and texture of the fresh-cut vegetables but also ensure their hygienic safety.

One way to overcome such potential food contamination is to treat the food with ionizing radiation. Food irradiation is one of the few techniques that address both food quality and safety, with the ability to control decay and food-borne microbial pathogens, and without appreciably affecting the sensory or other quality characteristics of food. Further, food irradiation can be employed after final food packaging, thus preventing cross-contamination post treatment during the manufacturing process [5–7]. Especially important for this study is that X ray is one type of ionizing radiation that can be used to irradiate food, and it has a similar penetration ability to gamma rays. X rays are widely used for the inspection and detection of foreign materials in packaged foods in the Republic of Korea, but it is not yet allowed for the purpose of food irradiation. However, the application of X ray for the purpose of food sterilization and insect pest control is expected to increase in the near future.

Therefore, this study examined the effects of X ray irradiation on the microbiological, physiochemical, and sensory quality characteristics of fresh vegetables such as carrot, green pepper, cherry tomato, and paprika to evaluate whether they could be used as a treatment to ensure the hygienically clean fresh-cut vegetables. In addition, sensory preference tests by cancer patients were performed to see if the irradiated vegetables would be acceptable as food for hospital patients.

10.2. MATERIALS AND METHODS

10.2.1. Sample preparation and X ray irradiation

Fresh carrots, green peppers, cherry tomatoes, and paprikas were purchased from a local market in Jeongeup, Republic of Korea. They were washed thoroughly, peeled and cut with a sterile knife, and packaged (10 g of food product, each in a sterilized plastic container measuring 10 × 10 × 3.5 cm) using a food packaging machine (EHQ-200N: Enterline, Gyeonggi, Republic of Korea). The packaged samples were then irradiated with varying exposure times to reach absorbed doses of 0.2, 0.4 and 0.6 kGy (160 kV, beam current 10 mA) using a cabinet X ray system (CP-160: Faxitron X ray LLC., Lincolnshire, IL, USA). Dosimetry was performed with a Bruker EMS PR analyser (Bruker Instruments, Rheinstetten, Germany) using alanine dosimeters (Bruker Instruments) inserted into the plastic container before X ray irradiation of the packaged samples. The actual measured doses were within 5% of the target doses.

10.2.2. Microbial analysis

To check the microbial level of X ray-irradiated fresh-cut vegetables, the number of aerobic or anaerobic bacteria such as total aerobic bacteria, yeasts and moulds, coliform group, *Escherichia coli* (*E. coli*), *Salmonella* spp., *Bacillus cereus* (*B. cereus*), *Staphylococcus aureus* (*S. aureus*), and *Clostridium perfringens* (*C. perfringens*) was measured. Specifically, 10 g of each sample was placed in a sterilized bag (10 x 15 cm; Sunkyun Co. Ltd., Seoul, Korea) with 90 mL of peptone water (0.1%, w/v) and homogenized by pummeling in a stomacher (Model 400; Tekmar Co., Los Angeles, CA, USA) for 2 min. The resulting supernatant was

diluted and then tested for microbial growth. The culture media used in these tests were: plate count agar (PCA, Difco Co., Detroit, MI, USA) for total aerobic bacteria; potato dextrose agar (PDA, Difco Co.) for yeasts and moulds; eosin methylene blue agar (EMB, Difco Co.) for the coliform group; 3M petrifilm (St. Paul, MN, USA) for *E. coli*; Salmonella Shigella agar (SSA, Difco Co.) for *Salmonella* spp.; mannitol egg yolk polymyxin agar (MYP, Difco Co.) for *B. cereus*; Baird-Parker agar (BPA, Difco Co.) for *S. aureus*, and; tryptose sulfite cycloserine agar (TSC, Oxoid, Hampshire, England) for *C. perfringens*. Each agar plate was seeded with 0.1 mL of diluted sample suspension. Incubation was allowed for 24 to 48 hours at 35°C for PCA, EMB, MYP, BPA and SSA agars, respectively, and for 72 hours at 25°C for PDA agar. TSC agar was also incubated for 48 hours at 35°C under anaerobic condition using an anaerobic jar (Oxoid), and 3M petrifilm was maintained for 48 hours at 35°C. Viable colonies on each plate or petrifilm were enumerated as log colony-forming units per gram (log CFU/g). To determine the sterilization of X ray irradiated fresh-cut vegetables, microbial growth tests were performed using the method of the Korea Food and Drug Administration (2010) as follows. Briefly, 10 g of each sample was homogenized in sterilized 90 mL 4 mM phosphate buffer (pH 7.4) using a stomacher. Then, 1 mL of each sample supernatant obtained was inoculated into three sterilized test tubes containing 9 mL thioglycollate (TG) medium (Difco) and allowed to incubate for 48 h at 35°C. Any tubes showing yellowish colour were considered as positive (+), indicating the presence of microorganisms in samples, whereas tubes showing no colour changes were considered as negative (–), indicating that samples were free of microorganisms.

10.2.3. Sensory analysis

Unless otherwise stated, sensory qualities of X ray irradiated fresh-cut vegetables were evaluated by a panel of ten trained individuals. Panel members were asked to rate the appearance, flavour, texture, taste and the overall acceptability of the food samples using a seven-point hedonic scale (1 = dislike extremely, 7 = like extremely). The same protocol was employed to rate the intensity of off-flavours (0 = none, 7 = extreme). Total scores were calculated as the mean of the panelist scores for each attribute.

10.2.4. Sensory survey of hospitalized cancer patients

The Institutional Review Board of Dongnam Institute of Radiological and Medical Sciences gave approval (No. D-1511-002-002) for a sensory survey of hospitalized cancer patients. The survey was performed using fresh-cut vegetables sterilized by X ray irradiation (0.4 kGy). There were 50 participants in total (42 female and 8 male). The sensory attributes of the fresh-cut vegetables were evaluated on the basis of colour, flavour, texture, taste, and overall acceptability. These sensory attributes were scored using a five-point hedonic scale (1 = very bad, 5 = very good). Total scores for each attribute were calculated as the mean of the cancer patients scores for each attribute.

10.2.5. Statistical analysis

All analyses were performed at least in triplicate. The study variables are expressed as the mean ± standard deviation (SD). Statistical significance of the data was analysed by one-way analysis of variance followed by Duncan's multiple-range test using Statistical Package for the Society Science (SPSS, window version 18.0, SPSS IBM., Chicago, IL, USA). Values were considered significant at $p<0.05$.

10.3. RESULTS AND DISCUSSION

10.3.1. Microbiological test of X ray irradiated fresh-cut vegetables

The microbial populations of carrot, green pepper, cherry tomato, and paprika samples as selected and prepared as fresh-cut vegetables are shown in Table 1. The initial total aerobic bacterial populations in carrot, green pepper, and paprika were 2.3, 3.3, and 1.6 log CFU/g, respectively, and was below the detection limit in cherry tomatoes (<1.0 log CFU/g). The growth of bacterial was not detected in paprika after irradiation to a dose of 0.2 kGy, and in carrots and peppers after 0.4 kGy of X ray irradiation. A similar tendency was observed with coliform bacteria. The initial coliform bacterial levels in carrot, green pepper, and paprika were 1.2, 2.7, and 1.1 log CFU/g, respectively, and coliforms were not found in cherry tomato (<1.0 log CFU/g). In addition, the growth of bacteria was not detected in carrot, pepper and paprika after 0.2 kGy of X ray irradiation. In contrast, microbial populations such as yeasts and moulds, *E. coli*, *Salmonella* spp., *B. cereus*, *S. aureus*, and *C. perfringens* were not observed in the fresh-cut vegetables. Moreover, to confirm the sterilization of X ray irradiated fresh-cut vegetables, microbial growth tests were performed. No growth of aerobic nor anaerobic microorganisms were observed in the irradiated fresh-cut vegetables at doses above 0.4 kGy. Therefore, the results suggest that the fresh-cut vegetables can be effectively sterilized at a dose of 0.4 kGy, and that they can be provided to hospitalized cancer patients as hygienically safe foods in terms of microbiological safety.

TABLE 1. MICROBIAL POPULATIONS IN FRESH-CUT VEGETABLES IRRADIATED WITH VARIOUS DOSES OF X RAY

Fresh-cut vegetables	Dose (kGy)	Viable cells (log CFU/g)								
		Total aerobic bacteria	Yeasts and moulds	Coliform group	*E. coli*	*Salmonella* spp.	*B. cereus*	*S. aureus*	*C. perfringens*	Microbial growth
Carrot	0.0	2.3±0.4	ND[1)]	1.2±0.2	ND	ND	ND	ND	ND	+[2)]
	0.2	1.0±0.0	ND	ND	ND	ND	ND	ND	ND	+
	0.4	ND	ND	ND	ND	ND	ND	ND	ND	-
	0.6	ND	ND	ND	ND	ND	ND	ND	ND	-
Green pepper	0.0	3.3±0.2	ND	2.7±0.1	ND	ND	ND	ND	ND	+
	0.2	1.5±0.5	ND	ND	ND	ND	ND	ND	ND	+
	0.4	ND	ND	ND	ND	ND	ND	ND	ND	-
	0.6	ND	ND	ND	ND	ND	ND	ND	ND	-
Cherry tomato	0.0	ND	ND	ND	ND	ND	ND	ND	ND	+
	0.2	ND	ND	ND	ND	ND	ND	ND	ND	-
	0.4	ND	ND	ND	ND	ND	ND	ND	ND	-
	0.6	ND	ND	ND	ND	ND	ND	ND	ND	-
Paprika	0.0	1.6±0.3	ND	1.1±0.2	ND	ND	ND	ND	ND	+
	0.2	ND	ND	ND	ND	ND	ND	ND	ND	-
	0.4	ND	ND	ND	ND	ND	ND	ND	ND	-
	0.6	ND	ND	ND	ND	ND	ND	ND	ND	-

[1)] Not detected within the detection limit (<1 log CFU/g).
[2)] Microbial growth was observed by colour change of TG medium after incubation at 37°C for 48 hours (positive).

10.3.2. Sensory test of X ray-irradiated fresh-cut vegetables

The sensory characteristics for 4 kinds of fresh-cut vegetables irradiated with various doses of X ray radiation are shown in Table 2. There were no significant differences in appearance preference and off-flavour intensity of the four fresh-cut vegetables in unirradiated (control) samples or X ray irradiated samples, while different results were observed for their flavour, texture, and taste preferences. Overall acceptance, which reflects each of the sensory quality attributes, was gradually decreased with increasing irradiation dose ($p<0.05$). However, cherry tomato was not significantly affected by X ray irradiation. Carrot and green pepper irradiated with a dose of 0.4 kGy or higher showed significantly lower overall acceptance than those of non-irradiated samples, and had lower overall acceptance from 0.2 kGy or higher. Nevertheless, the overall preferences of all four fresh-cut vegetables tested were scored above 5.5 on the seven-point scale. Therefore, even if they are irradiated up to 0.6 kGy, their sensory quality is considered to be above the acceptable level.

TABLE 2. SENSORY QUALITIES OF FRESH-CUT VEGETABLES IRRADIATED WITH VARIOUS DOSES OF X RAY

Fresh-cut Vegetables	Absorbed dose (kGy)	Attributes					
		Appearance	Flavour	Texture	Taste	Off-flavour	Overall acceptance
Carrot	0.0	6.5 ± 0.5^{NS}	6.0 ± 0.8^{NS}	6.5 ± 0.8^{a}	6.3 ± 0.5^{NS}	1.0 ± 0.0^{NS}	6.5 ± 0.5^{a}
	0.2	6.3 ± 0.5	5.9 ± 0.6	6.1 ± 0.8^{ab}	6.0 ± 0.5	1.0 ± 0.0	6.1 ± 0.6^{ab}
	0.4	6.4 ± 0.5	6.0 ± 0.8	5.6 ± 0.5^{b}	5.8 ± 0.9	1.0 ± 0.0	5.6 ± 0.9^{b}
	0.6	6.4 ± 0.5	6.0 ± 0.8	5.6 ± 0.7^{b}	5.5 ± 0.8	1.1 ± 0.4	5.6 ± 0.7^{b}
Green pepper	0.0	6.9 ± 0.4^{NS}	6.8 ± 0.5^{a}	6.8 ± 0.5^{a}	6.8 ± 0.5^{a}	1.0 ± 0.1^{NS}	6.8 ± 0.5^{a}
	0.2	6.9 ± 0.4	6.3 ± 0.7^{ab}	6.3 ± 0.5^{ab}	6.1 ± 0.4^{b}	1.0 ± 0.1	6.4 ± 0.5^{ab}
	0.4	6.9 ± 0.4	6.0 ± 0.8^{b}	5.9 ± 0.6^{b}	5.9 ± 0.6^{b}	1.0 ± 0.1	5.9 ± 0.6^{b}
	0.6	6.8 ± 0.5	6.1 ± 0.6^{ab}	5.8 ± 0.7^{b}	5.6 ± 0.7^{b}	1.0 ± 0.1	5.8 ± 0.7^{b}
Cherry tomato	0.0	7.0 ± 0.1^{NS}	6.8 ± 0.5^{a}	6.8 ± 0.5^{NS}	6.8 ± 0.5^{NS}	1.0 ± 0.1^{NS}	6.8 ± 0.5^{NS}
	0.2	7.0 ± 0.1	6.3 ± 0.5^{b}	6.4 ± 0.5	6.3 ± 0.5	1.0 ± 0.1	6.3 ± 0.5
	0.4	7.0 ± 0.1	6.4 ± 0.5^{ab}	6.3 ± 0.5	6.4 ± 0.5	1.0 ± 0.1	6.3 ± 0.5
	0.6	7.0 ± 0.1	6.1 ± 0.4^{b}	6.1 ± 0.8	6.3 ± 0.5	1.0 ± 0.1	6.3 ± 0.5
Paprika	0.0	6.6 ± 0.5^{NS}	6.5 ± 0.5^{a}	6.6 ± 0.5^{a}	6.5 ± 0.5^{a}	1.0 ± 0.1^{NS}	6.5 ± 0.5^{a}
	0.2	6.6 ± 0.5	5.9 ± 0.6^{b}	6.1 ± 0.4^{ab}	5.6 ± 0.5^{b}	1.0 ± 0.1	5.8 ± 0.5^{b}
	0.4	6.6 ± 0.5	5.9 ± 0.6^{ab}	5.9 ± 0.6^{b}	5.8 ± 0.5^{b}	1.0 ± 0.1	5.6 ± 0.5^{b}
	0.6	6.6 ± 0.5	5.8 ± 0.7^{b}	5.5 ± 0.8^{b}	5.5 ± 0.8^{b}	1.0 ± 0.1	5.5 ± 0.8^{b}

[NS] No significant within a column for each sample with 95% confidence level ($p<0.05$).
[a-b] No significant within a row followed by the different letter are significantly different ($p<0.05$).

10.3.3. Sensory survey of hospitalized cancer patients

The results of the sensory survey undertaken by the hospitalized cancer patients ($n = 50$) for 4 kinds of fresh-cut vegetables are shown in Table 3. In the sensory evaluation by cancer patients, all the sterilized fresh-cut vegetables were acceptable for consumption because all the attributes tested scored close to or above 4.0 on the five-point scale. This suggests that the fresh-cut vegetables irradiated with X ray provided the patients with a good sensory quality. However, carrot had the lowest average score of 3.94 among the four fresh-cut vegetables, indicating that the value might reflect the cancer patient's personal preference for the vegetable. In contrast, cherry tomato was the most preferred item with the highest average score of 4.21.

From these results, it is considered that fresh-cut vegetables sterilized with X ray irradiation (0.4 kGy) are acceptable for consumption by cancer patients.

TABLE 3. HOSPITALIZED CANCER PATIENTS SENSORY SURVEY OF FRESH-CUT VEGETABLES STERILIZED WITH 0.4 KGY X RAY IRRADIATION

Fresh-cut vegetables	Attributes					Average
	Colour	Flavour	Texture	Taste	Overall acceptance	
Carrot	3.90±0.86	3.86±0.86	3.98±0.74	3.98±0.71	4.02±0.68	3.94
Green pepper	4.28±0.64	3.98±0.77	4.14±0.67	4.14±0.70	4.14±0.64	4.14
Cherry tomato	4.28±0.61	4.16±0.71	4.16±0.71	4.24±0.62	4.22±0.68	4.21
Paprika	4.24±0.77	4.24±0.69	4.16±0.65	3.70±0.88	4.14±0.67	4.10

10.4. CONCLUSIONS

In conclusion, only total aerobic bacteria and coliform bacteria in fresh-cut carrot, green pepper, cherry tomato, and paprika were detected, and the growth of these bacteria was effectively controlled by X ray irradiation. In addition, X ray irradiation at a dose of 0.4 kGy completely sterilized the fresh-cut vegetables without significantly affecting their sensory quality. The results indicate that X ray irradiation can enable the inclusion of fresh-cut vegetables in the diets of hospitalized cancer patients. They also show that the sensory quality of sterilized fresh-cut vegetables is acceptable to cancer patients. Therefore, this study suggests that the sterilizing method is widely applicable to preparing the diets requiring sterile foods for cancer patients as well as other patients.

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11. EFFECTS OF X RAY IRRADIATION ON QUALITY CHARACTERISTICS OF STRAWBERRY DURING STORAGE

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Abstract

This study evaluated the effects of X ray irradiation (0–1 kGy) on quality parameters and sensory characteristics of Korean strawberries during storage at 15°C for 9 days. Irradiation significantly reduced the weight loss and decay rate of the fruit during storage ($p<0.05$). Their firmness decreased immediately after irradiation, but no significant changes occurred after 3 days. Neither irradiation nor storage period significantly affected the total soluble solids, pH, or titratable acidity. Irradiation also delayed their colour changes. Furthermore, irradiated strawberries showed improved sensory properties throughout storage. Thus, X ray irradiation was confirmed as suitable as a phytosanitary treatment for strawberries as well as an effective strategy for delaying the decay and negative quality changes of the fruits while extending their shelf life and maintaining their sensory quality.

11.1. INTRODUCTION

Strawberries are a rich source of antioxidants and vitamins and offer potential benefits in preventing various diseases such atherosclerosis, diabetes, and cancer. Strawberries are also regarded as a fruit that has health benefits and are well known and appreciated for their taste, shape, and visual appeal [1]. They are one of the most widely consumed fruits globally, with considerably high international sales [2], and are the main contributors to the Korean export of fresh agricultural products [3]. Strawberries are usually consumed in the fresh form. However, fresh strawberries are susceptible to postharvest deterioration and colour change due to microbial spoilage and this limits long-term storage and the retention of sensory attributes associated with freshness [2, 4], thus highlighting the need for advanced preservation techniques.

The increasing international trade of fresh agricultural products has necessitated the development of effective phytosanitary treatments to control dissemination of foreign pests, shelf-life extension, and microbial decontamination [5]. In recent years, irradiation has emerged as a potential eco-friendly alternative to conventional fumigation. Ionizing radiation is a non-thermal food preservation technology that can improve the food safety and shelf life of agricultural products. Irradiation to relatively low doses of ionizing radiation can be achieved within a short exposure time, it does not generate harmful residues nor requires load pretreatment or post-sterilization degassing/aeration [6]. Therefore, to overcome the limitations of chemical fumigant usage, the International Atomic Energy Agency and the International Plant Protection Convention recommend exposure to ionizing radiation as a phytosanitary treatment [7].

Currently, γ-radiation is mainly used for phytosanitary treatments or shelf-life extension of fresh agricultural products including strawberry because of its ability to penetrate through large volumes of pre-packaged products and suitability for commercial packaging [1, 8, 9]. However, γ-rays are produced by ^{60}Co, a radioactive material. In contrast, electron-beam (e-beam) or

X ray irradiation is not accompanied by the generation of radioactive material and, thus, does not result in environmental pollution [10]. Therefore, the use of e-beam or X ray irradiation for the purpose of phytosanitary treatment is gradually increasing.

In this study, the Korean strawberry variety “Maehyang” was exposed to X ray radiation to various doses and its effect on several representative quality indicators was investigated during storage at 15°C for nine days. These preliminary experiments on changes to the sensory attributes and ability to store the fruits were undertaken to assess the efficiency and applicability of X ray irradiation as a potential phytosanitary treatment for Maehyang strawberries.

11.2. MATERIALS AND METHODS

11.2.1. Strawberry procurement

Commercially ripened (70% red stage) strawberries (*F. ananassa* cv. Maehyang) for export purposes were purchased from the Sogok Dukcheon Agricultural Cooperative Export Group (Jinju, Republic of Korea). Uniformly sized fruits, free from apparent disease or injury were selected and placed in commercial packaging (50 cm x 30 cm x 11.5 cm cardboard boxes with lids) and immediately transported to the irradiation facility.

11.2.2. X ray irradiation

X ray irradiation was performed using an electron accelerator (MB10-8/635, Seoul Radiology Services Co., Eumseong, Republic of Korea) with a beam energy of 7 MeV and a conveyor speed of 0.891 m/minute. The samples were irradiated at the doses of 0.15, 0.4, 0.6, and 1 kGy, respectively. To deliver the target dose, multiple passes were performed. The actual absorbed doses were determined at three different heights within each box using alanine pellet dosimeters (Bruker Instruments, Rheinstetten, Germany) with a Bruker EMS 104 PR analyser (Bruker Instruments) at our Advanced Radiation Technology Institute (Jeongeup, Republic of Korea). Each actual dose (0.14, 0.39, 0.58, and 0.94 kGy) was within 7% of the respective target dose.

11.2.3. Determination of decay extent and weight loss

The extent of strawberry decay was calculated as the number of strawberries showing decay symptoms (rot, lesions, or visible fungal growth) divided by the total number of the fruits and was expressed as a percentage (%). Each treatment comprised eight replications, and each replication was performed using 20 fruits. The weight of each fruit was measured following treatment at day 0 and on different sampling days, with weight loss expressed as a percentage (%) of the initial weight.

11.2.4. Determination of total soluble solids, pH, titratable acid content, and firmness

Total soluble solids (TSS) were determined using a digital refractometer (Hi 96801, Hanna Instruments, Woonsocket, RI, USA). For pH measurements, 1 g of each sample were homogenized using 9 mL of distilled water and the resulting homogenate was analysed using a pH meter (Inolab, pH 7110, WTW, Weilheim, Germany). Titratable acid (TA) content was determined via titration with 0.1 N NaOH to an endpoint of pH 8.2 and expressed as a percentage of citric acid. Firmness, defined as the maximum penetration force (N), was measured by performing a penetration test with a 5 mm cylindrical probe on the skin of strawberries using a texture analyser (TA-XT2i, Stable Micro Systems, Godalming, UK) at a penetration depth and probe speed of 6 mm and 2.0 mm s^{-1}, respectively.

11.2.5. Determination of colour

Colour was measured using a chromometer (Chroma Meter, CM-5, Minolta Co., Ltd., Osaka, Japan) and expressed in terms of *L** (lightness or darkness), *a** (redness or greenness (-greenness to +redness)), and *b** (blueness or yellowness (-blueness to +yellowness)) values of the CIE colour system.

11.2.6. Sensory evaluation

Sensory quality evaluation was performed to determine the effect of X ray irradiation on consumer acceptance of strawberries during storage. Strawberry samples were coded using a three-number scheme and were randomly presented to a panel of 20 untrained individuals, and each one evaluated the fruit colour, flavour, texture, and overall acceptability based on a nine-point hedonic scale (1: extremely poor, 3: poor, 5: fair, 7: good, 9: very good).

11.2.7. Statistical analysis

All measurements were performed at least in triplicate, and the results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to calculate the statistical significance of data in conjunction with Duncan's multiple-range test using SPSS v. 18.0 software (SPSS Inc., Chicago, IL, USA). The values were considered significant at $p<0.05$.

11.3. RESULTS AND DISCUSSION

11.3.1. Effect of X ray irradiation on strawberry decay and weight loss

Prolonged storage of fruits and vegetables causes an enhanced rate of decay owing to the increased respiration activity. The decay rates of control and irradiated strawberries are shown in Table 1. It was observed that the storage period in conjunction with irradiation treatment exerted a significance influence on the decay rate. Generally, a higher decay rate was observed for the unirradiated control samples at each storage interval (days 3, 6 and 9 days). After 3 days of storage, the control samples exhibited a higher decay rate than the irradiated samples, with the decay rate of the irradiated samples decreasing linearly with the applied dose in the range of 0.15–1 kGy. However, the decay rates for irradiated samples were not significantly different at 3 days storage. Irradiated samples showed a similar trend after 6 days of storage, with dose-dependent decreases observed at 0.15, 0.4, and 0.6 kGy. However, the decay rate was slightly higher for samples irradiated at 1 kGy. The effect of X ray irradiation on the decay rate was most prominent for the strawberries subjected to 9 days of storage. Dose-pendent decreases were observed, with the dose of 1 kGy exerting the most significant effect on decay rate of irradiated samples (44.3%) as compared to control, which exhibited the highest decay rate (79.9%). These results are in agreement with previous findings [9], which reported decreases in the decay rate of strawberries after exposure to gamma irradiation. In particular, only 36.7% of strawberries irradiated at 0.9 kGy were rotted at the end of the storage period as compared to the control, which showed a decay rate of 86.7%.

TABLE 1. DECAY RATE AND WEIGHT LOSS OF X RAY IRRADIATED STRAWBERRY FRUITS DURING STORAGE

Irradiation dose (kGy)	Storage period (days)			
	0	3	6	9
Decay rate (%)				
0	0.0 ± 0.0^{d}	8.0 ± 8.0^{Ac}	33.9 ± 8.3^{Ab}	79.9 ± 7.5^{Aa}
0.15	0.0 ± 0.0^{c}	3.2 ± 3.2^{Bc}	26.2 ± 7.9^{Bb}	77.5 ± 7.4^{Aa}
0.4	0.0 ± 0.0^{c}	2.6 ± 2.6^{Bc}	23.2 ± 5.6^{Bb}	61.2 ± 8.1^{Ba}
0.6	0.0 ± 0.0^{c}	1.6 ± 2.2^{Bc}	19.4 ± 3.7^{Bb}	55.3 ± 7.8^{Ba}
1	0.0 ± 0.0^{c}	1.6 ± 2.2^{Bc}	20.8 ± 6.3^{Bb}	44.3 ± 4.9^{Ba}
Weight loss (%)				
0	0.0 ± 0.0^{d}	5.5 ± 1.1^{Ac}	8.3 ± 1.7^{Ab}	13.3 ± 1.7^{Aa}
0.15	0.0 ± 0.0^{d}	4.7 ± 1.0^{ABc}	7.2 ± 1.0^{ABc}	11.1 ± 1.7^{Ba}
0.4	0.0 ± 0.0^{d}	3.6 ± 1.2^{Bc}	6.9 ± 1.4^{Bb}	10.8 ± 1.7^{Ba}
0.6	0.0 ± 0.0^{d}	3.9 ± 0.8^{Bc}	6.8 ± 1.1^{Bb}	9.9 ± 1.7^{BCa}
1	0.0 ± 0.0^{d}	3.7 ± 1.3^{Bc}	5.4 ± 0.8^{Cb}	8.7 ± 1.2^{Ca}

Values indicated by different uppercase letters within the irradiation dose (A–B) and by different lowercase letters within the storage period (a–d) are significantly different at $p<0.05$ based on Duncan's multiple range test.

Weight loss is an important indicator of food stability during storage. Typically, a high rate of weight loss leads to drastic changes in the texture of food products. The weight losses of control and irradiated strawberries are shown in Table 1. It was found that both storage period and irradiation treatment exerted a significant ($p<0.05$) influence on weight loss in strawberries. The weight loss of irradiated samples tended to decrease as the irradiation dose increased, and the control exhibited the highest weight loss, regardless of the storage period. Considerable variation in the weight loss was observed for all samples during storage for 3, 6, and 9 days. After 9 days of storage, the maximum weight loss was observed for control samples (13.3%), whereas a weight loss of 8.7% was observed after 9 days for samples irradiated at 1 kGy. These results suggest that irradiation can reduce the weight loss in strawberries and hence improve the stability and texture as compared to unirradiated control samples.

11.3.2. Effect of X ray irradiation on strawberry total soluble solids, acidity and firmness

The TSS, pH, TA, and firmness results of strawberries are shown in Table 2. The TSS is indicative of the total sugar content of fruits and vegetables and is measured in terms of refractive index. The TSS values of control samples for all storage intervals were in the range of 7.2–7.5 °Brix. On day 0, irradiation at 0.15 kGy led to a slight increase in TSS, whereas after 6 and 9 days of storage the TSS values of both control and irradiated samples were not significantly different (Table 2). Compared with the control, further increasing the dose to 0.4–1 kGy did not cause any significant change in TSS of the irradiated samples, although slight non-significant increases were observed at 0.6 and 1 kGy. After 9 days of storage period, samples irradiated at 1 kGy exhibited a TSS value of 7.8 °Brix, equivalent to that of control samples.

Examination of pH revealed no significant variation between the unirradiated (control) and irradiated samples, which implied that X ray irradiation exposure exerts no influence on the pH value. The measurements of TA showed a tendency to decrease slightly with the increasing storage time. Compared with the control, the TA of the irradiated samples remained unaltered,

except for that of the sample irradiated at 1 kGy after 6 days of storage, which showed a slight increase.

TABLE 2. TOTAL SOLUBLE SOLIDS, ACIDITY, AND FIRMNESS OF X RAY IRRADIATED AND CONTROL STRAWBERRIES DURING STORAGE

Irradiation dose (kGy)	Storage (days)			
	0	3	6	9
Total soluble solids, TSS (°Brix)				
0 (Control)	7.5±0.8	7.3±0.7	7.3±0.7	7.2±1.0
0.15	7.8±0.8	7.7±0.8	7.3±0.7	7.3±0.7
0.4	7.2±1.0	7.5±0.6	7.2±0.6	7.8±0.7
0.6	7.5±0.9	7.6±0.6	7.6±0.7	7.5±0.7
1.0	7.8±0.8	7.3±0.5	7.3±0.8	7.8±1.5
pH				
0 (Control)	3.5±0.2	3.6±0.2	3.6±0.2	3.7±0.1
0.15	3.5±0.2	3.6±0.1	3.7±0.1	3.7±0.2
0.4	3.5±0.2	3.6±0.1	3.6±0.2	3.7±0.2
0.6	3.5±0.3	3.5±0.2	3.6±0.2	3.6±0.1
1.0	3.4±0.3	3.5±0.2	3.6±0.2	3.6±0.1
Titratable acid, TA (%)				
0 (Control)	0.9±0.1	0.9±0.1	0.8±0.1	0.8±0.1
0.15	0.9±0.1	0.9±0.1	0.8±0.1	0.8±0.1
0.4	0.9±0.1	0.9±0.1	0.8±0.1	0.8±0.1
0.6	0.9±0.1	0.9±0.1	0.8±0.1	0.8±0.1
1.0	0.9± 0.1	0.9±0.1	0.9±0.1	0.8±0.1
Firmness* (N)				
0 (Control)	5.2 ± 0.8^{Aa}	4.5 ± 0.8^{Ab}	3.4 ± 0.8^{c}	2.7 ± 0.9^{d}
0.15	5.1 ± 0.7^{Aa}	4.3 ± 0.6^{Ab}	3.3 ± 0.8^{c}	2.6 ± 0.9^{d}
0.4	3.9 ± 0.7^{BCa}	3.8 ± 0.9^{Ba}	3.2 ± 0.5^{b}	2.8 ± 0.7^{b}
0.6	3.7 ± 0.7^{BCa}	3.6 ± 0.6^{BCa}	3.3 ± 0.6^{a}	2.7 ± 0.9^{b}
1.0	3.4 ± 0.6^{Ca}	3.3 ± 0.5^{Ca}	3.1 ± 0.9^{a}	2.7 ± 0.6^{b}

* A–C, a–d Values followed by different uppercase letters (A–C) within a column and by different lowercase letters (a–d) within a row are significantly different at $p<0.05$ based on Duncan's multiple range test.

A decreasing trend was observed for the firmness of all samples with increasing storage time from 0 to 9 days irrespective of the X ray dose applied. Furthermore, compared with the control, a dose of 0.15 kGy did not significantly influenced the firmness ($p>0.05$). However, the samples irradiated at 0.4–1 kGy showed a dose-dependent decrease in firmness on day 0, and this trend was maintained until 3 days. After 6 days of storage, the firmness of irradiated samples was comparable to that of control samples. These results are similar to previous findings [11], which reported that exposure to applied doses of γ-irradiation at 0, 0.4, and 1 kGy had no significant effect on the TSS, pH, and firmness of Korean citrus fruits.

11.3.3. Effect of X ray irradiation on strawberry colour

Table 3 shows the *L**, *a**, and *b** values obtained for the X ray irradiated and non-irradiated control samples. On day 0, irradiation treatment did not significantly affect the fruit colour properties. After 3 days of storage, *L** value increased from 48.9 to 52.1, whereas *a** value tended to decrease with corresponding the increase in irradiation dose from 0.15 to 1 kGy. Thus,

the lightness increased with increases in applied dose, but the degree of redness decreased. After 6 and 9 days of storage, all irradiated samples showed significant increases in *L** and *b**, whereas *a** value showed a decreasing trend. The tristimulus colour of strawberries may be modified after exposure to irradiation, and it has been reported that irradiated foods of plant origin usually demonstrate higher colour intensity than non-irradiated samples [12]. These tristimulus colour changes might be a consequence of modifications to components of strawberry pigments because irradiation and the underlying mechanism may involve modulation of ethylene production and enzymatic activity [13]. Similar to our results, UV-C irradiation has also been reported to delay colour changes in a variety of agricultural products such as mangosteen, tomato, and strawberry [13–15].

TABLE 3. COLOUR OF X RAY IRRADIATED AND CONTROL STRAWBERRIES DURING STORAGE

Parameter	Irradiation dose (kGy)	Storage period (days)			
		0	3	6	9
*L**	0	68.5 ± 3.0^{a}	48.9 ± 1.2^{b}	38.7 ± 3.6^{c}	37.1 ± 1.9^{c}
	0.15	67.5 ± 1.6^{a}	51.7 ± 4.3^{b}	38.6 ± 0.9^{c}	37.6 ± 2.3^{c}
	0.4	67.3 ± 2.2^{a}	48.9 ± 6.8^{b}	39.6 ± 2.9^{c}	36.7 ± 0.7^{c}
	0.6	68.4 ± 3.6^{a}	48.0 ± 2.3^{b}	41.9 ± 3.5^{c}	37.2 ± 1.9^{d}
	1.0	67.9 ± 3.4^{a}	52.1 ± 5.0^{b}	41.5 ± 5.0^{c}	38.8 ± 0.5^{c}
*a**	0	7.4 ± 1.7^{c}	37.6 ± 0.5^{Ab}	39.4 ± 0.3^{Aa}	40.7 ± 0.7^{Aa}
	0.15	7.3 ± 2.0^{d}	36.0 ± 0.8^{ABc}	37.8 ± 1.0^{ABb}	40.3 ± 1.0^{Aa}
	0.4	8.1 ± 2.2^{c}	35.7 ± 2.5^{ABb}	37.5 ± 1.1^{ABab}	39.6 ± 1.1^{Aa}
	0.6	7.7 ± 2.0^{b}	33.4 ± 4.7^{BCa}	35.0 ± 3.6^{Ba}	37.9 ± 1.7^{Ba}
	1.0	7.9 ± 3.7^{c}	29.7 ± 3.5^{Cb}	35.0 ± 3.4^{Ba}	35.1 ± 1.5^{Ca}
*b**	0	25.3 ± 3.4^{b}	35.5 ± 1.5^{a}	26.4 ± 3.5^{Bb}	23.2 ± 1.3^{Bb}
	0.15	26.3 ± 2.5^{bc}	35.7 ± 3.4^{a}	29.0 ± 4.5^{ABb}	24.1 ± 2.2^{ABc}
	0.4	25.9 ± 2.0^{b}	33.6 ± 2.8^{a}	29.6 ± 4.8^{ABab}	25.6 ± 2.7^{ABb}
	0.6	25.6 ± 3.4^{b}	36.5 ± 3.4^{a}	34.1 ± 5.3^{Aa}	26.4 ± 1.6^{Ab}
	1.0	26.5 ± 2.7^{b}	35.1 ± 2.3^{a}	33.7 ± 6.1^{Aa}	27.1 ± 2.7^{Ab}

Note that, A–C, a–d values followed by different uppercase letters (A–C) within a column and by different lowercase letters (a–d) within a row are significantly different at $p<0.05$ based on Duncan's multiple range test.

11.3.4. Effect of X ray irradiation on sensory property of strawberry

The effects of X ray irradiation on the sensory properties of control and irradiated strawberries are shown in Table 4. It was evident that the storage period and irradiation treatment dose exerted a significant influence on the sensory attributes ($p<0.05$). On day 0, the colour of the irradiated samples remained unaltered. After 3 days of storage, the 0.15 and 0.4 kGy irradiated samples showed improvement in colour, whereas further increasing the dose led to decreases in the colour score. After 6 and 9 days of storage, a significant improvement in the colour score was found for the irradiated strawberry samples with increases in irradiation doses as compared to the control, which suggested that irradiation treatment was effective for improving the visual colour appeal, even after prolonged storage for 6 and 9 days.

TABLE 4. SENSORY SCORES OF X RAY IRRADIATED AND CONTROL STRAWBERRIES DURING STORAGE

Parameter	Irradiation dose (kGy)	Storage period (days)			
		0	3	6	9
Colour					
	0	3.7 ± 1.1^{c}	7.5 ± 1.2^{a}	5.9 ± 1.4^{Bb}	3.9 ± 0.7^{Bc}
	0.15	3.7 ± 1.1^{c}	7.6 ± 1.1^{a}	6.2 ± 1.0^{ABb}	4.1 ± 0.9^{BCc}
	0.4	3.7 ± 1.1^{d}	7.7 ± 0.8^{a}	6.7 ± 0.9^{ABb}	4.9 ± 1.1^{ABc}
	0.6	3.7 ± 1.1^{c}	6.8 ± 0.9^{a}	7.0 ± 0.5^{Aa}	5.4 ± 1.3^{Ab}
	1.0	3.7 ± 1.1^{c}	6.7 ± 1.2^{a}	7.1 ± 0.7^{Aa}	5.5 ± 1.1^{Ab}
Flavour					
	0	4.7 ± 1.1^{b}	7.3 ± 1.3^{a}	6.9 ± 1.4^{a}	4.6 ± 1.8^{b}
	0.15	4.4 ± 1.3^{b}	7.6 ± 1.1^{a}	7.0 ± 0.9^{a}	4.7 ± 1.8^{b}
	0.4	3.7 ± 1.1^{b}	7.4 ± 1.3^{a}	7.0 ± 1.1^{a}	4.8 ± 2.0^{b}
	0.6	3.6 ± 1.1^{c}	7.6 ± 1.1^{a}	7.2 ± 0.9^{a}	5.2 ± 2.0^{b}
	1.0	4.1 ± 1.0^{c}	7.4 ± 0.8^{a}	7.4 ± 0.8^{a}	5.6 ± 1.6^{b}
Texture					
	0	8.2 ± 0.6^{Aa}	7.4 ± 1.3^{a}	5.1 ± 1.1^{b}	4.8 ± 1.5^{b}
	0.15	7.9 ± 0.7^{ABa}	7.1 ± 1.9^{a}	5.6 ± 1.4^{b}	4.5 ± 1.6^{b}
	0.4	7.5 ± 0.8^{ABCa}	7.1 ± 2.1^{a}	5.6 ± 1.3^{b}	4.7 ± 1.6^{b}
	0.6	7.3 ± 0.8^{BCa}	7.0 ± 2.0^{a}	6.0 ± 1.5^{ab}	4.9 ± 1.4^{b}
	1.0	6.9 ± 1.1^{Ca}	6.8 ± 1.8^{a}	5.9 ± 1.6^{ab}	5.0 ± 1.4^{b}
Overall acceptance					
	0	4.3 ± 0.9^{b}	7.6 ± 1.6^{a}	6.5 ± 1.6^{a}	4.0 ± 1.2^{Bb}
	0.15	4.0 ± 0.8^{c}	8.1 ± 1.1^{a}	6.6 ± 1.3^{b}	4.3 ± 1.3^{Bc}
	0.4	4.0 ± 0.8^{c}	8.0 ± 0.9^{a}	6.5 ± 1.3^{b}	4.9 ± 1.1^{ABc}
	0.6	3.8 ± 0.9^{d}	7.9 ± 1.1^{a}	6.8 ± 0.8^{b}	5.7 ± 1.3^{Ac}
	1.0	3.5 ± 1.0^{d}	8.0 ± 0.8^{a}	6.8 ± 0.8^{b}	5.7 ± 1.2^{Ac}

$^{A–C, a–d}$ Values followed by different uppercase letters (A–C) within a column and by different lowercase letters (a–d) within a row are significantly different at $p<0.05$ based on Duncan's multiple range test.

Flavour was also affected by X ray irradiation. On day 0, irradiation treatment led to a slight decrease in the flavour scores as compared to the control; however, after 3 storage days, the 0.15 and 0.6 kGy irradiated samples showed improved flavour scores as compared to the control. After 6 and 9 days of storage, the control samples received flavour scores of 6.9 and 4.6, respectively, which were slight lower than those recorded for the 1 kGy irradiated samples (7.4 and 5.6 on day 6 and 9, respectively). Therefore, it may be inferred that irradiation treatment contributed somewhat to improving the strawberry flavour after 3, 6, and 9 days of storage.

The texture score of the irradiated samples tended to decrease depending on the irradiation dose after 0 and 3 days of storage as compared to the control. While the irradiated samples after 6 and 9 days of storage tended to show increased texture scores with the increase in applied dose in comparison with the control. These results implied that irradiation treatment had a positive impact of the fruit texture during extended storage.

As compared to the control on day 0, the overall acceptability of irradiated samples tended to decrease depending on dose. However, all the irradiated samples showed a gradual improvement in the overall acceptability after 3, 6, and 9 days of storage in a dose-dependent

manner when compared with the control. This improvement in overall acceptability suggested that irradiation treatment is quite effective for retaining or improving the sensory characteristics of strawberries during prolonged storage. Thus, X ray irradiation could potentially be utilized as an alternative for shelf life extension of perishable fruits to realize adequate consumer acceptability.

11.4. CONCLUSION

This study aimed to evaluate the effects of X ray irradiation on the quality parameters and sensory characteristics of strawberries during storage at 15°C for 9 days. As compared to the unirradiated control samples, X ray irradiation significantly reduced the weight loss and decay rate of strawberries during all storage periods. The firmness of the fruits showed an initial decrease after irradiation, but no significant changes were observed after 3 days of storage. Irradiation treatment and storage period had no significant effects on the TSS, pH, or TA of fruits, but the treatment delayed colour changes during storage. In addition, compared with the non-irradiated strawberry samples, the irradiated strawberries had improved sensory qualities after a complete storage period of nine days. These results suggest that X ray irradiation could be an effective strategy to extend the shelf life and maintain the sensory qualities of the fruits, irradiation delays the rate of decay and inhibits negative physicochemical changes in strawberries.

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12. ASSESSMENT OF ACTIVATION IN FOOD PRODUCTS IRRADIATED WITH HIGH ENERGY X RAYS

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Abstract

The aim of the study is to investigate the amount of potentially induced radioactivity in food after irradiation by high energy X rays produced by an electron accelerator. The ultimate objective is to quantify with a high precision the induced radioactivity as a function of accelerator and food characteristics. The approach consists of combining experimental measurements and Monte Carlo simulations of radiation-matter interactions, so as to define a protocol that will enable levels of induced radioactivity to be assessed. Such assessments may be important for controlling the irradiation processes.

12.1. INTRODUCTION

Over the last few decades, numerous studies have been devoted to determining energies suitable for radiation processing. Today electron beams are the most widely used of any method. However, the penetration of electrons in matter is generally small, thus making photons quite useful for multiple applications. Gamma ray photons emitted by radioactive sources (^{60}Co, ^{137}Cs) are limited to specific emission energies and are not easily controllable. Accelerator beams allow a more complete control of photon emissions and the possibility of stopping the irradiation if necessary. In addition, the energy spectrum of bremsstrahlung is continuous, covering a wider energy range than gamma rays. For these reasons, X ray photons produced by electron accelerators are very interesting for industrial processes. One of the main drawbacks of X ray beams is the low efficiency of electron conversion. Going to energies higher than 5 MeV will improve the quality of irradiation, while reducing the cost of utilization. Typically, for accelerators developed for these applications, the bremsstrahlung yield rises from 8% at 5 MeV electrons to 14% at 7 MeV, and the angular dispersion of the photon beam is reduced. The photon penetration in matter is also increased, leading to a better Dose Uniformity Ratio (DUR) and a reduction of the irradiation time. Increasing X ray energy thus allows a faster and more efficient irradiation process.

Radiation processing is subjected to international regulations and standards. The norms ISO 11137:2016; ISO 14470:2011 in force today provide recommendations permitting to satisfy requirements and guides of norms of dosimetry and its use in the development, validation and routine control of radiation processing. One of the risks associated with irradiation by ionizing

radiation is nuclear activation. Photons with energy of several MeV can indeed generate radioactive nuclei through photonuclear reactions. This corresponds to the absorption of a photon by a nucleus, with de-excites by emitting secondary particles such as neutrons or protons. The ejection of nucleons from a stable nucleus can then transform it into a radionuclide (direct activation). Around an energy of 10 MeV, the most probable process is the (γ,n) reaction because of the Coulomb barrier which strongly reduces the cross section of the other processes ((γ,p), (γ,α), etc.). Neutrons emitted by this (γ,n) reaction are generally called photoneutrons and can be captured by surrounding nuclei and lead to the production of other radionuclides (indirect activation). Different reactions can therefore occur in food irradiated with high-energy photons: emissions of photo-protons (γ,p) and photo-neutrons (γ,n), neutron captures (n,γ), activation of long-lived metastable states and activation of isomeric states via reactions (γ,γ0). Most of the nuclei resulting from (γ,p) and (γ,n) reactions are stable, and those few unstable nuclei formed are most often formed in very small quantities. The more important reactions to consider are neutron captures arising from (γ,n) processes and isomeric states.

The objective of the present project is to study the potentially induced radioactivity in food after an irradiation by high energy X rays. The ultimate objective is to quantify with a high precision the radioactivity that can be induced in food as a function of accelerator and food characteristics. The approach consists of combining experimental measurements and Monte Carlo simulations of radiation-matter interactions, so as to define a protocol that will enable levels of induced radioactivity to be assessed for a wide range of different foods.

12.2. MATERIALS AND METHODS

12.2.1. Production of photoneutrons

Photoneutrons resulting from photonuclear reactions are produced when a nucleus absorbs a high energy photon causing it to undergo an internal rearrangement to eject one or more particles (protons, neutrons, alpha, etc.). It is an evaporation process. Evaporated neutrons are emitted isotropically with average energies between 1 and 2 MeV. Photoneutrons can also be emitted by a direct emission process. A direct emission occurs when a photon interacts directly with one or several nucleons in the nucleus. Generally, these neutrons have an average energy of several MeV and their angular distribution has a sin (2θ) dependence relative to the direction of the incident photon.

Photons with an energy superior to the binding energy of the nucleons (between 2 and 10 MeV for most nuclei) can produce (γ,n), (γ,2n), or (γ,p) photonuclear reactions. The production of photoneutrons depends on the total cross-section and the binding energy of the nucleus concerned. The corresponding cross sections show several characteristic structures of which the giant dipole resonance (GDR) has a maximum around 20 MeV for light nuclei and 12 MeV for heavier nuclei. The kinetic energy of the neutron (E_n) can be estimated using the following formula (Equation 1):

$$E_n = \frac{A - 1}{A}\left[E_\gamma - E_{th} - \frac{E_\gamma^2}{2m_n c^2 (A - 1)}\right] + E_\gamma \sqrt{\frac{2(A - 1)(E_\gamma - E_{th})}{m_n c^2 A^2}}\cos\theta \qquad (1)$$

Where:

A is the mass number of the nucleus;
E_γ is the energy of the incident photon;
E_{th} is the threshold energy of the (γ,n) reaction;

$m_n c^2$ is the rest energy of the neutron, and;
θ is the angle of emission of the neutron relative to the direction of the incident photon.

Table 1 gives the threshold energies (E_{th}) of different photonuclear reactions for ^{13}C, a naturally occurring isotope of carbon with a mean natural abundance of 1.11%.

TABLE 1. PHOTONUCLEAR REACTIONS OF ^{13}C

	Threshold energy (MeV)								
Reaction	(γ,n)	(γ,α)	(γ,p)	(γ,np)	(γ,2n)	(γ,^{2}H)	(γ,^{3}He)	(γ,2p)	(γ,3n)
E_{th} (MeV)	**4.95**	**10.65**	**17.53**	**20.90**	**23.67**	**23.88**	**24.41**	**31.63**	**36.79**

Table 1 shows that with ^{13}C, (γ,n) reactions can occur in foods beginning at an X ray irradiation energy of 4.95 MeV. Reactions (γ,2n) and (γ,3n) are also possible with photons at higher energies of 23.67 and 36.79 MeV respectively. The production of photoneutrons is more probable than production of a charged particle (proton, alpha), which has to cross a Coulomb barrier.

12.2.2. Radioactivity induced by high energy photons

One of the reference publications in the field of radioactivity induced by irradiation is the IAEA report "Natural and Induced Radioactivity in Food" (IAEA-TECDOC-1287) [1]. This report proposes a complete calculation of the induced radioactivity, and the corresponding absorbed dose, for electron and X ray food irradiations. It concludes that food irradiated with X rays with energy up to 7.5 MeV to a dose of 30 kGy has a radioactivity well below natural radioactivity in non-irradiated food, with a theoretical maximum absorbed dose from ingestion between 10^{-4} and 10^{-3} mSv/year. The results obtained for irradiation with X rays of 7.5 MeV are of the same order of magnitude as those of other studies, such as the article by O. Grégoire *et al.* [2]. Although the results of these different studies are consistent with each other, it should however be noted that the theoretical calculations carried out were based on a certain number of approximations both at the level of the accelerator (parts, composition, energy spectrum of X rays, ...) and at the irradiated samples (geometry, composition, ...). The IAEA report itself states that "*as the neutron flux depends on many design parameters that may not have been anticipated in these estimates, the above calculations, estimates and recommendations should only serve as rough guide*" (section 13.4 p51). It is therefore important to complete the theoretical calculations with experimental measurements. The measurement of gamma-emitting radionuclides produced by photonuclear activation is generally possible by well-known gamma spectrometry techniques. But the experimental measurement of pure beta and alpha emitting radionuclides is much more complex. A hybrid solution then consists of using numerical methods, previously validated by experimental measurements, to estimate as precisely as possible the radioactivity induced during the X ray irradiation process. These numerical methods are based on both experimental data and nuclear models.

Several studies over the last few years have established databases containing all the experimental and evaluated data (theoretical models) of photonuclear cross sections. Evaluated databases (ENDF [3], JEFF [4], TENDL [5], and JENDL [6]) propose theoretical cross-sections produced by codes such as EMPIRE [7] and TALYS [8]. All of these data are regrouped in a single program, JANIS [9], permitting a comparison of experimental data and nuclear models for a large number of nuclei. Two types of codes are generally used to estimate the radionuclides associated with photonuclear reactions: analytical codes and Monte Carlo simulations. The

code ActiWiz [10] was for example developed by CERN to estimate the accelerator activation as a function of their components (compositions, geometries, etc.) and their uses. FISPACT-II [11] and CINDER-90 [12] are two other examples of analytical activation codes based on experimental and evaluated nuclear data. These codes permit evaluating the neutron activation for different radiation scenarios (fluence, irradiation time, cooling time, etc.), as also for radiological values of interest like activity and dose rate. Monte Carlo simulation of radiation-matter interactions is considered a powerful and practical tool to estimate all the radionuclides produced during X ray irradiations. Many past and current research projects seek to calculate by Monte Carlo simulation how photonuclear activation varies as a function of accelerator design, irradiation parameters, or characteristics of the irradiated objects. For the Monte Carlo simulation, the three principal codes used are MCNPX [13], Fluka [14] and GEANT4 [15]. Each method, however, offers its own modeling of photonuclear processes and/or neutron activation. It is therefore important to know the accuracy and the reliability of the calculational method considered. By studying the 3 main stages of calculations (photonuclear data and model, photoneutron production and activation), we have identified the most critical steps for the accuracy of the final results and propose a general methodology for a step-by-step validation of numerical photonuclear calculations for X ray applications.

12.3. RESULTS AND DISCUSSION

12.3.1. Monte Carlo photonuclear calculations

The two Monte Carlo codes selected for the present project are GEANT4 and MCNPX. These codes were used to model an accelerator (LINAC type) and to estimate the distribution of photoneutrons produced by both the accelerator and the irradiated sample. For the photonuclear processes, the most important physical data and/or models are photonuclear cross-sections and final state calculations. For the photoneutron production, one of the most critical parameters is the X ray energy spectrum produced by the accelerator. In addition, there is the geometry and the elemental composition of materials which participate both in the production and the thermalization of the photoneutrons (accelerator components, irradiated sample, etc.).

12.3.1.1. Photonuclear reactions

The first part of the study focused on the comparison of cross-sections used by simulation codes (MCNPX, GEANT4) with nuclear data available in the JANIS database. For this, we have selected the main light elements (including H, Be, C, O, etc.) and heavy elements (including W, Pb, Ta, etc.) that contribute to photonuclear reactions in industrial applications. Among other things, we were able to demonstrate that the parameterized model used by the GEANT4 code (G4PhotoNuclear) did not take into account the isotopic specificities of the radionuclides, which very often led to a poor calculation of the reaction threshold as well as to an unreliable estimation of the number of neutrons produced. This model was completed in 2018 (from version 10.04) by another model (LEND - Low Energy Nuclear Data) allowing access to the ENDF-BVII.1 nuclear database for photo-nuclear reactions with E_{gamma} <20 MeV (also used in the MCNPX code), which has greatly contributed to reducing the differences between the different Monte Carlo simulation codes in this energy range. The most obvious example (Fig. 1, top) is deuterium (^{2}H) which has a null photonuclear cross-section in the G4Photonuclear model (because it is taken identical to that of hydrogen (^{1}H)) while it is non-zero from an energy of around 2.22 MeV in the LEND model (which corresponds to the theoretical photonuclear threshold). Fig. 1 (bottom) shows another example of the possible differences between simulation codes and experimental data for ^{13}C. This demonstrates the need to verify the availability and precision of the photonuclear cross-sections used in the simulation codes for all the nuclei corresponding to the accelerator and food elemental compositions.

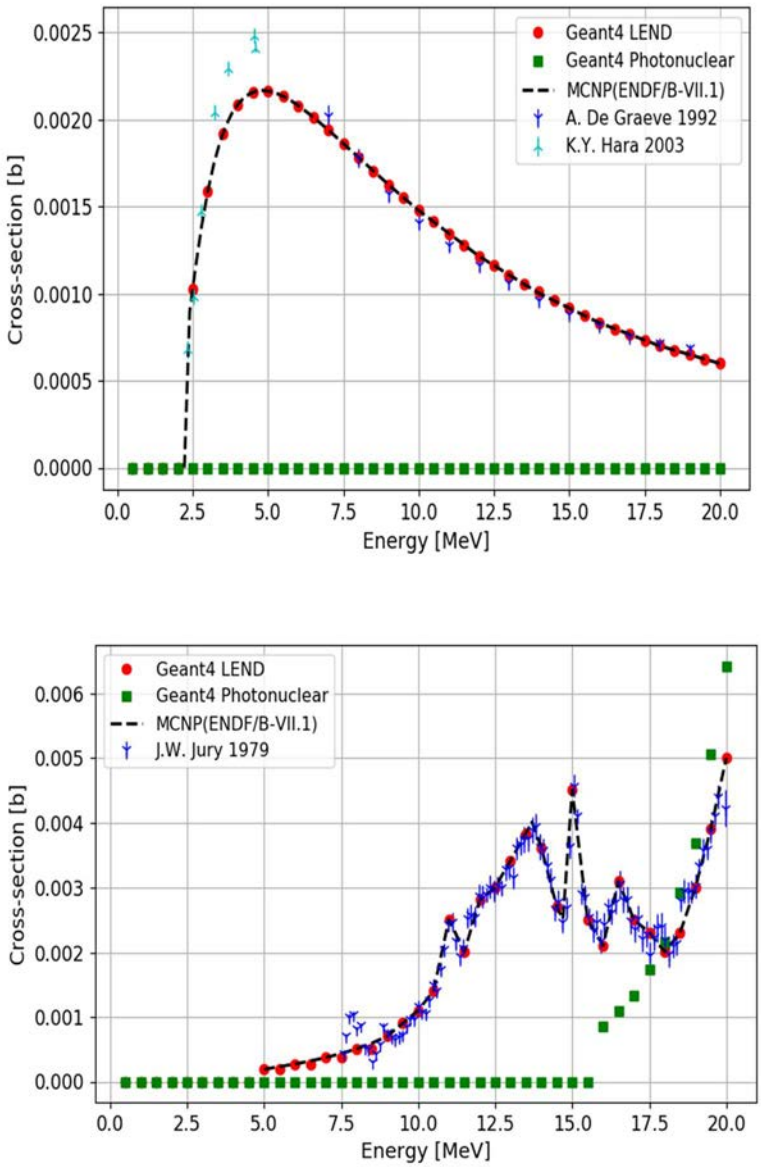

FIG. 1. Comparison of GEANT4 (G4Photonuclear and LEND models), MCNP(ENDF-BVII.1) and some experimental photonuclear cross-sections of ^{2}H (top) and ^{13}C (bottom). Experimental data are available on the JANIS database.

12.3.1.2. Photo-neutron production

In addition to the photonuclear cross-sections discussed previously, other parameters can strongly influence the production of photo-neutrons: final state models in simulation codes, X ray energy distributions and elemental composition. As for the cross-sections, it is therefore important to compare the neutron spectra obtained with the different simulation codes for different elements and different photon energies. For example, Fig. 2 shows that the production of photoneutrons by interaction of 20 MeV photons in ^{9}Be produces on average 30% more neutrons in the GEANT4 code compared to the MCNPX code, but that the energy peaks are comparable in both codes.

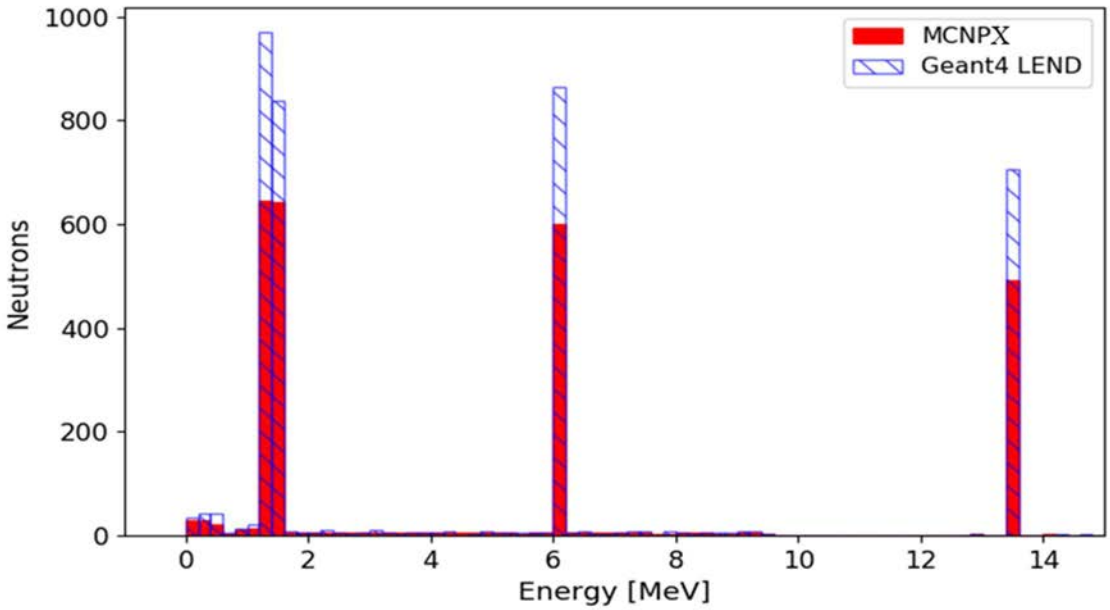

FIG. 2. Comparison of GEANT4 (LEND models) and MCNPX photoneutron spectrum corresponding to the irradiation of a ^{9}Be cube (1 mm^{3}) with 20 MeV photons.

Besides the modelling of the photonuclear processes themselves, one of the most critical parameters for the Monte Carlo simulations is the energy spectrum of the X rays emitted by the accelerator. As the activation thresholds for most of the elements are between 6.5 and 8 MeV, it is essential to validate the spectrum modelling precisely. However, there are currently few experimental methods that can accurately measure a neutron spectrum in this energy range. In radiotherapy, for example, the modelling of the accelerator is generally validated through experimental measurements of the dose distribution at depth and the lateral dose profile in a water phantom [16]. It is therefore common practice to adjust the energy of the electron beam to match the simulated and measured data, and to use, for example, an energy of 7 MeV or 14 MeV for an accelerator operating respectively theoretically at 6 MeV or 15 MeV. Although this adjustment method is valid for the Monte Carlo dose calculation, it presents many problems for the calculation of photonuclear activation. As only photons at the tail of the distribution of the energy spectrum contribute to photonuclear reactions, uncertainties in the shape of the spectrum and the maximum value of the energy can lead to large errors in the estimation of the number of photo-neutrons produced. Being part of the simulation, it is thus essential to validate experimentally the modelling of the X ray spectrum. The measurement of dose distributions (percentage depth dose, lateral profiles, etc.) in a water phantom, as done in radiotherapy, is a necessary but not sufficient step. For photonuclear processes, it is also necessary to be able to verify the maximum energy of the beam. One possibility would be to use photonuclear reactions with different thresholds and to measure radionuclides or secondary particle production.

The elemental composition of materials is another important source of uncertainty, both in terms of photonuclear processes (production of neutrons) and activation (production of radionuclides). The first difficulty arises from the lack of information from manufacturers on the exact composition of the materials used in the accelerator components. The second difficulty is linked to the presence of traces of activatable elements in the irradiated samples, present in a proportion of a few milligrams per kilogram of material (ppm). The calculation of photonuclear reactions with these trace elements by Monte Carlo simulation poses obvious problems of calculation time and statistical uncertainty.

12.3.2. Experimental validations

Monte Carlo simulations of the production and the thermalization of photoneutrons in the full irradiation setup (accelerator, sample, etc.) give access to the neutron fluence and energy at any point in space. The accuracy of these distributions is a critical parameter for the activation calculation and requires experimental validations.

12.3.2.1. Accelerator and X ray spectra

The first step in the validation process is related to the accelerator and X ray beam modeling. The accelerator design and the related dose distributions obtained from simulations can be validated by comparison with ionization chamber measurements. The X ray spectrum validation can be based on activation of materials with well-known characteristics (physical dimensions, composition, density, etc.) and analytical measurements of activation products by gamma spectrometry.

To illustrate this protocol, we proceeded in 4 steps:

- Modeling of a fully medical LINAC (target, collimators, shielding);
- Validation of the dose deposited at the isocenter;
- Selection of the element to be irradiated;
- Irradiation and activation measurements.

A detailed bibliographic survey has been performed to select an element that produces gamma emitters whose activity can be precisely measured by gamma spectrometry. The selection criteria retained depends on three parameters: high cross-section of gamma emitter production (σ_{prod}), high intensity of gamma ray emissions (I) and high gamma detection efficiency (ε_{detec}):

$$\sigma_{prod} \times I \times \varepsilon_{detec}$$

We have considered a “simple” case in order to have the best precision on our experimental results. The selected material, natural tantalum consisting of two isotopes ^{180m}Ta (0.012%) and ^{181}Ta (99.988%), was irradiated by X rays with a maximal energy of 15 MeV produced by a medical LINAC at the Paul Strauss Center (Strasbourg – France). The activation products are ^{180}Ta due to photo-neutron emission from ^{181}Ta and ^{182}Ta due to neutron capture by ^{181}Ta. The production of (n,γ) reactions was demonstrated. Fig. 3 (top) shows experimental measurements of ^{180}Ta and ^{182}Ta decays after an irradiation at 117 Gy. The experimental value of the ^{180}Ta period is estimated at 8.18 ± 0,47 h, which is comparable to the literature value of 8.15 h.

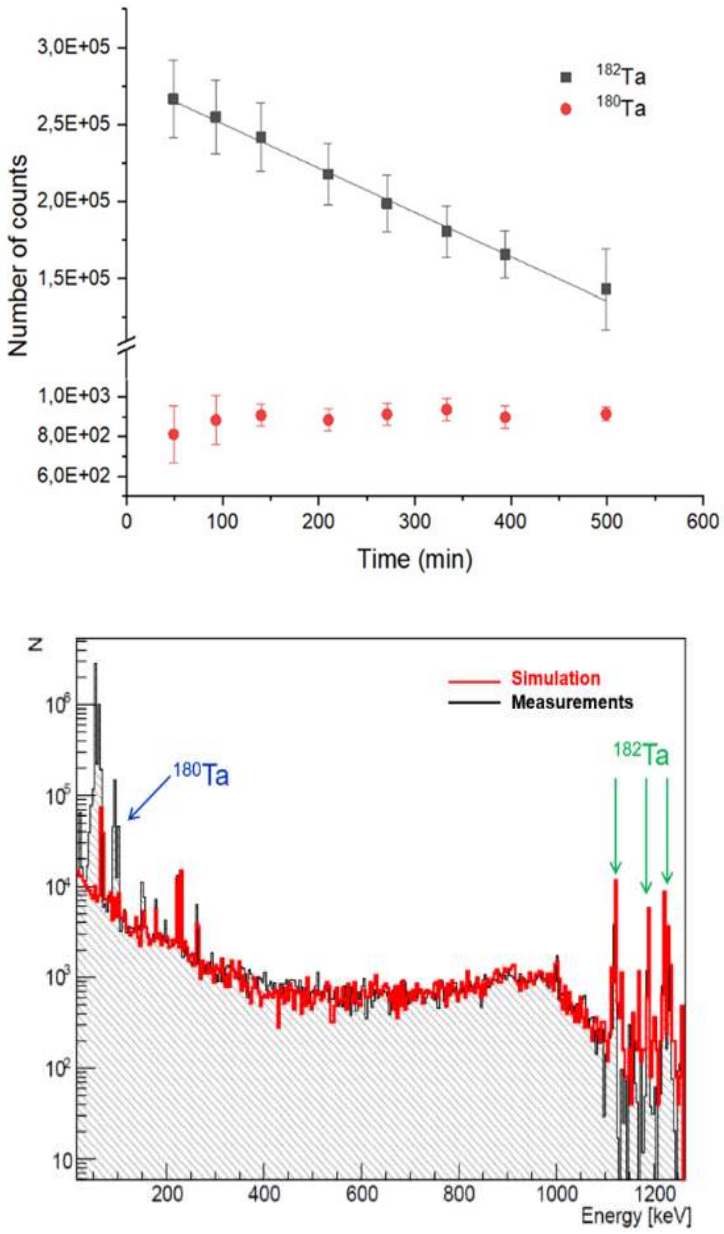

FIG. 3. Radioactive decay (top) and gamma emission spectrum (bottom) of ^{180}Ta and ^{182}Ta produced by the irradiation of tantalum plate with 15 MeV photons.

Gamma spectra of these activation products were obtained with a high-resolution gamma detector (HPGe) and compared to GEANT4 Monte Carlo results (Fig. 3, bottom). The Monte Carlo simulation predicts the production of ^{180}Ta and ^{181}Ta well, but from the comparison of the gamma spectra it can be seen that the calculated and experimental activities do not agree within 10%. The deviations can come from the different simulation steps described previously: photonuclear cross-sections, models of production of end states (photo-neutrons), modeling of the X ray energy spectrum. Experimental Ta activities can thus be used to improve the simulation until the results become compatible with the measurements. In addition to gamma spectrometry measurements, (γ,n) reactions with different thresholds can also be used to check the true maximum energy of the X ray beam. Reactions on ^{207}Pb (6.7 MeV), ^{117}Sn (6.9 MeV), ^{67}Zn (7.05 MeV) and ^{186}W (7.2 MeV) could be used for instance to check the true maximum energy of a beam with a nominal electron energy of 7 MeV.

12.3.2.2. Photo-neutron production

After working on the validation of the accelerator modeling and the X ray spectrum, it seems essential to compare simulated neutron distributions with experimental neutron measurements. Neutrons are classified according to their kinetic energy (thermal: E<1 eV, epithermal: 1 eV< E<10 keV Fast: >10 keV). The term "temperature" can also describe this energy representing thermal equilibrium between a neutron and a medium with a certain temperature. The measurement of neutrons, in particular thermal neutrons, around and inside irradiated objects is indeed an interesting method for globally validating the Monte Carlo simulation. The measurement of neutrons can indeed be considered as an experimental means of ensuring that the theoretical calculations of the photoneutron distributions obtained are acceptable. This point was also put forward in the IAEA report of 2002 [1], as follows:

« In X ray *facilities, it is important therefore to measure the neutron fluence in the food to assure compliance with the requirement that the food should not have any significant amount of any induced radioactivity ».*

This measurement can be done in different ways (activation of indium, vanadium or gold foils, Solid-State Nuclear Track Detectors (SNTD) such as CR39, real-time neutron detectors). The IAEA report puts a general limit on the fluence for the composition of the reference food (3×10^8 neutrons/cm^2.s), but limits should be established for each type of irradiated product and accelerator design.

Experimental measurements were carried out at the new feerix (Faisceau d'Electrons Et Rayonnement Ionisants X) facility of Aerial (Strasbourg-France). This facility is based on advanced technologies in a Rhodotron, an electron accelerator (up to 10 MeV), and high-energy X ray (up to 7 MeV) generation. This feerix facility permits studying, on two separate beamlines, all multisectoral applications of ionization. In this part of the study, we focused on the detection of neutrons induced by photonuclear reactions likely to occur by X rays of 7 MeV maximum energy. We were interested in a simple case of irradiation easily reproducible by Monte Carlo simulations. For this, we irradiated a water phantom to generate neutrons and thermalize them. In order to map the neutron flux, various passive detectors were placed at different locations from the incident photon beam.

The irradiation conditions were:

- Electron energy: 7 MeV;
- Beam width ± 30 cm from the top side of the phantom;
- Distance converter to front face phantom: 60 cm;
- Estimated dose by Alanine/EPR routine dosimetry system: 5.17 kGy (the accumulated dose was estimated using Alanine/EPR with an accuracy of 5%).

The phantom is a Plexiglas parallelepiped of 35 x 35 x 35 cm^3 filled with water. In order to thermalize the neutrons more efficiently, a polyethylene plate with dimensions of 88 x 49 x 2.5 cm^3 was placed on the front of the phantom (Fig. 4). SSNTD types CR39s from Technol Chiyoda, Japan, for passive neutron dosimetry, were placed at different locations on the water phantom to map fast and thermal neutrons. These detectors were placed on the front, lateral and back sides of the phantom as well as inside the phantom. All SSNTDs were etched at the same time in a potassium hydroxide solution (concentration of 30%) for 2.5 hours [17].

FIG. 4. View of the 35 x 35 x 35 cm^3 water phantom and solid-state nuclear track detectors (SSNTD).

The detector dimensions are 19 x 8.5 x 0.8 mm^3 with a density of 1.29 $g.cm^{-3}$. Each SSNTD is enclosed in a 1 mm thick plastic case consisting of two parts, one using a High Density Polyethylene (HDPE) converter for fast neutrons and the other reserved for thermal neutrons using a boron (BN in HDPE) converter. The surface area read on each side is 19.36 mm^2. Several readings were taken, averaging 3 to 5 times for each part. Table 2 reports the average values of the net trace density (D_{PE}: fast neutrons, D_{BN}: thermal neutrons) after subtraction of the background noise.

From these measurements one can conclude that the ratio of thermal to rapid components can vary from 1.24 to 4.64 depending on the position of the detectors. The results obtained with the various dosimeters thus show that neutrons were produced in sufficient quantities to allow validation of the Monte Carlo simulation with this kind of experimental set up. These results also highlight that the spatial distribution of photoneutrons is quite heterogeneous around and inside the water phantom. This must be taken into account in the activation calculations, in particular when the irradiated samples are themselves also inhomogeneous (geometry, compositions, etc.).

The experiments carried out thus illustrate two experimental methods using SSNTD and Alanine/EPR which can be used to validate the Monte Carlo modeling of the X ray spectrum and the Monte Carlo calculation of the spatial distributions of photoneutrons.

TABLE 2. RESULTS FROM SSNTDS PLACED AT VARIOUS LOCATIONS OF A 35 X 35 X 35 CM^3 WATER PHANTOM IRRADIATED WITH 7 MeV X RAYS (5.1 kGy)

Dosimeter number	Phantom Location	Tracks per unit area		DBN/DPE
		Net Density D_{PE} (cm^{-2})	Net Density D_{BN} (cm^{-2})	
F00753	Inside Centre	1491 (92)	3198 (117)	2.14
F00756		1676 (77)	5586 (176)	3.34
F00721	Inside Top	1563 (217)	2285 (272)	1.46
F00723		2218 (319)	4911 (155)	2.21
F00752	Front Side	2396 (160)	6066 (263)	2.53
F00758		2285 (248)	5267 (196)	2.30
F00720		1878 (155)	4994 (309)	2.66
F00757		2579 (248)	4540 (196)	1.76
F00729		2332 (253)	10833 (434)	4.64
F00728	Right Side	1625 (248)	5102 (387)	3.14
F00727		1346 (299)	3616 (330)	2.68
F00751	Left Side	1352 (258)	2296 (495)	1.70
F00722		1656 (227)	2363 (279)	1.43
F00726	Back	1254 (165)	3616 (201)	2.88
F00719		1383 (227)	2337 (237)	1.24

12.3.3. Activation calculations and measurements

After obtaining neutron distributions from Monte Carlo simulations, it is then possible to calculate the corresponding activation using analytical codes such as FISPACT-II or CINDER-90. Since analytical codes do not manage particle transport, Monte Carlo simulations must be used to model photo-neutron production and thermalization inside the irradiated object. By reconstructing the 3D distribution of thermal neutrons, it would then be possible to calculate in a very limited time the number and type of radionuclides produced from an analytical code, even for trace elements present in small proportions. It is also possible to use Monte Carlo simulation methods to calculate both the production of photo-neutrons and the corresponding neutron activation, but here the issue of long calculation times might be problematic.

12.3.3.1. Irradiation at the industrial facility STERIS

In this part of the research we studied the possibility of using a numerical framework (combining Monte Carlo and analytical codes) to determine the different radionuclides produced (and their relative activities) according to the beam characteristics, the material composition, and the accelerator geometry configuration for food irradiation with X rays higher than 5 MeV.

The first step of the work was to perform a 7 MeV X ray irradiation in the industrial facility of STERIS Applied Sterilization Technologies (Däniken, Switzerland). This company provides contract sterilization services, laboratory testing, and product and packaging testing services to medical device and pharmaceutical manufacturers. It uses a Rhodotron electron beam

accelerator to generate X rays with energies of 5 to 7.5 MeV. The Rhodotron is a recirculating electron accelerator marketed by IBA for industrial sterilization by ionizing radiation. X rays are produced by bremsstrahlung following the interaction of electrons with a 1.2 mm thick tantalum target sheet cooled by a 4 mm thick water sheet contained between the tantalum sheet and a 3 mm thickness of stainless steel [18].

Several food samples (rabbit food and black pepper powder from the Institute for Security Detection Technology of the People's Republic of China) were irradiated by X rays at 7 MeV with different doses ranging from 25 kGy to 120 kGy. The electron beam intensity and the deposited dose rate were respectively 50 mA and 5 Gy/min. The total absorbed dose was measured by the Alanine/EPR (Electron Paramagnetic Resonance) routine dosimetry system. Fig. 5 shows the irradiation geometry. The samples have been conditioned in 500 cc and counted by gamma ray spectrometry before irradiation to identify natural radioactivity. PN3-type polyallyl diglycol carbonate (PADC) solid state nuclear trace detectors were installed to estimate the ambient neutron dose during irradiation.

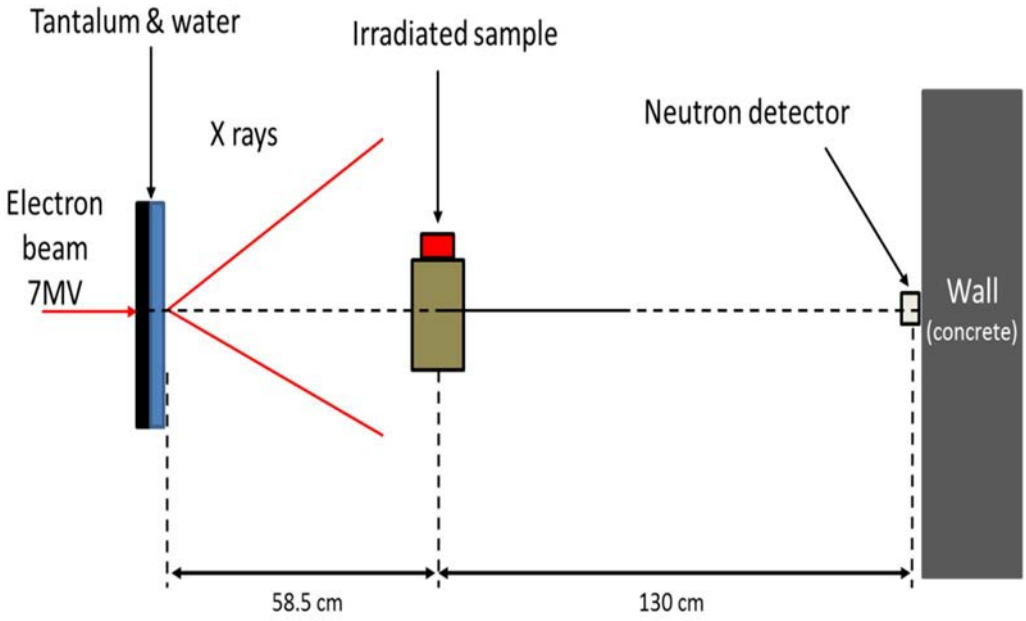

FIG. 5. Setup of the STERIS experiments.

After X ray irradiations, we measured the induced radioactivity using high-resolution gamma-ray spectrometry in situ (Falcon 5000 Portable HPGe-Based Radionuclide Identifier) and in the laboratory with a Compton-Suppressed System to reduce the background continuum for low-background counting. For the highest dose (120 kGy), we were able to measure and identify the artificial radionuclides produced during the irradiation as ^{14}C, ^{32}P, ^{31}Si, ^{24}Na, ^{42}K, ^{56}Mn, ^{135m}Ba and ^{87m}Sr (Table 3).

TABLE 3. EXPERIMENTAL AND SIMULATED RADIONUCLIDES PRODUCED FROM FOOD IRRADIATION WITH 7 MeV X RAYS (120 kGy)

Radionuclide	Half-life	Emission	γ-spectrometry	Simulation
^{87m}Sr	2.8 hours	γ	Yes	No
^{135m}Ba	1.2 days	γ	Yes	No
^{24}Na	15.0 hours	β, γ	Yes	Yes
^{42}K	12.4 hours	β, γ	Yes	No
^{56}Mn	2.6 hours	β, γ	Yes	Yes
^{14}C	5 700 years	β	No	Yes
^{32}P	14.3 days	β	No	Yes
^{31}Si	2.6 hours	β	No	Yes

The second step was to simulate the full irradiation process using MCNPX and GEANT4 Monte Carlo codes. Based on the manufacturers (IBA) information, simulations of the bremsstrahlung radiation produced by monoenergetic electron pencil beam in the converter configuration (Tantalum, water & steel) were compared with MCNPX and GEANT4 calculations. The maximum probability located around 350 keV as well as the global shape of the spectrum were found compatible between the two codes (Fig. 6).

The thickness of the tantalum sheet is calculated for a maximum conversion efficiency, i.e. an electron to photon efficiency of 14% at 7 MeV.

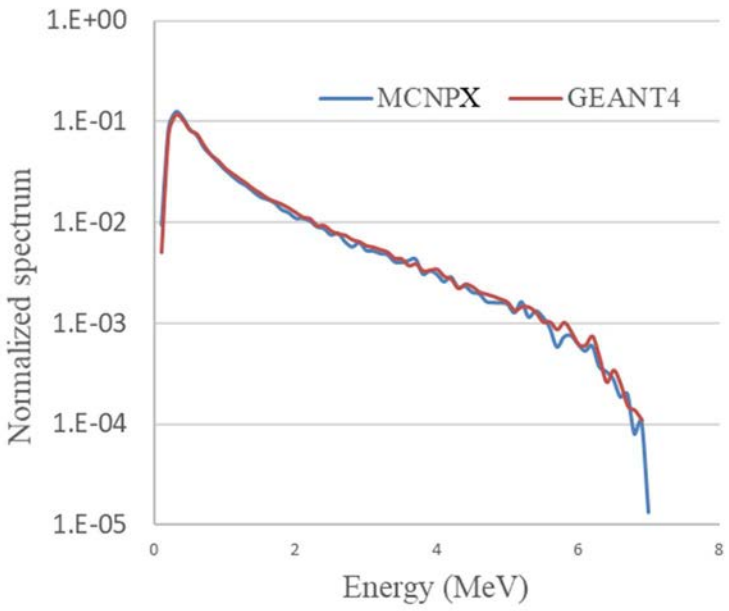

FIG. 6. X ray spectrum calculated from MCNPX and GEANT4 Monte Carlo codes for 7 MeV electron energy spectrum of the IBA Rhodotron.

For the estimation of induced activity, we implemented the activation code CINDER-90. It is a multigroup activation tool developed by LANL. It simulates neutron activation for neutrons up to 25 MeV. This autonomous code requires knowledge of the geometry of the device as well as the source and flux of neutrons used. The code package and its updated data libraries come with several subscripts that allow calculations of multi-cell problems in combination with the radiation transport code MCNPX via the *histp* option and the results of the F4 tally (energy

deposition by volume). CINDER allows the calculation of specific activities for different post-irradiations times and for each isotope present in the library (Z<104). Since version 2.7.0 of MCNPX, the libraries have been integrated. MCNPX can thus calculate the photonic spectra of radioactive decay of the elements resulting from the radiative capture.

The irradiated samples were defined following the composition reported on the food packaging (Ca, P, Na, Mn, Zn, etc.). The comparison between gamma spectrometry measurements and simulation results reveals two interesting points for the research project. Firstly, it shows that the elemental food composition must be known very precisely to be able to predict the type of induced radioactivity. The basic analysis furnished by the supplier was not sufficient for calculations because many trace elements were missing. Secondly, the results obtained prove that the Monte Carlo simulation is a powerful tool to predict not only gamma emitters but also beta and alpha emitters, which are more difficult to measure experimentally. This experiment also demonstrates that an irradiation of approximately 100 kGy at 7 MeV makes it possible to produce several radioelements of interest for the validation of the activation calculations.

12.3.3.2. Irradiation at the industrial facility feerix

Another experiment was planned at the feerix facility, this time using a cardboard box as the packaging material. Indeed, Monte Carlo simulation with modeling of such a setup with similar dimensions as for the water phantom, preicted that a significantly higher quantity of neutrons would be produced when using cardboard.

The aim of this experiment was to measure samples as they are usually irradiated in industrial conditions for sterilization. The cardboard box packaging consisted of successive layers of cut out cardboard packaging with a cylindrical hole at its centre (to accommodate the samples). The total dimensions of the cardboard box were 35 x 35 x 35 cm^3. The samples irradiated were silver and vanadium sheets, and rabbit food contained in a 500 ml borosilicate glass volumetric flask (Model SG500).

The irradiation conditions were as follows:

- Electron energy: 7 MeV;
- Static irradiation mode;
- Distance from X-ray converter to the front face of the phantom: 60 cm;
- Estimated dose by the Alanine/EPR routine dosimetry system per sample: 14 kGy for the vanadium, 11 kGy for the silver and 14 kGy for the rabbit food (accumulated absorbed dose was estimated using Alanine/EPR with an accuracy of 5%).

This experiment was simulated using GEANT4 Monte Carlo software (Fig. 7). Starting from a 7 MeV electron beam, the X ray spectrum was computed by modelling the full accelerator target.

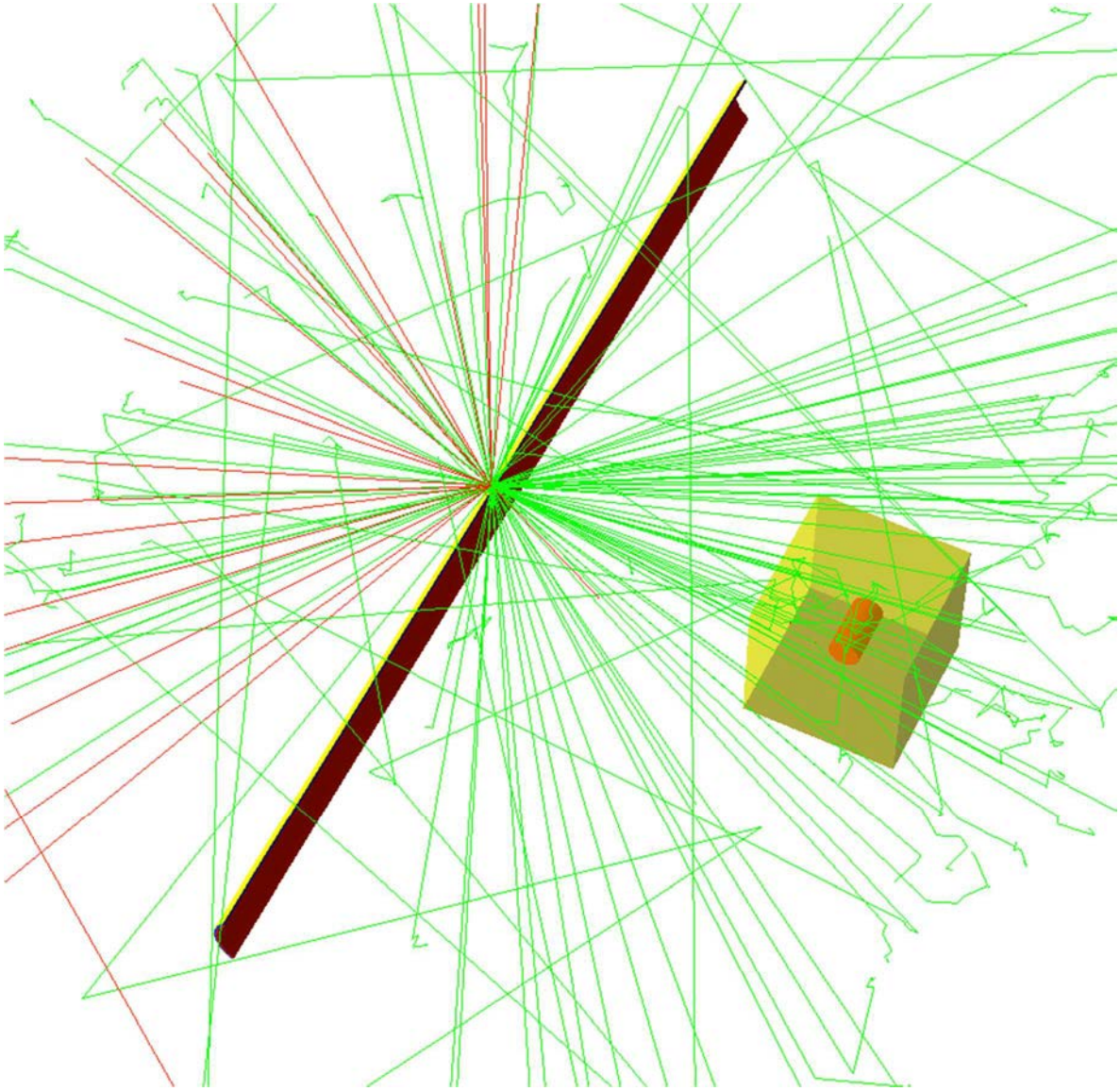

FIG. 7. GEANT4 simulation for 7 MeV X ray irradiations of food sample inside a cardboard box.

Neutron spectra were computed for both the cardboard box (surface and centre of the box), and the SG500 flask that contained the food sample (at the centre of the box) (Fig. 8).

The simulated neutron spectra at the centre of the box and in the SG500 flask were then normalized using beam current and irradiation duration, and used as the input parameter (neutron flux in $cm^{-2}.s^{-1}$) for FISPACT activation calculations.

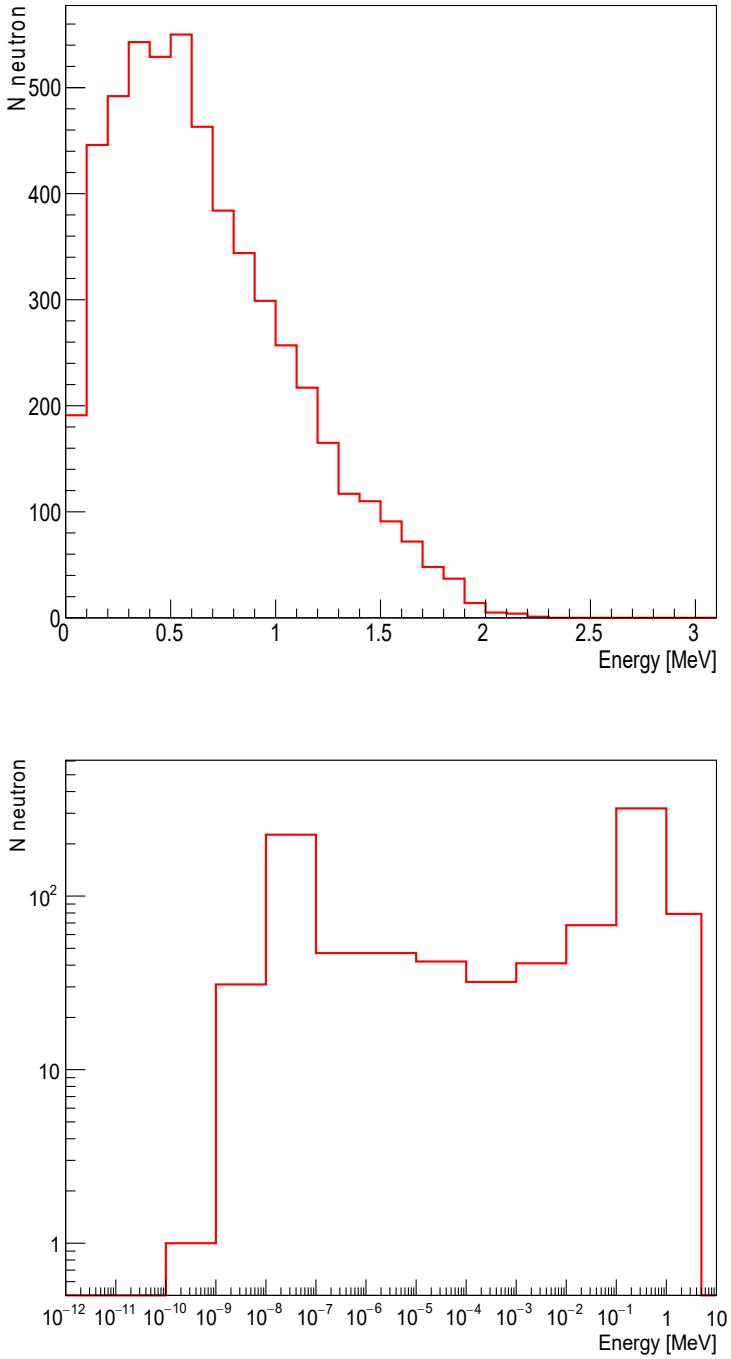

FIG. 8. Top: neutron spectrum at the surface of the cardboard box (GEANT4). Bottom: neutron spectrum inside the SG500 flask (at the centre of the cardboard box) (GEANT4).

12.3.3.3. Activation of silver and vanadium samples

FISPACT calculations

For FISPACT activation calculations on vanadium (V) and silver (Ag), natural compositions were used with the corresponding masses of the irradiated samples. Based on the previous Monte Carlo neutron spectra, FISPACT calculated V and Ag activities shown in Table 4.

TABLE 4. FISPACT CALCULATED ACTIVITIES OF VANADIUM AND SILVER SAMPLES

Radionuclide	Activity (Bq)	Half-life (s)
^{52}V	57.2	225.18 (30)
^{108}Ag	202	142.9 (7)
^{110}Ag	4637	24.56 (11)

Experimental measurements

After irradiations, we measured the induced radioactivity using high-resolution gamma-ray spectrometry in situ (Falcon 5000 Portable HPGe-Based Radionuclide Identifier). Fig. 9 shows the experimental gamma spectrum of the vanadium sample obtained after 10 minutes of irradiation.

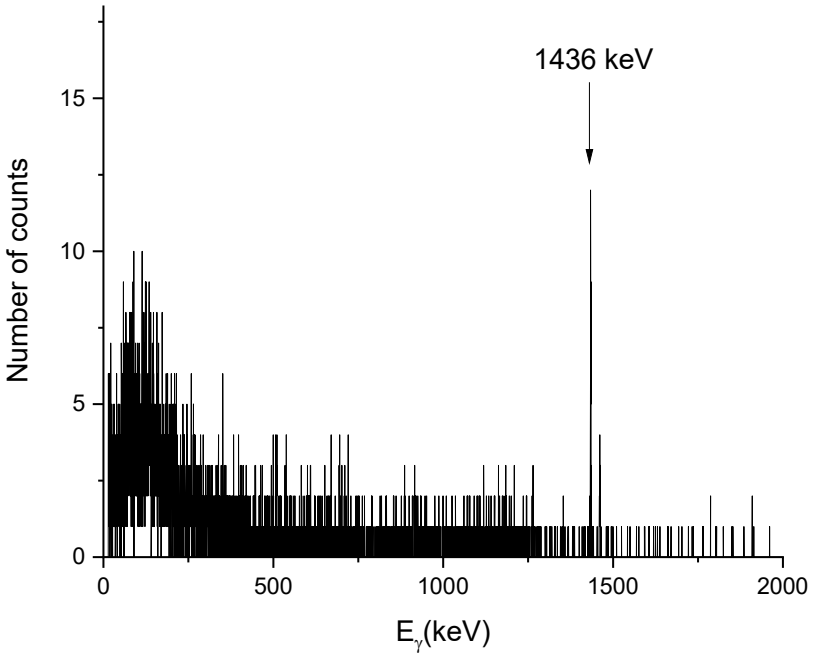

FIG. 9. Gamma ray spectrum of irradiated vanadium sample, with the main emission peak of ^{52}V at 1436 keV.

The activity induced by neutron interactions can be deduced from the main peak of the ^{52}V radionuclide produced at 1436.06 (1) keV, which has an emission intensity of 99.75 (25)%. The results of the measurements by gamma-ray spectrometry for the vanadium and silver samples are shown in Table 5.

TABLE 5. MEASURED ACTIVITIES OF VANADIUM AND SILVER SAMPLES

Radionuclide	Activity (Bq)
^{52}V	140 (30)
^{108}Ag	<1200
^{110}Ag	<1100

In the case of vanadium, the measured and predicted activities were of the same order of magnitude. In the case of silver, ^{108}Ag and ^{110}Ag were found below the detection limit of the system. Since around 4 minutes have elapsed between the end of irradiation and the beginning of the measurement, most of the ^{110}Ag had already decayed. The results cannot be directly compared, but peaks appeared at the energy of the gamma-ray of ^{108}Ag allowing us to suppose that the radioactivity of this radionuclide was just below the detection limit. Furthermore, these results have shown the advantage of an irradiated vanadium sample (compared to a silver one) for such experiments. Vanadium can be easily activated due to the high reaction cross-section and the measurement is more precise thanks to the half-life of its reaction products (^{52}V). Furthermore, these results show the advantage of an irradiated vanadium sample (compared to a silver one) for such experiments.

12.3.3.4. Activation of rabbit food

FISPACT calculations

In order to determine rabbit food elemental composition, the dried sample was first crushed and mineralized with HNO_3/HCl (0.3:1 v/v) in a digestion system (DigiPREP, SCP Sciences) then analysed by ICP-MS (7500ce, Agilent). However, results provided by this technique (Table 6 in µg/g) do not make it possible to determine the concentration of light elements hydrogen, carbon, nitrogen and oxygen. Thus, the rest of the concentration has been completed with these elements by taking the same relative concentrations as those indicated in the IAEA TECDOC of 2002 [1].

TABLE 6. ELEMENTAL COMPOSITION OF RABBIT FOOD

Element concentration (µg/g)								
Na	Mg	Al	Si	K	Ca	Sc	V	Cr
75.8	**94**	**1.3**	**4.9**	**275**	**482**	**0.02**	**0.22**	**0.97**
Mn	Fe	Co	Ni	Cu	Zn	As	Rb	Sr
0.01	**6.8**	**0.24**	**0.63**	**10**	**52**	**0.07**	**4.1**	**4.9**
Y	Zr	Nb	Mo	Ag	Cd	Cs	Ba	La
0.08	**0.58**	**0.18**	**1.03**	**0.09**	**0.03**	**0.03**	**3.5**	**0.06**
Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho
0.05	**0.01**	**0.04**	**0.008**	**0.003**	**0.009**	**0.001**	**0.008**	**0.002**
Er	Yb	Tl	Pb	Th	U			
0.006	**0.004**	**0.001**	**1.2**	**0.005**	**0.4**			

The input concentrations used in FISPACT for hydrogen, carbon, nitrogen and oxygen is presented in Table 7. All elements were input with their natural elemental composition.

TABLE 7. INPUT CONCENTRATIONS OF HYDROGEN, CARBON, NITROGEN AND OXYGEN

Elements	H	C	N	O
Concentration (%)	9.1	18.2	2.0	70.6

Based on the Geant4 Monte Carlo neutron spectrum corresponding to a 10 minutes 7 MeV irradiation of the setup, activities were computed by FISPACT for the rabbit food sample (Table 7). Only radionuclides with a specific activity above 1.0 x 10^{-6} Bq/g/kGy are listed. Below that value, we estimated the activity too low to be measurable by gamma-ray spectrometry.

TABLE 8. ESTIMATED ACTIVITIES OF RADIONUCLIDES IN RABBIT FOOD

Radionuclide	Specific activity (Bq/g/kGy)	Half-life	Main gamma energy line (keV)	Intensity (%)
^{110}Ag	2.2x10^{-2}	24.56 s	657.8	4.6
^{19}O	5.4x10^{-3}	26.91 s	197.1	95.9
^{66}Cu	3.7x10^{-3}	5.12 min	1039	9.23
^{108}Ag	1.3x10^{-3}	2.38 min	633	1.62
^{52}V	1.0x10^{-3}	3.75 min	1434	99.75
^{28}Al	9.0x10^{-4}	2.25 min	1779	100
^{49}Ca	5.0x10^{-4}	8.72 min	3084	90.72
^{69}Zn *	5.0x10^{-4}	56.4 min	318.4	0.001 2
^{24}Na	3.5x10^{-4}	14.96 h	1369	99.99
^{27}Mg	3.1x10^{-4}	9.46 min	843.8	71.8
^{16}N	2.3x10^{-4}	7.13 s	6129	67.0
^{42}K	1.7x10^{-4}	12.36 h	1525	17.9
^{64}Cu	1.2x10^{-4}	12.7 h	511	35.04
^{239}U	1.1x10^{-4}	23.46 min	74.66	51.6
^{165}Dy	5.0x10^{-5}	2.33 h	94.7	3.58
^{71}Zn	3.6x10^{-5}	2.45 s	511.6	32
^{88}Rb	2.6x10^{-5}	17.8 min	1836	22.4
^{139}Ba	1.6x10^{-5}	1.4 h	165.9	24
^{55}Cr *	9.3x10^{-5}	3.5 min	1528	0.037
^{101}Mo	4.3x10^{-6}	14.6 min	590.1	20.3
^{56}Mn	3.4x10^{-6}	2.58 h	846.8	98.85
^{69m}Zn	2.4x10^{-6}	13.76 h	438.6	94.77
^{233}Th	1.5x10^{-6}	22.15 min	29.37	2.17
^{161}Gd	1.1x10^{-6}	3.66 min	360.9	60.1

* Radionuclides decaying only or mainly by emitting β particles

Experimental measurements

After X ray irradiations, we measured the induced radioactivity in rabbit food samples using high-resolution gamma-ray spectrometry in the laboratory with a Compton-Suppressed System to reduce the background continuum for low-background counting. Fig. 10 shows the experimental gamma-ray spectrum of the rabbit food sample before and after irradiation. The two spectra were normalized with respect to the acquisition time (21602 s for the spectra after irradiation). Natural radioactivity was detected from the spectral acquisition of unirradiated samples before X ray irradiation, which comes from ^{40}K and the radionuclides in the natural decay chains of uranium and thorium. It concords with concentration of elements given in the Table 6, which is quite important in the case of uranium (0.4 μg/g).

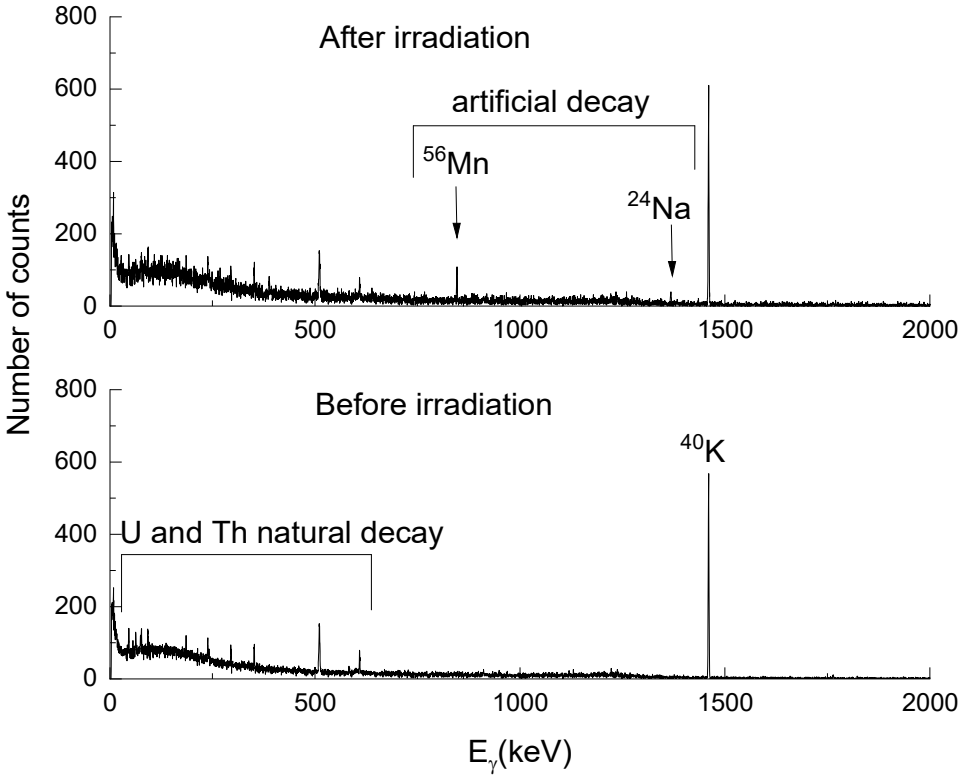

FIG. 10. The measured gamma emission spectrum of the rabbit food after 7 MeV X ray irradiation (top) and before irradiation (bottom).

Two radionuclides, ^{24}Na and ^{56}Mn, were observed experimentally that correspond to artificial activities induced by neutron interactions. The activity of ^{24}Na and ^{56}Mn is determined from their main peak respectively at 1368.630 (5) keV, which has an emission intensity of 99.9934 (5)% and at 846.7638 (19) keV, with an emission intensity of 98.85 (3)% [19].

The results of the gamma-ray spectrometry for the rabbit food sample are shown in Table 9.

TABLE 9. MEASURED ACTIVITIES OF ^{24}NA AND ^{56}MN

Radionuclides	Specific activity (Bq/kg)
^{24}Na	0.024 (16)
^{56}Mn	0.029 (14)

For ^{24}Na, the experimental specific activity is 0.024 (16) Bq/kg, i.e. 2.4 (16) 10^{-5} Bq/g. The predicted specific activity from FISPACT is 4.9 10^{-3} Bq/g (see Table 8) for a dose of 14 kGy. There is a large discrepancy (factor 100) between measurements and calculations.

In the case of ^{56}Mn, the experimental specific activity is 0.029 (14) Bq/kg, i.e. 2.9 (14) 10^{-5} Bq/g. From FISPACT, the estimated specific activity is 4.8 10^{-5} Bq/g (see Table 8) for a dose of 14 kGy. Experimental and calculated activation are in this case of the same order of magnitude. It should also be noted that most of the radionuclides obtained by FISPACT could not be measured because of their (very) short half-life's. The experimental measurement was started in the laboratory about 1 hour after the end of the irradiation. Some radionuclides with longer half-life's, such as ^{42}K or ^{165}Dy, could not be detected because of their very low intensity gamma emission.

The results obtained show the complexity of numerical activation calculations for the industrial food irradiation. For the same experiment, it is possible to obtain a satisfactory agreement between measurement and calculation for certain radioelements while having several orders of magnitude of difference for other radioelements. There can be many explanations for these differences (nuclear data, X ray spectrum, composition of the sample, etc.). The results obtained thus show the importance of an experimental step-by-step validation of the Monte Carlo simulation, as well as measurements of the spatial and energy distributions of the neutrons obtained by Monte Carlo simulation before using them as an input parameter for the activation calculation.

12.4. CONCLUSIONS

Monte Carlo simulations and analytical activation calculation codes are two powerful and practical tools to estimate all the radionuclides potentially produced during X ray irradiations. These tools can help to predict not only gamma emitters but also beta and alpha emitters which are more difficult to measure experimentally. The precision and reliability of the results is however very sensitive to several parameters, making it essential to set up a step-by-step experimental validation process. After studying the most critical points of the calculation chain (nuclear data, X ray spectrum, sample composition), this work has tested different experimental methodologies to validate both the Monte Carlo modeling of the irradiation setup and the activation calculations. The experimental measurement of photoneutrons around and in the irradiated sample seems to be one of the most reliable means of control. Beside the global validation of the Monte Carlo simulation, the spatial and energy distributions of photoneutrons are one of the fundamental parameters for the precision of activation calculations.

The next stage of the project would be to test the full protocol in a reference facility in order to validate dedicated software for quantitative calculation of the radioactivity produced in the food during an industrial irradiation.

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13. DEVELOPMENT OF A LOW ENERGY IN-LINE CABINET X RAY MACHINE FOR PHYTOSANITARY IRRADIATION

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Abstract

Phytosanitary irradiation (PI) treatment is a means to disinfest fresh host commodities of quarantine insect pests to overcome regulatory trade barriers for market access. In general, large-scale irradiation facilities are multipurpose and therefore designed to apply high doses (5–50 kGy) and may not be optimal for applying precisely lower doses (0.15–0.40 kGy) required for insect control in fresh commodities. Low energy (160–185 keV) cabinet irradiators, using a large surface area X ray emitter, may have unique applications where a relatively low volume of fresh produce must be treated at low doses for quarantine insect control. The design criteria for a low energy cabinet irradiator are that it should have excellent penetration to enable treatment of fruit in boxes, good dose uniformity to ensure treatment capability at doses up to 400 Gy but below the 1 kGy maximum, sufficient throughput for the target industry, low cost, portability, reliability during continuous use, and self-shielding. Sweet cherry is a possible application for a low energy cabinet X ray irradiator for insect disinfestation because the current quarantine treatment, methyl bromide fumigation, causes adverse effects on fruit quality. A cabinet X ray irradiator would be attractive to industry if it is suitable for installation in-line at a packinghouse near to where fruit are harvested. It might also allow treatment of fruit in a cold room to maintain the cold chain and thereby preserve fruit quality. A low energy cabinet X ray irradiator was developed by Applied Energy Devices (Albuquerque, NM, USA) for treatment of sweet cherry fruit in boxes and evaluated for dose uniformity and throughput.

13.1. INTRODUCTION

13.1.1. Background

Irradiation with gamma, electron beam or X rays is an effective and proven postharvest treatment to eliminate quarantine pests on fresh commodities [1, 2]. The movement of fresh commodities and access to markets can be restricted to prevent the spread and introduction of pests. Quarantine or phytosanitary treatments disinfest host commodities of insect pests. Treatments applied before the fresh commodities are exported or moved to areas where the pests do not occur are often the simplest approach to overcome restrictions and gain or retain market access [3]. The US Food and Drug Administration (FDA) has approved irradiation doses up to 1 kGy for preservation and disinfestation of fresh horticultural products such as fruits. The US Department of Agriculture (USDA) and the International Plant Protection Convention (IPPC) have approved specific and generic radiation doses for disinfestation such as the 150 Gy treatment for tephritid fruit flies. The USDA has also approved a generic radiation

dose of 400 Gy for all insects except pupae and adults of Lepidoptera, meaning phytosanitary irradiation treatments are available for nearly all fruit exports without further research. In addition to generic doses, irradiation has advantages over other phytosanitary treatments because it is 1) broadly effective against insects and other organisms at doses that do not harm fruit quality, 2) temperature insensitive, 3) able to treat fruit in its final packaging, and 4) fast (short treatment time).

Most large-scale irradiation facilities are multipurpose and designed to apply high doses of radiation (5–50 kGy) and may not be optimal for applying the low doses (0.05–0.40 kGy) required for insect control in fresh commodities. Also, large-scale high energy systems are costly. A small-scale low energy cabinet-style irradiator may be a sustainable and economically attractive method for phytosanitary treatment of fresh commodities for low volume fruit exports.

13.1.2. Cabinet X ray irradiator for phytosanitary irradiation

The design criteria for a low energy cabinet irradiator are that it should have excellent penetration to enable treatment of fruit in boxes, good dose uniformity to ensure treatment capability at doses up to 400 Gy but below the 1 kGy maximum, sufficient throughput for the target industry, low cost, reliability during continuous use, and self-shielding. In some cases, portability may be a desirable feature. Low energy X ray meets these criteria for a cabinet style system for phytosanitation.

The practicality of a low energy cabinet X ray irradiator (160–185 keV) for insect disinfestation depends on having an efficient large surface area X ray emitter. The target for treatment could be a single layer of fruit travelling on a conveyor in a packing line or multiple layers of fruit packed in boxes [3]. The large-surface area of the emitter would allow for treatment at close range over the full width of the conveyor carrying fruit or of the full width of a box of fruit. Standard small window point-source X ray tubes are not suitable for this application. A cabinet X ray irradiator will have limited throughput compared with a larger stand-alone high energy irradiator. However, a self-shielded cabinet-style irradiator may have unique applications where a relatively low volume of fresh produce must be treated at low doses for insect disinfestation. Several features make it attractive to the commercial fruit industry: 1) suitable for installation in-line or at a packinghouse, 2) close proximity allows treatment of fruit immediately after harvest, 3) potential for treatment of fruit in a cold room to maintain the cold chain, 4) portable if movement to an isolated domestic quarantine is required, 5) the grower or packer would have control of the treatment process, and 6) lower cost and smaller footprint than a high energy system. Irradiation is a cold process and generally has minimal adverse effects on fruit quality, and therefore is a safe alternative for fruit that are sensitive to methyl bromide or another postharvest treatment type.

A cabinet X ray system has many practical applications for phytosanitary treatment. For example, the California sweet cherry industry is interested in alternatives to methyl bromide for control of spotted wing drosophila in fruit exported to relatively small markets such as Australia, New Zealand and Mexico. New Zealand and Tasmania would like a portable X ray treatment option to move fruit out of quarantine no-export zones when they get incursions of Queensland fruit fly or other fruit flies [4]. Mexico would like to export dragon fruit to the USA using X ray treatment for Mexican fruit fly control. Australian sweet cherry growers currently ship fruit to several southeast Asian countries using a 3-week in-transit cold treatment which results in low quality fruit and would like to replace cold with faster X ray treatment. Fiji would like to use X rays to ship fresh fruit such as breadfruit and papaya to New Zealand. Any industry that faces quarantine restrictions due to insect pests may benefit from this technology.

13.1.3. Target application - California sweet cherries

Australia and several other countries (e.g. New Zealand, Mexico) have imposed restrictions on the importation of sweet cherries from California and the Pacific Northwestern USA due to possible infestation by the quarantine pest spotted wing drosophila (*Drosophila suzukii*) (Diptera: Drosophilidae). An irradiation treatment protocol (80 Gy) was developed for spotted wing drosophila [5], and quality studies have shown that sweet cherries are very tolerant of irradiation up to 500–600 Gy [6–10]. Currently California ships sweet cherries to Australia using methyl bromide fumigation for quarantine control of spotted wing drosophila, but methyl bromide turns cherry stems an undesirable brown colour which lowers overall quality. Methyl bromide treatment also cannot be done at cold temperatures, so treatment requires warming up the fruit and breaking the cold chain, which results in shorter shelf life for highly perishable sweet cherries. Irradiation does not cause quality loss and can be applied at cold temperatures, and therefore is an attractive alternative to current fumigation quarantine treatments. The portability of a cabinet X ray system also may be useful for dealing with domestic quarantines due to new quarantine pest incursions, as occurred with the detection of Oriental fruit fly in an important sweet cherry production area in California in 2011.

Designs for a packing house scale X ray tube irradiation system were developed by Applied Energy Devices, LLC with the idea of demonstrating the system to the California sweet cherry industry. A. Sambado & Son, Inc. (Linden, California), a large sweet cherry grower and packing house (Prima Frutta Packing and Primavera Marketing, Inc.), collaborated on the project as a target user and provided industry input on cherry packing and handling operations, box types, and throughput requirements that were used to designing the system. A. Sambado & Son also agreed to provide fruit, logistical support and space for a demonstration to the industry.

13.2. MATERIAL AND METHODS

13.2.1. Low energy cabinet X ray machine concept

The goal was to design a self-shielded cabinet-style irradiator using a large transmission surface X ray emitter capable of treating the full width of the box at close range in a single pass. Several commercial fruit boxes of different sizes used by the sweet cherry industry for exporting were selected as the target for treatment for testing purposes. The standard 8.2 kg box used to pack sweet cherries for export to Australia was used for throughput calculations. The 4-pi X ray emitter (Fig. 1) [11] was the basis for the cabinet X ray irradiator concept. This capsule shape

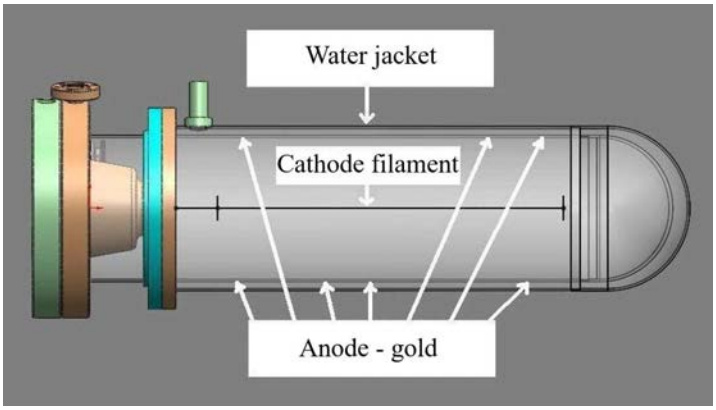

FIG. 1. The Rad Source 4-Pi X ray tube emits X rays in 360 degrees over a large surface area.

X ray tube not only emits X rays over a wide field but emits X rays in 360 degrees, which provides increased efficiency as boxes can be treated from two sides by passing over and then under the tube. The basic components of the cabinet X ray system are the X ray emitters with power supplies, conveyor, control system (computer interface), radiation measurement equipment, and shielding. The key performance attributes for evaluating a cabinet X ray system are dose rate, dose uniformity, and product handling efficiency.

13.2.2. Sweet cherry irradiation sensory test

The California cherry industry asked for proof of concept by way of irradiated fruit for sensory evaluation. Two sweet cherry irradiation quality tests were conducted. In the first test, newly harvested 'Bing' cherries delivered to the Prima Frutta packing house in California were shipped the same day to Atlanta and irradiated at Rad Source Technologies (Buford, Georgia) using two 4-Pi X ray tubes mounted in an experimental treatment cabinet (Fig. 2). This configuration is similar to what was envisioned for a cabinet X ray system at that time, except that the cherries were irradiated stationary, as a batch process, instead of moving on a conveyor belt as part of a continuous irradiation process.

FIG. 2. Experimental irradiation of sweet cherry boxes using two 4-Pi X ray *tubes (Rad Source Technologies, Buford GA, USA).*

The boxes were irradiated from one side then flipped and treated from the other side. Measurements of the dosages of radiation absorbed showed that the X rays had easily penetrated through the 18 lb box of fruit and had good uniformity. The cherries were repacked and shipped back to Prima Frutta for evaluation. However, the packed fruit were temporarily lost during transit and removed from refrigeration. After a two-day delivery delay, the fruit arrived in poor condition, apparent having experienced temperature abuse. Therefore, no quality assessments were conducted. The California cherry season ended soon afterward and therefore continued testing required sourcing fruit from a cherry production area further north.

In the second test, export quality 'Skeena' cherries were shipped under cold storage from Oregon to San Francisco, California, where they were irradiated at Nutek (e-beam irradiation,

Hayward, California, USA), and transported immediately to A. Sambado & Son two hours away for quality evaluation. A sensory 'taste test' panel comprised 15 employees at the packinghouse and included field, office and management personnel. Each food tester was presented with three bowls labelled A, B and C, each containing 4 to 5 cherries. Cherries in bowl A had been irradiated at 150 Gy, bowl B at 400 Gy, and bowl C were untreated (0 Gy). The taste tester was given three evaluation sheets (A, B, C), each with a 22 cm line on it with the word 'dislike' at the left end and 'like' at the right end. Taste testers were asked to evaluate the cherries in each bowl by judging appearance, texture, and taste and place a mark on the line corresponding to their overall degree of liking for each sample. The distance from the start of the line on the left to the mark was measured for each sample (A, B, and C) for each of the participants and averaged. Higher numbers indicated increased likeness. Likeness data were log transformed and subjected to analysis of variance (ANOVA) and means separation using a Tukey's HSD test.

13.3. RESULTS AND DISCUSSION

13.3.1. Low energy cabinet X ray irradiator

The first prototype of the cabinet X ray machine (Bugs I) was irreparably damaged during transportation from its construction site in New Mexico to A. Sambado & Son in California for testing, i.e., the lead cabinet was structurally compromised. The second prototype (BUGS II) was designed and built based on lessons learned to be much sturdier for improved portability. All components of BUGS II have been fabricated and assembled for testing by Applied Energy Devices (Albuquerque, NM). The 4-pi X ray tube was redesigned for increased efficiency and treatment of a larger target. The tube is roughly 90 cm long with a 50 cm emission surface and output is 160kV and 37 mA (6 kW) (Fig. 3). The current cabinet configuration uses two X ray tubes, with space available for a maximum of four tubes. A distilled H_2O/ethanol mixture is used as a coolant for X ray tubes. The X ray tubes are mounted in a lead-shielded (1.22 cm lead thickness) containment box, with conveyors moving fruit boxes into the containment area and over and under the X ray tubes during treatment (Fig. 4). The conveyor below the X ray tubes can be raised or lowered electrically to accommodate different box sizes. The distance from the surface of the box to the X ray emitter as it passes over and under is approximately 8 cm. The conveyor rollers above the X ray tubes are made of carbon fiber to reduce attenuation and improve dose delivery. A pulley system on sliding rails mounted on the ceiling of the shipping container allows for lifting and removing the lead panels to service the X ray emitters (Fig. 5). The X ray tubes are suspended from rails on wheels inside the containment chamber which allows them to be rolled back out of the chamber to facilitate servicing. A strip of eight Si PIN diodes (silicon radiation detectors) is attached above and parallel to each X ray tube which provides real-time measurement of power output along the tube and facilitates remote evaluation of tube performance.

FIG. 3. The capsule-type X ray *emitters in BUGS II. The conveyer system moves boxes of fruit over and then under the* X ray *tubes for treatment from the bottom and then the top of the box. The over-and-under design improves dose uniformity of the treatment and doubles the volume of fruit that can be treated per unit time compared to a point source* X ray *tube. The large surface area of the* X ray *emitter allows for complete coverage of the fruit box during each pass at close range.*

FIG. 4. BUGS II cabinet X ray *irradiator under construction by Applied Energy Devices (July 2018). The lead shielded treatment chamber with conveyor system is housed inside a 6.08 m shipping container.*

The system is housed inside a 6.08 m shipping container which is 2.86 m in height (Figs. 4 and 5). The weight of the shipping container is approximately 3,700 kg and the weight X ray machine is about 3,400 kg, for a total weight of 7,100 kg. The control room with computer interface is separated in the container from the main room containing the X ray machine by a door with interlocks to prevent entry during operation. The computer control system consists of a controlled area network (CAN) backbone with distributed processing for various electrical components (conveyors, power supplies, conveyor height adjustment, pin diodes) programmed in C. Sensors measure tube temperature and coolant flow, power output, and movement of boxes through the system, and interrupt the power supply and X ray emissions if normal tolerances are exceeded.

The customer must have 3-phase power, 208 volts, and approximately 15 kW for a two-X ray emitter system. Dose mapping in several box types suggests a dose uniformity ratio of less than 1.5. A dose of 150 Gy is delivered to an 8.2 kg box of sweet cherries in approximately 85 seconds, for an estimated throughput of 346 kg/hr. Throughput can be increased by increasing power. The current X ray tube uses an off-the-shelf 6-kW power supply (Spellman High Voltage Electronics Corp., Hauppauge, NY). Previous tests with the 4-Pi tube at Rad Source used a 10-kW power supply, and use of this power supply with higher power in our machine would decrease treatment time proportionally to 51 seconds per box and an estimated throughput of 576 kg/hr. The recommended treatment dose for spotted wing drosophila is expected to be 100 Gy. Irradiation treatment at this lower dose would further reduce the treatment time to 34 seconds for an estimated throughput of 865 kg/hr.

FIG. 5. The cabinet treatment chamber of BUGS II is shielded using lead panels (February 2020). A pulley system allows for removal of the front panels for access to the X ray *tubes and interior of chamber.*

13.3.2. Sweet cherry irradiation sensory test

The sensory panel was unable to distinguish between irradiated and unirradiated sweet cherries, or between the two levels of irradiation (Fig. 6). Cherries that had the 400 Gy treatment received higher average scores, indicating higher preference, but panel scores for the three differently treated cherries were not statistically different ($F_{2,44} = 1.01$, $P = 0.37$). Therefore, cherries irradiated at 150 and 400 Gy were indistinguishable from untreated cherries. This result (no effect of irradiation on quality) is consistent with previous fruit quality studies showing sweet cherries tolerate irradiation up to 500–600 Gy without significant quality losses. Fruit were held for an additional 1 week at 3ºC for continued evaluation of appearance and taste by several Prima Frutta Packing employees (including the President, Lawrence Sambado). Over the one week period, fruit quality remained high in all treatments. This result provided the California cherry industry with the confidence to continue funding research on irradiation and the development of in-line treatment options using the cabinet X ray irradiator concept.

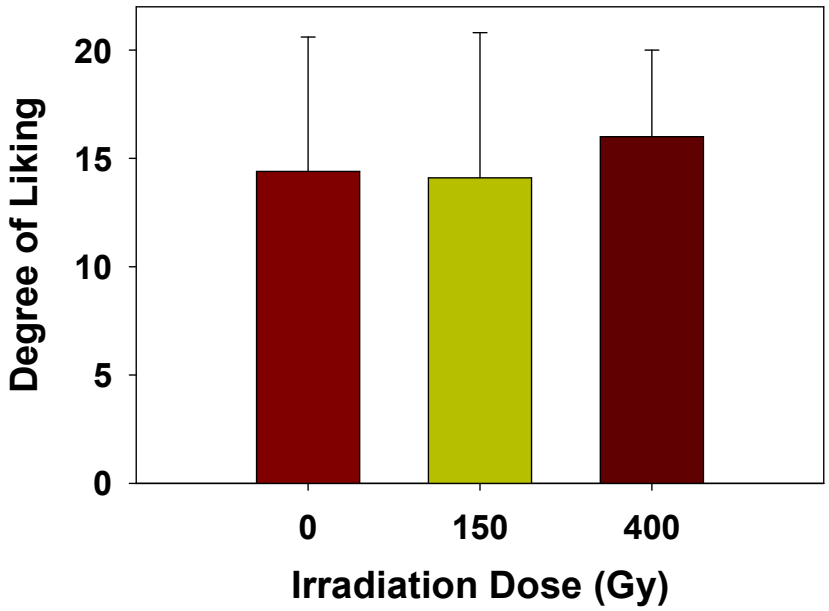

FIG. 6. The sensory panel of 15 taste testers could not differentiate between 0 (no irradiation), 150 Gy and 400 Gy treatments, indicating that irradiation had no detectable effect on overall cherry quality (taste, texture, appearance).

13.4. CONCLUSIONS

Our goal was to construct a self-shielded cabinet-style irradiator with a large transmission surface X ray emitter capable of treating the full width of fruit boxes of various sizes at close range in a single pass. Several commercial fruit boxes of different sizes used by the sweet cherry industry for exporting were selected as the target for treatment for testing purposes. Applied Energy Devices designed the conveyor system and X ray emitter to handle and treat a sweet cherry export box in consultation with the California fruit packer A. Sambado & Sons. The 4-pi X ray emitter used in the Rad Source RS 3400 blood irradiator was repurposed and modified for the cabinet irradiator. This X ray tube is an ideal design for a cabinet-style irradiator as it not only emits X rays over a wide field but emits X rays in 360 degrees, which provides increased efficiency as boxes can be treated from two sides by passing over and then under the tube. The basic components of the cabinet X ray system are the X ray emitters with power supplies, conveyor, control system (computer interface), radiation measurement equipment, and shielding. The next steps are to 1) finish construction of the machine and conduct further dose mapping studies, 2) install and demonstrate the machine at a large sweet cherry fruit packing operations (e.g. in California or Washington, USA), 3) obtain regulatory certification, and 4) conduct pilot scale shipments of irradiated fruit from the USA to Australia or elsewhere.

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Comparative Studies of Different Modes of Irradiation

14. WHICH DO YOU CHOOSE FOR A PHYTOSANITATION PROGRAM- ELECTRON BEAM OR X RAY TECHNOLOGY?

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Abstract

Ionizing radiation technologies such as gamma radiation from ^{60}Co, electron beam (eBeam) and X ray have been used around the world for phytosanitary treatment purposes for over two decades. X ray technology has been successfully used for over 20 years to treat fresh agricultural products shipped from Hawaii to mainland United States. Gamma radiation from ^{60}Co based technology is currently the mainstay for the phytosanitary treatment industry and has been for over a decade. This technology is widely used to treat fresh agricultural products for transboundary shipments in the Asia and the Pacific region, especially in Australasia. The volumes of agricultural products treated with ^{60}Co has seen strong growth year after year over many years. Presently, this technology is also used to treat agricultural products that are exported from Latin America and Asia into the United States. Electron beam (eBeam) technology has also been successfully demonstrated to treat mangoes that arrive from Asia and Mexico into the United States. The security and transportation challenges along with the replenishment costs associated with ^{60}Co, has spurred interest in the adoption of machine sources such as eBeam and X ray technologies. As indicated in our previous publications, the decision to adopt eBeam as compared to X ray technology should never be made without a deep understanding of the technology, the economics of the process and the capital and operating expenses [1, 2].

14.1. INTRODUCTION

The growing economies around the world have resulted in an ever-expanding population of consumers who can pay for an improved standard of living. Countries around the world have increasing numbers of people that can purchase premium quality fresh fruits and vegetables throughout the year. Therefore, there is an expanding increase in volumes of fresh fruits and vegetables that are shipped across continents to meet this growing demand. Countries in Asia and Latin America are now poised to become major exporters of fresh fruits and vegetables to countries around the world. However, fresh fruits and vegetables cannot be transported across international boundaries without meeting strict phytosanitary requirements and rules. The International Plant Protection Convention (IPPC), is an international agreement and an international standards setting body. The convention is focused on preserving standardization

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of plant health practices around the world to prevent the spread or accidental introduction of regulated pests and pathogens, and promoting safe trade. The agreement introduced International Standards for Phytosanitary Measures (ISPMs) as its main tool to achieve its goals, making the IPPC the global standard setting organization for plant health. The ISPMs are strict global standards governing the different phytosanitary treatment technologies such as methyl bromide fumigation, hot water treatment, cold temperature, and ionizing technologies for treating agricultural commodities in transboundary shipments [1].

To meet the growing demand of fresh agricultural products, countries around the world have targeted their fresh fruit and vegetable exports to be of high economic value, and, therefore of great strategic significance. This realization is clearly evident in the number of bilateral agreements signed around the world. For example, the USA has signed bilateral agreements with many countries and this has allowed US consumers to access exotic fruits and vegetables which hitherto were unavailable. The US Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS) has established protocols for the use of ionizing radiation for treating specific agricultural commodities from specific countries. Several research papers and consumer trials have documented that ionizing technologies such as ^{60}Co, eBeam and X ray have little or no minimal impacts of commodity quality [3, 4]. In fact, the use of these technologies can result in commodities with a higher sugar content and better flavour notes than if these commodities underwent legacy treatments such as hot water dips or methyl bromide fumigation. These ionizing technologies are already being used for shipments into the USA, between Australia and New Zealand and when products are exported into the People's Republic of China. Between 2007 and 2014, the total volume of agricultural products that entered the USA from overseas destinations increased 7000% from 195 000 kg to almost 14 million [5]. Exporting countries such as the Republic of India, Mexico, South Africa, the Kingdom of Thailand and the Socialist Republic of Viet Nam accounted for this steep increase in volumes. The present trends suggest that this increase will continue well into the future. According to the International Irradiation Association (iia, *sic*) recent data from the General Directorate of Plant Health of Mexico indicates that the volume of Mexican exports treated by ionizing technologies increased by 30% in 2020.

The increasing reliance on ionizing technologies for transboundary shipment of agricultural products suggests that the base for these technologies must increase considerably in the exporting country. Other than the United States which has a unique option of "treatment upon arrival", countries tend to require that the treatment must be accomplished in the exporting country. Therefore, the availability of these ionizing technologies must increase in the Asia Pacific and Latin American regions. Gamma radiation from ^{60}Co is undoubtedly the leading ionizing technology used in phytosanitary programs around the world. However, the challenges in securing and safeguarding ^{60}Co sources, the challenges in shipping the source around the world to replenish the facilities, and the cost of replenishment have exerted significant constraints on the expansion of this particular technology [6]. There is universal agreement that machine sources rather than ^{60}Co sources will be the technologies of the future. The International Atomic Energy Agency (IAEA) launched the worldwide research collaboration "DEXAFI" (Development of Electron Beam and X ray Applications for Food Irradiation) to coordinate research and development for the practical implementation of machine sources rather than ^{60}Co technology in the food processing industry.

There is already historical data documenting that machine sources such as X ray technology being successfully used for over 20 years for treating agricultural products from Hawaii to mainland United States. Electron beam technology has also been successfully demonstrated to treat mangoes that arrive from Asia and Mexico into the United States [7]. For these

technologies to rapidly expand in the exporting countries, the private industry needs to be involved in establishing phytosanitary treatment facilities for these facilities to be financially and technologically sustainable. To encourage and facilitate private investment to stimulate rapid expansion of these technologies detailed analyses of equipment capital costs, facility operating costs, throughput volumes, location of treatment facilities, and commodity seasonality are a pre-requisite. This report provides a case study on the choice of the technology and how decisions on the equipment specifications can have a significant impact on the treatment costs. It is the treatment costs that will in the final analysis dictate the financial sustainability of the phytosanitary treatment facility, as well as the wide-scale acceptance of this technology by the retailers.

14.2. BACKGROUND

The USDA-APHIS allows the ionization technology to be used "in-country", before the commodities are exported or "on arrival" in the USA, subject to the commodity meeting strict quarantine packaging, transportation, and handling requirements. The National Center for Electron Beam Research (NCEBR) situated on the Texas A&M University campus in College Station Texas is one of only three ionizing technology-based treatment facilities that are approved by the USDA-APHIS for phytosanitary treatment of regulated commodities on arrival in the U.S. The NCEBR uses high energy 10 MeV eBeam technology for delivering USDA-APHIS mandated minimum phytosanitary doses to imported commodities. NCEBR partnered with the world's largest retailer, Walmart, Inc, in a multi-year project to determine the feasibility of eBeam processing for phytosanitary treatment of imported Mexican mangoes. During this project, the packaging, the post-harvest handling, the transportation conditions were continually improved upon to enhance the quality of the fruit for retail sale. By the end of the three year project, NCEBR had treated millions of pounds of fresh mangoes (Ataulfo and Tommy Atkins cultivars) with a minimum dose of 200 Gy and a maximum dose of 1000 Gy.

14.2.1. Problem statement

Based on retailer feedback, it was noted that eBeam treated mangoes were of a superior quality (flavour profile) as compared to mangoes that were treated by hot water. Hot water is the legacy phytosanitary treatment technology for Mexican mangoes. The flavour profile of eBeam treated mangoes were better because the fruits could remain on the trees longer (thereby increasing Brix levels) since eBeam processing is gentler on the fruit as compared to the hot water treatment process. In short, hot water treatment does not yield a high-quality product. However, compared to hot water treatment, the retailer indicated that the eBeam processing costs (per pound) were more expensive than hot water treated mangoes because a) the specialized transportation requirements since "on-arrival" procedures called for refrigerated shipments, b) costs to process paperwork to meet Mexican and US border regulations, and 3) actual eBeam processing costs. The Mexican mangoes were shipped to NCEBR in 5-pound shipping cases packed in pallets on conventional freight (40 000 pound) shipping trucks. Since eBeam processing cannot treat entire pallets, NCEBR personnel had to unload the trucks, "de-palletize" the single cases and the single cases had to be placed within cardboard carriers on the conveyor system for eBeam processing (Fig. 1). There is significant labor required for "de-palletization", placing single boxes on the carriers and after eBeam processing, "re-palletizing" prior to loading back onto the trucks. The great advantage of eBeam processing namely speed was also the biggest challenge in using humans to unload the mango boxes off the conveyor belts for re-palletization. To keep up with the processing speed, multiple employees were needed to unload the boxes coming off the conveyor belt. Labor costs were found to be a significant component of the per pound processing costs. The challenge, therefore, was to reduce the

processing costs to make the costs comparable to the current hot water treatment. A bigger confounding challenge was that that it was almost impossible to obtain the actual hot water treatment costs from the mango packers since these costs can vary significantly depending on the volumes treated, signing of long-term contracts, etc.

FIG. 1. Photo showing the Mexican mangos arriving on pallets, then "de-palletized", and single cases placed on carriers on the eBeam processing conveyor system.

Moreover, this information was confidential. This was no different to NCEBR's eBeam processing costs for treating mangoes. The price per pound depended on the guaranteed treatment volumes, expectations for product turn-around time and other factors. Therefore, we estimated the per pound processing costs for various eBeam linear accelerator (LINAC) power configurations, various personnel staff numbers and an annual 2000 hour (single-shift) production scenario. In these studies, we assumed the use of an S-band LINAC (rather than a Rhodotron) and we used the estimated costs of these LINACs based on conversations with equipment suppliers as well as a published source for estimating capital equipment [2].

14.2.2. eBeam Facility Configuration and Associated Costs

Table 1. highlights the facility costs, annual amortized costs and variable costs associated with the three different LINAC power configurations. The maintenance costs, the interest rates and the twenty-year amortization are based on US values. The hourly labor costs and electricity costs were based on NCEBR data.

TABLE 1. PROJECTED COSTS ASSOCIATED WITH THREE DIFFERENT LINAC POWER CONFIGURATIONS

Parameter	LINAC POWER		
	5 kW	**20 kW**	**40 kW**
Facility Cost	US$6.3 million	US$7.7 million	US$8.4 million
Annual amortized cost	US$1.6 million	US$1.9 million	US$2.1 million
Background power usage	4 kW	16 kW	32 kW
Hourly staff numbers	20	80	200
Annual production hours	2000	2000	2000

It is evident that as the LINAC power increases, the throughput will also increase (Table 2). This necessitates a larger number of personnel in the facility to de-palletize and re-palletize the mango cases. The number of employees will increase tenfold when comparing the 5 kW LINAC facility as compared to the 40 kW LINAC facility. It is interesting that the facility costs remain low even for high powered facilities. The annual amortized cost of the facility is the contributor to the annual fixed operating costs. The total facility cost is assumed to be spread over 20 years (with interest) to avoid an overwhelming 1st year fixed cost followed by very low costs in the 2nd to 20th year.

14.2.3. eBeam Processing Cost Evaluation

With the construction of phytosanitary treatment-customized eBeam facilities, eBeam phytosanitary treatment costs can be made as low as 1.36 cents/pound (Table 2). In this analysis, beam power is used as a proxy for overall facility size. Table 2 shows the facility costs and the annual amortized costs and power usage associated with three different LINAC power configurations namely 5kW, 20 kW and 40 kW. The electricity costs were based on US $0.10 cents per kilowatt hour (kwh)

TABLE 2. PROJECTED ANNUAL EBEAM THROUGHPUT AND COSTS (FIXED AND VARIABLE) FOR PURPOSE-BUILT PHYTOSANITARY TREATMENT FACILITIES [REF: 2]

eBeam Power	Calculated Throughput	Variable costs	Fixed Costs	Per Pound processing costs
5 kW	31 680 tons	US$485 000	US$2.34 million	4.55 cents/pound
20 kW	126 720 tons	US$1.94 million	US$2.70 million	1.82 cents/pound
40 kW	254 440 tons	US$4.84 million	US$2.94 million	1.36 cents/pound

The high variable costs are primarily due to labor costs which as described earlier are due to eBeam technology's primary processing limitation which is the requirement for pallets to be "de-palletized" down into individual cases for eBeam processing. As the beam power increases, the facility throughput will increase substantially resulting in significant increase in work force to simply to de-palletize and re-palletize product. This work is physically demanding and must be maintained virtually non-stop to ensure that product continues to flow at a high rate through the electron beam. It is evident as facility size increases the staffing requirements increase linearly. These increases in staff size results in requirements for additional floor supervisors, increased restroom areas, bigger break rooms etc. Thus, it is evident that while throughput can

theoretically grow in linear proportion to beam power, this linear growth will require systemic changes to the design of a typical eBeam facility.

14.2.4. Facility Design Changes

Current eBeam processing facilities are often designed with a single conveyor system for products. This conveyor then passes through the electron beam, and products receive a dose of irradiation. While this configuration is well-suited to applications where the power of the electron beam itself limits product throughput, the single-conveyor design imposes substantial throughput restrictions on products that require only a low dose of irradiation. For example, at the NCEBR, at Texas A&M University, the 18kW accelerator could theoretically process nearly 100 000 lbs. of mango per hour. However, since the facility was originally not custom built for phytosanitary treatment, the conveyor system is not designed to keep pace with the high throughput afforded by the 18kW LINAC. Thus, these limitations have reduced the throughput rate by almost 83% due to the inability of the conveyor system to "keep pace" with the maximum output of the electron beam accelerator. One way to overcome this problem would be to have multiple parallel conveyor systems that make use of the beam power. It is evident that even the smallest of facilities (such as the 5kW facility) would require four or more conveyors passing through a wide-aperture electron beam to take full advantage of the power output potential of the electron beam. Therefore, in addition to changes to the conveyor system, reductions in the amount of labor required to de-palletize and re-palletize products would drastically improve per-pound processing costs. Currently, high labor requirements are the primary cost driver of electron beam processing. One can reduce labor costs with mechanized or automated processes. These would result in even more significant cost savings. However, automation will increase the up-front fixed facility costs. Based on these analyses, it is evident that mango phytosanitary treatment can be brought down as low as 1.36 cents/pound. Therefore, eBeam processing has the potential to disrupt current industry norms for mango phyotsanitation. However, current eBeam facilities are not being built for low dose processing. eBeam facilities custom-built for low dose processing can be a game changer.

14.2.5. Location of Phytosanitary Treatment Facilities

Besides paying attention to the facility/equipment configuration, greater attention needs to be paid to the location of such facilities. This is especially significant since the commodity is perishable, the primary growing locations will vary across a country during the particular fruit season. For example, in Mexico, there are at least four different mango producing areas. Deciding where to locate such phytosanitary treatment facilities can have a profound impact on the quality of the product. Recent studies, using advanced operations management theory, have revealed the critical importance of cooperation among all the entities in the produce phytosanitary supply chain [8]. Electron beam businesses can expect to see profits increased by almost 10% if there is strong cooperation among the different entities along the supply chain. Therefore, choosing the locations of eBeam phytosanitary treatment facilities based only on cursory maps of growing areas and transportation corridors and hubs can be fraught with serious financial ramifications.

14.3. CAN X RAY TECHNOLOGY REPLACE E-BEAM TECHNOLOGY?

There is already a low power (15 kW) X ray facility for phytosanitary treatment that has been in operation in Hawaii for the past 20 years. The financial detail of that privately owned facility is not publicly available. However, the Hawaii X ray facility caters to a very niche market to the extent that exporters of Hawaiian sweet potatoes etc. had no other option than to use the X ray facility. The primary disadvantage of X ray processing comparing to eBeam processing

is the low dose-rate of X rays systems. This implies that the throughputs are significantly lower than that of eBeam system. The medical industry is investing very heavily into X ray processing as a replacement of gamma ray ^{60}Co facilities. Therefore, major equipment manufacturers are catering to this need by building high power (between 100 kW and 1000 kW) X ray systems. The use of high-power X ray systems to process large bulk volumes of products in the irradiation area overcome the issue of relatively lower dose rates. Recently, a high power (~100 kW) X ray system was commissioned in Australia. Another facility is currently under construction in Mexico. Theoretically, X ray processing allows for pallet level irradiation treatment, thereby, avoiding the need for the excessive labor requirements that eBeam facilities need. However, the costs (capital and electricity costs) of X ray systems as compared to eBeam facilities can be steep. Also, since quick positive cash flow and a three- to five-year return of investment time frame are critical for investors, careful attention must be paid to ensure that the facility is continuously operating. This implies that there needs to be a continuous supply of product to be treated. However, fresh fruits and vegetables are extremely seasonal. It is too early to judge whether X ray-based phytosanitary processing will be economically feasible. However, the low dose of phytosanitary treatment suggests that if the facility and equipment are configured accurately, these facilities can be economically lucrative. Only time will tell.

14.4. CONCLUSION

Phytosanitary treatment to facilitate transboundary shipment of agricultural products is showing steep upward trends. Compared to legacy treatment technologies, ionizing technologies (gamma from ^{60}Co, eBeam and X ray processing) are gentler on the commodity and yield a higher quality product. Gamma radiation from ^{60}Co as a treatment technology is waning given the different challenges this technology faces. X ray technology avoids the excessive labor requirements of eBeam processing. Only time will tell whether X ray will become the go to choice for phytosanitary treatment or if bespoke eBeam facilities will be built specifically for phytosanitary treatment.

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15. PHYTOSANITARY TREATMENT FOR SOME EXPORTED VIET NAM FRESH FRUITS BY GAMMA AND HIGH ENERGY ELECTRON BEAM IRRADIATION

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Abstract

Tropical fruits such as green pomelo (Citrus maxima), king orange (the citrus hybrid Citrus reticulata × sinensis), star apple (Chrysophyllum caimito) and custard apple (Annona reticulata) are very important fruits in Viet Nam agriculture, due to their export potentials. However, postharvest losses caused by pests or pathogens can be an issue in their commercial production. Citrus bacterial canker (CBC), citrus tan spot disease, anthracnose and dieback diseases caused by *Xanthomonas* sp, *Phyllosticta citriasiana, Colletotrichum gloeosporioides,* and *Lasiodiplodia theobromae,* respectively are increasingly affecting crops. Therefore, these pathogens must be controlled before exportation. In these studies, the radio-sensitivity of these organisms to electron beam (EB) and gamma irradiation was investigated. The results showed that the radio-tolerance of *Xanthomonas* sp < *C. gloeosporioides* < *Phyllosticta citriasiana* < *Lasiodiplodia theobromae*, and the difference between gamma and electron beam radiation was insignificant. The D_{10} value for *Xanthomonas* sp was determined as 61 and 63 Gy for gamma and EB irradiation, respectively. The D_{10} value was 0.94 kGy and 1.1 kGy respectively for *Phyllosticta citriasiana*; 0.86 kGy and 0.9 kGy for *C. gloeosporioides,* and 0.95 kGy and 1.1 kGy for *Lasiodiplodia theobromae.* The *Xanthomonas* sp was completely controlled by both gamma and EB irradiation at generic quarantine dose of 0.4 kGy, but higher doses (4 and 5 kGy respectively for gamma and EB) were needed to inhibition spore germination and mycelial growth of the fungi and these higher doses would reduce fruit quality. Therefore, pretreatment with NaDCC or hot water was applied before irradiation to control the fungi at lower radiation doses. In this case, the D_{10} value reduced to 0.11 kGy and 0.12 kGy; 0.10 kGy and 0.11 kGy in combination hot water (50°C for two minutes) corresponding to gamma and EB for *C. gloeosporioides* and *Phyllosticta citriasiana,* respectively. For *Lasiodiplodia theobromae,* the D_{10} value reduced to 0.13 kGy and 0.19 kGy in combination with NaDCC (20 ppm). Thus, the combination of hot water (50°C for two minutes) or NaDCC (20 ppm) pre-treatment and irradiation treatment (0.4–0.6 kGy) in the range used for phytosanitary purposes can be applied to control the pathogens. Absorbed radiation dose distribution in the fruit commodities was also measured and the results indicated that green pomelo could not be treat by EB irradiation, whereas treatment by gamma ray still kept green pomelo in good quality after 30 days storage. High energy electron beams could be used to treat star apple and custard apple for phytosanitary purposes. There were no significant differences for the effects of EB and gamma ray radiations on the quality of irradiated star apples.

15.1. INTRODUCTION

The increasing globalization of trade in fresh commodities gives customers a large variety of fruits and vegetable to choose from and use all year-round. Customers desire freshness, variety, and year-round availability. To meet these demands, traders may source many different fresh commodities from different countries. This widespread trade, especially in fresh fruits, increases the potential for the global spread of invasive agricultural pests. The introduction of insect pests into a new area or region could damage agricultural production and effect the

natural environment resulting in significant economic losses. Therefore, many countries require quarantine treatments of fresh commodities such as fruits to minimize the risk of introducing new or regulated pests through importation. Traditionally, chemical fumigation has been applied to control pests and plant diseases associated with pests and prolong the useful product life of traded fruit. However, the use of chemicals can leave residues, may negatively affect the environment and workers. In addition, fumigation may take an appreciable time to complete. The fumigation treatment time can be significant, especially for products with a short useful product life such as fresh tropical fruit. Therefore, many researchers have tried to find new methods that can replace chemical fumigation, such as hot water dips, cold treatments, various heat treatments, irradiation, or combinations of these different methods. A quarantine treatment must provide a high level of confidence that efficacy is greater than 99.99% [1]. Irradiation is an effective phytosanitary treatment not only because it can disinfest a variety of different fresh commodities of great number of different quarantined pests, but also because it can control fruit borne pathogens and can treat fruits that are already packaged [2]. Many fruits are tolerant to radiation treatment up to a dose of 1 kGy and relatively low doses of radiation do not significantly affect nutrient content in fruits such as guava, grapes, and most citrus fruits [3, 4, 5]. Although a low dose of 0.4 kGy is recommended as a generic dose to control harmful insects and pests, many pathogens such as bacteria, fungi and virus are not controlled by doses of less than 1 kGy. However, irradiation treatment at higher doses may negatively influence to the fruit quality, therefore the maximum dose for phytosanitary treatments for commodities such as fresh fruits and vegetable is generally prescribed as 1 kGy. Thus, a combination of irradiation and other treatments (hot water, chemical, etc.) is needed to control pathogenic organisms effectively at the low radiation doses used for phytosanitary treatments and maintain superior fruit quality after treatment and during storage.

Currently, phytosanitary irradiation is approved and applied on a commercial scale. Three primary types of ionizing radiation; gamma, electron beam (EB) and X ray are approved to meet quarantine requirements for exporting fresh fruits to markets with strict access requirements. Many papers in the scientific literature report the use of irradiation to extend the post-harvest life of many tropical and subtropical fruits by preventing microbial growth that cause food deterioration [6]. Mangoes treated at a dose of 0.45 kGy by ^{60}Co gamma rays can arrest the development of pathogens without damaging nutrients [7]. Using gamma irradiation at a dose of 0.2 kGy can extend the shelf life of guava [8]. In addition, using sodium dichloroisocyanurate (NaDCC) combined with gamma radiation can be applied to preserve quality of postharvest paprika [9]. Measured firmness of custard apples was higher than the control when fruits were treated by 1-MCP 810 nL/L [10]. A gamma radiation dose of 1.5 kGy in combination with a treatment of 50 ppm benzyl adenine would extend the shelf-life of custard apple fruits by six days when stored post-treatment at ambient temperature with good pulp texture, flavour, colour and nutritional quality in comparison with non-irradiated custard apple [11]. Electron beams can be used for food irradiation at energy levels up to 10 MeV. Even though EB radiation does not penetrate into products as deep as gamma or X ray radiation, EB can be applied to relatively small dimensions of fruits and vegetables (~ 20–30 cm) via a two pass irradiation treatment (Irradiating first from one side of the package and then the opposite side) [12] and EB is more rapid than the other irradiation methods. So, when fresh fruits are treated by EB irradiation, a quick turn-around can be achieved, tons of product on a truck can be unloaded, processed, and reloaded for transport and, with good logistical arrangements, there is no need to place products in a large warehouse before treatment. Jung *et al.* [13] studied the comparative effect of gamma ray, X ray, and electron beam irradiation at dose range of 200–1000 Gy on the sensory properties of fresh Fuji apples and Niitaka pears. The authors reported that the sugar content of Fuji apples and Niitaka pears were not changed by the three types of radiations. Based on comparison with gamma and electron beam irradiation, the

authors identified the applicability of X ray irradiation as a phytosanitary treatment for Fuji apples and Niitaka pears [13]. Jung et al. [14] also investigated the effects of X ray, gamma ray, and EB irradiation (2–10 kGy) on the hygienic and physic-chemical qualities of red pepper powder. The study revealed that a dose of 6.0 kGy (for all radiation sources) reduced the total aerobic microbial population effectively without affecting major quality indicators. For example, the three radiation types did not change the pungency of red pepper powder based on the capsaicinoid content. The authors concluded that X ray can be used for the irradiation of dried condiments with the same effects as gamma rays and electron beams.

In our studies, the effects of EB and gamma irradiation on fungi *Lasiodiplodia theobromae*, (commonly found infesting star apple); fungi *Phyllosticta citriasiana; Colletotrichum gloeosporioides* and bacteria *Xanthomonas* sp, (commonly found infesting citrus fruits) were evaluated and compared. In addition, the synergistic effects of EB and gamma irradiation and retreatment on the quality of some fruits were also investigated.

15.2. MATERIALS AND METHODS

15.2.1. Materials

15.2.1.1. Sample preparation

Lò Rèn star apple fruits were harvested from 240 to 250 day after flowering. Fruit from the main harvest were readily available from commercial orchards that meet Global good agricultural practice (GAP) standards in the Vinh Kim commune, Chau Thanh district, Tien Giang province of the Socialist Republic of Viet Nam. Green pomelo and king orange were purchased at the Tan Trieu pomelo village, Dong Nai province and Thanh Hoi village, Hoa Binh province, and custard apple was bought in Tay Ninh province. All fruits were harvested in the afternoon and early next morning were immediately transported to the laboratory at the Research and Development Center for Radiation Technology (VINAGAMMA), Ho Chi Minh City, Socialist Republic of Viet Nam. After washing and trimming to remove any remaining stems, the standard fruits of good appearance were randomly assigned for treatment.

15.2.1.2. Equipment

- Irradiation facility: Electron beam accelerator UERL-10-15S2, 10 MeV, 15 Kw supplied by CORAD Co. Ltd., Russian Federation, at the Research and Development Center for Radiation Technology and Co-60 Issledavachel (Russian Federation) with source activity 300Ci at Nuclear Research Institute, Da Lat.
- Testing equipment: Colorimeter CR200, Minolta, Japan; portable refractometer (TIRBX32, Trans Instruments Pte Ltd., Singapore); stereomicroscope, UK, cold storage, thermo temperature.

15.2.2. Methods

15.2.2.1. Isolation of pathogenic organisms

Experiments used *Lasiodiplodia theobromae* that was isolated directly from infested star apples. For a pure culture, isolated individual conidia (fungal spores) were transferred to potato dextrose agar (PDA, Hi-Media, India). To harvest the conidia, about 10 mL of sterile distilled water (DW) was added to a culture dish and the conidia were gently harvested by filtration through four layers of gauze. The conidial suspensions were collected in sterile screw-cap test tubes containing 15 mL of sterile distilled water and filtered twice using sterile Pasteur pipettes

(4.62 mm) packed with glass wool. The procedure was used to remove mycelial fragments and conidial clumps [15]. Conidia concentrations were confirmed using a serial dilution technique. Experiments also made use of the fungi *Colletotrichum gloeosporioides* as collected from infested citrus fruits, using the same method as described above.

The bacteria *Xanthomonas* sp. was isolated from leaves and fruits which were naturally infected with the citrus canker pathogen at a citrus orchard in Dong Nai and Hoa Binh Province. Citrus canker lesions on leaves and fruits were cut into small pieces (5 × 5 mm) with a blade and sterilized surface with 1% sodium hypochlorite solution (NaClO) for at least 30 seconds, rinsed in sterile distilled water three times each for 1 minute. Then, the samples were sterilized again with 70% ethanol for 30 seconds and likewise rinsed. The samples were divided into four parts, placed in 5% of peptone solution, and shaken at room temperature for 2 hours at 70 rpm by a mechanical shaker. Aliquots (100 µl) of the solution were inoculated into tryptic soy agar medium at 30°C for 48 hours [16].

The fungi *Phyllosticta citriasiana* was isolated from infested pomelo fruits. The infested samples were cut into small pieces (5 x 3 mm) with a blade and sterilized surface with ethanol 70^{o} for 3 minutes, rinsed in sterile distilled water two times then left to dry at ambient temperature. Samples were transfer to water agar at 25^{o}C for 4–5 days. When the fungi appeared, they were transfer to potato dextrose agar culture (PDA, Hi-Media, India). A pine leaf was place on top of each culture and conidia were produced after 20 days at room temperature. This step was carried out at the department of plant pathology, Faculty of Agronomy, Viet Nam National University of Agriculture.

15.2.2.2. Irradiation of the suspension of fungi and bacteria

Experiments made use of aqueous suspensions of spores. Samples (5ml, ~10^5 CFU/mL for conidia and 10^7 CFU/mL for bacteria) contained in sterile screw-cap test tubes were exposed to either EB or gamma irradiation at the Research and Development Center for Radiation Technology (10 MeV and 15 kW linear accelerator) or gamma irradiation (Co-60 Issledavachel with source activity 300Ci) at Nuclear Research Institute, Da Lat. Fricke and B_3 film dosimeters were you for dosimetry. Following treatment, both irradiated and non-irradiated suspensions of fungi and bacteria were serially diluted with sterile water. After dilution, 1 ml µl of each dilute was spread on culture medium. After incubation at 20°C (for 3 days with bacteria and 5 day with fungi), the number of colonies were counted. Survival curves were constructed by plotting the survivor Log_{10}CFU/mL versus actual radiation dose measured by dosimetry. Radiation sensitivity was expressed in terms of D_{10} values. A D_{10} value is defined as the dose required to reduce a given population by 90% of its initial value. The D_{10} value was determined from the reciprocal of the slope for straight-line portion of the survival curve [17, 18].

15.2.2.3. Irradiation of fruits

The 10 MeV and 15 kW linear accelerator (UERL-10-15S2, Corad Services Ltd, Russian Federation) with two beam passes (one from the top and one from the bottom) were used for electron beam irradiation. Star apple, green pomelo, king orange and custard apple samples were treated with target absorbed doses of 0.4, 0.6, 0.8 and 1.0 kGy within 1 hour after sample preparation. The EB machine was set to deliver a constant dose of 0.4 kGy by running the conveyor belt at 2.7 m/minute. For gamma ray irradiation, all samples were exposed to a ^{60}Co source with an activity of 1.5 kGy/hour. The room temperature was approximately 26 ± 1^{o}C. For each dose, irradiation was repeated at least three times. After irradiation, fruits were stored at ambient conditions (temperature of 26 ± 1^{o}C and humidity of 85–90%). Colour, Brix degree, vitamin C content, pH, weight loss, percentage decay and sensory evaluations were performed

immediately after irradiation treatment (storage day zero) and at regular intervals over the storage period. Unirradiated samples were used as the control.

To determine the dose distribution inside and outside the star apple, green pomelo, king orange and custard apple, B3 film dosimeters (GEX Corp., USA) were placed in different locations on and in the fruits as described in Fig. 13. Fruits were then irradiated to the target dose of 0.4 kGy. After irradiation, the dosimeters were measured to calculate the dose variation within each box of fruit and within the fruit. Each experiment was repeated three times.

15.2.2.4. Quality evaluation of fruits

Colour measurement

The surface colour of star apple, green pomelo, king orange and custard apple was measured by using Minolta Chroma Meter (ModelCR400, Konica Minolta Co., Japan) chromameter in L*a*b* system and illuminant D65, 2° standard white standard tile. In this system, L* represents lightness (high values) and darkness (low values), +a* redness, -a* greenness, and +b* yellowness, -b* blueness [19]. Measurements were taken on 3 different points of each fruit, and the mean value was calculated.

Fresh weight loss and decay rate measurement

Weight loss (*WL*) was determined as a percentage of the initial fresh weight for each treatment. The change in weight was monitored during the storage period and *WL* was calculated according to Equation 1 [20].

$$WL\ (\%) = \frac{(Fresh\ weight - Weight\ at\ storage\ inerval)}{Fresh\ weight} \times 100 \qquad (1)$$

Any fruit showing signs of soft rot, a brown spot or mold, was considered as decayed. Decay percentage was calculated as Equation 2 [20].

$$Decay\ (\%) = \frac{Number\ of\ decayed\ fruit}{Total\ number\ of\ fruits\ tested} \times 100 \qquad (2)$$

Soluble solid (°Brix) and ascorbic acid content

The total soluble solid (TSS) content was determined with a handheld refractometer (TIRBX32, Trans Instruments Pte Ltd., Singapore). The readings for TSS were expressed as Brix. Flesh of the fruit was crushed and homogenized and then 1 ml of the homogenates was measured. Ascorbic acid content was determined according to the method of AOAC 67.21 [21].

Sensory testing

Sensory testing was performed by 10 panel members including students and staff at the VINAGAMMA center. Numerical values were assigned to each attribute on a 5-point scale where, 1 = very poor, 2 = poor, 3 = fair, 4 = good, 5 = excellent [22]. Scores from 2.5 to 5 were considered acceptable [23]. The sensory analysis was performed at 0, 3, 6, 9, and 12 days after irradiation.

15.2.2.5. Statistical analysis

Data were subjected to analysis of variance (ANOVA) at $P<0.05$ using SPSS 13.0 software and Duncan's multiple range tests were used to compare the differences among the mean values. Percentages of weight loss were arcsine transformed before analysis.

15.3. RESULTS AND DISCUSSION

15.3.1. Effect of high energy electron and gamma irradiation to bacteria and fungi infested on star apple and citrus fresh fruit

15.3.1.1. To Lasiodiplodia theobromae infested on fresh star apples

The fungi *Lasiodiplodia theobromae* was found to be sensitive to increasing doses of gamma or EB radiation. The log transformed number of viable fungi after each irradiation treatment was observed to have a linear dose response (P<0.05). The survival curves for conidiospores *Lasiodiplodia theobromae* following gamma and EB treatment are provided in Fig. 1. The D_{10} values can be derived from the gradient of the best fit linear regression to the data points and this calculation gives a D_{10} value of 0.95 kGy for gamma irradiation and 1.1 kGy for EB irradiation. However, these D_{10} values for both types of radiations are not significantly different (P<0.05) and therefore the D_{10} value is approximately 1.0 kGy.

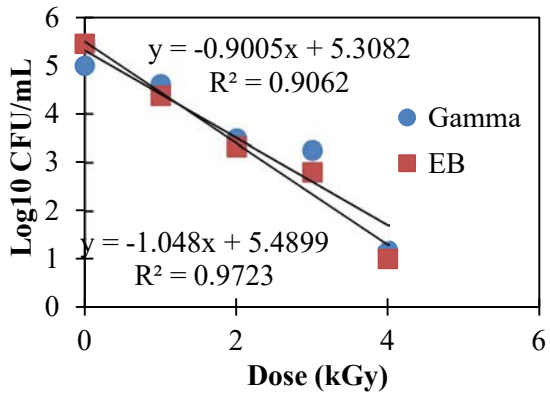


FIG. 1. Survivial curves for conidiospores Lasiodiplodia theobromae following gamma and EB treatment.

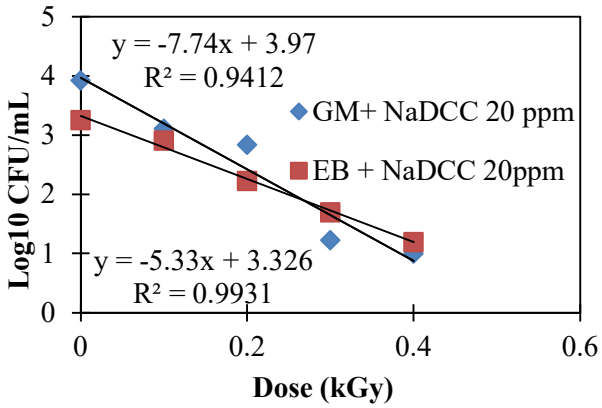


FIG. 2. Survival curves for conidiospores Lasiodiplodia theobromae following gamma and EB combined with NaDCC treatment.

The effect of a combined treatment using a chemical disinfectant plus irradiation (NaDCC and either gamma or EB irradiation) on *Lasiodiplodia theobromae* conidia is shown in Fig. 2. A synergistic effect was observed for these combined treatments against *Lasiodiplodia theobromae* conidia. The fungi *Lasiodiplodia theobromae* was more sensitive to the combination of treatments (NaDCC together with irradiation) in comparison to a treatment involving gamma irradiation (GI) or EB irradiation (EBI) alone. The D_{10} value was also significantly reduced to 0.13 kGy (for NaDCC + GI) and 0.19 kGy (for NaDCC + EBI) in combination. The calculated D_{10} values for NaDCC + GI and NaDCC + EBI are not significantly different (P<0.05). Thus, it was concluded that the use of combined treatment involving NaDCC and irradiation with ionizing radiation has a synergistic effect on reducing the irradiation dose required to eliminate this spoilage fungal species.

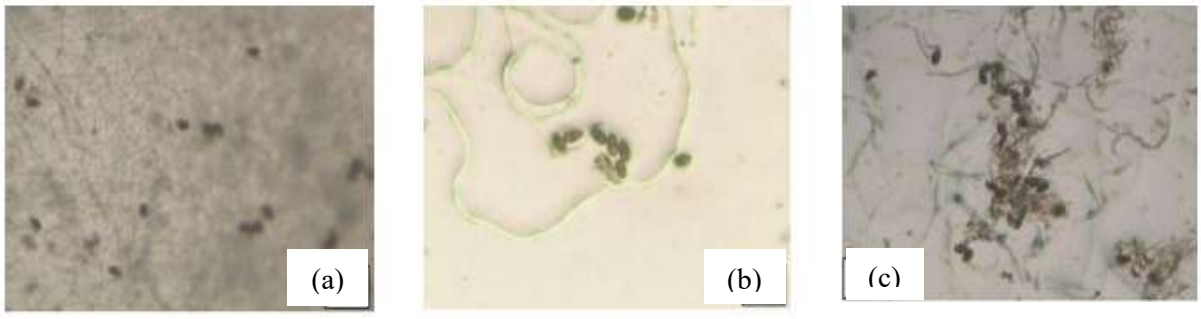


FIG. 3. Spores and hyphae of Lasiodiplodia theobromae under microscope in combined treatment of irradiations and NaDCC: 20ppm NaDCC + 0 kGy (a); 20ppm NaDCC + 0.4 kGy Gamma irradiation (b) & 20ppm NaDCC + 0.4 kGy EB irradiation (c).

15.3.1.2. Fresh pomelo and king orange infected with the bacteria Xanthomonas sp and fungi Phyllosticta citriasiana and Colletotrichum gloeosporioides.

Radiation sensitivity of Xanthomonas sp

The viability of the bacteria *Xanthomonas* sp. was found to be sensitive to irradiation and the number of colony forming units showed a marked decrease with increasing doses of ionizing radiation (Table 1), it was completely inactivated at a dose of 400 Gy for both gamma and EB irradiation ($P<0.05$). Similar results were obtained by Song et al. [24] for *Xanthomonas citri* subsp. *citri* with total inactivation at 400 Gy of X ray irradiation. It is well known that ionizing radiation results in inactivation of microorganisms including insects, fungi, bacteria and viruses [25]. Populations of bacterial such as *Escherichia coli, Salmonellae*, and *Campylobacter jejuni* are significantly reduced with increasing radiation doses and are completely inactivated at 2.5 kGy [26, 27]. The lethal dose for *Xanthomonas* sp was lower than that for *E. coli* which is known to be a microorganism sensitive to radiation [28]. Additionally in comparison to mold strains, the lethal doses for *Rhizopus stolonifer, Botrytis cinerea, Botrytis elliptica*, and *Aspergillus flavus* have been reported as 2.8 kGy, 4 kGy, 2 kGy, and 10 kGy, respectively [29, 30, 31, 32], which are much higher than the lethal dose for *Xanthomonas* sp. This high radiation sensitivity of *Xanthomonas* sp may be useful for treating exportation agricultural products in which elimination of *Xanthomonas* sp is required by using irradiation without any additional combined treatment.

TABLE 1. SUPPRESSION OF *XANTHOMONAS* SP POPULATION IN THE SUSPENSION BY GAMMA AND EB IRRADIATION WITH DIFFERENT DOSES

Dose (Gy)	Gamma irradiation		**EB irradiation**	
	Number of *Xanthomonas* sp. (Log CFU/ml)	Inhibition rate (%)	**Number of *Xanthomonas* sp. (Log CFU/ml)**	**Inhibition rate (%)**
0 (Control)	6.9 ± 0.10^{a}	–	**6.6 ± 0.06^{a}**	**–**
100	5.5 ± 0.21^{b}	20.30	**5.2 ± 0.15^{b}**	**21.21**
200	4.3 ± 0.15^{c}	37.68	**3.9 ± 0.10^{c}**	**40.90**
300	2.9 ± 0.15^{d}	57.97	**2.6 ± 0.06^{d}**	**60.60**
400	0^{e}	100	**0^{e}**	**100**

Means followed by different letters in the same column differ significantly according to Duncan's multiple range test (DMRT).

Survival curves were constructed by plotting viable numbers of *Xanthomonas* sp. As Log_{10}CFU/mL versus measured absorbed radiation dose (Fig. 4). Radiation sensitivity was expressed in terms of the derived D_{10} values. A D_{10} value is defined as the dose required to reduce a given population by 90% of its initial value. The D_{10} value was determined from the reciprocal of the slope for straight-line portion of the survival curve [33, 34]. A straight regression fit to the survival curves for *Xanthomonas* sp following gamma and EB irradiation in Fig. 4 was used to calculate D_{10} values of 61 Gy and 63 Gy for gamma rays and electron beams, respectively. However, there is no significant difference in these D_{10} values obtained for the two types of radiation ($P<0.05$) and therefore the D_{10} value is 62 Gy within experimental uncertainty. Our experimental results are similar to those obtained by others who report a D_{10} value of 69 Gy for *Xanthomonas citri subsp. Citri* when exposing to X ray radiation [16]. The D_{10} value for *Xanthomonas* sp was found to be relatively lower than those of other microorganisms such as *E. coli, Salmonella* spp., and *Yersinia enterocolitica* with D_{10} values of 360, 610, and 150 Gy, respectively [35]. Also, in the case of mold strains such as *A. flavus, B.cinerea*, and *Curvularia geniculata*, their D_{10} values of between 1.0 to 2.5 kGy are higher than that of *Xanthomonas* sp [36, 37]. Normally, the D_{10} values for viruses are higher than those of bacterial strains or fungi, which are calculated to range from 3 to 5 kGy [38]. These differences in the radiation sensitivity of pathogens may be due to their chemical and physical structure as well as their ability to recover from the radiation injury [39, 40]. The suppression of colony formation in the suspension of *Xanthomonas sp* on semi-selective medium when exposed to electron beams at a range of doses can be seen directly on culture plates as is shown in Fig 5.

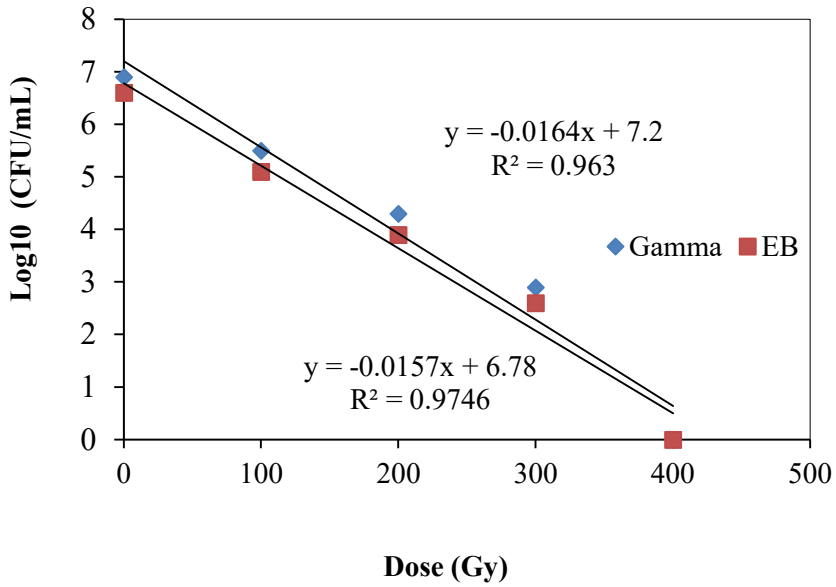
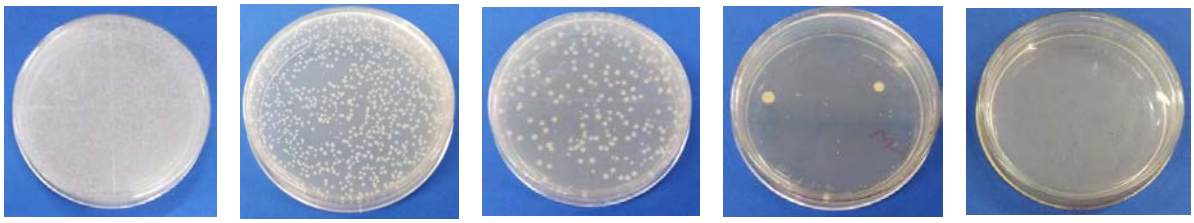


FIG. 4. Survival curves for Xanthomonas sp following gamma and EB treatment.



Control 100 Gy 200 Gy 300 Gy 400 Gy

FIG. 5. Suppression of colony formation in the suspension of Xanthomonas sp on semi-selective medium. From the left to right the suspension of Xanthomonas sp (~10^7 CFU/mL) was exposed to electron beams at doses of 0 (control); 100; 200; 300, and 400 Gy, from left to right respectively.

To check the symptoms citrus canker causing by *Xanthomonas* sp on citrus fruits, green skin pomelo and king orange fruits were washed under running water, and then dried and surface-sterilized with sodium hypoclorite 1% solution for 2 minutes to remove any dirt and microorganisms. The fruit samples were inoculated with 10 µl *Xanthomonas* sp. suspension at a concentration of 1×10^7 cfu/ml. After drying at room temperature, the citrus fruits were exposed to EB radiation at absorbed doses of 0, 100, 200, 300 and 400 Gy. The sample was kept at room temperature with RH 95% and inspected regularly over a period of 14 days to check for symptoms of citrus canker. The results (Fig. 6) showed that the symptoms of citrus canker did not appear on green skin pomelo and king orange treated at dose of 400 Gy after 14 days storage. For the unirradiated control samples and samples treated with doses of 100, 200 and 300 Gy, the symptoms occurred at the same time, especially in control samples and the samples treated at the lowed radiation dose of 100 Gy. This means that EB irradiation at dose of 400 Gy can destroy Xanthomonas sp causing citrus canker on citrus fruits.

Radiation sensitivity of Phyllosticta citriasiana

The viability of *Phyllosticta citriasiana* fungi were significantly decreased with increasing radiation doses ($P<0.05$). The survival curves for conidiospores of *P. citriasiana* following gamma and EB treatment are given in Fig. 7. Viable number of *P. citriasiana* spores decreased from values of 5.2 $\log_{10}$ CFU/mL and 5.0 $\log_{10}$ CFU/mL on unirradiated control samples to 3.1 $\log_{10}$ CFU/mL and 2.8 $\log_{10}$ CFU/mL when irradiated to a dose of 2.0 kGy of gamma and EB radiation, respectively. Viable spores of *P. citriasiana* were detected in samples treated to a dose of 5 kGy (Fig. 7a and 7b). The derived D_{10} values were 0.94 kGy and 1.1 kGy for gamma rays and electron beams, respectively. However, there was no significant difference in these D_{10} values for both types of radiation ($P<0.05$). Therefore, the D_{10} value is approximately 1.0 kGy. The effect of EB irradiation on spore germination, and growth of mycelium of *P. citriasiana* was also determined. Germination of *P. citriasiana* spores was significantly ($P<0.05$) inhibited at 2 kGy (Fig. 7b and 7c). And inhibition of mycelial growth gradually increased with increasing radiation doses and was completely inhibited at a dose of 5.0 kGy (Fig. 7b and 7d). The D_{10} value for *P. citriasiana* spores of approximately 1 kGy is comparable to the maximum dose of 1 kGy that is generally applied to phyosanitary irradiation. However, irradiation in combination with another treatment may be able to reduce this D_{10} value to doses that are normally used for phytosanitary treatments. Therefore, hot water treatment at 50°C for 2 minutes was applied before irradiation in order to develop a combination treatment to control *P. citriasiana* at lower radiation doses.

FIG. 6. Green skin pomelos and king oranges inoculated with 10 μl Xanthomonas sp. suspension at a concentration of 1×10^7 cfu/ml and irradiated by EB, immediately after irradiation on day zero of storage (top row) and after 14 days storage at room temperature (bottom row).

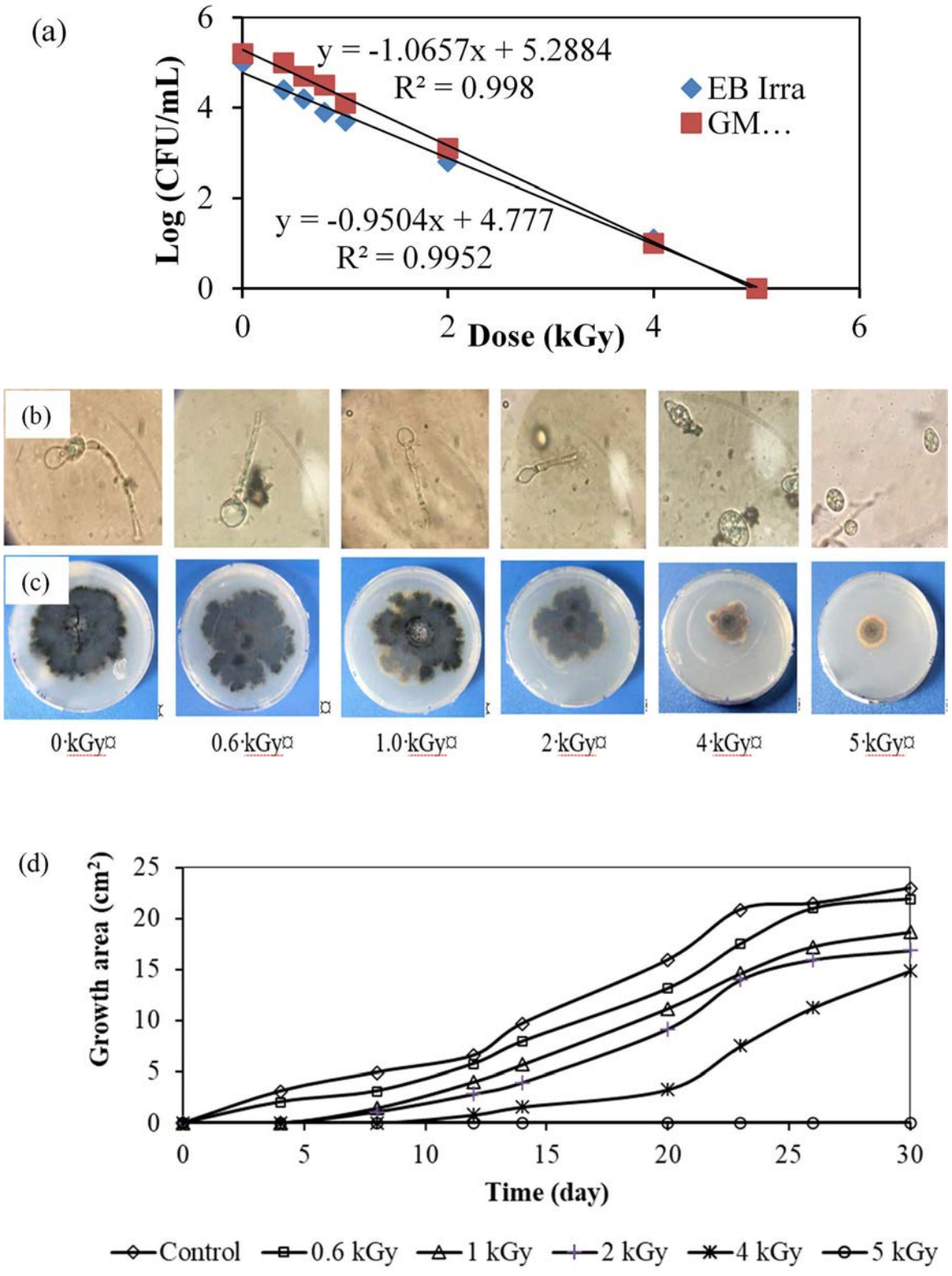


FIG. 7. (7a) Survival curves for P. citriasiana following gamma and EB treatment; (7b) Growth of P. citriasiana after exposes to various doses of EB irradiation; (7c) Changes in growth of P. citriasiana measured at irradiation doses; (7d) Area covered by fungal growth from storage day 0 to 30.

With a combination process, the radio-sensitivity of *P. citriasiana* after hot water treatment (50ºC, 2 minutes) was markedly increased in comparison with either gamma ray or EB irradiation alone. The D_{10} values derived from dose response plots of our experimental data (Fig. 8) were also significantly reduced from 0.94 kGy for gamma irradiation alone to 0.1 kGy for hot water plus gamma irradiation. For electron beam experiments the derived D_{10} values reduced from 1.1 kGy for EB alone to 0.11 kGy for hot water plus EB in combination. There was no significant difference in D_{10} values for both types of irradiation ($P<0.05$). Other researchers have investigated the use of hot water in combination with irradiation as a treatment against fungal pathogens that cause spoilage of apples, avocados, citrus, grapes, lychees, melons, peaches, strawberries and tomatoes [41]. A combined treatment of hot water (50ºC for 5 minutes) and irradiation was found to significantly reduce blue mold rot fungus *Penicillium Digitatum* on Grapefruit [42].

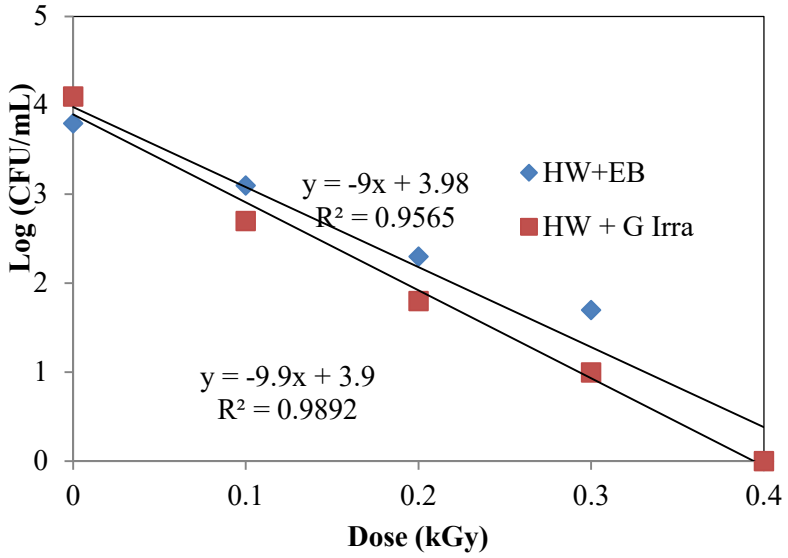


FIG. 8. Survival curves for conidiospores P. citriasiana following a combination treatment using hot water (HW) followed by irradiation with either an electron beam (EB) or gamma radiation from ^{60}Co (G Irra).

Green pomelo and king mandarin fruits were also inoculated with suspensions of *P. citricarpa* spores and the results of these investigations are presented in Fig. 9. No symptoms (no dark spots) appeared in green pomelo after 1 month and after 15 days on king orange. Other research involving the inoculation of Pera-Rio orange with *P. citricarpa* is reported by Ricardo et al. [43] and no symptoms associated with *P. citricarpa* appeared 55 days after inoculation of Pera-Rio orange. However, Citrus black spot symptoms appeared on 'Murcott' fruit (honey tangerine) five months after inoculation [44]. Based on these results and our findings it was concluded that the presence of *P. citriasiana* is difficult to observe by the appearance of black spots symptoms on the skin of ripe fruits. However, infections may arise in the planting and fruit growing stages of production. It would therefore be necessary to control *P. citriasiana* on fruits, to prevent the spread of disease and fruit spoilage.

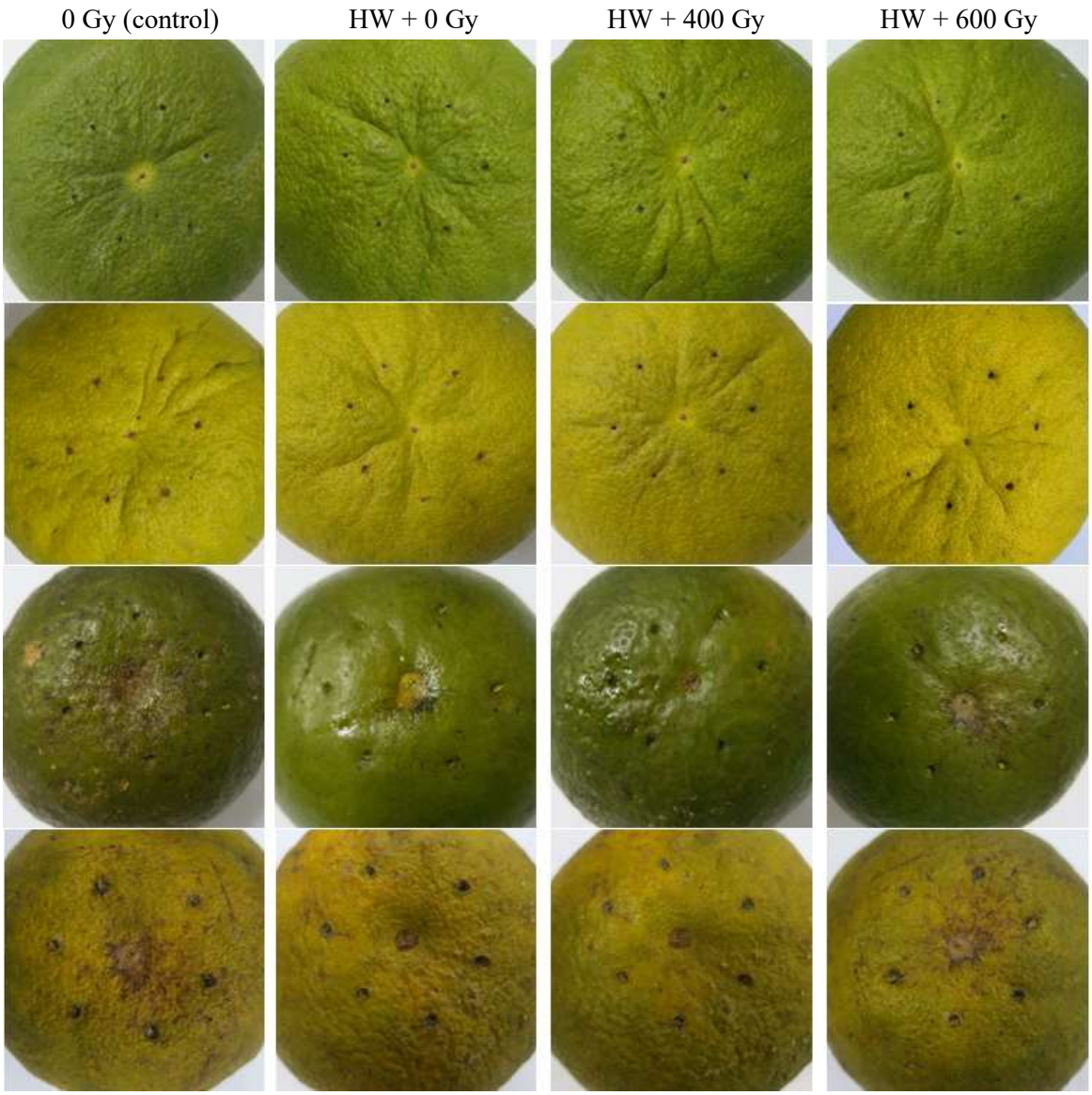


FIG. 9. Green skin pomelo and king orange inoculated with P. citriasiana (each hole was inoculated with 10 µl of 10^5 CFU/mL P. citriasiana spores) and subjected to hot water treatment (HW) at 50^oC for 2 minutes followed by irradiation to various doses of ionizing radiation. The top row is green skin pomelo on storage day zero, the second to top row is king orange on storage day zero. The third row down is green skin pomelo after one month of storage and the bottom row is king orange after 15 days of storage. There are no signs of citrus spot.

Radiation sensitivity of Colletotrichum gloeosporioides

The survival curves for conidiospores of *C. gloeosporioides* following gamma and EB treatment are given in Fig. 10(a). Based on the slope of the best fit linear regression lines in Fig. 10(a), the D_{10} values were calculated as 0.86 kGy and 0.90 kGy for gamma rays and electron beams, respectively. However, no significant difference of D_{10} values was obtained for both types of radiation ($P<0.05$). Therefore, the D10 value for irradiation treatment is approximately 0.88 kGy. The inhibition of mycelial growth gradually increased at higher radiation doses and was completely inhibited at a dose of 4.0 kGy (Fig. 10(b) and 10(c)). Similar results have been reported for combination treatments of stored apples, where conidial germination was reportedly stopped completely, resulting in no germ tube formation in *C. gloeosporioides* at dose of 4.0 kGy [45].

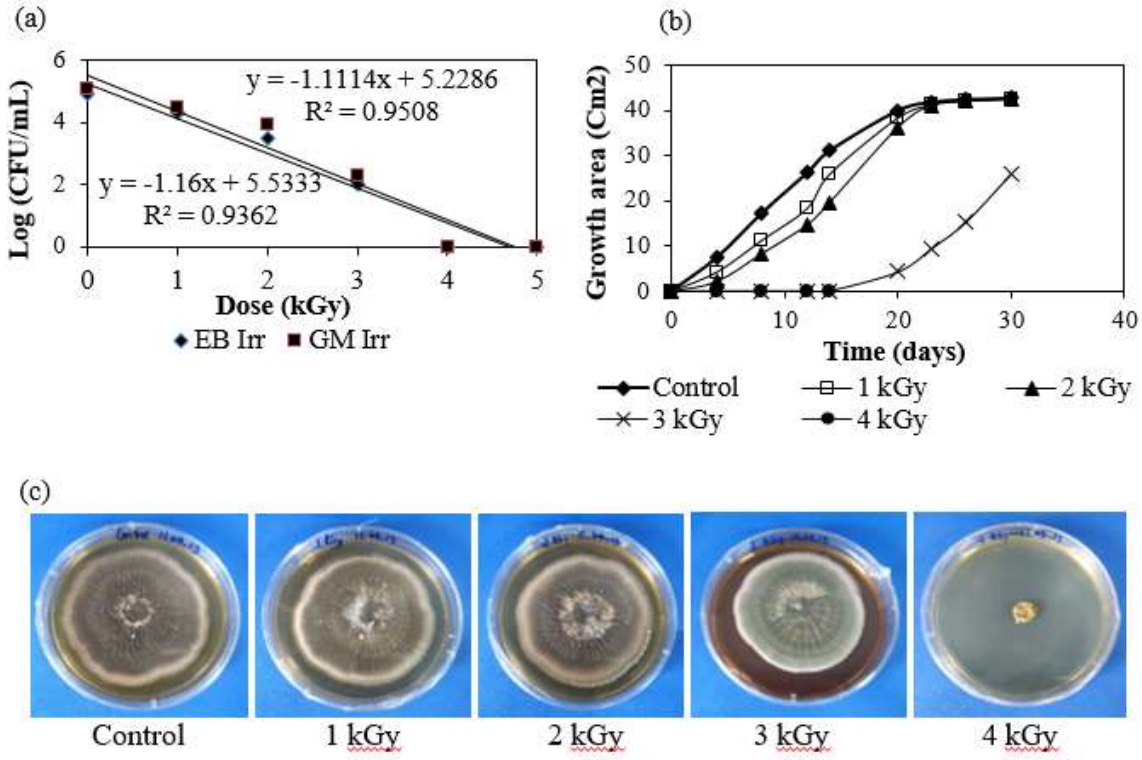


FIG. 10. (a) Survivial curves for C. gloeosporioides following gamma and EB treatment;
(b) Inhibition (%) means the percentage of fungal inhibition after 30 days;
(c) Changes in growth of C. gloeosporioides measured at irradiation doses.

The synergistic effect of combined treatment with hot water (50°C/2 minutes) and either gamma or EB irradiation on *C. gloeosporioides* conidia is shown in Fig. 11. As can be seen in Fig. 11, the synergistic effect was observed for combined treatment against *C. gloeosporioides* conidia. The sensitivity of *C. gloeosporioides* in combined treatment with hot water (50°C/2 minutes) and irradiation was markedly increased in comparison to the gamma (GI) or EB irradiation (EbI) alone. The D_{10} value was also significantly reduced to 0.11 kGy (for Hot water + GI) and 0.12 kGy (for Hot water + EbI) in combination. Similarly, there is no significant difference of D_{10} value for both types of irradiation ($P<0.05$). Effect of EB on survival of *C. gloeosporioides* in in vivo was showed in Fig. 12

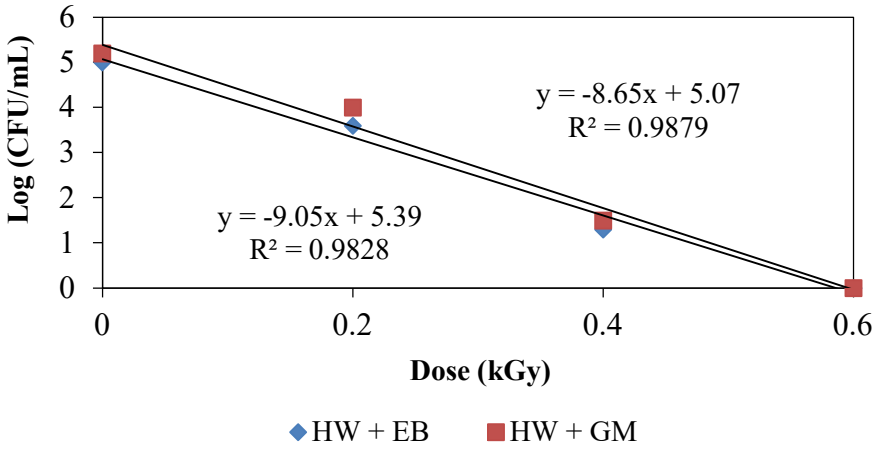


FIG. 11. Survival curves for conidiospores C. gloeosporioides following gamma and EB combined with hot water treatment with combination treatments of hot water (HW) plus electron beam (EB) or gamma (GM) irradiation.

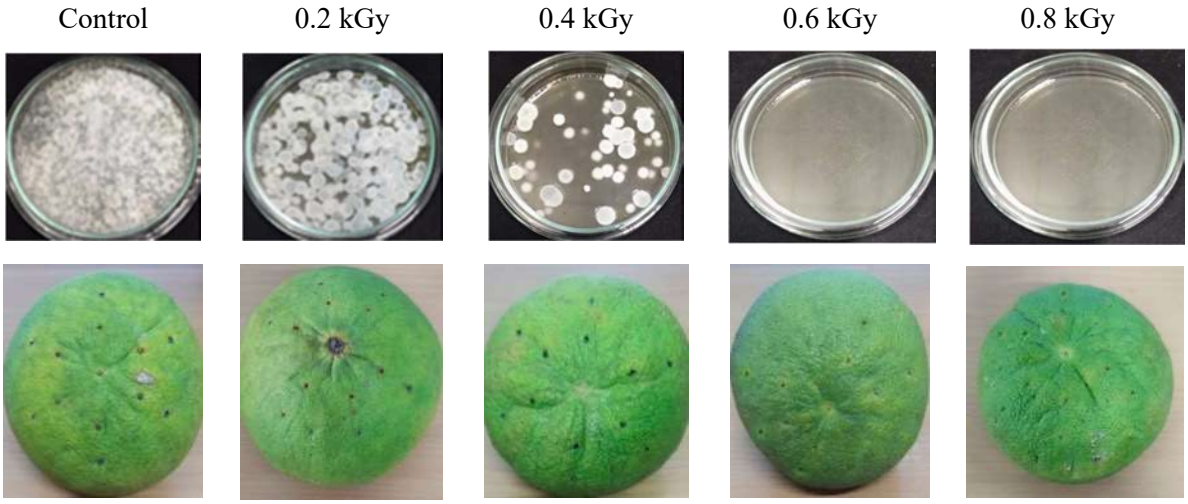


FIG. 12. Effect of EB on survival of C. gloeosporioides in invivo.

15.3.2. Dose distribution inside and outside of fruits

The diameter of tropical fruits can vary widely, for example for green pomelo the diameter can range from approximately 13 to 17 cm, for king oranges from 6 to 8 cm; for star apple from 5 to 7 cm and for custard apple from 7 to 10 cm. Moreover, the penetration of EB is very low when compared to gamma ray irradiation. It is important to ensure that EB can penetrate through the fruit commodity, so we investigated the dose distribution and uniformity inside and outside of fruits. Before treatment, film dosimeters were fixed inside and outside fruits as shown in Fig. 13 and the results of this dose mapping are given in Table 2. The maximum dose of 16.4; 20.3; 0.47 and 10.4 kGy and the minimum dose of 1.2; 10.6; 0.4 and 5.2 kGy were measured for green pomelo, king orange, star apple and custard apple, respectively. There results show that the dose inside and outside the fruits was different with dose uniformity ratios (DUR, maximum dose ÷minimum dose) of 13.7; 1.9; 1.2 and 2.0 respectively. The results indicated that green pomelo cannot be treat by EB irradiation because the DUR of 13.7 is too

large for the phytosanitary purpose. For example, to achieve a minimum treatment dose of 0.4 kGy with green pomelo the maximum dose received would be greater than 5 kGy and therefore well above the 1 kGy generally stipulated as the maximum for phytosanitary purposes. For king orange, star apple and custard apple the DURs are much lower and therefore these can be treated by EB. So, our following experiments used gamma irradiation to treat green pomelo and EB irradiation for the other fruits.

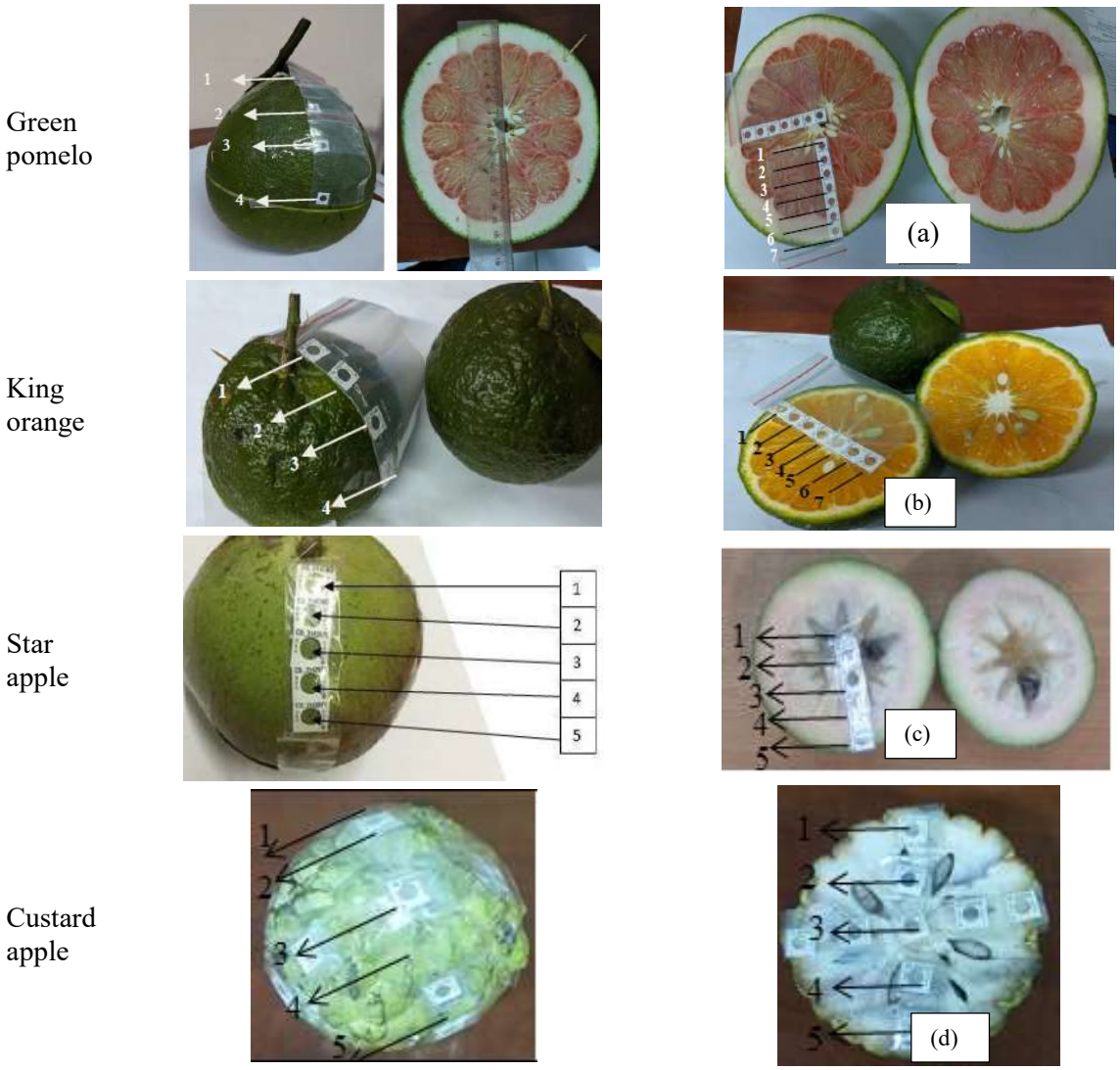


FIG. 13. Dose mapping of fruits for EB irradiation. Dosimeters were placed at locations on the outside, and inside of green pomelo (a), king orange (b); Lò Rèn star apple (c) and custard apple (d).

TABLE 2. DOSE UNIFORMITY RATIO (DUR) FOR TROPICAL FRUITS

Location	Green skin Pomelo		King orange		Star apple		Custard apple	
	Dose Inside of fruit (kGy)	Dose Outside of fruit (kGy)	Dose Inside of fruit (kGy)	Dose Outside of fruit (kGy)	Dose Inside of fruit (kGy)	Dose Outside of fruit (kGy)	Dose Inside of fruit (kGy)	Dose Outside of fruit (kGy)
1	1.2	7.1	17	10.9	0.42	0.4	7.1	9.2
2	9.0	8.4	14.1	10.6	0.44	0.4	5.2	5.4
3	9.0	13.1	13.7	11.3	0.42	0.42	5.2	5.2
4	8.6	12.7	15.5	20.2	0.47	0.42	6.7	5.5
5	8.7	–	20.3	–	0.44	0.42	7.2	10.4
6	14.6	–	13.0	–	–	–	9.3	–
7	16.4	–	12.7	–	–	–	–	–
DUR	**13.7**		**1.9**		**1.2**		**2.0**	

15.3.3. Evaluating the quality of fresh fruits after irradiation treatment

15.3.3.1. Star apple

Fresh weight loss of star apples

The percentage of weight loss of star apple fruits increased significantly with increasing storage time. Weight loss changed insignificantly at the doses 0.4 kGy and 0.6 kGy as compared with the control (non-irradiated) fruits for both types of irradiation (Table 3).

TABLE 3. COMPARISON OF EFFECTS OF GAMMA (GM) AND ELECTRON BEAM (EB) IRRADIATION ON THE WEIGHT LOSS (%) OF STAR APPLE DURING STORAGE (TEMP. OF 27 ±2°C; RH: 85 ± 5%)

Type of radiation*	Dose (kGy)	Weight loss (%)					Mean for the same radiation dose
		Storage time (days)					
		0	3	6	9	12	
EB	0	**0.03**	**0.2±0.01**	**0.27±0.02**	**0.41±0.05**	**0.42±0.04**	**0.27^{A}**
	0.4	**0.03**	**0.18±0.04**	**0.25±0.09**	**0.36±0.5**	**0.4±0.13**	**0.24^{A}**
	0.6	**0.03**	**0.23±0.07**	**0.26±0.06**	**0.38±0.1**	**0.46±0.13**	**0.27^{A}**
	0.8	**0.03**	**0.1±0.05**	**0.36±0.04**	**0.46±0.03**	**0.49±0.01**	**0.29^{B}**
	1.0	**0.03**	**0.23±0.11**	**0.37±0.09**	**0.47±0.12**	**0.66±0.09**	**0.35^{B}**
	Mean for the same storage time	**0.03^{a}**	**0.19^{b}**	**0.3^{c}**	**0.42^{d}**	**0.49^{e}**	–
GM	0	**0.03**	**0.22±0.07**	**0.31±0.06**	**0.4±0.04**	**0.48±0.03**	**0.3^{A}**
	0.4	**0.03**	**0.23±0.05**	**0.3±0.03**	**0.37±0.02**	**0.45±0.01**	**0.29^{A}**
	0.6	**0.03**	**0.22±0.04**	**0.33±0.03**	**0.38±0.03**	**0.49±0.02**	**0.3^{A}**
	0.8	**0.03**	**0.25±0.02**	**0.36±0.01**	**0.46±0.02**	**0.6±0.01**	**0.35^{B}**
	1.0	**0.03**	**0.23±0.05**	**0.38±0.01**	**0.49±0.03**	**0.67±0.02**	**0.37^{B}**
	Mean for the same storage time	**0.03^{a}**	**0.23^{b}**	**0.33^{c}**	**0.42^{d}**	**0.54^{e}**	–

Mean values followed by the same letter are not significantly different at P<0.05.
**EB, electron beam and GM, gamma ray*

Colour measurements of star apple fruits

The colour of fruit is a very important characteristic for consumer acceptance and the most evident indicator of their quality [46, 47]. Changes in the external colour of star apple fruits were monitored by measuring lightness (L*), and redness (a*), (Table 4). The measured colour values of fruit skins changed significantly during storage starting from storage day 6 to day 12. However, the difference of L value (indicated the light of fruit skin) similar for unirradiated control samples and electron beam irradiated (EBI) at all doses measured, however, with gamma irradiated samples (GI) differences in colour were significant from a dose of 0.6 kGy upwards (Table 4). The changes in L* values with irradiation dose were reported by Drake and Neven [47] in irradiated Bing'cherries and it was reported that L* values increased at irradiation doses greater than 0.15 kGy [47]. Moreno, Perez, Gomes, Da Silva, & Moreira, (2006) reported similar result in mango [48]. These results could be attributed to an increased polyphenoloxidase activity and the consequent oxidation of phenolic compounds giving rise to brown and dark pigmentation of the fruit [49]. The redness (a* values) of the star apple samples exposed to 0.8 kGy and 1.0 kGy was significantly ($P>0.05$) higher (more red) than the samples exposed to 0.6 kGy after 6 days storage. Discoloration of the star apple could be attributed to the water loss in the fruits. Similar results have been reported for blueberries irradiated with EB at dose of 1.1 kGy [50].

Soluble solids (^{o}Brix) of star apples

The soluble solids (^{o}Brix) of star apples did not changed between the irradiation doses for both types of ionizing radiation (Table 5), but the total soluble solid (TSS) decreased with increasing storage time. The result can be explained that the star apple is a non-climacteric fruit so it must mature on the tree before being harvested. At the time of mature, the TSS content of the fruit reaches to the greatest value. After harvesting, the TSS does not increase any more. In addition, the respiration of the fruits continues after harvest Similarly, Wall & Khan (2008) reported that TSS of dragon fruits was not affected by absorbed dose up to 0.8 kGy.

15.3.3.1. Custard apple

Evaluating the effect of EB irradiation on the quality of custard apple

Fresh custard apples were harvested in the afternoon from a Viet-GAP farm in Tay Ninh province (Viet Nam), about 120 kilometers far from HCM City, and transported to our laboratory in the early morning of the following day. Thirty-six custard apples with the same size were selected and used for each treatment in experiments. All the experiments were carried out in triplicate. Mean values of different physico-chemical parameters obtained from statistical analysis at the range of target doses after 4 days are presented in Table 6.

The radiation dose significantly affected the chemical parameters of custard apple. The TSS content of fruits increased with increasing radiation doses up to 0.6 kGy, the TSS (^{o}Brix) in all irradiated samples was higher than for the control samples (0 kGy). The increase in TSS of fruits might be due to delay in ripening, senescence, the enzymatic conversion of higher polysaccharides into simple sugars during ripening or the radiation induced hydrolysis of pectic substances [51]. The weight loss of fruits was found to increase significantly with increasing radiation dose. Weight loss (WL%) changed significantly at the doses of 0.4 kGy and 0.6 kGy as compare with the WL% at a dose of 0.2 kGy and 0 kGy control samples (non-irradiated). Similarly, acidity (%) and the a* value (a*, green to red) of control samples (unirradiated) were found to be significantly different to those of irradiated samples. Discoloration of the custard apple could be attributed to the browning reaction, and water loss in the fruits. A similar result was obtained in research into the effects of EB irradiation of star apples, in which discoloration of treated fruit's was reported to appear after 6 days storage at ambient temperature [52]. The

content of vitamin C decreased gradually with increase of radiation doses. The decreasing trend of vitamin C under higher doses may be due to the conversion of ascorbic acid into de-hydro ascorbic acid in the presence of enzyme ascorbinase in over ripe fruits [51]. However, the difference between vitamin C content for control samples and irradiated samples was not significant in the statistical analysis. In our observation, fungi had appeared on the control samples after 2 days of storage (Fig. 13) and in all the samples that had irradiation treatments after 4 days of storage (Fig. 14). However, levels of fungi growing on the irradiated fruits was observed to be less than on the control (unirradiated) samples. This result indicates that low radiation doses can inhibit development of fungi on custard apple fruits, but the dose levels used in these experiments were not sufficiently high enough to completely prevent fungal growth. Therefore, a pre-treatment method in combination with irradiation may be necessary to prevent fungal growth and extend the shelf life of custard apple fruits.

FIG. 13. Custard apple after 2 days at room temperature.
1) Control; 2) 0.2 kGy; 3) 0.4 kGy; 4) 0.6 kGy.

FIG. 14. Custard apple after 4 days at room temperature.
1) Control; 2) 0.2 kGy; 3) 0.4 kGy; 4) 0.6 kGy.

TABLE 4. COMPARISON OF EFFECTS OF GAMMA AND EB IRRADIATION ON THE COLOUR (L & a* VALUES) OF STAR APPLE DURING STORAGE TIME (TEMP. OF 27 ± 2°C; RH: 85 ± 5%)

Type of radiation*	Parameter	Dose (kGy)	Storage time. Day					Mean for the same radiation dose
			0	**3**	**6**	**9**	**12**	
EB	L*	0	62.04±1.14	60.55±3.04	58.2±5.33	56.05±4.15	40.54±6.96	55.48A
		0.4	58.95±1.35	60.71±6.01	57.9±3.12	57.24±5.72	38.45±4.43	54.65A
		0.6	61.61±0.81	61.64±1.73	56.74±0.53	53.63±2.16	40.06±5.57	54.74A
		0.8	63.37±5.14	60.97±1.84	60.31±4.28	51.18±2.87	47.99±0.79	56.76A
		1.0	63.14±6.86	63.16±1.08	57.92±1.53	49.47±2.75	45.88±0.91	55.91A
	Mean for the same storage time		61.82a	61.41a	58.21b	53.51b	42.58c	
	a*	0	-13.65±1.37	-11.67±3.77	-5.39±1.21	-0.31±3.35	3.56±2.28	-5.49A
		0.4	-12.61±1.03	-11.17±3.29	-5.00±2.76	-0.14±3.05	3.61±0.42	-5.06A
		0.6	-11.77±2.20	-10.25±3.24	-5.99±4.45	-0.68±3.55	4.80±3.11	-4.78A
		0.8	-12.73±1.80	-10.69±0.88	-3.56±3.48	4.00±0.59	4.80±1.58	-3.64AB
		1.0	-10.43±1.28	-10.21±1.31	-1.57±2.76	3.45±0.60	5.84±1.99	-2.58B
	Mean for the same storage time		-12.24a	-10.8a	-4.3b	1.26c	4.52d	
GM	L*	0	61.62±2.64	60.09±1.97	59.36±1.23	53.71±1.66	39.74±0.86	54.90A
		0.4	60.99±1.25	61.21±1.65	60.63±0.55	49.48±2.20	41.42±1.33	54.75A
		0.6	59.63±0.71	61.98±2.67	58.07±3.00	43.31±1.47	39.82±2.24	52.56B
		0.8	60.12±0.77	59.22±0.85	56.64±0.89	46.37±0.50	39.75±2.39	52.42B
		1.0	60.54±0.67	60.97±2.78	55.56±1.50	43.65±0.57	39.50±0.30	52.04B
	Mean for the same storage time		60.58a	60.69a	58.05b	47.30c	40.05d	
	a*	0	-12.28±1.50	-8.42±1.17	-5.45±1.34	6.32±0.74	4.38±1.25	-5.62A
		0.4	-8.37±0.75	-9.44±0.70	-5.65±0.80	0.43±1.95	5.32±1.07	-3.54B
		0.6	-10.51±1.89	-7.68±1.84	-6.43±1.26	2.47±0.91	4.38±0.67	-3.55B
		0.8	-9.27±1.82	-4.57±1.03	-4.69±1.10	6.23±1.72	2.15±0.90	-2.03C
		1.0	-9.93±1.70	-4.82±1.12	-6.70±1.40	3.96±0.32	5.15±1.72	-2.47C
	Mean for the same storage time		-10.07a	-6.99b	-5.78c	-1.35d	4.28e	

Mean values followed by the same letter are not significantly different at P<0.05.
**EB, electron beam and GM, gamma ray*

TABLE 5. EFFECTS OF EB AND GAMMA IRRADIATION ON THE SOLUBLE SOLIDS (^{O}BRIX) DURING STORAGE TIME (TEMPERATURE OF 27 ± 2 °C; RH: 85 ± 5%)

Type of radiation*	Dose (kGy)	Total soluble solids (^{o}Brix)					Mean for the same dose
		Storage time (days)					
		0	**3**	**6**	**9**	**12**	
EB	0	12±1.6	11.8±0.9	11.6±0.5	10.2±0.3	9.2±0.7	10.96A
	0.4	11.9±2	12.1±0.4	11.6±1.7	10.1±0.4	8.8±2.5	10.92A
	0.6	11.9±0.1	11.3±0.8	11.2±0.3	10.2±0.3	8.3±1.4	10.59A
	0.8	12.9±0.5	11.8±1.3	11.1±0.8	10.6±0.4	9.3±2.1	11.14A
	1.0	12.3±1.1	12.1±1.1	11.3±1	10.3±0.6	9.2±2	11.03A
	Mean for the same storage time	12.2a	11.83a	11.38a	10.28b	8.95c	–
GM	0	12±1.6	10.8±0.8	11.1±0.7	10.2±0.3	9±2	10.6A
	0.4	12.2±1.1	12.1±0.4	12±1.3	10.1±0.4	8.8±2.5	11A
	0.6	12.3±0.6	11.3±0.8	11.2±0.3	10.6±0.4	8.3±1.4	10.7A
	0.8	12.7±0.1	11.8±1.3	12.5±0.9	10.6±0.4	8.3±1.5	11.3A
	1.0	12.3±1.1	12.1±1.1	10.7±0.3	10.8±0.9	7.9±0.9	10.7A
	Mean for the same storage time	12.3a	11.6a	11.5a	10.4b	8.5c	–

Mean values followed by the same letter are not significantly different at P<0.05.
**EB, electron beam and GM, gamma ray*

TABLE 6. EFFECTS OF E-BEAM IRRADIATION ON THE QUALITY OF CUSTARD APPLE FRUIT AFTER 4 DAYS (TEMP. OF 27 ± 2°C; RH: 85 ± 5%)

Dose (kGy)	TSS (°Brix)	Acid (%)	Vitamin C (mg/100g)	L^*	a^*	Weight loss (%)
0	23.80±0.20a	0.16±0.04a	34.86±4.62a	47.02±3.27a	0.37±4.26a	0.19±0.01a
0.2	25.40±0.20b	0.34±0.00c	34.03±4.39a	46.14±4.06a	1.94±1.98b	0.21±0.01a
0.4	26.50±0.10c	0.29±0.04bc	25.18±4.18a	45.06±4.90a	2.18±0.86b	0.26±0.02b
0.6	27.80±0.20d	0.27±0.00b	27.39±3.80a	44.05±3.97a	1.86±1.88b	0.28±0.03c

Mean values within same a column followed by the same letter are not significantly different at P<0.05.

Effects of EB irradiation and observed appearance of fungi and pest insects infesting custard apples

The effect of EB irradiation on fungi and pest emergence on custard apples during storage is given in Table 7. We observed that fungi had appeared on control samples after 2 days but on irradiated samples fungi were observed after 4 days. This indicates that electron beam irradiation at low doses used for phytosanitary treatments (<1 kGy) cannot control fungi in infected fresh custard apples. Also, pest insects including larvae of fruit borer and flies were observed in the control fruits after 4 days. However, no pest insects were found to emerge from any of the irradiated (0.2 to 0.6 kGy) custard apple fruits. The results showed that EB irradiation at the range of dose from 0.2 kGy to 0.6 kGy can controlled pest insects but could not inhibit molds/fungi infected in custard apple.

TABLE 7. APPEARANCE OF FUNGI AND PEST INSECTS LOADING ON CUSTARD APPLE AFTER EB IRRADIATION DURING STORAGE TIME (36 FRUITS/ OBSERVATION TIME)

Dose (kGy)	Molds/fungi				**Pest insects**			
	2 days	4 days	6 days	8 days	2 days	4 days	6 days	8 days
0 (Control)	+	++	++	++	-	+	++	++
0.2	-	+	++	++	-	-	-	-
0.4	-	+	++	++	-	-	-	-
0.6	-	+	+	++	-	-	-	-

Key: (-), was not observed to appear. (+), observed to appear. (++) very apparent.

15.3.3.2. Pomelo fruits

Evaluating the effect of combination of pretreatment and Gamma irradiation at target doses on the quality of green pomelo

Green pomelo fruits were subjected to a combined treatment of hot water dipping (50ºC for 5 minutes) followed by EB irradiation. The disease index and weight loss (WL%) of green pomelo fruits changed with increasing storage time, similar results for the measured colour values (L*, a* and b*) of the fruit skin (Table 8). All samples were placed in storage for up to 30 days including 10 days at a temperature of 13°C with humidity of 85% and 20 days at room temperature (28 ± 2°C) to mimic temperature profiles when fruits are traded. At the end of the storage time (30 days) the colour of green pomelo became more yellow in all treatment (Fig. 15). The incidence of disease in samples that were processed with a combined treatment of hot water and irradiation was significantly difference in comparison with the others. The combined hot water plus irradiation treatment inhibited fungal growth. Although hot-water treatment may damage fruits to some degree, the change of colour in treated samples was not significant when compared with the change of colour of control samples. The hot water treatment was carried out at the low temperature (50ºC) and for a short time (2 minutes). Also, after treatment the fruits were immediately cooled in order to limit the damage to fruits.

TABLE 8. EFFECTS OF HOT WATER TREATMENT PLUS GAMMA IRRADIATION ON THE WEIGHT LOSS (%), COLOUR (L*, A* & B* VALUES) AND THE DISEASE INDEX OF GREEN SKIN POMELO DURING STORAGE TIME AT TRADING CONDITIONS

Parameter	Sample*	Storage time, day					Mean for the same dose
		0	**10**	**15**	**22**	**30**	
L*	Control	50.76±1.84	51.96±1.07	52.81±0.95	52.60±1.92	57.91±1.86	53.21^{A}
	HW-0 Gy	51.34±1.25	51.38±0.4	51.92±0.25	51.72±3.51	57.92±3.59	52.86^{A}
	HW-400 Gy	50.84±0.61	51.99±0.59	52.73±0.15	52.68±1.4	58.52±2.0	53.35^{A}
	HW-600 Gy	50.63±0.29	52.15±1.47	52.83±0.07	53.91±1.05	58.55±2.08	53.61^{A}
Mean for the same storage time		50.89^{a}	51.87^{ab}	52.57^{b}	52.72^{b}	58.22^{c}	
a*	Control	-15.68±0.38	-15.34±0.25	-13.92±0.33	-13.19±0.72	-9.59±1.05	-13.54A
	HW-0 Gy	-15.60±0.44	-15.55±0.21	-14.09±1.1	-13.22±1.11	-9.51±1.35	-13.59A
	HW-400 Gy	-15.57±0.60	-15.36±0.39	-14.07±0.66	-13.14±1.22	-9.51±1.35	-13.53A
	HW-600 Gy	-15.74±2.30	-15.48±0.05	-13.83±0.32	-13.17±0.45	-9.5±0.43	-13.54A
Mean for the same storage time		-15.65^{a}	-15.43^{a}	-13.98^{b}	-13.18^{b}	-9.53^{c}	
b*	Control	31.59±1.96	31.77±1.86	32.89±0.81	34.73±2.05	39.27±1.13	34.05AB
	HW-0 Gy	31.27±0.30	31.62±1.36	31.97±3.89	34.18±5.47	39.96±3.95	33.8A
	HW-400 Gy	31.45±0.57	32.26±0.59	32.58±0.24	35.09±1.31	39.67±1.14	34.21AB
	HW-600 Gy	31.88±0.86	32.36±0.94	33.92±1.72	35.23±2.35	41.13±0.61	34.90AB
Mean for the same storage time		31,55a	32.00a	32.84ab	34.81b	40.00c	
Disease	Control	0.71	1.22	1.46±0.21	1.87	1.95±0.14	1.44A
	HW-0 Gy	0.71	1.05±0.29	1.46±0.21	1.65±0.38	2.04±0.14	1.38A
	HW-400 Gy	0.71	1.05±0.29	1.34±0.21	1.22	1.46±0.21	1.16B
	HW-600 Gy	0.71	1.05±0.29	1.22	1.22	1.58	1.16B
Mean for the same storage time		0.71a	1.09b	1.37c	1.49c	1.76d	
Weight loss (%)	Control	0.02	0.02	0.07±0.01	0.21±0.01	0.34±0.01	0.22A
	HW-0 Gy	0.02	0.02	0.06±0.01	0.23±0.03	0.34±0.01	0.22A
	HW-400 Gy	0.02	0.02	0.07±0.01	0.23±0.01	0.34±0.01	0.23A
	HW-600 Gy	0.02	0.02	0.07±0.01	0.24±0.01	0.34±0.01	0.23A
Mean for the same storage time		0.02^{a}	0.02^{a}	0.07^{b}	0.23^{c}	0.34^{d}	

Mean values followed by the same letter are not significantly different at P<0.05.

* control samples were not treated, HW 0 kGy, hot water treated but unirradiated, HW-400 Gy, Hot water treated and irradiated to 400 Gy, HW-600 Gy, Hot water treated and irradiated to 600 Gy.

0 day after treatment

Control HW (50°C/2 minutes) HW + 400 Gy HW + 600 Gy

30 days after treatment

FIG. 15. Skin colour of pomelo fruit after 0 day and 30-day treatment by gamma irradiation.

A combination treatment using both hot water (50°C/2 minutes) and irradiation (absorbed dose of 400–600 Gy) was more effective at controlling *Xanthomonas* sp, *P. citriasiana* and *C. gloeosporioides* loaded on green pomelo skin (Table 9). The sensitivity of the microorganisms was markedly increased to combined treatments compared with treating with hot water only. This result was similar to the reports of research that used hot water to reduce symptoms of black spot on Valencia oranges and measured results for a starage time of up to two weeks [53].

TABLE 9. EFFECTS OF GAMMA IRRADIATION ON THE VIABLE NUMBERS OF *XANTHOMONAS* SP, *P. CITRIASIANA* AND *C. GLOEOSPORIOIDES* ON GREEN POMELO SKIN

Type of microorganism	Sample*	Numbers of organisms per unit area (CFU/cm^2)				
		Storage time (days)				
		0	**10**	**15**	**22**	**30**
P. citriasiana	Control	<10	<10	<10	<10	<10
	HW-0 Gy	ND	ND	<10	<10	<10
	HW-400 Gy	ND	ND	ND	ND	ND
	HW-600 Gy	ND	ND	ND	ND	ND
Xanthomonas sp	Control	$4.8x10^1$	$5.4x0^1$	$6.3x10^1$	$7.2x10^1$	$8.5x10^1$
	HW-0 Gy	<10	<10	$1.4x10^1$	$2.0x10^1$	$2.7x10^1$
	HW-400 Gy	ND	ND	ND	ND	ND
	HW-600 Gy	ND	ND	ND	ND	ND
C. gloeosporioides	Control	$4.0x10^1$	$5.0x10^1$	$5.5x10^1$	$6.2x10^1$	$7.1x10^1$
	HW-0 Gy	<10	<10	1.0×10^1	2.3×10^1	3.0×10^1
	HW-400 Gy	ND	ND	ND	ND	ND
	HW-600 Gy	ND	ND	ND	ND	ND

ND: No detected

* Control samples were not treated, HW 0 kGy, hot water treated but unirradiated, HW-400 Gy, Hot water treated and irradiated to 400 Gy, HW-600 Gy, Hot water treated and irradiated to 600 Gy.

The sensory attributes (colour, flavour, texture, taste and overall acceptability) of green pomelo fruits were measured by a sensory testing panel on storage days 0, 15, 22 and 30 (Table 10).

The results show that colour, texture, taste and flavour of green pomelo achieved the highest score of 5.0 in all samples until 15 days of storage. After 30 days of storage at trading condition, the colour of the segment of green pomelo was whiter, but still acceptable with the score 3.33 and the texture became firmer at the ends of the fruit segment but the texture was still acceptable for all samples. The results can explain by the loss of water during the storage time. Other researchers have reported research results for cut Cantaloupe melon using hot water dipping (76ºC for 3 minutes) combined with gamma irradiation at a dose of 0.5 kGy and this was not found to affected the colour or texture of the cut melon [54]. We found that the flavour and taste of green pomelo was not changed (panel score of 5.0) after 1 month of storage, when stored under conditions that mimicked trading conditions. Similar research results were reported for grapefruit where an irradiation at dose of 400 Gy was not found to affect sensory attributes of the fruit for up to 35 days of storage [55]. Other research has also reported that irradiation at a dose 750 Gy did not significantly affect flavor changes in gamma irradiated grapefruit [56]. Similarly, irradiated mandarin from Brazil and orange from Spain were reported to maintain an acceptable flavour when the fruits were irradiated to a dose of 500 Gy [57, 58]. Based on our experimental results, we conclude that the quality of green pomelo can be maintained for at least one month after hot water and irradiation treatment where the irradiation treatment is at the low levels typically used as a phytosanitary treatment.

TABLE 10. EFFECT OF GAMMA IRRADIATION ON SENSORY QUALITY OF GREEN POMELO AT TRADING CONDITION

Storage duration (days)	Sample	Colour	Flavour	Texture	Taste	Overall acceptability
0	Control	5a	5a	5a	5a	5a
	HW-0 Gy	5a	5a	5a	5a	5a
	HW-400 Gy	5a	5a	5a	5a	5a
	HW-600 Gy	5a	5a	5a	5a	5a
15	Control	5a	5a	5a	5a	5a
	HW-0 Gy	5a	5a	5a	5a	5a
	HW-400 Gy	5a	5a	5a	5a	5a
	HW-600 Gy	5a	5a	5a	5a	5a
22	Control	4.33a±0.27	5a	5a	5a	4a
	HW-0 Gy	4.33a±0.67	5a	5a	5a	4.33a±0.67
	HW-400 Gy	4.33a±0.57	5a	5a	5a	4.33a±0.57
	HW-600 Gy	4.33a±0.58	5a	5a	5a	4.33a±0.58
30	Control	3.33a±0.57	5a	4.33a±0.57	5a	3.33a±0.57
	HW-0 Gy	3.33a±0.57	5a	4a	5a	3.33a±0.57
	HW-400 Gy	3.33a±0.17	5a	4.67a±0.57	5a	3.67a±0.17
	HW-600 Gy	3.33a±0.27	5a	4.67a±0.57	5a	4a

Mean values followed by the same letter are not significantly different at $P<0.05$.

15.4. CONCLUSIONS

Our results suggested that low EB radiation doses, typical of phytosanitary irradiation treatments, can inhibit the development of *Xanthomonas* sp. bactria but this low dose treatment is not enough to completely control the fungi. But a combination of a pre-treatment combined with low dose irradiation can control fungi on fresh fruits. Pre-treating custard apple and star apple fruits with 20 ppm NaDCC followed by EB irradiation at 400–600 Gy can maintain the quality and extend the shelf life of these fruits and inhibit fungal growth. The overall quality and appearance of custard apples and star apples after the combination treatment were evaluated as being better than in the control samples (untreated NaDCC, non-irradiated). The size

(diameter) of green pomelo fruit means that it cannot be treated correctly by EB irradiation for phytosanitary purposes (the DUR is too large) and so gamma irradiation was chosen as the preferred method of irradiation of this fruit. In this case using a hot water (50°C/2 minutes) pre-treatment followed by low-dose gamma irradiation could completely inhibit fungal growth and the shelf life of green pomelo can be extended up to 30 days. The treatment was constrained to low doses typically used for phytosanitary (from 0.4 to 0.6 kGy). These results can be applied at an industrial scale for export purposes.

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16. USE OF IRRADIATION AS A PHYTOSANITARY TREATMENT OF FRESH PRODUCE AND STORED PRODUCTS EXPORTED BY THE ARAB REPUBLIC OF EGYPT

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Abstract

The Arab Republic of Egypt produces and exports many agricultural products such as fruits, vegetables, legumes, rice, etc. Many of these commodities are susceptible to attack by pest species that cause a great of economic losses and restrict exportation to other countries. In recent years, irradiation is being used increasingly as a phytosanitary treatment to control insect pests of traded agricultural products. Ionising radiation is an alternative to fumigants that may be harmful and damage the environment. This research work was carried out to evaluate the effect of irradiation on the various development stages of Peach Fruit Fly (*Bactrocera zonata*), Cowpea Seed Weevil (*Callosobruch maculatus*), Rice Moth (*Corcyra cephalonica*) and Fig Moth (*Ephestia cautella*). We also evaluated the impact of the effective phytosanitary irradiation dose on the quality attributes of host commodities.

In a large-scale confirmatory test, a gamma radiation dose of 150 Gy applied to 17 000 3rd instar larvae of *B. zonate* infested pomegranate fruits resulted in no adult emergence. This indicates that 150 Gy is required to provide quarantine security. Gamma irradiation dose of 650 Gy applied to 27 754 individuals of the adult species *C. maculatus* in cowpea seeds resulted in no completion of reproduction. Indicating that a dose of 650 Gy is required to control *C. maculatus* and provide quarantine security. A gamma irradiation dose of 350 Gy applied to

16 000 of *Corcyra cephalonica* pupae resulted in no egg hatching of the F1 generation, indicating that this dose was sufficient to provide quarantine security. A gamma irradiation dose of 400 Gy was required to provide quarantine security against *E. cautella*, based on the prevention of egg hatching from F1 generation when the most tolerant stage to irradiation (3-day-old pupae of *E. cautella*) is considered. When the most radio-tolerant life-stages of *B. zonata* and *C. maculatus* are considered and the prevention of adults emergence is used as a criterion for measuring the electron beam irradiation efficacy, electron beam irradiation (EBI) to doses of approximately 305 and 414 Gy (measured doses) are required to control these insects, respectively and provide quarantine security. Our results also indicate that all the above mentioned effective phytosanitary irradiation doses do not significantly affect the quality attributes of the host commodities studied (pomegranates, cowpeas and rice). X ray irradiation doses of approximately 238 and 300 Gy were required to control *B. zonata* and *E. cautella* respectively and provide quarantine security of export/ import/ fresh fruits.

16.1. INTRODUCTION

Agricultural products such as fresh fruits, vegetables and stored agricultural commodities produced in Egypt are susceptible to different invasive pest species. Some of these insects are of quarantine importance and have a large economic impact. The occurrence of these pests can restrict the movement of agriculture products. An example is the Peach Fruit Fly, *Bactrocera zonata,* it is one of the most important pests in agriculture and can be found in many countries including Egypt. It attacks a wide range of fruits such as peach, pomegranate, guava, apricot, fig, citrus, apples, etc. [1, 2]. The Cowpea Seed Weevil, *Callosobruchus maculatus* is another common pest insect of stored products ranked as the principal pest-harvest insect of cowpea seeds and other legumes. It originated in West Africa and has moved throughout the world with the trade of legumes, causing heavy losses of stored products each year [3]. The Rice Moth, *Corcyra cephalonica* is an economically important stored grain pest in Asia, Africa, North America and Europe. It can have major implications for rice, but also infests commodities such as wheat, maize, sorghum, dates, ground nuts, beans and millet [4]. The Fig Moth, *Ephestia cautella* , is also a significant pest, it can infest commodities such as figs and dates causing damage during the several steps of harvest, storage, processing and transportation [5]. The important factor that contributes to the seriousness of infestations of this moth is its ability to develop resistance to chemical insecticides [6].

The traditional phytosanitary treatment of choice used to control insect pests of fresh, stored products and other agriculture products in Egypt and many other countries is the use of chemical insecticides and fumigants such as methyl bromide and phosphine [1, 7]. However, the use of these insecticides and fumigants have many disadvantages including the presence of residues in treated food, environmental contamination, development of resistance in certain insects and the inability of the chemical to penetrate large fruits to kill larvae [8, 9]. Therefore, it is of ultimate importance to develop a safe, eco-friendly and economically feasible alternatives to these insecticides and fumigants. Ionizing radiation in the form of gamma radiation, electron beams and x rays are ow used in many countries as a safe and effective phytosanitary treatment to disinfest agriculture products. In recent years, the use of phytosanitary irradiation to control insect pests of agricultural commodities has expanded rapidly [10]. The main advantages of phytosanitary irradiation are the absence of any residues in treated food, few changes in the physiochemical properties and nutritive value of the commodity, no development of resistance by insects and its ability to treat food in final packing in pallet loads [11, 12].

Most food and agricultural products irradiated in the world are treated in facilities using gamma radiation from ^{60}Co. The relatively recent development of high-power, high-energy accelerators

to generate electron beams and x rays has made alternatives to gamma radiation for food treatments. The main advantage of these machine source technologies is that they use electricity instead of radioactive isotopes, allowing them to be switched on an off as needed [13, 9].

The main objectives of our research were to:

- Establish rearing facilities for *Bactrocera zonata*, *Callosobruchus maculatus*, *Corcyra cephalonica*, and *Ephestia cauttela*;
- Select a suitable dosimetry system for the various types of radiation and dose to be applied;
- Study the effect of gamma irradiation, electron beam and X ray on various development stages of the above mentioned insects;
- Define the most radiation tolerant life stage and establish the criterion for phytosanitary efficacy;
- Confirm the phytosanitary irradiation dose for each using a large- scale test;
- Evaluate the quality attributes of the main hosts (pomegranate fruits, cowpea seeds, rice) after irradiation to the phytosanitary dose levels.

16.2. MATERIALS AND METHODS

16.2.1. GAMMA IRRADIATION PROCESS

For all studied pests samples were expoused to ionizing radiation in in a ^{60}Co Gamma Chamber (4000A) at the National Center for Radiation Research and Technology (NCRRT). Over the duration of the experiments the average gamma dose rate was between 1.388–1.144 kGy/hour. Detailed dose mapping was conducted by the Department of Radiation Protection and Dosimetery at NCRRT. Alanine dosimeters, were used for the dose calibration of the irradiator and for measuring the average absorbed dose. The alanine dosimetry system used was traceable to a recognised standard (the UK National Physical Laboratory).

16.2.1.1. *Gamma irradiation of Bactrocera zonata*

Peach fruit fly, *B. zonata,* were obtained from a continuously reared strain (under conditions of 25 ± 2°C and 60–70% RH) maintained in the Entomology Laboratory at the Plant Protection Research Institute, Dokki, Giza, Egypt. Adults were reared in a cage (60 x 40 x 40 cm) with wooden frame and metal screen sides. Caged adults were provided with food consisting of sugar and protein hydrolyzate (1:3 W/W) in Petri dishes. Drinking water was available from a small plastic bottle. The cage also contained plastic fruits containing many small pores (as an oviposition receptacle). These plastic fruits were filled with 3 ml of sterilised water to receive eggs and prevent them from dehydrating.

Deposited eggs were collected from the oviposition receptacles at 12-hourly intervals. The eggs were transferred to plastic trays (15 x 20 x 10 cm). Larvae were reared in plastic trays, each containing 250 g of the artificial diet (a mixture of 330 g wheat bran, 84.5 g yeast, 84.5 g sugar, 3 g sodium benzoate, 3g citric acid and 500 ml water). The trays were then placed in a large wooden box (cage) with sand at the bottom in order to allow larvae to pupate.

The pomegranate fruits used for artificial infestation in these experiments were fresh, well ripened, healthy and free from microbial infestation or insect infestation. The collected eggs

were selected and place on card (black filter paper 2 x 2 cm) using a brush under a stereo microscope. The card with a specific number of eggs was used to infest pomegranate fruits. For irradiation of the egg life-stage in host fruits, infested pomegranate fruits were gamma irradiated at doses of 0 (control), 50, 100, 150, 200, 250, 300, 350 and 400 Gy. Five pomegranate fruits replicates with 50 eggs for each fruit were used in each dose level. After irradiation, the pomegranate fruits were transferred to plastic vials and stored in controlled environment cabinet (25 ± 2°C and 60–70% RH). All eggs were examined under a microscope on a daily basis to check the number of hatched eggs, pupation and adult emergency.

For irradiation of the larvae stage of Peach Fruit Fly in host fruits, each pomegranate fruit was infested with 1st, 2nd and 3rd instar larvae. The infested pomegranate fruits with larvae were irradiated at doses of 0 (control), 50, 100, 150, 200, 250, 300, 350 and 400 Gy. Each treatment had five pomegranate fruits replicates with 50 larvae of the 1st, 2nd or 3rd instar respectively. After irradiation, the pomegranate fruits were transferred to plastic vials and stored in controlled environment cabinet (25 ± 2°C and 60–70% RH). All infested pomegranate fruits were examined daily to check the larval mortality, the percentage of pupation and adult emergence.

Irradiation of the Peach Fruit Fly pupae life-stage was undertaken in test tubes. Pupae (either three-day or seven-day old pupae) were placed in a 10 cm high test tube. Pupae were irradiated at doses of 0 (control), 50, 100, 150, 200, 250, 300, 350 and 400 Gy. Each treatment was replicated five times with 50 pupae in each sample tube. After irradiation, the pupae were transferred to a cage (60 x 40 x 40 cm) with wooden frame and metal screen sides. The percentage of adult emergence was estimated from regular observations.

Large scale confirmatory tests were conducted by irradiating 100 late third instar larvae in each pomegranate fruit at 150 Gy (irradiation dose predicted to prevent adult emergence). This was repeated 170 times. As controls, 100 late third instars were put in pomegranate fruit and this was repeated 5 times. All irradiated and unirradiated infested pomegranate fruits were stored in controlled environment cabinet at 25 ± 2°C and 70 ± 5% RH. All infested pomegranate fruits were observed on daily basis to check the adult emergence.

To study the effect of phytosanitary irradiation dose on the firmness of the pomegranate fruits and different quality attributes of pomegranate juice, commercially ripe pomegranate (*Punica granatum*) fruits were obtained from a private farm located at Cairo-Alexandria Desert road. These good fresh fruits were irradiated at 150 Gy, whereas other fresh fruits were left without irradiation and served as Control Group. All irradiated and unirradiated fruits were stored at 5 ± 1°C and 80 ± 5% RH for three months.

Firmness of pomegranate fruits and quality attributes of pomegranate juice were assessed in unirradiated and irradiated pomegranate. Three unirradiated and irradiated pomegranate fruit samples were used for each test. The juice was prepared by direct squeezing in a commercial juicer. Fruit firmness was determined using Magness and Taylor-pressure tester with 5/16 inch plunger. The results were recorded as lb/inch^2. Acidity was measured by titration of 1 ml of pomegranate juice against 0.1 NaOH and expressed as percentage of citric acid [14]. Ascorbic acid of pomegranate juice was measured according to AOAC [14] and expressed as mg/100 ml fruit juice. Total anthocyanin content of the juice was determined according to the method described by Elfalleh, et al. [15] and expressed as mg/100 ml. Total carotenoid content was determined by spectrophotometer according to the method described by Sharon and Kahn [16] and the results were expressed as mg/100 ml juice. Total soluble solid (TSS) content was measured by a hand refractometer (Atago digital, Japan) and expressed as percentage according to the method by AOAC [14]. Total sugars and reduced sugar content were determined according to Miller [17]. The results were expressed as mg/100ml juice. Anti oxidant activity

was measured using 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging and determined according to the method described by Chrzczanowicz, et al. [18] and expressed as a percentage. Total polyphenols were determined using Folin-Ciocaltue reagent according to Elfalleh, et al. [19] and expressed as percentage.

Viable bacterial in pomegranate juice samples were assessed as total aerobic bacterial counts (TABC). The TABC were determined using a pour plate technique [20]. The inoculated plate was poured with plate count agar medium (PCA, Difco, USA) then incubated (30°C for 72 hours). The TABC were enumerated as colony forming units per gram (cfu/g) and expressed as log cfu/g. Total mold and yeast counts (TMY) were also determined in a similar way, using the pour plate technique. However, the plates were poured with Czapek,s yeast extract agar medium and then incubated (25°C for 72 hours). The Charm peel plate EC microbial test (kit: code: PP–EC–100k) was used to enumerate total coliform bacteria and *E. coli* according to the Charm Operators Manual: Inoculated plates were incubated (35 ± 1°C for 24 hours) after which the plates were inspected for colour stained colonies (red colonies represent total coliform bacteria, while blue-black colonies represent *E. coli*). This test has been certified by AOAC research institute as performance tested method #061501.

16.2.1.2. Gamma irradiation of Callosobruchus maculatus

The procedures are described in detail in our previous publication [21]. Cowpea seed weevil (*Callosobruchus maculatus*) were obtained from the Plant Protection Research Institute, Dokki, Giza, Egypt. Cowpeas used for the bioassay were first maintained in a freezer for 10 days in order to eliminate the risk of prior infestation. *C. maculatus* was reared on cowpea seeds in 500 ml glass jars. To each jar was added 300–400 adults (male and female) of *C. maculatus* (0–2-day-old) for laying eggs. The Jar opening was covered with muslin held firmly in place by rubber bands to prevent insect escape. The jars with insects were incubated for one week (28 ± 2°C and 75 ± 5% RH), afterwhich time the parent adults were removed (by sieve) and discarded.

For the production of one- and three-day old egss in studies of the irradiation of the egg life-stage, cowpea seeds (5 g) were infested by male and female couples (five males and five femails) of tested weevils. After one or three days the adults were removed, leaving the cowpea seeds infested with one or three-day-old eggs. These eggs were exposed to 0, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 Gy of gamma radiation (3 replicates for each treatment were used). The irradiated one and three-day-old eggs in each replicate were counted. The hatchability and adult emergence percentages were also determined.

For studies of the irradiation of larvae and pupae stages of the Cowpea seed weevil, cowpea seeds (400 g) were infested by two hundred tested insects. After three days the cowpea seeds were sieved thoroughly to remove and discard the weevils. Five grams of infested cowpea were separated for each treatment. Insects present as larvae were irradiated 13 days after infestation to doses of 0, 50, 100, 150, 200, 250, 300 and up to 850 Gy. Insects present as pupae were irradiated 17 days after infestation to doses of 0, 50, 100,150, 200, 250, 300 and up to 1250 Gy. The number of adult cowpea weevil that emerged were counted and the reduction in emergence numbers (percentage) from irradiated larvae and pupae were estimated. Five couples (males and females) resulting from each irradiated dose were separated and placed with 3 g of cowpea seeds. Infested cowpea seeds were stored in a controlled environment cabinet (28 ± 2°C and 75 ± 5% RH). The adult weevils that emerged (F1 progeny) were counted, and reduction in the F1 generation was determined as a percentage.

For irradiation of adult stage, 5 g of cowpea seeds were infested by 50 couples of *C. maculatus*. One-day-old adults were exposed to 0 (control), 50, 100, 150, 200, 250, 300, 350 and up to1350 Gy of gamma radiation (three replicates for each dose treatment). The mortality of irradiated adults was estimated as a percentage after 24 hours. Five live couples resulting from each irradiated dose were separated and put on 3 g of cowpea seeds. After three days, the cowpea seeds were sieved thoroughly in each treatment and the adult weevils were discarded. The eggs were counted in each treatment and the number of emerging adults was estimated by observation and counting.

Confirmatory tests were conducted by treating a large number (27,745 individual weevils) of male and female adults of *C. maculatus* taken from the stock colony at the dose proposed as the effective dose for a phytosanitary treatment (650 Gy) with the measure of efficacy being to prevent the production of F1 adults. In these experiments, approximately 700 one-day-old adults (male and female) were put on cowpea seeds (100 g) in ventilated containers for 1–2 days and irradiated at 650 Gy (39 replicates). The unirradiated control comprised 190 adults (male and female) placed in 100 g of cowpea seeds, and this was repeated 5 times. Parent adults were removed from the containers after 7 days, and the cowpea seeds were stored at 28 ± 2°C and 75 ± 5% RH for more 60 days. Any emergence of F1 adults was counted. The level of confidence associated with treating a large number of *C. maculatus* with zero survival was estimated by the equation: $C = 1 - (1 - Pu)^n$ where *Pu* (0.0001) is the acceptable level of survivorship and n is the number of treated insects [22].

To evaluate the effect of a phytosanitary irradiation dose of 650 Gy on the chemical, physical and microbiological quality of cowpea seeds, moisture, protein, lipids, ash and carbohydrate contents of irradiated and unirradiated cowpea seeds were determined according to the method of AOAC [14]. For investigations of changes to germination, groups of twenty-five cowpea seeds (irradiated or unirradiated) were put into each dish on top of moist paper. Three dishes were used for irradiated or unirradiated cowpea seeds were placed under the lights to allow the seeds' to germinate. After four and eight days, the numbers of germinated seeds were counted and data were expressed as percent germination. Hardness testing was carried out using a Penetrometer system (Digital Force Gauge Model FGN-20G, Nidec-Shimpo Corporation, Japan). Two hundred cowpea seeds (irradiated and unirradiated) were soaked for 1 hour in water, then cooked in 1250 ml of water. The average cooking time (minutes) for three replicates was recorded. The colour quantity of cowpea seeds in terms of hunter's L (lightness), a* (red – green coordinate, redness), b* (blue – green coordinate, yellowness) was determined according to Nieto-Sandoval *et al.,* [23]. Microbiological determinations were carried out on cowpeas in the same way as for pomegranate fruit samples as mentioned above.

16.2.1.3. Gamma irradiation of Corcyra cephalonica

Rice moth, (*Corcyra cephalonica*) were obtained from the Plant Protection Research Institute, Dokki, Giza, Egypt. Larvae were reared on an artificial diet (500 g wheat bran, 500 g flour maize, 250 ml glycerol, 125 g yeast, 250 ml natural honey and 250 g milk powder) [24]. Adult moths were introduced into chimney glass cages to lay eggs. The cage an opening at the top covered with gauze after placing moths inside the cage, and another opening at the bottom for collecting eggs. The bottom opening was covered with a coarse mesh gauze (wide holes to allow eggs to pass through). A Petri dish was placed under the cage to collect the eggs. Eggs were collected daily from the Petri dishes. All insect stages were reared in an incubator (30 ± 2°C and 65 ± 5% R.H.) in continuous darkness.

For irradiation of Rice Moth eggs, fifty eggs (three days old) were exposed to 0, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 and 550 Gy of gamma radiation (5 replicates for each

treatment). The number of hatching larvae in each replicate were counted and transferred to rearing media. The hatchability and adult emergence percentages were calculated. Five paired adults (male and female couples) resulting from each irradiated dose were introduced into chimney glass cages. Newly laid eggs were collected in petri dishes and counted daily and these data were used to calculate the hatchability percentage for the F1 generation.

For the irradiation of the Rice Moth larvae stage, 2nd instar larvae (10 days old) were exposed to 0, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 and 550 Gy of gamma radiation. Also, 4th larval instar (22 days old) were exposed to 0, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600 and 650 Gy (five replicates for each treatment were used, with 25 larvae in each replicate sample). The irradiated larvae for each treatment were transferred to rearing media. The number of viable and dead larvae were counted after seven days. The larval mortality and adult emergence were estimated. Five paired adults (male and female couples) resulting from each irradiated dose was introduced into chimney glass cages to breed and lay eggs. Newly laid eggs were collected in Petri dishes and counted daily. These data were used to calculate the percentage F1 generation egg-hatch.

Experiments investigating the effects of radiation of Rice Moth pupae involved exposing twenty-five pupae (five days old) per sample exposed to 0, 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 to 700 Gy of gamma radiation. (five replicate samples for each treatment were used). The numbers of emerging adult Rice Moths and percentage adult emergence was estimated. Five paired adults (male and female couples) resulting from each irradiated dose were introduced into chimney glass cages to breed. Newly laid eggs were collected and counted daily and the percent egg hatch for the F1 generation was determined.

For irradiation of adult stage Rice Moths, twenty-five adults (1–2 days old) were exposed to 0, 50, 100, 150, 200, 250, 300, 350 and 400Gy of gamma radiation (five replicates for each treatment were used). The number of dead adults were determined after five days. Five pairs (male and female couples) resulting from each irradiated dose experiment were introduced into chimney glass cages to breed. Newly laid eggs were collected and counted daily to calculate the percentage egg hatch for the F1 generation.

In large scale confirmatory tests, 16 500 male and female pupae of *C. cephalonica* stock colony were irradiated with the proposed irradiation dose (350 Gy) that produced zero egg hatch in the F1 generation when the most radio tolerant stage (pupae) were irradiated. In this experiment, approximately 550 pupae (male and female) from 5-day-old were place into ventilated containers and irradiated at 350 Gy. This was repeated 30 times. Control experiments (unirradiated pupae) involved five replicate samples, each of 100 pupae (male and female) resulting parent adults from the control experiment and from each irradiated dose were introduced into chimney glass cages to lay eggs. Newly laid eggs were collected and counted daily to derive the egg hatch data for F1 generation. The level of confidence (C) associated with treating a large number of *C. cephalonica* with zero survival was estimated using equation 1, where Pu (0.0001) is the acceptable level of survival and n is the number of treated insects [22].

$$C = 1 - (1 - Pu)^n \qquad (1)$$

To evaluate the effect of phytosanitary irradiation dose level (350 Gy) on some quality attributes of rice, the hulled rice was packed in a polyethylene bags (200 g per bag) and exposed to 350 Gy of gamma irradiation. The colour of de-hull rice was measured using a Portable Colour Analyser (Model: RGB-1002) equipped with three external phototransistors for measuring red (R), green (G) and blue (B) values as well as the HSL values: Hue, Saturation (Sat), Luminance

(Lum). Three replicates from each sample were used for these measurements. Two hundred grams of irradiated or non-irradiated hulled rice were cooked for 15 minutes in tap water and the percentage of absorbed water, increase in the rice volume and weight were determined [25]. Microbiological determinations were carried out as mentioned earlier.

16.2.1.4. Gamma irradiation of Ephestia cautella

Fig Moths (*Ephestia cautella*) were obtained from the Entomology Laboratory at Plant Protection Research Institute, Dokki, Giza, Egypt. Fig Moth larvae were reared in plastic jars of one litre volume on artificial diet consisting of ground wheat (250 g), ground sugar (25 g), dry yeast (25 g) and glycerol (37.5 g) [5]. Adult moths were introduced into chimney glass cages. The cage had two openings one at the top covered with a gauze after placing moths inside the cage, the other opening at the bottom of the cages was covered with gauze that had holes wide enough to allow eggs to pass through. A Petri dish was put under each cage to collect the eggs which passed through the gauze. Newly laid eggs were collected daily from the Petri dishes. All insect life stages were reared in an incubator at 27 ± 2°C and 65 ± 5% relative humidity in continuous darkness.

For the irradiation of Fig Moth eggs, each sample comprised thirty eggs (one day old) placed in a test tube and exposed to 0, 50, 75, 100, 150 and 200 Gy (5 replicate samples were used for each treatment dose). Similarly three day old eggs (thirty eggs per sample) were exposed to 0, 50, 75, 100, 150, 200, 250, 300, 350 and 400 Gy of gamma radiation (5 replicate samples for each treatment). The number of hatching larvae in each replicate were counted and transferred to date fruits. The percentage egg hatch and adult emergence were determined. Five pairs of Fig Moth adults (one male and one female in each replicate) resulting from each irradiation dose treatment were introduced into chimney glass cages. Newly laid eggs were collected in Petri dishes and counted daily to calculate the percentage egg hatch for the F1 generation resulting from irradiated eggs.

For the irradiation of the larvae stage, date fruits with 2nd instar larvae (6-day old Fig Moth larvae) and date fruits with 4th instar larvae (12-day old Fig Moth larvae) were exposed to 0, 50, 75, 100, 150, 200, 250, 300 and 350 Gy of gamma radiation (5 replicate samples were used for each treatment dose). The numbers of larvae in each replicate were 20 larvae. Larval mortality and adult emergence data were determined. Five adult Fig Moth pairs (one male and one female in each replicate) resulting from each irradiated dose treatment were introduced into chimney glass cages to breed. Newly laid eggs were collected and counted daily to determine the percent egg hatch for the F1 generation.

For the irradiation of Fig Moth pupae, twenty pupae (3 days old) were exposed to 0, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, and 500 Gy of gamma radiation (five replicates were used for each treatment). The number and percentage of emerging adults were recorded. Five adult Fig Moth pairs (one male and one female in each replicate) resulting from each irradiated dose treatment were introduced into chimney glass cages to breed. Newly laid eggs were collected and counted daily to determine the percent egg hatch for the F1 generation.

The irradiation of one day old adult Fig Moths involved samples of twenty adults (10 male and 10 female) being exposed to 0, 50, 100, 150, 200, 250 and 300 Gy of gamma radiation (five replicates for each treatment). The number of dead adults was determined after 5 days. Five couples of F1 adults (one male and one female in each replicate) resulting from each irradiation treatment dose were introduced into chimney glass cages. Newly laid eggs were collected and counted daily to determine the percent egg hatch for the F1 generation.

16.2.2. ELECTRON BEAM IRRADIATION PROCESS

Electron beam irradiation (EBI) was performed at the electron beam accelerator (ICT, VIVIRAD Co., France) located at the National Centre for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The details of general electron beam parameters and operating parameters during irradiation treatment are given below in Table 1. The samples of infested cowpeas or artificial feeding medium (5 g) were spread, in single layer in small plastic boxes (5 x 5 x 3 cm) with small holes punched for air movement. These small boxes were then arranged in a large plastic box (36 x 36 x 15 cm) covered with a plate (8 mm) of polymethyl methacrylate (PMMA) to attenuate the radiation field to reach the proposed doses (100, 125, 300, 400, 500 and 650 Gy). Gafchromic HD-V2 film dosimeters (Dynamic dose range 10 to 1000 Gy; lot #: 01091801) were used. These dosimeters were calibrated at NCRRT using a ^{60}Co-Gamma Cell 220 Excel irradiation unit (MDS Nordion, Canada). The absorbed dose rate to water at the center of gamma cell (GC) is calibrated by the National Physical Laboratory (NPL) in England using a transfer alanine dosimeter. The HD-V2 film dosimeters were calibrated in this GC at the centre in the dose range of 10 Gy to 1000 Gy and measured at 660 and 605nm using a UV-Vis spectrophotometer (UVICON 860, KONTRON Co. Ltd., Switzerland). The obtained calibration curve was then used to measure the absorbed dose imparted to samples of cowpea seeds or artificial feeding medium during electron beam irradiation. In order to establish the calibration curve, the absorbance of ten un-irradiated film dosimeters were measured at 660 and 605nm wavelengths (to determine the background absorbance) and then many HD-V2 film dosimeters were irradiated to different doses ranging from 10 Gy to 1 kGy (five dosimeters at each dose). The absorbance was measured at the same wavelengths of 660 and 605nm. Thin film alanine dosimetry was used to compare the absorbed dose measured by HD-V2 film dosimeters in electron beam for doses higher than 500 Gy. For every treatment, HD-V2 film dosimeters were placed with the infested cowpea or artificial feeding medium during irradiation and read by the UV-V is spectrophotometer to measure the actual absorbed dose.

TABLE 1. ELECTRON BEAM PARAMETERS

Parameter	General operating parameters	Settings used in experiments
Beam energy (MeV)	Up to 3	2.50
Beam current (mA)	Up to 30	0.50
Beam Power (kW)	90	1.25
Scan width (cm)	90	90
Distance between scanner and conveyor system (cm)	53	53
Scan speed (m/h)	Up to 16	6–16

16.2.2.1. Electron beam irradiation of Bactrocera zonata

For the electron beam irradiation of Peach Fruit Fly eggs, artificial rearing medium (5 g) in plastic vials were infested with one-day-old eggs and irradiated to proposed doses of 0.0, 100, 125, 300, 400, 500 and 650 Gy (that corresponded to measured doses of 0.0, 103.6, 123.3, 304.8, 414.3, 488.3 and 653.5 Gy). Each treatment had five replicates with 100 eggs each. After irradiation the plastic vials were stored in a controlled environment (25 ± 2ºC and 60–70% RH). The samples were examined under a microscope daily to check the number of hatched eggs, pupation and for adult emergence.

The irradiation of Peach Fruit Fly larvae was carried out in a similar way but with 5 g of artificial medium in plastic vials infested with 1st, 2nd or 3rd instar larvae. Samples were irradiated to proposed doses of 0.0, 100, 125, 300, 400, 500 and 650 Gy (that corresponded to measured doses of 0.0, 103.6, 123.3, 304.8, 414.3, 488.3 and 653.5 Gy). Each treatment had five replicates with 100 larvae each. After irradiation, the plastic vials were stored in a controlled environment (25 ± 2ºC and 60–70% RH). The samples were examined daily to check the larval mortality, percentage pupation and adult emergence.

For irradiation of pupae stage, Three or seven-day-old pupae were placed in plastic vials and irradiated to proposed doses of 0.0, 100, 125, 300, 400, 500 and 650 Gy (that corresponded to measured doses of 0.0, 103.6, 123.3, 304.8, 414.3, 488.3 and 653.5 Gy). Each treatment had five replicates with 50 pupae each. After irradiation the pupae were transferred to a wooden frame cage with metal screen sides (6 x 40 x 40 cm). The percentage adult emergence was determined.

16.2.2.2. Electron beam irradiation of Callosobruchus maculatus

For the irradiation of Cowpea seed weevil eggs, 5 g of cowpea seeds were infested by 5 couples (male + female) of insects. After three days the adults were removed, then the cowpea seeds infested with three-day-old eggs were exposed to the proposed doses 0.0, 100, 125, 300, 400, 500 and 650 Gy of electron beam (five replicates for each dose treatment were used). The irradiated three-day-old eggs in each replicate were counted; also, the hatchability and adult emergence percentages were estimated.

For the irradiation of larvae and pupae of Cowpea seed weevils, 400 g of cowpea seeds were infested by two hundred tested insects. After three days the infested cowpea seeds were sieved thoroughly, and the weevils were discarded. Five grams of infested cowpea was separated for each treatment. After 13 days of infestation, insects were treated at proposed doses 0.0, 100, 125, 300, 400, 500 and 650 Gy as larvae. After 17 days of infestation, insects were treated by 0.0, 100, 125, 300, 400, 500 and 650 Gy corresponded to measured doses of 0.0, 103.6, 123.3, 304.8, 414.3, 488.3 and 653.5 Gy as pupae. Adult emerging and reduction percentage from irradiated larvae and pupae was estimated. Five couples (males and females) resulting from each irradiated dose were removed and placed with 3 g cowpea. Infested cowpea was stored in controlled environment cabinet (28 ± 2ºC and 75 ± 5% RH) and the adult weevils that emerged (F1 progeny) were counted, and the percentage reduction of insect numbers in the F1 generation was determined.

For the irradiation of adult weevils, 5 g of cowpea seeds were infested by 25 couples (male and female) *C. maculatus*. Adults (one day old) were exposed to proposed doses of 0.0, 100, 125, 300, 400, 500 and 650 Gy corresponded to measured doses of 0.0, 103.6, 123.3, 304.8, 414.3, 488.3 and 653.5 Gy of electron beam radiation (5 replicate for each treatment were used). The mortality percentage of irradiated adults was determined after 24 hours. Five surviving couples (male and female) resulting from each irradiation treatment dose were removed and placed on 3 g cowpea seeds. After three days the cowpea seeds were sieved thoroughly in each treatment and the adult weevils were discarded. The eggs were counted in each treatment and the number of emerging adults was determined. Large-scale confirmatory tests were conducting by treating a large number of cowpea weevil adults with dose of electron beam radiation of 414.3 Gy. Approximately 700 adults (1-day-old) were counted and placed on 5 gs cowpea seeds (40 replicates were applied). In controls, 500 adults were counted and placed on 5 g of cowpea seeds (10 replicates were applied). Adults were removed from the containers after 7 days, and the treated and untreated cowpea seeds were held for additional 60 days and examined regularly for the emergence of F1 adults.

16.2.3. X ray irradiation process

X ray irradiation treatment was performed using an electron beam accelerator (3MeV, ICT, VIVARAD, Co., France) equipped with x ray convertor, located at the NCRRT, Nasr City, Cairo, Egypt. The infested mango fruits (individually) were arranged directly on the trays of facility conveyor system. The infested date fruits were placed in plastic boxes (5 x 5 x 3 cm) with small holes punched for air movement.

The Gafchromic HD-V2 Film dosimetry system was used (dynamic dose range 10 to 1000Gy; lot #: 01091801). Dosimeters (HD-V2 films) were calibrated at the NCRRT using a 60Co-Gamma Cell (220 Excel, MDS Nordion, Canada) as mentioned above. The dosimeter read out measurements were carried out after 24 hours to completely develop the colour of the active diacetylene materials on the HD-V2 films. The obtained calibration curve was utilized to measure the absorbed dose to samples of mango and dates fruits during X ray irradiation to a maximum dose of 400 Gy. In order to establish this calibration curve, un-irradiated HD-V2 film dosimeters (10 film dosimeters) were used to determine the absorbance before irradiation at 660 nm and 605 nm. After that, different HD-V2 films were irradiated at a series of absorbed doses from 10 Gy to 1000 Gy (five dosimeters per each dose set) and measured at the same wavelengths of analysis. Alanine pellets were used to compare and verify the absorbed dose measured by HD-V2 films exposed to X rays to doses higher than 50 Gy. With experiments on dates, HD-V2 films were placed on the top and bottom of dates during irradiation, and then read by the UV-Vis spectrophotometer 24 hours post irradiation to measure the actual dose delivered to the samples. However, for experiments with mangoes, the film dosimeters were inserted into the middle of the mango and also placed on the top and bottom of each mango fruit. The average absorbed doses were then calculated according to IAEA (2004). All samples were subjected to two-sided irradiation by the X ray beam.

16.2.3.1. X ray irradiation of egg and larval development stage of Bactrocera zonata

Before artificial infestation, the mango fruits used were inspected to make sure that they were fresh, well ripened, healthy, and free from microbial infestation or insect infestation. A soft brush was used to place the required number of Peach Fruit Fly eggs on card (black filter paper 2 x 2 cm) under a stereo microscope. Each mango fruit was infested using a card containing 100 eggs (one-day-old eggs).

Peach fruit fly eggs were irradiated in the host mango fruit to doses of 0 (control), 60.7, 137, 156.9, 237.7, 300.59 and 344.46 Gy (measured doses). Each treatment had five replicates with 100 eggs each replicate mango sample. After irradiation, the mango fruits were transferred to plastic vials and stored in a controlled environment cabinet (25 ± 2ºC and 60–70% RH). All eggs were examined under a microscope daily to check the number of hatched eggs, pupation and adult emergence.

Peach fruit fly larvae were also irradiated in host mango fruits. Each mango fruit was infested with larvae (1st or 2nd or 3rd instar larvae) and irradiated by x ray radiation to measured doses of 0 (control), 60.7, 137, 156.9, 237.7, 300.59 and 344.46 Gy. Each treatment had five replicates with 100 larvae each. After irradiation, the mango fruits were transferred to plastic vials and stored in controlled environment cabinet (25 ± 2ºC and 60–70% RH). All infested mango fruits were daily examined to check the larval mortality, percent pupation and adult emergence.

16.2.3.2. X ray irradiation of larval, pupal and adult development stages of Ephestia cautella

Fig moths (*Ephestia cautella*) were obtained from the Entomology Laboratory at the Plant Protection Research Institute, Dokki, Giza, Egypt. The larvae were reared in plastic jars of one litre volume, that contained date fruits that had their seeds removed (pitted dates). The reared adult moths were introduced into chimney glass cages to lay eggs. Each cage had two openings, the one at the top was covered with a gauze after placing moths inside the cage, while the other opening at the bottom covered with a gauze that had wide holes to allow eggs to pass through and into Petri dish collection vessels. Newly laid eggs were collected daily by retrieving the Petri dishes. All Fig Moth life stages were reared in an incubator at 27 ± 2°C and 65 ± 5% relative humidity in continuous darkness.

Fig Moth larvae (6 day old, 2nd instar larvae or 12 day old, 4th instar larvae) were irradiated in pitted date fruit. Samples were exposed to 0, 56.6, 104.9, 190.5, 264.3, 300, 350 and 400 Gy of x ray radiation (5 replicate for each treatment). There were 100 larvae in each replicate and samples were irradiated in plastic vials (11 cm length x 9 cm width x 2 cm height). Two-sided exposure was used, i.e., the x ray beam was first directed to the upper face of the sample and then to the lower face of the plastic vials. After irradiation, larvae mortality and adult emergence were recorded. Five pairs (one male and one female in each replicate) resulting from irradiated larvae at each irradiation dose were introduced into chimney glass cages to lay eggs. Newly laid eggs were collected and counted daily. Percent egg hatch for the F1 generation was determined.

For irradiation of Fig Moth pupae, each sample comprised one hundred pupae in a test tube. Three-day old pupae were exposed to 0, 56.6, 104.9, 190.5, 264.3, 300, 350 and 400 Gy of x ray radiation (five replicates for each treatment were used). The number of emerging adults and percentage adult emergence was recorded. Five pairs (one male and one female) resulting from each irradiation treatment dose were introduced into chimney glass cages to lay eggs. Newly laid eggs were collected and counted daily to derive the percent egg hatch for the F1 generation.

Adult Fig Moths (one day old) were also irradiated in test tubes, each sample comprised one hundred adults in a test tube (50 male and 50 female). Samples were exposed to 0, 56.6, 104.9, 190.5, 264.3, 300, 350 and 400 Gy of x ray radiation (in five replicates for each dose treatment). Mortality was determined after 5 days. Five pairs (one male and one female in each replicate) resulting from each irradiation dose treatment were introduced into chimney glass cages to lay eggs. Newly laid eggs were collected and counted daily to derive the percent egg hatch for the F1 generation.

16.2.4. Statistical analysis

One way analysis of variance (ANOVA) using SPSS (statistical package for social sciences, ver.17.0) was used to analyze all experimental data, except *Callosobruchus maculatus* with treated by gamma irradiation, and the significance among the samples was compared at $P \leq 0.05$. Results were expressed as mean ± SD (n = 5).

Our statistical analysis of data for *Callosobruchus maculatus* treated with gamma irradiation is described in a previous publication [21], all variables were tested to see if they met the assumption of normality and equality of variance. All except four variables met these assumptions. The non-parametric variables were subjected for various transformation techniques, but normality remained unsolved. Therefore, non-parametric analysis was used to analyze these data. Parametric variables distributions have been expressed in tables in the form

of Mean ± Standard Error of mean (SE) while non-parametric variables distributions were expressed in tables in the form of Median (First Quartile – Third quartile). Three statistical analyses have been used all over the data. Two Independent samples t test was used for comparing the 2 levels factors with parametric response while One Way ANOVA has been used for >2 levels factors with parametric response while >2 levels factors with non-parametric response, Kruskal Wallis One-way ANOVA on Ranks was used for this purpose. Post hoc tests were done for both parametric and non-parametric data at *P*-value <0.05. Significant results obtained from ANOVA test were further tested for multiple comparison testing using Holm-Sidak test as it is recommended over Tukey and Bonferroni tests. A significant result obtained from the Kruskal Wallis ANOVA on Ranks was further tested for multiple comparisons testing using Dunnett test for non-parametric data. All multiple comparisons were versus the control group. An asterisk was assigned for all significant groups against the control group (*P*-value <0.05). Different assumptions testing and statistical data analyses were carried out using Sigmaplot 12.5.

16.3. RESULTS AND DISCUSSION

16.3.1. Gamma irradiation

16.3.1.1. Effect of gamma irradiation on Bactrocera zonata and pomegranate host

Increasing doses of gamma radiation delivered to eggs of Peach Fruit Fly (*B. zonata*) significantly decreased the percent egg hatch (Table 2). An irradiation dose of 100 Gy to the egg life stage, prevented adult emergence and 150 Gy prevented pupation, while 200 Gy completely prevented egg hatching. This is in reasonable agreement with previous research that reported a significant reduction of *B. zonata* egg hatching after treatments with a gamma radiation dose of 70 Gy for either females or both sexes, and 90 Gy in case of treated males only [26].

TABLE 2. GAMMA IRRADIATION OF *B. ZONATA* EGGS IN HOST FRUIT AND EFFECT ON EGG HATCH, PUPATION AND ADULT EMERGENCE

Dose (Gy)	Mean number of eggs irradiated	Number of hatched eggs (mean±SD)	Egg hatch (%)	No. pupae (mean±SD)	Pupation (%)	No. emerging as adults (mean±SD)	Adult emergence (%)
0 (control)	50	48.5±2.4	97	45.75±2.9	94.3	42.75±2.1	93.4
50	50	17.75±2.6*	35.5	2.25±0.5*	12.7	1.25±1*	55.6
100	50	12.25±2.2*	24.5	1.25±1*	10.2	0±0	0.0
150	50	4±0.8*	8	0±0	0.0	0±0	0.0
200	50	0±0	0.0	0±0	0.0	0±0	0.0
250	50	0±0	0.0	0±0	0.0	0±0	0.0
300	50	0±0	0.0	0±0	0.0	0±0	0.0
350	50	0±0	0.0	0±0	0.0	0±0	0.0
400	50	0±0	0.0	0±0	0.0	0±0	0.0

* The mean difference is significant at the 0.05 level compare with control.

The results of irradiation on 1st, 2nd and 3rd instars indicate that increasing doses of gamma radiation significantly increased mortality and reduced pupation and adult emergence of Peach Fruit Fly (Tables 3, 4 and 5 respectively).

TABLE 3. GAMMA IRRADIATION OF 1ST LARVAL INSTARS OF *B. ZONATA* IN HOST FRUIT AND THE EFFECTS ON LARVAE MORTALITY, PUPATION AND ADULT EMERGENCE

Dose (Gy)	No. irradiated larvae (mean)	Number of dead larvae (mean±SD)	Larval mortality (%)	Number of pupae (mean±SD)	Pupation (%)	No. emerging as adults (mean±SD)	Adult emergence (%)
0 (control)	50	2.5±2.1	5	47.5± 2.5	95	47.5±2.5	100
50	50	45±1.6*	90	5±1.6*	10	0±0	0.0
100	50	48.75±1*	97.5	1.25±1*	2.5	0±0	0.0
150	50	50±0*	100	0±0	0.0	0±0	0.0
200	50	50±0*	100	0±0	0.0	0±0	0.0
250	50	50±0*	100	0±0	0.0	0±0	0.0
300	50	50±0*	100	0±0	0.0	0±0	0.0
350	50	50±0*	100	0±0	0.0	0±0	0.0
400	50	50±0*	100	0±0	0.0	0±0	0.0

* The mean difference is significant at the 0.05 level compare with control.

TABLE 4. GAMMA IRRADIATION OF 2ND LARVAL INSTARS OF *B. ZONATA* IN HOST FRUIT AND THE EFFECTS ON LARVAE MORTALITY, PUPATION AND ADULT EMERGENCE

Dose (Gy)	No. irradiated larvae (mean)	Number of. dead larvae (mean±SD)	Larval mortality (%)	Number of pupae (mean±SD)	Pupation (%)	No. emerging as adults (mean±SD)	Adult emergency (%)
0 (control)	50	1.75± 2.4	3.5	48.25±2.3	96.5	47.5±2.5	98.4
50	50	27.75±3.5*	55.5	22.25±3.5*	44.4	3±1.1*	13.5
100	50	34±2.8*	68	16±2.8*	32	0±0	0.0
150	50	34.75±1.3*	69.5	15.25±1.3*	30.5	0±0	0.0
200	50	36.75±1.3*	73.5	13.25±1*	26.5	0±0	0.0
250	50	40.5±1.2*	81	9.5±1.2*	19	0±0	0.0
300	50	50±0	100	0±0	0.0	0±0	0.0
350	50	50±0	100	0±0	0.0	0±0	0.0
400	50	50±0	100	0±0	0.0	0±0	0.0

* The mean difference is significant at the 0.05 level compare with control.

The 3rd instar larvae were found to be more radio-tolerant than the 1st and 2nd instars. These results are in agreement with the previous findings [27, 28]. In the present study, one hundred percent larvae mortality was achieved at 150, 300 and 350 Gy for the irradiation of the 1st, 2nd and 3rd instars, respectively. On the other hand, the radiation doses to 1st, 2nd and 3rd instars needed to fully prevent adult emergence are lower than those needed for full larvae mortality. The gamma radiation doses to 1st, 2nd and 3rd instars found to fully prevent adult emergence were 50, 100 and 150 Gy respectively. The trend is similar to that of other species, for example it has been reported that the third larvae instars of Mediterranean fruit fly (*Ceratitis capitata*) are more tolerant to gamma irradiation treatment, and that the dose of 50 Gy caused sterility to adult Mediterranean fruit fly emerging from all irradiated immature stages [29]. Others have also reported that tolerance to radiation increases with age and life-stage development of insects [30]. When irradiation of 1st instars larval stage was considered and the adult emergence was used as a criterion for measuring the efficacy of the radiation treatment, only 50 Gy was required, and when 100% larval mortality was used, the required effective dose was higher (350 Gy).

TABLE 5. GAMMA IRRADIATION OF 3RD LARVAL INSTARS OF *B. ZONATA* IN HOST FRUIT AND THE EFFECTS ON LARVAE MORTALITY, PUPATION AND ADULT EMERGENCE

Dose (Gy)	Mean number of irradiated larvae	Number of dead larvae (mean±SD)	Larval mortality (%)	Number of pupae (mean±SD)	Pupation (%)	Number emerging as adults (mean±SD)	Adult emergence (%)
0 (control)	50	2.5±1.1	5.0	47.5±2.1	95.0	47.5±2.1	100
50	50	16.5±6.3*	33.0	33.5±6.4*	67.0	11±0.2*	32.8
100	50	19.75±4.2*	39.5	30.25±4.2*	60.5	1±0.1*	3.3
150	50	28.75±5*	57.5	21.25±5*	42.5	0±0	0.0
200	50	30±2.9*	60.0	20±2.9*	40.0	0±0	0.0
250	50	32.5±1.7*	65.0	17.5±1.7*	35.0	0±0	0.0
300	50	35±2.6*	70.0	15±2.6*	30.0	0±0	0.0
350	50	50±0	100	0±0	0.0	0±0	0.0
400	50	50±0	100	0±0	0.0	0±0	0.0

* The mean difference is significant at the 0.05 level compare with control.

Adult emergence from irradiated three-day-old and seven-day-old pupae of *B. zonata* was investigated and at a dose of 100 Gy was observed to be 51.2% and 72%, respectively, However, the irradiation dose at 300 Gy and above resulted in no adult emergence (Table 6).

TABLE 6. GAMMA IRRADIATION OF THREE-DAY OLD AND SEVEN-DAY-OLD PUPAE OF *B. ZONATA* AND THE EFFECT ON ADULT EMERGENCE

Dose (Gy)	Mean number of irradiated pupae	3-day old pupae		7-day old pupae	
		Number of emergent adults (mean±SD)	Adult emergence (%)	Number of emergent adults (mean±SD)	Adult emergence (%)
0 (control)	50	48.6±2.2	97.2	46.8±3.4	93.6
50	50	32.6±5.2*	65.2	41±6.5*	82
100	50	25.6±4.7*	51.2	36±2.2*	72
150	50	22.6±1.3*	45.2	31±2.2*	62
200	50	8.8±2.3*	17.6	22±5.7*	44
250	50	5±0.7*	10	12±2.6*	24
300	50	0±0	0.0	0±0	0.0
350	50	0±0	0.0	0±0	0.0
400	50	0±0	0.0	0±0	0.0

* The mean difference is significant at the 0.05 level compare with control.

It was reported that the approved irradiation doses for controlling *B. cucurbita* and *C. dorsalis* were 210 and 250 Gy, respectively [31]. The results obtained by [32] revealed that the adult emergence percentages of *B. zonata* and *B. cucurbita* from one-day-old pupae irradiated at 80 Gy were only 53.75 and 57.00%, respectively. They also added that the difference in the adult emergence between the two insects may be due to the size of pupae as the effects of irradiation appeared unrelated to the size of the pupae and the pupae size of *B. cucurbita* was bigger than *B. zonata* pupae which resulted, comparatively, in a higher number of *B. cucurbita* adult emerging at the same radiation dose than *B.zonata.* It was found that exposure of *B. zonata*

5-day-old pupae to 90 Gy of gamma radiation led to decrease in the percentage of adult emergence which was 22.66% following irradiation [30].

In our experiment, no adults emerged from irradiating the late 3rd larval instars at 150 Gy. Science *B. zonata* occurs in fresh fruits as only eggs and larvae stages irradiation dose of 150 Gy was proposed to conduct large-scale tests to confirm the efficacy of that dose as a phytosanitary treatment. These tests were applied on a large number (17 000 late 3rd larval instar of *B. zonata* in pomegranate fruits). The irradiation dose of 150 Gy resulted in no adult emergence of F1 adultswith confidence level of 81.7% that the true survival of *B. zonata* was less than 0.0001 (Table 7).

TABLE 7. LARGE-SCALE CONFIRMATORY TEST OF IRRADIATING 3RD LARVAL INSTARS OF *B. ZONATA* IN HOST FRUIT TO PREVENT F1 ADULT EMERGENCE

Intended dose (Gy)	Measured dose (Gy)	Number of replicates	Number treated	Number of F1 adults that emerged
0 (unirradiated control)	–	5	500	480
150 Gy	151.2	170	17 000	0

Each one pomegranate (one replicate sample of 3rd instar larvae contained 100 individuals).

The International Plant Protection Convention has approved the generic dose of 150 Gy for fruit flies of the family Tephritidae and its development is discussed by Hallman [33]. Results in support of this generic dose can be found in the literature. For example, similar results were reported by Follett *et. al.* [31]; they found that an irradiation dose of 150 Gy applied to 92,660 melon fly late 3rd larval instars in papaya resulted in no survival of the adult stage, indicating that this dose is sufficient to provide quarantine security for export/import papaya. It was reported that, generally, within Diptera, Coleopteran and Himiptera, radiation dose varies widely among families and ranged from 20 to 200 Gy [34]. Although, [35] reported that 70–90 Gy was most effective irradiation dose range for *B. zonata*, our results discovered higher effective irradiation doses.

Irradiation of pomegranate fruits at 150 Gy did not significantly affect the quality attributes of its juice (total acidy, total ascorbic acid, total carotenoids content, total soluble solids, total sugar, Table 8). Meanwhile, phenolic content and antioxidant activity slightly increased and total anthocyanins decreased by about 4%. Fruit firmness did not change. These results are in close agreement with those of Shahbaz *et. al.* [36] who reported that the higher irradiation doses of 0.4, 1.0 and 2.0 kGy did not affect total sugars and total soluble solids of pomegranate juice. However, the reducing sugar was slightly increased. It was also reported that the total acidity of pomegranate juice remained unaffected at 0.4 kGy but significant decrease was observed at 1 and 2 kGy. Also, Ahmed *et. al.* [37] found no effect of 0.5 kGy irradiation dose on the total acidity, total ascorbic acid content and total sugars, although irradiation did reduce the sugar levels of Olinda Valencia orange samples. Higher irradiation doses (1, 2, 3, kGy) decreased acidity and ascorbic acid content. However, Aligourchl *et. al.* [38] reported that the irradiation dose of 0.5 kGy reduced anthocyanins content of pomegranate juice. El-Samahy *et. al.* [39] reported that gamma irradiation doses of 0.5 and 1.5 kGy had no effect on total sugar content of mangoes, but the content of reducing sugars were slightly increased.

TABLE 8. A PHYTOSANITARY IRRADIATION DOSE OF 150 GY TO POMEGRANATE FRUIT AND THE EFFECTS ON KEY QUALITY ATTRIBUTES OF POMEGRANATE JUICE IMMEDIATELY AFTER TREATMENT

Parameter	Unirradiated (0 Gy)	Irradiated (150 Gy)
Firmness (lb/inch^2)	18.733±0.57	19±0.436
Acidity (%)	0.646±0.007	0.671±0.02
Total Ascorbic acid (mg/100 ml)	14.643±0.450	14.34±0.563
Total anthocyanin (mg/100 ml)	41.613±0.417	41.273±0.683
Total carotenoids (mg/100 ml)	190.437±0.285	190.33±0.884
Total soluble solids (%)	19.903±0.257	19.6±0.223
Total sugars (%)	33.897±0.223	33.71±0.396
Total reduced sugars (%)	21.987±0.207	21.123±0.406
Antioxidant activity (%)	67.707±0.726	67.85±0.315
Polyphenolic content (%)	0.187±0.019	0.171±0.027

Note that there were no significant differences between unirradiated and irradiated samples. The mean differences between unirradiated control data and 150 Gy irradiated sample data were not found to be significant at the 0.05 confidence level.

Microbiological evaluations of foods are of prime importance because of the possible presence of harmful pathogens. The initial $\log_{10}$ counts of total aerobic bacteria, total molds and yeasts, total coliforms and *E. coli* were 3.25, 2.30, 1.90 and 1.11, respectively (Table 9). It is clear that total aerobic bacterial count was found to be higher than total molds and yeasts. Irradiation of pomegranate fruits at 150 Gy slightly reduced these counts, while *E. coli* in irradiated samples were below detectable levels. It is well known that gamma irradiation can be used to reduce numbers of viable microbes. For example, Parakash *et. al.* [40] reported that a radiation dose of 500 Gy could reduce the microbial counts of diced tomatoes without any adverse effects on sensory qualities. Studies of pomegranate juices by Aligourchl *et. al.* report that irradiation at 500 and 2 kGy reduced counts of total yeast and molds. Irradiation was also found to suppress the growth rate of yeasts and molds during storage at 4°C [38].

TABLE 9. EFFECT OF PHYTOSANITARY IRRADIATION DOSE (150 GY) ON THE MICROBIAL COUNT (LOG CFU/ML) OF POMEGRANATE JUICE IMMEDIATELY AFTER IRRADIATION

Microorganism	**Number of microorganisms ($\log_{10}$ CFU/g)**	
	Unirradiated (0 Gy)	Irradiated (150 Gy)
Total bacterial count	**3.25**	**3.20**
Total molds and yeast	**2.30**	**2.23**
Total coliform bacteria	**1.90**	**1.70**
E. coli	**1.11**	**<1.0**

16.3.1.2. Effects of gamma irradiation on Callosobruchus maculatus and the quality attributes of host cowpea seeds

The gamma radiation of one-day old eggs and the effects of egg hatching and adult emergence of the Cowpea Seed Weevil have been published previously [21] and are shown graphically in FIG. 1. the results for the irradiation of three-day old eggs are given in Table 10. These results show that the radiation sensitivity of *C. maculatus* eggs decreased as age increases. Although irradiation of one-day and three-day-old eggs at 450 and 500 Gy failed to prevent egg hatching, these doses completely prevented adult emergence from one-day old and three-day old eggs respectively. This indicates that these radiation doses prevent immature life stages from completing their development.

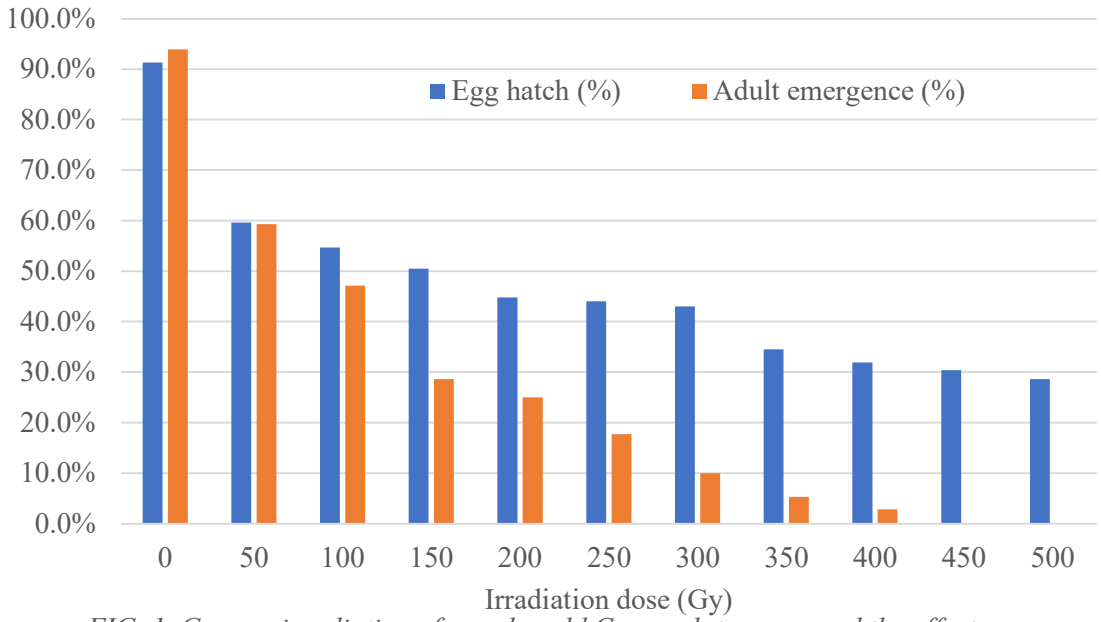

FIG. 1. Gamma irradiation of one-day old C. maculatus eggs and the effects on subsequent egg hatch and adult emergence

TABLE 10. GAMMA IRRADIATION OF THREE-DAY-OLD *C. MACULATUS* EGGS AND THE EFFECT ON EGG HATCH AND ADULT EMERGENCE

Dose (Gy)	Number of eggs irradiated (median, [P^{25}–P^{75}])	Number of hatched eggs (mean±SE)	Egg hatch (%)	Number of emergent adults (mean±SE)	Adult emergence (%)
0 (control)	158 [156–169]	146±9.713	92.4	138±2.887	94.5
50	230 [141–233]	150±7.937	65.0	97±4.726*	64.7
100	240 [233–262]*	146±2.309	60.8	90±7.095*	61.6
150	188 [175–189]	104±5.568*	77.7	54±5.132*	51.9
200	239 [237–250]*	125±3.844*	52.3	55±4.583*	44.0
250	201 [189–204]	69±2.082*	34.3	23±2.309*	33.3
300	209 [200–218]	66±5.033*	31.5	19±1.528*	28.8
350	144 [135–165]	42±1.732*	29.0	10±1.732*	23.0
400	199 [179–210]	54±4.359*	27.0	9±1*	16.7
450	265 [238–274]*	56±6.557*	21.0	3±0.577*	5.4
500	240 [233–256]*	35±3.464*	14.6	0.0	0.0
550	233 [230–242]	20±1.528*	8.6	0.0	0.0

* The mean difference is significant at the 0.05 level compare with control.

The larval stage was more tolerant to gamma radiation than eggs (Table 10). If the measure of treatment efficacy is based on the prevention of adult emergence as a result of irradiating larvae, the effective irradiation dose to control *C. maculatus* in cowpeas would be 850 Gy. When suppression of the F1 generation is used as the measure of efficacy, a 650 Gy dose to the larvae was effective at preventing the emergency of adult progeny in the F1 generation.

The results regarding the effects of gamma radiation on Cowpea Seed Weevil pupae are presented in Table 12, no adults were observed to emerge at or above a radiation doses of 1250 Gy. However, doses of 650 Gy and above to pupae (17-day old life stage) reduced egg hatch by two thirds and none of the F1 progeny emerged as adults.

TABLE 11. GAMMA IRRADIATION OF 13 DAY-OLD *C. MACULATUS* (LARVAL STAGE) AND THE EFFECT ON ADULT EMERGENCE AND ADULT EMERGENCE IN THE F1 GENERATION

Dose (Gy)	Number of emerging adults from irradiated larvae (mean±SE)	Reduction in number of emergent adults (%)	Number of emerging adult progeny in F1 generation (mean±SE)	Reduction in number of emergent F1 adults (%)
0 (control)	170±6.110	0.0	161±4.583	0.0
50	162±2.517*	4.7	109±1.528*	32.3
100	158±1*	7	99±3.215*	38.5
150	149±2.082*	12.4	92±2.082*	42.9
200	148±1.528*	12.9	83±2.082*	48.4
250	146±2*	14.1	67±1.856*	58.4
300	144±1*	15.3	63±2.646*	60.9
350	141±2.082*	17.1	60±1.732*	62.7
400	138±1*	18.8	53±1.528*	67.1
450	133±1.528*	21.8	47±1.528*	70.8
500	126±1.732*	25.9	39±2.082*	75.8
550	117±3.606*	31.2	30±3.606*	81.4
600	111±4.583*	34.7	9±1.155*	94.4
650	73±2.646*	57.1	0±0*	100
700	44±4.509*	74.1	0.0	100
750	20±1.155*	88.2	0.0	100
800	4±0.577*	97.6	0.0	100
850	0±0e	100	0.0	100

* The mean difference is significant at the 0.05 level compare with control.

The effects of irradiating adult weevils in terms of mortality, egg production and adult emergence from the F1 generation are shown in Table 13. The results of these experiments show that a high dose of 1.3 kGy is necessary for 100% mortality. The adult is the most radiation tolerant life stage (Table 13). However, the irradiation of adults to a 650 Gy dose prevented the emergence of adults in the F1 generation.

Other researchers have also studied the effect of different irradiation doses on *C. maculatus* and other closely related insects. A study by Tilton *et al* [41] indicated that the dose 500 Gy would virtually control all stored products pests by preventing their reproduction or adult emergence instead of providing acute mortality which would require much higher doses. In another study it by Sang *et al* reported that the irradiation of *C. maculatus* adults to 2 kGy resulted in approximately 50% acute mortality [42], and that immediate deaths were not observed up to a dose of 1 kGy. Other investigators [43] irradiated adults of *C. maculatus* on seeds of different cowpea varieties (Asontem, Nhyira, Nigerian and Togo varieties) and found that 100%

mortality was achieved at 750 Gy for Asontom variety, while irradiation doses of 1.0 and 1.5 kGy did not achieve 100% mortality for other varieties. Our results revealed that a dose of 650 Gy could be used as a phytosanitary treatment because this results in unsuccessful reproduction or fully prevents development after irradiating larvae, pupae or adults of *C. maculatus*.

Since *C. maculatus* occurs in cowpea in storage in the as eggs, larvae and pupa, the recommended effective irradiation dose to control this insect in cowpea could be 650 Gy. Our results are similar to those of Sutantawong *et. al*. [44] who reported that 100% of pupae mortality of *C. maculatus* and *C. chinensis* were found at 500 and 800 Gy, respectively. In contrast to these results, Dongre et. al. [45] reported that an irradiation dose of 100 Gy would prevent the reproduction of *Callosobruchus maculatus* adults.

TABLE 12. GAMMA IRRADIATION OF 17 DAY-OLD *C. MACULATUS* (PUPAE) AND THE EFFECT OF ON MATURATION AND F1 PROGENY

Dose (Gy)	Number emerged as adult from irradiated pupae (median, interquartile range [P^{25}–P^{75}])	Reduction in number of adults (%)	Number of emergent adults in F1 generation (mean±SE)	Reduction in number of F1 adults (%)
0 (control)	190 [188–201]	0.0	156±2.082	0.0
50	181 [175–181]	4.7	124±3.215*	20.5
100	170 [170–179]	10.5	99±3.055*	34.5
150	171 [168–174]	10	93±5.686*	40.4
200	164 [159–169]	13.7	86±2.082*	44.9
250	159 [155–175]	16.3	67±3.786*	57.1
300	159 [154–169]	16.3	69±2*	55.8
350	155 [153–160]	18.4	67±2*	57.1
400	152 [149–156]	20	65±2.517*	58.3
450	136 [136–142]	28.4	60±2*	61.5
500	119 [117–127]	37.4	44±3.215*	71.8
550	114 [114–123]	40	31±1.528*	80.1
600	79 [75–92]	58.4	11±1*	92.9
650	63 [60–69]	66.8	0.0*	100
700	53 [51–64]	72.1	0.0	0.0
750	49 [47–54]	74.2	0.0	0.0
800	47 [42–52]	75.3	0.0	0.0
850	33 [29–34]	82.6	0.0	0.0
900	25 [21–26]*	86.8	0.0	0.0
950	21[17–22]*	88.9	0.0	0.0
1000	18 [15–21]*	90.5	0.0	0.0
1050	15 [13–17]*	92.1	0.0	0.0
1100	7 [6–11]*	96.3	0.0	0.0
1150	2 [0–4]*	98.9	0.0	0.0
1200	2 [0–4]*	98.9	0.0	0.0
1250	0±0*	100	0.0	0.0

* The mean difference is significant at the 0.05 level compare with control.

In our experiment no emerging adult progeny (F1 generation) was seen at 650 Gy. Therefore, this irradiation dose was proposed as selected as a suitable for further validation as an effective treatment dose against this species. Further validation was undertaken by conducting large-scale tests with higher numbers of Cowpea Seed Weevil to confirm the efficacy of a 650 Gy dose as a minimum treatment dose for a quarantine treatment.

Tests were conducted on 27,745 adult *C. maulatus* in cowpea seeds irradiated at 650 Gy. This irradiation treatment dose resulted in no adult emergence from the F1 generation (no adult survivors) with a confidence level of approximately 93.77% (Table 14). These results indicate that 650 Gy was sufficient to provide quarantine security with high confidence. Results very similar to our were reported by Sutantawong [44] who found that no *C. maculatus* and *C. chinensis* adult emerged at 500 and 800 Gy, respectively. The irradiation dose (650 Gy) in our experiment is higher than the treatment doses proposed by other investigators [46, 47, 48]. The different results may be due to different environmental and physical factors that affect irradiation efficacy such as temperature and humidity, as reported by [49]. Other investigator found that the percent of mortality of *C. maculatus* in cowpea as a result of irradiation varied according to the cowpea cultivar [43].

TABLE 13. GAMMA IRRADIATION OF ADULT *C. MACULATUS* AND THE EFFECT ON MORTALITY, EGG PRODUCTION AND ADULT EMERGENCE FROM THE F1 GENERATION

Dose (Gy)	Mean Number of adults irradiated	Mortality (median and interquartile range, [P^{25}–P^{75}])	Adult mortality (%)	Number of eggs (median and interquartile range, [P^{25}–P^{75}])	Number of emerging adults in F1 generation (mean±SE)
0 (control)	100	0.0	0.0	173 [160–174]	137±3.215
50	100	0.0	0.0	82 [79–85]	48±2.517*
100	100	0.0	0.0	52 [52–58]	28±1.732*
150	100	0.0	0.0	29 [23–32]	13±1*
200	100	5 [2–5]	5	24 [24–27]	11±1.528*
250	100	6 [4–8]	6	13 [12–17]	5±0.577*
300	100	7 [5–9]	7	11 [8–11]	3±1*
350	100	9 [7–11]	9	7 [6–8]	2±0.577*
400	100	15 [11–16]	15	6 [4–8] *	3±0.577*
450	100	17 [14–17]	17	6 [3–6] *	3±1*
500	100	21 [17–22]	21	5 [2–5] *	3±1.155*
550	100	23 [20–26]	23	2 [2–5] *	2±0.577*
600	100	26 [25–33]	26	2 [1–3] *	1±0.577*
650	100	28 [27–29]	28	0.0	0.0
700	100	32 [25–33]	32	0.0	0.0
750	100	32 [30–37]	32	0.0	0.0
800	100	33 [33–36]	33	0.0	0.0
850	100	39 [32–40]	39	0.0	0.0
900	100	56 [49–60]	56	0.0	0.0
950	100	68 [63–79]	68	0.0	0.0
1000	100	71 [69–85]	71	0.0	0.0
1050	100	75 [73–77] *	75	0.0	0.0
1100	100	75 [74–76] *	75	0.0	0.0
1150	100	79 [77–85] *	79	0.0	0.0
1200	100	79 [77–81] *	79	0.0	0.0
1250	100	89 [85–90] *	89	0.0	0.0
1300	100	100 [100–100] *	100	0.0	0.0
1350	100	100 [100–100]*	100	0.0	0.0

* The mean difference is significant at the 0.05 level compare with control.

TABLE 14. LARGE-SCALE CONFIRMATORY TESTS OF IRRADIATING THE ADULT LIFE STAGE OF *CALLOSEBRUCHUS MACULATUS* (IN COWPEA HOST) TO PREVENT F1 ADULT EMERGENCE

Proposed dose (Gy)	Measured dose (Gy)	No. replicates	No. treated	No. F1 adults
0 (control)	Not irradiated	5	950	13.015
650 Gy	653.0	39	27 754	0

The effect of phytosanitary irradiation dose (650 Gy) on the major chemical composition of cowpea seeds (moisture, protein, lipid, ash and carbohydrate content) of irradiated cowpea seeds at zero time and after three months of storage were determined. The results indicate that there was no significant effect of irradiation at this dose level either immediately after irradiation (zero time) or after three months of storage at ambient temperature and humidity (Table 15). Many other researchers reported that the irradiation dose used for insect control in stored products (grains or legumes) has no significant effect on the major chemical constituents, of the host commodity at the irradiation dose required for controlling insects that infest stored products, but a few negative effects on commodity were also reported [50, 51, 52].

TABLE 15. EFFECT OF PHYTOSANITARY IRRADIATION DOSE (650 GY) ON THE MAJOR CHEMICAL CONSTITUENTS OF COWPEA

Constituent	Content immediately after irradiation (%)		Content after 3 months storage (%)	
	Unirradiated*	Irradiated*	Unirradiated*	Irradiated*
Moisture	7.9±0.096	7.68±0.19	7.17±0.4	7.13±0.15
Protein	15.74±0.57	15.9±1.01	15.73±0.35	15.13±0.30
Lipids	1.22±0.06	1.26±0.12	1.19±0.02	1.22±0.03
Ash	2.75±0.3	3±0.22	2.62±0.16	2.88±0.23
Carbohydrate	72.43±0.9	72.16±0.6	73.28±0.5	73.64±0.37

* The values are mean ± SD.

The effect of the 650 Gy phytosanitary irradiation dose on some properties of cowpea is very important for demonstrating the quality attribute of cowpea seeds. The irradiation dose (650 Gy) decreased cowpea seeds germination by only 4% in comparison with non-irradiated control (Table 16). Many researchers reported that seeds for planting should not be irradiated because irradiation reduces the germination ability of seed stocks and they will not grow normally [50, 53]. Also, irradiation of cowpea at 650 Gy had no significant effect on its hardness, while the cooking time of cowpea reduced slightly.

TABLE 16. EFFECT OF A PHYTOSANITARY IRRADIATION DOSE OF 650 GY ON SOME PROPERTIES OF COWPEA SEEDS

Parameter	Immediately after irradiation		After 3 months storage	
	Unirradiated (0 Gy)	Irradiated (650 Gy)	Unirradiated (0 Gy)	Irradiated (650 Gy)
Germination (%)	60	54.7	51.3	48
Hardness (N)*	60.2±2.1	60.4±6.7	60.2±3.6	60.4±1.9
Cooking time (minutes)*	48±4	45±5	46±4	43±3.6

* The values are mean ± SD.

Applying the phytosanitary irradiation dose of 650 Gy did not change colour of cowpea according to colour analysis using the Hunter test (Table 17). From our results, it is clear that irradiation at a phytosanitary dose level of 650 Gy did not significantly affect the major chemical constituents or properties of cowpea. Many researchers have reported that the relatively low irradiation doses used to control insects do not significantly change the quality of the food material or stored products [43, 51, 52]. Also, [54] found that irradiation at dose levels of 0.25, 0.5, 0.75, 0.10 and 1.5 kGy had no significant ($P>0.05$) effect on the sensory quality attributes like flavour, taste, texture, softness and colour of cowpea seeds. The results obtained by [52] indicate that irradiation at 250, 500 and 1000 Gy for chickpea, kidney beans and green lentils do not have significant effect on the level of riboflavin and thiamine. The sensory evaluation testers were not able to differentiate between the irradiated and the unirradiated control samples.

TABLE 17. EFFECT OF A PHYTOSANITARY IRRADIATION DOSE OF 650 GY ON THE COLOUR OF COWPEA SEEDS IMMEDIATELY AFTER IRRADIATION

Treatment	Red	Green	Blue	Hue	Sat	Lum
Control	248	242	182	37	194	202
Irradiated	248	241	181	37	182	201

The microbial load of cowpea (total aerobic bacterial counts, total molds and yeasts, total coliform bacteria and *E. coli* counts) were enumerated for unirradiated control samples and for samples irradiated to a dose of 650 Gy. Microbial counts were slightly reduced at the phytosanitary dose level 650 Gy (Table 18). *E. coli* was mostly eliminated at this irradiation dose besides providing quarantine security against phytosanitary treatment; irradiation can provide additional benefits to some agricultural commodities. Irradiation of agricultural products at less than 1 kGy has been shown to delay the ripening of some fruits, increase shelf-lite by reducing spoilage bacterial and controlling molds, and improving safety through the inactivation of radiosensitive pathogens [12, 55, 56]. In our studies, irradiation at 650 Gy slightly reduced the initial microbial level not only immediately after irradiation, but also during storage at ambient temperature for 3 months. This irradiation dose could reduce the count of *E. coli* contaminating cowpea seeds to below detectable level according to the methodology used.

TABLE 18. EFFECT OF PHYTOSANITARY IRRADIATION DOSE OF 650 GY ON THE MICROBIAL COUNTS OF COWPEA SEEDS

Microorganisms	**Number of microorganisms (log_{10} cfu/g)**			
	Immediately after irradiation		After 3 months storage	
	Unirradiated	Irradiated	Unirradiated	Irradiated
Total bacterial	**4.86**	**4.76**	**4.95**	**4.84**
Total molds and yeast	**4.18**	**3.07**	**3.40**	**3.32**
Total coliform bacteria	**2.41**	**2.30**	**2.34**	**2.27**
E. coli	**1.23**	**<1.00**	**<1.00**	**<1.00**

16.3.1.3. The gamma irradiation of Rice Moth (Corcyra cephalonica) and the effect on moth development and also rice as the host commodity

Our results documented the effect of gamma radiation on all developmental stages of the Rice Moth, *Corcyra cephalonica,* (1–2-day-old adults, 3-day-old eggs, 2nd instar larvae, 4th instar larvae and 5-day-old pupae):

(a) When adult Rice Moths (1 to 2-day-old) were irradiated, a dose level of at least 350 Gy was required to fully prevent the hatching of eggs laid by the F1 generation (Table 19);
(b) When 3-day-old eggs were irradiated, the dose level of 250 Gy prevented adult emergence but at dose level 200 Gy the hatchability percentage of F1 generation was zero (Table 20);
(c) When 2nd instar larvae were irradiated, the dose level of 200 Gy prevented the adult emergence but at the lower dose level of 150 Gy, the F1 egg hatch percentage was zero (Table 21);
(d) When 4th instar larvae were irradiated, the dose level of 550 Gy prevented adult emergence. However, 4th instar larvae irradiated to a dose level of 250 Gy or more, resulted in no egg hatching in the F1 generation. The 4th larval instar was more tolerant to radiation treatment than 2nd larval instar (Table 22);
(e) When 5-day-old pupae were irradiated, the dose level of 650 Gy prevented the adult emergence but at the dose level of 350 Gy, the hatchability percentage was zero at F1 generation (Table 23).

TABLE 19. GAMMA IRRADIATION OF THE ADULT LIFE STAGE OF *CORCYRA CEPHALONICA* AND THE EFFECT ON ADULT MORTALITY AND F1 GENERATION

Dose (Gy)	Mean number of irradiated adults	Number of dead adults (mean±SD)	Adult mortality (%)	**F1 generation**		
				Fecundity	Number of eggs that hatched (mean±SD)	Egg hatch (%)
0 (control)	25	1.4±0.5	5.6	**128.6±7.8**	**116.2±10.2**	**90.5**
50	25	17.2±0.8*	68.8	**111±9.9***	**57.8±3.7***	**52**
100	25	19.8±1.4*	79.2	**69.4±6.6***	**30.2±3.8***	**43.5**
150	25	20.2±1.3*	80.8	**50.6±8.1***	**19.2±1.8***	**37.9**
200	25	21.8±1.3*	87.2	**38.6±4.6***	**13.8±1.6***	**35.8**
250	25	22±2.2*	88	**24.8±5***	**7.2±1.5***	**29**
300	25	22.6±1.1*	90.4	**18.4±2***	**1±0.7***	**5.4**
350	25	23.8±1.1*	95.2	**8.4±1.1***	**0±0**	**0.0**
400	25	25±0.0*	100	**0±0**	**0±0**	**0.0**

* The mean difference is significant at the 0.05 level compare with control.

TABLE 20. GAMMA IRRADIATION OF THREE-DAY-OLD EGGS OF *CORCYRA CEPHALONICA* AND THE EFFECT ON EGG HATCH, EMERGENT ADULTS AND THE F1 GENERATION

Dose (Gy)	Mean number of eggs irradiated	Number of hatched eggs (mean±SD)	Egg hatch (%)	Number of adults that emerged (mean±SD)	Adult emergence (%)	**F1 generation**		
						Fecundity	Number of F1 egg hatch (mean±SD)	F1 egg hatch (%)
0 (control)	50	46.2±5.5	92.4	40.9±5.4	88.5	**92±7**	**84.2±12.5**	**91.5**
50	50	35.6±6.8*	71.2	29.4±5.4*	82.6	**73±11.3***	**52.6±17.4***	**72.1**
100	50	33.6±5.9*	67.2	19.4±2.3*	57.7	**37.2±11.9***	**23±7.5***	**61.8**
150	50	29.2±6.5*	58.4	6.0±1.3*	20.5	**10.2±3.2***	**3.2±1.2***	**31.4**
200	50	27.6±8.8*	55.2	2.6±1.4*	9.4	**2.6±1.3***	**0±0**	**0.0**
250	50	26.6±6.6*	53.2	0±0	0.0	**0±0**	**0±0**	**0.0**
300	50	24.2±4.7*	48.4	0±0	0.0	**0±0**	**0±0**	**0.0**
350	50	20.4±7.1*	40.8	0±0	0.0	**0±0**	**0±0**	**0.0**
400	50	13.9±9.1*	27.8	0±0	0.0	**0±0**	**0±0**	**0.0**
450	50	8.4±3.4*	16.8	0±0	0.0	**0±0**	**0±0**	**0.0**
500	50	0±0*	0.0	0±0	0.0	**0±0**	**0±0**	**0.0**

* The mean difference is significant at the 0.05 level compare with control.

TABLE 21. GAMMA IRRADIATION OF 2ND LARVAL INSTARS OF *CORCYRA CEPHALONICA* AND THE EFFECT ON LARVAL MORTALITY, ADULT EMERGENCE AND THE F1 GENERATION

Dose (Gy)	Mean number of irradiated larvae	Number of dead larvae (mean±SD)	Larval mortality (%)	Number of adults that emerged (mean±SD)	Adult emergence (%)	**F1 generation**		
						Fecundity	Number of F1 eggs that hatched (mean±SD)	F1 egg hatch (%)
0 (control)	25	1.0±0.1	4.0	20.4±1.1	85	**72.6±13**	**65.2±12.5**	**89.8**
50	25	9.4±1.5*	37.6	10.6±1.2*	67.9	**60.4±9.6***	**35.8±5.8***	**59.3**
100	25	14.4±2.4*	57.6	6.8±2.4*	64.2	**32.2±7.0***	**13±4.1***	**40.4**
150	25	20.2±1.3*	80.8	2.0±1.2*	41.7	**11.4±1.7***	**0±0**	**0.0**
200	25	21.2±0.8*	84.8	0±0	0.0	**0±0**	**0±0**	**0.0**
250	25	21.8±0.8*	87.2	0±0	0.0	**0±0**	**0±0**	**0.0**
300	25	24.0±0.1*	96.0	0±0	0.0	**0±0**	**0±0**	**0.0**
350	25	25±0.0*	100	0±0	0.0	**0±0**	**0±0**	**0.0**
400	25	25±0.0*	100	0±0	0.0	**0±0**	**0±0**	**0.0**

* The mean difference is significant at the 0.05 level compare with control.

TABLE 22. GAMMA IRRADIATION OF 4TH LARVAL INSTARS OF *CORCYRA CEPHALONICA* AND THE EFFECT ON LARVAL MORTALITY, ADULT EMERGENCE AND F1 GENERATION

Dose (Gy)	Mean number of irradiated larvae	Number of dead larvae (mean±SD)	Larval mortality (%)	Number of adults that emerged (mean±SD)	Adult emergence (%)	**F1 generation**		
						Fecundity	Number of F1 eggs that hatched (mean±SD)	F1 egg hatch (%)
0 (control)	25	0.6±0.5	2.4	23.6±1.1	96.7	**92.4±5**	**82.2±9.4**	**89**
50	25	2.4±1.1	9.6	22±1.6*	97.3	**83.2±11.4**	**51.6±8.6***	**62**
100	25	7.0±0.7*	28	15.4±1.5*	85.6	**68.4±11.9***	**24±3.2***	**35.1**
150	25	9.6±1.5*	38.4	10.2±0.8*	66.2	**49±8.7***	**15±3.2***	**30.6**
200	25	10.8±1.3*	43.2	8.8±1.6*	62	**22.4±1.8***	**3.4±0.5***	**15.2**
250	25	11.2±2.3*	44.8	8.0±1.3*	60.6	**5.8±1.6***	**0±0**	**0.0**
300	25	13.8±2.7*	55.2	6.6±1.5*	59	**0±0**	**0±0**	**0.0**
350	25	14.85±1.5*	59.4	5.6±1.1*	55.2	**0±0**	**0±0**	**0.0**
400	25	21.2±1.8*	84.8	1.6±1.3*	42.1	**0±0**	**0±0**	**0.0**
450	25	21.6±1.1*	86.4	1.2±0.4*	35.3	**0±0**	**0±0**	**0.0**
500	25	22.4±1.5*	89.6	0.6±0.2*	23.1	**0±0**	**0±0**	**0.0**
550	25	25.0±0.0*	100	0±0	0.0	**0±0**	**0±0**	**0.0**
600	25	25.0±0.0*	100	0±0	0.0	**0±0**	**0±0**	**0.0**
650	25	25.0±0.0*	100	0±0	0.0	**0±0**	**0±0**	**0.0**

* The mean difference is significant at the 0.05 level compare with control.

According to our result, the most tolerant stage to gamma radiation was the Rice Moth pupae (Table 23). A dose level of at least 350 Gy was found to prevent eggs laid by the F1 generation from hatching. Therefore, the irradiation dose of 350 Gy was proposed for large scale tests to confirm the efficacy of this applying dose for controlling *C. cephalonica* in rice.

Others have used the prevention of F1 generation egg hatching as the measure of efficacy for a phytosanitary treatment when late pupae are irradiated [57]. Also, [58] reported that irradiation of *C. cephalonica* eggs at 100 Gy resulted in 100% mortality of hatched larvae in 14 days and 100% mortality of 1–7-day-old pupae. The female Rice Moth, *C.cephalonica*, has been found to be sterilized with as little as 100 Gy [59]. Also, *C. cephalonica* weevil in stored rice irradiated at 200 Gy was found to result in 99% mortality three weeks [60]. Other investigator reported that the dose of 500 Gy would control virtually all stored products pests by preventing completion of reproduction or adult emergence instead of providing adult mortality [41]. It is also reported that the irradiation dose of 205 Gy prevented *C. cephalonica* F1 egg hatch [61]. Also, [62] found that the irradiation dose of 500 Gy prevented *C. cephalonica* F1 egg hatch.

TABLE 23. GAMMA IRRADIATION OF THE PUPAL STAGE OF *CORCYRA CEPHALONICA* AND THE EFFECT ON ADULT EMERGENCE AND F1 GENERATION

Dose (Gy)	Mean number of irradiated pupae	Number of emergent adults (mean±SD)	Adult emergence (%)	**F1 generation**		
				Fecundity	Number of eggs that hatched (mean±SD)	Egg hatch (%)
0 (control)	25	23.4±0.9	93.6	**107.8±9.5**	**99.8±9.8**	**92.6**
50	25	20±0.7*	80	**77.2±14.2**	**49.6±4.4***	**64.2**
100	25	18.2±1.9*	72.8	**54.8±10.6**	**30.2±6.8***	**55.1**
150	25	15.6±1.8*	62.4	**50.4±9.4***	**20.8±3.9***	**41.3**
200	25	15.4±0.9*	61.6	**38.8±8.8***	**13.8±2.8***	**35.6**
250	25	11.6±1.1*	46.4	**32±7.4***	**10.8±2.3***	**33.75**
300	25	11±1.6*	44	**23.2±5.8***	**1.8±0.8***	**7.8**
350	25	9.4±1.7*	37.6	**20.6±5.1***	**0±0**	**0.0**
400	25	8.2±0.8*	32.8	**11.2±3.1***	**0±0**	**0.0**
450	25	6.2±1.8*	24.8	**0±0**	**0±0**	**0.0**
500	25	3.2±1.3*	12.8	**0±0**	**0±0**	**0.0**
550	25	1.8±0.8*	7.2	**0±0**	**0±0**	**0.0**
600	25	0.8±0.4*	3.2	**0±0**	**0±0**	**0.0**
650	25	0±0	0.0	**0±0**	**0±0**	**0.0**
700	25	0±0	0.0	**0±0**	**0±0**	**0.0**

* The mean difference is significant at the 0.05 level compare with control.

It has been reported that the control of some lepidoptera and most mites require a minimum radiation dose of about 300 Gy for phytosanitary control [63]. Abdalla [64] found that Adult emergence was greatly affected when full gown pupae of *C. cephalonica* where irradiated to dose levels 350 and 700 Gy. Ignatowicz [47] reported that no adults develop after the irradiation of immature *C. cephalonica* (eggs, larvae and pupae) to doses of 80 and 100 Gy. The tested three doses (150, 300 and 450 Gy) of gamma radiation against full-grown pupae of *C. cephalonica* indicated that the dose 450 Gy was the most effective dose, where it completely prevented the laid eggs from hatching. The percent of both pupation and adult emergence

decreased as irradiation dose increased [65]. According to the previous studies, irradiation dose required to control *C. cephalonica* ranged from 100 to 650 Gy.

Our results revealed that the irradiation dose of 350 Gy for the prevention of F1 laid eggs from hatching was sufficient to prevent *C. cephalonica* from completing its development. This irradiation dose is within the reported range of previous studies. Therefore, a minimum radiation dose of 350 Gy was used to perform a further large-scale test to confirm its efficiency as a phytosanitary treatment.

The large-scale confirmatory test data (Table 24) indicated that 350 Gy irradiation dose applied to 16500 pupae resulted in no F1 laid hatching (prevent F1 egg hatch) with a confidence level of 80.797%. Thus, when measuring efficacy of irradiation, based on the prevention of F1 generation egg hatch, the dose 350 Gy will be sufficient to provide quarantine security.

TABLE 24. LARGE SCALE CONFIRMATORY TESTS OF A PHYTOSANITARY IRRADIATION DOSE OF 350 GY TO PUPAE OF *CORCYRA CEPHALONICA* TO PREVENT F1 EGG HATCH

Dose/Gy		Number of replicates	Number of irradiated pupae	Number of eggs	Number of eggs laid by the F1 generation that hatched
Dose targeted	Average measured dose				
0 (control)	Not irradiated	5	500	1965	1820
350	340–361	30	16 500	49	0

Sehgal *et al* [61] reported that an irradiation dose of 205 Gy applied to late pupae of *C. cephalonica* prevented F1 egg hatch. Also Etman *et al* [62] reported that an irradiation dose of 500 Gy applied to male pupae of *C. cephalonica* resulted in no egg hatching in the F1 generation. Farghaly *et al* [65] found that a gamma radiation dose of 450 Gy applied to full grown male and female pupae of *C. cephalonica* completely prevented the laid eggs from hatching. Hammad *et al*, [21] found that no adults emerged from F1 deposited eggs when larvae or pupae of *Callosobruchus maculatus* were exposed to 650 Gy.

Irradiation is increasingly being used as a phytosanitary measure; it is a safe alternative to harmful chemical insecticides. Irradiation can be applied to stored products and fresh horticultural products to control quarantine or regulated pests [63, 66, 67, 68]. In addition to ensuring that the treatment dose is effective against the pests, it is also important to consider the effect of phytosanitary irradiation dose levels on the sensory and quality attributes of the food products. Therefore, we investigate the effects of irradiation (350 Gy) on hulled rice quality indicators, such as colour, cooking time and some microbiological aspects.

Our results (Table 25) revealed no difference for Hunter test colour parameters between non-irradiated (0.0Gy) and irradiated (350 Gy) hulled rice samples. All measured colour parameters of the irradiated samples were almost the same to those of the control. This is similar to our results for cowpeas (Table 17) where the colour analysis of irradiated and unirradiated samples were also similar. Other researchers have also examined the irradiation of cowpea seeds with gamma irradiation doses of 250, 500, 750 and 1000 Gy was not significantly ($P \geq 0.5$) affect the physical parameters studied [54]. They also found that there was no significant ($p \geq 0.05$) effect of the irradiation on the sensory attributes like flavour, taste, softness and colour of the treated samples. Also, [21] found that all the physical and chemical characteristics of cowpea seeds were not-significantly ($p \leq 0.05$) affected by the irradiation dose of 650 Gy. In

general, the scientific literature indicates that radiation dose levels used for disinfestation do not significantly affect the host commodity. For example, the effects on the major constituents, nutritive value and organoleptic properties of the food products at these relatively low doses are generally insignificantly for most agricultural commodities [51, 52, 69, 70].

TABLE 25. THE EFFECT OF A PHYTOSANITARY TREATMENT DOSE OF 350 GY ON THE COLOUR OF HULLED RICE

Treatment	Red	Green	Blue	Hue	Sat	Lum
Control	254±0.0	254±0.0	234±2.6	39±0.0	210±0.0	232.7±3.2
Irradiated	254±0.0	253.7±0.6	234±2.6	39±0.0	210±0.0	230.7±1.5

* The mean difference is significant at the 0.05 level compare with control.

Our results for the effects of irradiation on rice cooking parameters (Table 26) indicated that the irradiation dose of 350 Gy (effective against the Rice Moth) did not affect the amount of absorbed water; the percentage of absorbed water amount was the same as in the control samples. However, a slight increase in the volume (5.9%) and weight (0.7%) of irradiated cooked rice were recorded. This indicates that a 350 Gy irradiation dose, slightly improved rice quality. Other researchers have suggested that gamma irradiation up to 1 kGy can be used to improve rice eating or cooking quality [71].

TABLE 26. THE EFFECT OF A PHYTOSANITARY IRRADIATION DOSE OF 350 GY ON THE COOKING PARAMETERS OF HULLED RICE

Samples	Cooking time (min)	Amount of absorbed water (%)	Increase in volume (%)	Increasing in weight (g)
Control (0 Gy)	15	300	320	241.80
Irradiated (350 Gy)	15	300	340	243.58

Results od our microbiological analyses of hulled riced samples are given in Table 27. These show that the initial microbial count of rice was high, indicating contamination with microbes. This high level of contamination could be attributed to high natural microflora of the rice as well as general conditions during their harvesting, drying, and handling. An irradiation dose of 350 Gy very slightly reduced total bacteria counts and counts of yeasts and molds. The count of coliform bacteria was 93 and 75 CFU/g in control and irradiated samples, respectively indicating a slight reduction, but levels of *E. coli* were less than the detectable level in both control and irradiated samples.

TABLE 27. THE EFFECT OF A PHYTOSANITARY IRRADIATION DOSE OF 350 GY ON THE MICROBIAL QUALITY OF HULLED RICE

Microorganisms	**Number of microorganisms (cfu/g)**	
	Control (0.0 Gy)	Irradiated (350 Gy)
Total bacterial counts	**$2.9x10^6$**	**$2.8x10^6$**
Total molds and yeast	**$2.55x10^4$**	**$2.47x10^4$**
Total coliform bacteria	**93**	**75**
E. coli	**<10**	**<10**

Many investigators have reported that irradiation doses used for insect disinfestation of food slightly reduce the microbial counts [40, 72]. For example, it has been reported that an irradiation dose of 1 kGy decreased the total bacterial count of dates from $1.4x10^3$ CFU/g in control samples to 3.0 x 10^2 CFU/g in irradiated samples [73]. Others [74] have found that the initial total bacterial counts of the Fard date to be high (4.95 log_{10}CFU/g) and electron beam irradiation at 0.5, 1.0, 2.0 kGy to slightly reduced levels to 4.8, 4.7 and 4.65 log_{10}CFU/g, respectively. Several studies have indicated the effectiveness of higher irradiation doses (>2 kGy) for reducing microbial counts to a much greater extent and for the elimination of foodborne pathogens.

16.3.1.4. Effect of gamma irradiation on Ephestia cautella development stages

A summary of the research results for the gamma irradiation of Fig Moth eggs (*Ephestia cautella*) is presented in Table 28. These results show that an irradiation dose of 150 Gy prevented adult emergence from irradiated eggs (one-day old), lower irradiation dose (100 Gy) prevented egg hatching of eggs laid by the F1 generation. Results for the irradiation of three-day-old eggs (Table 29) indicate that they were more radiotolerant than one-day-old eggs, since the higher irradiation dose of 250 Gy prevented adult emergence, while 100 Gy irradiation dose also prevented egg hatching of the F1 generation laid eggs.

Results for the irradiation of Fig Moth larvae are given in Table 30 for the 2nd instar larval form and in Table 31 for the 4th instar life stage. These data indicate that the 4th instar larval form was more radiation tolerant than the 2nd larval instar. An irradiation dose of 250 Gy almost prevented adult emergence from irradiated 4th larval instars but the F1 adult forms that did emerge did not produce viable eggs. Eggs produced by the F1 generation did not hatch after a gamma radiation dose of 250 Gy was delivered to the 4th instar.

Results for the irradiation of *E. cautella* pupae are given in Table 32 and revealed that the most radiotolerant stage of *E. cautella* was the 3-day-old pupae. An irradiation dose of 400 Gy and almost completely prevented adult emergence and prevented eggs laid by the F1 generation from hatching. If the prevention of egg hatching from F1 generation is used as a criterion of measuring the efficacy of irradiation, 400 Gy is required. Irradiation of adult stage at 300 Gy (Table 33) resulted in 80% adult mortality, while only 150 Gy prevented F1 generation hatch (prevent F1 egg hatch). Similar results have been found by [75] who reported that a dose of 200 and 250 Gy applied to younger and late larval stage of *E. Kuenhnella* completely prevented adult emergence, respectively, indicating that the younger larval instars were more sensitive to irradiation than older ones. Also [76] reported that gamma irradiation doses required to inhibit development of eggs of *Ploidia inerpunctella* and *E. cautella* were 450 and 300 Gy, respectively. Early [77] investigated the effect as of six gamma irradiation doses in the range of 50 to 1000 Gy against all life stages of *E. cautella* and found that the development of adults from irradiated eggs and larvae was prevented at 200 and 300 Gy, respectively

TABLE 28. GAMMA IRRADIATION OF ONE-DAY-OLD EGGS OF *EPHESTIA CAUTELLA* AND THE EFFECT ON EGG HATCH, ADULT EMERGENCE AND F1 GENERATION

Dose (Gy)	Mean number of eggs irradiated	Number of hatched eggs (mean±SD)	Egg hatch (%)	No. emerging adult (mean±SD)	Adult emergence (%)	**F1 generation**		
						Fecundity	Number of hatched eggs (mean±SD)	Egg hatch (%)
0 (control)	30	27.8±1.3	92.7	25±1.9	83.3	**170.8±21.4**	**148±15.4**	**87.1**
50	30	17.5±2.4*	58.3	11.6±2.7*	38.6	**93±18.5***	**46.8±7.2***	**50.3**
75	30	10.8±2.2*	36.0	6±1.6*	20	**39±7.9***	**10.4±3.2***	**26.7**
100	30	5.2±2.9*	17.3	2±0.7*	6.7	**14±4.9***	**0±0**	**0.0**
150	30	1±0.7*	3.3	0±0	0.0	**0±0**	**0±0**	**0.0**
200	30	0.0±0.0*	0.0	0±0	0.0	**0±0**	**0±0**	**0.0**

* The mean difference is significant at the 0.05 level compare with control.

TABLE 29. GAMMA IRRADIATION OF THREE-DAY-OLD EGGS OF *EPHESTIA CAUTELLA* AND THE EFFECT ON EGG HATCH, ADULT EMERGENCE AND F1 GENERATION

Dose (Gy)	Mean number of eggs irradiated	Number of hatched eggs (mean±SD)	Egg hatch (%)	Number of adults that emerged (mean±SD)	Adult emergence (%)	**F1 generation**		
						Fecundity	Number of hatched eggs (mean±SD)	Egg hatch (%)
0 (control)	30	27±1.8	90	25±1.6	83.3	**171±15**	**149.8±9.4**	**87.6**
50	30	21±1.5*	70	16±3.2*	53.3	**80±12.8***	**50.8±11***	**63.5**
75	30	16±2.3*	53.3	11.2±2.4*	37.3	**39.4±6.8***	**6.2±1.9***	**15.7**
100	30	9.2±0.8*	30.7	4.2±1.9*	14	**28.8±5.4***	**0±0**	**0.0**
150	30	4.8±1.6*	16	0.8±0.8*	2.7	**8.6±8.8***	**0±0**	**0.0**
200	30	3±1.5*	10	0.4±0.5*	1.3	**0±0**	**0±0**	**0.0**
250	30	0.8±0.8*	2.7	0±0	0.0	**0±0**	**0±0**	**0.0**
300	30	0.4±0.5*	1.3	0±0	0.0	**0±0**	**0±0**	**0.0**
350	30	0.2±0.4*	0.7	0±0	0.0	**0±0**	**0±0**	**0.0**
400	30	0±0*	0.0	0±0	0.0	**0±0**	**0±0**	**0.0**

* The mean difference is significant at the 0.05 level compare with control.

TABLE 30. GAMMA IRRADIATION OF 2ND LARVAL INSTARS OF *EPHESTIA CAUTELLA*

Dose (Gy)	Mean number of irradiated larvae	Number of dead larvae (mean±SD)	Larval mortality (%)	Number of emerging adults (mean±SD)	Adult emergence (%)	**F1 Generation**		
						Fecundity	Number of hatched eggs (mean±SD)	Egg hatch (%)
0 (control)	20	0.0±0.0	0	18.6±1.14	93	**145.8±21.4**	**136.6±19.3**	**93.7**
50	20	1.2±0.4	6	12.6±2.1*	63	**80.8±11.9***	**40.6±6.6***	**50.2**
75	20	4.2±1.3*	21	9±2.24*	45	**52.4±7.7***	**22.4±3.8***	**42.7**
100	20	5.8±1.5*	29	5.2±1.3*	26	**18.8±3***	**5.6±1.1***	**29.8**
150	20	10.6±1.1*	53	1.4±1.14*	7	**7±2.5***	**1.4±1.7***	**20**
200	20	15.4±1.1*	77	0±0	0.0	**0±0**	**0±0**	**0.0**
250	20	18.4±1.34*	92	0±0	0.0	**0±0**	**0±0**	**0.0**
300	20	18.8±1.3*	94	0±0	0.0	**0±0**	**0±0**	**0.0**
350	20	19.2±1.1*	96	0±0	0.0	**0±0**	**0±0**	**0.0**

* The mean difference is significant at the 0.05 level compare with control.

TABLE 31. GAMMA IRRADIATION ON 4TH LARVAL INSTARS OF *EPHESTIA CAUTELLA*

Dose (Gy)	Mean number of irradiated larvae	Number of dead larvae (mean±SD)	Larval mortality (%)	Number of emerging adults (mean±SD)	Adult emergence (%)	**F1 Generation**		
						Fecundity	Number of hatched eggs (mean±SD)	Egg hatch (%)
0 (control)	20	0.0±0.0	0.0	18±1.6	90	**174.6±16.5**	**162.6±17.7**	**93.14**
50	20	0.2±0.4	1.0	15.2±0.8*	76	**105.6±9.3***	**54±13.3***	**51.14**
75	20	2±1.6*	10	13±1.9*	65	**74.4±8.2***	**31.6±5***	**42.5**
100	20	4.8±0.8*	24	10.8±1.3*	54	**60.6±9.9***	**20.4±2.7***	**33.7**
150	20	9.4±1.14*	47	6.4±1.1*	32	**35.8±6.3***	**10.6±2.7***	**29.6**
200	20	14±1.6*	70	1.2±0.8*	6	**15.4±9.2***	**3.2±0.8***	**20.8**
250	20	16±1.2*	80	0.4±0.5*	2	**1.8±2.5***	**0±0**	**0.0**
300	20	17.6±1.14*	88	0±0	0.0	**0±0**	**0±0**	**0.0**
350	20	19±1 *	95	0±0	0.0	**0±0**	**0±0**	**0.0**

* The mean difference is significant at the 0.05 level compare with control.

TABLE 32. GAMMA IRRADIATION OF THE PUPAE STAGE OF *EPHESTIA CAUTELLA* AND THE EFFECT ON ADULT EMERGENCE AND THE F1 GENERATION

Dose (Gy)	Mean number of irradiated pupae	Number of emergent adults (mean±SD)	Adult emergence (%)	**F1 generation**		
				Fecundity	Number of eggs hatched (mean±SD)	Hatchability (%)
0 (control)	20	18.6±1.14	93	**124.8±17.7**	**104.2±10.8**	**83.5**
50	20	15.4±0.9*	77	**95±11.8***	**72.2±4.9***	**76**
75	20	11±2.2*	55	**70.8±16.1***	**46.4±5.2***	**65.5**
100	20	10±2.1*	50	**49.8±6.1***	**29.2±5.2***	**58.6**
150	20	6±2.9*	30	**30.4±7.8***	**16.4±5.3***	**53.9**
200	20	4.2±1.9*	21	**21±6.8***	**7.8±2.7***	**37.1**
250	20	2±1.1*	10	**13.4±4.3***	**4.6±1.1***	**34.3**
300	20	1±1.6*	5	**10.2±6.6***	**2.8±1.9***	**27.5**
350	20	0.6±0.5*	3	**4.2±4***	**1±1***	**23.8**
400	20	0.2±0.4*	1	**0±0**	**0±0**	**0.0**
450	20	0.0±0.0	0.0	**0±0**	**0±0**	**0.0**
500	20	0.0±0.0	0.0	**0±0**	**0±0**	**0.0**

* The mean difference is significant at the 0.05 level compare with control.

TABLE 33. GAMMA IRRADIATION OF THE ADULT STAGE OF *EPHESTIA CAUTELLA* AND THE EFFECT ON MORTALITY AND THE F1 GENERATION

Dose (Gy)	Mean number of irradiated adults	Number of dead adults (mean±SD)	Adult mortality (%)	**F1 generation**		
				Fecundity	Number of eggs hatch (mean±SD)	Egg hatch (%)
0 (Control)	20	0.0±0.0	0.0	**149±43.9**	**127.8±35.4**	**85.8**
50	20	3.6±1.14*	18	**121.8±50**	**59.2±29.8***	**48.6**
100	20	4.2±1.3*	21	**84.6±19.2***	**8.8±2.9***	**10.4**
150	20	5.4±1.3*	27	**65.2±11.4***	**0±0**	**0.0**
200	20	9.2±0.8*	46	**38.6±18.6***	**0±0**	**0.0**
250	20	14.8±1.5*	74	**16.6±9.5***	**0±0**	**0.0**
300	20	16±0.7*	80	**15.6±11.1***	**0±0**	**0.0**

* The mean difference is significant at the 0.05 level compare with control.

16.3.2. EFFECT OF ELECTRON BEAM IRRADIATION

Research investigated the effects of electron beam irradiation (EBI) on *Bactrocera zonata* and *Callosobruchus maculatus*.

16.3.2.1. Effect of electron beam irradiation on development stages of Bactrocera zonata

When one-day-old eggs were irradiated by an electron beam, an irradiation dose of 304.8 Gy prevented adult emergence, whereas irradiation dose of 414.3 Gy prevented egg hatching and pupation (Table 34).

TABLE 34. ELECTRON BEAM IRRADIATION OF ONE-DAY-OLD EGGS (*N*=100) OF *B. ZONATA* IN ARTIFICIAL MEDIA

Dose (Gy)	Number of hatched eggs (mean±SD)	Egg hatch (%)	Number of pupae (mean±SD)	Pupation (%)	Number of emergent adults (mean±SD)	Emergent adults (%)
0 (control)	99.4±0.9	99.4	98±1.1	98.6	96.8±1.6	98.8
103.6	24.6±2.1*	24.6	3.8±0.8*	15.4	1.8±1.1*	47.4
123.3	13.6±2.7*	13.6	2±1*	14.7	0.8±0.8*	40
304.8	4.8±1.8*	4.8	1.2±0.8*	2.5	0±0	0.0
414.3	0±0	0.0	0±0	0.0	0±0	0.0
488.3	0±0	0.0	0±0	0.0	0±0	0.0
653.5	0±0	0.0	0±0	0.0	0±0	0.0

Note that the mean number of irradiated eggs (*N*) at each treatment dose was 100 (*N*=100).
* The mean difference is significant at the 0.05 level compare with control.

When the 1st larval instars were irradiated with an electron beam, an irradiation dose of 123.3 Gy prevented adult emergence, while irradiation dose of 304.3 Gy caused 100% larval mortality and zero pupation (Table 35).

TABLE 35. ELECTRON BEAM IRRADIATION OF 1ST LARVAL INSTARS (*N*=100) OF *B. ZONATA* IN ARTIFICIAL MEDIA

Dose (Gy)	Number of dead larvae (mean±SD)	Larval mortality (%)	Number of pupae (mean±SD)	Pupation (%)	Number of emergent adults (mean±SD)	Emergent adults (%)
0 (control)	3.4±2.1	3.4	96.6± 2.1	96.6	92.2±2.7	95.4
103.6	82.8±2.3*	82.8	17.2±2.3*	17.2	8.2±1.5*	47.7
123.3	94.8±2.3*	94.8	5.2±2.3*	5.2	0±0	0.0
304.8	100±0*	100	0±0	0.0	0±0	0.0
414.3	100±0*	100	0±0	0.0	0±0	0.0
488.3	100±0*	100	0±0	0.0	0±0	0.0
653.5	100±0*	100	0±0	0.0	0±0	0.0

Note that the mean number of irradiated larvae (*N*) at each treatment dose was 100 (*N*=100).
* The mean difference is significant at the 0.05 level compare with control.

When the 2nd larval instars were irradiated with an electron beam, an irradiation dose of 123.3 Gy prevented adult emergence, whereas 414.3 Gy resulted in 100% larval mortality and zero pupation (Table 36).

TABLE 36. ELECTRON BEAM IRRADIATION OF 2ND LARVAL INSTARS (*N*=100) OF *B. ZONATA* IN ARTIFICIAL MEDIA

Dose (Gy)	Number of dead larvae (mean±SD)	Larval mortality (%)	Number of pupae (mean±SD)	Pupation (%)	Number of emergent adults (mean±SD)	Emergent adults (%)
0 (control)	3.6± 1.1	3.6	96.4±1.1	96.4	94±3.1	97.5
103.6	95.6±1.1*	95.6	4.4±1.1*	4.4	2.6±1.1*	59.1
123.3	97.6±1.5*	97.6	2.4±1.5*	2.4	0±0	0.0
304.8	98.8±1.3*	98.8	1.2±1.3*	1.2	0±0	0.0
414.3	100±0.0*	100	0±0	0.0	0±0	0.0
488.3	100±0.0*	100	0±0	0.0	0±0	0.0
653.5	100±0.0*	100	0±0	0.0	0±0	0.0

Note that the mean number of irradiated larvae (*N*) at each treatment dose was 100 (*N*=100).
* The mean difference is significant at the 0.05 level compare with control.

When the 3rd larval instars were irradiated with an electron beam, an irradiation dose of 304.8 Gy prevented adult emergence, whereas 100% larval mortality and zero pupation required 653.5 Gy (Table 37).

TABLE 37. ELECTRON BEAM IRRADIATION OF 3RD LARVAL INSTARS (*N*=100) OF *B. ZONATA* IN ARTIFICIAL MEDIA

Dose (Gy)	Number of dead larvae (mean±SD)	Larval mortality (%)	Number of pupae (mean±SD)	Pupation (%)	Number of emergent adults (mean±SD)	Emergent adults (%)
0 (control)	1.2±1.8	1.2	98.8±1.8	98.8	96.8±1.6	98.0
103.6	75.8±5.5*	75.8	24.2±5.5*	24.2	11.4±1.1*	47.0
123.3	90.4±3*	90.4	9.6±3*	9.6	1.2±0.8*	12.5
304.8	92.2±2.5*	92.2	7.8±2.5*	7.8	0±0	0.0
414.3	96.2±1.9*	96.2	3.8±1.9*	3.8	0±0	0.0
488.3	97.8±2.8*	97.8	1.4±1.1*	1.4	0±0	0.0
653.5	100±0.0*	100	0.0±0.0*	0.0	0±0	0.0

Note that the mean number of irradiated larvae (*N*) at each treatment dose was 100 (*N*=100).
* The mean difference is significant at the 0.05 level compare with control.

When one-day-old, three-day-old and seven-day-old pupae were irradiated with an electron beam, adult emergence was prevented at 103.3, 123.3 and 653.5 Gy, respectively (Table 38).

TABLE 38. ELECTRON BEAM IRRADIATION OF ONE-DAY_OLD, THREE-DAY-OLD AND SEVEN- DAY-OLD PUPAE OF *B. ZONATA*

Dose (Gy)	1 day old		3 day old		7 day old	
	Number of emergent adults (mean±SD)	Emergent adults (%)*	Number of emergent adults (mean±SD)	Emergent adults (%)*	Number of emergent adults (mean±SD)	Emergent adults (%)*
0 (control)	48±2.3	96	48.8±1.8	97.6	49.6±0.5	99.2
103.6	0±0	0.0	0.8±0.8*	1.6	47.8±2.7	95.6
123.3	0±0	0.0	0±0	0.0	44.8±3.4*	89.6
304.8	0±0	0.0	0±0	0.0	41.6±2.1*	83.2
414.3	0±0	0.0	0±0	0.0	30.2±2.4*	60.4
488.3	0±0	0.0	0±0	0.0	10.8±5.2*	21.6
653.5	0±0	0.0	0±0	0.0	0.0±0.0*	0.0

* The number of irradiated pupae (*N*) at each treatment dose was 50 (*N*=50).
** The mean difference is significant at the 0.05 level compare with control.

The sensitivity of larvae and pupae to EBI decreased as their age increased. The 3rd larval instars and 7-day-old pupae were the radiation tolerant stages. However, the 7-day-old pupal stage does not usually exist in fresh fruit hosts (peach, mango, guava, pomegranate, etc.). Therefore, the third instar larval stage is considered the most radiation tolerant stage for the purpose developing a phytosanitary treatment for shipments of fresh commodities. When this most radio tolerant life stage is considered and adult emergence was used as the criterion for measuring the effective phytosanitary irradiation dose, 304.8 Gy was required, which was higher than that reported by the other researchers with gamma radiation of other fruits flies and higher than the 150 Gy we found for gamma irradiated *B. Zonata* 3rd instars in pomegranate fruit (Table 5 and Table 7). However, in the EBI experiments 123.3 Gy was the next lowest dose that was applied below 304.8 Gy. Hence, from the EBI experiments the camparable phytosanitary treatment dose for electron beam irradiation is expected to be greater than 123.3 Gy but less than or equal to 304.8 Gy.

Other researchers have reported that currently used phytosanitary treatments rely on minimum irradiation doses at at least 210 Gy, 220 Gy and 225 Gy, to protect against *B.cucurbita, Ceratitis capitata* and *B. dorsalis* respectively for fresh fruits and vegetables shipped from Hawaii to continental USA [31]. It is also reported that a dose of 232 Gy was recommended to disinfest any fruits from oriental fruit moth under ambient and hypoxic atmosphere [78], although lower irradiation doses were also proposed [79]. Our research results with the effect of gamma radiation on *B. zonata* revealed that irradiation dose of 150 Gy applied to 17 000 late 3rd instars of *B. zonata* resulted in no adult emergence in the F1 generation. This indicate that a gamma irradiation dose of only 150 Gy is sufficient for providing quarantine security of traded pomegranate which was lower than the EBI dose needed for providing security of pomegranate. The results of the present study indicated that EBI of 1-day-old and 3-day-old pupae of *B. zonata* with 103.6 Gy resulted in zero and 2% adult emergence, respectively. On the other hand, [30] found that gamma irradiation dose of 90 Gy applied to *B. zonata* pupae (5 days old) decrease adult emergence to 22.66%. It was also found that gamma radiation of one-day-old pupae of *B. zonata* and *B. cucurbitae* at 80 Gy reduced adult emergence from 83.25% and 87.5% (non-irradiated pupae) to 53.75% and 57%, respectively [32]. Our EBI results indicate

that, when one-day-old and three-day-old pupae were irradiated and the adult emergence was used as the criterion for measuring efficacy of the treatment, irradiation doses of 103.6 and 123.3 Gy were required, respectively.

16.3.2.2. Effect of electron beam irradiation on development stages of Callosobruchus maculatus

An EBI dose of 304.8 Gy completely prevented adult emergence of the F1 generation when three-day-old eggs were irradiated (Table 39).

TABLE 39. ELECTRON BEAM IRRADIATION OF THREE-DAY-OLD EGGS OF *C. MACULATUS*

Dose (Gy)	Number of eggs irradiated (mean±SD)	Number of eggs that hatched (mean±SD)	Egg hatch (%)	Number of emergent adults (mean±SD)	Adult emergence (%)
0 (control)	25.8±5.2	23.2±4.5	90.0	20.4±3.8	88.0
103.6	22±2.5	11.2±2.6*	51.0	6.4±2.3*	57.1
123.3	22.4±3.9	10.2±2.9*	45.5	5.2±2.7*	51.0
304.8	20.6±2.7	8.2±2.4*	39.8	0±0	0.0
414.3	26.2±3	6.6±2.2*	25.2	0±0	0.0
488.3	25.8±5.3	3.4±1.1*	13.2	0±0	0.0
653.5	23.8±7.7	0.0±0.0*	0.0	0±0	0.0

* The mean difference is significant at the 0.05 level compare with control.

Results of experiments where larval and pupal stages of cowpea weevil (*C. maculatus*) were treated by EBI are provided in Table 40 and Table 41 respectively. A dose of only 103.6 Gy was found to completely prevented F1 generation adult emergence when either larvae or pupae were irradiated by electron beam.

TABLE 40. ELECTRON BEAM IRRADIATION OF THE LARVAL STAGE OF *C. MACULATUS*

Dose (Gy)	Number of adults that emerged from irradiated larvae (mean±SD)	Reduction (%)	**F1 generation**	
			Number of adults that emerged from eggs laid by the F1 generation (mean±SD)	Reduction (%)
0 (control)	31.4±9.1	0.0	**52.2±6.2**	**0.0**
103.6	3.4±2.5*	89.2	**0±0**	**100**
123.3	1.4±1.3*	95.5	**0±0**	**100**
304.8	0±0	100	**0±0**	**100**
414.3	0±0	100	**0±0**	**100**
488.3	0±0	100	**0±0**	**100**

Note that larvae (and pupae) develop inside the cowpea host commodity, and therefore the initial numbers exposed to the electron beam are not known. Prior to the experiment, 400 g of cowpea seeds were infested with two hundred adult insects. After three days the infested cowpea seeds were sieved thoroughly to remove external insects, and these were discarded. Aliquots (5 g) of these cowpeas, infested with larvae and pupae were removed and used in each radiation dose treatment.

* The mean difference is significant at the 0.05 level compare with control.

TABLE 41. ELECTRON BEAM IRRADIATION OF THE PUPAE OF *C. MACULATUS*

Dose (Gy)	Number of adults that emerged from irradiated pupae (mean±SD)	Reduction in pupae (%)	**F1 generation**	
			Number of emergent adult progeny (mean±SD)	Reduction in adult progeny (%)
0 (control)	36±3.5	0.0	**42.8±8.7**	**0.0**
103.6	15.4±5.5*	57.2	**0±0**	**100**
123.3	14±3.4*	61.1	**0±0**	**100**
304.8	3.4±2.9*	90.5	**0±0**	**100**
414.3	0±0	100	**0±0**	**100**
488.3	0±0	100	**0±0**	**100**
653.5	0±0	100	**0±0**	**100**

Note that the pupae develop inside the cowpea host commodity, and therefore the initial numbers exposed to the electron beam are not known. Prior to the experiment, 400 g of cowpea seeds were infested with two hundred adult insects. After three days the infested cowpea seeds were sieved thoroughly to remove external insects, and these were discarded. Aliquots (5 g) of these cowpeas, infested with larvae and pupae were removed and used in each radiation dose treatment.

* The mean difference is significant at the 0.05 level compare with control.

When adult *C. maculatus* were irradiated, an EBI dose of 414.3 Gy completely prevented adult emergence of F1 generation adults (Table 42). These results indicate that the adult stage was the most tolerant to EBI. Based on the prevention of F1 generation when the most radio tolerant stage (adult) is irradiated, 414.3Gy is required as a phytosanitary irradiation dose for quarantine and security treatment.

TABLE 42. ELECTRON BEAM IRRADIATION OF THE ADULT LIFE STAGE OF *C. MACULATUS* AND THE EFFECT ON MORTALITY, NUMBER OF EGGS AND EMERGENCY F1 ADULTS

Dose (Gy)	Mean number of irradiated adults	Number of dead adults (mean±SD)	Adult mortality (%)	Number of eggs (mean±SD)	Number of emergent adults in the F1 generation (mean±SD)
0 (control)	50	0.0	0.0	56.8±11.8	46.2±1.3
103.6	50	3.4±2.6*	6.8	47.2±9*	30.4±2.3*
123.3	50	5.6±1.1*	11.2	43.4±4.7*	19.4±5*
304.8	50	12.4±2.9*	24.8	9.4±3*	2.4±1.14*
414.3	50	15.8±3*	31.6	0±0	0±0
488.3	50	22.2±6.6*	44.4	0±0	0±0

* The mean difference is significant at the 0.05 level compare with control.

In the present study, an EBI dose of 488.3 Gy resulted in 44.4% adult mortality (Table 42). These results are in agreement with those obtained by Sang *et. al.* [42] where immediate death of *Callosobruchus maculatus* adult males or females was not observe until doses of 150 Gy and 500 Gy when irradiated by electron beam. Sang *et. al.* commented that EBI at a dose of 2 kGy resulted in approximately 50% acute mortality, indicating that the adult stage was the most tolerant to EBI. By comparison, our experiments of gamma irradiation of adult *C. maculatus* indicated that 1.0 kGy and 1.3 kGy resulted in 71% and 100% acute mortality respectively (Table 13). A gamma irradiation dose of at least 650 Gy was required for quarantine treatment

of cowpea seed, when prevention of emergence of the F1 generation was used as the measure of efficacy of the treatment (Table 14). In the present study with EBI, a dose 414.3 Gy was needed for prevention of F1 generation indicating that EBI was more effective at controlling cowpea weevil than gamma irradiation. Darfour *et. al.* [43] investigated different varieties of cowpea seeds (ASONTEM, NHYIRA, NIGERIA, TOGO) infested with *Callosobruchus maculatus* adults. Samples were gamma irradiated to doses of 0.25, 0.5, 0.75, 1.0 and 1.5 kGy [43]. An irradiation dose of 0.75 kGy was found to caused 100% mortality in ASONTEM variety, whereas irradiation doses of 1.0 and 1.5 kGy did not achieve 100% mortality in other cowpea seeds varieties up to six days after irradiation. Almost similar results to our results have shown by [44] who reported that gamma irradiation dose of 500 Gy resulted in 100% mortality of *C. maculatus* larvae and pupae stages and these experiments involved stored mung bean irradiated by gamma irradiation. Whereas Follett *et. al.* [48] irradiated rice weevil, *Sitophilus oryzae*, with X ray and found that only 120 Gy arrested the development of rice weevil and sterilized its adults, thus providing quarantine security.

Data from large-scale tests where an electron beam was used to deliver a dose of 414.3 Gy to 28 000 adults of *C. maculatus* confirmed that this dose resulted in no adult emergence of the F1 generation (Table 43).

TABLE 43. LARGE-SCALE CONFIRMATORY TESTS IRRADIATING ADULT STAGE OF *CALLOSEBRUCHUS MACULATUS* WITH ELECTRON BEAM IN COWPEA

Measured dose	Number of replicates	Total number irradiated	Number of F1 adults
0 (control)	10	5000	4097
414.3 Gy	40	28 000	0

Our results in this study indicate that EBI is effective against *C. maculatus* and *B. zonata* and provide new information on the effect of different EBI doses applied to the different life stages of these insects. But further investigations are needed to confirm the efficacy of EBI as a phytosanitary treatment to control *C. maculatus* and *B. zonata* in their hosts to provide quarantine security. Our results revealed that the effectiveness of EBI against *C. maculatus* was lower than that of gamma radiation. The lower effect of EBI on the control of insect pests as compared with gamma radiation was also reported by other investigators [81, 82]. However, contrary to the findings for *C. maculatus,* our results also indicate that the EBI of *B. zonata* was more effective than the gamma radiation of this species.

16.3.3. EFFECT OF X RAY IRRADIATION

16.3.3.1. Effect of X ray irradiation on egg and larval development stages of Bactrocera zonata

Research results summarized in Table 44 show that X ray irradiation of *B. zonata* eggs in host fruits (mango fruits) significantly decreased the egg hatch percentage. The numbers of *B. zonata* eggs that hatched decrease as the dose of X ray radiation increased. X ray irradiation of eggs at 156.9 Gy decreased the percentage of hatchability to 8.8 and prevented pupation, whilst 237.8 Gy (the next highest radiation dose we studied) completely prevented egg hatch. On the other hand, irradiation of eggs at the dose of 137.0 Gy completely prevented adult emergence.

TABLE 44. X RAY IRRADIATION OF EGGS (*N*=100) OF *B. ZONATA* IN MANGO FRUITS AND THE EFFECTS ON EGG HATCH, PUPATION AND ADULT EMERGENCE

Dose (Gy)	Number of hatched eggs (mean±SD)	Egg hatch (%)	Number of pupae (mean±SD)	Pupation (%)	Number of adults that emerged (mean±SD)	Adult emergence (%)
0 (control)	98.8±1.3	98.8	97±1.2	98.2	95.2±0.8	96.1
60.7	59.8±2.7*	59.8	49.8±0.8*	83.3	17±0.3*	34.1
137	22.6±3.3*	22.6	10.2±1.7*	49.1	0±0	0.0
156.9	8.8±1.8*	8.8	0±0	0.0	0±0	0.0
237.8	0±0	0.0	0±0	0.0	0±0	0.0
300.6	0±0	0.0	0±0	0.0	0±0	0.0
344	0±0	0.0	0±0	0.0	0±0	0.0

Note that the mean number of irradiated eggs (*N*) at each treatment dose was 100 (*N*=100).
* The mean difference is significant at the 0.05 level compare with control.

The effect of X ray irradiation on the 1st larval instars (Table 45) indicate that 100% larval mortality and zero pupation was achieved at 300.6 Gy, while irradiation of 1st larval instars at only 60.7 Gy completely prevented adult emergence.

TABLE 45. X RAY IRRADIATION OF 1ST LARVAL INSTARS OF *B. ZONATA* (*N*=100) IN MANGO FRUITS

Dose (Gy)	Number of dead larvae (mean±SD)	Larval mortality (%)	Number of pupae (mean±SD)	Pupation (%)	Number of emergent adults (mean±SD)	Adult emergence (%)
0 (control)	2.8±0.8	2.8	97.2±1.7	97.2	92.2±1.5	94.9
60.7	53.8±3.8*	53.8	46.2±3.7*	46.2	0±0*	0.0
137	70±2.9*	70.0	30±3.8*	30.0	0±0	0.0
156.9	85.8±3.4*	85.8	14.2±3.4*	14.2	0±0	0.0
237.8	98.4±2.1*	98.4	1.6±0.07*	1.6	0±0	0.0
300.6	100±0*	100	0±0*	0.0	0±0	0.0
344	100±0	100	0±0	0.0	0±0	0.0

Note that the mean number of irradiated 1st instar larvae (*N*) at each treatment dose was 100 (*N*=100).
* The mean difference is significant at the 0.05 level compare with control.

When X ray irradiation of the 2nd larval instars in mango fruits were investigated, an irradiation dose of 344.4 Gy resulted in 100% larval mortality and zero pupation. An irradiation dose of 156.9 Gy to 2nd instars completely prevented adult emergence (Table 46).

TABLE 46. X RAY IRRADIATION OF 2ND LARVAL INSTARS OF *B. ZONATA* (*N*=100) IN MANGO FRUITS

Dose/Gy	Number of dead larvae (mean±SD)	Larval mortality (%)	Number of pupae (mean±SD)	Pupation (%)	Number of emergent adults (mean±SD)	Adult emergence (%)
0 (control)	1.2± 0.3	1.2	98.8±1.3	98.8	97.2±2	98.3
60.7	27.4±2.3*	27.4	72.6±2.3*	72.6	6.8±2.2*	9.3
137	38.6±2.7*	38.6	61.1±2.7*	61.1	4.6±1.1*	7.5
156.9	55.8±2.8*	55.8	44.2±2.8*	44.2	0±0	0.0
237.8	73±2.2*	73	27±2.2*	27	0±0	0.0
300.6	92.2±2.3*	92.2	7.8±2.3*	7.8	0±0	0.0
344	100±0.0	100	0±0	0.0	0±0	0.0

Note that the mean number of irradiated 2nd instar larvae (*N*) at each treatment dose was 100 (*N*=100).
* The mean difference is significant at the 0.05 level compare with control.

Irradiation of the 3rd larval instars in mango fruits with 344.4 Gy also caused 100% larval mortality and zero pupation, while irradiation dose of 237.8 Gy resulted in no adult emergence, with the next lowest dose studied of 156.9 Gy having an adult emergence of 23.1% (Table 47).

TABLE 47. X RAY IRRADIATION OF 3RD LARVAL INSTARS OF *B. ZONATA* (*N*=100) IN MANGO FRUITS

Dose (Gy)	Number of dead larvae (mean±SD)	Larval mortality (%)	Number of pupae (mean±SD)	Pupation (%)	Number of emergent adults (mean±SD)	Adult emergence (%)
0 (control)	1.1±1.1	1.1	98.8±1.1	98.8	95.6±4.3	96.8
60.7	72.8±3.7*	72.8	24.2±2.5*	24.2	12±2.9*	49.6
137.0	81.2±2.4*	81.2	18.8±2.4*	18.8	5±1.2*	26.6
156.9	89.6±2.7*	89.6	10.4±2.7*	10.4	2.4±0.3*	23.1
237.8	93.2±1.8*	93.2	6.8±1.6*	6.8	0±0	0.0
300.6	96.6±3.2*	96.6	3.4±0.2*	3.4	0±0	0.0
344.0	100±0.0	100	0±0	0.0	0±0	0.0

Note that the mean number of irradiated 3rd instar larvae (*N*) at each treatment dose was 100 (*N*=100).
* The mean difference is significant at the 0.05 level compare with control.

In general, it was observed that as the X ray irradiation dose increased larval mortality increased, pupation and adult emergence decreased. The 3rd larval instar of *B. zonata* was found to be the life stage most tolerant to X rays. When the most radio-tolerant stage is considered and the prevention of adult emergence was used as the criterion for measuring phytosanitary irradiation efficacy the effective treatment doses for gamma, EBI and X ray irradiation were identified in our experiments as 150 Gy (Table 5 and Table 7), 304.8 Gy (Table 37) and 237.8 Gy (Table 47) respectively, to control *B. zonata* and provide quarantine security of pomegranate and mango fruits. This indicates that gamma radiation was the most effectiveness to control *B. zonata* and provide quarantine security followed by X ray irradiation and EBI.

16.3.3.2. Effect of X ray irradiation on larval, pupal and adult development stages of Ephestia cautella

Individually date fruits were artificially infested with larvae (2nd or 4th instars), 3-days-old pupae or one-day-old adults (in the test tube) of *E.cautella.* The infested dates were kept as controls (0 Gy dose) or exposed to different doses of X ray radiation. The delivered doses of X ray were measured at 56.6, 104.9, 190.5, 264.3, 300, 350 and 400 Gy. From the obtained results, the required X ray irradiation dose to prevent adult emergence from irradiated 2nd larval instars was 300 Gy (Table 48), and 350 Gy (Table 49) was required to prevent adults' emergence from irradiated 4th larval instars. On the other hand, only X ray irradiation dose 190.5 Gy was required to prevent eggs hatchability of F1 generation for both 2nd and 4th larval instars.

TABLE 48. X RAY IRRADIATION OF 2ND LARVAL INSTARS OF *EPHESTIA CAUTELLA* (*N*=100)

Dose (Gy)	Dead larvae (mean±SD)	Larval mortality (%)	Emergent adults (mean±SD)	Adult emerg. (%)	**F1 generation**		
					Fecundity	Egg hatch (mean±SD)	Egg hatch (%)
0 (control)	0.0±0.0	0	94.8±1.6	94.8	**137.4±2.9**	**130.0±3.2**	**94.6**
56.6	4.6±0.9*	4.6	35.2±3.3*	35.2	**30.6±1.1***	**13.0±2.1***	**42.5**
104.9	8.0±1.4*	8.0	22.8±3.3*	22.8	**23.4±4.8***	**7.6±2.3***	**32.5**
190.5	10.0±2.8*	10.0	19.6±5*	19.6	**0.0**	**0.0**	**0.0**
264.3	23.2±3.3*	23.2	3.2±1.1*	3.2	**0.0**	**0.0**	**0.0**
300	35.6±3.6*	35.6	0.0	0.0	**0.0**	**0.0**	**0.0**
350	40.0±1.8*	40.0	0.0	0.0	**0.0**	**0.0**	**0.0**
400	67.2±1.8*	67.2	0.0	0.0	**0.0**	**0.0**	**0.0**

Note that the mean number of irradiated 2nd instar larvae (*N*) at each treatment dose was 100 (*N*=100).
* The mean difference is significant at the 0.05 level compare with control.

TABLE 49. X RAY IRRADIATION OF 4TH LARVAL INSTARS OF *EPHESTIA CAUTELLA* (*N*=100).

Dose (Gy)	Dead larvae (mean±SD)	Larval mortality (%)	Emergent adults (mean±SD)	Adult emerg. (%)	**F1 generation**		
					Fecundity	Hatched eggs (mean±SD)	Egg hatch (%)
0 (control)	0.0±0.0	0	93.2±3	93.2	**140.4±6.7**	**131.8±4.6**	**93.9**
56.6	2.4±1.7*	2.4	29±2.6*	29.0	**36.0±4.3***	**19.8±2.6***	**55.0**
104.9	4.8±1.1*	4.8	32.8±3.3*	32.8	**34.8±4.4***	**15.0±4.3***	**43.1**
190.5	7.6±1.7*	7.6	17.6±3.6*	17.6	**8.6±1.2***	**0±0**	**0.0**
264.3	20±4.9*	20	6.0±2*	6.0	**0.0**	**0±0**	**0.0**
300	26.4±2.2*	26.4	3.6±1.1*	3.6	**0.0**	**0±0**	**0.0**
350	40.8±1.8*	40.8	0±0	0.0	**0.0**	**0±0**	**0.0**
400	57.2±3.9*	57.2	0±0	0.0	**0.0**	**0±0**	**0.0**

Note that the mean number of irradiated 4th instar larvae (*N*) at each treatment dose was 100 (*N*=100).
* The mean difference is significant at the 0.05 level compare with control.

The results in Table 50 indicate that increasing X ray irradiation doses applied to *E.cautella.* pupae decreases the number of adults that emerge. An X ray irradiation dose of 300 Gy to pupae prevented egg hatch of F1 generation.

Table 51 indicates that the percentage of adult mortality increased as irradiation dose increased. Irradiation dose of 400 Gy caused 97.8% mortality. While only irradiation dose of 264.3 Gy prevented eggs hatchability of F1 generation. According to these results it could be concluded that pupal stage was the most tolerant to X ray irradiation treatment.

TABLE 50. X RAY IRRADIATION OF THE PUPAL STAGE OF *EPHESTIA CAUTELLA*

Dose (Gy)	Mean number of irradiated pupae	Emergent adults (mean±SD)	Adult emergence (%)	**F1 generation**		
				Fecundity	Number of hatched eggs (mean±SD)	Egg hatch (%)
0 (control)	100	92±0.4	92	**127.4±8.3**	**120±6.6**	**94.2**
56.6	100	78.4±4.6*	78.4	**73±7.9***	**54±2.9***	**74**
104.9	100	68.8±2.8*	68.8	**47.4±6***	**29.6±5.8***	**62.4**
190.5	100	64.6±3.8*	64.6	**24.2±4.8***	**11.2±3.3***	**46.3**
264.3	100	57.6±3.2*	57.6	**16.4±3.4***	**4.6±1.3***	**28**
300	100	46.4±1.6*	46.4	**9.4±2.2***	**0±0**	**0.0**
350	100	38.4±1.1*	38.4	**0±0**	**0±0**	**0.0**
400	100	32.8±3.3*	32.8	**0±0**	**0±0**	**0.0**

* The mean difference is significant at the 0.05 level compare with control.

TABLE 51. X RAY IRRADIATION OF THE ADULT STAGE OF *EPHESTIA CAUTELLA*

Dose (Gy)	Mean number of irradiated adults	Number of dead adults (mean±SD)	Adult mortality (%)	**F1 generation**		
				Fecundity	Number of hatched eggs (mean±SD)	Egg hatch (%)
0 (control)	100	0.0±0.0	0.0	**132.2±8.4**	**125.4±6.3**	**94.9**
56.6	100	7.6±3.1*	7.6	**92±5.4***	**56±5.5***	**60.8**
104.9	100	13.6±3.6*	13.6	**69.6±9.7***	**33.6±4.8***	**34.7**
190.5	100	26.4±4.6*	26.4	**57±4.5***	**13.2±2.1***	**23.2**
264.3	100	44.8±2.2*	44.8	**26.4±2.6***	**0.0**	**0.0**
300	100	65.6±4.9*	65.6	**14.6±1.3***	**0.0**	**0.0**
350	100	88.8±3.1*	88.8	**0.0**	**0.0**	**0.0**
400	100	97.8±3*	97.8	**0.0**	**0.0**	**0.0**

* The mean difference is significant at the 0.05 level compare with control.

When the most radio-tolerant stage is considered and the prevention of F1 generation hatch is used as a criterion for measuring the phytosanitary irradiation effectiveness, an X ray irradiation dose of 300 Gy was required to control *E.cautella* and provide quarantine security for exportation or importation of dates in comparison to gamma irradiation dose of 400 Gy (Table 32). When the prevention of F1 generation egg hatch (prevent F1 hatch) was used to measure phytosanitary irradiation efficacy of *E.cautella*, gamma irradiation dose of 400 Gy and X ray irradiation dose of 300 Gy were required. These doses were sufficient to control *E.cautella* and provide quarantine security of export dates. This indicates that X ray irradiation was more effective than gamma irradiation.

16.4. CONCLUSIONS

The results obtained in this research investigation revealed that electron beam and X ray irradiation doses required to control *B. zonata* and provide quarantine security of export/import

fresh fruits were 304.8 Gy and 237.8 Gy, respectively in comparison to 150 Gy of gamma radiation. The required electron beam and gamma irradiation doses to control *C.maculatus* were 414.3 and 650 Gy, respectively. X ray irradiation doses required to control *E.cautella* and provide quarantine treatment was 300 Gy compared to 400 Gy of gamma irradiation.

In general, the three types of ionizing radiations studies were very effective at controlling the pest studied and could be used to provide a quarantine treatment. It is obvious from the results that different insects and development stages vary in their response to the three types of ionizing radiation. The effectiveness of phytosanitary irradiation on insects is varied depending on the species, stage, age, and types of ionizing radiation. The younger metamorphic stages of insects are most sensitive to irradiation than the older stages. The difference between our results on the effect of irradiation on the development stages of the tested insects and those obtained from the other previous reported could be due to the difference in the age of the studied development stage at the time of irradiation, irradiation dose, environmental condition, and some physical factors.

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17. THE EFFECTS OF GAMMA IRRADIATION ON INSECTS: A COMPARATIVE STUDY USING DIFFERENT DOSE RATES

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Abstract

In this research, we examined the effects of gamma irradiation as a phytosanitary treatment on wax moth, *Galleria mellonella* L. late pupae. In addition, we examined the effects of low and high dose rates (approximately 4 Gy/minute vs. 330 Gy/minute) of gamma irradiation on wax moth and the Khapra beetle, *Trogoderma granarium* eggs. Results showed that the radiosensitivity of *G. mellonella* late pupae increased with increasing radiation dose and the severity of the effect depends on the criterion used for measuring effectiveness. Results of studies comparing the effects of low and high dose rates on egg hatch in *G. mellonella* and *T. granarium* did not show any significant difference.

17.1. INTRODUCTION

World trade in agricultural products is growing commercially. Expanding international trade in these products increases the risk of transporting pests of phytosanitary importance to countries where they do not exist. Quarantine pests can seriously disrupt agricultural trade between countries, but acceptable phytosanitary treatments (PT) will neutralize the risks associated with the spread of pests and can enable trade that would otherwise be restricted. Phytosanitary treatments, such as fumigation, heat, cold storage, and ionizing radiation, particularly gamma radiation, have been used for decades to disinfest agricultural products before export to areas where such pests are under quarantine. Among the phytosanitary treatments, ionizing radiation has several advantages including broad spectrum activity against insect pests, fast application, it leaves no residues on the treated product, does not induce resistance in pest populations and has minimal adverse effects on the quality of the treated commodities. The radio isotope ^{60}Co is the main source for gamma radiation that is used to irradiate agricultural products. However, ^{60}Co is increasingly difficult to obtain. Ionizing radiation generated by other sources can be used. For example, electron accelerators employ electricity to generate ionizing radiation in the form of electron beams. Electron beams from such machine sources might be more acceptable to the consumer and have characteristics that can make them easier to incorporate into production systems.

Phytosanitary irradiation (PI) relies on delivering a specific treatment dose of ionizing radiation to an agricultural product: The minimum dose received by the commodity must be greater than the dose specified as efficacious for the phytosanitary treatment, regardless of it being by gamma, electron beam or X ray irradiation. One hypothesis is that there could potentially be differences between using gamma, electron beam or X rays for PI because they have very different dose rates. With heat treatments for example, the rate of heat application as a phytosanitary treatment is known to affect efficacy [1] and researchers have questioned if there is a dose rate effect for PI [2, 3]. Electron beam irradiation from machine sources have high dose rates and transfer energy to a product at a rate that is several hundred times faster than gamma rays from ^{60}Co sources. It is crucial to know if differences in dose rates can affects PI because it could lead to treatment failure and/or commodity damage [4, 5] and treatment

failure in PI may go unnoticed because there is no independent verification of efficacy for PI as there is for other commercial phytosanitary treatments.

It has been reported that the electron beam irradiation of insects is slightly less efficacious than gamma ray irradiation [6]. Some researchers, on the other hand, have report that electron beam irradiation is more efficacious than electron beam [7, 8]. It should be pointed out, however, that real comparisons between the effects of the two types of ionizing radiation on insects are rarely, if ever, done. In fact, most of the available data on the effects of the different types of ionizing radiation on a particular insect species were obtained by different researches working in different labs, using different sources of ionizing radiation, different dosimetry systems, different strains of insects and different laboratory procedures. Therefore, it is difficult to make a conclusion unless the work is done by the same researcher using the same insect colony, the same dosimetry system and the same experimental procedure.

The objectives of this research project were to obtain more reliable data on the effects of e-beam and gamma radiation with high and low dose rates, respectively, on insects. The two kinds of ionizing radiation were to be tested on a particular developmental stage of two insects of phytosanitary importance, specifically, the wax moth, *Galleria mellonella* L. and the Khapra beetle, *Trogoderma granarium.* Unfortunately, the e-beam machine in our institute (SAEC) suffered from a breakdown that left it out of use; something could not be fixed during the course of this study due the sanctions imposed on Syria.

Consequently, the objectives of the study were modified to:

(a) Study the effects of gamma radiation on wax moth late pupae;
(b) Study the effects of gamma irradiation dose rates (low and high) on wax moth and Khapra beetle eggs.

17.2. MATERIALS AND METHODS

A. Studying the effects of gamma radiation on *G. mellonella* late pupae

17.2.1. Establishing *G. mellonella* lab rearing

The wax moth colony was maintained in a growth chamber under constant temperature and relative humidity. The colony originated from larvae collected from infested bee hives at several locations near the city of Damascus (Syria). Rearing conditions were maintained at 32 ± 1°C, 60 ± 5% RH and total darkness. To maintain vigour, *G.mellonella* males from the local natural populations in the same area were periodically introduced into the colony. Wax moth larvae were reared on a diet composed of their naturally occurring larval food (Pollen, honey and beeswax) in 19 x 14 x 5 cm plastic dishes containing each about 400 g of the rearing medium. With such a diet, mature larvae leave the diet and pupate on the upper surface of the medium. When close to maturation, folded paper strips ("larval holding strips") were placed at the top of the rearing media to provide pupation sites for mature larvae searching for a place to pupate. Mature larvae were collected daily and incubated under the previously mentioned conditions (32 ± 1°C, 60 ± 5% RH and total darkness) for pupation and adult emergence. Emerging adults were transferred into 15 x 10 cm cylindrical polyethylene jars for mating and oviposition (oviposition cages). An accordion folded paper strips were provided for oviposition and the jar openings were covered with a fine mesh to prevent the escape of moths from the jars. The strips of folded paper were collected daily and replaced with new ones. The collected paper strips carrying *G. mellonella* eggs were incubated at 32 ± 1°C and 60 ± 5% RH and transferred into a rearing medium 5 days later.

17.2.2. Establishing *G. mellonella* developmental rate

Eggs of G. *mellonella*, maintained under constant temperature and relative humidity (32 ± 1°C, 60 ± 5% RH and total darkness), were collected at 24 hourly intervals and incubated under the same conditions until eggs hatched (32 ± 1°C and 60 ± 5% RH). Samples of eggs were checked daily and the time for 50% egg hatch was noted. Newly born larvae were planted on the surface of the rearing diet and incubated under the same conditions mentioned above to support larval development. Mature larvae leaving the diet and searching for a place to pupate were collected using the "larval holding strips", the number of insects was recorded, and 50% larval maturation was calculated. Larval holding strips were incubated under the same previously mentioned conditions and the number of formed pupae was recorded. Formed pupae were transferred into the oviposition cages and incubated under the same conditions for the colony. Adult emergence was recorded daily and the developmental time of larvae to pupae and pupae to adults was calculated at 50% pupation and adult emergence.

17.3. STUDYING THE EFFECTS OF GAMMA RADIATION ON *G. MELLONELLA* LATE PUPAE

17.3.1. Obtaining *G. mellonella* late pupae

When larvae finished feeding, strips of sterile cardboard paper (25 x 2.5 cm) folded at 1 cm intervals to take a zigzag form, were distributed at the top of the rearing medium. Mature larvae leaving the medium and searching for a place to pupate, spun their cocoons in the folds of the paper strips "pupal holding strips". The "pupal holding strips" were collected at 24 hour intervals, incubated under the same environmental conditions (32 ± 1°C, 60 ± 5% RH and total darkness) for pupation and 7 days later, the pupae (late pupae) were prepared for irradiation (pupation takes about 8 days).

17.3.2. Preparing pupae for irradiation

The "pupal holding strips" were opened on day 7, pupae were carefully removed using forceps, examined, sexed [9] and each sex (males or females) was placed separately in 9 cm diameter Petri dishes (20 pupae from each sex). Care was taken to include only undamaged, healthy looking insects of a uniform stage of development and any underdeveloped pupae were excluded. To reduce temperature fluctuations during transportation to and from the gamma cell, the Petri dishes were placed inside an insulating box.

17.3.3. Irradiation

Wax moth late pupae were exposed to gamma radiation dosages in a gamma cell supplied with a Co-60 source around the cylindrical (15 x 25 cm) irradiation chamber (Issledovatel Gamma Irradiator). The average dose rate at the time of irradiation was approximately 5.00 Gy/minute with a factor of homogeny (the dose uniformity ratio of the maximum divided by the minimum received dose) of about 1.14 and the absorbed dose was calibrated using a Fricke solution. Four Petri dishes were irradiated simultaneously at each dose level (n=4). The Petri dishes were placed at the centre of the irradiation chamber and irradiated with 50, 100, 150, 200, 250, 300, 350 and 400 Gy.

17.3.4. Effects of gamma radiation on adult emergence

Following irradiation, the Petri dishes containing the pupae were returned immediately to the laboratory and incubated at 32 ± 1°C, 60 ± 5% RH and total darkness for adult emergence.

The dishes were checked daily, emerging adults (from each sex) were removed and their number was recorded. Percentage adult emergence was calculated by dividing the number of emerging adults by the number of irradiated pupae.

17.3.5. Effects of gamma radiation on fecundity and egg hatch

Emerging *G. mellonella* adults from the previous experiment were crossed to untreated insects of the opposite sex by confining them inside oviposition cages. Ten irradiated insects of one sex were placed in an oviposition cage with an equal number of non-irradiated insects of a similar age and the opposite sex. The cages were placed randomly on shelves in the rearing room under the same conditions for the colony. Eggs were deposited on folded paper strips "ovipositon paper strips" that were replaced daily for 4 days. The "ovipositon paper strips" carrying eggs were incubated under the same previously mentioned conditions of temperature, humidity and photoperiod (32 ± 1°C, 60 ± 5% RH and total darkness), and placed at the appropriate age (about 144 hour-old), on the surface of the larval rearing diet in 19 x 14 x 5 cm plastic dishes. The dishes were incubated under the same rearing conditions for the colony. Five days later, the "oviposition paper strips" were removed and examined under a binocular microscope for egg hatch. The number of deposited and hatched eggs in each replicate and treatment was recorded. Percentage egg hatch was calculated by dividing the number of hatched eggs in each replicate on the total number of deposited eggs.

17.3.6. Effects of gamma radiation on F1 survival

After recording egg hatch, the dishes containing diet with larvae resulting from the different crosses were incubated under the same temperature and relative humidity for larval and pupal development (32 ± 1°C, 60 ± 5% RH and total darkness). Four weeks later, “larval holding strips”, were distributed at the top of the rearing medium to collect mature larvae leaving the medium and searching for a place to spin their cocoons. The dishes were covered with fine muslin mesh to prevent emerging moths from leaving the dishes. The dishes were examined daily and emerging adults in each dish were removed, counted and their number recorded. Percentage larval survival to the adult stages was calculated by dividing the number of recorded adults by the number of hatched eggs.

17.4. STUDYING THE EFFECTS OF DOSE RATES ON WAX MOTH AND KHAPRA BEETLE EGG HATCH

17.4.1. Studying the effects of dose rates (low and high) on wax moth egg hatch

17.4.1.1. Obtaining G. mellonella eggs of certain age

To obtain *G. mellonella* eggs of a certain age, about 20 pairs (males and females) of 1–2 day old wax moth adults were placed in "oviposition cages". Eggs were deposited on folded paper strips "ovipositon paper strips" that were replaced daily. The "ovipositon paper strips" carrying eggs were incubated under the same previously mentioned conditions of temperature, humidity and photoperiod (32 ± 1°C, 60 ± 5% RH and total darkness), and irradiated at the appropriate age (121–144 hour-old).

17.4.1.2. Preparing G. mellonella eggs for irradiation

Folded paper strips holding *G. mellonella* eggs (total of 50 eggs/rep.) were placed in Petri dishes 9 cm in diameter. Care was taken to include only typical shape good looking eggs and extra

ones were carefully removed. The dishes containing the eggs were placed inside an insulating box and transferred to the gamma cell for irradiation.

17.4.1.3. Irradiation

To compare the effects of low and high dose rates on egg hatch, two Co-60 gamma irradiation sources were used in this study; one with a low dose rate (gamma cell) and the other with a high dose rate (Semi-commercial irradiation facility). The average dose rate of the first source (Issledovatel Gamma Irradiator, Techsnabexport Co. Ltd. USSR) at the time of irradiation was approximately 4.35 Gy/minute with a dose uniformity ratio (maximum dose/minimum dose) of about 1.14. The average dose rate of the second source (Co-60 Semi commercial gamma irradiation facility) at the irradiation point, however, was approximately 330 Gy/minute (greater than 75 times the dose rate of the gamma cell) and the absorbed dose was calibrated with Fricke solution. At each dose level, and for each dose, 4 Petri dishes containing each 50 wax moth eggs 121–144 hour-old were irradiated simultaneously (n=4). Irradiation dosages ranged between 100 and 400 Gy at 100 Gy increments.

17.4.1.4. Effects of dose rates on egg hatch

Following irradiation, the paper strips carrying irradiated eggs were returned immediately to the laboratory and incubated under the same conditions for the colony (32 ± 1°C and 50 ± 5% RH). The eggs were placed on the surface of the rearing diet in 19 x 14 x 5 cm plastic dishes. Five days later, the paper strips were removed and examined under a binocular microscope. The number of hatched eggs was recorded and percentage egg hatch was calculated by dividing the number of hatched eggs on the total number of irradiated ones.

17.4.2. Studying the effects of dose rates (low and high) on Khapra beetle egg hatch

17.4.2.1. Rearing T. granarium

Khapra beetle larvae were obtained from infested grains taken from one of the grain storage facilities in Damascus in the summer of 2009. The larvae were reared on broken wheat (cracked wheat) in polyethylene jars covered with a fine mesh inside a climatic chamber [10]. Cultures were maintained at 35 ± 1ºC, 40 ± 5% rh and total darkness; under these conditions, eggs start to hatch in 3 days.

17.4.2.2. Obtaining T. granarium eggs of a certain age

Khapra beetle virgin adults were obtained by isolating male and female pupae from the stock culture. To obtain eggs of a certain age, about 50 pairs of 1–2 day-old adults were released in a Petri dish (9 cm in diameter), provided with few wheat grains as an oviposition stimulus. The insects were left for 24 hours for egg laying after which they were transferred into a new clean Petri dish. The eggs were incubated at 35 ± 1ºC and irradiated at the appropriate age (49–72 hour-old). Before irradiation, the dishes were examined, eggs were counted, 50 good looking eggs of a typical shape were left and the rest were removed without disturbing the other eggs. A grid of one centimetre squares on a transparent plastic sheet was used to facilitate counting.

17.4.2.3. Irradiation

Petri dishes containing *T. granarium* eggs were exposed to doses of gamma radiation using either of the same two gamma irradiation sources mentioned before (Issledovatel Gamma

Irradiator, Techsnabexport Co. Ltd. USSR and Semi-commercial irradiation facility at the same dose rates of 4.35 Gy/minute and 330 Gy/minute respectively). At each dose level, 4 Petri dishes carrying fifty *T. granarium* eggs (49–72 hour-old) were irradiated simultaneously (*n*=4). Irradiation dosages ranged between 25 and 100 Gy at 25 Gy increments.

17.4.2.4. Effects of dose rates on egg hatch

After irradiation, the Petri dishes containing the eggs were returned immediately to the laboratory and incubated at 35 ± 1ºC for egg hatch. Each dish was provided with about 1 g of broken wheat at the periphery of the dish where there were no eggs so newly born larvae were able to feed and continue their development. When one-week old, irradiated eggs were examined under a binocular microscope for egg hatch. The number of hatched eggs was recorded, and percentage egg hatch was calculated by dividing the number of hatched eggs on the total number of irradiated ones.

17.5. DATA ANALYSIS

Data from the various experiments were subjected to analysis of variance. Means were separated, at the 5% level of probability, by Fisher's protected least significant difference (PLSD) test. In addition, % hatch in eggs irradiated at similar doses with low and high dose rate sources were subjected to the *t* test.

17.6. RESULTS AND DISCUSSION

17.6.1. Studies on the developmental rate of *G. mellonella* under lab. conditions

Results of studies on the developmental rate of *G.mellonella* under our lab conditions (32 ± 1°C, 60 ± 5% RH and total darkness) are presented in Table 1. The results clearly show that *G. mellonella* development takes, under our lab. conditions, about 8 weeks. From those, about 6.5 days for the eggs stage, 4 weeks for the larval stage and 8 days go for the pupal stage. These results are in general agreement with data reported by other investigators [11, 12, 13]. Deviations may be related to strain differences, different rearing techniques and differences in diet composition, particularly nutrients.

TABLE 1. DURATION OF *G. MELLONELLA* DEVELOPMENTAL STAGES UNDER OUR LABORATORY REARING CONDITIONS (32 ± 1°C, 60 ± 5% RH AND TOTAL DARKNESS)

Stage of development	Duration of development (days)
Eggs	6.5
Larvae	28
Pupae	8

17.6.2. Studies on the effects of gamma radiation on *G. mellonella* late pupae

The results of the effects of gamma radiation on *G. mellonella* late pupae are presented in Figs. 1–4. Fig. 1 presents data on the effects of gamma radiation on adult emergence in *G. mellonella* late pupae. The results do not indicate any negative effects on adult emergence at the examined dosages (P>0.0001).

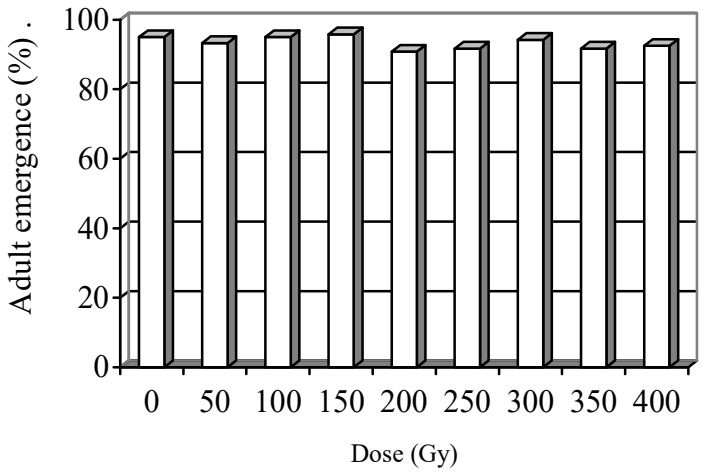

FIG. 1. Effects of gamma irradiation on adult emergence in G. mellonella late pupae.

Data on the effects of gamma radiation on female fecundity (mean number of eggs per female) are reported in Fig. 2. The results show that, within the 0 to 400 y dose range investigated, no effect on female fecundity is noticed when irradiated males were crossed to normal females (non-irradiated females). When normal males were crossed to irradiated females, however, the effect was obvious, particularly at 250 Gy and higher doses; irradiation caused a consistent decrease in the mean number of deposited eggs/females. For instance, while the mean number of produced eggs/female in the 1st kind of crosses (IM X NF) at 400 Gy was about 276 eggs/female, this number decreased to about 35% in crosses where females were the irradiated sex (NM X IF).

The effects of gamma irradiation on fertility of irradiated insects (males or females) crossed to un-irradiated individuals of the opposite sex are shown in Fig. 3. The results indicated that increasing irradiation dose caused consistent decrease in female fertility whether irradiated or crossed to irradiated male. The effect, however, was more severe when females were irradiated. For instance, fertility of irradiated males exposed to 200 Gy and crossed to un-irradiated females was about 37% and at 400 Gy dose, fertility was about 3%. In comparison, fertility of irradiated females exposed to 200 Gy and crossed with un-irradiated males was reduced to less than 12% and no egg hatch was noted at 250 Gy dose.

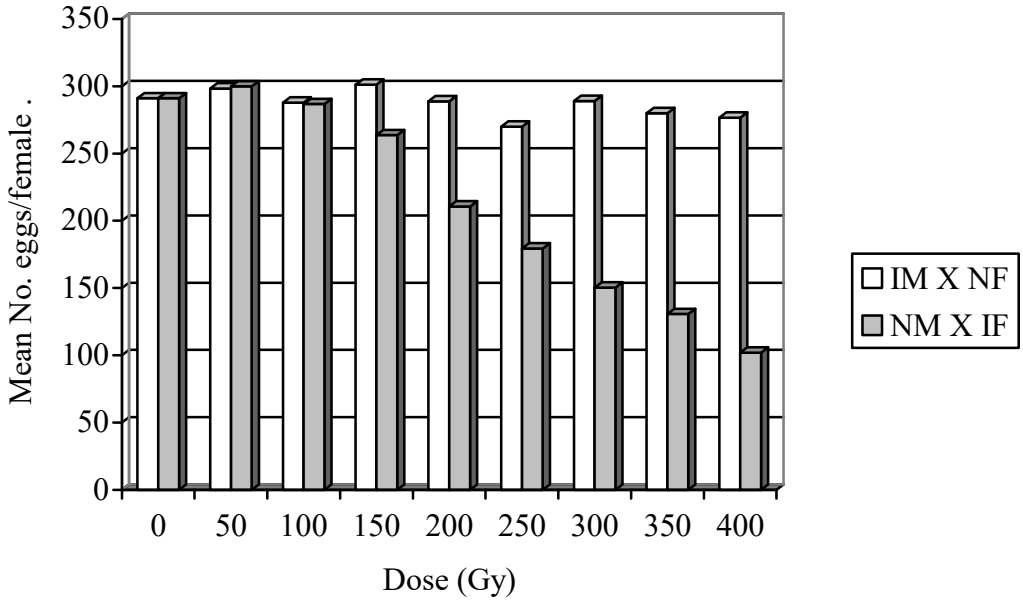

FIG. 2. Effects of gamma irradiation on fecundity in G. mellonella late pupae. Irradiated male crossed with normal female (IM X NF) and normal male crossed with irradiated female (NM X IF).

The effects of gamma irradiation on pupation of F1 insects (insects resulting from crossings between irradiated and normal insects) are presented in Fig. 4. The results presented in Fig. 4 clearly indicate that increasing irradiation dose consistently decreases the percentage of insects that were able to reach the pupal stage. The effect, however, was more severe when females were the irradiated sex. For instance, while 250 Gy dose caused complete death of F1 insects before pupation when females were irradiated (IF X NM), about 22% of F1 insects were able to reach the pupal stage when male pupae were irradiated (IM X NF).

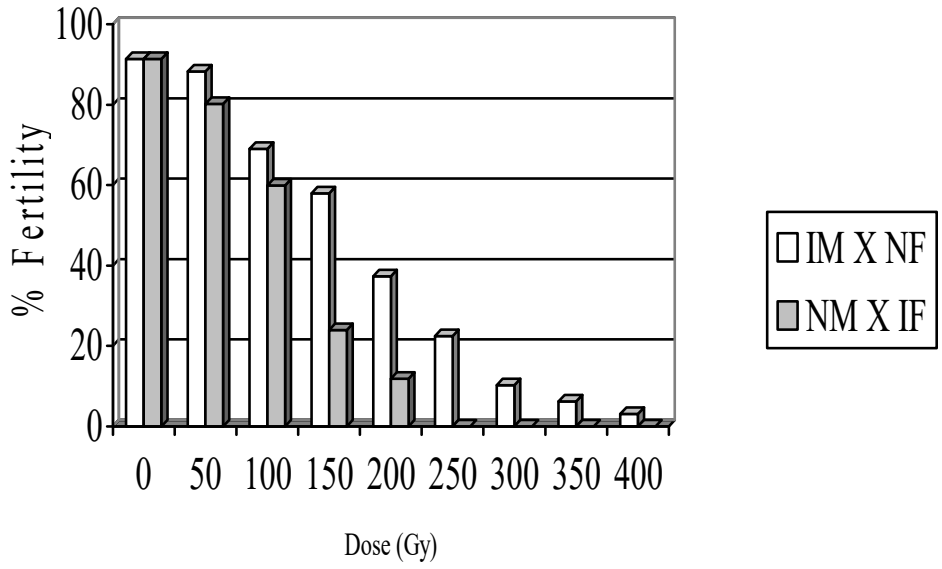

FIG. 3. Effects of gamma irradiation on fertility in G. mellonella late pupae.

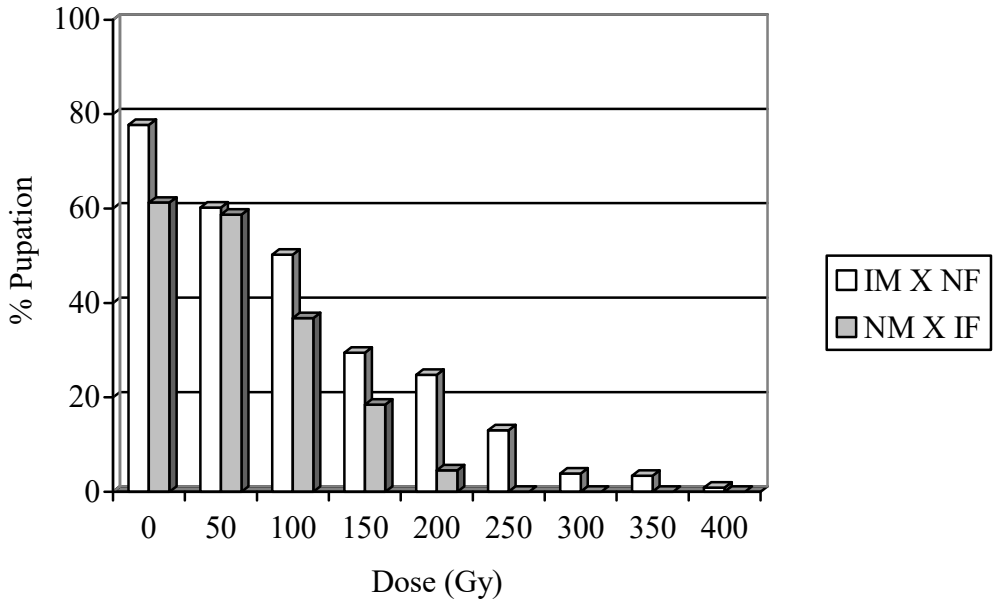

FIG. 4. Effects of gamma irradiation on pupation of F1 insects in wax moth late pupae.

Fig. 5. presents data on adult emergence of F1 insects resulting from parents irradiated in the late pupal stage. Similar to effects on pupation in F1 insects, the data shows that increasing irradiation dose caused consistent decrease in the % of insects that were able to reach the adult stage and that the effect was little higher when females were irradiated.

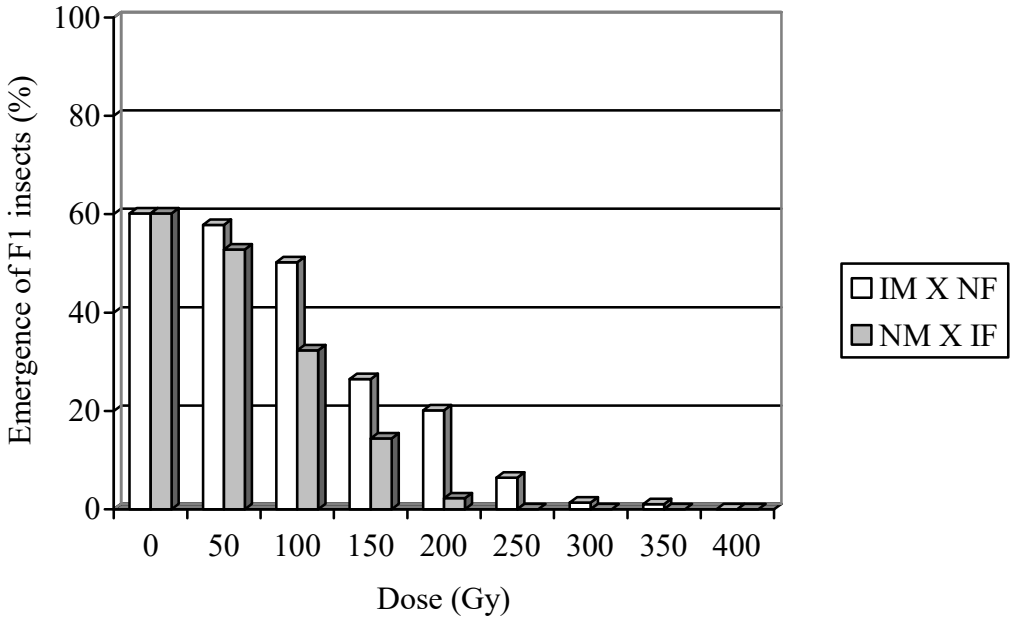

FIG. 5. Effects of gamma irradiation on adult emergence of F1 insects in wax moth late pupae.

Generally, the results of this study show that the radiosensitivity of *G. mellonella* late pupae increased with increasing radiation dose and the severity of the effect depends on the criterion used for measuring effectiveness. More specific, *G. mellonella* late pupae required a high dose when "prevention of adult emergence" is used as the criterion for measuring effectiveness; 400 Gy did not significantly affect adult emergence. This indicates that preventing *G. mellonella* late pupae from reaching the adult stage requires a dose of ionsing radiation that is relatively too high (>400 Gy). The same could be said about egg hatch; 400 Gy did not completely stop egg hatch in crosses where males were the irradiated sex (IM X NF). However, when survival of F1 insects to the adult stage is used as the measure of efficacy [14], the results were more promising. A dose of 400 Gy applied to *G. mellonella* late pupae completely prevented any resulting insects from reaching the adult stage.

In summary, the results of this study provide data on the radiosensitivity of *G. mellonella,* late pupae. They also show that the use of gamma radiation as a phytosanitary treatment for honeycombs infested with *G. mellonella* late pupae requires a relatively low dose (250–400 Gy), provided that prevention of F1 insects from reaching the adult stage is used as the criterion for measuring effectiveness. This dose (250–400 Gy), depending on the criterion used for measuring effectiveness, is less than the suggested generic phytosanitary irradiation dose of 400 Gy for Lepidopteran larvae [15] and much lower than the maximum allowed dose for irradiation of food products in the USA [16]. It should be pointed out however, that a dose of 250–400 Gy may allow some F1 eggs to hatch and resulting larvae to develop causing some damage to stored honeybee combs. Although these F1 larvae would not reach maturity, such latent damage might not be acceptable.

17.7. STUDIES ON THE EFFECTS OF DOSE RATES ON WAX MOTH AND KHAPRA BEETLE EGG HATCH

17.7.1. Studies on the effects of dose rates on wax moth egg hatch

Results on the effects of low and high dose rates of gamma radiation on egg hatch in 121–144 hour-old *G. mellonella* eggs are presented in Fig. 6. This Figure clearly shows that, in both cases (irradiation at low and high dose rates) the radiosensitivity of *G. mellonella* eggs decreased with increasing radiation dose. A dose of 100 Gy significantly reduced egg hatch and 400 Gy completely prevented it. These results are in agreement with those reported by Mansour [17]. They are different, however, from those reported by Milcheva [18]. Part of the differences in egg radiosensitivity with the current study may be related to differences in dose rates, experimental techniques and genetic variability among different strains [2]. In addition, the actual delivered dose by Milcheva [18] was not measured and, consequently, dosimetry cannot be excluded as one of the possible factors in explaining the differences between the two studies. It should be pointed out, however, that it is difficult to relate all of these differences (65 Gy vs. 400 Gy) to the above-mentioned factors. In fact, Lepidopteron, due to their holokinetik chromosomes, are the most tolerant insects to ionizing radiation and species belong to the family Pyralidae, where this insect belongs, are the most tolerant ones in this order [19]. For instance, 350 Gy was needed to prevent egg hatch in "about to hatch" *E. kuehniella* eggs [20] and likewise with the Indian-meal moth *Plodia interpunctella* [21]. A slightly lower dose (300 Gy) was also required to cause a similar response in the almond moth, *E. cautella*, when eggs were irradiated [22].

The results in Fig. 6 also show that dose rate had no significant effect on radiation sensitivity. Statistical analysis showed that percentage egg hatch, at the examined dosages, were not significantly different regardless of the dose rate used; low and high dose rates had a similar effect. These results may seem to contradict results obtained by several previous researchers

[23–27]. Results obtained by these authors show that dose rate has a significant effect and that high dose rate gives a more radiation induced effect than low dose rate treatment whereas in this study no significant difference in effect was observed for the two different dose rates used.

17.7.2. Studies on the effects of dose rates on Khapra beetle egg hatch

Fig. 7 presents data on the effects of two different dose rates on egg hatch in 49–72 hour-old Khapra beetle eggs ("about to hatch" eggs). Similarly, the Figure clearly shows that the radiosensitivity of *T. granarium* eggs decreased, in both cases (irradiation at low or high dose rates), with increasing radiation dose. A dose as low as 25 Gy significantly reduced egg hatch and 100 Gy dose completely prevented it. These data are in agreement with those reported in earlier studies [23–27], our data indicate that dose rate had no significant effect on egg hatch, at the examined dosages and for the two dose rates studied.

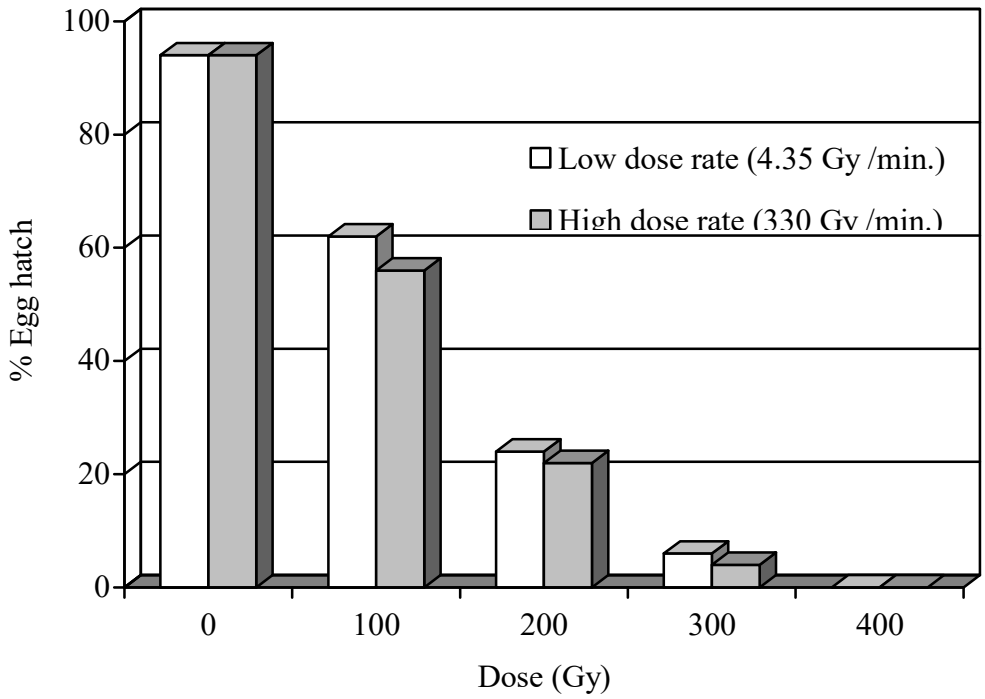

FIG. 6. Effects of low and high dose rates of gamma radiation on egg hatch in G. mellonella eggs irradiated few hours before egg hatch (121–144 hour-old).

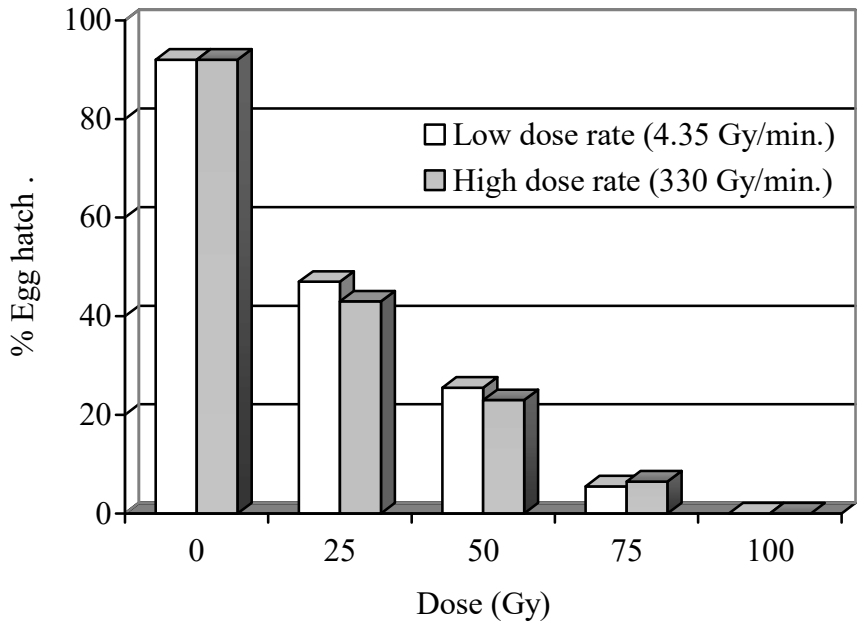

FIG. 7. Effects of gamma irradiation on egg hatch in T. granarium eggs irradiated few hours before egg hatch (49–72 hour-old).

Dose rate has frequently been cited as one of the factors that affect the degree of damage caused by ionizing radiation to biological systems; the higher the dose rate the more is the biological effect [23–27]. The classical dose rate effect results, as often explained, from the repair of DNA damage that occurs during radiation exposure [26]. It should be pointed, however, that the dose rate effect is most dramatic between 0.01 and 1 Gy/minute [26]. Above this dose rate range, the survival curve changes only a little, if at all, with dose rate [24]. As phytosanitary irradiation treatments generally involve much higher doses (hundreds of grays in most cases) that are usually delivered at much higher dose rates, the dose rate effect may not be an important factor to consider in phytosanitary irradiation.

17.8. CONCLUSIONS

Results on the effects of gamma irradiation on wax moth late pupae showed that the radiosensitivity of *G. mellonella* increased with increasing radiation dose. In addition, the results of studies comparing the effects of low and high dose rates on egg hatch in *G. mellonella* and *T. granarium* did not show any significant differences.

The results of this study also show that the use of gamma irradiation as a disinfestations treatment for honeybee combs potentially infested with *G. mellonella* late pupae is possible. The radiation disinfestations technique is safer than treating with chemicals and the required dose should not cause any undesirable changes in the treated product. The technique is very reliable, provides a quick and homogenous treatment and there is no fear of the pest developing resistance. The results also show that dose rates, within the range used for phytosanitary treatment, have no significant effect on the efficacy of the treatment.

ACKNOWLEDGEMENT

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18. COMPARATIVE STUDY OF DIFFERENT IRRADIATION TECHNOLOGIES (GAMMA, ELECTRON BEAM AND X RAYS) TO TREAT MEALS-READY-TO-EAT AND FRUITS AND VEGETABLES

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Abstract

Low (0.1, 0.5, 1 kGy), medium (2, 4, 6, 8 kGy) and high (11, 12, 13 kGy) radiation doses were applied for shelf extension of ready-to-eat meals (meals ready to eat, MREs) and for the preservation of fruits and vegetables using gamma (^{60}Co source), electron beam and X ray radiation. Their effects of the different modes of irradiation were compared for proximate composition and microbial analysis of the irradiated samples of MREs and fruits and vegetables. All the three mentioned irradiation technologies showed similar results for the same level of applied irradiation doses. In the low dose range the 1 kGy treatment gave best results in terms of microbial growth retardation of the irradiated MRE samples. Sprout inhibition for bulb and tuber foods (onion and potato) was successfully achieved at a dose of 50 Gy without significant changes in nutritional value. The astringency of mature fruits was removed by gamma or X ray irradiation at a dose of 0.9 kGy. In the medium dose range, 8 kGy was the most appropriate irradiation dose for shelf-life extension of the MRE resulting in a sixty-day storage period at ambient temperature. MRE samples (a spicy cooked beef dish, beef keema: 49% meat, 13% oil, 13% onion, 23% tomato, 2% spices by weight) were prepared and vacuum packed in 100 g pouches. Packed MRE samples were irradiated by gamma rays, X rays and electron beams at doses of 11, 12, 13 kGy by using ^{60}Co gamma irradiation source, gamma radiation source (Hungarian Made ISSO GAMMA LL Type) having a dose rate 3.60 kGy/hour and irradiation time of the samples were 3 hours and 3 minutes, 3hours and 20 minutes and 3hours and 37 minutes respectively for the mentioned target doses at an ambient temperature 28^{o}C. Electron beam and X ray irradiation was performed with an accelerator (10 MeV, for electrons beams and 5MV for X rays). The beam currents were 5.5, 6, and 6.5 mA for 11, 12, and 13 kGy respectively. Total Viable Counts (TVC) significantly decreased in MREs irradiated to these high doses. The overall observed TVC was 4.69 to 4.21 $\log_{10}$CFU/g for control and irradiated MRE samples respectively at the time equivalent to immediately after irradiation. However, control samples of MREs spoiled by day 30 ($\geqslant$8.0 $\log_{10}$CFU/g) whereas irradiated MRE samples had approximately 4.6 and 5.1 $\log_{10}$CFU/g TVC by storage day 60 and 90 respectively.

18.1. INTRODUCTION

Read to eat meals (meals ready to eat, MRE) are prepared as food for special target groups like immunocompromised cancer patients, emergency and disaster victims of earthquake and flood etc. The pre-packaged food can be highly sterilized with ionizing radiation, which kills microbes that cause spoilage and robust packaging protects the MRE from contamination post treatment. Shelf-life extension of MRE through using this nuclear related technique is a physical

process as compared to other processing technologies. The use of ionizing radiation has an advantage in that it does not significantly increase temperature of the irradiated food as compared to other processing techniques like heating. Thus, the irradiated MRE maintains its flavour, taste and nutritional value at least up to three months storage at room temperature [1].

In order to utilize irradiation as tool for food safety in the country, food irradiation activities were initiated by Pakistan Atomic Energy Commission (PAEC). NIFA, Peshawar is the only institute in the Islamic Republic of Pakistan conducting research and development work in the discipline of food irradiation. A ^{60}Co gamma irradiator was installed at NIFA by IAEA in 1983. The main focus was to develop techniques for the conservation of food resources, to minimize post-harvest losses and enhance food safety, security and trade. The majority of food and agricultural products treated by irradiation are processed in facilities using gamma irradiation from ^{60}Co as the source of ionizing radiation. With the advent of machine generated radiation sources like electron beams and X rays, the focus of food product irradiation is shifting as more commercial scale electron beam and X ray facilities are being established.

The standards of the Codex Alimentarius Commission currently limit the maximum electron energy and nominal X ray energy for the purpose of food irradiation, for example, X rays generated from machine sources operated at or below an energy level of 5 MeV, and electrons generated from machine sources operated at or below an energy level of 10 MeV. In this study we have compared the effects of electron beam and X ray irradiation of food to those of gamma irradiation, as these relatively novel sources of ionizing irradiation are utmost importance for the future and a comparison will help support uptake by the food industry. It is prerequisite need of internationally coordinated research to stimulate the development of machine sources and to establish the conditions that could broaden the choice of technologies to irradiate food. The aim of this CRP was therefore to coordinate research and development (R&D) activities that are prerequisite for practical implementation of process using novel radiation technologies and to unlock the potential of machines for the radiation treatment of agricultural and food products [2].

Presently, food trade from Pakistan is encountering various barriers to SPS (Sanitary and Phytosanitary) and Technical Barriers to Trade (TBT) agreements of WTO. To overcome the quarantine related problems in food exports a viable alternative of chemical fumigants is needed, an important feature of irradiation is its ability to achieve different types of beneficial effects (sanitary, phytosanitary and shelf-life extension) on a wide range of different products. The data generated regarding use of food irradiation using ^{60}Co as sanitary and phytosanitary treatment is beneficial to food exports for using the commercial irradiators being installed in the country. These new commercial irradiators include accelerators. Therefore, this R&D work was carried out using electron beam and X ray radiation. In addition, post-harvest handling, quality issues and the preservation of fruits and vegetables is of prime importance and great economic value, especially to the forthcoming WTO regulations [3].

18.2. MATERIALS AND METHODS

18.2.1. Sample preparation

Fresh raw meat was purchased from the local market, after cleaning and physical examination the following steps were followed in the preparation of the beef keema meal and irradiation of this MRE.

18.2.1.1. Cooking

The meat was cooked with oil, onion, tomato, spices and garlic at 70ºC for 20 minutes. The cooking time needed to reach 70ºC internal core temperature was optimized by trial and error. After cooking, the samples were cooled at room temperature to 25ºC. The temperature was monitored with scientific thermometer (made of USA ACC2457).

18.2.1.2. Packing

Cooked meal ready to eat (MRE) samples were packed in vacuum tetra pack aluminum pouches using a vacuum sealer machine (HENKELMAN, Model: B100608477) according to the weight composition of 20 g meat, 5 g oil, 10 g onion, 10 g tomato, 2.5 g spices and 2.5 g garlic and transfer for irradiation after cooling to the normal temperature.

18.2.1.3. Irradiation

Packaged MRE samples were irradiated by gamma rays, X rays and electron beams at doses 0, 11, 12, 13 kGy by using ^{60}Co gamma irradiation source (Hungarian Made ISSO GAMMA LL Type) installed at Nuclear Institute for Food and Agriculture (NIFA) Peshawar, Pakistan (Fig. 1), having dose rate 3.60 kGy/hour and irradiation time of the samples were 3 hours and 3 minutes, 3 hours and 20 minutes and 3 hours and 37 minutes respectively for the mentioned target doses at an ambient temperature of 28ºC. Fricke Dosimetry System is used for the dosimetry of the radiation source.

FIG. 1. Gamma radiation source used to irradiated MRE samples at NIFA, Peshawar.

Electron beam and X ray irradiation was performed at a commercial irradiation facility with a DD type Electron-Beam-Accelerator (10 MeV, 5MV) at the Pak Electron Beam Irradiation (Pvt.) Ltd. Port Qasim, Karachi Pakistan (Fig. 2). The beam currents were 5.5, 6, and 6.5 mA for 11, 12, and 13 kGy, respectively.

FIG. 2. The irradiation of MRE Samples at Pak electron beam, Karachi.

18.2.2. Microbiological analysis

18.2.2.1. Microbiological Assessment

The irradiated vacuum packed MRE samples in polyethylene pouches were analysed for total viable count fortnightly for a period of 90 days storage at room temperature.

18.2.2.2. Total Viable Counts

Total viable microbial counts (TVC) were determined by the dilution plate method using nutrient agar media: 10 g of sample was taken in 90 ml sterilized peptone water and thoroughly mixed (1:10) dilution. Further dilutions were made in similar way as (1:102), (1:103) and (1:104) respectively. Nutrient agar medium was used as culture media. The culture media was sterilized and cooled back to 45°C in a water bath. One millilitre of each dilution of diet samples (in triplicate) was poured into sterilized petri plates. After this, 15–20 ml of nutrient agar medium was added to each plate. The plates were shaken and kept for some time to solidify the culture media, inverted and incubated for 24–48 hours at 27°C. The colonies were counted by means of colony counter and TVC was calculated by multiplying average number of colonies by the dilution factor and reported as number of colonies per gram of sample [4].

18.2.3. Proximate compositional analysis of MRE

Proximate analyses (Weende analyses) were performed for the determination of moisture, crude protein (total nitrogen), crude fibre, ash and nitrogen-free extract content of the control and irradiated MRE samples [5].

18.2.3.1. Crude Protein%

Method

(a) Weighed 1 g of sample and placed in the Kjeldahl flask.
(b) Added 1 g mixture of potassium sulphate & mercuric oxide and 7 ml concentrated sulphuric acid.
(c) Flask is placed in the digester for the digestion, retained until the solution is clear; continue to heat 30 minutes more.
(d) Leave to cool, gradually adding approximately 90 ml distilled, de-ionized water.
(e) Quickly connected the flask to the distillation unit, heated and collected the ammonia in 50 ml titration flask containing boric solution.
(f) At the end of distillation, removed the receptor flask, rinse the end of the condenser and titrated the solution with the standard hydrochloric acid (HCl) solution.

18.2.3.2. Crude Ash%

Method

(a) Weighed 2–5 g of dry sample in a crucible.
(b) Placed the crucible in a furnace and heat at 550°C for 12 hours.
(c) Leave to cool and transfer to a dryer.
(d) Carefully weighted the crucible again with the ash.

18.2.3.3. Crude Moisture%

Method

(a) Weighed out approx. 5–10 g of MRE grounded samples.
(b) Placed the sample in drying oven at 105 °C for at least 12 hours.
(c) Allowed the sample to cool in dryer.
(d) weighed again, avoided the samples from exposing to the atmosphere.

18.2.3.4. Crude Fat%

Method

(a) Weighed 3–5 g of dried MRE samples in an extraction thimble, handling it with tongs and placed in the extraction unit.
(b) Connected the flask containing petroleum ether at 2/3 of total volume to the extractor.
(c) Brought to boil and adjust heat to obtain about 10 refluxes per hour.
(d) Evaporated the ether by evaporator.
(e) Cooled the flasks in a dryer and weighed them.

18.2.4. Astringency removal from persimmon fruit

Irradiation doses of 0.3, 0.6, 0.9, and 1.2 kGy were applied to persimmon fruit samples in three replicates after sorting and proper packing (Figs. 3 and 4) using gamma radiation (^{60}Co) and electron beam or X ray radiation technology. The effect of radiation on the physiochemical properties (total phenols, ascorbic acid, acidity and total soluble solids) of persimmon fruits were studied by the Folin Ciocalteu method.

FIG. 3. Sorting of Persimmon Fruits.

FIG. 4. Packing of Persimmon Fruits for Irradiation.

18.2.5. Sprout inhibition in bulbs and tubers

Storage stability and nutritional analysis of gamma-ray and X ray irradiated local variety of potato ‘Cardinal’ and onions (Fig. 5) were carried out to evaluate effect of both gamma and

X ray irradiation technologies on sprout inhibition. The potato samples were irradiated with doses of 50, 100 and 150 Gy by applying the above-mentioned technologies. The samples were stored at ambient temperature for at least 60 days.

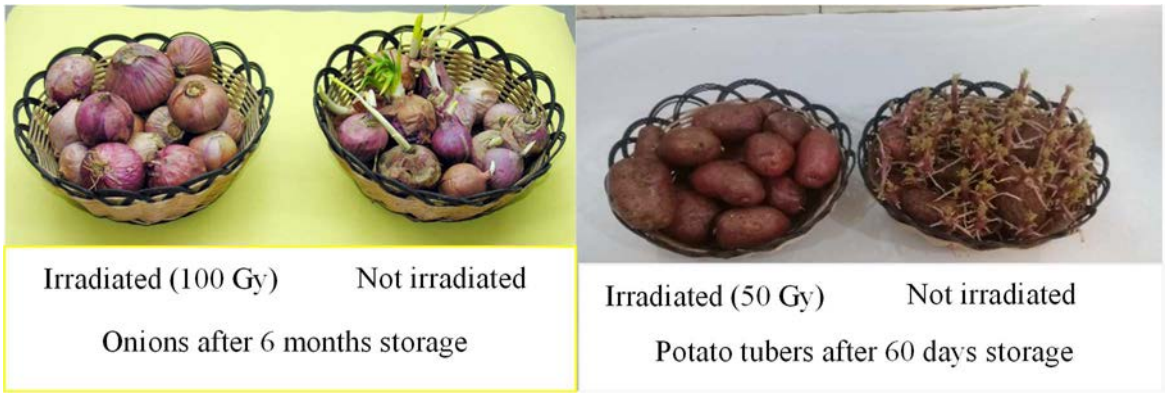

FIG. 5. Sprout inhibition in bulb and tuber foods.

18.3. RESULTS AND DISCUSSION

18.3.1. Beef keema MRE

There was no significant difference in crude protein, moisture, ash, fat and nitrogen contents of irradiated and control (non-irradiated) samples among all treatments over the 90-day storage period at 28ºC. Slight variations in moisture content of irradiated samples were observed during storage at room temperature (28ºC). High dose irradiation using gamma, electron beam or X rays was found to be equally as effective at maintaining the microbial counts below the permitted level during the entire storage period.

Irradiation doses of 11, 12 and 13 kGy from gamma, electron beam and X rays were applied to MRE samples, sensory evaluation of irradiated MRE food were carried out (Table 1). No significant change was observed for the different modes of irradiation technologies (gamma, electron beam and X rays) and high energy X ray irradiation may serve as an alternative to gamma or electron beam irradiation. Of the irradiation doses studied, the 12 kGy dose treatment showed good overall acceptability of the irradiated MRE samples. Proximate compositional analysis of the irradiated MRE food showed slight variations among the treatments during the storage period of 90 days. The differences in protein, moisture, ash, fat and nitrogen contents between irradiated and non-irradiated samples among all treatments were not found to be significant. Variations in the moisture content of irradiated samples were observed at room temperature at the third month of the storage period. The TVC value immediately after irradiation and at intervals during the 90-storage time are shown in Table 2. Irradiation decreased TVC significantly from an overall observed value of 4.69 $\log_{10}$CFU/g in unirradiated control samples to approximately 4.21 $\log_{10}$CFU/g in irradiated MRE samples at the start of the storage test. In general, the TVC increased with increasing storage period (up to 90 days). The TVC of cooked meat increased dramatically by a factor of 100 (2 log unit increase) from storage day 0 to storage day 15 and by day 30 these unirradiated control samples were spoiled. In contrast, none of the irradiated samples spoiled over the duration of the storage test but the TVC of all irradiated samples increased by approximately a factor of 10 (1 log unit increase) from day 0 to day 90 of the storage test. No significant differences were observed between the

different irradiation technologies (gamma, electron beam or X ray) for all the treatments during the entire storage period of the irradiated MRE food at ambient temperature.

18.3.2. Sensory evaluation

The MREs were prepared by heating and were evaluated for their sensory qualities (appearance, Odor, texture and overall acceptance) by a panel of 10 judges during 90-day storage at room temperature. Each sample was evaluated by using a 9-point hedonic scale (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much and 1 = dislike extremely). Sensory evaluation was on the initial day and repeated at 30, 60 and 90 days, respectively. The samples were coded using standard random numbers. The panelists were free to judge samples using up to three tastings. These sensory tests were performed under normal laboratory light conditions at a room temperature of 28ºC.

TABLE 1. OVERALL ORGANOLEPTIC EVALUATION RESULT

	Storage (Days)	Control (0 kGy)	Electron beam			X ray			Gamma-ray		
			11 (kGy)	12 (kGy)	13 (kGy)	11 (kGy)	12 (kGy)	13 (kGy)	11 (kGy)	12 (kGy)	13 (kGy)
Appearance	0	9.0	8.9	8.7	8.7	8.9	8.7	8.6	8.8	8.7	8.7
	30	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	8.2	7.2
	60	6.2	6.2	6.2	6.2	6.2	6.2	6.1	6.2	6.2	6.2
	90	5.1	5.1	5.1	5.1	5.2	5.1	5.1	5.1	5.1	5.1
Odour	0	9.0	8.7	8.7	8.7	8.8	8.7	8.6	8.8	8.7	8.7
	30	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
	60	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1
	90	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1
Texture	0	9.0	8.9	8.7	8.7	8.7	8.7	8.7	8.8	8.7	8.7
	30	7.1	7.1	7.1	7.1	7.2	7.1	7.1	7.1	7.1	7.1
	60	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1
	90	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1
Overall Score	0	9.0	8.8	8.7	8.7	8.8	8.7	8.6	8.9	8.7	8.7
	30	6.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
	60	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1
	90	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1

18.3.3. Crude Protein%

This study showed that, 11, 12 and 13 kGy doses induce only a small breakdown of food proteins into lower molecular weight protein parts and amino acids (Fig. 6). Also, the reported decrease in pectinase activity which is the most sensitive enzyme to irradiation at 20 kGy was in the range 20% to 50%. As a result, experiments indicated that such treatments cause less chemical reactions than steam heat sterilization [6].

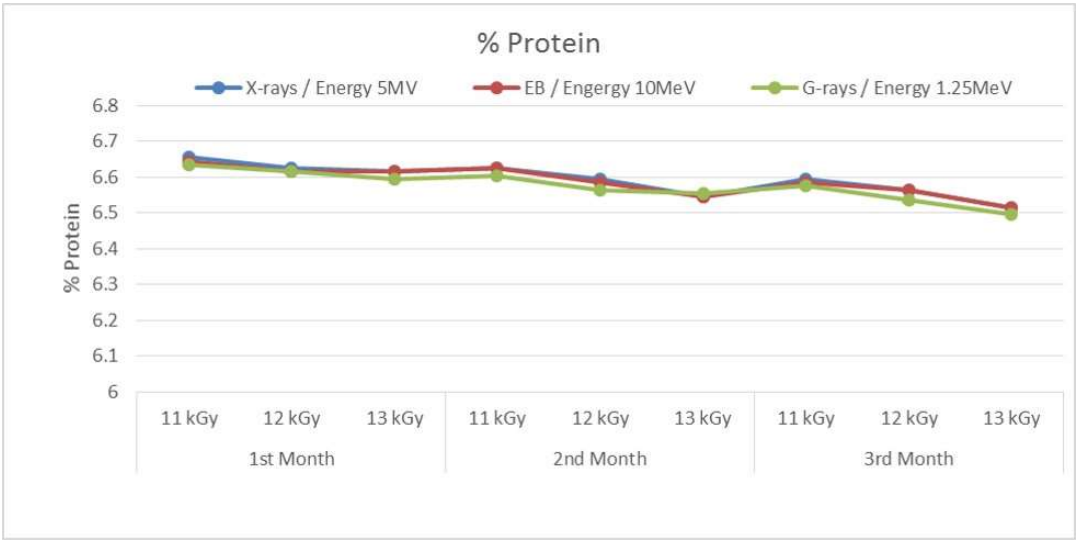

FIG. 6. Percentage protein of irradiated MRE samples over the storage period.

18.3.4. Crude ash

The ash content of meat after irradiation is presented in graphical form below (Fig. 7). The result showed that the ash content of meat was dose dependent of irradiation and decreased.

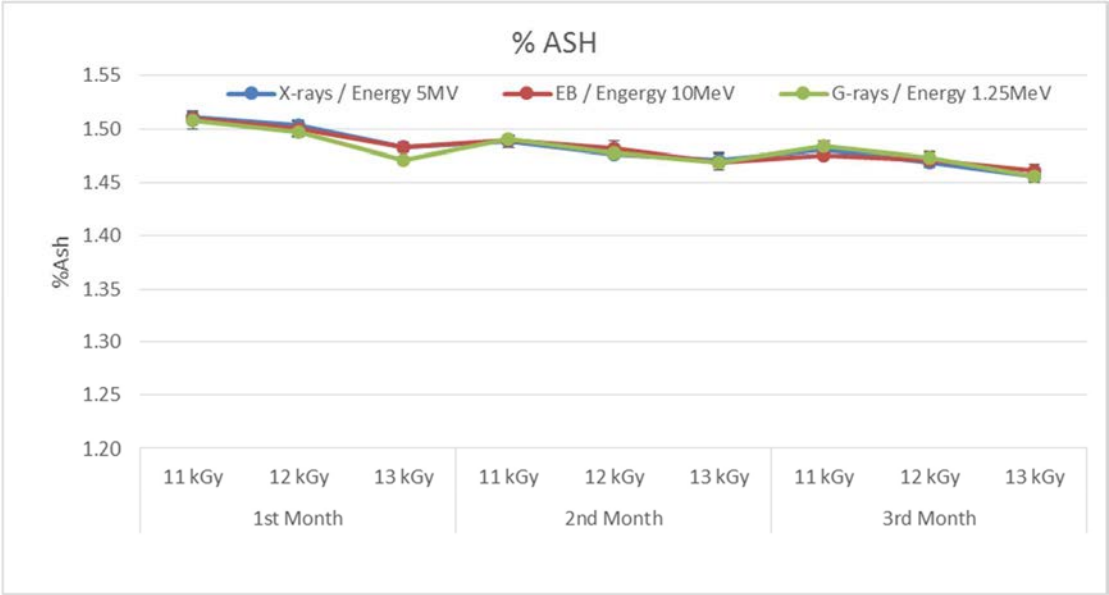

FIG. 7. Shows % ASH of irradiated MRE samples during storage period.

18.3.5. Crude %moisture

Our results indicate that moisture content increases with irradiation doses 11, 12 and 13 kGy having corresponding increase in values of water content 0.1% and 0.3% for 12 and 13 kGy as compared to 11 kGy for the first month of storage at room temperature (Fig. 8). During the second and third month, storage period the moisture content decreases with the passage of time for two months.

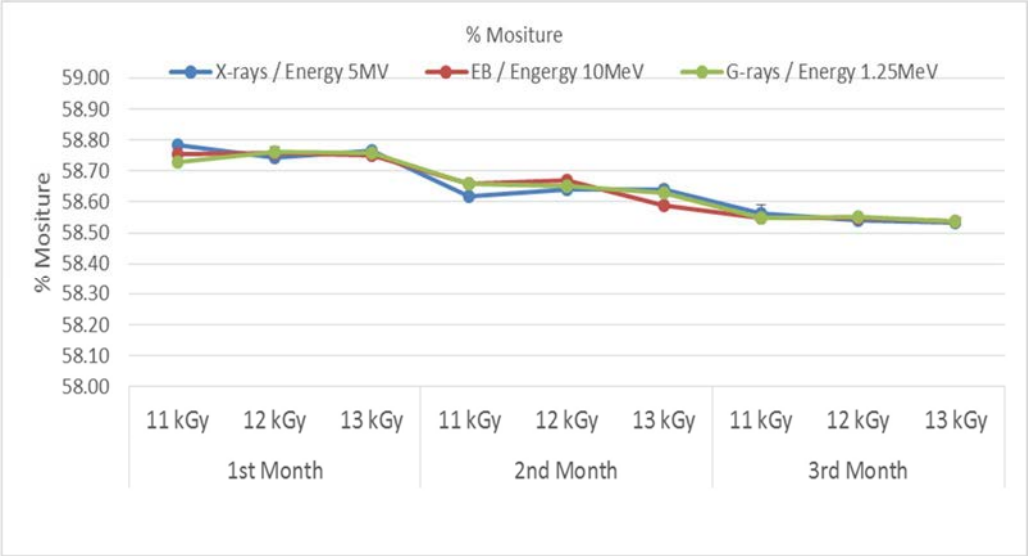

FIG. 8. Shows % Moisture of irradiated MRE samples during storage period.

18.3.6. Crude fat%

In the irradiated MRE samples the content fat% were slight increase for doses from 11 kGy and 12 kGy to 13 kGy by 0.10% for the first month independently of storage temperature. The variation in the fat% of the irradiated samples were observed in the range 0.11% to 0.20% for the second and the third month storage as compared to the first month storage at room temperature (Fig. 9). The high radiation doses cause more changes in lipids, mainly in the matrix with unsaturated fatty acids, which are more susceptible to oxidation [7].

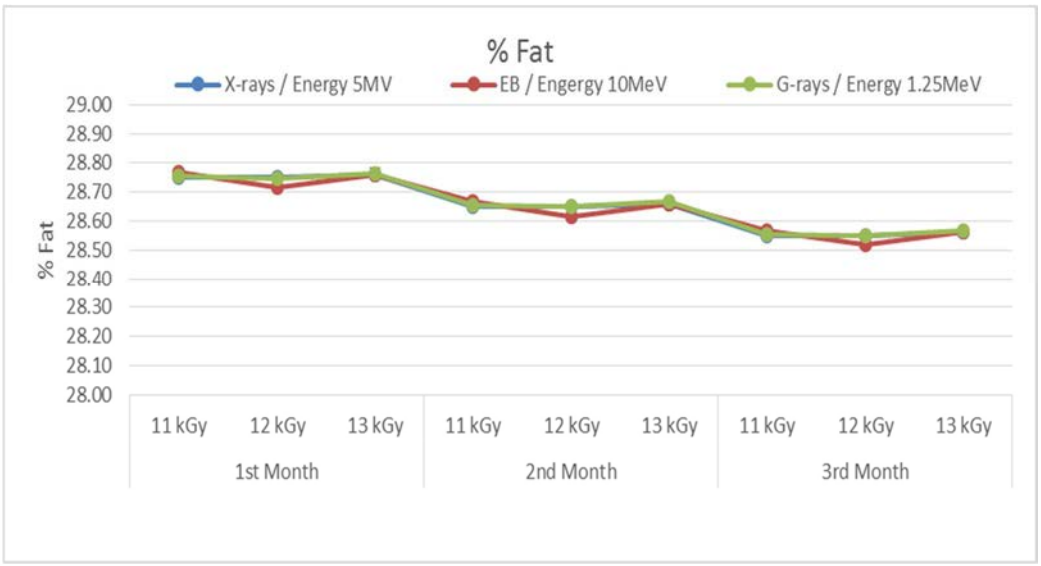

FIG. 9. Shows % Fat of irradiated MRE samples during storage period.

18.3.7. Crude nitrogen%

During the storage period of second and third month slight decrease was observed in the nitrogen component at room temperature the volatile basic nitrogen is related to protein breakdown and the observed increase through the storage periods may be attributed to the formation of ammonia or other basic compounds due to microbial activity (Fig. 10) [8].

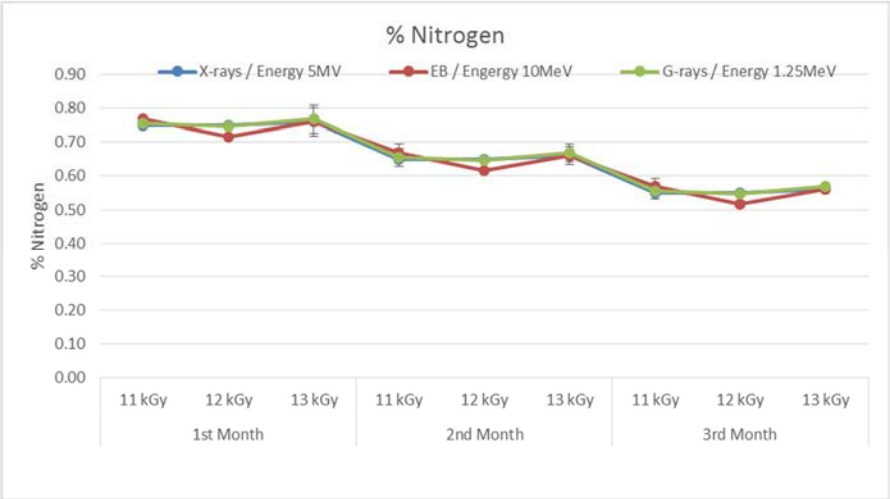

FIG. 10. Shows % Nitrogen of irradiated MRE samples during storage period.

18.4. MICROBIOLOGICAL ANALYSIS

18.4.1. Total viable count

Total viable count (TVC) values were determined and the values after irradiation and at different days-of-storage intervals are shown in Table 2. Irradiation decreased the TVC significantly from the overall observed value was 4.7 $\log_{10}$CFU/g for unirradiated control samples on day zero to approximately 4.1 $\log_{10}$CFU/g for 13 kGy irradiated MRE samples on day zero. The TVC of all samples increased over the storage period of up to 90 days. Unirradiated control samples increased from 4.7 to 6.7 $\log_{10}$CFU/g from day 0 to day 15 and had spoiled by day 30. Spoilage was defined as results of total bacterial populations greater than or equal to 8 $\log_{10}$CFU/g [9]. The TVC of all irradiated samples increased at a much slower rate than the control samples and regardless of irradiation dose were 5.1 to 5.2 $\log_{10}$CFU/g by day 90. This indicates that irradiation to 11 to 13 kGy has considerably extended the useful shelf-life of the MRE.

TABLE 2. MICROBIAL COUNTS OF READY TO EAT MEALS (MRE) AFTER GAMMA-RAY, ELECTRON BEAM AND X RAY IRRADIATION DURING STORAGE AT 28ºC ROOM TEMPERATURE

Storage period (days)	Total viable counts ($\log_{10}$CFU/g)									
	Control (0 kGy)	Electron beam			X ray			Gamma-ray		
		11 (kGy)	12 (kGy)	13 (kGy)	11 (kGy)	12 (kGy)	13 (kGy)	11 (kGy)	12 (kGy)	13 (kGy)
0	**4.7**	**4.3**	**4.2**	**4.1**	**4.3**	**4.2**	**4.1**	**4.3**	**4.2**	**4.1**
15	**6.7**	**4.4**	**4.2**	**4.2**	**4.4**	**4.3**	**4.2**	**4.4**	**4.3**	**4.2**
30	NC	**4.3**	**4.4**	**4.3**	**4.4**	**4.4**	**4.3**	**4.5**	**4.4**	**4.2**
45	NC	**4.6**	**4.5**	**4.4**	**4.6**	**4.5**	**4.4**	**4.6**	**4.4**	**4.4**
60	NC	**4.7**	**4.6**	**4.5**	**4.6**	**4.6**	**4.5**	**4.6**	**4.6**	**4.5**
75	NC	**4.8**	**4.7**	**4.5**	**4.8**	**4.6**	**4.8**	**4.9**	**4.8**	**4.7**
90	NC	**5.2**	**5.1**	**5.1**	**5.2**	**5.2**	**5.1**	**5.1**	**5.1**	**5.1**

NC, not countable because the samples had spoiled ($\geqslant$8.0 $\log_{10}$CFU/g).

18.5. CONCLUSION

The effect of high dose irradiation using gamma rays, electron beams and X rays was investigated on the physical, chemical, and bacteriological parameters of vacuum-packed beef MRE after 90 days of storage at room temperature. All samples were packaged in polyethylene bags containing aluminum layer to exclude light. Compositional analysis including protein, ash, moisture, fat and nitrogen along with microbial analysis were carried out during the storage period of three months at room temperature. The preservation method used was effective in maintaining the microbial counts below the permitted level during the entire storage period. This study concluded that, among the treatments studied, high dose irradiation with storage at room temperature showed potential for the preservation of MRE products made from beef meat, to provide foods safe for consumption.

ACKNOWLEDGEMENTS

We are highly thankful to International Atomic Energy Agency (IAEA) for the financial support for this R&D work carried out under the Coordinated Research Project contract agreement 19231 with Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan Atomic Energy Commission (PAEC). We also appreciate and acknowledge the irradiation services provided by Pak Electron Beam Irradiation Pvt, Ltd, Karachi Pakistan.

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Dose Intercomparison Exercise

19. IRRADIATION AND DOSE MEASUREMENT PRACTICES – DOSIMETRY INTER-COMPARISON STUDY

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Abstract

The results of an irradiation and dose measurement inter-comparison study are presented. This study was undertaken in the framework of CRP D61024 on the Development of Electron Beam and X ray Applications for Food Irradiation (DEXAFI). Fourteen laboratories from twelve countries participated in this intercomparison exercise. The main purpose of the inter-comparison was to check or improve the level of confidence in the doses stated in the reports and publications of the CRP participants.

The method used for this study was based on verifying the accuracy and precision of the participant's dosimetry systems by comparison to a reference dosimetry system with guaranteed traceability.

Two performance indicators were evaluated: (1) Ability of participants to meet pre-set dose values, and; (2) Ability of the participants to measure doses actually applied.
Half of the participants demonstrated that both, their irradiation facility and dosimetry system are well qualified and calibrated with traceability to an international or national absorbed dose standard.

Participants were encouraged to perform an audit of their metrology system(s).

19.1. INTRODUCTION

When collaborative research is performed in the field of radiation processing, especially when different irradiation modalities are used, it is of prime importance to efficiently control and measure the key parameter of the process, namely: the absorbed dose.

Comparison of the research outputs, moreover between different laboratories, is relevant only if:

- Dose measurements are traceable to (inter)national standards;
- Radiation sources are well characterized and qualified;
- Dose distributions inside the treatment units (samples, products, …) are assessed and DUR (Dose Uniformity Ratio) minimized.

As a first step in this direction, the laboratories involved in the DEXAFI CRP decided to conduct two dose inter-comparison studies.

Proficiency testing programs by inter-laboratory comparisons have proven to be a useful tool to give opportunity for those that participate to demonstrate their technical competence and ensure the quality and traceability of their measurements. Participation may also help to review procedures and may identify problems related to the performance of personnel, equipment calibration and the adequacy of methods. The main purpose of this inter-comparison was to provide an opportunity for participants to check or improve the level of confidence in the doses

stated in their reports and publications [1]. The method used in the study was based on the verification of the accuracy and precision of the participant's dosimetry systems compared to a reference dosimetry system with guaranteed traceability.

Thus, this inter-laboratory comparison exercise was developed to evaluate two performance indicators:

Part 1: The ability of participants to meet pre-set dose values (targeted doses);

Part 2: The ability of the participants to measure doses actually applied.

Fourteen laboratories from twelve countries volunteered and participated in this exercise. Confidentiality was ensured by keeping participants' names, data and results anonymous.

It should also be noted that this exercise does not concern the dose given to an irradiated product, but the dose given and measured by dosimeters.

19.2. MATERIALS AND METHODS

19.2.1. Testing dosimeters

The proficiency test reference dosimeters used were alanine dosimeters (containing 4 pellets each) and these were sent to each participant. These reference dosimeters were provided by Aerial [2].

Only one of the provided alanine dosimeters was irradiated for each target dose value, and if necessary one routine dosimeter from the participant was also irradiated.

Aerial sent an invitation email to each of the DEXAFI participants that was willing to participate in the inter-comparison, and this was followed up with a mail package containing:

- 3 alanine dosimeters (each dosimeter containing four alanine pellets),
- 1 alanine dosimeter to preserve as control, which should not be irradiated,
- 1 irradiation form to be filled in by participant,
- 1 proficiency test presentation document.

Each participant was asked to measure and record the minimum dosimeter temperature measured at the start of irradiation process, using a calibrated thermometer. The were also instructed to record the maximum dosimeter temperature during irradiation as measured or evaluated throughout the irradiation process, using a thermometer, thermolabel, or other proceedures [2].

19.2.2. Irradiation geometry

In this study, irradiation modalities were limited to gamma (^{60}Co source), 10 MeV electrons and X rays (1 MV accelerating voltage minimum). Dosimeters were to be irradiated to doses (equivalent to water) of 1 kGy, 5 kGy and 10 kGy.

For photon irradiation [3]:

- To ensure electronic equilibrium, alanine and participant´s dosimeters were enclosed in 3 to 5 mm thickness water equivalent material such as polystyrene, PE or PMMA.

Alanine dosimeters as supplied were already placed in Delrin holders of 3 mm wall thickness.

For electron beam irradiation [4]:

- Alanine dosimeters were placed in square shaped polystyrene holders;
- During irradiation, dosimeter label was to be placed toward electron beam.

For both irradiation types, the irradiation geometry and container phantom design was to be defined by the participant. It must ensure that both, Alanine reference dosimeter and routine dosimeter from participant get the equivalent dose.

Measurements of dose from routine dosimeters belonging to the participant´s dosimetry system were made using the technique and equipment used by the participant in their normal routine work.

Participants were invited to return to Aerial the irradiated alanine reference dosimeters and the control dosimeter, along with the filled in “irradiation form" after irradiation was completed. The “irradiation form” was completed by participants as a way of providing the results of their measured dosimeter’s dose, temperature of alanine dosimeter before irradiation and maximum temperature during irradiation, irradiation date, irradiation duration, radiation type, participants details, related details and any other comments.

The key parameter to determine in this part of the study was the delivered dose to the dosimeters and the ability to target a given dose.

19.2.3. Statistical evaluation

Statistical software (XLSTATS v. 2018.4 (Addinsoft)) was used for calculating Z scores (corrected data minus outliers) and Mandel’s h and k test statistics [5].

19.2.3.1. Performance evaluator

Performance was evaluated according to the Z performance parameter (Equation 1):

$$Z = \frac{xi - X}{\sqrt{u(xi)^2 + u(X)^2}} \qquad (1)$$

Where:

xi is the dose value of the dosimeter irradiated by the participant and measured by Aerial (the reference laboratory);

X is the dose value given by the participant;

u(xi) is the uncertainty (k=2) on the dose measured by Aerial (the reference laboratory);

u(X) is the uncertainty (k=2) on the dose declared by the participant.

The Z score criteria for determining if participant’s results were acceptable were:

The result is considered acceptable if $-1 \leq Z \leq 1$;

The result is considered questionable if $1 < |z| < 2$;

The result is not acceptable if $|z| \geq 2$.

19.2.3.2. Mandel's h & k statistics

The h and k test statistics were calculated as they are measures for data consistency, particularly useful for this type of inter-laboratory study.

By studying the data deviations and accuracy, the performance of a laboratory can be established in terms of its reliability and errors.

The k value compares the repeatability standard deviation of a laboratory data set with the average of the repeatability standard deviations of all other laboratories. From the k value, we can evaluate the spread of the data set and its precision. This test statistic reflects the single lab's repeatability against the average repeatability of all participating laboratories. The larger the k value, the bigger is the data deviation, indicating the poorer the precision.

The h test statistic is used to examine the consistency of inter-laboratory data, confirming if any laboratory data is an outlier. In other words, it is to indicate the accuracy of a laboratory result against the other results reported.

The h test statistic value reflects the deviation of a single laboratory's mean test results from the overall mean results obtained from all participating laboratories. The larger the h value, the bigger the deviation, the poorer is the accuracy of that single laboratory.

19.3. RESULTS AND DISCUSSION

The results presented here are the outcomes of a second round of inter-comparisons within the DEXAFI CRP. The goal of this inter-comparison exercise was to evaluate the technical competence of the participants and to ensure the quality and traceability of both, their dose measurements using their own routine dosimetry system and their irradiation process control. Thus, the results are presented in two parts: The first one will discuss "assigned dose" versus "applied dose" (irradiation process control) and the second part discusses "measured dose" versus "applied dose" (routine dosimetry). Outlier tests were perofrmed (Grubbs test; α=5%) and outliers were excluded in all results given hereunder.

19.3.1. Assigned versus applied dose

Participants were asked to irradiation Alanine dosimeters to 3 target doses (1, 5 and 10 kGy). Figs. 1, 2 and 3 below show the Z Scores for all included laboratories (excluding outliers).

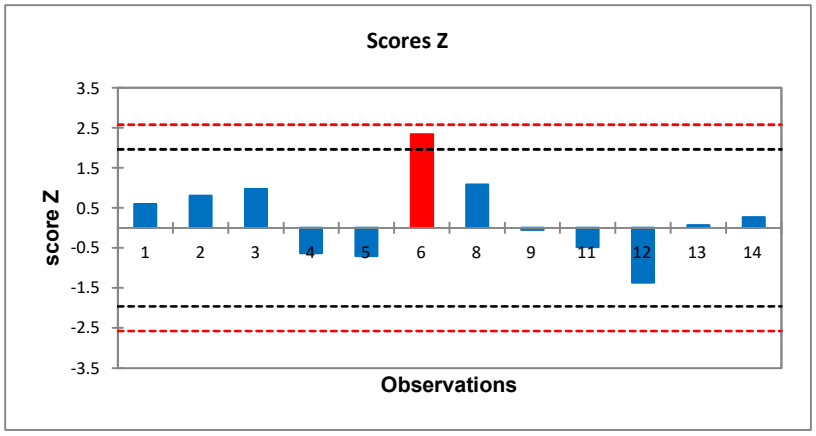

FIG. 1. The Z scores for 1 kGy assigned versus applied dose.

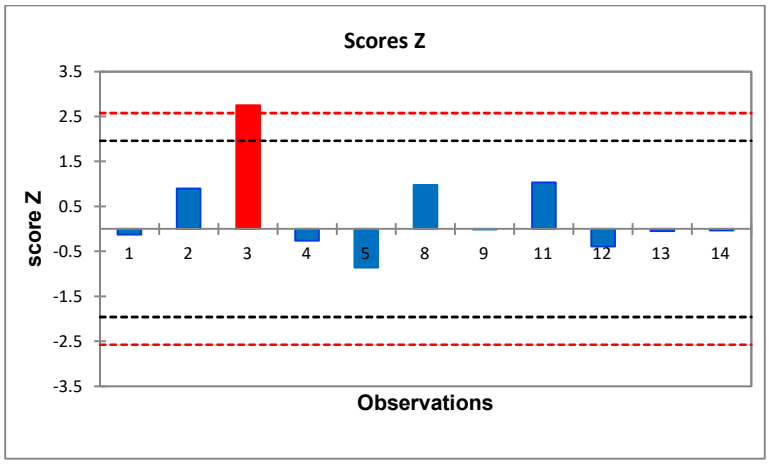

FIG. 2. The Z scores for 5 kGy assigned versus applied dose.

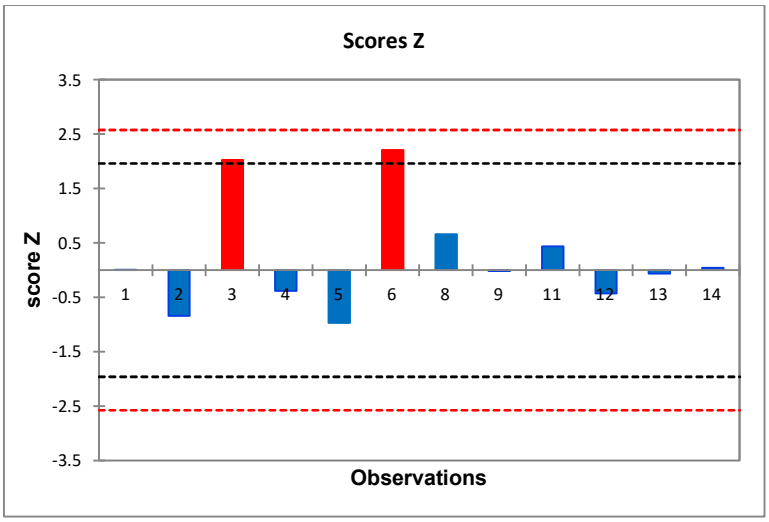

FIG. 3. The Z scores for 10 kGy assigned versus applied dose.

The Z scores of two participating laboratories (laboratory3 and laboratory 6 highlighted in red) indicate questionable results at the lower assigned dose of 1 kGy and not acceptable results for the higher assigned doses, demonstrating their difficulty to target a preset dose with acceptable accuracy.

19.3.2. Measured versus applied dose

Participants were asked to measure, with their routine dosimetry system, the actual dose given to the Alanine dosimeters while irradiating them at 3 different doses (1, 5 and 10 kGy). Figs. 4, 5 and 6 show the Z scores for all included laboratories (excluding outliers).

These data, excluding outliers, indicate that the only one participant laboratory shows questionable results (laboratory 6, highlighted in red). This might explain why the irradiation test at targeted doses (Figs. 1-3) was unacceptable as well for this laboratory.

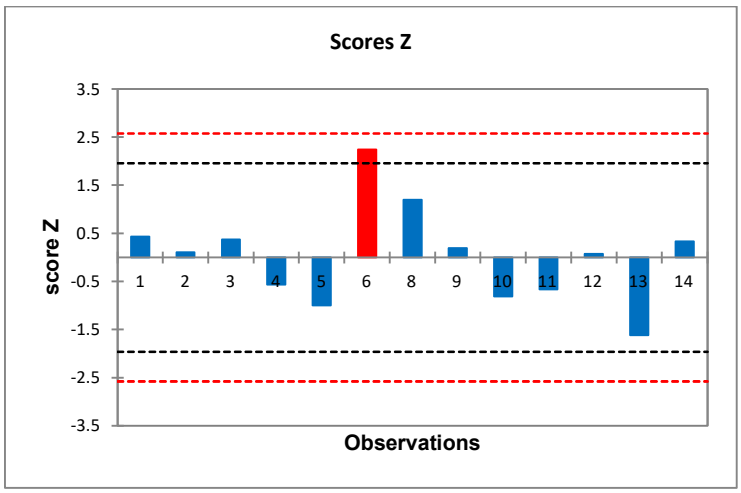

FIG. 4. The Z scores for 1 kGy irradiation measured with participant's routine dosimetry system and compared to actual alanine dose.

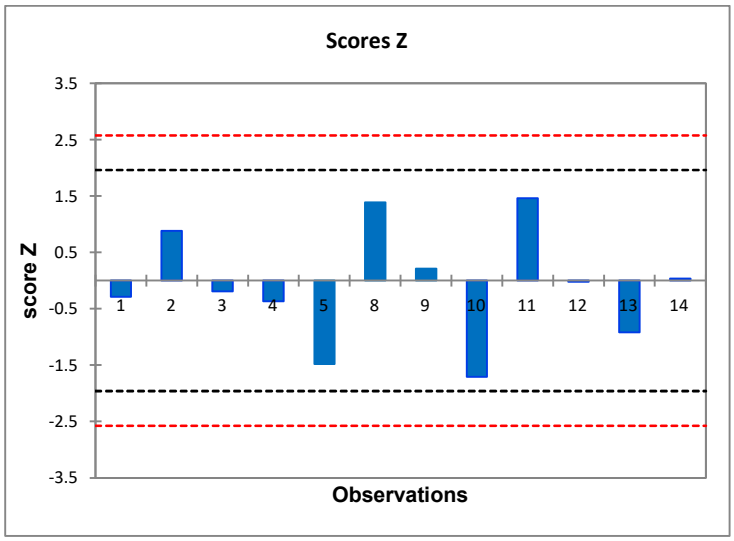

FIG. 5. The Z scores for 5 kGy irradiation measured with participant's routine dosimetry system and compared to actual alanine dose.

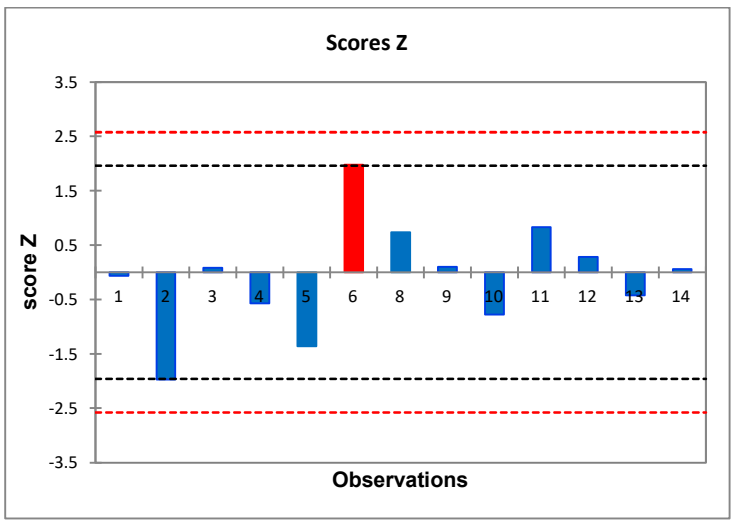

FIG. 6. The Z scores for 10 kGy irradiation measured with participant's routine dosimetry system and compared to actual alanine dose.

19.3.3. Mandel's h and k statistics for measured versus applied dose analysis

Deviations in percentage of participant's measured versus applied doses were investigated here. Table 1 shows all calculated deviations.

TABLE 1. PERCENT DEVIATION BETWEEN MEASURED AND APPLIED DOSE

Participant	Deviation at 1 kGy (%)	Deviation at 5 kGy (%)	Deviation at 10 kGy (%)
1	4.77	-1.97	-0.74
2	1.15	5.93	-22.65
3	4.12	-1.31	0.93
4	-6.23	-2.49	-6.59
5	-11.0	-10.0	-15.56
6	24.8	24.1	22.74
7	35.71	27.34	28.20
8	13.24	9.35	8.43
9	2.1	1.4	1.11
10	-8.95	-11.53	-8.95
11	-7.37	9.84	9.48
12	0.83	-0.174	3.20
13	-17.86	-6.206	-4.85
14	3.67	0.215	0.64

Note that participants 2, 11 and 6, 7 were excluded from Mandel's h and k statistics test respectively.

19.3.3.1. Mandel's h statistics–trueness test

Fig. 7 gives the h statistic calculated for each laboratory. Data for laboratories 2 and 11 were excluded from Mandel's h test calculations. Except for the excluded participant's results, the laboratories show acceptable results in terms of trueness. None of the twelve laboratories show h values higher than the h critical value for consistency (h_{crit} is 1.829). If the h value is greater than h_{crit}, it is concluded that the mean result given by the laboratory concerned is not accurate and reliable. However, when looking at the table of deviations (Table 1), there are several participants with measure doses that are more than 10% different from the actual alanine measured doses as determined by the reference laboratory. This would indicate that there may be dosimetry system calibration issues and issues related to traceability to a national or international dose standard.

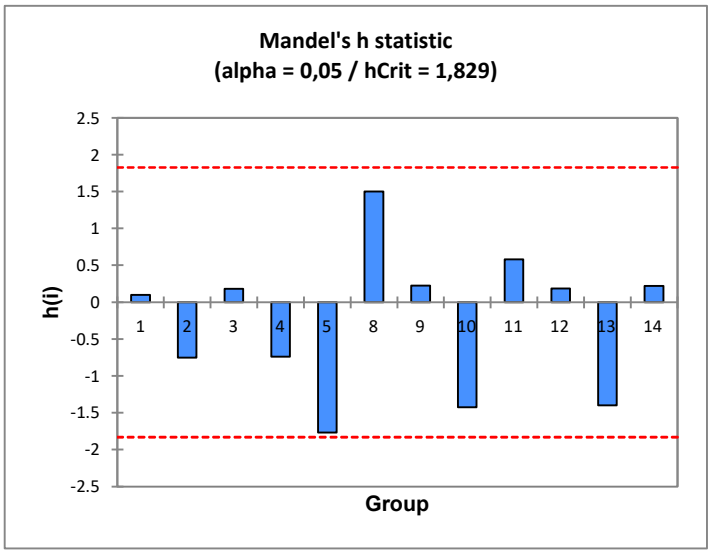

FIG. 7. Mandel's h statistics results.

19.3.3.2. Mandel's k statistics – reproducibility test

Fig. 8 presents the Mandel's k statistic for each laboratory except participants 6 and 7. These results indicate that only a few of the participant were giving reproducible dose measurement results i.e. with a low k value with less than 5% variability in the results. One participant (laboratory 13, highlighted in red) had a high k statistic for the low dose measurement of 1 kGy. This would indicte an inadequacy between the dose measured and the sensitivity of the implemented dosimetry method.

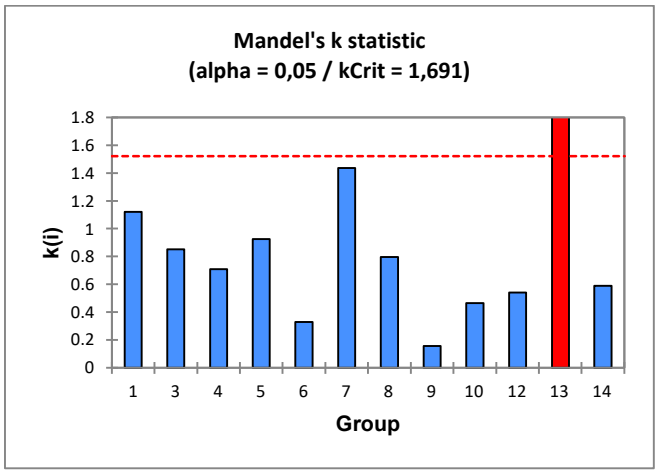

FIG. 8. Mandel's k statistics results.

19.3.4. Z performance parameter

The analysis here takes into account the dose measurement uncertainty given by each participant which helps in assessing the equivalency or significant deviation between the actual dose given by alanine dosimetry (Applied dose) and the dose measured by the participant (measured dose). Table 2 presents each participant's measured doses and stated uncertainties within two standard deviations (k=2). Fig. 9 is a plot of Z scores for each laboratory and is based on the data in Table 2.

TABLE 2. MEASURED DOSES WITH STATED UNCERTAINTIES

Participant	**Applied**		Measured		Z
	Dose (kGy)	Uncertainty, k=2 (kGy)	Dose kGy	Uncertainty, k=2 (kGy)	
1	**1.08**	**0.04**	1.03	0.03	1.0
1	**4.93**	**0.14**	5.03	0.03	-0.7
1	**10.00**	**0.28**	10.10	0.07	-0.3
2	**1.11**	**0.03**	1.10	0.12	0.1
2	**5.47**	**0.15**	5.15	0.03	2.1
2	**8.83**	**0.25**	10.83	0.10	-7.5
3	**1.14**	**0.03**	1.09	0.06	0.7
3	**6.46**	**0.18**	6.54	0.39	-0.2
3	**12.80**	**0.36**	12.70	0.76	0.1
4	**1.37**	**0.04**	1.45	0.12	-0.7
4	**4.86**	**0.14**	4.98	0.40	-0.3
4	**9.47**	**0.27**	10.09	0.81	-0.7
5	**1.80**	**0.05**	2.00	0.00	-3.9
5	**4.45**	**0.12**	4.89	0.00	-3.6
5	**7.82**	**0.22**	9.04	0.00	-5.6
6	**1.33**	**0.04**	1.00	0.10	3.1
6	**6.59**	**0.18**	5.00	0.20	5.8
6	**13.10**	**0.37**	10.10	0.20	7.1
7	**1.87**	**0.05**	1.20	0.19	3.4
7	**6.96**	**0.19**	5.06	0.34	4.9
7	**13.90**	**0.39**	10.00	0.62	5.4
8	**10.90**	**0.39**	10.00	0.56	1.3
8	**1.15**	**0.04**	1.00	0.06	2.1
8	**5.52**	**0.24**	5.00	0.27	1.4
9	**9.97**	**0.28**	9.86	0.42	0.2
9	**4.99**	**0.14**	4.92	0.21	0.3
9	**0.99**	**0.03**	0.97	0.04	0.4
10	**0.32**	**0.01**	0.34	0.02	-1.3
10	**0.35**	**0.01**	0.39	0.02	-1.6
10	**0.49**	**0.01**	0.54	0.03	-1.2
11	**10.60**	**0.37**	9.60	0.10	2.6
11	**5.55**	**0.16**	5.00	0.20	2.2
11	**0.93**	**0.03**	1.00	0.10	-0.7
12	**0.81**	**0.02**	0.80	0.04	0.2
12	**4.79**	**0.13**	4.80	0.22	0.0
12	**9.40**	**0.26**	9.10	0.42	0.6
13	**1.01**	**0.03**	1.19	0.07	-2.3
13	**4.97**	**0.14**	5.28	0.32	-0.9
13	**9.91**	**0.28**	10.39	0.62	-0.7
14	**1.04**	**0.03**	1.00	0.01	1.2
14	**4.98**	**0.14**	4.97	0.10	0.1

Z scores were calculated based on these tabulated data and are plotted in Fig. 9.

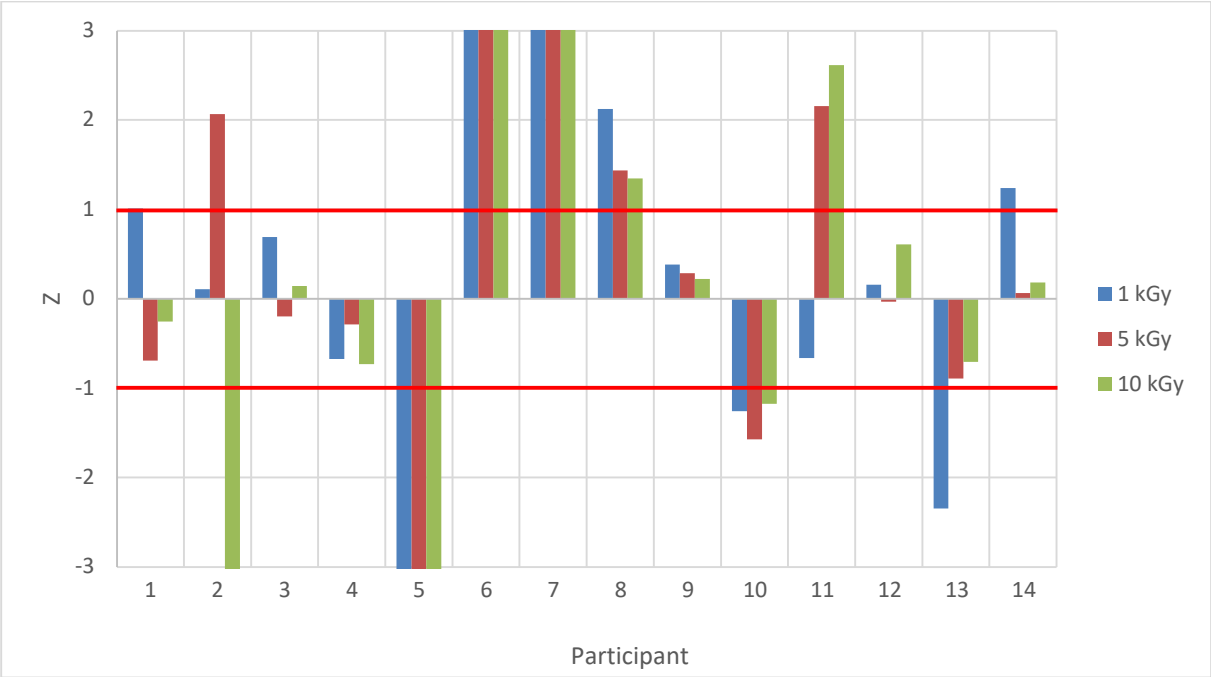

FIG. 9. Z performance parameter for each participant.

When considering the participant's stated dose measurement uncertainty, Fig. 9 indicates that seven out of the fourteen participating laboratories in this study were able to demonstrate their capability at accurately and consistently measuring the dose given to a dosimeter during irradiation.

19.4. CONCLUSION

Over the time frame of the DEXAFI CRP two irradiation/dosimetry inter-comparison exercises were performed. Only the results of the second study are given in this report because there was no significant improvement between the two studies.

The main purpose of this inter-comparisons was to check and help improve the level of confidence in the doses stated by each participant in their reports and publications. The study method was based on verifying the accuracy and precision of the dosimetry systems of the participants compared to an international standard dosimetry system with guaranteed traceability.

This exercise evaluated two performance indicators:

- The ability of participants to meet preset dose values (targeted doses);
- The ability of the participants to measure doses actually applied.

In the light of the results obtained, half of the participants demonstrated that both their irradiation facility and dosimetry system are well qualified and calibrated with traceability to an (inter)national absorbed dose standard. Other participants showed large deviations which may be linked to the use of inadequate dosimetry systems and/or the lack of traceable calibration.

Participants were encouraged to perform an audit of their metrology system(s). A non-exhaustive list of questions that should be answered while auditing the dosimetry laboratory is given here [6]:

- Is dosimetry system calibration performed? When?
- What is the reference dosimetry system used?
- Is it traceable to certified/approved calibration lab?
- Is it in house/ in plant calibration? In calibration irradiation plant? In industrial irradiation plant?
- Does irradiation geometry allow that reference dosimeter dose is equivalent to routine dosimeter dose?
- Has calibration verification been performed?
- Is measurement instrumentation calibrated/verified?
- Did the dosimetry laboratory assess the dose measurement uncertainty?

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Illkirch, France (French Republic): 27–31 March 2017
Bangkok, Kingdom of Thailand: 11–15 June 2018
Virtual (internet): 7–14 April 2021

Consultancy Meeting

Vienna, Republic of Austria: 26–30 May 2014

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